Appendix 2

Preamble

On the request of the inquiry and clarifying on my previous submission, Appendix 2 summarizes allegations of research misconduct by RM. Notably, section 1 concerns publications by RM produced at NTU. Points listed below show breach of NTU Research Integrity Policy. An asterisk (*) indicates suspected breach of NTU Research Integrity Policy. A dagger (†) indicates breach or suspected breach of research integrity in The University Code of Conduct and policies herein other than the Research Integrity Policy. References are cited as numbers in square brackets. The order of presentation of publications in section 1 is not based on date of publication, journal impact factor, or magnitude and seriousness of evidence. Sections 1.4. to 1.12. address specific issues related to research integrity in RM's publications. They are presented in the order in which they were processed. Sections 1.1 to 1.3 concern the general quality of the data. Allegations not related to RM's publications are in sections 2 to 4.

Contents

1. Publications

1.1. Behavioral video analyses

In all subsequent sections on publications by RM produced at NTU, videos of behavioral testing suffer one or more of the below, with additional details under the relevant publication [abbreviations: Elevated Plus Maze (EPM); Object Recognition Task (ORT); Open Field Test (OFT)]:

a. There is no animal in view.
b. The file is corrupt.
c. The video is of poor quality as to be unusable.
d. The view is from one side rather than from above. This is very unusual and questionable for obvious reasons (such as observing the animal in the closed arms of the EPM and the open arm on the other side of the field of view), and is a unique and novel method in the context of most tests used by RM. In some experiments 2 cameras on opposite sides complement each other. In some, not.
e. When the view is from above, it is not perpendicular. The angle of view is so skewed it does not allow for analysis of reported parameters or rough estimates, or at best yields questionable data. This is because the researcher analyzing the video manually, or in the case of two articles using ANY-maze software, cannot correct errors of perspective when calculating movement of an animal in/out of a zone. Automated correction requires careful measurement and reconstruction of experimental conditions, and is not applicable to RM's data and reporting.
f. Whether from the side or from above, parts or essential parts of the experimental field are out of view.
g. The presence of a large number of other animals in the experimental room is clearly heard in background audio. It does not sound like rats in one or two cages. It sounds like a whole rack of cages of excited rats. This renders an experiment very poor or without meaning [1].
h. Ambient light can be: apparently off, brightly on, red, coming directly from a bright lamp, or alternating between on and off as the researcher removes one animal and puts in another. Turning lights on and off every 3 or 5 minutes is very upsetting for rats. If the lights were turned off, they should stay off. More so if the researcher is forced to keep the whole group of animals in an experiment in the same room the researcher is doing the experiment. It is common for researchers interested in rodent behavior or just well-being to carry a small and dimmed torch, preferably the headlamp kind so both hands are free. As RM is interested in anxiety-like behavior, stress, and effect of environment, and RM's published methods invariably report testing in extremely low light conditions, I wonder why she does not possess a box of constantly replenished head-lamps for use by her researchers.
i. Experimental data is not shown or 'flashed' to the camera between animals.
j. Two researchers are seen in the background, chatting, directly adjacent to the experimental field. This is, of course, unacceptable for behavioral rat work [1].

1.2. Image and neuron analyses

Where applicable to RM's publications:

a. A note of clarification on the *camera lucida* used by RM in her studies of neuronal structure. The camera lucida is composed of mirrors and was used in the past by microscopists to help them draw what they are seeing through the lens. It reflects what they are seeing onto the table and to the side of the microscope, so the researcher can then 'trace' the object of interest on plain paper. The camera lucida is also called a drawing tube, and I prefer this term to avoid any ambiguity about the potential presence of an impartial recording device.

b. For imaging dendritic spines, RM reports using x1000 magnification with an upright microscope from Olympus, model BX43. It is customary to mention the magnification of the objective lens (among other qualities) which RM partially does (to the best of my knowledge) in two articles published from Singapore. I'm assuming she is otherwise informing the reader of final magnification (one may guess it's x100 for the objective lens, x10 for the eyepiece, total x1000). The alternative, digital zoom, is of course not applicable with a drawing tube. Further details are in the sections below notably section 1.8.3.
c. I have never actually seen a drawing tube, neither before I came to RM's lab nor after. I am not sure I would recognize one if I did. I think it is still possible to purchase a used drawing tube. In principle, it is possible to install a drawing tube in the Olympus BX43 (for example see [2]). The latest brochure for the BX43 is available from Olympus. It appears Olympus presently does not supply nor support drawing tubes.
d. On hearsay, researchers at RM's lab have not been using a drawing tube. They have been drawing free hand with pencil-and-paper by looking through the eyepiece.

1.3. Data selection*†

Evidence is below that data from the same cohorts of animals were used in different publications. To the best of my knowledge this is not completely unethical if: (i) the research questions and objectives are pre-defined and different between two (or more) published studies; (ii) the information that the same dataset is being used in more than one study is disclosed to the journal on submission and mentioned in the article; (iii) co-authorship is agreed upon and all contributors are acknowledged; and (iv) funding is accurately reported. I understand this question is raised with large, expensive, and varied datasets from human populations. In RM's publications and as presented below, this question is at best one of duplicate, multiple, and redundant (*salami*) publications, at worst of fabrication and misrepresentation.

Sometimes male animals go into one study, females into another. Sometimes one brain region is reported for one study, another in a second and third study. Often, criteria for selection are not explicit. Often, outliers are removed at an early stage of analysis, or then later in the process.

It is not clear how research projects are divided, or sliced, between co-authors *vs.* a mention or not in acknowledgements. RM can be corresponding author on one article, her spouse AV on another (not always, see below and references). No article declared competing or conflicting interest. Since AV and RM are married, I wonder if this is not competing or conflicting with the interests of: (i) the people who do the work which is then sliced and for which they may or may not be acknowledged; (ii) RM's affiliation with NTU when producing sub-standard or allegedly falsified and/or misrepresented publications; (iii) funding bodies, for whom grant allocation is unacknowledged, untransparent, or open to question of misuse.

N.B. I had and have no qualms working in a lab under a married couple who collaborate to produce knowledge. Collaborating for other reasons with potentially harmful consequences on immediate and extended society, I disagree with.

1.4. Paper I [3]

Title: Enriched environment facilitates anxiolytic efficacy driven by deep-brain stimulation of medial prefrontal cortex
Authors: Bhaskar Y, Lim LW, and Mitra R
Journal: Frontiers in Behavioral Neuroscience
Year of publication: 2018
URL: https://www.frontiersin.org/article/10.3389/fnbeh.2018.00204
DOI: 10.3389/fnbeh.2018.00204

1.4.1. Falsification and misrepresentation

The cohort of animals used in this study [3] were also used in another study and article published by LWL in co-authorship with AV and others [4]. These animals received different stimulation protocols and BrdU, and so the article [3] was falsified and misrepresented. Article [4] also states that these animals were tested in the Morris water maze.

1.4.2. Peer review†

The article was reviewed by two reviewers who are co-authors from the same institute [5-20]. The editor of the article is a co-author of both reviewers [21-31]. This is unethical [32,33] and may implicate a paper mill [34]. See also section 1.6.8. In my opinion as well as others (*ibid*) the review process for this article is faulty.

1.4.3. Electrode placement*

Electrodes were not implanted into ventromedial prefrontal cortex (vmPFC) according to the coordinates stated. The deviation from published coordinates for vmPFC in animals of comparable age and weight is big [35-42]. The same coordinates were used in the article produced by NTU and co-authored by LWL, AV, and others [4]. Other articles by LWL not produced at NTU (but see also section 1.4.8.) use the same or slightly different coordinates to target regions alternately referred to as vmPFC or prelimbic cortex [43-47]. For comparison see [48-53]. Literature LWL states as the source for coordinates in various articles is unrelated to vmPFC [47,54].

1.4.4. Reversed day-night cycle*†

It is not mentioned that animals housed under standard conditions were not housed under reversed cycle.

1.4.5. Stimulation protocol*

The description is vague, electronic parameters are lacking, see for example [55]. The protocol cannot be replicated. LWL himself emphasizes the importance of these parameters, methodologically and physiologically, in other publications, some of which are analogous [56-59]. What is reported in the protocol used is in contradiction to recommendations made in reviews co-authored by LWL (*ibid*). One of the reviews in which LWL is co-author was heavily criticized by others for many, huge, and indefensible errors [60].

1.4.6. Behavioral testing (manual)

See section 1.1. For EPM: (i) it is not possible to assess "….presence of the whole body including head, four paws, and at least the base of tail inside the open arm…" [3]; and (ii) it is not possible to assess head dips. For ORT: (i) experiments were done under red light but this is not mentioned in the methods. It should have been considering rat acuity of vision in red light, see for example [61,62]; (ii) there is alternately one, two, or no discernable object, the methods described for this task in [3] and [4] are different but neither are ORT; (iii) the position of the object(s) is difficult to assess but appears to be random; (iv) it is not stated how 'exploration' was assessed. Simple automation which was not

done for this test is highly recommended for reproducibility and replicability see [63]; and (v) number of experiments do not match reported.

1.4.7. Statistics

Outliers were removed and not reported. There is no randomization protocol. This study was stated to be confirmatory, but there is no evidence of the same. "…Several endpoints exhibited significant departure from normality…" [3] and both parametric and nonparametric tests are used. An apt description for the data analysis is found in the reviewer comments on the article published by LWL, AV, and others [4]: "…I felt that the presentation of the correlation data was a bit **muddled**. I did not see a lot of value in computing an entire correlation matrix on this dataset with a relatively low N. A more useful approach might be to simply explore hypothesis-driven correlations…" (emphasis added).

1.4.8. Funding*†

Article [3] was funded by "…Ministry of Education, Singapore (#RG 46/12) to RM…". In article [4], only "…the Singapore Lee Kuan Yew Research Fellowship (M4080846.080) that awarded to LWL…" is acknowledged. I am unable and unwilling to decipher where LWL was at what time, his affiliation, and by whom he was funded including MoE [43,47,45,64-66].

1.5. Paper II [67]

Title: Dendritic architecture of principal basolateral amygdala neurons changes congruently with endocrine response to stress
Authors: Hegde A, Soh Yee P, and Mitra R
Journal: International Journal of Environmental Research and Public Health
Year of publication: 2017
URL: https://www.mdpi.com/1660-4601/14/7/779.
DOI: 10.3390/ijerph14070779

1.5.1. Behavioral testing (manual)

See section 1.1. In the OFT: (i) the central zone is not demarcated; and (ii) light is much higher than "…10 lux at center and 3–4 lux at the periphery…" [67]. Note the name 'OFT' here is used to refer to that test in a way that is generally understood and recognized. In contrast, see section 1.8.2.

1.5.2. Predator odor (bobcat urine/manual)*

There is no evidence of this. It is not stated that aversion was "…quantified as occupancy of the bisect containing odor (76 cm x 9 cm) relative to the total area of the arena (chance = 47.2%, based on the area of the bisect containing predator odor vis-à-vis total area of the arena)…" [67] real-time. The source of bobcat urine is unknown. See sections 1.6.3, 1.8.2, and 1.10.3.

1.5.3. Analysis of neurons (manual)

There is no evidence that "…Camera Lucida tracings of BLA neurons were produced using 400x magnification (Olympus Bx43, Tokyo, Japan)…" (*sic*) [67], there is no record of scanned images of the tracings produced. In the article pencil-and-paper drawn "…representative…neuronal tracings…" are shown (*ibid*).

1.5.4. Statistics

It is not clear how data was selected. Outliers were removed and not reported.

1.5.5. Salami publications*†

It is possible this article [67] is the control group data of separate studies by RM, AV, and others [68] and/or studies by AV and a co-author [69,70]. Data may have been taken from/given to [68] (section 1.6) and/or [71] (section 1.8). See also section 1.10.3.

1.6. Paper III [68]

Title: Effects of stress or infection on rat behavior show robust reversals due to environmental disturbance *or* [version 2; peer review: 2 approved]
Authors: Abdulai-Saiku S, Hegde A, Vyas A, Mitra R
Journal: F1000Research
Year of publication: 2017 *or* 2018
URL: https://f1000research.com/articles/6-2097/v1#DS0 *or* https://f1000research.com/articles/6-2097/v2.
DOI: 10.12688/f1000research.13171.1 *or* 10.12688/f1000research.13171.2

1.6.1. Euthanasia

The animals were apparently killed by either decapitation or cardiac perfusion.

1.6.2. Falsification and misrepresentation

In a rebuttal, RM implicates herself while proving to the reviewer that data interpretation is correct. RM writes: "…Toxoplasma-induced loss of fear once construction project abated. Toxoplasma effects were eventually published (reference 7 in the revised bibliography; DOI: 10.1016/j.bbi.2017.04.005). Same set of experimenters conducted experiments before, during and after the construction project…" [68]. RM is not co-author on this second tranche, only AS and AV [70]. If research question and objective were different in these two studies, there is one additional outcome in [70] compared to [68].

1.6.3. Predator odor (bobcat urine/automated)*

ANY-maze tracking files were not preserved. It is not clear why two tests of predator odor were done for this study. The rectangular test is preferred by RM in [67], the circular one in [71]. Here [68], we have both, apparently done sequentially and for no interpretable justification. Tests for rectangular arena appear incomplete. The circular arena looks much bigger than 1 m in diameter - see sections 1.5.2, 1.8.2 and 1.10.3. See also section 1.1.

1.6.4. Behavioral testing (manual)

See section 1.1. For EPM, though RM notes in a rebuttal that they "…revised the manuscript to include date for percentage open arm time…" (*sic*), I am not certain anything can be made out.

1.6.5. Serological examination*

There is no method nor data on this. The same is absent in the article [68]. Congruent data can be found in [70], but it is not serological it is genetic.

1.6.6. Design*

It is puzzling. Male and female animals were habituated over 3 days to the rectangular arena then stressed with predator odor, then repeat the same with the circular arena. Significantly, only female animals were infected with *T. gondii* see [70].
In a rebuttal, RM states: "…Unfortunately, we did not record videos for habituation sessions. We have earlier shown that Toxoplasma infection does not affect locomotion or exploration in open field arena…" [68] with no reference. Whether arena or OFT, why not call it a test of anxiety-like behavior

(as the reviewer was suggesting) instead of habituation, record videos, analyze them on the spot with ANY-maze, and see what happens?
Regarding design, a fresh cohort is introduced, and their male progeny are maternally separated. How is the new animals being mated and maternal separation of the progeny related to the previous set of experiments? The only way to link them is to a research question which arose due to construction. If females were infected prior to construction for whatever reason, then females should have been infected after construction to test its effect. If males were the research subject all along, then they should have been the group getting *T. gondii*. In any case it appears the males were stressed [68] and then later the females were ovariectomized [70].

1.6.7. Statistics*†

There is no evidence of code nor key for blinded experiments. It is not clear how the data was selected.

1.6.8. Suspect peer review*†

One reviewer of this article [68] is co-author with the editor of RM's paper [3] on many articles as follows: [72-80]. This reviewer is also co-author of a reviewer of RM's article [3] with or without the editor as follows: [25,27,28,26,29,30,81,31,21]. The text from the second reviewer on this article [68] is entertaining. RM's rebuttal is also good.

1.6.9. Funding*†

This article was funded by MoE grants grant RG136/15 and RG 46/12. The second tranche was funded by MoE grant RG136/15 only, but it is the same project. RM said so herself, see section 1.6.2.

1.7. Paper IV [82]

Title: Complex housing causes a robust increase in dendritic complexity and spine density of medial prefrontal cortical neurons
Authors: Ashokan A, Lim JWH, Hang N, Mitra R
Journal: Scientific Reports
Year of publication: 2018
URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5943332/
DOI:10.1038/s41598-018-25399-4

1.7.1. Falsification and misrepresentation

Under *Author Contributions* the article states: "…R.M. conceptualized, planned, analyzed and wrote the script. A.A., N.H. and J.L. executed, analyzed and edited the script…" [82]. The dataset for the script is from another work but this is not reported in the methods nor the results.

1.7.2. Statistics

In the first "…**Data analysis and Statistics**…" section (emphasis in the original, p.3), apparently "…Mean for each animal, across multiple neurons analyzed, was used as biological replicate for statistical analysis. Statistical significance for comparisons between simple and complex housing was calculated using unpaired two-tailed Student's t-test… The standardized effect size was calculated using Cohen's d^{13}…" [82] and so on until the end of the paragraph. The use of the term *biological replicate* here is inappropriate and misleading. I think what RM really means is 'animals from another study'. I do not understand why all the following statistical methods were used in this analysis: (i) 2-tailed Student's t-test; (ii) Cohen's *d* and subsequent comparisons of its negative values; (iii) "… Mean inter-group difference was also calculated with 95% confidence intervals…"; and (iv) repeated measure analysis of variance for Sholl analysis [82]. I think what RM means here by 'standardization' is 'removing outliers'.

1.7.3. Analysis of neurons (manual)*†

The article states that: "…Two-dimensional profiles of dendritic arbors were drawn at 400X magnification using a camera lucida attachment on the optical microscope (Olympus BX43, Japan). Dendritic profiles were then scanned along with a calibrated scale for subsequent analysis (300 dpi, 8-bit grayscale, tiff) using a freely available image processing package…". Why were the dendritic profiles *drawn* and then *scanned* if one may just as well replace the camera lucida with a real one, or use one of many available at SBS and NTU, and put the same images and many more in the image processing package right away?

"…Dendritic spines were manually counted at 1000X magnification using an oil-immersion objective lens…" (*sic*) [82]. See sections 1.2 and 1.8.3.

1.8. Paper V [71]

Title: Short environmental enrichment in adulthood reverses anxiety and basolateral amygdala hypertrophy induced by maternal separation
Authors: Koe AS, Ashokan A, Mitra R
Journal: Translational psychiatry
Year of publication: 2016
URL: https://www.nature.com/articles/tp2015217
DOI: 10.1038/tp.2015.217

1.8.1. Euthanasia

Apparently these animals were killed by decapitation. See section 1.6.1.

1.8.2. Misrepresentation of behavioral data (manual)

In this article, the OFT becomes "…an open circular arena (radius=120 cm…". OFT almost always (in recent decades) refers to a rectangular or square box of some kind. RM describes the OFT herself in [67] as "…A square-shaped open field…constructed from Plexiglas…". Why is the normally square OFT now circular? The data here comes from the circular arena in [68]. Were the animals being habituated before predator exposure in [68], or being tested for anxiety-like behavior in a circular OFT in [71]?

The circular arena, whether for anxiety-like behavior, habituation or predator exposure, can be around 2.4 m in diameter, certainly not 1 m (see sections 1.6.3. and 1.10.3., perhaps radii and diameters were confused?). It's hard to say how big it was, the angle is so skewed, among other things (see section 1.1.). I think the circular arena/OFT might have been even bigger than 2.4 m or else the researcher is a very small person. Strangely, this circular OFT re-appears in RM's article [83], but this time it has shrunk to the proportions reported in [68], 1 m in diameter. And it materialized for the first reported time in Perspex form [83].

It is interesting to note that RM neglects to mention anywhere in the article *when* these 'predator odor circular OFT' tests were done. In other words, the *age of rats* at the time of testing is unknown (see also section 1.8.7.). In addition, the article states: "…Time spent in the center of the field was quantified as the reciprocal proxy of the anxiety (center defined as a concentric circle to the arena with 0.33 m radius). Total distance travelled during the trial was also quantified as a measure of locomotion…" [71]. There is no mention of software.

Leaving analysis of time in the center zone aside – I know some researchers use sticky notes on the screen to demarcate a line, a zone might be more difficult, but never mind. I am curious, how does RM quantify total distance manually? Does the researcher, for example, use a piece of string to follow the animal around on the screen, leading more string out as the animals moves around? Then measure the string lead out as a guesstimate of total distance travelled. If the angle of view had been perpendicular, one could have even made a guesstimate of actual distance travelled in meters.

This was not necessary because this data [71] was taken from animals tracked in ANY-maze in [68]. But we encounter a problem. The tracking files were not preserved – see section 1.6.3. These files would have allowed for calculation of any parameter a reviewer may ask for or RM may conceptualize. As luck would have it, the researcher in [68] initially set the software to calculate total distance travelled, not locomotion. There is no shame (or maybe not much) in reporting total distance travelled as an indication of how locomotive the rat has been in the test. It's called ambulatory or total distance travelled. Locomotion *per se* is something different. For example, an interesting study published in 1969 describes *in rectangular* OFT: "…The openfield measure (OF) was number of squares entered per 10 min period… A sheet of…oilcloth ruled into 16 1-ft squares comprised the floor of the cage…" [84]. With the tracking files, calculating locomotion any way one cares to define it is not a problem, a few clicks and we're done. Without the files, we need to somehow "…also [quantify]…" total distance travelled "…as a measure of locomotion…" [71]. This is important for research integrity because in the results RM writes: "…Time spent in the center of the open-field arena and total locomotion in the arena were quantified…" [71]. Whatever was analyzed wasn't significant, which is odd see section 10.1., and nevertheless "…Failure to mention any of these procedures is distressing…" [84].

In another article [83] RM defines *locomotion* as "…total time spent by animals being mobile in the arena was also quantified as an index of locomotion…". [(Different readout = same readout) = neither readout]. In principle, if one knows *distance travelled* and *time of travelling*, one may calculate *speed*. Speed is commonly distance over time, but I do not know how this can be applied to RM's analyses and why RM didn't think of speed as an indicator of locomotion in a (three) study (studies) reporting on locomotion. Software or no. And they're statistically pre-planned.

1.8.3. Suspected fabrication and/or misrepresentation of neuron analysis (manual)*

This article states in the methods for neuron analysis (manual): "…Complete stellate or pyramidal-like neurons in the BLA, consisting of the lateral and basal nuclei, were selected for tracing using a microscope (Olympus BX43, Tokyo, Japan, × 40 objective lens) with the aid of a camera lucida. For each animal, 10 − 11 neurons were drawn to yield a representative sample of BLA neurons for each group. Custom-designed macros embedded in ImageJ (http://rsb.info.nih.gov/ij/) were used for analysis of scanned images…Using the same microscope (Olympus BX43, 1.3 numerical aperture, ×100 objective lens), all protrusions from dendrite, irrespective of morphological characteristics, were counted as spines…" [71]. Note that the objective lens is mentioned here, compare for example with [67] and [82].

So the objective lens RM has been using was reported in the present article [85] to have magnification x100 and numerical aperture 1.3. The numerical aperture was reported without lens magnification in another article [86]. In all other publications with histology, we may be informed of magnification alone, or magnification and the fact that it is an oil-immersion type lens. Why is this information not all in one place like it should be, and out of respect for the community who use her major tool in the investigation of structural plasticity?

A x100 objective lens numerical aperture 1.3 oil-immersion sounds as a start ideal for visualizing dendritic spines, one important detail which is customary to report missing though. The only objective lens fitting this description and presently available from Olympus is UPLFLN100XO2. But it's semi-apochromatic (FL), so it's less than ideal for the Golgi staining. The better choice I think would be the apochromatic UPLXAPO100XO, which has a numerical aperture of 1.45. The now out-of-production Olympus Plan Apo x100 is an oil-immersion objective and has a numerical aperture of 1.3. Perhaps she has one of those, or another brand, or maybe she was bequeathed by a mentor one of the precious and classic Leitz apochromatic x100. A drawback with the Plan Apo x100 is its short working distance (0.09 mm), which would probably be annoyingly limiting for the present experiments, in that measuring dendritic spines will be limited to the surface of the slice. It's spring-loaded, of course, in case the researcher is eager to go deeper into the slice and hits the coverslip – at that working distance it's quite likely to happen often especially in unexperienced or unpracticed hands. With new researchers counting spines for the work, how often does RM have to send the lens for polishing?

Working a microscope with a x100 objective of any kind requires special preparations, such as measures against vibration and casual disturbance, and which are not in place at RM's lab. I do not think there should be another lab at NTU that has this unique, expensive, and, may I say, obsolete combination of drawing tube *plus* x100 oil immersion objective lens numerical aperture 1.3 *minus* digital camera and computer. In my opinion, this conjecture is easy to falsify. For example: I did not recently check so is there now a x100 apo- or semiapo-chromatic oil immersion objective at NTU core imaging facilities? If yes, attaching a drawing tube remains problematic, of course, because the in-house digital arrangements would need to be removed before RM's imaging and re-installed after by an expert from the microscope supplier. Potential collaborators were not acknowledged in RM's articles.

When someone tells me they're *looking* at dendritic spines, they have my attention because I know how hard and expensive that is. If the financial and technical cost of magnifying to x1000 were made, why not go that little extra step and use a digital camera, which anyways should be part of such a rig for a multitude of reasons? Then we can use Imaris, expensive and powerful software available at NTU, to analyze many features of many dendritic spines in these high-magnification images. Instead, we count them manually and come out the other end with nothing to show how hard we've worked – just a number in an Excel sheet. Needless to say, data yield from software is orders of magnitude higher than manual counting. There is no contraindication to digitizing the process, it is well-applied to Golgi staining, and would allow one to use more modest magnifications [87,88]. One also then gets to produce attractive images to impress one's colleagues during meetings, put in publications, and send to present and potential funding bodies.

1.8.4. Data interpretation*†

According to Supplementary Figure 3, environmental enrichment and maternal separation have no effect on "…prelimbic medial prefrontal cortex neurons…" [71]. I don't know what 'prelimbic medial prefrontal cortex' refers to in the brain. I tried Googling it. Why is it necessary to refer to whatever brain region was analyzed in this awkward manner? According to article [82], environmental enrichment *does* have an effect on medial prefrontal cortex. According to article [3], deep brain stimulation of medial prefrontal cortex *potentiates* anti-anxiety effects of environmental enrichment. Also according to article [3], the stimulating electrodes were placed in vmPFC, or perhaps it was prelimbic cortex (see section 1.4.3.). In the present article [71] RM needs to say they looked at neurons in this part of the brain and did not see any significant changes with stress and environmental enrichment, or else it's a headache to explain why not. Hence, 'prelimbic medial prefrontal cortex'.

1.8.5. Terminal sample collection*†

The article states: "…On PN84, rats were killed by decapitation. Terminal trunk blood was collected…" [71]. Why was blood not collected from the reportedly unanesthetized and decapitated rat? In other words, why guillotine its head *then* slice its abdomen? Needless to say, the former procedure produces copious amounts of blood, and of higher quality than the latter (see section 3.1.).

1.8.6. Behavioral testing (manual)

In the methods for EPM: "…Dim light was shone directly onto each open arm (6 lux)…". I disagree. I would say 'strong light (*c.a.* 100 – 300 lux) was shone directly into the camera'. See section 1.1.

1.8.7. Supplementary data

In addition to the absence of any information on the postnatal date of *circular* OFT testing, Supplementary Figure 1 is incorrect. According to the text, behavioral testing needed 5 days. According to Supplementary Figure 1, behavioral testing needed 2 days.

Supplementary Figure 2 looks doctored to me. Perhaps fabricated. Regardless of conditions, the rats are not gaining weight fast enough before 7 weeks of age, and gaining weight too quickly after 9 weeks of age.

1.8.8. Funding*†

Apparently this research was funded by NTU (M4081146). How this entangles the produced article with various authors, affiliations, grants, and IACUC approvals in other articles and which share data is complex. Reconstruction of what happened when and where will require time, computational capacity, and suspension of judgement on what is possible. Hitherto, I will not comment on funding acknowledged in articles.

1.9. Paper VI [85]

Title: Short-term environmental enrichment is sufficient to counter stress-induced anxiety and associated structural and molecular plasticity in basolateral amygdala
Authors: Ashokan A, Hegde A, Mitra R
Journal: Psychoneuroendocrinology
Year of publication: 2016
URL: https://www.sciencedirect.com/science/article/abs/pii/S0306453016300993
DOI: 10.1016/j.psyneuen.2016.04.009

1.9.1. Behavioral testing (manual?)*†

See section 1.1. The article states: "…Trials were videotaped and coded before offline analysis…". Is that manually? Or offline analysis using a software?

1.9.2. Analysis of neurons (manual)*†

We are informed that "…Two-dimensional traces of BLA neurons (10 neurons per animal) were obtained at 400X magnification using a camera lucid attachment on the optical microscope (Olympus BX43, Japan)…" [85]. I thought about it carefully and decided the information that the traces obtained from a camera were two-dimensional is not trivial. It has immediate consequences, in this particular case that the parameters 'obtained' from the 'traces' cannot be measured, only 'estimated', see below. Perhaps a reviewer asked for z-stacks. In my opinion, this information also has broader consequences implying 'I, RM, bully my researchers into using pencil-and-paper for unknown reasons, and later conceptualize convoluted language to poorly conceal the fact in publications'.

Please forgive me for re-iterating the obvious, that at a basic level for *this kind of* Scholl analysis, "…Performing this process by hand is time-consuming and introduces inherent variability due to inconsistency and experimenter bias…" [89].

Those remarkable two-dimensional tracings are then, strangely, scanned "…for subsequent computerized estimation of dendritic arbors using custom-designed routine embedded in ImageJ (http://rsb.info.nih.gov/ij/). Dendritic length and number of branch points were quantified as function of radial distance from the cell soma (Sholl'sanalysis, Shankaranarayana Rao et al., 2001; Vyas et al., 2002)…" [85]. In the former reference, Shankaranarayana Rao et al. [90] use tracing paper as an aid to count dendritic branching points and dendritic intersections. They are two different things. RM's subsequent statement in [85] is therefore odd: "…This is represented as both total branch points and segmental branch points at radial distance in Results…". Why is historically well-defined data violated in this manner? Only branch points are reported in Results. What does 'total' refer to here? And 'both' – branching points and intersections, or one or both of those lumped together with distance? That can't be, because whatever outcome is obtained is plotted against distance from the center (soma). The weblink provided is generic, there is no 'custom-designed routine'. Did RM feel a need to inform the reader where to find ImageJ?

Ending this section of the methods is a statement I am guessing was put there as part of a rebuttal from RM: "…Since morphology and spine analysis were done independently but from the same slides for BLA, there is a good chance of an overlap among the cells that underwent analysis for both morphology and spine analysis…" [85]. Given RM's penchant for orthogonal analyses, I wondered why

she did not provide a coefficient or index or something to indicate the power behind this "…good chance of overlap…" (*ibid*). I tried to do a rough estimate based on the average size of a pyramidal neuron in basolateral amygdala (BLA) of adult rat, neuron density in BLA per μm^3, that 1-3% of neurons are stained with Golgi [87], diameter of view at x400, number of brain slices with BLA when sliced at 100 μm, number of slices placed on a slide according to lab tradition, the area of BLA in the coronal section where it is largest, the reported number of animals in the study, and the number of neurons analyzed. I gave up after 1 hour. For the purpose of this inquiry, please take me at my word for the time being that the chances for two 'independent' analyses to happen across the same neuron is very low indeed. And that's just at magnification x400. When magnification is presumably raised to x1000, I think the very small chance of someone measuring spines from exactly the same neuron ('overlapping' RM had called it) someone else had analyzed earlier falls even more. I would be surprised if the chances are not negligibly above zero. She must have not been serious when she wrote that?

1.10. Paper VII [86]

Title: Housing environment influences stress-related hippocampal substrates and depression-like behavior
Authors: Ashokan A, Hegde A, Balasingham A, Mitra R
Journal: Brain research
Year of publication: 2018
URL: https://www.sciencedirect.com/science/article/abs/pii/S0006899318300350
DOI: 10.1016/j.brainres.2018.01.021

1.10.1. Falsification – forced swimming test and behavior

These videos are surprisingly good. Some are a little out of focus and in some the angle is a little off; the serious concerns are as follows.

The lighting is not 5 lux as reported. More like 500 lux. It's hard to guess, but it's *very* bright, even for a room with the ceiling lights on.

RM's writes: "…Animals were individually placed in a glass cylinder filled with water…" [86]. The animals were tested in groups of four. From the author of this test, to the best of my knowledge first published 1977, animals are to be visually shielded from one another if they are to be tested in groups [91,92]. This was not done. In a more recent description for the protocol for mice, the authors write: "…While the dividers we use prevent mice from seeing each other during the test, and the white noise generator suppresses audible vocalizations, our set-up does not prevent all ultrasonic or olfactory cues from being transmitted…" [93]. We now know that for the much more social rats, audible and inaudible vocalizations during this stressful test will influence behavioral outcome [94-96].

I have no idea what "…Animals tested in forced swim test, and baseline immobility were raised in separate batches…" [86] means. Baseline immobility in the forced swimming test is what one measures *before* administering an antidepressant, see section 1.10.2. Apparently an automated system was used (Laboras, Metris, Hoofddorp, Netherlands) but the dimensions reported (37 x 21 x 24 cm) are very wrong, even if the mouse system was used. And then "…Immobility in non-stressed animals, with or without enrichment, was also examined in a novel rectangular arena…" [86]. Wasn't the device enough? Which data are we seeing? Importantly, what does this data mean? In the forced swimming test I know, but on dry ground I do not, and could not find any references. Why were animals raised in separate batches for the same experiment?

1.10.2. Misrepresentation – Statistics

Concerning analysis of data from the forced swim test, RM writes in the relevant parts of the methods and results: "…Effect of sensory enrichment…or the interaction between stress and enrichment…did

not reach statistical significance. Orthogonal planned comparisons revealed significant stress-induced reduction in body weight gain in absence or presence of enrichment…Stress did not cause significant change in immobility in presence or absence of enrichment…Latency to the first immobility event did not exhibit statistically significant intergroup differences during ANOVA…or planned comparisons…Similarly, time spent attempting to climb the vertical walls did not exhibit statistically significant differences in ANOVA…or planned comparisons…" [86].

So what was significant? "…Planned comparisons revealed that stress increased the total number of immobility events in absence of enrichment…but not in presence of enrichment…" [86].

Is number of immobility events a readable and dependable outcome of this simple test? Is it valid to say anything about depressive-like behavior without a positive control group – in other words, without administering an antidepressant and re-testing? So far, I think the answers are 'probably not' and 'no' [97-100]. And please note, here it is number of immobility events alone, with no significance in the primary readout, time in immobility, and a secondary one, climbing. RM's statement in the discussion "…Data presented here show that enrichment reduces depressive like behavior in rats, evidenced by reduction of immobility in Porsolt forced swim task…" [86] does not seem to be backed up by dis-ambiguous use of terminology, and robust technical and statistical methods.

Planned comparisons are very much used by RM and here she writes: "…These planned comparisons were guided by *a priori* experimental interest in the possibility of sensory enrichment rescuing the effects of stress…" (italics in the original) [86]. In several places in the text, RM emphasizes the "…the longer time window in this design…" [86], perhaps as a justification for the number and sequence of cohorts described in the methods – which, incidentally and in contradiction to the statement just quoted, started environmental enrichment *on the same day* as chronic immobilization. As these were planned, and one may not ethically apply a planned comparison twice to the same data, it was either impressive foresight or else very fortuitous that the experimenter measured number of immobility events, in addition to time in immobility and other outcomes, on the first analysis. Manually, of course.

RM writes: "…Non-orthogonal comparisons were avoided in order to preclude spurious effects…". This confused me. Were they planned or not? Does this mean that non-orthogonal comparisons do not preclude spurious effects? So what does significance mean in non-orthogonal comparisons? Why is a justification needed at all if planned and orthogonal comparisons are allowed and were done? (P.S. RM uses the same phrase in [85])

1.10.3. Falsification and misrepresentation – salami publications

RM in co-authorship with others and AA as first author produced articles from an experiment and published the findings separately for prefrontal cortex and basolateral amygdala. A third was produced with ASK as first author, RM as last and corresponding author, and AA as middle author, the same study but with the findings on hippocampus. In these and other articles detailed in this section, the main manipulation is environmental enrichment, and is referred to in the titles as 'housing environment', 'short-term environmental enrichment', 'early-life short-term environmental enrichment', and 'complex housing'. In addition to misrepresentation and falsification, this is concerning because accurate terminology is required for scientific investigation. Furthermore, it is unusual because if RM is as concerned with her 'lab culture' as she claims (*Appendix.pdf*, Appendix C) then it follows that the PI would work towards establishing a style of reference to a method or manipulation and it's reporting. For example, in addition to what the manipulation is (environmental enrichment), control group animals can be called 'standard', 'not enriched', 'animal-facility reared', or 'simple' depending on the article by RM.

The datasets are mixed and matched with one or more behavioral correlate confused with an animal model and which are misrepresented, mistermed, misused, or all three. Briefly the present article used datasets in which environmental enrichment rats and/or their control group counterparts were used and correlated with a behavioral test and/or neuronal analysis as follows: (i) histological neuron analysis after enrichment [82]; (ii) anxiety-like behavior after maternal separation in a cohort of males

OR predator odor exposure in an experimentally unrelated cohort of adult females all with no enrichment [68]; (iii) histological neuron analysis after exposure to predator odor with no enrichment [67]; (iv) anxiety-like behavior after chronic immobilization stress with enrichment [85]; (v) anxiety-like behavior after chronic immobilization stress with enrichment – please note this is not a typographical error RM's articles referred to are different [86]; and (vi) anxiety-like behavior after maternal separation with enrichment [83].

Call something different here (e.g. predator odor in the circular arena *vs.* OFT, serological *vs.* genetic), add a little thing there (e.g. mRNA or ELISA assay) and it spirals to other references, more than one mentioned in this submission, by AV and others.

Fundamental physiological contradictions emerge in RM's salami publications such as outlined in section 1.8.4. Here [86], she uses an obscure and barely significant datum from a poorly done test all to say that it's about something new (depression-like behavior) somewhere new (hippocampus). Assuming a project along the lines of 'using behavioral testing and histology to assess the effect of environmental enrichment on the defense response': a study of plasticity and neural correlates of fear in prefrontal cortex, hippocampus, and basolateral amygdala is highly relevant [101-110] and could have been ethically arranged in multiple publications. Adding a test to a dataset does not make a new animal model from an old one. Slicing the behavioral data is not meaningful, and not reporting that data is from another study is unethical.

1.11. Paper VIII [83]

Title: Early-life short-term environmental enrichment counteracts the effects of stress on anxiety-like behavior, brain-derived neurotrophic factor and nuclear translocation of glucocorticoid receptors in the basolateral amygdala
Authors: Hegde A, Suresh S, Mitra R
Journal: Scientific reports
Year of publication: 2020
URL: https://www.nature.com/articles/s41598-020-70875-5
DOI: 10.1038/s41598-020-70875-5

1.11.1. Falsification and misrepresentation

See section 1.10.3.

1.11.2. Suspected fabrication and/or misrepresentation of BDNF and GR quantification*†

To the best of my knowledge, Abcam does and did not produce horse anti-mouse IgG secondary antibodies used in this study for brain-derived neurotrophic factor (BDNF) quantification. Santa Cruz discontinued production of primary antibodies against glucocorticoid receptor (GR) used in this study for quantification of GR. The last publication listed on the company website using rabbit anti-GR (P-20): sc-1002 was published in 2015. The last publication listed using rabbit anti-GR (H-300): sc-8992 was published in 2017. The last publication listed on the company website using rabbit anti-GR (M-20): sc-1004 was published in 2017.

Immunofluorescence is not an appropriate technique for quantification of BDNF [111,112]. Immunohistochemistry of BDNF is used for localization. Thus far I have found two other articles estimating BDNF quantity with immunofluorescence alone, one quantifying BDNF as weak, moderate, or strong in human brain, the other by comparative quantification of the positive neuron with a negative control in the same field of view in cultured cells [113,114]. Quantification of GR with immunofluorescence is challenging, used in human studies, requires extensive controlling, and often combined with other methods of quantification [115-117].

I do not understand anything on these quantifications from the methods in this article [83] nor its Supplementary Figure 2. The image-data files were not preserved. To batch-process image files in an

ImageJ script one may need to collect the image files in a folder without any image-data files, but storage requirements for image-data files are negligible. On hearsay, images are not batch-processed in ImageJ at the RM lab, they are put into a script one-by-one. In any case, the software used for BDNF quantification is unknown. It was reported in [83] that ImageJ was used for Western blot analysis, not confocal images. GR quantification was done manually by counting GR over DAPI. I do not understand why Western blot was used on those samples in the presence of superior and well-described quantification techniques, including used by RM in [85] and which would have made it either unnecessary to quantify with fluorescence, or supported the data.

1.11.3. Erratum

I do not think the data in Figure 4(B) matches the images in Figure 4(A).

1.12. Paper IX [118]

Title: Prefrontal-hippocampus plasticity reinstated by an enriched environment during stress
Authors: Wu Y, Mitra R
Journal: Neuroscience Research
Year of publication: 2020
URL: http://www.sciencedirect.com/science/article/pii/S0168010220304041
DOI: 10.1016/j.neures.2020.07.004

This worried me. What are "…Non-survival electrophysiological measurements…" [118]?

I do not think it is possible to measure field excitatory post-synaptic potentials (fEPSP) in intact brain because: (i) voltage is potential difference, so there must be something to measure the recorded signal against, which in slice work is ground or zero (the potential difference across the intact blood-brain barrier is unknown); (ii) to say it is excitatory or inhibitory with confidence one must first measure reversal potential; and (iii) when using some kind of wire (or even fancy electrode) stuck into brain, one does not know for sure if the changes in recorded voltage are pre- or post-synaptic in origin.

It is therefore generally agreed that changes in voltage measured from brain *in vivo* are more accurately called local field potential. The reference in the methods is [119] and refers to another by a co-author [120]. In [119], the authors awkwardly avoid emphasizing *excitatory*. In [120] the signal measured is referred to as *evoked*. This is significant in light of evidence below. For the sake of argument, let's assume for a moment that any voltage measured was indeed excitatory and post-synaptic.

What does 0.033 Hz refer to? It can't be the stimulating frequency, that was 250 Hz. Is it a cut-off frequency?

I'm impressed that an A-M Systems Model 1800 amplifier was used. It is old, but good for simple stimulation protocols such as used for electromyography or as a teaching tool. To measure local field potential or putative fEPSP, it is inadequate. Perhaps with the right preamplifier or headstage, it just *might* work. It would not work with the headstage that comes with the amplifier, modified or not. Frankly I would not bother trying, even if a clever engineer custom-made a preamplifier for me with an appropriate input resistance. *In vivo* electrophysiology experiments are not easy, it defeats the purpose to use such a device. But these were "…Non-survival electrophysiological measurements…" [118] not *in vivo*, so I'm missing information vital for replication. Was the signal digitized or was a paper-chart recorder used? If it was digitized, with what and what software was used for analysis?

The electrodes used were apparently sourced from Kopf Instruments. But Kopf Instruments do not produce electrodes. Were they Rhodes electrodes, originally made by Kopf? Or were Kopf Instruments an intermediary supplier for WPI or MicroProbes? That might be the case for the stimulating electrode, SNE-100. I can't guess for the recording electrode, SNE-300. But it *is* stated the recording electrode was unipolar, so there must be a reference electrode. Obviously, if there is no reference

electrode, there is no potential difference and no signal. What was the nature of the reference electrode? Where was it put?

As mentioned in Appendix K (*Appendix.pdf*), the Supplementary Data file doesn't work.

Who is YW I wonder? Both the stimulating (SNE-100) and recording (SNE-300) electrodes were used in an article co-authored by YW [121] in which YW's affiliations were: (i) Department of Pharmacology, Yong Loo Lin School of Medicine, National University Health System, NUS, Centre for Life Sciences; (ii) Neurobiology and Ageing Programme, Life Sciences Institute, NUS; and (iii) Singapore Institute for Neurotechnology (SINAPSE). But I could not find YW. In another article on which YW is co-author, the same stimulating electrode is used [122]. Both the stimulating and recording electrodes were also used in another article by one of YW's co-authors [123]. Both electrodes are used in YW's Master's thesis [124] and *there* we find the reference for 'fEPSP' and the method from YW's co-authors [121]. But fEPSP was never measured. Neither in [121] nor in YW's thesis according to the methods and results in the thesis [124]. Explicitly in the former and incorrectly in the latter, fEPSP denoted field *evoked* potential. They're about long-term potentiation. But whether excitatory *vs.* evoked and pre- *vs.* post-synaptic becomes of secondary importance.

I think it is important to establish what "…Non-survival electrophysiological measurements…" [84] are. If RM did *in vivo* experiments without IACUC approval, and then convolutes the language to indicate otherwise, that's bad but would not surprise me in light of the evidence above and see also section 3.1. below. If she has conceptualized a novel neither *in vivo* nor *in* vitro, some kind of *in limbo* method for electrophysiology measurements, she should share it with us.

2. NTU AUP: A19027

I was asked to mention this.

a. The Project Title is *Neurobiology of Resilience*. The funding source is the *Defining the brain circuitry defects that cause dementia* (AcRF Tier 3) grant. Another funding source is *Connectivity of ventromedial prefrontal cortex in simple and complex housing* living *environment* (AcRF Tier 1). Funding status is awarded with expiry date 30-June-2023.
b. I was surprised when I read this application for several reasons. Foremost, it appears that the application is for one project, in line with international standards of ethics (one project, one application). However, in my opinion as a physiologist and the reality imposed by RM, the application is for two separate research projects. I had very quickly checked NTU IACUC Guidelines, and interpreted it as OK to have two projects in one application as a series of related experiments.
c. It is evident in what regard RM holds NTU research policy and ethical standards. RM was not honest with me and SBS HR about the budget available for my salary (*Appendix.pdf*, Appendix D). She was also not honest about the budget available for the research I came for (*Appendix.pdf*, Appendix D). RM falsely accused NTU IACUC of giving incorrect information (*Appendix.pdf*, Appendix H). RM's regard for standards is also evident in the application, for example, section *(6) Animal Use and 3Rs*. She put two projects in one application – perhaps she was not feeling well, but it should have weighed on her mind and she should have asked me to separate the projects – immediately. Unfortunately, this question is rendered rhetorical by the following section.

3. Present research misconduct activity

3.1. Animal experiments

I mentioned in a comment in my previous submission (*Appendix.pdf*, Appendix I) that trunk blood was to be collected from aged mice belonging to another PI. At this stage of the inquiry it is known to whom the mice belong. This 'experiment' was done in my absence: I took annual leave on that and the preceding day (see section 3.2.). I would like to clarify:

a. This terminal sample collection from these animals is in principle against NACLAR Guidelines. It is not possible to construe a research question, objective, plan, methodological constraint, or other objective reason so that this terminal sample collection from these animals would be allowed under NACLAR Guidelines. It is also not possible to re- or mis-interpret NACLAR Guidelines so such terminal sample collection would be allowed. The Guidelines are very clear as they relate to the present.
b. Needless to say, NTU IACUC were unaware of these experiments and would not have allowed violation of NACLAR Guidelines. Other major and highly unethical inconsistencies between the submitted AUP and these specific experiments are obvious in the submitted AUP.
c. The method is not fine. Trunk blood in this case means cutting the mouse down its front side. A small plastic tube is then used to collect some tens of microliters of blood from the pool that gathers in the abdominal and thoracic areas of the excoriated mouse, and the corpse is discarded. Trunk blood is dirty, we don't know what it's been in contact with – lung, intestine, liver, skin and fur? The method of killing these animals, wholesale with CO_2, might be generally accepted among some authors but it is really not OK. The downstream effects of CO_2 on pH, potassium, calcium, and so on, are very well-known, and so one should think very carefully before using CO_2 to kill an animal for a terminal sample. More so if there is no reason not to.
d. I won't kill several dozens of animals for no reason (see section 3.2.). Since I know nothing about the research question and objective, I would have killed them for the sake of, as far as I know, RM's whim. And a few drops of bad-quality blood. Not even brain, in a huge study on dementia. From aged and genetically modified mice that don't belong to RM. This a couple of hours after I've put these animals through RM's experimental protocol in which the behavior being tested is not tested. To later spend weeks analyzing the meaningless tests with a stopwatch when the same data and much more can be obtained instantly using a software available at no cost to RM in the same room the experiments were done. And during those couple of hours between behavioral (un)testing and butchering, she later decides and 'updates' me through the Research Assistant (see *Appendix.pdf*, Appendix H), attend an online meeting with herself with an unknown and unpredictable agenda.
e. If the cost of keeping a cage of 4 mice is $15 per day, then it's about $800 for one of these mice. That's about $25,000 of Singapore's money down the drain on a Friday afternoon with RM. And that's just cage costs. My duty and responsibilities assigned to me as a researcher are first to Singapore, not RM.

3.1. Communication, update

In section 3.1.2. below is the email I received from RM in the afternoon of Monday 14th of September with the Research Assistant cc-ed, and my reply. In section 3.1.1. below is the email I sent to RM morning of the same day. In section 3.1.3. is evidence of the phone call I received from the Research Assistant also the same day. In this phone call, I was made aware that RM was upset with the email I had sent earlier in the day.

3.1.1. Email to RM Monday 4th of September morning

From: Mohamed Mustafa Mahmoud Helmy <mohd.mustafa@ntu.edu.sg>
Date: Monday, September 14, 2020 at 8:44 AM
To: "Rupshi Mitra (Asst Prof)" <RMitra@ntu.edu.sg>
Subject: Re: Meeting 03.09.2020

Hello Rupshi,

I had requested meetings with an agenda to discuss experimental plans many times. On two occasions, I requested to meet with an agenda to discuss organizational challenges. We had a meeting on Wednesday 3rd of September, the minutes of this meeting are below, but the agenda is still unknown to me. The conclusion of this meeting denied me any accessibility to information and resources I need to meet my duties and responsibilities, and to preserve research integrity. The content of what I wish to communicate and a summary of it is in text below.

CONTENT

1. **Training**
   I was surprised you thought I need to be trained how to manually analyze videos of rodent experiments. You wrote to Maria at SingHealth yourself describing my extensive teaching and research expertise. Your argument that 'you have no published material on the light-dark box therefore you need training' is puzzling, may you please clarify?

2. **Manual *vs.* automatic analysis**
   I think that analyzing behavioral videos manually for days and weeks for one outcome when it can be done in ANY-maze in minutes for many outcomes and at no financial cost is a waste of time and energy. The urgency to generate data from behavioral videos is not aligned with the fact that the same data plus much more can be obtained instantly at the site of experiment with no further effort nor delay. Why am I not allowed to use ANY-maze?

3. **Experimental protocol and manual analysis**
   - Resileo laboratory protocol presently states 10 lux is to be used in the light compartment of the light-dark box (LDB). The least amount of light used in the light compartment that I could find in the literature is 100 lux, and about 50 lux

would then be measured in the dark compartment from scattered light. Most authors use between 200 to 500 and up to 1000 lux in the light compartment. High light levels are in line with the LDB as a test of anxiety-like behavior, but clearly current Resileo protocol is using LDB to test something different. As I do not know the research question, I cannot guess why 10 lux is used here. I would need a justification for doing the experiment in this way.

- You wanted me to measure *number of entries into light* and *number of entries into dark*. These two parameters will always be either identical or the difference between them will be one (1). May you please clarify: (i) The meaning of these parameters as opposed to simply measuring transitions in LDB; (ii) Why *number of entries into light* and *number of entries into dark* were not included in the first analysis; (iii) Why measuring *number of entries into light* and *number of entries into dark* was not part of my training and subsequent random checks by Shruti if accuracy is concerned?
- You wanted me to statistically analyze behavioral data from Batch 1. I would need a justification, because I think statistical analysis of a batch in a cohort in an ongoing experiment is unethical.

4. **CORT**

   I had sent you a sheet with a table summarizing articles infusing corticosterone in the brain, corticosterone supplier, cost, and estimated time of delivery. In the same email, I requested we please meet to plan for experiments. Subsequently you: (i) Told me that we will meet after I reviewed articles infusing corticosterone into brain when you already had the sheet in an attachment; (ii) Told me to summarize information in the sheet which I did with more text in an email than there was in the table; (iii) Told me to order corticosterone immediately in addition to a solvent without specifying which one and which can cost anything between $100 and $3500; (iv) Asked about supplier and cost, information which was already in the sheet with weblinks.

   May you please clarify: (i) How ordering corticosterone is a priority to meeting to plan on what to do with the corticosterone and how to do it; (ii) Why these experiments are pursued since the associated grant is expiring in December; (iii) How I was meant to place an order with Sigma-Aldrich immediately without the information I would need to place an order in Ariba; (iv) Why procurement is now one of my duties after you had previously assured me it was not?

5. **Grant and research allocation**

   As I am now working in the T3 grant, the time-frame shift you had said meant the Neurobiology of Stress research has to end in December is no longer a problem?

6. **Sample collection**

   The samples to be collected on Friday 11th September were trunk blood from aged mice, including genetically modified. From the method to be applied and housing conditions, I deduced that there is no contraindication to collecting brain in addition to other samples. From the fact that we do not own any cages at LKC, I deduced that the mice belong to another PI. Since neuroscience is the field, I do not understand why brain will not be collected from these precious animals. I am doubtful CO_2 is the

ideal method for terminal sample collection. I would need a justification for such tasks where the 3Rs Principle is not upheld and NACLAR Guidelines are not followed.

7. **Finalized review**

In our meeting you said you would upload the review in two hours but I did not receive a notification email. Perhaps *Frontiers in Neuroendocrinology* does not send notification emails to co-authors?

8. **Novel review/opinion**

I looked through relevant emails and notes you sent in *situational.docx* and was able to rule out certain lines of fear research, but was unable to locate a particular topic in animal and translational fear research to review/opinion. I had previously sent two documents with related references. I asked if the topic is situational reminders in animal models of PTSD and have not yet received a reply. I asked during the meeting if I may meet with Shruti and Archana to coordinate the work and make a timeline, and was prohibited from doing so. I would need further instructions on the topic to review or the opinion to be expressed and my contribution to it.

9. **Co-authorship**

I would appreciate it if we please make an agreement on co-authorship for my contribution to the T3 grant and novel review/opinion.

10. **Communication**

I have been receiving my duties and times for online meetings from Shruti. May you please clarify why I am not cc-ed in schedule and meeting planning?

SUMMARY

I understand it is your wish to discuss research question, objective, protocol, and plan, my duties and responsibilities, and plans for co-authorship at a later date.
After careful consideration and discussion with others, I am concerned that working in the T3 grant research without an informed question, objective, protocol, and plan is breaching Research Integrity Policy, and without informed duties and responsibilities is breaching The University Code of Conduct. Please share this information with me. Please note this information is essential.

Kind regards,
Helmy

From: Mohamed Mustafa Mahmoud Helmy <mohd.mustafa@ntu.edu.sg>
Date: Thursday, September 3, 2020 at 10:09 PM
To: "Rupshi Mitra (Asst Prof)" <RMitra@ntu.edu.sg>
Subject: Meeting 03.09.2020

Hi Rupshi,

Thank you for meeting with me today, below are the minutes.

1. BLA review
   I will today send the material I generated for our review and the to-do list for finalizing to you to upload to *Frontiers in Neuroendocrinology.*

2. Behavioral experiments
   Analysis will be done manually and ANYMAZE will not be used. I will be informed on research question, objective, and behavioral outcomes after my training. Co-authorship will also be addressed at a later point.

3. Situational review/opinion
   Address points in your email on the topic and notes in *situational.docx.*

4. Office computer
   I follow-up with Alex to replace my desktop with a new one.

Kind regards,
Helmy

3.1.2. Emails from/to RM with the Research Assistant cc-ed on Monday 4th of September afternoon

From: Mohamed Mustafa Mahmoud Helmy <mohd.mustafa@ntu.edu.sg>
Date: Monday, September 14, 2020 at 1:57 PM
To: "Rupshi Mitra (Asst Prof)" <RMitra@ntu.edu.sg>, Suresh Shruti <shruti.suresh@ntu.edu.sg>
Subject: Re: Absent/ Instructions

Hi Rupshi,

I am waiting for your instructions as detailed in the email I sent to you this morning at 08.44, subject *Re: Meeting 03.09.2020*.

I am excellently well, thank you. There was and is no need for medical advice nor certificate.

Kind regards,
Helmy

From: "Rupshi Mitra (Asst Prof)" <RMitra@ntu.edu.sg>
Date: Monday, September 14, 2020 at 1:31 PM
To: Mohamed Mustafa Mahmoud Helmy <mohd.mustafa@ntu.edu.sg>, Suresh Shruti <shruti.suresh@ntu.edu.sg>
Subject: Re: Absent

Hi

How are you today? Hope you are feeling better and in lab. If you are still not feeling well, do seek medical advice and send a medical certificate to me/our office. Do apply for MC online too.

Bests
Rupshi

From: Mohamed Mustafa Mahmoud Helmy <mohd.mustafa@ntu.edu.sg>
Sent: Wednesday, September 9, 2020 11:45 AM
To: Rupshi Mitra (Asst Prof) <RMitra@ntu.edu.sg>; Suresh Shruti <shruti.suresh@ntu.edu.sg>
Subject: Absent

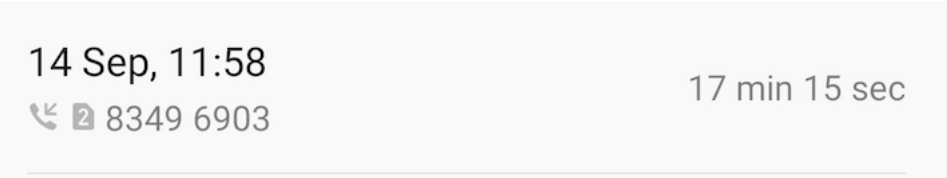
Hi

I'm really not feeling well and need to stay home.

I'll fill in online for leave 10.10 – 11.10 and/or inform HR ASAP.

Helmy

3.1.3. Phone call from the Research Assistant



4. Research misconduct in addition to publications*†

I hope evidence presented in *Appendix.pdf* should illustrate other breaches or suspected breaches of research integrity not related to already published articles, namely:

a. Haphazard design and implementation of research.
b. Questionable methods and record keeping.
c. Lack of professional courtesy, honesty, and good stewardship.
d. Disregard of objective evidence in preference to her opinion in research matters.
e. Matters of co-authorship.
f. External research collaborations.
g. Unethical conduct and absence of trust and respect in her professional relationships.
h. Commitment to excellence and proper use of resources.

At this stage of the inquiry I realize that the quantity of evidence in *Appendix.pd* may have been excessive and difficult to reconstruct from how research integrity was breached in ways not related to published articles. Please allow me to relate events indicating research misconduct listed above (a. to h.) in the form of a short narrative (of course, any evidence not in *Appendix.pdf* is available to the inquiry):

I came to Singapore to engage in research activity and produce knowledge at NTU for important challenges facing society using cutting edge technology in an atmosphere of safety, integrity, and transparency. I asked RM if I can start working on a review or grant, and if I can start animal work with my previous RCULAC.

During the Circuit Breaker I worked on a review on the subject of RM's expertise, neuroplasticity and the basolateral amygdala (BLA). *Appendix.pdf* shows how her contribution was at best meaningless, at worst obstructive and negative. Managerial matters aside, I am happy to do the research with little guidance, but it is her role as supervisor to give this little guidance or not get in the way.

For example, my initial outline for the review (*Review BLA outline v1.do*cx) did not include anything on anti-depressants and the BLA, it's a minefield for both RM and myself in terms of expertise (actually, now that I've looked at that document again…it looks a lot like an outline of the finished product. Just the arrangement of topics is different). In an online meeting after I sent this document to RM, she rolled back progress and told me to start with a 'general literature search', kind of 'to see what's out there on BLA'. At the time, I thought to myself 'She's the expert, my outline must have been deficient'. What followed is documents and documents of references, abstracts, outlines, proposed figures, possible journals, feedbacks, updates, and online meetings, phone calls, WhatsApp messages, and so on. In building a new outline for the review, many new and irrelevant topics entered the discussion, including anti-depressants. It took me much time and effort to come to the independent conclusion that 'I will not include anything about anti-depressants and the BLA in this review. This is because pharmacologic manipulation of neurotransmitters is only indirectly related to post-stress BLA hypertrophy and has brain-wide effects. Since even direct mechanisms underlying BLA hypertrophy are poorly understood, talking about anti-depressants is unhelpful, and the subject of an speculative review on its own'. RM should have not introduced anti-depressants into the review outline, nor subsequently encouraged a continuation of their presence. Even better, she could have just said '*Review BLA outline v1.do*cx looks good, carry on' to begin with.

From *Appendix.pdf*, the reader may see she was unable to decide if 'environmental enrichment' or EE, an area she is well-published in, is in or out. In light of section 1 above, perhaps this is understood.

After the Circuit Breaker I started building a setup for animal neuroscience surgery. We had group meetings, and I shared documents on technical specifications of products, proposed plans, and so on. At some point, AV and I met and made concrete steps forward on what we need to buy (RM withheld permission for this meeting until the last minute). I felt the interaction with AV was pleasant and constructive. I very gladly agreed to train his staff for animal surgery, and implied I am also happy to continue helping them in their own work as needed outside working hours.

I met with representatives of suppliers. I made concrete lists of what we need to buy, streamlined the planned animal surgery experiments, and potentially saved 20-40% of a bill in the fifteen- to twenty-thousand dollar range by finding alternative solutions and products. I wrote plans for experiments for RM, and pleaded to meet her at the time and place of her choosing to discuss these plans. I tried to make these plans look less laughable with RM's demands for things like metabolism and inflammation and histology and electrophysiology all to be included in the same plan. When AV commented in an email that our 'plan' is very ambitious, RM immediately sent me a directive by email that I immediately elaborate on how I would 'hypothetically' put *all* these plans into effect immediately, like tomorrow (I am not exaggerating nor being polemical. This happened). All I could do was to work on a useless document containing mostly her own suggestions and amazing ideas she 'brought up' in an online meeting (the realistic parts were already and sufficiently elaborated for further discussion). I wrote how I would hypothetically approach this plethora of research, using my own expertise or in collaboration with others at NTU. I restrained myself from saying or writing: 'How and why am I supposed to immediately and concretely elaborate on execution of an unrealistic and extremely ambiguous multitude of plans that exist only in your head, RM? And which are based on a research question and objective which, to the best of my knowledge, do not exist anywhere, RM?'

Then there was the Roozendaal Affair, the 'email query' I sent to an author in The Netherlands. I was only doing what I was told – by that time I had stopped thinking what was necessary or meaningful. She sent me an email that was very unprofessional, to say the least. I don't think it's OK for a PI at NTU to send an email to her Research Fellow basically saying: 'you silly boy, it took me 5 minutes to figure out what took you days, do your homework first and do it properly before embarrassing us all in front of world-leading authorities and revealing our plans, here's a paper and link to what was worrying you so much'. Communication issues aside, as we are here focused on issues of research integrity: (i) I contacted the world-leading authority on her own demand for an unknown research question regarding an outdated research method; (ii) the query was her own and was unnecessary had she looked at the research plans I sent and which offer alternatives (I would never willingly put ethanol into brain during brain research); (iii) there were no plans to reveal; and importantly (iv) the paper and link she sent contained nothing on what she's talking about.

At that point, my research integrity and sense of duty to the University as regards to research compelled me to argue back objectively. So she threatened to terminate my contract, cancelled the research two laboratories and their group members were working on, was dishonest about the reasons for cancellation of this research, demoted me to an assistant of an assistant, told me to train how to use a stopwatch, and that using software for analysis, research question, objective, plan, protocol and co-authorship were none of my concern. My duties and responsibilities as Research Fellow were also none of my concern. All this was to remain none of my concern until I'm done with my training and subsequent experiments. *And* she'll take the review I wrote immediately, and that's none of my concern too, of course. As if to reinforce my silencing, she communicated my tasks to me through the Research Assistant.

I was disappointed that I had the opportunity to join NTU, only to be silently trained on the research of using a stopwatch. Nevertheless, I understood there was no formal breach of NTU Research Integrity Policy, and so I continued doing as I was told.

But in doing what I was told, over the course of two weeks I realized that: (i) RM's published material is almost certainly research misconduct (see section 1); and (ii) I will become implicated in research misconduct (see sections 2 and 3).

Needless to say, I have neither training nor power to translate the normative and empirical implications of the NTU Research Integrity Policy and The University Code of conduct as regards to RM's research activities and my involvement, only to signal something's wrong. If it's about creative and technical excellence in scientific research and supervision of research, there is evidence for concern. If it's about ethical conduct of research activities at NTU to produce knowledge and publish articles, there is evidence to the contrary.

I trust in the Office and Persons to whom this is addressed, and hope I provided sufficient clarification for the inquiry into research-related matters of my previous submission.

5. References

Note on the references: To revert quickly to the inquiry, I did not change the letters in the titles all into small or capital. DOI is included where applicable to the reference, and/or URL. I hope the references are accurate, and apologize in advance for any irregularity.

1. Whishaw IQ, Kolb B. (2005). *The behavior of the laboratory rat: a handbook with tests*. Ian Q. Qhishaw and Bryan Kolb Eds. Oxford University Press. ISBN-13: 9780195162851. doi:10.1093/acprof:oso/9780195162851.001.0001

2. BX43/BX46/BX53 brochure date unknown. https://lri.se/onewebmedia/LRI_Olympus%20BX43BX46BX53%20brochure.pdf.

3. Bhaskar Y, Lim LW, Mitra R. (2018). Enriched Environment Facilitates Anxiolytic Efficacy Driven by Deep-Brain Stimulation of Medial Prefrontal Cortex. *Frontiers in Behavioral Neuroscience* **12** (204). https://www.frontiersin.org/article/10.3389/fnbeh.2018.00204. doi:10.3389/fnbeh.2018.00204

4. Liu A, Jain N, Vyas A, Lim LW. (2015). Ventromedial prefrontal cortex stimulation enhances memory and hippocampal neurogenesis in the middle-aged rats. *Elife* **4**:e04803. https://elifesciences.org/articles/04803. doi:10.7554/eLife.04803

5. Hill R, Kouremenos K, Tull D, Maggi A, Schroeder A, Gibbons A, Kulkarni J, Sundram S, Du X. (2020). Bazedoxifene–a promising brain active SERM that crosses the blood brain barrier and enhances spatial memory. *Psychoneuroendocrinology*:104830. https://www.sciencedirect.com/science/article/pii/S0306453020302523. doi:10.1016/j.psyneuen.2020.104830

6. Du X, Hill RA. (2019). Hypothalamic-pituitary-gonadal axis dysfunction: An innate pathophysiology of schizophrenia? *General and Comparative Endocrinology* **275**:38-43. https://www.sciencedirect.com/science/article/pii/S0016648018305422. doi:https://doi.org/10.1016/j.ygcen.2019.02.009

7. Du X, Mccarthny CR, Notaras M, Van Den Buuse M, Hill RA. (2019). Effect of adolescent androgen manipulation on psychosis-like behaviour in adulthood in BDNF heterozygous and control mice. *Hormones and Behavior* **112**:32-41. https://www.sciencedirect.com/science/article/pii/S0018506X18304628. doi:https://doi.org/10.1016/j.yhbeh.2019.03.005

8. Schroeder A, Nakamura JP, Hudson M, Jones NC, Du X, Sundram S, Hill RA. (2019). Raloxifene recovers effects of prenatal immune activation on cognitive task-induced gamma power. *Psychoneuroendocrinology* **110**:104448. https://www.sciencedirect.com/science/article/pii/S0306453019309722. doi:https://doi.org/10.1016/j.psyneuen.2019.104448

9. Grech AM, Du X, Murray SS, Xiao J, Hill RA. (2019). Sex-specific spatial memory deficits in mice with a conditional TrkB deletion on parvalbumin interneurons. *Behavioural Brain Research* **372**:111984. https://www.sciencedirect.com/science/article/pii/S0166432819305339. doi:https://doi.org/10.1016/j.bbr.2019.111984

10. Nakamura JP, Schroeder A, Hudson M, Jones N, Gillespie B, Du X, Notaras M, Swaminathan V, Reay WR, Atkins JR, Green MJ, Carr VJ, Cairns MJ, Sundram S, Hill RA. (2019). The maternal immune activation model uncovers a role for the Arx gene in GABAergic dysfunction in schizophrenia. *Brain,*

Behavior, and Immunity **81**:161-171. https://www.sciencedirect.com/science/article/pii/S0889159119301837. doi:https://doi.org/10.1016/j.bbi.2019.06.009

11. Mccarthny CR, Du X, Wu YC, Hill RA. (2018). Investigating the Interactive Effects of Sex Steroid Hormones and Brain-Derived Neurotrophic Factor during Adolescence on Hippocampal NMDA Receptor Expression. *International Journal of Endocrinology* **2018**:7231915. https://doi.org/10.1155/2018/7231915. doi:10.1155/2018/7231915

12. Schroeder A, Notaras M, Du X, Hill RA. (2018). On the developmental timing of stress: delineating sex-specific effects of stress across development on adult behavior. *Brain sciences* **8** (7):121. https://www.mdpi.com/2076-3425/8/7/121. doi:10.3390/brainsci8070121

13. Du X, Serena K, Hwang WJ, Grech AM, Wu YWC, Schroeder A, Hill RA. (2018). Prefrontal cortical parvalbumin and somatostatin expression and cell density increase during adolescence and are modified by BDNF and sex. *Molecular and Cellular Neuroscience* **88**:177-188. https://www.sciencedirect.com/science/article/pii/S104474311730310X. doi:https://doi.org/10.1016/j.mcn.2018.02.001

14. Schroeder A, Hudson M, Du X, Wu YWC, Nakamura J, Van Den Buuse M, Jones NC, Hill RA. (2017). Estradiol and raloxifene modulate hippocampal gamma oscillations during a spatial memory task. *Psychoneuroendocrinology* **78**:85-92. https://www.sciencedirect.com/science/article/pii/S0306453016309878. doi:10.1016/j.psyneuen.2017.01.022

15. Notaras M, Du X, Gogos J, Van Den Buuse M, Hill RA. (2017). The BDNF Val66Met polymorphism regulates glucocorticoid-induced corticohippocampal remodeling and behavioral despair. *Translational Psychiatry* **7** (9):e1233-e1233. https://doi.org/10.1038/tp.2017.205. doi:10.1038/tp.2017.205

16. Du X, Hill RA. (2016). The Potential of Gonadal Hormone Signalling Pathways as Therapeutics for Dementia. *Journal of Molecular Neuroscience* **60** (3):336-348. https://link.springer.com/article/10.1007/s12031-016-0813-9. doi:10.1007/s12031-016-0813-9

17. Du X, Hill RA. (2015). 7,8-Dihydroxyflavone as a pro-neurotrophic treatment for neurodevelopmental disorders. *Neurochemistry International* **89**:170-180. https://www.sciencedirect.com/science/article/pii/S0197018615300231. doi:https://doi.org/10.1016/j.neuint.2015.07.021

18. Wu YWC, Du X, Van Den Buuse M, Hill RA. (2015). Analyzing the influence of BDNF heterozygosity on spatial memory response to 17β-estradiol. *Translational Psychiatry* **5** (1):e498-e498. https://doi.org/10.1038/tp.2014.143. doi:10.1038/tp.2014.143

19. Du X, Wu YC, Hill RA. (2014). BDNF–TrkB signaling as a therapeutic target in neuropsychiatric disorders. *Journal of Receptor, Ligand and Channel Research* **7**:61-79. https://pdfs.semanticscholar.org/dc44/c25e86a29dceb100afb06e658d95e91a34b8.pdf. doi:10.2147/JRLCR.S50404

20. Wu YC, Du X, Van Den Buuse M, Hill RA. (2014). Sex differences in the adolescent developmental trajectory of parvalbumin interneurons in the hippocampus: a role for estradiol. *Psychoneuroendocrinology* **45**:167-178. https://www.sciencedirect.com/science/article/pii/S0306453014001127. doi:10.1016/j.psyneuen.2014.03.016

21. Du X, Pang T, Hannan A. (2013). A Tale of Two Maladies? Pathogenesis of Depression with and without the Huntington's Disease Gene Mutation. *Frontiers in Neurology* **4** (81). https://www.frontiersin.org/article/10.3389/fneur.2013.00081. doi:10.3389/fneur.2013.00081

22. Hill RA, Klug M, Kiss Von Soly S, Binder MD, Hannan AJ, Van Den Buuse M. (2014). Sex-specific disruptions in spatial memory and anhedonia in a "two hit" rat model correspond with alterations in hippocampal brain-derived neurotrophic factor expression and signaling. *Hippocampus* **24** (10):1197-1211. https://doi.org/10.1002/hipo.22302. doi:10.1002/hipo.22302

23. Klug M, Hill RA, Choy KHC, Kyrios M, Hannan AJ, Van Den Buuse M. (2012). Long-term behavioral and NMDA receptor effects of young-adult corticosterone treatment in BDNF heterozygous mice. *Neurobiology of disease* **46** (3):722-731. https://www.sciencedirect.com/science/article/abs/pii/S0969996112000861. doi:10.1016/j.nbd.2012.03.015

24. Pang T, Du X, Catchlove W, Renoir T, Lawrence A, Hannan A. (2013). Positive environmental modification of depressive phenotype and abnormal hypothalamic-pituitary-adrenal axis activity in female C57BL/6J mice during abstinence from chronic ethanol consumption. *Frontiers in Pharmacology* **4** (93). https://www.frontiersin.org/article/10.3389/fphar.2013.00093. doi:10.3389/fphar.2013.00093

25. Pang TY, Renoir T, Du X, Lawrence AJ, Hannan AJ. (2013). Depression-related behaviours displayed by female C57BL/6J mice during abstinence from chronic ethanol consumption are rescued by wheel-running. *European Journal of Neuroscience* **37** (11):1803-1810. https://doi.org/10.1111/ejn.12195. doi:10.1111/ejn.12195

26. Du X, Leang L, Mustafa T, Renoir T, Pang TY, Hannan AJ. (2012). Environmental enrichment rescues female-specific hyperactivity of the hypothalamic-pituitary-adrenal axis in a model of Huntington's disease. *Translational Psychiatry* **2** (7):e133-e133. https://doi.org/10.1038/tp.2012.58. doi:10.1038/tp.2012.58

27. Howard ML, Palmer SJ, Taylor KM, Arthurson GJ, Spitzer MW, Du X, Pang TY, Renoir T, Hardeman EC, Hannan AJ. (2012). Mutation of Gtf2ird1 from the Williams–Beuren syndrome critical region results in facial dysplasia, motor dysfunction, and altered vocalisations. *Neurobiology of disease* **45** (3):913-922. https://www.sciencedirect.com/science/article/pii/S0969996111003858. doi:10.1016/j.nbd.2011.12.010

28. Kiriazis H, Jennings NL, Davern P, Lambert G, Su Y, Pang T, Du X, La Greca L, Head GA, Hannan AJ, Du X-J. (2012). Neurocardiac dysregulation and neurogenic arrhythmias in a transgenic mouse model of Huntington's disease. *The Journal of Physiology* **590** (22):5845-5860. https://doi.org/10.1113/jphysiol.2012.238113. doi:10.1113/jphysiol.2012.238113

29. Renoir T, Pang TYC, Zajac MS, Chan G, Du X, Leang L, Chevarin C, Lanfumey L, Hannan AJ. (2012). Treatment of depressive-like behaviour in Huntington's disease mice by chronic sertraline and exercise. *British Journal of Pharmacology* **165** (5):1375-1389. https://doi.org/10.1111/j.1476-5381.2011.01567.x. doi:10.1111/j.1476-5381.2011.01567.x

30. Renoir T, Zajac MS, Du X, Pang TY, Leang L, Chevarin C, Lanfumey L, Hannan AJ. (2011). Sexually Dimorphic Serotonergic Dysfunction in a Mouse Model of Huntington's Disease and Depression. *PLOS ONE* **6** (7):e22133. https://doi.org/10.1371/journal.pone.0022133. doi:10.1371/journal.pone.0022133

31. Pang TYC, Du X, Zajac MS, Howard ML, Hannan AJ. (2009). Altered serotonin receptor expression is associated with depression-related behavior in the R6/1 transgenic mouse model of Huntington's disease. *Human Molecular Genetics* **18** (4):753-766. https://doi.org/10.1093/hmg/ddn385. doi:10.1093/hmg/ddn385

32. Rockwell S. (2006). Ethics of peer review: a guide for manuscript reviewers. *URL: http://ori. dhhs. gov/education/products/yale/prethics. pdf.[Accessed on: 01-09-2013]*.

33. National Academies of Sciences, Engineering, and Medicine. (2017). Fostering integrity in research. https://www.ncbi.nlm.nih.gov/books/NBK475953/. doi:10.17226/21896

34. COPE. Promoting integrity in scholarly research and its publication. https://publicationethics.org/.

35. Coutureau E, Esclassan F, Di Scala G, Marchand AR. (2012). The Role of the Rat Medial Prefrontal Cortex in Adapting to Changes in Instrumental Contingency. *PLOS ONE* **7** (4):e33302. https://doi.org/10.1371/journal.pone.0033302. doi:10.1371/journal.pone.0033302

36. Almeida-Santos AF, Moreira FA, Guimaraes FS, Aguiar DC. (2017). 2-Arachidonoylglycerol endocannabinoid signaling coupled to metabotropic glutamate receptor type-5 modulates anxiety-like behavior in the rat ventromedial prefrontal cortex. *Journal of Psychopharmacology* **31** (6):740-749. https://doi.org/10.1177/0269881117704986. doi:10.1177/0269881117704986

37. Torres-Sanchez S, Perez-Caballero L, Mico JA, Celada P, Berrocoso E. (2018). Effect of Deep Brain Stimulation of the ventromedial prefrontal cortex on the noradrenergic system in rats. *Brain Stimulation* **11** (1):222-230. https://www.sciencedirect.com/science/article/pii/S1935861X17309348. doi:https://doi.org/10.1016/j.brs.2017.10.003

38. Hamani C, Machado DC, Hipólide DC, Dubiela FP, Suchecki D, Macedo CE, Tescarollo F, Martins U, Covolan L, Nobrega JN. (2012). Deep brain stimulation reverses anhedonic-like behavior in a chronic model of depression: role of serotonin and brain derived neurotrophic factor. *Biological psychiatry* **71** (1):30-35. https://pubmed.ncbi.nlm.nih.gov/22000731 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5756076/. doi:10.1016/j.biopsych.2011.08.025

39. Papp M, Gruca P, Lason M, Niemczyk M, Willner P. (2019). The role of prefrontal cortex dopamine D2 and D3 receptors in the mechanism of action of venlafaxine and deep brain stimulation in animal models of treatment-responsive and treatment-resistant depression. *Journal of Psychopharmacology* **33** (6):748-756. https://doi.org/10.1177/0269881119827889. doi:10.1177/0269881119827889

40. Hamani C, Amorim BO, Wheeler AL, Diwan M, Driesslein K, Covolan L, Butson CR, Nobrega JN. (2014). Deep brain stimulation in rats: different targets induce similar antidepressant-like effects but influence different circuits. *Neurobiology of disease* **71**:205-214. https://pubmed.ncbi.nlm.nih.gov/25131446 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5756089/. doi:10.1016/j.nbd.2014.08.007

41. Hill DF, Parent KL, Atcherley CW, Cowen SL, Heien ML. (2018). Differential release of dopamine in the nucleus accumbens evoked by low-versus high-frequency medial prefrontal cortex stimulation. *Brain stimulation* **11** (2):426-434. https://pubmed.ncbi.nlm.nih.gov/29239776 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6779483/. doi:10.1016/j.brs.2017.11.010

42. Chang CH, Chen MC, Qiu MH, Lu J. (2014). Ventromedial prefrontal cortex regulates depressive-like behavior and rapid eye movement sleep in the rat. *Neuropharmacology* **86**:125-132. https://pubmed.ncbi.nlm.nih.gov/25036609 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4188719/. doi:10.1016/j.neuropharm.2014.07.005

43. Huguet G, Kadar E, Temel Y, Lim LW. (2017). Electrical stimulation normalizes c-Fos expression in the deep cerebellar nuclei of depressive-like rats: implication of antidepressant activity. *The Cerebellum* **16** (2):398-410. https://link.springer.com/content/pdf/10.1007/s12311-016-0812-y.pdf. doi:10.1007/s12311-016-0812-y

44. Tan S, Poon CH, Chan Y-S, Lim LW. (2019). Deep brain stimulation of the prelimbic cortex disrupts consolidation of fear memories. *IBRO Reports* **6**:S66. https://www.sciencedirect.com/science/article/pii/S2451830119302699. doi:https://doi.org/10.1016/j.ibror.2019.07.217

45. Lim LW, Janssen MLF, Kocabicak E, Temel Y. (2015). The antidepressant effects of ventromedial prefrontal cortex stimulation is associated with neural activation in the medial part of the subthalamic nucleus. *Behavioural Brain Research* **279**:17-21. https://www.sciencedirect.com/science/article/pii/S0166432814007372. doi:https://doi.org/10.1016/j.bbr.2014.11.008

46. Janssen M, Lim L, Kocabicak E, Temel Y. (2015). Chapter 4: The antidepressant effects of ventromedial prefrontal cortex is associated with neural activation in the medial part of the subthalamic nucleus. Doctoral thesis of Janssen M? In *Selective stimulation of the subthalamic nucleus in Parkinson's disease*. Universiteit Maastricht. https://www.researchgate.net/profile/Marcus_Janssen/publication/275153084_Selective_stimulation_of_the_subthalamic_nucleus_in_Parkinson's_disease_Dream_or_near_future/links/5533aef90cf27acb0dedf209/Selective-stimulation-of-the-subthalamic-nucleus-in-Parkinsons-disease-Dream-or-near-future.pdf#page=73.

47. Lim LW, Prickaerts J, Huguet G, Kadar E, Hartung H, Sharp T, Temel Y. (2015). Electrical stimulation alleviates depressive-like behaviors of rats: investigation of brain targets and potential mechanisms. *Translational Psychiatry* **5** (3):e535-e535. https://doi.org/10.1038/tp.2015.24. doi:10.1038/tp.2015.24

48. Fucich EA, Paredes D, Saunders MO, Morilak DA. (2018). Activity in the Ventral Medial Prefrontal Cortex Is Necessary for the Therapeutic Effects of Extinction in Rats. *The Journal of Neuroscience* **38** (6):1408-1417. https://www.jneurosci.org/content/jneuro/38/6/1408.full.pdf. doi:10.1523/jneurosci.0635-17.2017

49. Tsutsui-Kimura I, Ohmura Y, Izumi T, Kumamoto H, Yamaguchi T, Yoshida T, Yoshioka M. (2013). Milnacipran enhances the control of impulsive action by activating D1-like receptors in the infralimbic cortex. *Psychopharmacology* **225** (2):495-504. https://doi.org/10.1007/s00213-012-2835-5. doi:10.1007/s00213-012-2835-5

50. Luque-García A, Teruel-Martí V, Martínez-Bellver S, Adell A, Cervera-Ferri A, Martínez-Ricós J. (2018). Neural oscillations in the infralimbic cortex after electrical stimulation of the amygdala. Relevance to acute stress processing. *Journal of Comparative Neurology* **526** (8):1403-1416. https://doi.org/10.1002/cne.24416. doi:10.1002/cne.24416

51. Scarlata MJ, Lee SH, Lee D, Kandigian SE, Hiller AJ, Dishart JG, Mintz GE, Wang Z, Coste GI, Mousley AL, Soler I, Lawson K, Ng AJ, Bezek JL, Bergstrom HC. (2019). Chemogenetic stimulation of

the infralimbic cortex reverses alcohol-induced fear memory overgeneralization. *Scientific Reports* **9** (1):6730. https://doi.org/10.1038/s41598-019-43159-w. doi:10.1038/s41598-019-43159-w

52. Sotres-Bayon F, Diaz-Mataix L, Bush DEA, Ledoux JE. (2009). Dissociable Roles for the Ventromedial Prefrontal Cortex and Amygdala in Fear Extinction: NR2B Contribution. *Cerebral Cortex* **19** (2):474-482. https://doi.org/10.1093/cercor/bhn099. doi:10.1093/cercor/bhn099

53. Reznikov R, Bambico FR, Diwan M, Raymond RJ, Nashed MG, Nobrega JN, Hamani C. (2018). Prefrontal Cortex Deep Brain Stimulation Improves Fear and Anxiety-Like Behavior and Reduces Basolateral Amygdala Activity in a Preclinical Model of Posttraumatic Stress Disorder. *Neuropsychopharmacology* **43** (5):1099-1106. https://doi.org/10.1038/npp.2017.207. doi:10.1038/npp.2017.207

54. Tan SKH, Vlamings R, Lim L, Sesia T, Janssen MLF, Steinbusch HWM, Visser-Vandewalle V, Temel Y. (2010). Experimental Deep Brain Stimulation in Animal Models. *Neurosurgery* **67** (4):1073-1080. https://doi.org/10.1227/NEU.0b013e3181ee3580. doi:10.1227/NEU.0b013e3181ee3580

55. Schor JS, Nelson AB. (2019). Multiple stimulation parameters influence efficacy of deep brain stimulation in parkinsonian mice. *The Journal of Clinical Investigation* **129** (9):3833-3838. https://doi.org/10.1172/JCI122390. doi:10.1172/JCI122390

56. Tan SZK, Fung M-L, Koh J, Chan Y-S, Lim LW. (2020). The Paradoxical Effect of Deep Brain Stimulation on Memory. *Aging and disease* **11** (1):179-190. https://pubmed.ncbi.nlm.nih.gov/32010491 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6961776/. doi:10.14336/AD.2019.0511

57. Hescham S, Lim LW, Jahanshahi A, Steinbusch HW, Prickaerts J, Blokland A, Temel Y. (2013). Deep brain stimulation of the forniceal area enhances memory functions in experimental dementia: the role of stimulation parameters. *Brain stimulation* **6** (1):72-77. https://www.sciencedirect.com/science/article/pii/S1935861X12000162. doi:10.1016/j.brs.2012.01.008

58. Hescham S, Lim LW, Jahanshahi A, Blokland A, Temel Y. (2013). Deep brain stimulation in dementia-related disorders. *Neuroscience & Biobehavioral Reviews* **37** (10, Part 2):2666-2675. https://www.sciencedirect.com/science/article/pii/S0149763413002054. doi:https://doi.org/10.1016/j.neubiorev.2013.09.002

59. Heng BC, Lim LW, Wu W, Zhang C. (2016). An overview of protocols for the neural induction of dental and oral stem cells in vitro. *Tissue Engineering Part B: Reviews* **22** (3):220-250. https://www.liebertpub.com/doi/abs/10.1089/ten.TEB.2015.0488. doi:10.1089/ten.teb.2015.0488

60. Páll O, Varga B, Collart-Dutilleul P-Y, Gergely C, Cuisinier FJG. (2017). Re:“An Overview of Protocols for the Neural Induction of Dental and Oral Stem Cells In Vitro” by Heng et al.(Tissue Eng Part B 2016; 22: 220–250). *Tissue Engineering Part B: Reviews* **23** (6):570-576. https://www.liebertpub.com/doi/full/10.1089/ten.teb.2016.0512. doi:10.1089/ten.teb.2016.0512

61. Rocha FaDF, Gomes BD, Silveira LCDL, Martins SL, Aguiar RG, De Souza JM, Ventura DF. (2016). Spectral Sensitivity Measured with Electroretinogram Using a Constant Response Method. *PLOS ONE* **11** (1):e0147318. https://doi.org/10.1371/journal.pone.0147318. doi:10.1371/journal.pone.0147318

62. Niklaus S, Albertini S, Schnitzer TK, Denk N. (2020). Challenging a Myth and Misconception: Red-Light Vision in Rats. *Animals* **10** (3):422. https://www.mdpi.com/2076-2615/10/3/422. doi:10.3390/ani10030422

63. Gulinello M, Mitchell HA, Chang Q, Timothy O'brien W, Zhou Z, Abel T, Wang L, Corbin JG, Veeraragavan S, Samaco RC, Andrews NA, Fagiolini M, Cole TB, Burbacher TM, Crawley JN. (2019). Rigor and reproducibility in rodent behavioral research. *Neurobiology of Learning and Memory* **165**:106780. https://www.sciencedirect.com/science/article/pii/S1074742718300017. doi:https://doi.org/10.1016/j.nlm.2018.01.001

64. Hescham S, Jahanshahi A, Meriaux C, Lim LW, Blokland A, Temel Y. (2015). Behavioral effects of deep brain stimulation of different areas of the Papez circuit on memory- and anxiety-related functions. *Behavioural Brain Research* **292**:353-360. https://www.sciencedirect.com/science/article/pii/S0166432815300589. doi:https://doi.org/10.1016/j.bbr.2015.06.032

65. Tan D, Soh LJT, Lim LW, Daniel TCW, Zhang X, Vyas A. (2015). Infection of male rats with Toxoplasma gondii results in enhanced delay aversion and neural changes in the nucleus accumbens core. *Proceedings of the Royal Society B: Biological Sciences* **282** (1808):20150042. https://doi.org/10.1098/rspb.2015.0042. doi:10.1098/rspb.2015.0042

66. Lim LW, Shrestha S, Or YZ, Tan SZK, Chung HH, Sun Y, Lim CL, Khairuddin S, Lufkin T, Lin VCL. (2016). Tetratricopeptide repeat domain 9A modulates anxiety-like behavior in female mice. *Scientific Reports* **6** (1):37568. https://doi.org/10.1038/srep37568. doi:10.1038/srep37568

67. Hegde A, Soh Yee P, Mitra R. (2017). Dendritic architecture of principal basolateral amygdala neurons changes congruently with endocrine response to stress. *International journal of environmental research and public health* **14** (7):779. https://www.mdpi.com/1660-4601/14/7/779. doi:doi.org/10.3390/ijerph14070779

68. Abdulai-Saiku S, Hegde A, Vyas A, Mitra R. (2017). Effects of stress or infection on rat behavior show robust reversals due to environmental disturbance. *F1000Research* **6**. https://f1000research.com/articles/6-2097/v2. doi:10.12688/f1000research.13171.1

69. Tan D, Vyas A. (2016). Toxoplasma gondii infection and testosterone congruently increase tolerance of male rats for risk of reward forfeiture. *Hormones and Behavior* **79**:37-44. https://www.sciencedirect.com/science/article/pii/S0018506X16300204. doi:https://doi.org/10.1016/j.yhbeh.2016.01.003

70. Abdulai-Saiku S, Vyas A. (2017). Loss of predator aversion in female rats after Toxoplasma gondii infection is not dependent on ovarian steroids. *Brain, Behavior, and Immunity* **65**:95-98. https://www.sciencedirect.com.remotexs.ntu.edu.sg/science/article/abs/pii/S0889159117301071. doi:10.1016/j.bbi.2017.04.005

71. Koe AS, Ashokan A, Mitra R. (2016). Short environmental enrichment in adulthood reverses anxiety and basolateral amygdala hypertrophy induced by maternal separation. *Translational psychiatry* **6** (2):e729-e729. https://www.nature.com/articles/tp2015217. doi:10.1038/tp.2015.217

72. Pang TYC, Stam NC, Nithianantharajah J, Howard ML, Hannan AJ. (2006). Differential effects of voluntary physical exercise on behavioral and brain-derived neurotrophic factor expression deficits in huntington's disease transgenic mice. *Neuroscience* **141** (2):569-584. https://www.sciencedirect.com/science/article/pii/S0306452206004933. doi:https://doi.org/10.1016/j.neuroscience.2006.04.013

73. Zajac MS, Pang TYC, Wong N, Weinrich B, Leang LSK, Craig JM, Saffery R, Hannan AJ. (2010). Wheel running and environmental enrichment differentially modify exon-specific BDNF expression in

the hippocampus of wild-type and pre-motor symptomatic male and female Huntington's disease mice. *Hippocampus* **20** (5):621-636. https://doi.org/10.1002/hipo.20658. doi:10.1002/hipo.20658

74. Pang TYC, Hannan AJ. (2013). Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. *Neuropharmacology* **64**:515-528. https://www.sciencedirect.com/science/article/pii/S0028390812002870. doi:https://doi.org/10.1016/j.neuropharm.2012.06.029

75. Short AK, Fennell KA, Perreau VM, Fox A, O'bryan MK, Kim JH, Bredy TW, Pang TY, Hannan AJ. (2016). Elevated paternal glucocorticoid exposure alters the small noncoding RNA profile in sperm and modifies anxiety and depressive phenotypes in the offspring. *Translational Psychiatry* **6** (6):e837-e837. https://doi.org/10.1038/tp.2016.109. doi:10.1038/tp.2016.109

76. Renoir T, Pang TYC, Mo C, Chan G, Chevarin C, Lanfumey L, Hannan AJ. (2013). Differential effects of early environmental enrichment on emotionality related behaviours in Huntington's disease transgenic mice. *The Journal of Physiology* **591** (1):41-55. https://doi.org/10.1113/jphysiol.2012.239798. doi:10.1113/jphysiol.2012.239798

77. Shirbin CA, Chua P, Churchyard A, Lowndes G, Hannan AJ, Pang TY, Chiu E, Stout JC. (2013). Cortisol and depression in pre-diagnosed and early stage Huntington's disease. *Psychoneuroendocrinology* **38** (11):2439-2447. https://www.sciencedirect.com/science/article/pii/S0306453012003551. doi:10.1016/j.psyneuen.2012.10.020

78. Lim NKH, Hung LW, Pang TY, Mclean CA, Liddell JR, Hilton JB, Li Q-X, White AR, Hannan AJ, Crouch PJ. (2014). Localized changes to glycogen synthase kinase-3 and collapsin response mediator protein-2 in the Huntington's disease affected brain. *Human Molecular Genetics* **23** (15):4051-4063. https://doi.org/10.1093/hmg/ddu119. doi:10.1093/hmg/ddu119

79. Rogers J, Vo U, Buret LS, Pang TY, Meiklejohn H, Zeleznikow-Johnston A, Churilov L, Van Den Buuse M, Hannan AJ, Renoir T. (2016). Dissociating the therapeutic effects of environmental enrichment and exercise in a mouse model of anxiety with cognitive impairment. *Translational Psychiatry* **6** (4):e794-e794. https://doi.org/10.1038/tp.2016.52. doi:10.1038/tp.2016.52

80. Mazarakis NK, Cybulska-Klosowicz A, Grote H, Pang T, Van Dellen A, Kossut M, Blakemore C, Hannan AJ. (2005). Deficits in Experience-Dependent Cortical Plasticity and Sensory-Discrimination Learning in Presymptomatic Huntington's Disease Mice. *The Journal of Neuroscience* **25** (12):3059-3066. https://www.jneurosci.org/content/jneuro/25/12/3059.full.pdf. doi:10.1523/jneurosci.4320-04.2005

81. Du X, Pang TY. (2015). Is Dysregulation of the HPA-Axis a Core Pathophysiology Mediating Co-Morbid Depression in Neurodegenerative Diseases? *Frontiers in Psychiatry* **6** (32). https://www.frontiersin.org/article/10.3389/fpsyt.2015.00032. doi:10.3389/fpsyt.2015.00032

82. Ashokan A, Lim JWH, Hang N, Mitra R. (2018). Complex housing causes a robust increase in dendritic complexity and spine density of medial prefrontal cortical neurons. *Scientific reports* **8** (1):1-9. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5943332/. doi:10.1038/s41598-018-25399-4

83. Hegde A, Suresh S, Mitra R. (2020). Early-life short-term environmental enrichment counteracts the effects of stress on anxiety-like behavior, brain-derived neurotrophic factor and nuclear translocation of glucocorticoid receptors in the basolateral amygdala. *Scientific reports* **10** (1):1-13. https://www.nature.com/articles/s41598-020-70875-5. doi:10.1038/s41598-020-70875-5

84. Leavitt FI. (1969). Drug-induced modifications in sexual behavior and open field locomotion of male rats. *Physiology & Behavior* **4** (5):677-683. https://deepblue.lib.umich.edu/bitstream/handle/2027.42/32912/0000292.pdf?sequence=1.

85. Ashokan A, Hegde A, Mitra R. (2016). Short-term environmental enrichment is sufficient to counter stress-induced anxiety and associated structural and molecular plasticity in basolateral amygdala. *Psychoneuroendocrinology* **69**:189-196. https://www.sciencedirect.com/science/article/abs/pii/S0306453016300993. doi:10.1016/j.psyneuen.2016.04.009

86. Ashokan A, Hegde A, Balasingham A, Mitra R. (2018). Housing environment influences stress-related hippocampal substrates and depression-like behavior. *Brain Research* **1683**:78-85. http://www.sciencedirect.com/science/article/pii/S0006899318300350. doi:https://doi.org/10.1016/j.brainres.2018.01.021

87. Zaqout S, Kaindl AM. (2016). Golgi-Cox Staining Step by Step. *Frontiers in neuroanatomy* **10**:38-38. https://pubmed.ncbi.nlm.nih.gov/27065817 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4814522/. doi:10.3389/fnana.2016.00038

88. Dickstein DL, Dickstein DR, Janssen WGM, Hof PR, Glaser JR, Rodriguez A, O'connor N, Angstman P, Tappan SJ. (2016). Automatic Dendritic Spine Quantification from Confocal Data with Neurolucida 360. *Current protocols in neuroscience* **77**:1.27.21-21.27.21. https://pubmed.ncbi.nlm.nih.gov/27696360 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5113738/. doi:10.1002/cpns.16

89. O'neill K, Akum B, Dhawan S, Kwon M, Langhammer C, Firestein B. (2015). Assessing effects on dendritic arborization using novel Sholl analyses. *Frontiers in Cellular Neuroscience* **9** (285). https://www.frontiersin.org/article/10.3389/fncel.2015.00285. doi:10.3389/fncel.2015.00285

90. Rao BS, Laxmi T, Meti B, Raju T. (2001). Subicular lesions cause dendritic atrophy in CA1 and CA3 pyramidal neurons of the rat hippocampus. *Neuroscience* **102** (2):319-327. https://www.sciencedirect.com/science/article/abs/pii/S0306452200004620. doi:10.1016/S0306-4522(00)00462-0

91. Porsolt RD, Bertin A, Jalfre M. (1977). Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* **229** (2):327-336. https://pubmed.ncbi.nlm.nih.gov/596982/.

92. Porsolt RD, Brossard G, Hautbois C, Roux S. (2001). Rodent Models of Depression: Forced Swimming and Tail Suspension Behavioral Despair Tests in Rats and Mice. *Current Protocols in Neuroscience* **14** (1):8.10A.11-18.10A.10. https://doi.org/10.1002/0471142301.ns0810as14. doi:10.1002/0471142301.ns0810as14

93. Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD. (2012). The mouse forced swim test. *Journal of visualized experiments : JoVE* (59):e3638-e3638. https://pubmed.ncbi.nlm.nih.gov/22314943 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3353513/. doi:10.3791/3638

94. Saito Y, Yuki S, Seki Y, Kagawa H, Okanoya K. (2016). Cognitive bias in rats evoked by ultrasonic vocalizations suggests emotional contagion. *Behavioural Processes* **132**:5-11. http://www.sciencedirect.com/science/article/pii/S037663571630198X. doi:https://doi.org/10.1016/j.beproc.2016.08.005

95. Laplagne DA, Elías Costa M. (2016). Rats Synchronize Locomotion with Ultrasonic Vocalizations at the Subsecond Time Scale. *Frontiers in Behavioral Neuroscience* **10** (184). https://www.frontiersin.org/article/10.3389/fnbeh.2016.00184. doi:10.3389/fnbeh.2016.00184

96. Monfils MH, Agee LA. (2019). Insights from social transmission of information in rodents. *Genes, Brain and Behavior* **18** (1):e12534. https://doi.org/10.1111/gbb.12534. doi:10.1111/gbb.12534

97. Molendijk ML, De Kloet ER. (2015). Immobility in the forced swim test is adaptive and does not reflect depression. *Psychoneuroendocrinology* **62**:389-391. https://www.sciencedirect.com/science/article/pii/S0306453015009014. doi:doi.org/10.1016/j.psyneuen.2015.08.028

98. Slattery DA, Cryan JF. (2012). Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nature Protocols* **7** (6):1009-1014. https://doi.org/10.1038/nprot.2012.044. doi:10.1038/nprot.2012.044

99. Ramos-Hryb AB, Bahor Z, Mccann S, Sena E, Macleod MR, Lino De Oliveira C. (2019). Protocol for a systematic review and meta-analysis of data from preclinical studies employing forced swimming test: an update. *BMJ Open Science* **3** (1):e000043. https://openscience.bmj.com/content/bmjos/3/1/e000043.full.pdf. doi:10.1136/bmjos-2017-000043

100. Kara N, Stukalin Y, Einat H. (2018). Revisiting the validity of the mouse forced swim test: Systematic review and meta-analysis of the effects of prototypic antidepressants. *Neuroscience & Biobehavioral Reviews* **84**:1-11.

101. Singewald N, Holmes A. (2019). Rodent models of impaired fear extinction. *Psychopharmacology* **236** (1):21-32. https://doi.org/10.1007/s00213-018-5054-x. doi:10.1007/s00213-018-5054-x

102. Lopresto D, Schipper P, Homberg JR. (2016). Neural circuits and mechanisms involved in fear generalization: Implications for the pathophysiology and treatment of posttraumatic stress disorder. *Neurosci. Biobehav. Rev.* **60**:31-42. https://www.sciencedirect.com/science/article/pii/S0149763415300300. doi:https://doi.org/10.1016/j.neubiorev.2015.10.009

103. Ortiz S, Latsko MS, Fouty JL, Dutta S, Adkins JM, Jasnow AM. (2019). Anterior Cingulate Cortex and Ventral Hippocampal Inputs to the Basolateral Amygdala Selectively Control Generalized Fear. *The Journal of Neuroscience* **39** (33):6526-6539. https://www.jneurosci.org/content/jneuro/39/33/6526.full.pdf. doi:10.1523/jneurosci.0810-19.2019

104. Dejean C, Courtin J, Rozeske RR, Bonnet MC, Dousset V, Michelet T, Herry C. (2015). Neuronal Circuits for Fear Expression and Recovery: Recent Advances and Potential Therapeutic Strategies. *Biol. Psychiatry* **78** (5):298-306. https://www.sciencedirect.com/science/article/pii/S0006322315002553. doi:https://doi.org/10.1016/j.biopsych.2015.03.017

105. Sotres-Bayon F, Cain CK, Ledoux JE. (2006). Brain Mechanisms of Fear Extinction: Historical Perspectives on the Contribution of Prefrontal Cortex. *Biol. Psychiatry* **60** (4):329-336. https://www.sciencedirect.com/science/article/pii/S000632230501293X. doi:https://doi.org/10.1016/j.biopsych.2005.10.012

106. Henigsberg N, Kalember P, Petrović ZK, Šečić A. (2019). Neuroimaging research in posttraumatic stress disorder – Focus on amygdala, hippocampus and prefrontal cortex. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **90**:37-42. http://www.sciencedirect.com/science/article/pii/S0278584618305050. doi:https://doi.org/10.1016/j.pnpbp.2018.11.003

107. Sierra-Mercado D, Padilla-Coreano N, Quirk GJ. (2011). Dissociable Roles of Prelimbic and Infralimbic Cortices, Ventral Hippocampus, and Basolateral Amygdala in the Expression and Extinction of Conditioned Fear. *Neuropsychopharmacology* **36** (2):529-538. https://doi.org/10.1038/npp.2010.184. doi:10.1038/npp.2010.184

108. Sangha S, Diehl MM, Bergstrom HC, Drew MR. (2020). Know safety, no fear. *Neurosci. Biobehav. Rev.* **108**:218-230. http://www.sciencedirect.com/science/article/pii/S0149763419300491. doi:https://doi.org/10.1016/j.neubiorev.2019.11.006

109. Orederu T, Schiller D. (2018). Fast and slow extinction pathways in defensive survival circuits. *Current Opinion in Behavioral Sciences* **24**:96-103. http://www.sciencedirect.com/science/article/pii/S2352154617302723. doi:https://doi.org/10.1016/j.cobeha.2018.06.004

110. Kinlein SA, Karatsoreos IN. (2020). The hypothalamic-pituitary-adrenal axis as a substrate for stress resilience: Interactions with the circadian clock. *Front. Neuroendocrinol.* **56**:100819. http://www.sciencedirect.com/science/article/pii/S0091302219300846. doi:https://doi.org/10.1016/j.yfrne.2019.100819

111. Naegelin Y, Dingsdale H, Säuberli K, Schädelin S, Kappos L, Barde Y-A. (2018). Measuring and Validating the Levels of Brain-Derived Neurotrophic Factor in Human Serum. *eNeuro* **5** (2):ENEURO.0419-0417.2018. https://pubmed.ncbi.nlm.nih.gov/29662942 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5898630/. doi:10.1523/ENEURO.0419-17.2018

112. Kolbeck R, Bartke I, Eberle W, Barde YA. (1999). Brain-derived neurotrophic factor levels in the nervous system of wild-type and neurotrophin gene mutant mice. *J Neurochem* **72** (5):1930-1938. https://onlinelibrary.wiley.com/doi/full/10.1046/j.1471-4159.1999.0721930.x. doi:10.1046/j.1471-4159.1999.0721930.x

113. Lavezzi AM, Corna MF, Matturri L. (2014). Disruption of the brain-derived neurotrophic factor (BDNF) immunoreactivity in the human Kölliker-Fuse nucleus in victims of unexplained fetal and infant death. *Frontiers in Human Neuroscience* **8** (648). https://www.frontiersin.org/article/10.3389/fnhum.2014.00648. doi:10.3389/fnhum.2014.00648

114. Larimore JL, Chapleau CA, Kudo S, Theibert A, Percy AK, Pozzo-Miller L. (2009). Bdnf overexpression in hippocampal neurons prevents dendritic atrophy caused by Rett-associated MECP2 mutations. *Neurobiology of disease* **34** (2):199-211. https://pubmed.ncbi.nlm.nih.gov/19217433 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2726722/. doi:10.1016/j.nbd.2008.12.011

115. Sarabdjitsingh RA, Isenia S, Polman A, Mijalkovic J, Lachize S, Datson N, De Kloet ER, Meijer OC. (2010). Disrupted Corticosterone Pulsatile Patterns Attenuate Responsiveness to Glucocorticoid Signaling in Rat Brain. *Endocrinology* **151** (3):1177-1186. https://doi.org/10.1210/en.2009-1119. doi:10.1210/en.2009-1119

116. Wang Q, Van Heerikhuize J, Aronica E, Kawata M, Seress L, Joels M, Swaab DF, Lucassen PJ. (2013). Glucocorticoid receptor protein expression in human hippocampus; stability with age.

Neurobiology of Aging **34** (6):1662-1673. http://www.sciencedirect.com/science/article/pii/S0197458012005969. doi:https://doi.org/10.1016/j.neurobiolaging.2012.11.019

117. Sarabdjitsingh RA, Meijer OC, De Kloet ER. (2010). Specificity of glucocorticoid receptor primary antibodies for analysis of receptor localization patterns in cultured cells and rat hippocampus. *Brain Research* **1331**:1-11. http://www.sciencedirect.com/science/article/pii/S0006899310006256. doi:https://doi.org/10.1016/j.brainres.2010.03.052

118. Wu Y, Mitra R. (2020). Prefrontal-hippocampus plasticity reinstated by an enriched environment during stress. *Neuroscience Research*. http://www.sciencedirect.com/science/article/pii/S0168010220304041. doi:https://doi.org/10.1016/j.neures.2020.07.004

119. Rocher C, Spedding M, Munoz C, Jay TM. (2004). Acute Stress-induced Changes in Hippocampal/Prefrontal Circuits in Rats: Effects of Antidepressants. *Cerebral Cortex* **14** (2):224-229. https://doi.org/10.1093/cercor/bhg122. doi:10.1093/cercor/bhg122

120. Jay TM, Burette F, Laroche S. (1995). NMDA Receptor-dependent Long-term Potentiation in the Hippocampal Afferent Fibre System to the Prefrontal Cortex in the Rat. *European Journal of Neuroscience* **7** (2):247-250. https://doi.org/10.1111/j.1460-9568.1995.tb01060.x. doi:10.1111/j.1460-9568.1995.tb01060.x

121. Rajkumar R, Wu Y, Farooq U, Tan WH, Dawe GS. (2016). Stress activates the nucleus incertus and modulates plasticity in the hippocampo-medial prefrontal cortical pathway. *Brain Research Bulletin* **120**:83-89. http://www.sciencedirect.com/science/article/pii/S0361923015300496. doi:https://doi.org/10.1016/j.brainresbull.2015.10.010

122. Farooq U, Rajkumar R, Sukumaran S, Wu Y, Tan WH, Dawe GS. (2013). Corticotropin-releasing factor infusion into nucleus incertus suppresses medial prefrontal cortical activity and hippocampo-medial prefrontal cortical long-term potentiation. *European Journal of Neuroscience* **38** (4):2516-2525. https://doi.org/10.1111/ejn.12242. doi:10.1111/ejn.12242

123. Ghaeminia M, Rajkumar R, Koh H-L, Dawe GS, Tan CH. (2018). Ginsenoside Rg1 modulates medial prefrontal cortical firing and suppresses the hippocampo-medial prefrontal cortical long-term potentiation. *Journal of Ginseng Research* **42** (3):298-303. http://www.sciencedirect.com/science/article/pii/S1226845316303189. doi:https://doi.org/10.1016/j.jgr.2017.03.010

124. You W. (2014). Role of the nucleus incertus in cognition. Master's thesis. National University of Singapore. https://core.ac.uk/download/pdf/48799243.pdf.