

Appendix 3

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1. Paradigms

Experimental paradigms used in RM's articles are listed in **Table 1** with: (i) the description of the paradigm; (ii) an abbreviation for the paradigm used in **Table 4**; (iii) how the paradigm is referred to by RM in the title, method, results, or figures in

different articles; and (iv) the number of articles using the paradigm. In RM's articles, to the best of my understanding, predator odor is both paradigm and method, so it is listed in both **Table 1** with paradigms and **Table 2** with methods

Table 1. Paradigms			
Experimental paradigm or group	Abbreviation	How it is referred to in the data (figures, methods, results, title)	Number of articles used
Environmental enrichment 14 – 16 days	E	<i>Environmental enrichment</i>	6 out of 8 articles
		<i>Short-term environmental enrichment</i>	
		<i>Early-life short-term environmental enrichment</i>	
		<i>Complex housing</i>	
		<i>Complex environment</i>	
		<i>Enriched environment</i>	
		<i>Enriched</i>	
Chronic immobilization stress 2 hours for 10 days started same day as enrichment	S	<i>Stress</i>	3 out of 8 articles
Control	C	<i>Animal facility rearing (ARF)</i>	7 out of 8 articles
		<i>Standard</i>	
		<i>Not enriched</i>	
		<i>Non-enriched</i>	
		<i>Simple</i>	
Maternal Separation PN2-14 once/day for 3 hours	M	<i>Stress</i>	3 out of 8 articles
		<i>Pre-weaning stress</i>	
		<i>Anxiety</i>	
		<i>Stress-induced anxiety</i>	
		<i>Maternal separation</i>	
Predator odor	P	<i>Predator odor exposure</i>	2 out of 8 articles
		<i>Predator stress</i>	
		<i>Stress</i>	
		<i>Stressor</i>	
Deep Brain Stimulation	D		1 out of 8 articles
Toxoplasma gondii	T	<i>Infected</i>	1 out of 8 articles

2. Methods

RM's articles obfuscate apparatus, methods, results, and data interpretation. In **Table 4** methods are referred to and grouped as they are generally understood. As such, the methods and an abbreviation for each are listed in **Table 2** next to terms and variations used in RM's articles for the same. The text in italics in **Table 2** is copied from RM's articles. This text refers to a method or test, and a definition of

the readout as given by RM. To the best of my ability, the readout as finally reported by RM in an article is in **Table 4**. The list of terms and definitions for a test used by RM in **Table 2** is not exhaustive and does not include variations of interpretations of those tests in RM's articles, for example in the Discussion of those articles.

Table 2. Experiments				
Method	Abbreviation	RM nomenclature or operational definition	Comment	
Behavior				
Home cage emergence	HCE	<i>Latency to leave the cage</i>		
		<i>Latency to emerge from the home cage</i>		
		<i>Latency to emerge from the home cage into a novel environment</i>		
		<i>Stress-induced anxiolysis</i>		
Object Recognition Task	ORT	<i>Object recognition task</i>	Used in one study.	
		<i>Memory performance</i>		
		<i>Novel object recognition</i>		
		<i>Exploration of novel and familiar objects was quantified over a period of 180 s. Object recognition was quantified as exploration of novel objects relative to the sum of exploration for novel and familiar objects</i>		
Elevated Plus Maze	EPM	<i>Relative open arm exploration</i>	This is consistently referred to as EPM by RM across articles, but I cannot comment on why the common and simple readouts were not used for this test, nor if RM's operational definitions are the same. The readouts are different from one article to another, listed to the best of my ability in Table 4 .	
		<i>Exploration in open and enclosed arms was quantified for five minutes each</i>		
		<i>Open arm exploration (entries and occupancy time) relative to the sum of open and enclosed arm explorations was used as an index for anxiety. Mean of percentage open arms entries and percentage open arms time was subtracted from 100 to derive an index for anxiety</i>		
		<i>Manually analysed to quantify the percentage open-arm time and open-arm entries, relative to the sum of open and enclosed arm exploration / with decreased open-arm entries and time indicative of heightened anxiety</i>		
		<i>Open arm exploration (entries and occupancy time) relative to sum of open and enclosed arm exploration was used as an index for anxiety</i>		
		<i>Open arm exploration in an elevated plus maze (EPM) was studied as an index of anxiety (lower exploration = greater anxiety). It was defined as mean of % open arm entries and % open arm time (relative to sum of open and enclosed arm entries and time)</i>		
		<i>general locomotion tested on the EPM</i>		
	Head dips		<i>Risk assessment</i>	Separate sections in articles and broad interpretations are based on this. It is still EPM.
			<i>Exploratory behavior</i>	
			<i>Goal-directed behavior</i>	
		<i>Absence indicates passive behavior</i>		

Method	Abbreviation	RM nomenclature or operational definition	Comment
Behavior			
Open Field Test	OFT-R/C	<i>Open field test</i>	This is reported square in some articles, circular in others denoted in Table 4 by OFT-R or OFT-C respectively. OFT-C is reported big or small as detailed in <i>Appendix2.pdf</i> .
		<i>Open field</i>	
		<i>Arena</i>	
		<i>Novel rectangular arena</i>	
		<i>A square-shaped open field was constructed / Time spent in the central part of the open field (33 cm x 33 cm) was measured as a proxy for lower anxiety-like behavior</i>	
Predator odor	PO-R/C	<i>Time spent in the centre of the field was quantified as the reciprocal proxy of the anxiety (centre 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</i>	This is used as a stress paradigm as indicated in Table 1 in some articles, as a method in other articles. Predator odor exposure in a rectangular arena is denoted by PO-R, and circular by PO-C.
		<i>Time spent in the center of the arena was quantified from recorded videos. The center was defined as a concentric circular region of interest with a radius of 0.33 m. In a subset of animals, total time spent by animals being mobile in the arena was also quantified as an index of locomotion</i>	
		<i>Predator odor exposure</i>	
		<i>Predator stress</i>	
		<i>Stressor</i>	
		<i>Open field</i>	
		<i>Open field arena</i>	
		<i>Maze</i>	
		<i>Rectangular arena</i>	
		<i>Circular arena</i>	
Golgi staining	DL	<i>Maze</i>	
		<i>Innate aversion</i>	
		<i>Occupancy of the bisect containing odor 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)</i>	
		<i>Aversion to cat odor was quantified as percentage time in bisect containing cat odor relative to total trial duration</i>	
		<i>Center time in open field</i>	
Dendritic Length	DB	<i>Percentage time in bisect containing cat odor relative to total trial duration</i>	
		<i>Total dendritic length</i>	
		<i>Total number of branch points</i>	
		<i>Number of branch points</i>	
		<i>Dendritic arborization</i>	
		<i>Dendritic complexity</i>	
Dendritic Branching Points	DB	<i>Pyramidal or modified pyramidal spiny projection neurons</i>	
		<i>Sholl analysis</i>	

Method	Abbreviation	RM nomenclature or operational definition	Comment	
Golgi staining				
contd. Dendritic Branching Points	DB	Primary dendrites		
		Mean of these dendritic segments on different neurons was used to estimate spine density for each animal		
		Number of primary dendrites was estimated by counting dendritic intersection with a circular region of interest (radius = 30 μm) centered on the cell soma. A Sholl analysis was further conducted for PrL neurons by overlaying multiple circular regions of interest centered on the cell soma and successively increasing the radius in step size of 10 μm . Number of intersections made by the dendritic arbors was then quantified as a function of radial distance from the soma		
		Dendritic length and number of branch points were quantified as function of radial distance from the cell soma		
		Dendritic architecture of PrL neurons was further analyzed as a function of radial distance from soma using Sholl analysis		
Dendritic spines	DS	Spine density	Manually counted	
		Spine count		
		Spine density per 60 μm		
		Scholl's both total branch points and segmental branch points at radial distance		
		Primary and secondary dendrites		
		Primary dendrites		Dendrites directly originating from the cell soma
				spine density (1/100 μm) counted along 80 μm stretch of dendrite
				spine density (1/100 μm) counted along 60 μm stretch of dendrite
		Secondary dendrites		the first branch emerging from the primary dendrite
				those originating from primary dendrites
spine density (1/100 μm) counted along 80 μm stretch of dendrite				
	spine density (1/100 μm) counted along 60 μm stretch of dendrite			
Dendritic Intersections		Dendritic intersections	Though reported, I cannot find it.	
		Segmental branch points		
		Sholl analysis		
Brain region				
Basolateral amygdala	BLA			
Prelimbic cortex	PL	Prelimbic medial prefrontal cortex		
		Prelimbic cortex		
		Prelimbic region of medial prefrontal cortex		
Infralimbic cortex	IL	Infralimbic region of medial prefrontal cortex		
Medial prefrontal cortex	mPFC			
Hippocampus	HP			

Method	Abbreviation	<i>RM nomenclature or operational definition</i>	Comment
Other			
Corticosterone	CORT		These are mentioned here for abbreviations in Table 4 .
Enzyme-linked immunosorbent assay	ELISA		
Glucocorticoid receptor	GR		
Mineralocorticoid receptor	MR		
Brain-derived neurotrophic factor	BDNF		
Polymerase chain reaction	PCR		
Immunohistochemistry	IHC		
Western blot	WB		

3. Article data

Table 4 summarizes article paradigm, method and readout, statistics, significance and effect size reporting, and unknown data in RM's publications. All text in italics in **Table 4** is quoted from the articles. This includes statistics used, the descriptive text is abridged from the articles. **Table 3** is a legend for **Table 4**.

- **Animals:** all results reported in all experiments were from adult male Wistar rats 6 to 8 weeks, except in Abdulai-Saiku et al. 2017, the sex is denoted in **Table 4** along with paradigm by ♂ or ♀.

- **Euthanasia:** when mentioned, animals were killed by decapitation and/or cardiac perfusion. This is related to present work and RM's AUP A:19027. See **Table 6**.
- **Articles:** those are the same as discussed in *Appendix2.pdf*, primary articles (not reviews) produced by RM at NTU.

Table 3. Legend					
Article Paradigm	Method	Readout reported	n (significance)	Statistics reported, figure data	Unknown
			Paradigm 1/paradigm 2/etc. (C: control) Number of animals used in a paradigm (or neurons) Significance reporting		
Article reference Paradigm	Method 1	<i>Readout (article text)</i>	X/X/X/X	Abridged text from article on statistics and figure data reporting if present.	Non-exhaustive list of: - Unreported methods or results. - Information mentioned said to be reported in part of the article but not found. - Methods information mentioned in another article by RM but not in this one. - Contradictory information in the article.
	Method 2	<i>Readout 1</i>	X/X/X/X		
		<i>Readout 2</i>	X/X/X/X		
		<i>Readout 3</i>	X/X/X/X		
		Legend			
		Numbers in red indicates statistical significance reported in Figure	X/X/X/X		
		Numbers in blue indicates effect size reported in results (not figure)	X/X/X/X		
		Numbers highlighted in yellow indicates contradictory reports of significance or effect size in results and figure	X/X/X/X		
	Text highlighted in green indicates data reported in results but not methods, or methods but not results	X/X/X/X			

Table 4. Data					
Article Paradigm	Method	Readout reported	n (significance)	Statistics reported, figure data	Unknown
			CC/MC/EE/ME		
Koe et al. 2016 E, M	HCE	Emergence latency	12/12/11/14	Normality using Shapiro-Wilk; non-parametric for intergroup comparisons (Kruskal-Wallis one-way analysis of variance); Mann-Whitney U 2-tailed if significant intergroup; C vs. MS ± EE; resultant Type 1 errors adjusted with Bonferroni; effect with non-parametric Cliff's delta. Figures depict median and interquartile range except for DL and DS average value/animal/n unique neurons	<ul style="list-style-type: none"> - Timing of OFT (age of rats at time of OFT) - Objective lens interface - Coordinates for histology - Software for analyzing distance travelled - Calibrated scale for drawing tube scans - Neuron sampling method - Neuron data analysis script
	EPM	Open arm entries (%)	14/10/15/10		
		Time spent in open arms (%)	14/10/15/10		
		Number of head dips	14/10/15/10		
	OFT-C	Time spent in open field center	14/10/15/10		
		Distance travelled / locomotion	?/?/?/?		
	DL-BLA	Total dendritic length 10-11 or 8-11 neurons/animal	6/9/9/9		
	DB-BLA	Total number of branch points 10-11 or 8-11 neurons/animal	6/9/9/9		
	DS-BLA	Spine density 6-10 neurons/animal/60 μm neuron, primary	6/9/9/9		
		Spine density 6-10 neurons/animal/60 μm neuron, secondary	6/9/9/9		
DL-PL	Total dendritic length 8 neurons/animal	6/9/9/9 4/10/7/9			
DB-PL	Total number of branch points 8 neurons/animal	6/9/9/9 4/10/7/9			
CORT-ELISA	Serum corticosterone	9/9/9/9			
			CC/SE/EE/SE		
Ashokan et al. 2016 E, S	EPM	% open arm exploration (%)	17/6/8/8	2-way ANOVA (inter-subject source of variance = stress and EE); planned comparison for stress ± EE (± EE); two orthogonal comparisons Sidak's multiple comparison. Figures represent mean and SEM and mean inter-group differences and SEM.	<ul style="list-style-type: none"> - Number of animals for results - Kit used for corticosterone analysis - Mean inter-group differences and SEM - Neuron sampling method - Objective lens - Method of Scholl analysis - Neuron data analysis script
		Enclosed entries	17/6/8/8		
		Number of head dips	17/6/8/8		
	DL-BLA	Dendritic length	8-7/7/7/7		
	DB-BLA	Number of branch points 8-10 neurons per animal	8-7/7/7/7		
		Number of branch points vs. distance from soma	8-7/7/7/7		
	DS-BLA	Spine density per 60 μm neuron Primary and secondary simultaneously	8-7/7/7/7		
	CORT-ELISA	Corticosterone measurement	8/7/6/6		
BDNF-PCR	BDNF mRNA abundance	4/4/4/4			
Adrenal wt.	Adrenal weight	?/?/?/?			

Article Paradigm	Method	Readout reported	n (significance)	Statistics reported, figure data	Unknown		
			P				
Hegde et al. 2017 P	EPM	% Open arm time	9	<i>Jackknife resampling model; Spearman's rank correlation coefficients for pairs of endpoints; principal component analysis; dimension reduction; factor loading exceeded/less 0.5 for component/alternative; 4/3 endpoints load on the 1st/2nd principal component.</i> <i>Figures depict raw data median %/unit/principal component</i>	<ul style="list-style-type: none"> - All data from control group (n=6) - Data from predator exposure in rectangular arena - Source of bobcat urine - Objective lens - Neuron data analysis script 		
		% Open arm entries	9				
		Number of enclosed arm entries	9				
	PO-R	Occupancy of bisect containing odor relative to the total area 47% chance	?				
	OFT-R	Center time in open field	10				
	DB-BLA	Median number of branch points	10				
	DL-BLA	Median dendritic length	10				
	DS-BLA	Primary and secondary dendrites Median spines on primary dendrites	8				
CORT-ELISA	Corticosterone: post-open field, post-EPM, post-sacrifice	10					
			♂C/M♂				
Abdulai-Saiku et al. 2017 P, M, T	EPM	Entries in open arms (%)	8/8	<i>Probability type 1 error unpaired two-tailed Student's t-test; standardized effect size Cohen's d; values magnitude 0.8 robust; negative d correspond comparisons mean of respective controls; mean intergroup 95% confidence intervals; univariate analysis of variance % open arm entries employing number of enclosed entries as a covariate.</i> <i>Figures presented as mean SEM, along with individual values for each animal for each endpoint</i>	<ul style="list-style-type: none"> - Source of cat urine - Mean and SEM - Method and data for serological exam 		
		Time in open arms (%)	8/8				
		Head dips	?/?				
	HCE	Escape latency (s)	8/8				
						♀C/♀P	
	PO-R	Time spent in cat bisect (%)	8/7				
	PO-C	Time spent in cat bisect (%)	10/9				
?	Serological exam	?					
			CC/SC/EE/SE				
Ashokan et al. 2018a E, S contd.	Forced swimming test x2 days	Time spent immobile (s) (trial 2)	8/8/8/6 or 8	<i>2-way ANOVA to estimate main effects; orthogonal planned comparisons between stressed and non-stressed in either enriched or non-enriched using independent sample t-test.; planned comparisons guided by a priori interest; non-enriched and enriched groups in absence of stress not compared using planned comparisons; eta squared.</i> <i>Figures represent mean and SEM.</i>	<ul style="list-style-type: none"> - Primary antibody catalogue - Secondary antibody for DCX - Number of sections for BrdU and DCX - Combining Golgi and IHC method - Device for measuring immobility data - Neuron data analysis script - First trial swimming test - Number of sections for counting BrdU - Lens objective magnification 		
		Latency to immobility	?				
		Climbing	?				
		Number of immobility events/episodes (Trial 2)	8/8/8/6 or 8				
	?	Baseline immobility - device	?				
	OFT-R	Baseline immobility, Total number of sorties	?				
		Baseline immobility, Total time spent immobile (s)	8/8/8/8				
Baseline immobility, Total distance travelled		?					

Article Paradigm	Method	Readout reported	n (significance)	Statistics reported, figure data	Unknown
contd. Ashokan et al. 2018a E, S	DS-HP	Total number of spines on primary dendrite across 60 μm in CA3, 2 neurons /animal/group (spine density /60 μm)	50/49/38/43		- Calibrated scale for drawing tube scans
	BrdU HP-ventral	Enumerated in 10 sections, 4-6 animals	?		
	BrdU HP-dorsal	Enumerated in 10 sections, 4-6 animals, dorsal dentate gyrus/CA3	4/5/5/6/		
	Doublecortin	Enumerated in 8 sections for each animal	?		
	GR-PCR	PCR real-time fluorescence Raw values abundance mean	4/4/4/4		
	MR-PCR	PCR real-time fluorescence Raw values abundance mean	3/4/4/4		
			C/E		
Ashokan et al. 2018b E	DL-PL	Total dendritic length (μm)	8/4	Mean for each animal, across multiple neurons analyzed; unpaired two-tailed Student's t-test; standardized effect size using Cohen's d values above magnitude of one robust; negative d values correspond to the comparisons where mean of complex housing greater than simple housing; mean inter-group difference 95% confidence intervals; repeated measure analysis of variance for Sholl analysis for number of intersections as a function of radial distance from the soma. Data presented as mean SEM along with individual values.	- Enrichment protocol - Objective lens magnification - Neuron data analysis script - Calibrated scale for drawing tube scans
		Total number of intersections	8/4		
		Sum of intersections (#)			
	DB-PL	Primary branches (#)	8/4		
		Maxima for the number of the intersection, maximum intersections (#)	8/7		
		Radius for the maxima of the intersection, radius max intersection (μm)	8/7		
		Intersections (#)	8/7		
	DS-PL	1° spine density (1/100 μm) counted along 80 μm stretch of dendrite	8/4		
		2° spine density (1/100 μm) counted along 80 μm stretch of dendrite	8/4		
	DL-IL	Total dendritic length	6/6		
	DB-IL	Total number of intersections Sum of intersections	6/6		
	DB-IL	Primary branches (#)	6/6		
DS-IL	1° spine density (1/100 μm) counted along 60 μm stretch of dendrite	6/6			
	2° spine density (1/100 μm) counted along 60 μm stretch of dendrite	6/6			

Article Paradigm	Method	Readout reported	n (significance)	Statistics reported, figure data	Unknown
			CC/DC/EE/DE		
Bhaskar et al. 2018 E, D	HCE	Ranks, home cage emergence	10/9/10/8	Normality Shapiro-Wilk test significant departure from normality; nonparametric statistics intergroup comparisons Kruskal–Wallis; each endpoint rank-transformed across four groups; two planned comparisons set before data collection started; orthogonal comparisons, no group more than one comparison; independent sample Student's t for parametric planned comparisons of rank transformed data; planned comparisons buttressed by effect size data using Cohen's d	<ul style="list-style-type: none"> - Power source for deep brain stimulation - Some parameters of deep brain stimulation - Values for individual animals - Mean and SEM for figures
	EPM	Ranks, EPM anxiety index	10/9/10/8		
		Ranks, head dips	10/9/10/8		
	ORT	Ranks, novel object recognition	10/9/10/8		
			CC/MC/EE/ME		
Hegde et al. 2020 E, M	OFT-C	Time in center (s)	12/8/8/6	Outliers removed using ROUT method with a max false discovery rate 1%27; main effects estimated using a two-way analysis of variance; omnibus post-hoc comparisons not conducted to avoid multiple pair-wise contrasts; two orthogonal planned comparisons constructed using independent sample t-test between non-stressed controls and stressed animals under animal-facility rearing and between non-stressed controls and stressed animals reared in enriched housing	<ul style="list-style-type: none"> - Softwares for behavioral analysis and image analysis - Number of animals for neuron analysis and IHC - Primary and secondary antibodies - Objective lens - Calibrated scale for drawing tube scans - Assessment for GR promoter methylation.
		Time mobile (s)	6/5/9/6		
	CORT-ELISA	Baseline corticosterone	6/5/9/6		
	Adrenal wt.	Wet adrenal weights mg/kg bd. wt.	6/5/9/6		
	DS-BLA	Spine density Averaged primary secondary 60 μm	60/60/60/60		
	GR-IHC	Intra-nuclear GR	24/22/24/24		
	GR-WB	GR relative intensity AU	6/6/6/6		
	GR-?	GR promoter methylation	5/5/5/5		
	BDNF-IHC	BDNF rise constant AU	24/24/24/26		
BDNF Y max AU		22/21/24/24			
BDNF-WB	BDNF relative intensity AU	6/6/6/6			
Wu and Mitra 2020	This article violates known ethical, physiological, and physical laws and so was excluded from this analysis.				

4. Duplicate publications

Articles with datasets possible to extract from one study are shaded the same color.

Table 5. Duplicate publications						
Acknowledgement	RM's funding	RM's paper	AV's paper	Shared authors	AV's funding	Comments
Ling, NY	NTU M4081146	<i>Koe et al., 2016</i>				
Vyas, A						
Bhaskar, Y	NTU	<i>Ashokan et al., 2016</i>				
Vyas, A	MoE RG 46/12					
Ashokan, A	NTU	<i>Hegde et al., 2017</i>				
Vyas, A	MoE RG46/12					
	MoE RG136/15	<i>Abdulai-Saiku et al., 2017</i>	<i>Singh et al., 2020</i>	Abdulai-Saiku, A	MoE RG136/15	The methods are incomplete, but there is a typo: testosterone cannot be dissolved to 25 mM in absolute ethanol (3% reported). Maybe it's 25 µM.
	MoE RG46/12		<i>Abdulai-Saiku and Vyas, 2017</i>	Abdulai-Saiku, A		There might be a readout novel to RM for PO-R (Entries into bisect) in AV's paper.
	MoE RG 46/12 to RM	<i>Bhaskar et al., 2018</i>	<i>Liu et al., 2015</i>	Lim, LW	NTU, Lee Wei Lim, Lee Kuan Yew Research Fellowship M4080846.080	
Koe, A	MoE RG 46/12	<i>Ashokan et al., 2018a</i>				
Vyas, A	NTU					
	MoE RG 46/12 to RM	<i>Ashokan et al., 2018b</i>				
Vyas, A	MoE RG 144/17 to RM	<i>Hegde et al., 2020</i>				
			<i>Tan et al., 2015</i>	Lim, LW	MoE: DT, LJTS, LWL, AV Duke-NUS block funding: TCWD, XZ	Are these different studies?
			<i>Tan and Vyas, 2016a</i>		MoE RG52/14 to TD and AV	
			<i>Tan and Vyas, 2016b</i>		MoE RG52/14	
			<i>Tong et al., 2019</i>	Abdulai-Saiku, A	MoE RG136/15	These two articles look quite similar.
			<i>Tong et al., 2020</i>	Abdulai-Saiku, A	Human Frontier Science Program RGP0062/2018	

5. AUP: A19027

Table 6. AUP: A19027		
Section	RM	Comment
6a. Avoiding duplication	<p>“...We do not initiate any project unless it is sufficiently novel, first because it will not be published since it cannot add any new information to the field, second, do not need/plan to use animals just to check something already published and replicated before. Thus when we initiate a project the maximum value is in its novelty where most of the work is likely first time, with some replicability options. Since our work is about animal models of psychopathology, measure of behavioural output is crucial. However the final read-out of projects also critically involve other important measures of physiology, protein and gene regulation. So there is minimal overlap between the main questions of different projects, hence minimising duplication of earlier work...” (emphasis added)</p>	Please see Table 4 .
6e. Reduction	<p>“...I have a good approximation of the number of animals needed for optimal behavioural output... we calculated approximate number of animal required through statistical power analysis...”</p>	<p>“...Please bring down the increase in animal numbers to less than 20% of the current approved animal numbers, which is less than 180 mice to qualify as a minor amendment...” [For Revision, ICCO, 28-Aug-2020]</p>
Description of non-surgical procedures	<p>“...The lack of use of anesthesia for decapitation is important in experiments (described in part 11) where mRNA levels are measured/ visualized. The animal when presented an odor, will process the sensory information in specific areas of the brain that will elicit a change at the gene expression level- either turning on some genes or turning off other genes. Thus, the use of anesthesia can be a confounding factor as this will also elicit its own changes at the gene expression level. Hence, it would be difficult to separate the effect of the odor and anesthesia. To reduce pain and discomfort during this procedure, Harvard restraint bags will be used to hold down the animal and a quick and smooth decapitation (taking less than a minute that also serves to reduce discomfort) with the guillotine will be done...”</p>	<p>I had been hoping RM neglected to mention anesthetic overdose for killing animals. In light of evidence presented here, notably Tables 1, 4 and 5, I think this might not be the case.</p>
12b. Genetically Modified Organisms (GMOs) (i) Is Recombinant DNA or transgenic animal used? GMAC approval must be obtained and attached to this section tab.	<p>“...No...”</p>	<p>I do not think this is true.</p>

6. References

1. Abdulai-Saiku, S., Hegde, A., Vyas, A., and 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> 10.12688/f1000research.13171.1
2. Abdulai-Saiku, S., and 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/science/remotex.ntu.edu.sg/science/article/abs/pii/S0889159117301071> 10.1016/j.bbi.2017.04.005
3. Ashokan, A., Hegde, A., and 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> 10.1016/j.psyneuen.2016.04.009
4. Ashokan, A., Hegde, A., Balasingham, A., and Mitra, R. (2018a). Housing environment influences stress-related hippocampal substrates and depression-like behavior. *Brain research* **1683**, 78-85. <https://www.sciencedirect.com/science/article/abs/pii/S0006899318300350> 10.1016/j.brainres.2018.01.021
5. Ashokan, A., Lim, J.W.H., Hang, N., and Mitra, R. (2018b). Complex housing causes a robust increase in dendritic complexity and spine density of medial prefrontal cortical neurons. *Scientific reports* **8**, 1-9. <https://www.nature.com/articles/s41598-018-25399-4> 10.1038/s41598-018-25399-4
6. Bhaskar, Y., Lim, L.W., and Mitra, R. (2018). Enriched Environment Facilitates Anxiolytic Efficacy Driven by Deep-Brain Stimulation of Medial Prefrontal Cortex. *Frontiers in Behavioral Neuroscience* **12**. <https://www.frontiersin.org/article/10.3389/fnbeh.2018.00204> 10.3389/fnbeh.2018.00204
7. Hegde, A., Soh Yee, P., and 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**, 779. <https://www.mdpi.com/1660-4601/14/7/779> doi.org/10.3390/ijerph14070779
8. Hegde, A., Suresh, S., and 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**, 1-13. <https://www.nature.com/articles/s41598-020-70875-5> 10.1038/s41598-020-70875-5
9. Koe, A.S., Ashokan, A., and Mitra, R. (2016). Short environmental enrichment in adulthood reverses anxiety and basolateral amygdala hypertrophy induced by maternal separation. *Translational psychiatry* **6**, e729-e729. <https://www.nature.com/articles/tp2015217> 10.1038/tp.2015.217
10. Liu, A., Jain, N., Vyas, A., and Lim, L.W. (2015). Ventromedial prefrontal cortex stimulation enhances memory and hippocampal neurogenesis in the middle-aged rats. *Elife* **4**, e04803. <https://elifesciences.org/articles/04803> 10.7554/eLife.04803
11. Singh, D.K., Hari Dass, S.A., Abdulai-Saiku, S., and Vyas, A. (2020). Testosterone Acts Within the Medial Amygdala of Rats to Reduce Innate Fear to Predator Odor Akin to the Effects of *Toxoplasma gondii* Infection. *Frontiers in Psychiatry* **11**. <https://www.frontiersin.org/article/10.3389/fpsy.2020.00630> 10.3389/fpsy.2020.00630
12. Tan, D., Soh, L.J.T., Lim, L.W., Daniel, T.C.W., Zhang, X., and 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**, 20150042. <https://doi.org/10.1098/rspb.2015.0042> 10.1098/rspb.2015.0042
13. Tan, D., and Vyas, A. (2016a). Infection of male rats with *Toxoplasma gondii* induces effort-aversion in a T-maze decision-making task. *Brain, Behavior, and Immunity* **53**, 273-277.

14. Tan, D., and Vyas, A. (2016b). 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>
<https://doi.org/10.1016/j.yhbeh.2016.01.003>

15. Tong, W.H., Abdulai-Saiku, S., and Vyas, A. (2019). Testosterone Reduces Fear and Causes Drastic Hypomethylation of Arginine Vasopressin Promoter in Medial Extended Amygdala of Male Mice. *Frontiers in Behavioral Neuroscience* **13**.
<https://www.frontiersin.org/article/10.3389/fnbeh.2019.00033>
10.3389/fnbeh.2019.00033

16. Tong, W.H., Abdulai-Saiku, S., and Vyas, A. (2020). Medial Amygdala Arginine Vasopressin Neurons Regulate Innate Aversion to Cat Odors in Male Mice. *Neuroendocrinology*. <https://www.karger.com/DOI/10.1159/000508862>
10.1159/000508862

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