Under or Absent Reporting of Light Stimuli in Testing of Anxiety-Like Behaviors in Rodents: The Need for Standardization

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Behavioral neuroscience tests such as the Light/Dark Test, the Open Field Test, the Elevated Plus Maze Test, and the Three Chamber Social Interaction Test have become both essential and widely used behavioral tests for transgenic and pre-clinical models for drug screening and testing. However, as fast as the field has evolved and the contemporaneous involvement of technology, little assessment of the literature has been done to ensure that these behavioral neuroscience tests that are crucial to pre-clinical testing have well-controlled ethological motivation by the use of lighting (i.e., Lux). In the present review paper, N = 420 manuscripts were examined from 2015 to 2019 as a sample set (i.e., n ∼ 20–22 publications per year) and it was found that only a meager n = 50 publications (i.e., 11.9% of the publications sampled) met the criteria for proper anxiogenic and anxiolytic Lux reported. These findings illustrate a serious concern that behavioral neuroscience papers are not being vetted properly at the journal review level and are being released into the literature and public domain making it difficult to assess the quality of the science being reported. This creates a real need for standardizing the use of Lux in all publications on behavioral neuroscience techniques within the field to ensure that contributions are meaningful, avoid unnecessary duplication, and ultimately would serve to create a more efficient process within the pre-clinical screening/testing for drugs that serve as anxiolytic compounds that would prove more useful than what prior decades of work have produced. It is suggested that improving the standardization of the use and reporting of Lux in behavioral neuroscience tests and the standardization of peer-review processes overseeing the proper documentation of these methodological approaches in manuscripts could serve to advance pre-clinical testing for effective anxiolytic drugs. This report serves to highlight this concern and proposes strategies to proactively remedy them as the field moves forward for decades to come.

Keywords: light/dark test, open field, elevated plus maze, three chamber social interaction test, Lux/lighting, behavioral neuroscience tests, rodent pre-clinical screening/testing, ethologically motivated controls
INTRODUCTION

Over the last decade, there has been a rapid advancement of a more integrative and interdisciplinary neuroscience that has sought to explore structure-function relationships either through brain mapping, (neuro) imaging, establishing and refining the connectome, and/or using cross-species comparisons to better understand the uniqueness of the human brain that orchestrate behavioral outputs (Koch et al., 2010; Goulas et al., 2014; Miranda-Dominquez et al., 2014; Wang et al., 2014; Snyder and Bauer, 2019). Despite these exceptional advancements, prior to this recent boom in the big-technology and big-data revolution in the field of neuroscience, there remained a series of challenges faced by behavioral neuroscientists in trying to address longstanding issues in: relating behavioral observations back to molecular biological targets and exercising caution that molecular data cannot replace the unique adaptive behavioral processes of organisms in response to changing environments (Lederhendler and Schulkin, 2000), mining large datasets (Akil et al., 2011) and in particular bridging data between psychology, ethology, and neuroscience (Marshall, 2009; Gomez-Marin et al., 2014), with long-term goals in understanding the subtleties of social behaviors (Adolphs, 2010; Insel, 2010) that can be complex in nature (Cacioppo and Decety, 2011), and to remain grounded in our understanding of reinforcement learning, conditioning, and its neural correlates (Maia, 2009). What is more concerning is that these challenges remain decades later with little advancement and standardization in their use within the field to address such a critical problem.

This situation may be partially explained by many non-traditionally trained behaviorists engaging in basic behavioral neuroscience tests in an effort to characterize and/or determine traits of specific locomotor activities (i.e., through the use of the Open Field) and anxiety-like behaviors (i.e., through the use of the Elevated Plus Maze, Zero Maze, Light/Dark Box or Light/Dark Test, and the Three Chamber Social Interaction Test). This situation has resulted in an unfortunate reduction in the quality of behavioral neuroscience research (Bespalov and Steckler, 2018) and efforts by other groups to try to make behavioral tests they design more accessible through open-source applications are noteworthy (White et al., 2019). Notably, without ensuring both generalizability and reliability training through open-source efforts to standardize the field, one must seriously question the behavioral neuroscience datasets they review as their interpretations can be quite variable. There seems to be a tendency that as advancements in (bio) technology are made, it inadvertently creates a widening gap between re-establishing important links of behavioral bioassays in neuroscience (Brown and Bolivar, 2018). Thus, researchers in the field that employ behavioral tests to better describe neurobiological phenomena associated with a broad range of animal model systems and associated ecological contexts to diversify the translations of their work (Mathuru et al., 2020) should undergo more training in traditional behaviorism (Thompson, 1994; Moore, 2011) to prevent creating barriers in integrating behavioral and neural datasets (Thompson, 1994; Moore, 2002; Carobrez and Bertoglio, 2005; Ortu and Vaidya, 2016).

One of the basic tenets of the Open Field Test, the Elevated Plus Maze, the Zero Maze, the Light/Dark Test, or the Light/Dark Box, is the use of an aversive bright light to serve as an establishing operation to induce anxiogenic behaviors, whereas, in contrast, the Three Chamber Social Interaction Test uses dim lighting to increase socialization to induce anxiolytic behaviors. These ethologically derived concepts surrounding the intentional utilization of light as the motivational stimuli serve as the foundational basis by which all interpreted anxiety-like behaviors in rodents are reliably confirmed through pre-clinical behavioral neuroscience testing procedures. Thus, the rodent's sensitivity to lighting (i.e., changes in Lux) can serve to set the floor and ceiling thresholds for anxiogenic responses in both typical wild type, transgenic, and/or other mutant rodent models engineered to exhibit face and construct valid social and other associated anxiety-like behavioral traits. However, despite cases where revisiting standards for testing anxiety-like behaviors for the Elevated Plus Maze over a 20-year period have been reported in the literature to increase future face, construct, and predictive validity within an ethological context (Ortu and Vaidya, 2016), it unfortunately, failed to include lighting controls in its 2-decade review of the experimentation. These observations led to the following question: How many publications for these behavioral neuroscience tests actually report the Lux values as their ethological stimulus controls for inducing anxiogenic and/or anxiolytic responses? This question is important to consider, especially if the behavioral neuroscience field continues to produce large amounts of publications without the standardization of Lux through clearly defined controlled lighting as the main experimental control to be the anxiogenic/anxiolytic stimulus to motivate rodents. Furthermore, when such standardization is established, it, in turn, increases the integrity of the science, the internal and external validity, and translational meaning of the work that has and continues to be published and the work forthcoming. Thus, the present study sought to determine what proportion of research studies actually report the lighting/lux values in their reports in an effort to revisit the standards for behavioral neuroscience testing of anxiety-like behaviors in rodents.

METHOD

Database Keyword Search

In order to determine the frequency of publications that used the Open Field Test, the Elevated Plus Maze, the Zero Maze, the Light/Dark Box, or the Light/Dark Test, and the Three Chamber Social Interaction Test, a keyword search for these exact terms were done using Elsevier’s Science Direct for the years 2009–2019 covering a decade of research published through this outlet. The Elsevier’s Science Direct Article Database was used as it had to most journal subscriptions (i.e., 4,620 journals) to cover a broad range to obtain a representative sample of the behavioral neuroscience tests understudy. The years were truncated not to go beyond 2019 to avoid any publication data being skewed due to the coronavirus (COVID-19). However, some papers in
2019 at the time of the search were either in press/forthcoming or published online ahead of print and their actual publication dates occurred in 2020. The total number of the articles returned that met the inclusion criteria were then used to evaluate the trends of the use of each test in the behavioral neuroscience field over the last decade.

**Refined Search and Inclusion/Exclusionary Criteria**

Next, for each of the types of behavioral tests of interest (i.e., Light/Dark Box, the Open Field, the Elevated Plus Maze, and the Three Chamber Social Interaction Test) from 2015 to 2019, covering the latter half of the decade (i.e., since there was a consistent upward trend in these latter years). Using this time-period, the first 22–23 publications from each year met the inclusion criteria were then used to evaluate the trends of the use of each test in the behavioral neuroscience field over the last decade.
Camp et al., 2018; Varghese et al., 2018; Walia et al., 2018, 2019a,b; Wang S. et al., 2018; Wang et al., 2018a,b; Wille-Bille et al., 2018; Zahra et al., 2018; Zhang and Yao, 2018; Zhou et al., 2018; Al-Harraisi et al., 2019; Arnold et al., 2019; Atigari et al., 2019; Bahi and Dreyer, 2019; Basaure et al., 2019; Borrow et al., 2019; Burns et al., 2019; Caliskan et al., 2019; Dempsey et al., 2019; Dixon and Hughes, 2019; Dougherty et al., 2019; Ebrahimi-Ghiri et al., 2019; Elhady et al., 2019; Estrada-Camarena et al., 2019; Faure et al., 2019; Fisch et al., 2019; Freels et al., 2019; Garbarino et al., 2019; García-Díaz et al., 2019; García-Ríos et al., 2019; Gebert and Hannan, 2019; Hatcher et al., 2019; Herbst et al., 2019; Hetzel et al., 2019; Jalilzad et al., 2019; Kosel et al., 2019; Kruse et al., 2019; Kumar et al., 2019; Laureano-Melo et al., 2019; Lee et al., 2019; Lin T. et al., 2019; Lin Y. et al., 2019; Liu et al., 2019; Lopes Andrade et al., 2019; Lovelock and Deak, 2019; Malikowska-Racina et al., 2019; Marks et al., 2019; Matsuou et al., 2019; Medawar et al., 2019; Miao et al., 2019; Miguel et al., 2019; Moreira et al., 2019; Morgan et al., 2019; Munshi et al., 2019; Nakazawa et al., 2019; Neuwirth et al., 2019b; Peleh et al., 2019; Peng et al., 2019; Queiroz et al., 2019; Samad et al., 2019; Sapozhnikova et al., 2019; Scholl et al., 2019; Suleymanova et al., 2019; Tartaglione et al., 2019; Tillmann and Wegener, 2019; Tillmann et al., 2019; Trofimiuk et al., 2019; Tsatsakis et al., 2019; Victoriano et al., 2019; Vieira et al., 2019; Wąsik et al., 2019; Wang G. et al., 2019; Wang L. et al., 2019; Wang H. et al., 2019; Winther et al., 2019; Xiao et al., 2019; Yang et al., 2019; Yuan et al., 2019; Zaccarelli-Magalhães et al., 2019; Zare et al., 2019; Zoeram et al., 2019; Bond et al., 2020; Ng et al., 2020) in which a full PDF was freely accessible were downloaded and their methods section were examined for clear reporting of the ethological lighting used (i.e., ~ ranging from 300 to 1,000 Lux to ensure to light an aversive stimulus for all tests but the Three Chamber Social Interaction Test). The Three Chamber Social Interaction Test requires a low-light stimulus in order to promote social behaviors in approaching other rodents as an anxiolytic stimulus (i.e., ~ ranging from 0 to 30 Lux to prevent freezing and other immobility behaviors that would otherwise interfere with testing). Data were included if the publication reported lighting measures, whereas if the publication did not, it was excluded (i.e., this also excluded any other work that previously cited other work). The number of remaining articles that met the criteria for (i.e., this also excluded any other work that previously cited other work). The number of remaining articles that met the criteria for (i.e., this also excluded any other work that previously cited other work). The number of remaining articles that met the criteria for (i.e., this also excluded any other work that previously cited other work). The number of remaining articles that met the criteria for (i.e., this also excluded any other work that previously cited other work).
Publications Meeting Criteria for Ethologically Controlled Lighting

Lastly, from the $n = 351$ selected publications, using the reporting of lighting as the next inclusion criteria, resulted in $n = 159$ publications returned across the behavioral tests (see
FIGURE 1 | This figure illustrates the flow chart of how the 420 behavioral articles that were searched for to generate an equal representative sample (n = 105 articles of each behavioral test) comprising the Light/Dark Test, Open Field Test, Elevated Plus Maze Test, and the Three Chamber Social Interaction Test were sampled from 2015 to 2019 (n = 20–22 per year; upper dark gray rectangles). Of the N = 420 sampled, only n = 351 met the criteria for animal behavioral test relevance. The publications were then examined for meeting the inclusion criteria for reporting a lighting/Lux value for ethological controls for either anxiogenic or anxiolytic responses (middle light gray rectangles). Next, the refined number of articles were then examined for meeting the inclusion criteria for reporting accurate ranges of lighting/Lux for ethologically relevant stimuli motivation purposes that align with the test’s purpose in the field of behavioral neuroscience (lower light gray rectangles). Through the criteria used, 11.9% of the sampled articles across the behavioral tests were published using appropriate ethological motivational principles (white lowest rectangle) and only n = 50 met the full inclusion criteria, with the Light/Dark Test having better reporting (62.25%), followed by the Open Field (30.09%), then the Elevated Plus Maze (41.41%), and finally the Three Chamber Social Interaction Test with the worst (34.69%) of the respective samples.

Figure 1 middle light gray rectangles). Next, a new refinement criteria of whether the publications clearly noted the use of Lux for the evaluation of ethologically controlled lighting were determined. From this new refinement criteria (see Figure 1 lower gray rectangles) the following data indicate the included number of publications from the previously selected n = 159: the Light/Dark Box Test [n = 29 (Banaskowski et al., 2015; Bentea et al., 2015; Liu et al., 2015; Sauce et al., 2015; Zhang C. et al., 2015; Zhang et al., 2016; Acevedo et al., 2016; Christensen et al., 2016; Farajdokht et al., 2016; Fernandez et al., 2016; Hicks J. A. et al., 2016; Labots et al., 2016; Miranda-Morales and Pautassi, 2016; Vogt et al., 2016; Benoit et al., 2017; Borbely et al., 2017; Chandra Sekhar et al., 2017; Khalil and Fendt, 2017; Makinson et al., 2017; Rogers et al., 2017; Sirohi et al., 2017; Heinla et al., 2018; Keenan et al., 2018; Mahmoudi et al., 2018; Wille-Bille et al., 2018; Zhang and Yao, 2018; Laureano-Melo et al., 2019; Morgan et al., 2019; Tillmann et al., 2019)], the Open Field Test [n = 10 (Zhang C. et al., 2015; Estork et al., 2016; Casarrubea et al., 2017; Kawabe, 2017; Khalil and Fendt, 2017; Kunishi et al., 2017; Blume et al., 2018; Pereia-Rodriguez et al., 2018; Struntz and Siegel, 2018; Hetzler et al., 2019)], the Elevated Plus Maze [n = 4 (Zhang C. et al., 2015; Estork et al., 2016; Wang S. et al., 2018; Moreira et al., 2019)], and the Three Chamber Social Interaction Test [n = 9 (Langley et al., 2015; Lee J. et al., 2015; Nakamura K. et al., 2015; Ferri et al., 2016; Kerr et al., 2016; Mihara et al., 2017; BenekeReddy et al., 2018; Crestani et al., 2018; He et al., 2018)]. Thus, from the original N = 420 publications sampled, only n = 50 met the criteria for ethologically controlled lighting across the behavioral tests evaluated herein, representing 11.9% of the sample studied (see Figure 1 white lowest rectangle).

Statistical Analyses
The descriptive statistics for the number of articles that met the aforementioned inclusion criterion were processed using SPSS version 24 (IBM®, Armonk, NY, United States). The data regarding the number of publications for the Light/Dark Test, Light/Dark Box Test, and the Open Field Test (Figure 2A) and the Elevated Plus Maze, Zero Maze Test, and the Three Chamber Social Interaction Test (Figure 2B) from 2009 to 2019 that were populated from Elsevier’s Science Direct search engine are depicted in Figure 2. The refined data that met the Lux criteria were depicted as Box and Whisker Plots showing the distribution of Lux used in each test (Figure 3). The mean is represented as (X) the median represented as the line within the boxes (-), the inter-quartile ranges (IQRs) were represented as the lower portion of the whisker to the box (IQR 1), the...
lower box to the median (IQR 2), the median to the upper box (IQR 3), and the upper box to the upper whisker (IQR 4; Figure 3).

RESULTS
Trends in the Frequency of Behavioral Tests Used in Publications Over the Last Decade
The publications that were sampled for the behavioral tests of interest over the last decade showed that a large number of publications used the Light/Dark Test ($\text{Mean} = 34,138.55$; $SD = 9,489.16$) and/or the Light/Dark Box Test ($\text{Mean} = 8,383.64$; $SD = 2,194.19$) along with the Open Field test ($\text{Mean} = 51,209.5$; $SD = 14,107.1$; see Figure 2A). Additionally, the use of these behavioral tests increased from 2009 to 2019 to the following levels: the Light/Dark Test (43.45%), the Light/Dark Box Test (45.37%), and the Open Field Test (43.13%). These three behavioral tests represented the most utilized in the neuroscience field. In contrast, the last decade showed a moderate number of publications that used the Elevated Plus Maze ($\text{Mean} = 1,013.82$; $SD = 151.85$) and/or the Zero Maze ($\text{Mean} = 372.55$; $SD = 70.54$) along with the Three Chamber Social Interaction Test ($\text{Mean} = 986.91$; $SD = 291.46$; see Figure 2B), which had the lowest representation. This latter point is most likely due to this behavioral test being the more recent to be introduced to and adopted within the behavioral neuroscience field. Moreover, the use of these behavioral tests increased from 2009 to 2019 to

![Figure 2](image-url)
was in the proper range to motivate the animal to respond and ethologically relevant anxiogenic light stimuli (i.e., lighting ethological motivational principles. The data showed a consistent stimuli parameters being properly used in the field under can help facilitate the assessment of anxiogenic vs. anxiolytic ceiling threshold parameters for establishing operation for their Social Interaction Tests, respectively. This was done to illustrate Open Field, the Elevated Plus Maze, and the Three Chamber distribution of Lux ranges reported for the Light/Dark Test, the data were compiled into a box and whisker plot to depict the criteria for ethologically relevant use of light stimuli (Lux) reported for the Light/Dark Test (L/D T), Open Field Test (OF), the Three Chamber Social Interaction Test (3-CSIT), and the Elevated Plus Maze Test (EPM). The data are presented as Box and Whisker Plots where the mean is represented as (X) the median represented as the line within the boxes (–), the inter-quartile ranges (IQRs) are represented as the lower portion of the whisker to the box (IQR 1), the lower box to the median (IQR 2), the median to the upper box (IQR 3), and the upper box to the upper whisker (IQR 4). The figure also shows a gray dashed line at 300 Lux indicating a threshold for anxiogenic behaviors that occur with light stimuli above this value (red arrow), whereas anxiolytic behaviors occur with light stimuli below the 25 Lux gray dashed line (green arrow). There was one reported outlier for the OF with a Lux of 1,400 reported, otherwise the L/D T, OF, and EPM for the studies that met criteria used comparable Lux as an anxiogenic stimulus range and the 3-CSIT Lux reported were within the anxiolytic stimulus range.

FIGURE 3 | This figure illustrates the number of publications that met the criteria for ethologically relevant use of light stimuli (Lux) reported for the Light/Dark Test (L/D T), Open Field Test (OF), the Three Chamber Social Interaction Test (3-CSIT), and the Elevated Plus Maze Test (EPM). The data are presented as Box and Whisker Plots where the mean is represented as (X) the median represented as the line within the boxes (–), the inter-quartile ranges (IQRs) are represented as the lower portion of the whisker to the box (IQR 1), the lower box to the median (IQR 2), the median to the upper box (IQR 3), and the upper box to the upper whisker (IQR 4). The figure also shows a gray dashed line at 300 Lux indicating a threshold for anxiogenic behaviors that occur with light stimuli above this value (red arrow), whereas anxiolytic behaviors occur with light stimuli below the 25 Lux gray dashed line (green arrow). There was one reported outlier for the OF with a Lux of 1,400 reported, otherwise the L/D T, OF, and EPM for the studies that met criteria used comparable Lux as an anxiogenic stimulus range and the 3-CSIT Lux reported were within the anxiolytic stimulus range.

the following levels: the Elevated Plus Maze (62.29%), the Zero Maze (56.73%), and the Three Chamber Social Interaction Test (42.07%).

Ethologically Relevant Findings From the Included Publications Reported Lux

The n = 50 publications that were included in the final analyses were compiled into a box and whisker plot to depict the distribution of Lux ranges reported for the Light/Dark Test, the Open Field, the Elevated Plus Maze, and the Three Chamber Social Interaction Tests, respectively. This was done to illustrate how researchers in the field are setting the lighting floor and ceiling threshold parameters for establishing operation for their behavioral tests, and when summarized in this illustrative way, can help facilitate the assessment of anxiogenic vs. anxiolytic stimuli parameters being properly used in the field under ethological motivational principles. The data showed a consistent and ethologically relevant anxiogenic light stimuli (i.e., lighting was in the proper range to motivate the animal to respond appropriately) for the Light/Dark Test (Mean Lux = 593.41; Median = 550; SD = 245.82), the Open Field (Mean Lux = 476.5; Median = 300; SD = 345.32), the Elevated Plus Maze (Mean Lux = 337.5; Median = 325; SD = 47.87), and the Three Chamber Social Interaction Test (Mean Lux = 12.67; Median = 10; SD = 8.53; see Figure 3).

DISCUSSION

The results from reviewing the literature on the proper use of lighting controls for ethological motivation in behavioral neuroscience testing revealed that as per this subsample, the majority of the publications did not report Lux or evidence of proper controls for lighting over the last half-decade. This highlights some rather serious concerns for the field, as current researchers using behavioral testing techniques, and prospective training of the next generation of behavioral neuroscientists will need to address this matter head-on. A main tenant in any science is the use of proper controls, minimizing threats to internal validity, and certainly having the foresight to limit and whenever possible, attempt to eliminate extraneous variables. In doing so, the behavioral work conducted will have an increased probability of external, face, construct, and predictive validity. This latter point prevents the unnecessary use of duplication of work, addressing ethical concerns with reducing the number of rodents required for testing research hypotheses, and ultimately serves to advance science in a more efficient and meaningful way; especially, in the behavioral neuroscience field (Russell and Burch, 1959; National Research Council of The National Academies, 2003; Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, Division on Earth and Life Studies, & National Research Council, 2010; Cardon et al., 2012; Office of Laboratory Animal Welfare, 2015). It is important to state that a limitation of the research conducted herein is that the results are limited to an editorial group sampled from one type of journal database and it is possible that the same findings may occur when sampled from other journal databases, but this remains to be tested. Notably, the findings from this study indicate that 11.9% of the sampled papers reported proper use of the Lux in their behavioral testing and this would allow other researchers to evaluate the quality of ethological motivational principles within their tests for determining how it would influence their work. The remaining 88.1% of the papers either failed to mention lighting stimuli or used incorrect lighting measures (i.e., anxiogenic light stimuli in an anxiolytic test or anxiolytic light stimuli in an anxiogenic test). The consequences of not mentioning light conditions or using incorrect lighting are quite different and the proportion of each case remains unknown. Thus, authors are strongly encouraged to measure the lighting for each behavioral test they conduct on anxiety-like behaviors to address this issue. Further, knowing the lighting conditions and then considering the anxiety-like behaviors will serve to aid authors in confirming or disconfirming whether they have observed the animals’ responses to be consistent with what is expected for a given behavioral test when conducted within the appropriate Lux range consistent with prior reports.
From the publications that met the criterion (Figure 3), there seems to be a consistent range of Lux being used for the behavioral tests as follows: the Light/Dark Box Test (300–1,000 Lux), the Open Field Test (300–635 Lux), the Elevated Plus Maze (300–400 Lux), and the Three Chamber Social Interaction Test (0–24 Lux), respectively. In particular, the anxiogenic behavioral tests (i.e., the Light/Dark Box Test, the Open Field Test, and the Elevated Plus Maze) were shown to have publications reporting Lux within overlapping ranges (Garcia et al., 2005, 2011; Miller et al., 2021; Shoji and Miyakawa, 2021). This is a good sign that some researchers are conscientious of the ethological motivational factors and by using the same and/or approximate ranges of Lux that overlap, it permits the ability to have an external, face, construct, and predicative validity for these tests. In addition, the anxiolytic Three Chamber Social Interaction Test showed a broad low range of Lux to promote mobility as it is a key principle and motivating factor to ensure rodents are comfortable and will attempt to engage in movement related to the social operationally defined dependent measures for the respective test. Moreover, what can be extracted from this study is that when researchers use these behavioral tests, they should first determine their lighting stimuli in their respective behavioral testing rooms. If need be, a commercial Lux meter can be easily purchased from the internet (e.g., for $20-$25 USD from www.amazon.com). Thus, there is no cost-prohibitive factor in securing a simple, yet critical, piece of equipment for determining Lux prior to starting a behavioral research study.

However, even though the articles that met the inclusion criteria for each test show logical use of Lux for conducting each of the behavioral tests reviewed, there are still some concerns in variability that arise and should caution how future work should be reported and reviewed carefully. For example, the consistent reporting of species used and sex studied are paramount in understanding anxiety-like behaviors both within and between species as well as sex within a given species. In further review of the articles that met the inclusion criteria (see Table 1) illustrates the following variability in sex reporting that was found: Elevated Plus Maze (25% for males, 25% for females, and 50% for both males and females); Three Chamber Social Interaction Test (50% for males, 25% for both males and females, 12.5% for females, and 12.5% did not specify any sex); Open Field Test (60% for males and 40% for both males and females); and Light/Dark Test (62.07% for males, 10.34% for females, and 24.14% for both males and females, and 3.45% did not specify any sex). Moreover, the variability in species reporting that was found indicated a range of rats, mice, voles, and gerbils. To this end, there is also a need to be cautious of the diurnal/nocturnal biological rhythms, the age, generation, and whether or not the animals were subjected to drug compounds or other genetic manipulation prior to anxiety-like behavioral testing. These factors are equally important to consider whilst ensuring that any extrapolations from testing these particular animal species and manipulated models should include a clear and consistent practice of reporting the sex, testing both sexes, and noting the Lux used.

Another factor for consideration is the issue of testing time (i.e., duration) of the anxiety-like behaviors in these species and sex used as pre-clinical models. In a review of the articles that met the inclusion criteria, it was also observed that variability ranging from as low as 4 min to as high as 30 min was used, but on average many studies reported use of 5 min to 10 min across all behavioral tests evaluated. Another concern arises that in the case of most animals, novelty to a new environment may induce natural neophobic traits that would cause the anxiogenic effect of just being exposed to a new testing environment. Thus, when constraining a behavioral test to provide an index or screening of anxiety-like behaviors, these tests may be skewed towards inflating the anxiogenic response profile as they do not offer ample time for habituation to a novel environment to be assessed carefully. In contrast, when prolonging a behavioral test of anxiety-like behaviors to run longer than 10 min, the inverse problem arises whereby the test may be skewed towards inflating the anxiolytic response profile as they offer too much time to habituate and remain uninterested in the novel environment to be assessed carefully. Thus, the 10 min behavioral testing time (i.e., duration) for a single test session for an animal is the recommended time (i.e., the first 5 min are used for screening anxiogenic responses and the last 5 min are used for screening anxiolytic responses).

Further, lighting becomes equally important when rodents are subjected to sequential behavioral tests that may compound or cause carryover anxiogenic effects [i.e., using the Open Field, then the Elevated Plus Maze, then the Two-Day Hole Board Test, or Context Fear Conditioning Tests, etc. (Neuwirth et al., 2013, 2015, 2017, 2019a; Neuwirth, 2014; Budylin et al., 2019)] and knowing the Lux measures can help to standardize such testing procedures to limit or as best limit artificially inflating the rodent’s anxiogenic neurobehavioral profiles. This is important as behavioral pharmacology or psychopharmacology that is used for such pre-clinical testing may then show either false-positives or false-negatives since they may represent more of an exaggerated behavioral phenotype than a well-intended pre-clinical animal model for screening new anxiolytic medications. In some cases, it may actually behoove researchers to employ the Open Field using an anxiolytic Lux stimulus (i.e., 0–24 Lux consistent with the Three Chamber Social Interaction Test) to promote behavioral movement in a baseline screening effort to assess for traits of hyperactivity or hypoactivity prior to being sequentially tested in the Elevated Plus Maze (Neuwirth et al., 2019a). This thoughtful and intentional behavioral testing methodology may help to better scrutinize whether immobility, freezing, or lack of exploration in the open arms of the Elevated Plus Maze were due to hypoactivity traits that were missed in a prior Open Field Test under anxiogenic lighting, in comparison to the rodent being hyperactive in the Open Field the prior day under anxiolytic lighting. The latter would provide insight into the clear environmental stimuli (e.g., the Elevated Plus Maze) and the lighting stimuli (i.e., anxiogenic effects), creating through the proper ethological motivational controls an actual anxiogenic behavioral response specific to the combined stimuli and not stimuli that were carried over. Additionally, through standardizing the anxiogenic and anxiolytic lighting stimuli for behavioral tests, such lighting parameters can be generalized.
TABLE 1 | Variations in behavioral tests that met criteria for the Lux reported to motivate anxiety-like behaviors.

| Behavioral test                        | Animal model                                                                 | Sex     | Lux reported |
|----------------------------------------|-------------------------------------------------------------------------------|---------|--------------|
| Elevated Plus Maze Test (EPM)          | Long Evans rats (Neuwirth et al., 2019b)                                      | M and F | 300          |
|                                        | Balb/c mice (Estork et al., 2016)                                            | M       | 400          |
|                                        | Balb/c mice and Swiss Webster mice (Moreira et al., 2019)                    | M and F | 250–300      |
|                                        | Mongolian gerbils and Sprague Dawley rats (Wang S. et al., 2018)              | M       | 350          |
| Three Chamber Social Interaction Test  | Mandar in vole (Wang L. et al., 2019)                                         | F       | 20           |
| (3-CSIT)                               | Swiss Webster mice (Crestani et al., 2018)                                    | M       | 20           |
|                                        | Emx.1Cremice & Sprague Dawley rats (Senekareddy et al., 2018)                | N/S     | 24           |
|                                        | C57Bl/6J mice and A/J mice (Mhara et al., 2017)                               | M       | 3–5          |
|                                        | C57Bl/6J mice (Ferri et al., 2016)                                            | M       | 5            |
|                                        | Sprague Dawley rats (Kerr et al., 2016)                                       | M and F | 0            |
|                                        | C57Bl/6J mice and BTBR T + Tpr3tf/J mice (Langley et al., 2015)               | M and F | 20           |
|                                        | C57Bl/6J mice, BTBR T + Tpr3tf/J mice, 129SvL/SvJ mice (Zhang W. et al., 2015) | M       | 16           |
|                                        | Sprayce xg-deficient mice and WT mice (Nakamura K. et al., 2015)              | M       | 10           |
| Open Field Test (OF)                   | Balb/c mice (Estork et al., 2016)                                            | M       | 300          |
|                                        | Sprague Dawley rats (Kunishita et al., 2017)                                  | M       | 300          |
|                                        | C57Bl/6J mice (Khalil and Fendt, 2017)                                        | M and F | 300          |
|                                        | Wistar rats (Casarrubea et al., 2017)                                         | M       | 300          |
|                                        | Wistar rats (Kawabe, 2017)                                                    | M and F | 300          |
|                                        | Sprague Dawley rats (Blume et al., 2018)                                      | M and F | 250–300      |
|                                        | Sprague Dawley rats (Robinson et al., 2018)                                  | M       | 530          |
|                                        | California mice (Perea-Rodriguez et al., 2018)                                | M       | 1,400        |
|                                        | Long Evans rats (Hetzler et al., 2019)                                        | M       | 635          |
|                                        | Long Evans rats (Neuwirth et al., 2019b)                                      | M and F | 300          |
| Light/Dark Test (L/D)                  | CD-1 mice (Banaskowski et al., 2015)                                         | M       | 900          |
|                                        | ICR mice (Liu et al., 2015)                                                   | M       | 300          |
|                                        | C57Bl/6J mice (Zhang C. et al., 2015)                                         | M       | 600          |
|                                        | L heterozygous (L1+/-) mice and WT (L1 +/+) mice (Sauce et al., 2015)         | F       | 300          |
|                                        | XCT/- mice and xCT +/- mice with a C57BL/6J genetic background (Bentea et al., 2015) | M       | 700          |
|                                        | Sprague Dawley rats (Christensen et al., 2018)                                | F       | 1,000        |
|                                        | B6 mice (Hicks J. A. et al., 2016)                                            | M       | 700          |
|                                        | ICR mice (Zhang et al., 2016)                                                 | M and F | 500          |

(Continued)
TABLE 1 | Continued

| Behavioral test | Animal model | Sex | Lux reported |
|-----------------|--------------|-----|--------------|
|                 | C57BL/6 N mice (Vogt et al., 2016) | M   | 600          |
|                 | Wistar rats and Sprague Dawley rats (Fernandez et al., 2016) | M   | 400          |
|                 | C57BL/6J/GtAsd mice & C57BL/6NCrl mice (Labots et al., 2016) | M   | 650          |
|                 | Wistar rats (Acevedo et al., 2016) | M and F | 400          |
|                 | Wistar rats (Miranda-Morales and Pautassi, 2016) | M and F | 400          |
|                 | Wistar rats (Farajdokht et al., 2016) | M   | 1,000        |
|                 | B6.129S6-HorTmr1Ywa Orexin-deficient mice (Khalil and Fendt, 2017) | M and F | 310          |
|                 | SP/NKA, HK1, or the NK1 receptor gene-deleted (Tacr1-/-), Tac1 and Tac4 gene-deficient (Tac1-/- and Tac4 -/-) mice, and C57 BL/6 mice (Borbely et al., 2017) | M   | 800          |
|                 | Swiss Webster mice (Genolet et al., 2017) | M   | 1,000        |
|                 | CD-1 out-bred mice (Makinson et al., 2017) | M and F | 300          |
|                 | Balb/c mice (Chandra Sekhar et al., 2017) | M   | 500          |
|                 | C57BL/6J mice (Rogers et al., 2017) | N/S | 700          |
|                 | Long Evans rats (Sirohi et al., 2017) | M   | 600          |
|                 | Wistar rats (Mahmoudi et al., 2018) | M   | 1,000        |
|                 | C57BL/6Arc mice, SJL mice, Swiss Webster mice, SJL/BL6 mice, C57BL/6N mice, CD-1 mice, and Swiss Webster mice (Keenan et al., 2018) | M | 934          |
|                 | C57BL/6N/Hsd mice, and Balb/cOlaHsd mice (Heinla et al., 2018) | F   | 550          |
|                 | Wistar rats (Wille-Bille et al., 2018) | M and F | 400          |
|                 | ICR mice (Zhang and Yao, 2018) | M   | 400          |
|                 | Swiss Webster mice (Laureano-Melo et al., 2019) | M and F | 400          |
|                 | ICR mice (Morgan et al., 2019) | M    | 350          |
|                 | Wistar rats (Tillmann et al., 2019) | M    | 350          |

Note: N/S, not stated in article.

over into novel or other behavioral tests to induce the same ethological motivational factors to make the rodents elicit specific and well-controlled behavioral responses, that in turn, can be treated with anxiolytic drugs for pre-clinical testing. Thus, with proper care and consideration for standardizing Lux as a measure in all behavioral neuroscience research design and then subsequent testing, better and more rapid advancements into anxiolytic drugs in the pre-clinical stage may become a reality for next-generation behavioral neuroscientists.

CONCLUSION

The field of behavioral neuroscience has had many challenges to overcome during the last few decades. One factor studied herein was the proper use of ethologically motivated Lux/lighting stimuli in the Light/Dark Test, the Open Field, the Elevated Plus Maze (i.e., for proper anxiogenic controls), and the Three Chamber Social Interaction Test (i.e., for proper anxiolytic controls). In a review of a sample set of $N = 420$ publications using these tests (i.e., $n = 100–102$ per test with approximately $n = 20–22$ sampled from each year from 2015 to 2019 prior to the pandemic), there were only $n = 50$ publications that were properly done and specified in their reports the use of Lux. This suggests that if this sample set were to be generalized back to the total publications in the literature that approximately 11%–12% of publications use proper ethologically motivated controls in their behavioral tests. This raises serious concerns for researchers in the area of behavioral neuroscience and perhaps the following
in and employ behavioral neuroscience techniques and methods researchers have the utmost responsibility that if they are to work animal models to be included in their pre-clinical studies. Lastly, needs to be more cognizant and intentional in increasing female their differences in neurophysiological systems. Thus, the field to drugs and drugs may need to be designed specifically to over men. Moreover, males and females respond differently susceptibility, vulnerability, and diagnoses of disorders in women shows largely a male animal model dominated literature. The amount of reports on both male and female animal behaviors that neuroscience methods to consistently report Lux to interpret the other challenges left unaddressed in the area. The goal should be to address one challenge in the behavioral neuroscience field have been actually approached or achieved in a direct effort to occur in another decade to determine whether such standards have been actually approached or achieved in a direct effort to address one challenge in the behavioral neuroscience field so that other precious efforts and resources can be devoted to other challenges left unaddressed in the area. The goal should be for the field to reach 80%–85% of publications using behavioral neuroscience methods to consistently report Lux to interpret the ethologically motivated controls from each report. Additionally, researchers in the field should also seek to achieve an equal amount of reports on both male and female animal behaviors that should also reach 80%–85% of reports as the current situation shows largely a male animal model dominated literature. The latter is a critical point as the clinical literature reports greater susceptibility, vulnerability, and diagnoses of disorders in women over men. Moreover, males and females respond differently to drugs and drugs may need to be designed specifically to their differences in neurophysiological systems. Thus, the field needs to be more cognizant and intentional in increasing female animal models to be included in their pre-clinical studies. Lastly, researchers have the utmost responsibility that if they are to work in and employ behavioral neuroscience techniques and methods in the field then it is their duty to upskill and reskill themselves in fully understanding the importance of ethologically motivated controls for their behavioral tests. This would be no different for someone needing to know the basic principles of proteins to conduct proper Western blots, or reporting the degrees of freedom for any statistical test in a manuscript. So too, the Lux measure ought to be reported in all behavioral neuroscience manuscripts as they should not be an exception. In closing, researchers in this area can find invaluable information from seminal books on behavioral neuroscience testing from a range of classics to modern resources to help fill in any gaps that one might have (Green and Swets, 1966; Underwood, 1966; Gordon et al., 1968; Richelle and Lejune, 1980; Martin, 1997; Plomin et al., 2001; Harrington, 2011; Conn, 2017; Kolb and Whishaw, 2017; Commings, 2018).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MV (Open Field Test), ZH-M (Elevated Plus Maze/Zero Maze), JO (Light/Dark Test and/or Light/Dark box), and OL (Three Chamber Social Interaction Test) conducted the literature search, the sample collection method, and organized and tabulated the data from the respective publications of the behavioral tests reported herein. LN wrote the manuscript. MV, ZH-M, OL, and LN approved the final version of the manuscript to be submitted for publication. All authors contributed to the article and approved the submitted version.

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REFERENCES

Abdellatif, A., Omar, E. H., and Halima, G. (2017). The neuronal basis of copper induced modulation of anxiety state in rat. Acta Histochem. 119, 10–17. doi: 10.1016/j.acthis.2016.10.003
Acevedo, M. B., Fabio, M. C., Fernandez, M. S., and Pautassi, R. M. (2016). Anxiety response and restraint-induced stress differentially affect ethanol intake in female adolescent rats. Neuroscience 334, 259–274. doi: 10.1016/j.neuroscience.2016.08.011
Adolphs, R. (2010). Conceptual challenges and directions for social neuroscience. Neuron 65, 752–767. doi: 10.1016/j.neuron.2010.03.006
Akbar, S., Subhan, F., Karim, N., Aman, U., Ullah, S., Shahid, M., et al. (2017). Characterization of 6-methoxyflavanone as a novel anxiolytic agent: a behavioral and pharmacokinetic approach. Eur. J. Pharmacol. 801, 19–27. doi: 10.1016/j.ejphar.2017.02.047
Akl, H., Martone, M. E., and van Essen, D. C. (2011). Challenges and opportunities in mining neuroscience data. Science 331, 708–712. doi: 10.1126/science.1199305
Al-Harrasi, A., Khan, A., Rehman, N. U., Al-Shidhani, S., Karim, N., Khan, I., et al. (2019). Evidence for the involvement of a gabaergic mechanism in the effectiveness of natural and synthetically modified incense derivatives in neuropharmacological disorders: a computational and pharmacological approach. *Phytochemistry* 163, 58–74. doi: 10.1016/j.phytomedia.2019.04.007

Alihaif, Y., Bagdas, D., Jackson, A., Park, A. J., and Damaj, I. M. (2017). Assessment of nicotine withdrawal-induced changes in sucrose preference in mice. *Pharmacol. Biochem. Behav.* 161, 47–52. doi: 10.1016/j.pbb.2017.08.013

Allah Yar, R., Akbar, A., and Iqbal, F. (2015). Creatine monohydrate supplementation for 10 weeks mediates neuroprotection and improves learning/memory following neonatal hypoxia ischemia encephalopathy in female albino mice. *Brain Res.* 1395, 92–100. doi: 10.1016/j.brainres.2017.08.013

Alves, C. D. S., Frias, H. V., Kirsten, T. B., Cordeiro, F., Bernardi, M. M., and Sufredini, I. B. (2018). *Lofia operculata* fruit aqueous extract induces motor impairments, anxiety-like behavior and testis damage in rats. *J. Ethnopharmacol.* 222, 52–60. doi: 10.1016/j.jep.2018.04.044

Amodeo, L. R., Wills, D. N., Sanchez-Alavez, M., Nguyen, W., Conti, B., and Ehlers, C. L. (2018). Intermittent voluntary ethanol consumption combined with ethanol vapor exposure during adolescence increases drinking and alters other behaviors in adulthood in female and male rats. *Alcohol* 73, 57–66. doi: 10.1016/j.alcohol.2018.04.003

Amos-Kroohs, R. M., Bloor, C. P., Qureshi, M. A., Vorhees, C. V., and Bernard, P., et al. (2015). Effects of developmental exposure to manganese and/or low iron diet: changes to metal transporters, sucrose preference, and neuronal activity. *Neuropharmacology* 105, 478–486. doi: 10.1016/j.neuropharm.2016.02.010

Bątinić, B., Stanković, T., Stephen, M., Kodali, R., Tiruveedhula, V., Li, G., et al. (2018). Attaining in vivo selectivity of positive modulation of α3/2 GABA receptors in rats: a hard task. *Eur. Neuropsychopharmacol.* 28, 903–914. doi: 10.1016/j.euroneuro.2018.05.014

Bausch, A. E., Ehinger, R., Straubinger, J., Zerfass, P., Nann, Y., and Lukowski, R. (2017). Loss of sodium-activated potassium channel slack and FMRP differentially affect social behavior in mice. *Neuroscience* 384, 361–374. doi: 10.1016/j.neuroscience.2018.05.040

Benkareddy, M., Stachiak, T., Bruns, A., Knoflach, F., Von Kienlin, M., Künckeke, E., et al. (2018). Identification of a corticohabenular circuit regulating socially directed behavior. *Biol. Psychiatry* 83, 607–617. doi: 10.1016/j.biopsych.2017.10.032

Benoit, S., Chaumontet, C., Schwarz, J., Cabir-Kiefer, C., and Tome, D. (2017). Mapping in mice the brain regions involved in the anxiolytic-like properties of a caspiotaxine, a triptic peptide derived from bovine asp-2ase. *J. Funct. Foods* 38, 464–473. doi: 10.1016/j.jff.2017.09.014

Bentea, E., Demuyser, T., Van Liefferinge, J., Albertini, G., Deneyer, L., Nys, J., et al. (2015). Absence of system xc- in mice decreases anxiety and depressive-like behavior without affecting sensorimotor function or spatial vision. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 59, 49–58. doi: 10.1016/j.pnpbp.2015.01.010

Bernard, P., Castano, A., Beitzel, C., Carlson, V., and Benke, T. (2015). Behavioral changes following a single episode of early-life seizures support the latent development of an autistic phenotype. *Epilepsy Behav.* 44, 78–85. doi: 10.1016/j.yebeh.2015.01.006

Bertolus, J. B., Nemeth, G., Makowska, I. J., and Weary, D. M. (2015). Rat aversion to sevoflurane and isoflurane. *Appl. Anim. Behav. Sci.* 164, 73–80. doi: 10.1016/j.applanim.2014.12.013

Bespalov, A., and Steckerl, T. (2018). Lacking quality in research: Is behavioral neuroscience affected more than other areas of biomedical science? *J. Neurosci.* 380, 4–9. doi: 10.1016/j.neurem.2017.10.018

Biagioli, A., Anjos-Garcia, T., Ullah, F., Fisher, I., Falconi-Sobrinho, L., Freitas, R., et al. (2016). Neurotochemical validation of an experimental apparatus to evaluate oriented and non-oriented escape behaviours: comparison between the open field arena with a burrow and the circular enclosure of an open-field test. *Behav. Brain Res.* 298, 65–77. doi: 10.1016/j.bbr.2015.10.059

Bialuk, I., Taranta, A., and Winnicka, M. (2018). IL-6 deficiency alters spatial memory in 4- and 24-month-old mice. *Neurobiol. Learn. Mem.* 155, 21–29. doi: 10.1016/j.nlm.2018.06.006

Blankenship, P., Cherep, L., Donaldson, T., Brockman, S., Trainer, A., Yoder, R., et al. (2017). Otothlì dysfunction alters exploratory movement in mice. *Behav. Brain Res.* 325, 1–11. doi: 10.1016/j.bbr.2017.02.031

Blume, S., Nam, H., Luz, S., Bangasser, D., and Bhatnagar, S. (2018). Sex- and age-dependent effects of orexin 1 receptor blockade on open-field behavior and neuronal activity. *Neuroscience* 381, 11–21. doi: 10.1016/j.neuroscience.2018.04.005

Boddren, C., Siestrup, S., Palme, R., Kaiser, S., Sachser, N., and Richter, S. (2018). Evidence-based severity assessment: impact of repeated versus single open-field testing on welfare in C57BL/6J mice. *Behav. Brain Res.* 336, 261–268. doi: 10.1016/j.bbr.2017.08.029

Bond, C. M., Johnson, J. C., Chaudhary, V., McCarthy, E. M., McWhorter, M. L., and Woehrle, N. S. (2020). Perinatal fluoxetine exposure results in social deficits and reduced monoamine oxidase gene expression in mice. *Brain Res.* 1727:146282. doi: 10.1016/j.brainres.2019.06.001

Bonuti, R., and Morato, S. (2018). Proximity as a predictor of social behavior in rats. *J. Neurosci.* Methods 293, 37–44. doi: 10.1016/j.jneumeth.2017.08.027
emotional behavior in rats using the open field and elevated plus maze tests. *J. Ethnopharmacol.* 168, 45–49. doi: 10.1016/j.ep.2015.03.053

Garbarino, V. R., Santos, T. A., Nelson, A. R., Zhang, W. Q., Smolik, C. M., Javors, M. A., et al. (2019). Prenatal metformin exposure or organic cation transporter 3 knock-out curbs social interaction preference in male mice. *Pharmacol. Res.* 140, 21–32. doi: 10.1016/j.phrs.2018.11.013

Garcia, A. N., Bezner, K., Depena, C., Yin, W., and Gore, A. C. (2017). Effect of different illumination levels on rat behavior in the elevated plus-maze. *Physiol. Behav.* 85, 265–270. doi: 10.1016/j.physbeh.2005.04.007

Garcia, A. M., Cardenas, F. P., and Morato, S. (2005). Effect of different chronic fluoxetine, reboxetine and venlafaxine on open-field behavior and spatial memory in rats. *Behav. Brain Res.* 281, 43–54. doi: 10.1016/j.bbr.2014.12.023

Green, D. M., and Swets, J. A. (1966). *Signal Detection Theory and Psychophysics*. New York, NY: John Wiley and Sons, Inc.

Gubert, C., and Hannan, A. J. (2019). Environmental enrichment as an experience-dependent modulator of social plasticity and cognition. *Brain Res.* 1717, 1–14. doi: 10.1016/j.brainres.2019.03.033

Haleem, D. J., Inam, Q., and Haleem, M. A. (2015). Effects of clinically relevant doses of methylenephendate on spatial memory, behavioral sensitization and open field habituation: a time related study. *Behav. Brain Res.* 281, 208–214. doi: 10.1016/j.bbr.2014.12.031

Hanssen, A., Almeida, F., Bandiera, S., Pulcinelli, R., Fragoso, A., Schneider, R., et al. (2017). Taurine restores the exploratory behavior following alcohol withdrawal and decreases BDNF mRNA expression in the frontal cortex of chronic alcohol-treated rats. *Pharmacol. Biochem. Behav.* 161, 6–12. doi: 10.1016/j.pbb.2017.09.001

Harrington, M. (2011). *The Design of Experiments in Neuroscience*, 2nd Edn. Thousand Oaks, CA: Sage Publishers

Hatcher, K., Willing, J., Chiang, C., Rattan, S., Flaws, J., Mahoney, M., et al. (2019). Exposure to di-2-ethylhexyl phthalate transgenerationally alters anxiety-like behavior and amygdala gene expression in adult male and female mice. *Physiol. Behav.* 207, 7–14. doi: 10.1016/j.physbeh.2019.04.018

He, Z., Guo, Q., Yang, Y., Wang, L., Zhang, S., Yuan, W., et al. (2018). Pre-weaning paternal deprivation impairs social recognition and alters hippocampal neurogenesis and spine density in adult mandarin voles. *Neurobiol. Learn. Mem.* 155, 452–462. doi: 10.1016/j.nlm.2018.09.006

Hegde, S., Ji, H., Oliver, D., Patel, N., Poupore, N., Shuttman, M., et al. (2016). PDE11A regulates social behaviors and is a key mechanism by which social experience sculpts the brain. *Neuroscience* 355, 151–169. doi: 10.1016/j.neuroscience.2016.08.019

Heinla, I., Ahlgren, J., Vasar, E., and Voikar, V. (2018). Behavioural characterisation of C57BL/6N and BALB/c female mice in social home cage - effect of mixed housing in complex environment. *Physiol. Behav.* 188, 32–41. doi: 10.1016/j.physbeh.2018.01.024

Henbid, M., Marks, W., Collins, M., Cain, S., Soutch, T., Howland, J., et al. (2017). Sociality impairments in genetic absence epilepsy rats from strabagloss: reversal by the t-type calcium channel antagonist Z944. *Exp. Neurol.* 296, 16–22. doi: 10.1016/j.expneurol.2017.06.022

Herbst, L., Gaithger, T., Siqueira, A., Joca, S., Sampao, K., Beijamini, V., et al. (2019). New evidence for refinement of anesthetic choice in procedures preceding the forced swimming test and the elevated plus-maze. *Behav. Brain Res.* 368:111897. doi: 10.1016/j.bbr.2019.04.011

Hetzler, B. E., McIester-Davis, L. W. Y., and Tenpas, S. E. (2019). Methylphenidate and alcohol effects on flash-evoked potentials, body temperature and behavior in Long-Evans rats. *Physiol. Behav.* 198, 121–126. doi: 10.1016/j.yhbeh.2016.06.010

Hicks, J. A., Hatzidis, A., Arruda, N. L., Gelineau, R. R., Monteiro De Pina, I., Hetzler, B. E., Mclester-Davis, L. W. Y., and Tenpas, S. E. (2019). Methylphenidate illumination levels on rat behavior in the elevated plus-maze. *Physiol. Behav.* 171–177. doi: 10.1016/j.physbeh.2010.09.032

Hicks, J. A., Holtz, B. E., Mosquera, D., Falbo, N., Amador, M., and Lee, R. G. (2018). NOAEL-dose of a neonicotinoid pesticide, clothianidin, acutely induce anxiety-like behaviors and amygdala gene expression in adult male mice. *Physiol. Behav.* 207, 7–14. doi: 10.1016/j.physbeh.2019.04.018

Holubová, A., Mikulecká, A., Pometlová, M., Nohejlová, K., and Šlamberová, R. (2018). Long-term early life adverse experience impairs responsiveness to exteroceptive stimuli in adult rats. *Behav. Processes* 149, 59–64. doi: 10.1016/j.beproc.2018.02.005
Horii, Y., and Kawaguchi, M. (2015). Higher detection sensitivity of anxiolytic effects of diazepam by ledge-free open arm with opaque walled closed arm elevated plus maze in male rats. Behav. Brain Res. 294, 131–140. doi: 10.1016/j.bbr.2015.07.059

Horsley, R., Lhotkova, E., Hajkova, K., Jursak, B., Kuchar, M., Palenicek, T., et al. (2018). Detailed pharmacological evaluation of methoxetamine (MXE), a novel psychoactive ketamine analogue—behavioural, pharmacokinetic and metabolic studies in the Wistar rat. Brain Res. Bull. 126, 102–110. doi: 10.1016/j.brainresbull.2016.05.002

Hsieh, L., Wan, J. H., Miyares, L., Lombroso, P. J., and Bordey, A. (2017). Outbred CD1 mice are as suitable as inbred C57BL/6J mice in performing social tasks. Neurosci. Lett. 637, 142–147. doi: 10.1016/j.neulet.2016.11.035

Hughes, R. N., and Hamilton, J. J. (2018). Sex-dependent modification by chronic caffeine of acute methamphetamine effects on anxiety-related behavior in rats. Behav. Brain Res. 350, 38–39. doi: 10.1016/j.bbr.2018.02.013

Hughes, R. N., and Hancock, N. J. (2016). Strain-dependent effects of acute caffeine on anxiety related behavior in P2VG/c long-evans and wistar rats. Pharmacol. Biochem. Behav. 140, 51–61. doi: 10.1016/j.pbb.2015.11.005

Insel, T. R. (2010). The challenge of translation in social neuroscience: a review of oxytocin, vasopressin and affiliative behavior. Neurosci. 65, 768–779. doi: 10.1016/j.neuren.2010.03.005

Iqbal, S., Ali, M., and Iqbal, F. (2015). Long term creatine monohydrate supplementation, following neonatal hypoxic ischemic insult, improves neumuscular coordination and spatial learning in male albino mouse. Brain Res. 1603, 76–83. doi: 10.1016/j.brainres.2014.10.006

Jacobskind, J. S., Rosinger, Z. J., Gonzalez, T., Zuloaga, K. L., and Zuloaga, D. G. (2017). Sub-anxiolytic and anxiolytic-like activity of sodium selenite after acute treatment in mice. Pharmacol. Rep. 69, 276–280. doi: 10.1016/j.pharep.2016.11.005

Kerr, D. M., Giltmartin, A., and Roche, M. (2016). Pharmacological inhibition of fatty acid amide hydrolase attenuates social behavioural deficits in male rats prenatally exposed to valproic acid. Pharmacol. Res. 113, 228–235. doi: 10.1016/j.phrs.2016.08.033

Ketcha Wanda, G. J. M., Djougue, S., Gamo, F. Z., Ngitedom, S. G., and Njamen, D. (2015). Anxiolytic and sedative activities of aqueous leaf extract of Dichrocephala integrifolia (Asteraceae) in mice. J. Ethnopharmacol. 176, 494–498. doi: 10.1016/j.jep.2015.11.035

Khali, R., and Fendt, M. (2017). Increased anxiety but normal fear and safety learning in orecin-deficient mice. Behav. Brain Res. 320, 210–218. doi: 10.1016/j.bbr.2016.12.007

Khali, S., Khalifa, H., Abdel-Motal, S., Mohammed, H., Eleya, W., Mahmoud, et al. (2018). Spirulina platensis attenuates the associated neurobehavioral and inflammatory response impairments in rats exposed to lead acetate. Ecotoxicol. Environ. Saf. 157, 255–265. doi: 10.1016/j.ecos.2018.03.068

Kigar, S. L., Chang, L., and Auger, A. P. (2015). Gadd45b is an epigenetic regulator of juvenile social behavior and alters local pro-inflammatory cytokine production in the rodent amygdala. Brain Behav. Immun. 46. 60–69. doi: 10.1016/j.bbi.2015.02.018

Koch, K., Wagner, G., Dahmke, R., Schachtzabel, C., Gullmar, D., Reichenbach, J. R., et al. (2010). Structure-function relationships in the context of reinforcement-related learning: a combined diffusion tensor imaging-functional magnetic resonance imaging study. Neuroscience 168, 190–199. doi: 10.1016/j.neuroscience.2010.03.026

Kochenberger, L., Levone, B., Da Silva, E., Tascheto, A., Terenzini, M., Paschoalini, M., et al. (2014). The microinjection of a cannabinoid agonist into the accumbens shell induces anxiogenesis in the elevated plus-maze. Pharmacol. Biochem. Behav. 124, 160–166. doi: 10.1016/j.pbb.2014.05.017

Kohl, B., and Whishaw, I. (2017). Brain Behaviour: Revisiting the Classic Studies. Thousand Oaks, CA: Sage Publications.

Komaki, A., Hoseini, F., Shahidi, S., and Baharlouei, N. (2016). Study of the effect of extract of Thymus vulgaris on anxiety in male rats. J. Tradit. Complement. Med. 6, 257–261. doi: 10.1016/j.jtcme.2015.01.001

Kosar-Nasab, M., Shokouhi, G., Ghorbanibaghjo, A., Abbasi, M. M., and Salari, A. (2018). Anxiolytic and antidepressant-like effects of silimarín compared to diazepam and fluoxetine in a mouse model of mild traumatic brain injury. Toxicol. Appl. Pharm. 338, 159–173. doi: 10.1016/j.taap.2017.11.012

Kose, F., Munoz, P. T., Yang, J. R., Wang, A. A., and Franklin, T. B. (2019). Age-related changes in social behaviours in the 5xFAD mouse model of Alzheimer’s disease. Behav. Brain Res. 362, 160–172. doi: 10.1016/j.bbr.2019.01.029

Kratzmann, N., Getselter, D., and Elliott, E. (2016). Sodium butyrate attenuates social behavior deficits and modifies the transcription of inhibitory/excitatory genes in the frontal cortex of an autism model. Neuropharmacology 102, 136–145. doi: 10.1016/j.neuropharm.2015.11.003

Kruse, M., Vadillo, M., Miguel Fernandez, A., Rey, M., Zanutto, B., and Coirini, H. (2019). Sucrose exposure in juvenile rats produces long-term changes in fear memory and anxiety-like behavior. Psychoneuroendocrinology 104, 300–307. doi: 10.1016/j.psyneuen.2019.03.016

Kumar, D., Gupta, S., Ganeshpurkar, A., Singh, R., Kumar, D., Das, N., et al. (2019). Biological profiling of piperazinediones for the management of anxiety. Pharmacol. Biochem. Behav. 176, 63–71. doi: 10.1016/j.pbb.2018.11.009

Kumar, H., and Sharma, B. (2016a). Memantine ameliorates autistic behavior, biochemistry blood brain barrier impairments in rats. Brain Res. Bull. 124, 27–39. doi: 10.1016/j.brainresbull.2016.03.013

Kumar, H., and Sharma, B. (2016b). Minocycline ameliorates prenatal valproic acid induced autistic behavior, biochemistry and blood brain barrier impairments in rats. Brain Res. 1630, 83–97. doi: 10.1016/j.brainres.2015.10.052

Kumar, H., Sharma, B., and Sharma, B. (2015). Benefits of agomelatine in behavioral, neurochemical and blood brain barrier alterations in prenatal valproic acid induced autism spectrum disorder. Neurochem. Int. 91, 34–45. doi: 10.1016/j.neuint.2015.10.007

Kunishi, H., Ichisaka, S., Yamamoto, M., Ikubo, N., Matsuda, S., Futora, E., et al. (2017). Early deprivation increases high-leaning behavior, in the open field test in rats. Neurosci. Res. 123, 27–35. doi: 10.1016/j.neures.2017.04.012

Kyratsou, E., Manfri, G., Spadaro, J., Nguyen, H., Harst, I., and Homberg, J. (2017). Anxiety and risk assessment-related traits in a rat model of Spinocerebellar ataxia type 17. Behav. Brain Res. 321, 106–112. doi: 10.1016/j.bbr.2016.12.023
Labots, M., Zheng, X., Moattari, G., Lozeman-van’t Klooster, J. G., Baars, J. M., Hesseling, P., et al. (2016). Substrain and light regime effects on integrated anxiety-related behavioral z-scores in male C57BL/6 mice hypomagnesaemia has only a small effect on avoidance behavior. Behav. Brain Res. 306, 71–83. doi: 10.1016/j.bbr.2016.01.060

Lamentagne, S., Olmstead, M., and Menard, J. (2016). The lateral septum and anterior hypothalamic axis in tandem to regulate burying in the shock-probe test but not open-arm avoidance in the elevated plus-maze. Behav. Brain Res. 314, 16–20. doi: 10.1016/j.bbr.2016.07.034

Langley, E., Krykbaeva, M., Blusztnaj, J., and Mellott, T. (2015). High maternal restraint stress on social behaviors and the number of hypothalamic oxytocin neurons in male rats. Neuropeptides 60, 21–28. doi: 10.1016/j.npep.2016.08.011

Li, K., Lund, E., and Voigt, J. (2016). The impact of early postnatal environmental enrichment on maternal care and offspring behaviour following weaning. Behav. Processes 122, 51–58. doi: 10.1016/j.beproc.2015.11.008

Lin, T., Lo, Y., Lin, H., Li, S., Lin, S., Chen, K. C., et al. (2019). MR imaging central thalamic deep brain stimulation restored autistic-like social deficits in the rat. Brain Stimul. 12, 1410–1420. doi: 10.1016/j.brs.2019.07.004

Lin, Y., Lin, Y., Chen, K. C., Yang, Y. K., and Hsiao, Y. (2019). Collapsin response mediator protein 5 (CRMP5) causes social deficits and accelerates memory loss in an animal model of Alzheimer’s disease. Neuropharmacology 157:107673. doi: 10.1016/j.neuropharm.2019.107673

Listowska, M., Glac, W., Grembecka, B., Grzybowska, M., and Wrona, D. (2015). Changes in blood CD4+T and CD8+ T lymphocytes in stressed rats pretreated chronically with desipramine or more pronounced after chronic open field stress challenge. J. Neuroimmunol. 282, 54–62. doi: 10.1016/j.jneuroim.2015.02.015

Liu, J., Zhao, W., Yang, Y., Shi, J., Liu, Q., Liu, G., et al. (2015). GABA and 5-HT systems are implicated in the anxiety-like effect of spinosin in mice. Pharmacol. Biochem. Behav. 128, 41–49. doi: 10.1016/j.pbb.2014.11.003

Liu, L., Zhang, L., Wang, T., and Chen, L. (2019). Dopamine D1 receptor in the medial prefrontal cortex mediates anxiety-like behaviors induced by blocking glutamatergic activity of the ventral hippocampus in rats. Brain Res. 1704, 59–67. doi: 10.1016/j.brainres.2018.09.024

Livingston-Thomas, J. M., Jeffers, M. S., Nguemeni, C., Shoichet, M. S., Morrishead, C. M., Corbett, D., et al. (2015). Assessing cognitive function following medial prefrontal stroke in the rat. Behav. Brain Res. 294, 102–110. doi: 10.1016/j.bbr.2015.07.053

Lopes Andrade, A., Dias Ribeiro Figueiredo, D., Torequl Islam, M., Viana Nunes, A., Da Conceição Machado, K., Da Conceição Machado, K., et al. (2019). Toxicological evaluation of the biflavonoid, agathisflavone in albino Swiss mice. Biomed. Pharmacother. 110, 68–73. doi: 10.1016/j.biopha.2018.11.050

López Rivilli, M., Turina, A., Bignante, E., Molina, V., Perillo, M., Briñon, M., et al. (2018). Synthesis and pharmacological evaluation of pyrazol(4,3-c)quinolines as high affinity GABAA-R ligands and potential anxiolytics. Bioorg. Med. Chem. 26, 3967–3974. doi: 10.1016/j.bmc.2018.11.005

López-Cruz, L., Carbó-Gas, M., Pardo, M., Bayarrí, P., Valverde, O., Ledent, C., et al. (2017). Adenosine A2A receptor deletion affects social behaviors and anxiety in mice: involvement of anterior cingulate cortex and amygdala. Behav. Brain Res. 321, 8–17. doi: 10.1016/j.bbr.2016.12.020

Loveck, D. F., and Deak, T. (2019). Acute stress imposed during adolescence yields heightened anxiety in Sprague Dawley rats that persists into adulthood: sex differences and potential involvement of the medial amygdala. Brain Res. 1723:146392. doi: 10.1016/j.brainres.2019.14.6392

Macedo, G., Moreira, G., Domínguez, L., Favoretto, C., Suchecki, D., Quadros, L., et al. (2018). Consequences of continuous social defeat stress on anxiety- and depressive-like behaviors and ethanol reward in mice. Horm. Behav. 97, 154–161. doi: 10.1016/j.yhbeh.2017.10.007

Machado, T., Alves, G., Quinteiro-Filho, W., and Palermo-Neto, J. (2017). Cohabitation with an Ehrlich tumor-bearing cagemate induces immune but not behavioral changes in male mice. Physiol. Behav. 169, 82–89. doi: 10.1016/j.physbeh.2016.11.022

Mahmoud, J., Mohaddes, G., Erfani, M., Sadigh-Eteghad, S., Karimi, P., Rajabi, M., et al. (2018). Cerebrolysin attenuates hyperalgesia, photophobia and neuroinflammation in a nitroglycerin-induced migraine model in rats. Brain Res. Bull. 140, 197–204. doi: 10.1016/j.brainresbull.2018.05.008

Maia, T. V. (2009). Reinforcement learning, conditioning and the brain: successes and challenges. Cogn. Affect. Behav. Neurosci. 9, 343–364. doi: 10.3758/CABN.9.4.343

Makinson, R., Lloyd, K., Rayasam, A., McKee, S., Brown, A., Barila, G., et al. (2017). Evaluation of the activity of bupropion hydrochloride in male CD-1 mice. Neurosci. Lett. 610, 1–6. doi: 10.1016/j.neulet.2016.11.011

Malikowska, M., and Saat, K. (2017). Evaluation of the activity of hypoxia in a mouse model of posttraumatic stress disorder using forced swim and elevated plus maze tests. Eur. Neuropsychopharmacol. 27, S768–S769. doi: 10.1016/S0924-977X(17)31403-7
National Research Council of The National Academies (2003). Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. Washington, DC: National Academies Press.

Neuwirth, L. S. (2014). The characterization of Pb2+ toxicity in rat neural development: an assessment of Pb2+ effects on the GABA shift in neural networks and implications for learning and memory disruption. Dissertations, Theses, and Capstone Projects. CUNY Academic Works. Available online at: https://academicworks.cuny.edu/ge_etds/82/.

Neuwirth, L. S., Emenike, B. U., Barrera, D. E., Hamed, N., Ribi, S., Dacius, T. F., Jr., et al. (2019a). Assessing the anxiotypic properties of tauire-derived compounds in rats following developmental lead exposure: a neurodevelopmental and behavioral pharmacological pilot study. *Adv. Exp. Med. Biol.* 1155, 801–819. doi: 10.1007/978-981-13-8023-5_69

Neuwirth, L. S., Masood, S., Anderson, D. W., and Schneider, J. S. (2016b). The effect of 2 phenylethynyl butyltellurium in mice. *Neuropharmacology* 113, 502–510. doi: 10.1016/j.neuropharm.2015.05.002

Ortu, D., and Vaidya, M. (2016). The challenges of integrating behavioral and neural data: bridging and breaking boundaries across levels of analysis. *Behav. Alan.* 40, 209–224. doi: 10.1007/s00416-016-0074-5

Palotai, M., and Telegdy, G. (2016). Anxiolytic effect of the GPR103 receptor agonist peptide P550 (homolog of neuropeptide 26RFa) in mice. Involvement of neurotransmitters. *Peptides* 82, 20–25. doi: 10.1016/j.peptides.2015.10.004

Peleh, T., Ike, K. G. O., Wams, E. J., Lebois, E. P., and Hengerer, B. (2019). The reverse translation of a quantitative neuropsychiatric framework into preclinical studies: focus on social interaction and behavior. *Neurosci. Biobehav. Rev.* 97, 96–111. doi: 10.1016/j.neubiorev.2018.07.018

Peng, H., Tsai, T., Huang, W., Wu, H., and Hsu, K. (2019). Probic treatment restores normal developmental trajectories of fear memory retention in maternally separated infant rats. *Neuropharmacology* 153, 53–62. doi: 10.1016/j.neuropharm.2019.04.026

Perea-Rodriguez, J., Zhao, M., Harris, B., Raqueno, J., and Saltzman, W. (2018). Behavioral and endocrine consequences of placotaphia in male California mice (*Peromyscus californicus*). *Physiol. Behav.* 188, 283–290. doi: 10.1016/j.physbeh.2018.02.022

Pereira, C., Pardo, M. R., Morales, Y., Dominguez, N., Arnau, M. R., and Borjes, R. (2015). Mice lacking chronogranins exhibit increased aggressive and depression-like behaviour. *Behav. Brain Res.* 278, 98–106. doi: 10.1016/j.bbr.2014.09.022

Plomin, R., DeFries, J. C., McClearn, G. E., and McGuffin, P. (2001). *Behavioral Genetics*. 4th Edn. New York, NY: Worth Publishers.

Provenzano, G., Chelini, G., and Bozzi, Y. (2017). Genetic control of social behavior: Lessons from mutant mice. *Behav. Brain Res.* 325, 237–250. doi: 10.1016/j.bbr.2016.11.005

Pyrydellis, M., Pautassi, R., and Justel, N. (2016a). Open field exposure facilitates recovery from an aversive emotional event: involvement of adrenergic and cholinergic transmitter systems. *Neurosci. Lett.* 633, 202–209. doi: 10.1016/j.neulet.2016.09.015

Pyrydellis, M., Pautassi, R., Mustaca, A., and Justel, N. (2016b). Cholinergic transmission underlies modulation of frustration by open field exposure. *Pharmacol. Biochem. Behav.* 140, 8–16. doi: 10.1016/j.pbb.2015.10.017

Purvis, E., Klein, A., and Ettenberg, A. (2018). Lateral habenular norepinephrine contributes to states of arousal and anxiety in male rats. *Behav. Brain Res.* 347, 108–115. doi: 10.1016/j.bbr.2018.03.012

Queiroz, M. P., da Silva Lima, M., Tavares de Melo, M. F. F., Bertozzo, C. C. M. S., Fernandes de Araújo, D., Guerra, C. G. B., et al. (2019). Maternal supplementation with conjugated linoleic acid reduce anxiety and lipid peroxidation in the offspring brain. *J. Affect. Disord.* 243, 75–82. doi: 10.1016/j.jad.2018.09.020

Quines, C. B., Da Rocha, J. T., Sampaio, T. B., Pesarico, A. P., Neto, J. S., Zeni, G., et al. (2015). Involvement of the serotoninergic system in the anxious-like effect of 2 phenylethyl butyrylfflurium in mice. *Behav. Brain Res.* 277, 221–227. doi: 10.1016/j.bbr.2014.05.071

Rafati, A., Erfanizadeh, M., Noorafshan, A., and Karbalay-Doust, S. (2015). Effect of 2-phenylethynyl butyltellurium on the cerebellar structure and behavioral characteristics in rats. *Neuropharmacology* 82, 20–25. doi: 10.1016/j.neuropharm.2015.11.001

Raulinh, A., and Curran-Raulh, M. (2018). 17 β-Estradiol exacerbates methamphetamine-induced anxiety-like behavior in female mice. *Neurosci. Lett.* 681, 44–49. doi: 10.1016/j.neulet.2018.05.025

Reilly, M., Weeks, C., Topper, V., Thompson, L., Crews, D., and Gore, A. (2015). The effects of prenatal PCBs on adult social behavior in rats. *Horm. Behav.* 73, 47–55. doi: 10.1016/j.yhbeh.2015.06.002

Reimer, A., de Oliveira, A., Diniz, J., Hoexter, M., Chiavegatto, S., and Brandão, M. (2015). Rats with differential self-grooming expression in the
elevated plus-maze do not differ in anxiety-related behaviors. Behav. Brain Res. 292, 370–380. doi: 10.1016/j.bbr.2015.06.036
Reinhart, F., Massi, N., Torres, N., Chabrol, C., Molet, J., Johnstone, D., et al. (2017). The behavioural and neuroprotective outcomes when 670nm and 810nm near infrared light are applied together in MPTP-treated mice. Neurosci. Res. 117, 42–47. doi: 10.1016/j.neures.2016.11.006
Richelle, M., and Leyune, H. (1980). Time in Animal Behavior. Oxford, England: Pergamon Press.
Rico, J., Penagos-Gil, M., Castañeda, A., and Corredor, K. (2016). Gerbils exhibit stable open-arms exploration across repeated testing on the elevated plus-maze. Behav. Processes 122, 104–109. doi: 10.1016/j.beproc.2015.11.017
Rilett, K., Friedel, M., Ellefjood, J., Mackenzie, R., Lerch, J., and Foster, J. (2015). Loss of T cells influences sex differences in behavior and brain structure. Brain Behav. Immun. 46, 249–260. doi: 10.1016/j.bbi.2015.02.016
Robinson, L., Spruit, B., and Riedel, G. (2018). Between and within laboratory reliability of mouse behaviour recorded in home-cage and open-field. J. Neurosci. Methods 300, 10–19. doi: 10.1016/j.jneumeth.2017.11.019
Rodrigues Tavares, L., Baptista-de-Souza, D., and Canto-de-Souza, A. (2016). The benzodiazepine midazolam acts on the conditioned contextual stimuli. Pharmocol. Biochem. Behav. 159, 76–83. doi: 10.1016/j.pbb.2017.07.011
Sanna, M. D., Ghelardini, C., Thurmond, R. L., Masini, E., and Galeotti, N. (2017). Behavioural phenotype of histamine H4 receptor knockout mice: focus on central neuronal functions. Neuropharmacology 114, 48–57. doi: 10.1016/j.neuropharm.2016.11.023
Santangelo, A., Provensi, G., Costa, A., Blandina, P., Ricca, V., Crescimanno, G., et al. (2017). Brain histamine depletion enhances the behavioural sequences complexity of mice tested in the open-field: partial reversal effect of the dopamine D2/D3 antagonist sulpiride. Neuropharmacology 113, 533–542. doi: 10.1016/j.neuropharm.2016.11.007
Santos, P., Herrmann, A., Benvenuti, R., Noetzold, G., Gongio, F., Gama, C., et al. (2017). Anxiolytic properties of N-acetylcysteine in mice. Behav. Brain Res. 317, 461–469. doi: 10.1016/j.bbr.2016.10.010
Sapozhnikova, T., Borisевич, V., Kireeva, D., Gubrakhanmanova, S., Khisamutdinova, R., Makara, N., et al. (2019). Effects of novel hexahydropyrimidine derivatives as potential ligands of M1 muscarinic acetylcholine receptor on cognitive function, hypoxia-induced lethality and oxidative stress in rodents. Behav. Brain Res. 375:112109. doi: 10.1016/j.bbr.2019.11.010
Saré, R., Levine, M., Hildreth, C., Picchioni, D., and Smith, C. (2016). Chronic sleep restriction during development can lead to long-lasting behavioural effects. Physiol. Behav. 155, 208–217. doi: 10.1016/j.physbeh.2015.12.019
Sauce, B., Wass, C., Netrakanti, M., Saylor, J., Schachner, M., and Matzel, L. D. (2015). Heterozygous L1 deficient mice express an autism-like phenotype. Behav. Brain Res. 292, 432–442. doi: 10.1016/j.bbr.2015.05.040
Schambra, U., Nunley, K., Harrison, T., and Lewis, C. (2016). Consequences of low or moderate prenatal ethanol exposures during gestation or neuroinflammation for open field activity and emotionality in mice. Neurotoxicol. Teratol. 57, 39–53. doi: 10.1016/j.ntt.2017.05.001
Scheib, C., Cseko, K., Borbely, A., Abraham, I., Csernus, V., Gaszner, B., et al. (2017). Higher susceptibility of somatostatin 4 receptor gene-deleted mice to chronic stress induced behavioral and neuroendocrine alterations. Neurosci. 346, 320–336. doi: 10.1016/j.neuroscience.2017.01.039
Scheinert, R. B., Haeri, M. H., Lehmann, M. L., and Herkenham, M. (2016). Therapeutic effects of stress programmed lymphocytes transferred to chronically stressed mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 70, 177–185. doi: 10.1016/j.pnpbp.2016.04.010
Schindler, N., Mayer, J., Saenger, S., Gimsa, U., Walz, C., Brenmoehl, J., et al. (2017). Phenotype analysis of male transgenic mice overexpressing mutant IGFBP-2 lacking the Cardin-Weintraub sequence motif: reduced expression of synaptic markers and myelin basic protein in the brain and a lower degree of anxiety-like behaviour. Growth Horm. IGF Res. 33, 1–8. doi: 10.1016/j.ighir.2016.11.003
Scholl, J., Alzal, A., Fox, L., Watt, M., Forster, G., and Scholl, J. (2019). Sex differences in anxiety-like behaviors in rats. Physiol. Behav. 211, 112670–112670. doi: 10.1016/j.physbeh.2019.112670
Schroeder, A., Buret, L., Hill, R., and van Den Buiase, M. (2015). Gene-environment interaction of reelin and stress in cognitive behaviours in mice: implications for schizophrenia. Behav. Brain Res. 287, 304–314. doi: 10.1016/j.bbr.2015.03.063
Screwen, L. A., and Dent, M. L. (2018). Preference in female laboratory mice is influenced by social experience. Behav. Processes 157, 171–179. doi: 10.1016/j.beproc.2018.09.011
Serafini, K., Russo, P., Fernandes, C., Gianlorenzo, A., and Mattioli, R. (2016). Intra-amigdala microinjections of chlorpromazine impair memory formation or memory retrieval in anxiety- and fear-mediated models. Brain Res. Bull. 125, 127–133. doi: 10.1016/j.brainresbull.2016.06.006
Shafia, S., Vafaei, A. A., Samaei, S. A., Bandegi, A. R., Rafiei, A., Valadan, R., et al. (2017). Effects of moderate treadmill exercise and fluoxetine on behavioural
and cognitive deficits, Hypothalamic-Pituitary-Adrenal Axis dysfunction and alterations in hippocampal BDNF and mRNA expression of apoptosis related proteins in a rat model of post-traumatic stress disorder. *Neurobiol. Learn. Memory* 139, 165–170. doi: 10.1016/j.nlm.2017.01.009

Sheth, S. K. S., Li, Y., and Shaw, C. A. (2018). Is exposure to aluminum adjuvants associated with social impairments in mice? A pilot study. *J. Inorg. Biochem.* 181, 96–103. doi: 10.1016/j.jinorgbio.2017.11.012

Shimizu, T., Minami, C., and Mitani, A. (2018). Effect of electrical stimulation of the infralimbic and prelimbic cortices on anxiety-like behavior of rats during the elevated plus-maze test, with particular reference to multiunit recording of the behavior-associated neural activity. *Behav. Brain Res.* 353, 168–175. doi: 10.1016/j.bbr.2018.07.005

Shoji, H., and Miyakawa, T. (2021). Effects of test experience, closed-arm wall color and illumination level on behavior and plasma corticosterone response in an elevated plus maze in male C57BL/6j mice: a challenge against conventional interpretation of the test. *Mol. Brain* 14:34. doi: 10.1186/s13041-020-00721-2

Stohn, J. P., Martinez, M. E., and Hernandez, A. (2016). Decreased anxiety-like behavior in the open field test, corticosterone and hippocampal pyrosine hydroxylase in adolescent and adult mice. *Behav. Brain Res.* 348, 211–218. doi: 10.1016/j.bbr.2018.04.019

Subramaniam, S., Magen, I., Bove, N., Zhu, C., Lemesre, V., Dutta, G., et al. (2018). Chronic nicotine improves cognitive and social impairment in mice overexpressing wild type α-synuclein. *Neurobiol. Dis.* 117, 170–180. doi: 10.1016/j.nbd.2018.05.018

Suleymanova, E., Borisova, M., and Vinogradova, L. (2019). Early endocannabinoid system activation attenuates behavioral impairments induced by initial impact but does not prevent epileptogenesis in lithium-pilocarpine status epilepticus model. *Epilepsy Behav.* 92, 71–78. doi: 10.1016/j.yebeh.2018.12.001

Taherichadornshin, H., Cheragh-Birjandi, S., Ramezani, S., and Abtahi-Eivary, S. (2017). Comparing sprint and endurance training on anxiety, depression and its relation with brain-derived neurotrophic factor in rats. *Behav. Brain Res.* 329, 1–5. doi: 10.1016/j.bbr.2017.04.034

Tarland, E., and Brosda, J. (2018). Male rats treated with subchronic PCP show intact olfaction and enhanced interest for a social odour in the olfactory habituation/dishabituation test. *Behav. Brain Res.* 345, 13–20. doi: 10.1016/j.bbr.2018.02.023

Tartaglione, A. M., Schiavi, S., Calamandrei, G., and Trezza, V. (2019). Prenatal valproate in rodents as a tool to understand the neural underpinnings of social dysfunctions in autism spectrum disorder. *Neuropsychopharmacology* 159:107477. doi: 10.1016/j.nuepharm.2018.12.024

Tavares, L., Baptista-de-Souza, D., and Canto-de-Souza, A. (2018). Activation of 5-HT2C (but not 5-HT1A) receptors in the amygdala enhances fear-induced antinociception: blockade with local 5-HT2C antagonist or systemic fluoxetine. *Neuropharmacology* 135, 376–385. doi: 10.1016/j.neuropharm.2018.03.008

Telenis, A., and Margarity, M. (2015). Phobos: a novel software for recording rodents’ behavior during the thigmotaxis and the elevated plus-maze test. *Neurosci. Lett.* 599, 81–85. doi: 10.1016/j.neulet.2015.05.045

Thompson, R. F. (1994). Behaviorism and neuroscience. *Psychol. Rev.* 101, 259–265. doi: 10.1037/0033-295x.101.2.259

Thompson, T., Grabowski-Boase, L., and 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

Tillmann, S., Skibdal, H. E., Christiansen, S. H., Gotzsche, C. R., Hassan, M., Mathé, A. A., et al. (2019). Sustained overexpression of neurepoptide S in the amygdala reduces anxiety-like behavior in rats. *Behav. Brain Res.* 367, 28–34. doi: 10.1016/j.bbr.2019.03.039

Tillmann, S., and Wegener, G. (2019). Probiotics reduce risk-taking behavior in the Elevated Plus Maze in the Flinders Sensitive Line rat model of depression. *Behav. Brain Res.* 359, 755–762. doi: 10.1016/j.bbr.2018.08.025

Toma, W., Kyte, S. L., Bagdas, D., alkhlaf, A., Alsharabi, S. D., Lichtman, A. H., et al. (2017). Effects of paclitaxel on the development of neuropathy and affective behaviors in the mouse. *Neuropsychopharmacology* 117, 305–315. doi: 10.1016/j.nuepharm.2017.02.020

Torres, L., Danver, J., Ji, K., Miyauchi, J., Chen, D., Anderson, M., et al. (2016). Dynamic microglial modulation of spatial learning and social behavior. *Brain Behav. Immun.* 55, 6–16. doi: 10.1016/j.bbi.2015.09.001

Tothova, L., Babickova, J., Borbelyova, V., Filova, B., Sebekova, K., and Hodosy, J. (2018). Chronic nicotine improves cognitive and social impairment in mice overexpressing wild type α-synuclein. *Neurobiol. Dis.* 117, 170–180. doi: 10.1016/j.nbd.2018.05.018

Tsuchiya, A., Tsylko, N., Docea, A., Shestakova, S., Sidorova, Y., Petrov, N., et al. (2019). The effect of chronic vitamin deficiency and long term very low dose exposure to 6 pesticides mixture on neurological outcomes—a real-life risk
similation approach. Toxicol Lett. 315, 96–106. doi: 10.1016/j.toxlet.2019.07.026

Uemura, M., Asano, T., Hikawa, R., Yamakado, H., and Takahashi, R. (2017). Zonisamide inhibits monoamine oxidase and enhances motor performance and social activity. Neurosci. Res. 124, 23–32. doi: 10.1016/j.neures.2017.05.003

Underwood, B. J. (1966). Problems in Experimental Design and Inference: Workbook for the First Course in Experimental Psychology, New York, NY: Meredith Publishing Company.

Upadhyay, P., Sadhu, A., Singh, P., Agrawal, A., Ilango, K., Purohit, S., et al. (2018). Revalidation of the neuroprotective effects of a United States patented polyherbal formulation on scopolamine induced learning and memory impairment in rats. Biomed. Pharmacother. 97, 1046–1052. doi: 10.1016/j.biopharm.2017.11.008

Vázquez-León, P., Campos-Rodríguez, C., González-Pliego, C., and Miranda-Páez, A. (2018). Differential effects of cholecystokinin (CCK-8) microinjection into the ventrolateral and dorsolateral periaqueductal gray on anxiety models in Wistar rats. Horm. Behav. 105, 101–111. doi: 10.1016/j.yhbeh.2018.10.003

Vázquez-León, P., Mendoza-Ruiz, L., Juan, E., Chamorro-Cevallos, G., and Miranda-Páez, A. (2017). Analgesic and anxiolytic effects of [Leu31,Pro34]-neuropeptide Y microinjected into the periaqueductal gray in rats. Neuropeptides 66, 81–89. doi: 10.1016/j.npep.2017.10.001

Van Camp, G., Cigalotti, J., Bouwalerh, H., Mairesse, J., Gatta, E., Palanza, P., et al. (2018). Consequences of a double hit of stress during the perinatal period and midlife in female rats: mismatch or cumulative effect. Psychoneuroendocrinology 93, 45–55. doi: 10.1016/j.psyneuen.2018.04.004

van Den Boom, B., Pavlidi, P., Wolf, C., Mooij, A., and Willuhn, I. (2017). Automated classification of self-grooming in mice using open-source software. J. Neurosci. Methods. 289, 48–56. doi: 10.1016/j.jneumeth.2017.05.026

Varghese, R., Majumdar, A., Kumar, G., and Shukla, A. (2018). Effects of early pubertal exposure to di-(2-ethylhexyl) phthalate on social behavior of mice. Horm. Behav. 80, 117–124. doi: 10.1016/j.yhbeh.2016.01.012

Wang, R., Xu, X., and Zhu, Q. (2016b). Pubertal exposure to di-(2-ethylhexyl) phthalate influences social behavior and dopamine receptor D2 of adult female mice. Chemosphere 144, 1771–1779. doi: 10.1016/j.chemosphere.2015.10.062

Washington, J., Kumar, U., Medel-Matus, J., Shin, D., Sankar, R., and Mazarati, A. (2015). Cytokine-dependent bidirectional connection between impaired social behavior and susceptibility to seizures associated with maternal immune activation in mice. Epilepsy Behav. 50, 40–45. doi: 10.1016/j.yebeh.2015.05.040

Wei, D., Allsop, S., Tye, K., and Piomelli, D. (2017). Endocannabinoid signaling in the control of social behavior. Trends Neurosci. 40, 385–396. doi: 10.1016/j.tins.2017.04.005

Wen, D., Zhao, P., Hui, R., Wang, J., Shen, Q., Gong, M., et al. (2017). Hydrogen-rich saline attenuates anxiety-like behaviors in morphine-withdrawn mice. Neuropharmacology 118, 199–208. doi: 10.1016/j.neuropharm.2017.03.029

Wensheng, Y., Jie, K., Guoliang, Z., Shuangcheng, L., Yunxiao, K., Lei, W., et al. (2015). The effects of gondadectomy and binge-like ethanol exposure during adolescence on open field behaviour in adult male rats. Neurosci. Lett. 604, 52–57. doi: 10.1016/j.neulet.2015.07.039

White, S. R., Amarante, L. M., Kravitz, A. V., and Laubach, M. (2019). The future is open: open-source tools for behavioral neuroscience research. eNeuro 6:ENEURO.0223–19.2019. doi: 10.1523/ENEURO.0223-19.2019

Wille-Bille, A., Miranda-Morales, R. S., Pucci, M., Bellia, F., D’Addario, C., and Pautassi, R. M. (2018). Prenatal ethanol induces an anxiety phenotype and alters expression of dynorphin & nociceptin/orphanin FQ genes. Prog. Neuropsychopharmacol. Biol. Psychiatry 85, 77–88. doi: 10.1016/j.pnpbp.2018.04.005

Winther, G., Eskelund, A., Bay-Richter, C., Elving, B., Muller, H. K., Lund, S., et al. (2019). Grandmaternal high-fat diet primed anxiety-like behaviour in the second generation female offspring. Behav. Brain Res. 359, 47–55. doi: 10.1016/j.bbr.2018.10.017

Wscieklicka, T., de Barros Viana, M., Le Sueur Maluf, L., Pouza, K., Spadari, R., and Céspedes, I. (2016). Alcohol consumption increases locomotion in an open field and induces Fos-immunoreactivity in reward and approach/withdrawal-related neurocircuits. Alcohol. 50, 73–82. doi: 10.1016/j.alcohol.2015.11.005

Wu, H., Wang, X., Gao, J., Liang, S., Hao, Y., Sun, C., et al. (2017). Fingolimod (FTY720) attenuates social deficits, learning and memory impairments, neuronal loss and neuroinflammation in the rat model of autism. Life Sci. 173, 43–54. doi: 10.1016/j.lfs.2017.01.012

Xiao, X., Xu, X., Li, F., Xie, G., and Zhang, T. (2019). Anti-inflammatory treatment with β- aarone improves impairments in social interaction and cognition in MK-801 treated mice. Brain Res. Bull. 150, 150–159. doi: 10.1016/j.brainresbull.2019.05.017

Wang, A., Dai, Z., Gong, G., Zhou, C., and He, Y. (2014). Understanding structural-functional relationships in the human brain: a large-scale network perspective. Neuroscientist 21, 290–305. doi: 10.1177/1073858414537660

Wang, S., Feng, D., Li, Y., Wang, Y., Sun, X., Li, X., et al. (2018). The different baseline characteristics of cognitive behavior test between Mongolian gerbils and rats. Behav. Brain Res. 352, 28–34. doi: 10.1016/j.bbr.2017.09.042

Wang, L., He, Z., Zhu, Z., Yuan, W., Cai, W., Li, L., et al. (2019). The serotonin system in the hippocampus CA3 involves in effects of CSDS on social recognition in adult female mandarin voles (Microtus mandarinus). Prog. Neuro-Psychopharmacol. Biol. Psychiatry 95:109704. doi: 10.1016/j.pnpbps.2019.109704

Wang, H., Pei, D., Yang, R., Wen, C., Ye, Y., Peng, S., et al. (2019). Prenatal maternal vaginal inflammation increases anxiety and alters HPA axis signaling in adult male mice. Int. J. Dev. Neurosci. 75, 27–35. doi: 10.1016/j.jdevneu.2019.04.001

Wang, R., Xu, X., Weng, H., Yan, S., and Sun, Y. (2016a). Effects of early pubertal exposure to di-(2-ethylhexyl) phthalate on social behavior of mice. Horm. Behav. 80, 114–124. doi: 10.1016/j.yhbeh.2016.01.012
Xu, H., Liu, L., Tian, Y., Wang, J., Li, J., Zheng, J., et al. (2019). A disinhibitory microcircuit mediates conditioned social fear in the prefrontal cortex. *Neuron* 102, 668–682.e5. doi: 10.1016/j.neuron.2019.02.026

Xu, X., Zhang, H., Shou, X., Li, J., Jing, W., Zhou, Y., et al. (2015). Prenatal hyperandrogenic environment induced autistic-like behavior in rat offspring. *Physiol. Behav.* 138, 13–20. doi: 10.1016/j.physbeh.2014.09.014

Yamamoto, H., Kamegaya, E., Hagino, Y., Takamatsu, Y., Sawada, W., Matsuwasa, M., et al. (2017). Loss of GluN2D subunit results in social recognition deficit, social stress, 5-HT2C receptor dysfunction and anhedonia in mice. *Neuropharmacology* 112, 188–197. doi: 10.1016/j.neuropharm.2016.07.036

Yang, Y., Qin, J., Chen, W., Sui, N., Chen, H., and Li, M. (2015). Behavioral and pharmacological investigation of anxiety and maternal responsiveness of postpartum female rats in a pup elevated plus maze. *Behav. Brain Res.* 292, 414–427. doi: 10.1016/j.bbr.2015.07.011

Zare, Z., Tehrani, M., Rezaei, N., Dana Ghalebarzand, B., and Mohammadi, M. (2015). Theta frequency prefrontal-hippocampal driving relationship. *Frontiers in Molecular Neuroscience* | www.frontiersin.org 24

Zhan, Y. (2015). Theta frequency prefrontal-hippocampal driving relationship during free exploration in mice. *Neuroscience* 300, 554–565. doi: 10.1016/j.neuroscience.2015.05.063

Zhang, M., Ji, M., Zhao, Q., Jia, M., Qiu, L., Yang, J., et al. (2015). Neurobehavioural abnormalities induced by repeated exposure of neonatal rats to sevoflurane can be aggravated by social isolation and enrichment deprivation initiated after exposure to the anaesthetic. *Br. J. Anaesth.* 115, 752–760. doi: 10.1093/bja/aev339

Zhang, W., Smolik, C., Barba-Escobedo, P., Gamez, M., Sanchez, J., Javors, M., et al. (2015). Acute dietary tryptophan manipulation differentially alters social behavior, brain serotonin and plasma corticosterone in three inbred mouse strains. *Neuropharmacology* 90, 1–8. doi: 10.1016/j.neuropharm.2014.10.024

Yeung, M., Treit, D., and Dickson, C. (2016). Ventral hippocampal histamine increases the frequency of evoked theta rhythm but produces anxiolytic-like effects in the elevated plus maze. *Neuropharmacology* 106, 146–155. doi: 10.1016/j.neuropharm.2015.09.024

Yoshimi, N., Futamura, T., and Hashimoto, K. (2015). Improvement of dizecline-induced social recognition deficits in mice by brexpiprazole, a novel serotonin-dopamine activity modulator. *Eur. Neropsychopharmacol.* 25, 356–364. doi: 10.1016/j.eunephar.2014.12.014

Yu, C., Mei, X., Zheng, Y., and Xu, D. (2015). Tauine zinc solid dispersions protect against cold restraint stress-induced gastric ulceration by upregulating HSP70 and exerting an anxiolytic effect. *Eur. J. Pharmacol.* 762, 63–71. doi: 10.1016/j.ejphar.2015.05.033

Yuan, H., Ni, X., Zheng, M., Han, X., Song, Y., and Yu, M. (2019). Effect of caltopol on behavior and neurodevelopment in an ADHD rat model. *Biomed. Pharmacother.* 118:109033. doi: 10.1016/j.biopha.2019.109033

Zaccarelli-Magalhães, J., Sandinib, T. M., Ramos de Abreu, G., Petrocelli, B. M., Moreira, N., Reis-Silva, T. M., et al. (2019). Prolonged exposure of rats to varenclines increases anxiety and alters serotoninergic system, but has no effect on memory. *Pharmacol. Biochem. Behav.* 181, 1–8. doi: 10.1016/j.pbb.2019.03.009

Zagorac, O., Kovacs, A., László, K., Ollmann, T., Péczely, L., and Lénárd, L. (2015). Effects of direct QRFQ-26 administration into the medial hypothalamic area on food intake in rats. *Brain Res. Bull.* 118, 58–64. doi: 10.1016/j.brainresbull.2015.09.004

Zahra, A., Jiang, J., Chen, Y., Long, C., and Yang, L. (2018). Memantine rescues prenatal citrullinopam exposure-induced striatal and social abnormalities in mice. *Exp. Neurol.* 307, 145–154. doi: 10.1016/j.expneurol.2018.06.003

Zare, Z., Tehrani, M., Rezaei, N., Dana Ghalebarzand, B., and Mohammadi, M. (2019). Anxiolytic activity of paraxonox is associated with alterations in rat brain glutamatergic system. *Neurotoxicol. Teratol.* 71, 32–40. doi: 10.1016/j.neuotox.2018.12.001

Zhan, Y. (2015). Theta frequency prefrontal-hippocampal driving relationship during free exploration in mice. *Neuroscience* 300, 554–565. doi: 10.1016/j.neuroscience.2015.05.063

Zhang, C., Chua, B. E., Yang, A., Shabanpoor, F., Hossain, M. A., Wade, J. D., et al. (2015). Central relaxin-3 receptor (RXFP3) activation reduces elevated, but not basal, anxiety-like behaviour in C57BL/6J mice. *Behav. Brain Res.* 292, 125–132. doi: 10.1016/j.bbr.2015.06.010

Zhang, H., Li, H., Dai, Y., Xu, X., Han, S., Zhang, R., et al. (2015). Electroacupuncture improves the social interaction behavior of rats. *Physiol. Behav.* 151, 485–493. doi: 10.1016/j.physbeh.2015.08.014

Zhang, M., Ji, M., Zhao, Q., Jia, M., Qiu, L., Yang, J., et al. (2015). Neurobehavioural abnormalities induced by repeated exposure of neonatal rats to sevoflurane can be aggravated by social isolation and enrichment deprivation initiated after exposure to the anaesthetic. *Br. J. Anaesth.* 115, 752–760. doi: 10.1093/bja/aev339

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