

Supplementary Information

Table S1: Factors with references embedded for the Open Field protocol

Category	Factor	Literature/Reference
Generic - Design Rigor	Establishing whether an experiment informs a formal knowledge claim or not	
	Blinding during experiment and during outcome assessment and analysis	10.1186/1744-9081-7-48
	Randomization of allocation and documentation of method used	
	Sample size and rationale behind sample size	
	Criteria for outliers	
	Inclusion and exclusion criteria or data censoring	10.3389/fneur.2020.00650
	Matching or balancing sex of animals/treatment of allocation	10.1186/1744-9081-7-48
	Addressing confounds with treatment/setting/co-morbidities	
Generic - <i>in vivo</i>- Subjects	Age	https://doi.org/10.2466/pms.1977.45.3f.1059 10.1002/npr2.12052
	Body Weight	10.3390/ani6010004 10.1258/002367796780744901 10.1007/s11011-017-0140-z
	Strain - incl. Substrain	10.1007/s10517-015-2821-0 10.1538/expanim.60.111
	Breeder	https://www.frontiersin.org/articles/10.3389/fnins.2015.00424/full https://doi.org/10.1016/j.physbeh.2012.12.019
	Transport from Breeder	
	Standardisation of husbandry practice	10.1111/gbb.12149 https://doi.org/10.1016/j.neuroscience.2013.02.012 https://doi.org/10.1016/j.physbeh.2017.04.009 10.1111/j.1601-183X.2005.00121.x
	Acclimatization to holding facility prior to experimentation	
	Sex	https://doi.org/10.1016/S0031-9384(01)00494-2 https://doi.org/10.1002/ajmg.c.31565 https://doi.org/10.1016/j.neuroscience.2013.02.012 10.1016/j.bbr.2011.03.038
	Estrous cycle	10.1111/j.1467-9450.1994.tb00946.x https://doi.org/10.1016/S0031-9384(01)00494-2 https://doi.org/10.1111/j.1601-183X.2006.00249.x
	Gonadal Hormones	10.1016/j.psyneuen.2017.10.007 10.1016/j.yhbeh.2008.10.010 10.1016/s0006-8993(02)03567-9 10.1016/s0306-4522(98)00341-8 10.1037/a0012749 10.1016/s0003-3472(72)80145-3 10.1037/0735-7044.118.2.306 10.1016/0031-9384(73)90124-8
	Weaning age	https://doi.org/10.1371/journal.pone.0167652

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	Gut Microbiome	10.1038/s41398-018-0240-5
	Specific Pathogen Free	10.1111/nmo.12110 10.1016/j.psyneuen.2014.01.014 10.1080/01616412.2019.1675021 10.1371/journal.pone.0201829
	Description of controls and their suitability for the experiment	10.1073/pnas.0912955107 https://doi.org/10.1371/journal.pbio.3000411
	Recording of physiological variables during experiment	10.1186/1744-9081-7-48
Generic- <i>in vivo</i>- Housing	Home cage type (Size, Color, etc)	10.1016/j.physbeh.2005.01.008 10.1016/S0166-2236(00)01718-5 10.1016/S0006-8993(98)00735-5
	Home cage bedding type	10.1037/com0000147 10.1016/j.physbeh.2013.08.003
	Enrichment	https://doi.org/10.3389/fnbeh.2014.00257
	Room Temperature	10.1016/j.pbb.2006.10.005
	Room Humidity	
	Light intensity/type	10.1111/j.1601-183X.2005.00121.x
	Circadian Rhythm/Light Cycle	10.3791/51785 10.18632/aging.100142 10.1016/j.bbr.2009.07.001 10.1016/j.neures.2007.06.1474 10.1034/j.1601-183x.2003.00002.x
	Acoustic noise (also radio)	10.1258/la.2009.0080098 PMID: 20587160
	Ultrasound noise	
	Vibration noise	
	Group or isolated housing	10.1111/j.1601-183X.2004.00106.x https://doi.org/10.3389/fnbeh.2014.00257 https://doi.org/10.1101/2020.04.28.066704 10.1016/s0278-5846(99)00081-0
	Food type and dispenser and if there were any restrictions on diet	10.1002/brb3.708 10.1016/j.physbeh.2005.06.013 10.1152/physiolgenomics.00018.2020 10.1016/j.physbeh.2005.07.008 10.1016/j.brainres.2012.06.004 10.3390/ani6010004 10.1007/s11011-017-0140-z 10.1016/j.neulet.2018.01.025
	Food type of dams before conception, during gestation or lactation	10.1016/j.neuroscience.2013.02.044 10.1016/j.psyneuen.2015.10.020 10.1016/j.neuint.2010.04.009 10.1016/j.bbr.2008.03.021 10.1080/1028415X.2017.1354958
	Cleaning frequency, bedding transfer	10.1258/la.2009.0080098
	IVC/isolator or open cages	10.1016/j.physbeh.2013.10.019 10.1111/gbb.12564 10.3390/ani10040746
Generic- <i>in vivo</i> - Handling	Sex of Experimenter	10.1038/nmeth.2935
	Lab policy on use of perfume/skin care	

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	Researcher experienced/not	10.1111/j.1601-183X.2005.00121.x 10.1016/j.bbr.2014.06.017
	Stress history of the animal (early life/chronic/acute etc.)	10.1097/WNR.0000000000000243 10.1016/0149-7634(81)90005-1 10.1016/j.neuroscience.2005.06.068
	Habituation to handling	https://doi.org/10.1016/S0091-3057(97)00502-9 https://doi.org/10.1016/S0091-3057(02)00789-X https://doi.org/10.1038/s41598-020-60530-4
	Handling method	10.1037/com0000147
Test conditions		
Test room	Temperature	10.1016/j.jtherbio.2019.102458 https://doi.org/10.1016/j.applanim.2016.08.005
	Humidity	10.3791/51785
	Background noise level	10.1177/0023677217711966 https://doi.org/10.1111/j.2044-8295.1971.tb02034.x 10.1016/0376-6357(94)90011-6
	Illumination Level	https://doi.org/10.1016/j.applanim.2016.08.005 https://link.springer.com/article/10.1007%2F978-3-319-00572-6_9 10.1016/j.brainresbull.2006.12.009 https://doi.org/10.1016/0031-9384(94)00317-3
	Odour	10.1016/S0091-6773(72)80214-1
	Cleaning agents (type, concentration, and frequency of cleaning)	10.3791/51785
	Time of testing (morning vs afternoon)	10.1037/a0021200 https://doi.org/10.1038/s41598-019-44705-2 10.3389/fphar.2019.00237
	Frequency of testing	10.2466/pms.1998.86.3c.1179 10.1016/j.bbr.2011.11.042 10.2466/pms.1998.86.3c.1179 10.1111/j.2044-8295.1972.tb01312.x 10.1016/S0003-3472(73)80047-8
	Transportation from holding facility	10.1258/la.2009.0080098 ;
	Acclimatization to test room and duration of test	https://doi.org/10.1371/journal.pone.0048414
	Bedding material in the test box	10.1016/j.pbb.2010.05.013
Administration of drugs	Name and type of drug	10.1016/S0014-2999(03)01272-X 10.1016/s0278-5846(99)00081-0 10.1016/j.bbr.2014.06.017
	Formulation of drug solution	
	Drug Naïve	10.1007/s002130050738 10.1016/j.neulet.2007.12.020 10.1016/j.peptides.2009.02.002 10.1016/j.alcohol.2017.04.005 10.1016/j.euroneuro.2015.10.002 10.2174/1570162x13666150121105221
	Administration route	10.1055/s-2006-941557

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	Volume (ml/kg)	
	Vehicle and vehicle volume	
	Frequency of dosing and dosing regimen	10.1017/S1461145711001283 10.1016/s0024-3205(03)00612-x
Recording & Scoring	Video Angle	
	Time of Recording (similar to time of testing)	https://doi.org/10.1038/s41598-019-44705-2 10.3389/fphar.2019.00237 10.1037/a0021200
	Duration of recording	
Data analysis	Software acquisition and analysis settings	10.1016/j.jneumeth.2017.05.026
	Manual/Automatic	10.4137/JCNSD.S13194 10.1038/s41386-020-0776-y
	Analysis parameters	
Statistical analysis	Multiple raters	10.1038/s41386-020-0776-y
	Pre-defined statistical analysis	10.1016/s0278-5846(99)00081-0 10.3389/fnbeh.2017.00026
	Appropriate choice of statistical method	10.1016/s0278-5846(99)00081-0
	Definition of unit of analysis	
	Precision of effect size	
Equipment	Apparatus Construction (floor surface, color, size)	https://doi.org/10.1152/physiolgenomic.s.90207.2008 https://link.springer.com/article/10.1007%2F978-94-007-2269-7
	Thermometer	
	Camera and computer type	10.3758/s13428-017-0904-8 ; 10.1038/s41386-020-0776-y 10.1016/j.jneumeth.2017.05.026
	Analysis Software	https://doi.org/10.1089/zeb.2018.1662
	Infrared fine tuning	10.1016/j.physbeh.2016.02.014

Table S2: List of factors for the Western Blotting protocol

Category	Factor
Tissue harvest conditions	Method utilised for collection of tissue (perfusion/CO2/decapitation)
	Method for preservation of tissue (flash frozen/fixation)
	Tissue fasted
Sample Preparation	Multiple freeze thaw cycles affecting degradation
	Method of tissue homogenisation
	Buffer utilised for tissue homogenisation and sample preparation
	Fractionation procedure

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	Protease inhibitors
	Temperature
	Storage
Sample quantification	Buffer compatibility
	Protein quantification method
Polyacrylamide gel	Type of gel utilised
	Manufacturer
	% gel
	Age/ lot
Membrane	Membrane type
	Age/lot
	Manufacturer
Sample loading	Amount of protein loaded onto gels
Transfer conditions	Protein size
	Transfer buffer
	Transfer time
	Current/voltage
Blockng solution	Type of blocking solution
	Concentration
	Cross-reactivity
Primary antibody	Specificity
	Titer
	Affinity
	Incubation time
	Source animal
	Concentration
	Lot
	Temperature
Secondary antibody	HRP conjugate enzyme activation level and activity
	Source animal
	Concentration
	Temperature
	incubation time,
Washing	Buffer

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	Frequency
	Volume
	Duration
Normalisation	Loading control utilised
	Housekeeping proteins
Detection	Detection method
	Substrate type, lot, sensitivity
	Age of substrate
	Film age
	Type of imaging instrument
	Exposure time
Quantification	Quantification (densitometry)
	Software used
	Background subtraction
	Signal saturation
Test conditions	Inter-operator variability