IICARus resource

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General

- This is a study of ***IN VIVO*** reporting **ONLY**. Therefore, only the *in vivo* experiments should be assessed against the checklist even if a manuscript also contains *in vitro* experiments.

  Studies that are in vivo are performed within a whole living organisms. In the situation that samples are taken from the animals after the experimental interventions have been carried out in vivo (i.e. blood samples, brain slices, DNA from transgenic animals) this would still be classified as an in vivo study. Similarly, if the experiment involves the creation of transgenic animals and no further interventions are carried out ex vivo/in vitro then this would also be in vivo.

- The checklist is organised in sections and the reviewer should look within the corresponding section in the manuscript to answer the checklist item (i.e. items under introduction need to be found in the introduction section of the manuscript). However, **for the methods and results** sections these can be interchangeably searched to answer the checklist items.

- Items need to be reported for all *in vivo* outcomes – err on the side of caution if in doubt. Check the drop-down options available to see which questions have a 'partially' option.

- For questions relating to **Landis criteria** (randomisation, blinding, sample size calculation, and reporting of exclusions) please copy and paste where you took this information from in the manuscript into the comments sections. If it is large text indicate which section (i.e. methods, results etc.) or page number it has been taken from. This will aid discrepancy checks down the line as each paper will be scored by two reviewers with a third senior reviewer assessing discrepancies.

- The questions relating to Landis criteria are **marked by a star** in the training. These need to be answered correctly before you can pass the training.

- When answering a question, if you feel reasonably **uncertain** about whether you have interpreted the paper correctly please copy and paste the corresponding text which you believe answers the question.

- NB: In some of the training papers the use of +/- has been replaced with a 6 in the conversion process. We believe this is quite obvious in the manuscripts and is usually used when saying mean +/- s.e.m etc. or if unusually large numbers seem to have been reported.

Species

Please indicate which species is/are used in this manuscript.

Please select from the drop down list the species used in the manuscript as part of the in vivo experiment. If more than one species are reported then select all that apply. If the species is not on the list then please tick 'other' and contact us with the new species name (https://ecrf1.clinicaltrials.ed.ac.uk/iicarus/Home/Contact).

Humans

Please indicate whether or not the manuscript also includes human subjects.

This is not relevant to the rest of the review process which only applies to the *in vivo* animal experiments but is merely to indicate whether or not a study also includes human subjects.

Select 'Yes' if the study includes human study (i.e. human subjects or tissue of human origin) which may include human samples such as tissue slice (ex vivo or in vitro). It would not be classified as a human study simply if the study used cell lines of human origin and 'No' should be selected in this case.

Note: only relevant in the review process, not scored as part of training.

Drop down options: Yes or No

Title

1. Title

Items under title need to be found in the title of the manuscript.

1.1. Is the species of animal studied reported in the title?

Have the authors stated the species used in the study in the title. This must be clearly interpretable.

- *Example*: Thoracic cage plasticity in prepubertal New Zealand white **rabbits** submitted to T1-T12 dorsal arthrodesis: computed tomography evaluation, echocardiographic assessment and cario-pulmonary measurements. (Canavese et al., 2013).

Drop down options: Yes or No

1.2. Is the biological mechanism, disease or pathophysiology studied, reported in the title?

Experiments may be investigating a normal physiological process e.g. neurodevelopment and/or a disease or condition e.g. diabetes, Alzheimer's, addiction etc. In addition, there may be instances where a specific biological process is being investigated such as the effect of a particular intervention/exposure on potassium channels or the role of a particular gene for example through the use of knock out animals. Select 'Yes' if it is clear that either biological mechanism or disease or pathophysiology studied is reported. Select 'No' if not.

- *Example*: Thoracic cage plasticity in prepubertal New Zealand white rabbits submitted to T1-T12 **dorsal arthrodesis**: computed tomography evaluation, echocardiographic assessment and cardio-pulmonary measurements. (Canavese et al., 2013).
- In Vivo Detection of Age- and Disease-Related Increases in Neuroinflammation by 18F-GE180 TSPO MicroPET Imaging in Wild-Type and **Alzheimer's** Transgenic Mice. (Liu et al., 2015)
- Depot delivery of dexamethasone and cediranib for the treatment of brain tumor associated edema in an intracranial rat **glioma model.** (Ong et al., 2015)

Drop down options: Yes or No

1.3. Is the intervention or exposure reported in the title?

The intervention or exposure needs to be as specific as possible. However, if a paper is studying the effect of multiple antidepressants then to state the general term 'antidepressants' in the title is acceptable. If it is however looking at only one antidepressant (e.g. fluoxetine) then the specific name needs to be in the title, rather than the general category. The exposure could be the generation of a model vs no model induction (see section 7 for more information).

If the paper is describing for instance the negative (or positive) effects of a particular exposure (i.e. anaesthetics) then they should report the exposure clearly; this could also include exposure such as environmental enrichment.

You would select 'Not Applicable' when a paper is only looking at, for instance, the effects of genetic modification i.e. using knock out mice. Similarly if they are looking at a normal biological process and there are not types of interventions or exposures in the experiment then also select 'Not Applicable'.

- *Example*: Depot delivery of **dexamethasone** and **cediranib** for the treatment of brain tumor associated edema in an intracranial rat glioma model. (Ong et al., 2015)

Drop down options: Yes, Not Applicable, and No.

Abstract

Items under abstract need to be found in the abstract section of the manuscript.

2. Abstract

2.1. Is the objective or hypothesis given in the abstract?

The objective needs to be a clear statement that suitably describes the questions they set out to answer or a hypothesis which describes what is to be tested through study and experimentation.

Drop down options: Yes or No.

2.2. Is the biological mechanism, disease or pathophysiology studied, reported in the abstract?

See explanations under Title section for 1.2.

Drop down options: Yes or No

2.3. Is the intervention or exposure reported in the abstract?

See explanations under Title section for 1.3.

Drop down options: Yes, Not Applicable, and No.

2.4. Is the species or strain studied stated anywhere in the abstract?

Definition of "species": Organisms of the same species have more characteristics in common than they do with organisms of a different species and can interbreed to produce fertile offspring.

Definition of "strain": a group of animals that are genetically uniform. For example:-

- Species: Mice
- Strain: The different strains are identified with specific letter-digit combinations; for example C57BL/6 and BALB/c.

Drop down options: Yes or No.

2.5. Are the key methods of the study briefly summarised?

The abstract should include a brief summary of the key methods, if this is reported select 'Yes' and if not select 'No'. This is not an assessment of the quality of the summary but whether the authors have described this section in the abstract or not.

Drop down options: Yes or No.

2.6. Are the principal findings of the study briefly summarised?

The abstract should include a brief summary of the principal findings, if this is reported select 'Yes' and if not select 'No'. This is not an assessment of the quality of the summary but whether the authors have described this section in the abstract or not.

Drop down options: Yes or No.

2.7. Are the conclusions of the study briefly summarised?

The abstract should include a brief conclusion of the study, if this is reported select 'Yes' and if not select 'No'. This is not an assessment of the quality of the summary but whether the authors have described this section in the abstract or not.

Drop down options: Yes or No.

Introduction

Items under introduction need to be found in the introduction section of the manuscript.

3. Background

3.1. Do the authors refer to previous work in the literature relating to this field?

For instance, do they reference other authors who have worked on similar areas of study such as shown in this example:

- "Several studies have shown that the commonly used inhalation anaesthetic isoflurane may induce caspase activation, apoptosis, Ab oligomerization and accumulation, neuro-inflammation, tau protein hyper phosphorylation, mitochondrial dysfunction, and impairment of learning and memory ([15,16,17,18,19,20,21,22,23], reviewed in [24,25])."

Select 'Yes' if there is reference to previous work in the literature relating to the field of study and 'No' if not.

Drop down options: Yes or No.

3.2. Is a statement reported about the rationale for using that animal species or animal disease model to address the scientific objectives?

Authors should provide a rational for using a specific species of animal or a rational for using a specific disease model for their experiments. Select 'Yes' if a rationale has been provided or 'No' if it has not. Examples include:

- "We chose this animal model as it has been used extensively to screen potential anti-HIV drugs and has been validated with a number of clinically approved anti-HIV drugs such as AZT and PMPA (tenofovir) that have relatively broad antiretroviral activity. (Clouser et al., 2011)"
- "For this purpose, we selected a pilocarpine model of epilepsy that is characterized by robust, frequent spontaneous seizures acquired after a brain insult [15, 16, 17, 18] well-described behavioral abnormalities [18] and poor responses to antiepileptic drugs [19]. (Hunt et al., 2013)"

Drop down options: Yes or No.

3.3. If applicable to the research question, is there a statement describing the relevance of the study to human biology?

If there has been reference to how this study might contribute to human biology select 'Yes' and 'No' if not.

- These animals recapitulate several key features of human temporal lobe epilepsy, the most common type of epilepsy in adults [1, 2]. (Hunt et al., 2013)
- ...detailed understanding of the underlying tissue-level changes is essential to facilitate therapeutic applications. (Tseliou et al, 2014)
- We hypothesized that gemcitabine would be an alternative to hydroxyurea that could be translated to clinical use for the treatment of retroviral infections when used in combination with current anti-retroviral therapies. (Clouser et al., 2011)

Select 'Not Applicable' if for example, research pertains to improving understanding of animal biology for veterinary advancement.

Drop down options: Yes, Not Applicable, or No.

4. Objectives

4.1. Is the objective or hypothesis given in the introduction?

As for Abstract section (2.1)

The objective needs to be a clear statement that suitably describes the questions they set out to answer or a hypothesis tested through study and experimentation.

- In the present studies, we set out to determine the effects of propofol on the isoflurane-induced caspase-3 activation in human neuroglioma cells and in the brain tissues of neonatal mice. (Zhang et al., 2011)
- We hypothesized that n-3 PUFAs supplementation during pregnancy and lactation could protect against neurotoxicity in neonatal rats exposed to sevoflurane anesthesia. (Lei et al., 2013)

Drop down options: Yes or No.

Methods/Results

The checklist is organised in sections and the reviewer should look within the corresponding section in the manuscript to answer the checklist item (i.e. items under introduction need to be found in the introduction section of the manuscript). However, **for the methods and results** sections these can be interchangeably searched to answer the checklist items. This includes figure and/or table legends and supplementary methods or results.

5. Ethical Statement

5.1. Does the manuscript include an explicit statement of approval?

This should usually be described in the methods and will include a statement whether the experiment was **approved** (select 'Yes' if reported). This will vary in wording, some examples are shown below:

- Animal experimentation at the CNIO, Madrid, was performed according to protocols **approved** by the CNIO-ISCIII Ethics Committee for Research and Animal Welfare (CElyBA).
- All experiments involving mice were conducted in accordance to policies and procedures described in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were **approved** by the Animal Care and Use Committee at The Jackson laboratory.

If these type of statements are not reported select 'No'.

Drop down options: Yes or No.

5.2. Does the manuscript identify the committee(s) approving the study protocol?

This should usually be described in the methods and will include a statement about if the experiment was **approved by <Name of> committee** (select 'Yes' if reported). This will vary in wording, some examples are shown below:

- Animal experimentation at the CNIO, Madrid, was performed according to protocols **approved by the CNIO-ISCIII Ethics Committee for Research and Animal Welfare** (CElyBA).
- All experiments involving mice were conducted in accordance to policies and procedures described in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were **approved by the Animal Care and Use Committee at The Jackson laboratory**.

If these type of statements are not reported select 'No'.

Drop down options: Yes or No.

5.3. Does the manuscript name the international, national or institutional guidelines followed?

This should usually be described in the methods and will include a statement about how the study was carried out in accordance/compliance with <Name of> a named guidelines (select 'Yes' if reported). This will vary in wording, here is an example of this type of statement:

- All experiments involving mice were conducted **in accordance** to policies and procedures described in the **Guide for the Care and Use of Laboratory Animals of the National Institutes of Health** and were approved by the Animal Care and Use Committee at The Jackson laboratory.

If this type of statement is not reported select 'No'.

Drop down options: Yes or No.

5.4. Does the manuscript report a protocol / permit number?

Examples include:

- This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Minnesota (**Permit Number: 27-2956**).
- Affidavit of Approval of Animal Use **Protocol No. 2008121108**.
- The animal protocol was approved by Massachusetts General Hospital Standing Committee (Boston, Massachusetts) on the Use of Animals in Research and Teaching (**Approval ID: 2006N000219**).

If a protocol or permit number have been provided select 'Yes' and if not then select 'No'.

Drop down options: Yes or No.

6. Study Design

6.1. Are the total number of experimental and control groups reported?

This refers to the number of experimental groups and is not to be confused with the reporting of the number of animals within a group. For example, "Rats were randomly assigned to one of three experimental groups" you would select "Yes". The number of groups can either be explicitly stated or it is clear as the groups are listed in the same sentence/paragraph or it is obvious from a table or figure. If this is not the case then select 'No'.

- *Example*: **Three groups** of 20 mice each were studied: A. Wild type fed AIN 93 G diet; B. Wild type fed AIN 93 G diet supplemented with 0.1% quinine HCl; C. Wild type fed AIN 93 G diet supplemented with 0.01% quinine HCl. (Cettour-Rose et al., 2013)

Drop down options: Yes or No.

6.2. Is the experimental unit stated?

The experimental unit is the smallest division of the experimental subjects such that any two experimental units can receive different treatments.

For example if an intervention is dietary and all animals receiving one dietary treatment are housed together then the experimental unit is the cage of rats. If an intervention is to administer a drug to a rat then the experimental unit is the rat as any two rats could have received a different intervention. For a paper to be awarded this checklist item it either needs to explicitly state 'the experimental unit is…' or imply the unit e.g. 'the intervention was given at the level of the individual animal'.

- In the study, n refers to number of animals, with five acquisitions from each slice, with a maximum of three slices obtained from each experimental animal used for each protocol (six animals each group). (Grasselli et al., 2013)

Even if the experimental unit is obvious if there is no reference to it in the paper then do not award this mark. This is accounted for by question 6.8.

If you select "Not Applicable" please provide justification in the comments box.

This website helps explain the experimental unit: http://www.3rs-reduction.co.uk/html/3__the_experimental_unit.html

Drop down options: Yes, Not Applicable, or No.

6.3. If the experimental unit is not stated is it clear what it is?

If the paper is awarded 'No' for 6.2 but the experimental unit is obvious award the paper 'Yes'.

- Each animal receives an intervention or exposure – Exp.Unit = animal
- All animals in a single cage receive the same intervention or exposure – Exp.Unit = cage
- Fish in a tank – Exp.Unit = tank
- In a crossover experiment an animal could be given a treatment for a period, then rested and given a different treatment for a period. It is assumed that the treatment doesn't alter the animal, so it has to be very mild – Exp.Unit = animal for period of time
- Pregnant female is treated with the test compound or a placebo. The pregnant females are killed at about mid-gestation and the pups are weighed, measured and studied for abnormalities – Exp.Unit = pregnant females

For scoring purposes if you answered 'Yes' to 6.2 then select 'Not Applicable' for this question.

Drop down options: Yes, Not Applicable, or No.

6.4. Is randomisation reported to assign animals to experimental group?

Select 'Yes' if randomisation has been reported to allocate the animals to their experimental group even if the exact method of randomisation is not described in detail; this will be dealt with later in the review process.

No mention of randomisation is taken to mean that no randomisation has been carried out, select 'No'.

It is unlikely this would be 'Not Applicable'; in the case that you believe this to be the case please explain in the comments section.

Please copy and paste the relevant and corresponding text in the comments section if the answer to this item is either 'Yes' or 'Partially'.

Drop down options: Yes, Partially, Not Applicable, or No.

6.5. If randomisation was NOT done, does the paper mention randomisation at all?

If a paper HAS reported randomisation then the answer to this is 'Not Applicable'.

Select 'Yes' if a statement describing randomization even if simply to state that randomization was not possible was stated (this includes if randomization was stated but no methods were provided).

Please copy and paste the relevant and corresponding text in the comments section if the answer to this item is 'Yes'.

Drop down options: Yes, Not Applicable, or No.

6.6. Are assessors blinded for at least one of the outcomes measured?

This refers to the blinded assessment of outcomes where an assessor will not have information regarding the animal's group allocation when assessing outcomes be they histological, behavioural etc. In some instances it may not be possible to blind this part of an experiment for instance where genetically modified animals are a different colour behavioural assessments cannot be blinded.

Please copy and paste the relevant and corresponding text in the comments section if the answer to this item is 'Yes'.

Drop down options: Yes, Not Applicable, or No.

6.7. Does the manuscript include a statement about blinding even if no blinding was done?

Select 'Yes' if a statement describing blinding even if simply to state that blinding was not possible was stated. Select 'No' if 6.3 is 'No' and there were no statements regarding blinding in the manuscript. If a paper has reported blinding then the answer to this is 'Not Applicable'.

Please copy and paste the relevant and corresponding text in the comments section if the answer to this item is 'Yes'.

Drop down options: Yes, Not Applicable, or No.

7. Experimental Procedures

Traditionally in clinical research the research questions follow a PICO approach where P: population, I (or E): intervention (or Exposure), C: control or comparator, and O: outcome. This is often similar for animal, in vivo, studies, where P: species. This can help when thinking about answering section 7.

When surgery is used for as part of an 'intervention', outcome measure assessment or model induction, fill out the information in section 7.5. If surgery was performed as part of organ harvesting (e.g., histological assessment), select 'Not Applicable' for section 7.5.

	Example 1	**Example 2**	**Example 3**	**Example 4**
P	Rat with stroke (model induction (7.1.8), surgical procedures (7.5))	Pig obesity model	Mouse	Rat
I/E	Treatment (7.1)	Bariatric surgery (Surgical intervention (7.1.9), surgical procedures (7.5))	Generation of transgenic mouse (i.e. to model Alzheimer's disease) (7.1, where applicable)	Rat with peripheral nerve ligation (7.1.8, 7.5) + treatment (7.1) (intervention 1) Rat with peripheral nerve ligation (7.1.8, 7.5) + vehicle control (7.1) (intervention 2)
C	Vehicle (7.3)	Sham surgery (7.3)	Wild type (7.3)	Rat with peripheral nerve ligation (7.1.8, 7.5) + vehicle control (7.3) (comparator 1) Rat with sham surgery (comparator 2) (7.3)
O	Infarct volume	Weight loss	Performance in water maze	Hyperalgesia

- **Section 7.1-7.4**
  - **Encompass experimental procedures relating to conventional treatment intervention vs control, exposure vs. no exposure, and model induction vs no induction (referred to here as experimental groups (7.1&7.2) / comparator (7.3&7.4) groups for simplicity).**
  - The manuscript needs to have reported for each item to receive a 'Yes'. If it is 'Not Applicable' for one and 'Yes' for another then select 'Yes' and likewise, if it is 'Not Applicable' for one and 'No' for another then select 'No'.
  - For the intervention/exposure groups 7.1 relates to *how* procedures were carried out and 7.2 relate to *when, where and why*. Similarly, for the

control/comparator groups and 7.3 relates to *how* procedures were carried out and 7.4 relate to *when, where and why*.
    - If there is no intervention or exposure or non-surgical model induction reported for a particular study then the below items will be 'Not Applicable'.
- Any surgical procedure, except for organ harvesting (e.g., histological assessment) is covered in section 7.5.
- Transgenic manipulations are covered in section 8.6.

7.1. For each experimental group, is the:

7.1.1. **Vehicle(s)** reported?

A vehicle is a carrier or inert medium used as a solvent (or diluent) in which the medicinally active agent is formulated and or administered i.e. phosphate buffered saline (PBS). An intervention/exposure may be added to animal food and in this case consider the diet as the vehicle. If a vehicle is not required for example when using interventions which are in liquid form or exposures such as environmental enrichment then the answer is 'Not Applicable'.

Drop down options: Yes, Not Applicable, or No.

7.1.2. Vehicle **volume(s)** reported?

Select 'Yes' if the authors have reported the volume (e.g. 100 µL) for the vehicle. If 7.1.1 is 'Not Applicable' this question would also be 'Not Applicable'. Select 'No' if the volume is not reported.

Drop down options: Yes, Not Applicable, or No.

7.1.3. Intervention **dose(s)** reported?

Dose should describe the quantity of intervention or exposure delivered to the experimental group i.e.100mg/kg or 2hrs/day.

Drop down options: Yes, Not Applicable, or No.

7.1.4. **Route(s)** of administration reported?

Common examples include:

- Oral route: swallowed by mouth as a pill, liquid, tablet etc.
- Rectal route: suppository inserted into the rectum.
- Intravenous route: injected into vein with a syringe.
- Intramuscular route: injected into muscle through skin with a syringe.
- Topical route: applied to skin.

Drop down options: Yes, Not Applicable, or No.

7.1.5. **Site(s)** of administration reported?

The site of administration refers to the part of the body of the animal that the intervention/exposure is administered i.e. the tail vein, a specified muscle etc. If it is clear site what the site is via the route of administration i.e. Intraparietal injection, where the site would be parietal lobe then you would select 'Yes'.

Drop down options: Yes, Not Applicable, or No.

7.1.6. **Frequency** of administration reported?

If it is obvious that the frequency is once then select 'Yes' (such as a surgery). Frequency could be described as once, twice, per day, per week etc. If the intervention is diet related and they have reported for instance *ad libitum* access then also select 'Yes'.

Drop down options: Yes, Not Applicable, or No.

7.1.7. **Supplier(s)** reported?

Have the authors clearly stated where they sourced their intervention or exposure and detailed the company name. If it was generated in house then select 'Yes'. If they only report the supplier for the in vitro experiment, do not assume the same supplier for the in vivo experiment and select 'No'.

Drop down options: Yes, Not Applicable, or No.

7.1.8. If a **surgical procedure** was carried out was it part of **model induction**?

Surgical model induction is the use of a surgical procedure to induce a disease or disease characteristics in an animal. It could have been carried out on all animals in the experiment or only on the 'intervention' group. Surgical model induction could include, for instance, the surgical occlusion of an artery to induce a model of ischemia.

If there has been a surgical procedure as part of model induction select 'Yes'. Select 'No' if surgery has been used for any other reason and 'Not Applicable' if surgery is not reported in the paper.

Drop down options: Yes, Not Applicable, or No.

7.1.9. If a **surgical procedure** was carried out was it part of either **treatment or outcome measurement(s)**?

This could include surgery as a form of treatment e.g. a surgical intervention such as bariatric surgery in models of obesity. Surgical interventions as part of an outcome measurement may include the insertion of an electrode into the brain for single cell recordings, most commonly in non-human primates.

If there has been a surgical procedure as part of the experimental intervention or outcome measurement select 'Yes'. Select 'No' if surgery has only been used for model induction and 'Not Applicable' if surgery is not reported in the paper.

Please note that if any surgery is to examine a body part after the intervention (such as organ harvesting) this is not an intervention and would therefore be 'Not Applicable'.

Drop down options: Yes, Not Applicable, or No.

7.2. Experimental group(s) procedure(s) – when, where, why

For the intervention or exposure group, assess whether the reporting of the when, where and why of the intervention or exposure procedure(s) is clear.

7.2.1. Does the study describe **when** the intervention/exposure group procedures were carried out?

For 'When' if the authors report any of the below then select 'Yes', examples include:

- Time of day that intervention or exposure is carried out.
  - Except the burrowing assay, which was conducted from the beginning of the dark cycle, all other behavioural experiments were conducted in the light phase. (Huang et al., 2013)
- Age of animals at the time of intervention or exposure.
- Time before or after model induction that the animals receive the intervention or exposure.

Drop down options: Yes, Not Applicable, or No.

7.2.2. Does the study describe **where** the experimental group procedure(s) were carried out?

Is the location of where the intervention or exposure procedures were performed described? This could include the home cage, an anaesthetising chamber, the laboratory, or water maze? If it is clear where the experiment took place then select 'Yes'. If it is unclear then select 'No'.

It is unlikely that the answer to this would be Not Applicable however, if you feel that for a paper that this is true then please provide reasoning in the comments section.

Drop down options: Yes, Not Applicable, or No.

7.2.3. Is any **rationale** for the use of the intervention/exposure reported?

Is a rationale provided with regards to the use of the intervention or exposure (this can include dose, dosing schedule, vehicle, route of administration, choice of specific anaesthetic). Any one rationale is sufficient to select 'Yes'. If no explanations have been provided select 'No'.

- *Example:* The subcutaneous (s.c.) route may be used for agents to prolong duration of action, the intravenous route (i.v.) avoids issues of first pass metabolism (number of doses restricted due to potential damage to veins), the intraperitoneal (i.p.) route using 27-30g needles when repeated injections are required, or by oral (p.o.) gavage in volumes of less than 5ml/kg. (Al-Izki et al., 2012)

Drop down options: Yes, Not Applicable, or No.

7.3. For the comparator group(s):

7.3.1. Is the control reported?
7.3.2. Is the control dose or volume reported?
7.3.3. Is the control route of administration reported?
7.3.4. Is the control site of administration reported?
7.3.5. Is the frequency of administration reported?
7.3.6. If a control (e.g. sham) surgical procedure was carried out do they describe the methods used?

Same as for section 7.1, but for the control or comparator group. For these questions the **information needs to be explicitly mentioned for the control group.** Do not assume that the control group information is the same as the intervention group.

If there is no control exposure or intervention reported when there could have been, then select 'No' for question 7.3.1 and 'Not Applicable' for the following questions. If the control is no exposure/intervention or it is no model induction then select 'Not Applicable'.

If animals act as their own control (i.e. baseline compared to intervention/exposure or contralateral vs ipsilateral) then the answers can be the same as for intervention or exposure group for many of the question. For 7.3.6. If a control (e.g. sham) surgical procedure was carried out do they describe the methods used?

Sometimes (but not always), the vehicle used to deliver the treatment is also given, without the treatment, to the control group.

Drop down options: Yes, Not Applicable, or No.

7.4. Comparator(s) procedure(s) - when, where, why

7.4.1. Does the manuscript describe when any control/comparator intervention procedures were carried out?
7.4.2. Does the manuscript describe where any control/comparator intervention procedures were carried out?
7.4.3. Is any rationale for the use of the control/comparator group reported?

Same as for section 7.2, but for the control or comparator group. For these questions the **information needs to be explicitly mentioned for the control group.** Do not assume that the control group information is the same as the intervention group.

Drop down options: Yes, Not Applicable, or No.

7.5. Surgery and surgical anaesthesia

This section (7.5) relates to any surgical procedure carried out in the manuscript. This can be a surgical procedure to induce a model, treat the animals or measure an outcome. If general anaesthesia is administered in the absence of other concurrent surgical procedures, this falls under non-surgical anaesthesia (i.e. only the experimental group is exposed to general anaesthesia). If no surgical procedures were performed, select 'Not Applicable' for all questions in section 7.5.

7.6. Is surgical **anaesthesia** use reported?

Select 'Yes' if the authors have reported surgical anaesthesia for all surgical procedures, 'Partially' if they have only reported it for a proportion of procedures and 'No' if it has not been reported.

- *Example*: All rats were chronically implanted with epidural silverball electrodes under inhalation **anesthesia** (isoflurane 2–3% mixed with 30% oxygen (O2) and 70% nitrous oxide (N2O)). Prior to surgery, rats were given an i.p. injection of 5 mg/kg Carprophen (Rimadyl) as analgetic (Jung et al., 2013)

Example of scenario for selecting 'Partially': reported for surgery as a model induction but not for surgery as part of a treatment intervention or only reported for experimental group but not for controls (excluding sham).

Drop down options: Yes, Partially, Not Applicable or No.

7.6.1. Is the anaesthesia **route** reported?

If the authors have reported the route that the anaesthetic has been given for all surgical procedures then select 'Yes', if only reported for a proportion of surgical procedures select 'Partially' and select 'No' if not reported. If % of anaesthetic in O2 is given then select 'Yes' however, inhalation but if the authors do not mention either inhalation or % isoflurane in O2, this is not sufficient and you should select 'No'.

- *Example*: All rats were chronically implanted with epidural silverball electrodes under **inhalation** anesthesia (isoflurane 2–3% mixed with 30% oxygen (O2) and 70% nitrous oxide (N2O)). Prior to surgery, rats were given an i.p. injection of 5 mg/kg Carprophen (Rimadyl) as analgetic. (Jung et al., 2013)

Example of scenario for selecting 'Partially': reported for surgery as a model induction but not for surgery as part of a treatment intervention or only reported for experimental group but not for controls (excluding sham).

Drop down options: Yes, Partially, Not Applicable or No.

7.6.2. Is the **anaesthetic** reported?

If the authors have reported the anaesthetic's name for all surgical procedures then select 'Yes', select 'Partially' if it has only been reported for a proportion, and select 'No' if it has not been reported.

- *Example*: All rats were chronically implanted with epidural silverball electrodes under inhalation anesthesia (**isoflurane** 2–3% mixed with 30% oxygen (O2) and 70% nitrous oxide (N2O)). Prior to surgery, rats were given an i.p. injection of 5 mg/kg Carprophen (Rimadyl) as analgetic. (Jung et al., 2013)

Example of scenario for selecting 'Partially': reported for surgery as a model induction but not for surgery as part of a treatment intervention or only reported for experimental group but not for controls (excluding sham).

Drop down options: Yes, Partially, Not Applicable or No.

7.6.3. Is the anaesthesia **dose or concentration** reported?

If the authors have reported the anaesthetic's dose or concentration then select 'Yes', select 'Partially' if it has only been reported for a proportion, and select 'No' if it has not been reported.

- *Example*: All rats were chronically implanted with epidural silverball electrodes under inhalation anesthesia (isoflurane **2–3% mixed with 30% oxygen (O2) and 70% nitrous oxide (N2O)**). Prior to surgery, rats were given an i.p. injection of 5 mg/kg Carprophen (Rimadyl) as analgetic. (Jung et al., 2013)

Example of scenario for selecting 'Partially': reported for surgery as a model induction but not for surgery as part of a treatment intervention or only reported for experimental group but not for controls (excluding sham).

Drop down options: Yes, Partially, Not Applicable or No.

7.6.4. Are the **methods used** for surgical procedures clearly described?

Have the methods for all surgical procedures been described in detail (e.g. where the experimenter cut, what instruments were used, what nerve/muscle/bone they modified). If a description of the methods used has been provided for all surgical procedures then select 'Yes', select 'Partially' if they have only been reported for a proportion of surgical procedures. If they just state for instance that surgery was carried out but no description of the procedure then select 'No'.

Example of scenario for selecting 'Partially': reported for surgery as a model induction but not for surgery as part of a treatment intervention or only reported for experimental group but not for controls (excluding sham).

Drop down options: Yes, Partially, Not Applicable or No.

7.6.5. Are the **suppliers** for any specialist surgical equipment reported?

The authors should state the suppliers for their surgical equipment. If a supplier is reported for any surgical equipment select 'Yes' and 'No' if they do not.

Drop down options: Yes, Not Applicable or No.

7.6.6. Is the **monitoring of at least one physiological parameters** during surgical anaesthesia reported?

Have the authors measured for example blood pressure, or temperature, or respiration rate etc. during the anaesthesia procedure.

Drop down options: Yes, Not Applicable, or No.

7.6.7. Is the use of an **analgesic**, or a reason why analgesic was not used, reported?

For studies involving surgical procedures do the authors report the use or explicitly state not using analgesia after surgical operation? Select 'Yes' if reported, 'Partially' if only reported for a proportion of surgical procedures, and 'No' if not reported.

If they have reported the monitoring of at least one physiological parameter for any surgical procedure then select 'Yes' and if not then select 'No'.

Drop down options: Yes, Not Applicable, or No.

7.7. Euthanasia

7.7.1. Is euthanasia, sacrifice etc. reported?

Animals are often euthanized at the end of a study for the purpose of sample collection or post-mortem examination. Animals may also be euthanized because they are experiencing pain, high levels of toxicity or distress during the experiment. Select 'Yes' if euthanasia is reported and 'No' if it is not.

Drop down options: Yes or No.

7.7.2. Is the method of euthanasia reported?

Select 'Yes' if the method of euthanasia has been reported. Common methods may include, but are not limited to, the following:

- Carbon dioxide (CO2) (i.e. in a CO2 chamber).
- Sodium Pentobarbital 100 or > mg/kg IV, IP.
- Commercial Euthanasia Solution (Sodium pentobarbital 390 mg + sodium phenytoin 50 mg/ml) (e.g. Beuthanasia®, Euthasol®, Fatal-Plus®, Somlethal®) 0.22 ml/kg IV, IP (~86 mg/kg pentobarbital).
- Decapitation or cervical dislocation of anesthetized animals.
- Cervical dislocation of conscious mice by individuals that have demonstrated a high degree of technical proficiency. In lieu of demonstrated technical competency, animals must be unconscious or anesthetized.
- Exsanguination under anaesthesia.

Select 'Yes' if the method of euthanasia has been reported. Select 'No' if euthanasia was reported but no method described and select 'Not Applicable' if euthanasia was not reported and therefore no method is required.

If you have selected 'No' for 7.6.1 then select 'Not Applicable' for this question.

Drop down options: Yes, Not Applicable, or No.

8. Experimental Animals

8.1. Is the animal species reported?

Organisms of the same species:

- Have more characteristics in common than they do with organisms of a different species.
- Can interbreed to produce fertile offspring.

Common species used in *in-vivo* experiments:

- Mouse, Rat, Cat, Chicken, Cow, Dog, Drosophila, Sheep, Frog, Gerbil, Guinea Pig, Hamster, Monkey, Pig, Rabbit, Zebrafish

Drop down options: Yes or No.

8.2. Is the strain of the animals reported?

A strain is a group of animals that are genetically uniform. Mouse strains can be inbred, mutated or genetically engineered, while rat strains are usually inbred. For example there are several thousand different strains of knockout mice and many are named after the gene that has been inactivated.

- Male **C57BL/6J** mice (25.3 ± 1.4 g), aged 8–12 weeks, were included (n = 40). (Van Dijk et al., 2013)

Drop down options: Yes or No.

8.3. Is the sex of the animals reported?

The sex needs to be explicitly stated for all the experiments. If it reports 'both sexes were used' but does not state the exact number of males and females then this is not enough information and you should select 'No' and similarly if they only report the sex for one experiment and not others. If it is clear that it is either male (i.e. investigation of testies etc.) or females (i.e. investigation related to ovaries etc.) then select 'Yes' but only when 100% confident that this is true. For some species the authors may state that during this age period it is not possible to determine the sex and in this case select 'Not Applicable'.

- *Example*: **Male** C57BL/6J mice (25.3 ± 1.4 g), aged 8–12 weeks, were included (n = 40). (Van Dijk et al., 2013)

Drop down options: Yes, Not Applicable, or No.

8.4. Is the age of the animals reported?

The age of the animals needs to be reported as a median or mean (with a range) OR an exact age for each animal (if same litter then might be they say for instance mice 6 days old).

- *Example*: Male C57BL/6J mice (25.3 ± 1.4 g), aged **8–12 weeks**, were included (n = 40). (Van Dijk et al., 2013) – **Partially**
- If (Van Dijk et al., 2013) had reported a mean i.e. 10 weeks **(range: 8-12 weeks)** then they would have received – **Yes**

Drop down options: Yes (mean/median with range OR exact ages), Partially (mean or median or range only), No.

8.5. Is the weight of the animals reported?

The weight of the animals needs to be reported as a median or mean (with a range) OR an exact weight for each animal.

- *Example*: Male C57BL/6J mice (**25.3 ± 1.4 g**), aged 8–12 weeks, were included (n = 40). (Van Dijk et al., 2013)

Drop down options: Yes (mean, median with range OR exact weights), Partially (mean or median or range only), No.

8.6. For studies using transgenic animals, do the authors report

- The genetic modification status (knockout, overexpression etc.),
- The genotype (homozygous, heterozygous) and
- The manipulated gene(s)?

Each of these three items have to have been reported to be awarded a 'Yes'.

- *Example*: We used 5XFAD mice that **co-overexpress FAD mutant forms of human APP** (Swedish mutation: K670N, M671L; Florida mutation: I716V; London mutation: V717I) and **presenilin 1** (PS1) (**M146L and L286V mutations**) transgenes under transcriptional control of the neuron-specific Thy-1 promoter (Oakley et al., 2006; Ohno et al., 2006, 2007). 5XFAD transgenic line (Tg6799) was maintained by crossing **hemizygous** transgenic mice with C57Bl/6 breeders (Taconic, Hudson, NY, USA). (Devi and Ohno *et al*., 2016)

✓ **The genetic modification status**
✓ **The genotype**
✓ **The manipulated gene(s)**

This paper would receive a 'Yes' for this question. If they have reported a proportion of the items but not all then select 'Partially'. If they haven't reported any of the items then select 'No'. If the study is not looking at genetically modified animals then select 'Not Applicable'.

Drop down options: Yes, Partially, Not Applicable, No.

8.7. Are the animals used in the study reported to be drug or test naïve prior to treatment or testing?

This means that the animals used have not received a drug in any previous experiments or that they have never for example been exposed to a behavioural test in any previous experiments. The authors should clearly say whether the animals are either drug or test naïve to be awarded a 'Yes'. Cannot assume that animals have not been exposed in utero or as neonates, the authors have to report that they are naïve prior to treatment or testing.

Drop down options: Yes or No.

8.8. Is the source/supplier of the animals reported?

The source or supplier of the animals should reported to receive a 'Yes'. If they are bred in house then also select 'Yes'. If the source or supplier has not been provided then select 'No'.

Drop down options: Yes or No.

8.9. Is the health status of the animals reported?

The health status of the animal refers to if they have i.e. any pathogens, disease etc.

- *Example*: Vendor health reports indicated that the rats were free of known viral, bacterial and parasitic pathogens. (Katayama et al., 2013)

Drop down options: Yes or No

9. Housing and Husbandry

Section 9 relates to the normal housing/husbandry of animals. Intervention such as environmental enrichment or diet changes should not be assessed in this section but rather section 7.

9.1. Housing

9.1.1. Is the biosecurity level of the **facility** reported?

Type of facility refers to the type of **animal house** the animals are kept in, which is different from 9.1.2, which refers to the type of cage or housing the animals are kept in. This could be a specific pathogen free animal house. Another example could include:

- Germfree/axenic, Gnotobiotic, Specific-pathogen-free (SPF), optimal hygiene conditions (OHC) or conventional.
- Physical containment (PC) facilities: Under the legislation, the Regulator has issued guidelines specifying the requirements for certification of each type of facility (laboratory, plant and animal, etc) to physical containment levels 1, 2, 3 or 4, which must be met before a facility can be certified [Australia].

Select 'Yes' if the type of facility has been reported and 'No' if it has not.

Drop down options: Yes or No.

9.1.2. Is the type of **cage or housing** reported?

The type of cage or housing the animals are kept in needs to be reported e.g. description of size, ventilation, or established cage type etc.

- Prior to surgery the animals were housed pairwise in **type 4 cages** filled with Lignocel® (hygiene animal bedding) enriched with next boxes and horizontal tubes for climbing. (Jung et al., 2013)

If cage size or ventilation are not described, and the cage type is not specifically identified, this is not sufficient.

If the experiment includes fish and therefore a tank has been used then select 'Not Applicable'.

Drop down options: Yes, Not Applicable, or No.

9.1.3. Is the **bedding material** reported?

If the authors report bedding in any way then select 'Yes' if not then select 'No'.

- All mice were allowed free access to water and a maintenance diet containing 0.75% calcium (EU Rodent Diet 22%; PMI Nutrition Inter- national, LLC, Brentwood, MO, USA) in a 12-hour light/dark cycle, with room temperature at 21±2 °C. **All cages contained** wood shavings, **bedding** and a cardboard tube for environmental enrichment. (Meakin et al., 2013).
- Animals were housed with an inverse 12 hours day-night cycle with lights on at 8:30pm in a temperature (22±1°C) and humidity (55±5%) controlled room. Prior to surgery the animals were housed pairwise in type 4 cages filled with **Lignocel®**

(hygiene animal bedding) enriched with next boxes and horizontal tubes for climbing. (Jung et al., 2013).

If the experiment includes fish and therefore a tank has been used then select 'Not Applicable'.

Drop down options: Yes, Not Applicable, or No.

9.1.4. Is the number of **cage companions** reported?

Select 'Yes' if the paper describes how many animals were caged together (if study includes fish then this would refer to how many fish were in the same tank). Select 'No' if they have not reported this.

- Prior to surgery the animals were **housed pairwise in type 4 cages** filled with Lignocel ® (hygiene animal bedding) enriched with next boxes and horizontal tubes for climbing. (Jung et al., 2013).

Drop down options: Yes or No.

9.2. Husbandry conditions

9.2.1. Are the **light/dark cycle** conditions reported?

A 14-hour light/10-hour dark cycle or 12 light/12 dark cycle is commonly used. Select 'Yes' if the light / dark cycle used has been reported and 'No' if it has not.

- All mice were allowed free access to water and a maintenance diet containing 0.75% calcium (EU Rodent Diet 22%; PMI Nutrition Inter- national, LLC, Brentwood, MO, USA) **in a 12-hour light/dark cycle**, with room temperature at 21±2 °C. All cages contained wood shavings, bedding and a cardboard tube for environmental enrichment. (Meakin et al., 2013).

Drop down options: Yes or No.

9.2.2. Is the **temperature** reported?

This is in the context of husbandry conditions and thus refers to the temperature of the room or cage that the animals are housed in and not the temperature of the animals. Select 'Yes' if they have reported the room/cage/tank temperature and 'No' if not (this includes if they simply say controlled temperature but do not specify the temperature).

- All mice were allowed free access to water and a maintenance diet containing 0.75% calcium (EURodentDiet 22%; PMI Nutrition Inter- national, LLC, Brentwood,MO, USA) in a 12-hour light/dark cycle, **with room temperature at 21±2 °C**. All cages contained wood shavings, bedding and a cardboard tube for environmental enrichment. (Meakin et al., 2013).

Drop down options: Yes or No.

9.2.3. For experiments involving fish, is the **quality of the water** reported?

Providing details such as the pH of the water in the tanks, dechlorinated water, and details around transporting water into and around an aquatic system. Select 'Yes' if they describe anything relating to the quality of the water. Select 'No' if water quality is not reported.

If the study does not involve fish select 'Not Applicable'.

Drop down options: Yes, Not Applicable, or No.

9.2.4. For experiments involving fish, is the **tank dimensions** or **materials**?

If the authors have provided details about the size of the tank or the materials used for the tank then select 'Yes' if not then select 'No'.

If the study does not involve fish select 'Not Applicable'.

Drop down options: Yes, Not Applicable, or No.

9.2.5. Is the type of **food** provided reported?

Select 'Yes' if the authors have reported the type of food that the animals have been provided with including if they simply report that the animals received regular laboratory chow. Select 'No' if they have not reported the type. This refers to food provided before any experimentation i.e. if the study is looking at special diets then this does not count.

- All mice were allowed **free access** to water and a maintenance diet containing 0.75% calcium (EURodentDiet 22%; PMI Nutrition Inter- national, LLC, Brentwood,MO, USA) in a 12-hour light/dark cycle, with room temperature at 21±2 °C.

Drop down options: Yes or No.

9.2.6. Are the conditions around **access to food** reported?

If the authors report how food was provided to the animals select 'Yes' (e.g. ad libitum) and 'No' if not.

- All mice were allowed **free access** to water and a maintenance diet containing 0.75% calcium (EURodentDiet 22%; PMI Nutrition Inter- national, LLC, Brentwood,MO, USA) in a 12-hour light/dark cycle, with room temperature at 21±2 °C.

Drop down options: Yes or No.

9.2.7. Are the conditions around **access to drinking** water reported?

If the authors report how water was provided to the animals select 'Yes' (e.g. ad libitum) and 'No' if not.

- All mice were allowed **free access** to water and a maintenance diet containing 0.75% calcium (EURodentDiet 22%; PMI Nutrition Inter- national, LLC, Brentwood, MO, USA) in a 12-hour light/dark cycle, with room temperature at 21±2 °C.

If the experiment includes fish and therefore a tank has been used then select 'Not Applicable'.

Drop down options: Yes, Not Applicable, or No.

9.2.8. Is any **environmental enrichment** reported?

Environmental enrichment is the process of providing a stimulating environment to promote species-typical behaviour, allow a degree of control and choice in behaviour, and to enhance well-being. If environmental enrichment has been described with relation to housing and husbandry (not as an exposure) then select 'Yes' and 'No' if they have not.

Drop down options: Yes or No.

9.3. Welfare-related assessments and interventions

9.3.1. Have they reported any welfare assessment or intervention before, during, or after the experiment?

The term 'welfare assessment' applies not only to monitoring animals for signs of pain, suffering and distress associated with procedures, but also to the routine assessment of all animals to check for any health or welfare problems.

There are six 'high level' categories:

1. **Appearance**, including body, coat and skin condition; for example unkempt coat, porphyrin staining
2. **Body functions**, such as reduced food intake, changes in body temperature
3. **Environment** within the enclosure; for example, nest quality, consistency of faeces
4. **Behaviours**, including social interaction, posture, gait, and undesirable behaviours such as stereotypies
5. **Procedure-specific** indicators, for example, tumour size in cancer studies
6. **Free observations**, for observers to enter their own text should they see an indicator of suffering that was not predicted

- *Example*: During the postoperative period, **pain was relieved** by a subcutaneous administration of carprofen (Rimadyl, Pfizer Animal Health, West Dundee, Great Britain; 5 mg/kg twice daily for 5 days). An intramuscular injection of enrofloxacin (Baytril_ 5 %, Bayer Animal Health, Kiel, Germany; 5 mg/kg twice daily) was administered for the **prevention of infection** during the week following surgery. (Canavese et al., 2013)

Welfare assessment needs to be distinguishable from studying drug outcomes. For example, looking at drug toxicity is not the same as assessing animal welfare as animal welfare is not the primary outcome of this outcome measure. For example the statement 'weighed daily to assess if loss of 15% or more of body weight, then euthanized due to toxicity' would not count as welfare assessment as the primary outcome of this is to assess drug toxicity not to assess welfare.

Select 'Yes' if any welfare assessment or intervention before, during, or after the experiment has been reported. Select 'No' if this has not been reported.

Drop down options: Yes or No.

10. Sample Size

10.1. Is the total number of animals used in the study reported?

The total number of animals used in an experiment must be either explicitly stated or able to be quickly and easily calculated from a table or from a list of number of animals in all groups within the same paragraph or sentence.

- **Twenty eight healthy rats** were divided into four groups of seven each. Animals of group I received distilled water (0.1 ml/day) and served as control, whereas group II animals received only ISO at 100 mg/kg. Animals of group III and IV were treated

with test alkaloid at pre-standardized dose of 40 mg/kg (p.o.) daily for 7 days. (Panda et al., 2013)

Simply reporting the number of animals contributing to each outcome in each group is not sufficient, unless there is also a clear description of the number of animals excluded from those analysis.

Drop down options: Yes or No.

10.2. Is the number of animals in each experimental group reported?

Select 'Yes' if the exact number, not range, of animals per group have been reported and 'No' if the number has not been reported or if only a range has been reported. Please note the 'Partially' option, which should be selected if the number of animals in each experimental group has not been reported for all experiments. If it is unclear whether the n numbers presented refer to all groups then also select 'Partially'.

- Twenty eight healthy rats were divided into **four groups of seven each.** Animals of group I received distilled water (0.1 ml/day) and served as control, whereas group II animals received only ISO at 100 mg/kg. Animals of group III and IV were treated with test alkaloid at pre-standardized dose of 40 mg/kg (p.o.) daily for 7 days. (Panda et al., 2013)

Specifically, this related to the number of animals allocated to the experimental groups, not the number of animals for which outcome is assessed.

Drop down options: Yes, Partially, or No.

10.3. Is a sample size calculation reported?

Powerful experiments are ones that have the maximum chance of detecting a true treatment effect. Power can be achieved by using appropriate numbers of animals (sample size). If the authors state that they have used X number of animals to obtain sufficient power for their experiment then select 'Yes'. If sample size is not reported then select 'No'.

Please paste the relevant and corresponding text in the comments section if the answer to this item is **'Yes'**.

Drop down options: Yes or No.

10.4. Is the basis for the sample size calculations (effect size, variance, power) presented?

Sample size should be determined using a formal method such as power analysis. To select 'Yes', studies should give an explanation of their sample size – this should be a statistically based calculation to justify their sample size number and authors should report at least the following information: **effect size, variance, power,** see items bolded in the example below. If the authors mention that their study was appropriately powered but do not present how this was quantified select 'No'. Select 'Partially' if only for example 1 out of two experiments were powered to detect an effect (i.e. this could be a sample size calculation for only one out of multiple independent in vivo experiments or in vitro experiments).

Example: Sample size calculations were performed in STATA/IC 10 (StataCorp, College Station, Texas, USA) with the sampsi function. Stratified meta-analysis of

hypothermia treatment in SHRs (10) reported a **normalized mean effect size of 49% [standard deviation (SD) = 28%].** To reject the null hypothesis that pethidine does not attenuate this effect, we predicted a normalized mean effect size of hypothermia in the presence of pethidine of 29%. To achieve **power = 0 ·8 and alpha = 0 05** to detect this difference would require a total of 60 animals. (Sena et al., 2013)

If your response to 10.3 is 'No' then select 'Not Applicable'. It is unlikely this would be 'Not Applicable' for other reasons, in the case that you believe this to be the case please explain in the comments section.

If the method for determining the sample size has been sufficiently described select 'Yes' if not then select 'No'.

Please paste the relevant and corresponding text in the comments section if the answer to this item is either 'Yes' or 'No'.

Drop down options: Yes, Partially, or No.

10.5. Is the number of independently replications of experiments reported?

If all of the experiments described are stated to have been replicated, and the exact number (not a range) of times it was replicated is reported, select 'Yes'. When replication is reported but not explicitly for all relevant experiments or the number of times that it was replicated is not stated then select 'Partially' and if none of the experiments were replicated select 'No'.

Often the replication of experiments will be described as follows:

- …experiments were carried out in triplicate or duplicate etc. or,
- …the experiment was repeated twice etc.

Select 'Not Applicable' if an experiment was not repeated, but they performed a sample size calculation and the experiment was adequately powered.

Drop down options: Yes, Partially, Not Applicable, or No.

11. Allocating animals to experimental groups

Allocation is the process by which experimental units are assigned to experimental groups. It can be achieved using various strategies for example a complete randomisation, a block randomisation or randomisation within matched pairs.

11.1. Is allocation concealment reported?

A technique used to prevent selection bias by concealing the allocation sequence from those assigning animals to intervention groups, until the moment of assignment. Allocation concealment prevents researchers from (unconsciously or otherwise) influencing which animals are assigned to a given intervention group. If authors has stated that allocation concealment was carried out for all experiments select 'Yes'. In addition, if they have reported that no allocation concealment was carried out for experiments and give a reason for this then select 'Yes'.

If it has only been reported for a proportion of experiments select 'Partially'. If it has not been reported in any way select 'No'.

Please paste the relevant and corresponding text in the comments section if the answer to this item is either 'Yes' or 'Partially'.

Drop down options: Yes, Partially, Not Applicable, or No.

11.2. Are the methods of allocation to group (i.e. randomisation, matching) described?

Select 'Yes' if full details of the **methods** used to allocate animals to experimental group has been described for each experiment. It can be achieved using various strategies for example a complete randomisation, a block randomisation or randomisation within matched pairs. More information - https://eda.nc3rs.org.uk/experimental-design-allocation

- *Example:* For experiments using animals without surgery, rats were ranked in ascending order according to pre-test levels of burrowing and allocated to treatment groups in order i.e. rat 1 group 1, rat 2 group 2, rat 3 group 3, rat 4 group 1, etc., thus ensuring the median of each group was similar prior to testing (Andrews et al., 2011).

'Partially' should be selected if a method of allocation (including randomisation or matching methods) has only been described for a proportion of experiments reported. The absence of statement is taken to mean that no procedural allocation method has been carried out, and you should select 'No'.

Methods of randomisation include:

- **Simple randomisation:** For experiments with only two groups (e.g. control versus treatment), the simplest form of randomisation is to flip a coin and the side of the coin determines which group the animal is assigned to (head = control, tails = treatment). Simple randomisation can also be done by shuffling a pack of cards (red = control, black = treatment) or throwing a dice (odd = control, even = treatment). **Computer generated:** Consider for example an experiment with 3 treatment groups (1: control, 2: low dose and 3: high dose) with 10 animals per group. The function =Rand() in Excel can be used to generate a column of random numbers in column A. Column B would contain ten 1's, ten 2's and ten 3's for each of the treatment groups and column C would contain unique identification numbers for each of the 30 animals. Sorting columns A and B by the order of column A will randomise the order of column B and each animal of column C will be allocated into treatment 1, 2 or 3 at random.
- **Other methods:** Numbers out of a hat, Latin squares, etc.

It is unlikely this would be 'Not Applicable' in the case that you believe this to be the case please explain in the comments section.

Please paste the relevant and corresponding text in the comments section if the answer to this item is either 'Yes' or 'Partially'.

Drop down options: Yes, Partially, Not Applicable, or No.

11.3. Is the order in which animals receive treatments defined?

Select 'Yes' if there is a description of the order in which different experimental groups are treated and 'No' if not. This may also be illustrated by a flow diagram.

There could be experiments where an animal is its own control. In these cases the sequence that an animal will be treated are evident so will not be reported and should be marked as 'Not Applicable'.

Drop down options: Yes, Not Applicable, No.

11.4. Is the order in which outcomes are assessed in different animals reported?

Select 'Yes' if there is a description of the order in which different experimental groups are assessed and 'No' if not. This may also be illustrated by a flow diagram.

There could be experiments where an animal is its own control. In these cases the sequence that an animal will be assessed are evident so will not be reported and should be marked as 'Not Applicable'.

Drop down options: Yes, Not Applicable, No.

12. Experimental outcomes

12.1. Are outcomes reported identified as being either primary or secondary?

If the authors clearly indicate what their primary and/or secondary outcomes are then select 'Yes' if not then select 'No'.

- … [(1)]**whether URB597, a selective inhibitor of FAAH, increases retinal ganglion cell (RGC) survival in an axotomy model of optic nerve injury**, and [(2)]**to determine the contribution of CB1 and CB2 to the survival-promoting effects of URB597 in the retina.** (Slusar et al., 2013)
- Two primary outcome measures were analyzed: [(1)]**overall performance on the MWM (days 12- 16)** and [(2)]**the numbers of surviving CA2-3 cells**. In addition, three secondary outcome measures were evaluated: terminal performance in the MWM (days 15-16), rate of learning the MWM (slope of days 12-14), and MWM probe trial. (Wang et al., 2013)

Drop down options: Yes or No.

12.2. Is at least one outcome measure described?

To be scored a 'Yes' a paper needs a relatively comprehensive description of an outcome measure and the methodology involved.

Drop down options: Yes or No.

13. Statistical Methods

13.1. Is at least one outcome measure associated with at least one statistical test?

If the authors explicitly associate a statistical test used for a defined outcome select 'Yes' and 'No' if not.

- *Example*: Kaplan–Meier survival analysis was performed and student t-test for normally distributed data (i.e., body weight, ABGA results, blood pressure, heart rate, body temperature, asphyxia time, CPR duration, and lactate) or Mann–Whitney U tests for non-normally distributed data (i.e., western blot results and immunohistochemistry results) were performed to compare the differences of

baseline characteristics, and expressions of cleaved caspase-3 and acetylated histone H3. For NDS, repeated measures analysis of variance test and Bonferroni posthoc test was performed. (Hyuk et al., 2013)

Drop down options: Yes or No.

13.2. Is the unit of analysis for at least one tests explicitly specified?

The unit of analysis is the major entity that is being analysed in a study. Select 'Yes' if this is specified and 'No' if it is not.

This answer of this question may differ from question 6.2 as a paper may state the experimental unit but not the unit of analysis or vice versa. The paper must specifically state the unit of analysis to gain a "Yes".

If mice in a cage are given a treatment in the diet, the cage of animals rather than the individual animal is the experimental unit as mice in the cage cannot have different treatments. This means that the p-values in the statistical analysis may be incorrect if it is assumed that the mouse is the experimental unit (and not the cage). In this case the statistical analysis should normally be done using the mean of all the animals in the cage, but this must be explicitly stated.

- *Example:* For each test, the experimental unit was an individual animal. (Podrini et al., 2013)

Drop down options: Yes or No.

13.3. Does the publication include a method to assess whether the data meet the assumptions of the statistical tests used?

Some assumptions that could be tested for are distribution (e.g. normality of distribution), homogeneity of variances, linearity and independence of data. If authors report a method or test used to assess any assumptions of the statistical tests they use, then the answer is 'Yes' if not then select 'No'.

- *Example:* Test for normality was performed by Kolmogorov–Smirnov test. (Hyuk et al., 2013)

Drop down options: Yes or No.

Results / Methods

The checklist is organised in sections and the reviewer should look within the corresponding section in the manuscript to answer the checklist item (i.e. items under introduction need to be found in the introduction section of the manuscript). However, **for the methods and results** sections these can be interchangeably searched to answer the checklist items. This includes figure and/or table legends and supplementary methods or results.

14. Numbers Analysed

14.1. Is the number of animals for each group reported for each analysis?

Authors should report the number of animals in each group included in each analysis. If a paper reports for example, "3 animals per group were used for all analyses performed" in

the methods or results, this would be sufficient for selecting 'Yes' even if n values aren't reported later in the paper.

However, if the n value for experimental groups is reported in the methods but they don't say that the same number was used for analysis and do not report n values in figures, results, supplementary documents etc. then you cannot assume the same number was used and 'No' should be selected; likewise if no n values are reported at all. If the n value is only reported for some of the analyses, select 'Partially'. Please refer to the figure legends, tables and potential supplementary information when answering this question.

- *Example*: Sixteen MPV-positive samples were from the group of young adults, with a positive rate of 13.9% (16/115), and the other four positive samples were detected in the middle-aged adult group, with a positive rate of 11.4% (4/35) (Table 1). (Wang et al., 2013)
- The impaired cytotoxicity of ΔrtxA1 compared with that of the WT strain shows that cytotoxicity at 120 min is due principally to RtxA1. V. vulnificus cytotoxicity is inhibited more markedly by 1/4 MIC of ciprofloxacin than by 1/4 MICs of cefotaxime or minocycline (n=12 per group).

Please paste the relevant and corresponding text in the comments section if the answer to this item is either 'Yes' or 'Partially'.

Drop down options: Yes, Partially, or No.

14.2. Are the reasons for the exclusion of animals (for any outcome) given?

The paper would be marked as 'Yes' if reasons for any exclusions are given, or if any criteria for exclusions is given. For example 'any animals not reaching a baseline value were excluded (n=2)' or 'all animals reached a baseline value and were therefore included in the analysis' (as the latter implies that if they had not reached this baseline value the animals would have been excluded).

- *Example*: Thirty-eight rats were utilized for this study and 30 were included and completed. Eight animals were excluded, including 5 rats which were not resuscitated (persistent VF during ECLS) and another 3 rats were excluded because of instrumentation or technical failure during animal preparation. (Rungatscher et al., 2013).

Please paste the relevant and corresponding text in the comments section if the answer to this item is 'Yes'.

Drop down options: Yes or No.

15. Outcomes and Estimation

15.1. Are findings presented with a measure of precision?

Measures of precision include standard deviation, standard error of the mean and co S.D, S.E.M, and C.I). All figures where this is appropriate should have an error of precision to qualify for 'Yes'.

Drop down options: Yes, Not Applicable, or No.

15.2. Is the measure of precision defined?

The measure of precision (e.g. error bars) should be defined. This could be on the graph, in the figure legend or in the methods or results text. Please select the partially option if not all error bars are defined. If the error bars are defined in the methods (e.g. 'data are presented as mean ± SD') then it can be assumed that this covers all measure of precision presented and therefore can be marked as 'Yes'.

Drop down options: Yes, Partially, Not Applicable, or No.

16. Adverse Events

16.1. Is there a statement indicating that adverse events occurred or did not occur for at least one experimental group?

Adverse events may occur in an experiment if for example, animals do not survive a surgery or become seriously ill due to adverse events of the intervention.

- *Example*: "One animal did not eat, and became febrile and lethargic on postoperative day (POD) 2. The animal failed to improve with IV antibiotic administration and was euthanized on POD 4. A second animal developed a fever lasting 2 days (POD 2 to POD 3) and was treated with an extended course of norfloxacin from POD 1 to POD 4."(Merrifield, Wagh &Thompson, 2006)

Select 'Yes' if there is a statement describing adverse events, or lack thereof. Select 'No' if not or no statement.

Drop down options: Yes or No.

16.2. Does the paper describe any refinements to the experimental design to reduce adverse events?

A description of any modifications to their experimental design in order to prevent or reduce adverse effects is required to receive a 'Yes'. They would also receive a 'Yes' if they stated that no modifications were required for X reason. If no modifications have been described then select 'No'

- *Example*: As mortality with multiple surgeries was significantly higher in aged animals than young animals, aged animals only underwent a single surgery in which RGCs were labelled either from the SC (aged control nonaxotomized animals) or from the optic nerve stump at the time of axotomy. (Slusar et al., 2013)

Drop down options: Yes or No.

Discussion

Items under discussion need to be found in the discussion section of the manuscript.

17. Interpretation/Scientific Implications

17.1. Are the results interpreted in the context of other studies in the literature?

For these questions the paper needs to refer to the study hypothesis or objective (not necessarily explicitly, but authors have to interpret their results in context of what they set out to do) and in the context of the existing literature. For the latter, this is quite an open

question, so for consistency if any mention is made to existing literature (e.g. 'as shown by x, in agreement with x, in contrast with x') then mark this as 'Yes'.

- *Example*: The aim of our study was to verify if the pharmacological manipulation of the endocannabinoid system could be effective in the modulation of abnormal eating behaviour developed by female rats in a confirmed rat model of BED, in which binge eating behaviour is induced in animals by giving them a sporadic (3 days week-1) and limited (2 h) access to a high-fat diet (margarine) in addition to a continuous access to chow and water (HR group). In these animals, the intake of margarine becomes significantly greater than those of animals with limited daily access to margarine (LR group), and remains stable over prolonged periods of time (Corwin and Buda-Levin, 2004; Corwin and Wojnicki, 2006).In our study, the effect of rimonabant on the bingeing group could be related to its capacity to block dopamine release in the nucleus accumbens shell that might be induced by the consumption of margarine, and by a possible enhancement in the tone of the endocannabinoid system. Chronic exposure to high-fat palatable diet was found to decrease the expression of CB1 receptors in the nucleus accumbens (Harrold et al., 2002). Accordingly, Bello et al. (2012) reported a reduction in CB1 receptor density in the same central area in an animal model of BED. (Scherma et al., 2013)

Drop down options: Yes or No.

17.2. Are the limitations of the study design and/or execution discussed?

It should be quite clear in the discussion when the authors are discussing limitations to their study. If they describe limitations select 'Yes' and 'No' if they do not.

- *Example*: A limitation of this study is the fact that the estrous cycle stage of the female rats was not determined, since the stage of the estrus cycle at the time of tissue collection could potentially have affected gene expression levels. (Ong et al., 2013)

Drop down options: Yes or No.

17.3. Are any implications of the experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research discussed?

For the publication to be awarded the mark for implications of the research methods or findings for the 3Rs, the 3Rs (replacement, refinement, reduction) do not have to be explicitly mentioned but there needs to be some reference to how this research could lead to replacing animal research with a substitute method, lead to refining of an animal model or how to reduce the number of animals used in research.

- *Example*: The new apparatus shows potential for considerably **reducing** the number of animals used in memory tasks designed to detect potential amnesic properties of new drugs…approximately 43,000 animals have been used in these tasks in the past 5 years but with the application of the continual trials apparatus we estimate that this could have been reduce to 26,000. (Ameen-Ali et al., 2013).

Drop down options: Yes or No.

18. Generalisability/ Translation

18.1. Is there a statement about how the findings of this study might translate to other species or systems, such as any relevance to human biology?

To qualify for this checklist item, the paper needs to make a reference how these *in vivo* results will translate in the future in something useful for human, or perhaps a more sophisticated animal model of the disease or condition being studied.

- *Example*: Establishing anaesthesia-independent settings for probing rodent analogues to the human MMN are important for facilitating the detection of therapeutic targets at the cellular level. Knowledge of these targets is likely to help guiding the development of drugs for treating the disorders that have been shown to be accompanied with reduced MMN responses, such as schizophrenia. (Jung et al., 2013)

Drop down options: Yes or No.

19. Funding

19.1. Do the authors report funding source(s)?

The authors should provide all funding sources. E.g. this work was supported by a grant from CIHR.

Drop down options: Yes or No.

19.2. Do the authors include the grant number (grant #)?

The authors should provide **at least one** grant number. E.g. this work was supported by a grant from CIHR (#12345).

Drop down options: Yes or No.

19.3. Has the role of the funders been reported?

There should be a description of how the funders were/were not involved in the study. E.g. the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Drop down options: Yes, Not Applicable, or No.

19.4. Is there a statement of competing or conflict of interests?

A statement regarding conflicts of interests should be presented, this includes reporting that no conflict of interest exists.

Drop down options: Yes or No.