**Editorial comments:**  
  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

**The manuscript has been proofread and please be noted that the entire manuscript has been extensively edited according to the reviewer’s comments.**

2. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”

**The copyright permission has been uploaded to the EM account.**

3. Please provide an email address for each author.

**We are sorry for the negligence. The email address for co-authors has been added in line 19 – 21.**

4. Please expand your Introduction to include the following: The advantages over alternative techniques with applicable references to previous studies; Description of the context of the technique in the wider body of literature; Information that can help readers to determine if the method is appropriate for their application.

**The Introduction section has now been expanded as required.**

5. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

**The protocol has been revised as required.**

6. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See examples below:  
1.1: Please specify the size and setup of the behavioral room.

**This issue has been addressed in Line 95 – 100 in the revised manuscript (1.1.1).**  
1.2.1: Where is the camera located?

**This issue has been addressed in Line 102 in the revised manuscript (1.1.2).**1.3.1: Please specify the age, gender and strain of mouse. How many mice are kept in one unit/cage?

**This issue has been addressed in Line 108 (1.2.1) in the revised manuscript.**  
  
1.3.4: What is used to clean?

**The cleaning procedure is in 1.2.4., line 124 – 126.**

2.1.6.2: Please describe how to divide the arena. Is this achieved by software? Please add more specific details (e.g. button clicks for software actions).

**This part has been revised as advised in line 156 – 168 (2.1.4.).**

2.2.1: A scheme showing the elevated plus maze and the starting position of the mouse would be helpful with the protocol.

**The scheme is now in Figure 1C.**

2.3.1, 2.4.1: Similarly, a scheme showing the test/chamber set up and positions of mouse and two objects would be helpful.

**The scheme for the social interaction test and novel object recognition test is now in Figure 1 B & D, respectively.**

2.5.2: Please specify the apparatus. Where is it placed?

**The apparatus for rotarod test was a commercially available one, IITC Roto-Rod Apparatus. It is placed on the bench without direct bright lighting in the behavioral room. (section 2.2.2., line 176 – 178)**

2.7.2: Where is the mouse placed in the apparatus, in the center or at the edge?

**The mouse is put in the center of the tank for forced swim test (section 2.5.2., line 233).**

7. Please combine some of the shorter Protocol steps so that individual steps contain 2-3 actions and maximum of 4 sentences per step.

**Thanks for the instruction. We have revised the protocol as required.**

8. Please include single-line spaces between all paragraphs, headings, steps, etc.

**Thanks for the instruction. We have revised the protocol as required.**

9. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

**We have highlighted the steps for filming in the revised protocol.**

10. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

**Thanks for the reminding. We have highlighted as required.**

11. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

**Thanks for the reminding. We have highlighted as required.**

12. Figure 2: Please split the figure into two figures. Please describe different panels in the figure legend.

**Thanks for the instruction. We have made the revision as required.**

13. Figures 2 and 3: Please define all error bars and asterisk symbols in the figure legend.

**We are sorry for missing this information in the first edition of the manuscript. These information has been added in the revised manuscript.**

14. Figure 3: What do the labels 1 and 2 refer to? Please explain. What do numbers 17 and 20 in panel A represent?

**As shown in the figure, 1 and 2 means trial 1 and 2, which are the first trial in the naïve mice and when repeated tested in the experienced mice. We have explained it in the figure legend.**

15. While the results present data of mice exposed to silica nanoparticles, the protocol does not mention about this at all. Please consider including a brief description of exposing mice to silica nanoparticles in the protocol.

**We appreciate your suggestion., we mentioned in 1.2.2. to perform administration, such as intranasal instillation of silica nanoparticles, post test in the revised manuscript.**

16. Discussion: Please discuss any limitations of the technique.

**We have added this part in the discussion, line 512 – 527.**

17. References: Please do not abbreviate journal titles.  
**Thanks for the reminding. This issue has been taken care of.**

**Reviewers' comments:**  
  
Reviewer #1:  
  
Manuscript Summary:  
You and colleagues suggest a series of behavioral tests to measure behavioral changes in neurodegeneration.  
  
Although they mention tests commonly used in Alzheimer's and Parkinson's studies, a better detailed discussion regarding other tests such as the Dark-Light box test to measure anxiety or Sucrose Preference Test to measure anhedonic behavior are necessary. Particularly because these test are less invasive.  
  
A point that catches the attention is that they indicate that the tests can be repeated. However, as one reads the manuscript, it realizes that there are aspects that the authors go through. For example, there are tests that are not as repeatable as the elevated plus maze, since the mice would lose the motivation for novelty (Figure 3C). Moreover, the authors make a comparison of the repeatability of the tests (FIG.3). And in 3 of the 4 tests (Fig. 3A, B and D) they do not find statistically significant differences. On the other hand the example that they use, the C57 mouse, apparently is not so good model for the Morris water maze because it is a rodent that is able to remember the location of the platform 1 month later (Fig. 3D).  
**Thanks for the insightful comments.**

**We have discussed and explained why choosing open field test and elevated plus maze test over dark/light box test in line 433 - 448. We used open field test and elevated plus maze test to analyze anxiety in mice. Open field test was initially used to describe emotionality in mice. The central area duration reveals the level of anxiety-like behavior. In addition, the open field test is also the habituation for the following tests in the battery. Elevated plus maze test is a well-established test for anxiety in mice. This test has excellent validity. Therefore, results in these two tests could be reference for each other. The principle of dark/light box test is similar to these tests, which is the conflict between the curiosity to novel environment and the avoidance to aversive environment such as the open, elevated and / or illuminated environment in these tests. To increase the efficiency of the battery, we did not initially include the dark/light box test. Sucrose preference test is a well-known test for studying anhedonia in mice after expose to stress, such as social defeat, or unpredictable stress. Its protocol often requires days of habituation to individually housing, which may also be a stress stimulus. Adding sucrose preference test in the battery may further extend the experimental period. This part has been included in the discussion Line 464 – 467.**

**We highlighted the key points that can increase the repeatability of the test in the young adult C57Bl/6 mice. Open field test and rotarod test measure the motor function. When repeatedly tested every month, the performance of the normal young adult mice was quite stable. Social interaction test, novel object recognition test and the elevated plus maze test were motivated by novelty. Maintaining the novelty, which means using novel helper in the social interaction test, new pairs of objects in the novel object recognition test and novel novel behavioral room in the elevated plus maze test, greatly helped to increase the repeatability of the test. Figure 3A and B demonstrated stable performance in these tests when maintaining novelty; in contrary, Figure 3C showed significantly decreased exploration to the open field when performing the experiment in the same behavioral room even after 1 month.**

**Figure 3D showed that C57 mice cannot be repeatedly tested in the Morris water maze. Therefore, we used naïve mice each time when repeatedly tested.**

Major Concerns:  
  
Line 55-65. Authors referred to elevated plus maze test for anxiety, why they did not consider the dark-light box test.  
**We have addressed this issue in line 433 – 448 in the Discussion part and in the response to the reviewer’s comments above.**

If mice are nocturnal animal why the experiments were made during light cycle? The authors do not believe that this can influence behavioral tests. In what time of the day behavioral tests were conducted.

**Thanks for bringing up this point. We performed all the handling and behavioral tests from 7 am to 7 pm. This arrangement was made according to Deacon. Nature Protocols. 2006. Doi:10.1038/nprot.2006.120., which recommended to conduct behavioral tests during the light cycle. This is because mice are easily roused during the day, especially when hungry or in novel environment (apparatus or novel social partner). If left unattended, they could go back to a light sleep. This feature allows the mice to accomplish the tests, hence the author stated that it was unnecessary to inverting the light-dark cycle during most of the behavioral tests. Considering tests in our battery all contains novel environment / apparatus / partner, or have motivation, we complied with the suggestion in this protocol.**

Line 126: time in the central area of OF was an indicator of anxiety. Can the authors please give a more detailed explanation of why considered this point? Why they did not consider time in the corners as an indicator of anxiety  
**We used central area duration as an indicator of anxiety, following the protocol by Deacon. Nature Protocols. 2006. Open field test was initially developed to study emotionality of mice. Similar as other tests for anxiety, such as elevated plus maze test and dark/light box test, open field test exploits the motivational conflict between exploration of a novel area and aversion to open space. Anxious mice reduce exploration to the central zone and stay longer in the corners and near the walls. Noteworthy, studying anxiety with open field test relies on the locomotor, because measuring anxiety is confounded by activity of mouse. In our protocol, we introduce the mouse into the arena next to the wall. Therefore, staying in the central area had to be the consequence of movement / exploration. Based on this thought, we used central area duration to indicate anxiety-like behavior.**

Why authors performed the Elevated plus maze test in the same day that the OF. Elevated plus maze measure anxiety, is it possible that animals are more anxious because they were submitted to OF. How long mice stay in the home cage after OF and before Elevated plus maze?  
**This arrangement was suggested in Walf A.A, et al,. Nat Protoc. 2013., which stated that performing elevated plus maze test after open field test could increase the exploration to the open arm. Therefore, we performed elevated plus maze test after all the mice has finished open field test on the same day. The interval between these two tests ranges from 1 to 3 hours.**

**In the elevated plus maze test, the mouse faces the conflict between exploration of a novel area and aversion to an unknown, open and elevated space. The conflict is stronger than in the open field test, as the difference between open and closed space was much more drastic. As reviewed in literature (Walf A.A, et al,. Nat Protoc. 2013.), pre-exposure to a novel environment, such as open field, could be habituation of the elevated plus maze test, hence increase the likelihood of entering the open arms. The authors also conducted elevated plus maze test alone or following open field test, and did not find anxiogenic effect in the latter arrangement. Therefore, we also arranged the elevated plus maze test after open field test.**

Why the authors no considered the Novel Local Recognition version as the second part of the Novel object recognition test (see Rivera et al. 2016, 2018).  
**Thanks for the suggestion. Morris water maze test and the novel object recognition test are included in the battery to study cognition. These two tests are distinct in the principle, protocol and motivation, which could test different cognitive domains. Novel local recognition test is the other version of the novel object recognition test. However, due to the limitation of time, we did not include novel local recognition in the test battery.**

I think it is better calculated the preference of the novel object as tnew/(tnew + told). See Rivera et al. 2016, 2018.  
**We agree that using tnew/(tnew+told) may be easier to be understood than discrimination index, whose calculation was shown in Leger M, Nat Protoc. 2013.. We have made the revision in line 267 – 269 accordingly.**

In social interaction test, why authors used juvenile mouse as helper? Social interaction protocols indicate: for control mouse use a mouse of the same background, age, gender, and weight, without any prior contact (not littermates) with the subject mouse.

**Thanks for the question. Social interaction test studies the sociability of the subject mouse by allowing it to freely interact with an imprisoned novel conspecific. The result can be easily interpreted as the social interaction is initiated by the subject mouse and the interaction is limited to odder and sound. Thus, this method prevents the aggressive behavior that may affect the social interaction behavior. Our protocol was the same as** **Poon DC, et al,. 2016; which are similar to Felix-Ortiz AC, et al,. 2014, J Neuroscience; Lin YT, et al,. 2018, J Neuroscience; Zhan Y, et al,. Nature Neuroscience. 2014. etc.. In these studies, the subject mouse was introduced to a novel juvenile conspecific of the same gender. Comparing to adult stranger, juvenile is smaller, which should be more attractive than adult same-sex conspecific. Moreover, juvenile is sexually immature. Therefore, the social interaction activity is unlikely to be affected by the sexual factor. Based on these thought, we used the protocol with juvenile helper.**   
  
When the authors explain the relevance results the explication of the tests was very different from the order given in section 2. I suggest changing the order of behavioral test similar to the Representative result section. In addition, it was clearer if the cognition tests were explained together.

**We sincerely appreciate your suggestion and have made the revision accordingly.**  
  
Social interaction test is a double test able to measure social affiliation and cognition (measured as short-term social memory). Why the authors did not consider measuring the social memory. In my opinion, measuring the social memory is biologically more relevant that measuring cognition with inanimate object such NOR

**Thanks for the constructive suggestion. Social interaction test can be used to study sociability and social memory. We did not initially include social memory test to ensure our battery could cover different different behavioral domains with considerable throughput. Although social memory is only one step addition to the social interaction test, the results may be not as easy to interpret, as the behavior in the social memory test is likely affected by the sociability of the subject mouse. Moreover, this battery was designed for preliminary behavioral phenotyping, social memory test could be included in further analysis. Nevertheless, the biological importance of social memory is not ignorable, we have added your suggestion in the revised manuscript (line 518 – 519).**  
  
I do not understand why the protocol of social interaction test is shorter. Only 3 min. Please consider more time for interaction (10 to 20 min).

**The protocol of social interaction test is adopted from Poon DC, et al,. 2015 and Felix-Ortiz AC, 2014, Journal of Neuroscience. Other studies, such as Lin TY, et al, 2018, Journal of Neuroscience; also used similar interaction time. According to our observation presented below, normal C57 mice showed higher preference to the helper at the beginning than the ending of the test (one-way ANOVA, p < 0.05). The explanation is quite simple, as the helper gradually losses novelty to the test mouse with the time goes by. Therefore, to increase the sensitivity of the test, we choose to use the protocols with less testing time. Nevertheless, we have included this point in the manuscript to thoroughly introduce social interaction test.**  


For the analysis of social interaction time, I suggest consider the Recognition index as the quotient of the time the mice spent with helper divided by the sum of the time spent with helper and the empty chamber. See Rivera et al. 2016 and 2018.

**Thanks for the suggestion. We have made the revision in the manuscript (line 207 – 208) as required.**  
  
I strongly suggest that the authors review the protocol of the second part of social interaction test (Rivera et al. 2016 and 2018 or <https://www.jove.com/video/2473/assessment-of-social-interaction-behaviors)>.

**Thanks for the constructive suggestion. We have added this part in the revised edition of our manuscript (line 512 – 521).**  
  
Fig. 2C there was a lot of variability in the control group during the 2sd month of treatment. An explanation could be that they used the same juvenile (line 176) and the control animals were no longer interested in interacting with a known animal?

**Thanks for point it out. Indeed, the subject mouse may lose curiosity to the juvenile when met again. Being aware of this issue, we used different novel juvenile as helpers in trials at different time point. The same juvenile was used in the same trial as part of the parallel experimental condition and this is what we meant in line 176 (original manuscript). We have addressed this issue in the main text to avoid misunderstanding.**

**The data variation was indeed quite a lot. However, this was due to individual difference as some mice in the control group was highly interested in the helper.**   
  
Fig 2D, why authors conclude that 2-months exposure to silica nanoparticles resulted in anxiety? I did not see any differences between control and NP groups.

**We concluded that 2-months exposure to silica nanoparticles resulted in anxiety based on the results in elevated plus maze test (Figure 2E), because comparing to control mice, mice exposed to silica nanoparticles for 2 months showed significantly less exploration to the open arm in the elevated plus maze test. Figure 2D was the central area duration in the open field test, which was used as an indicator of anxiety and showed similar trend of reduced central area exploration in the nanoparticle-exposed mice. We are sorry for the misleading expression and has made revision in the main content (line 352 – 354).**

The authors give a quite detailed explanation of the repeatability of the tests, in figures 3A and 3B, there are no statistical differences. Then, I do not understand the relevance of these results.

**Figure 3A and 3B showed that the performance of the same group of mice did not vary when repeatedly tested in social interaction test and the novel object recognition test, respectively, indicating these two tests can be repeatedly tested.**

According to the explanation of the repeatability in the elevated plus maze, it is possible that the differences detected in fig. 2E are due to the animals losing the desire to explore an environment that they already know?

**Thanks for the question. Figure 2E demonstrated decreased exploration to the open arm in the nanoparticles-exposed mice comparing to the control mice.** **Mice in both treatment groups were tested in parallel and the data was normalized to control group at each time point. Therefore, the difference was not likely due to the experimental protocol.**   
  
Legend of fig 2 means that for the Morris water maze the animals used during month 1 were different from animals used in month 2?  
**Yes, different animals were used in Morris water maze test in different time point.**

Age, sex, species, and strain differences influence MWM performance, a more detailed explanation in the legend of the figures is necessary to understand the context of the experiment (perhaps in the legend of Fig. 1)

**Thank you for the instruction. The information has been added in the revised manuscript (line 108).**  
  
Minor Concerns:  
  
Line 74: ….is correct: ….in the open field arena in the open field?  
**Thanks for the correction. We have made the revision accordingly.**

Line 87: 15 to 30 min for habituate animals to the experimental environment? There is some way to prove that the habituation in the experimental room of 15-30 min is enough?  
**When placed in a novel environment, mice tend to explore for a while, and then reduce the exploration. Such reduction of movement and exploration is defined as habituation. According to Deacon RMJ. Nature Protocols. 2006., habituation after transportation is recommended to be 5 to 30 min. We give it 15 to 30 min for habituation, which normally meaning that the experimenter leaves the room until all mice are settled down in the home cage without climbing up and down.**

Is it possible that authors calculate the speed and total distance traveled in the OF arena?

**Thanks for the suggestion. We adopted the open field test from Deacon RMJ. Nature Protocols. 2006., which counted number of squares entered as total distance traveled. Nowadays, the total distance traveled and speed can be analyzed by several software. We have introduced SMART in the main text (2.1.4.3. line 167 – 168).**   
  
Line 133: What are the differences between point 2.1.2 and point 2.1.4?

**Thanks for the reminder. We have revised these points.**  
  
Line 137: The authors wanted to say 2.1.2  
**Thanks for the reminder. We have made the correction accordingly.**

In Novel object recognition test, why the authors did not consider measure familiarization time with the objects as a way to control the test  
**Thanks for the suggestion. Familiarization time is a useful parameter. The total time reveals the exploration of the mouse, the ratio between time exploring left and right objects reveals the spatial bias in the behavioral room. We have added this point in the protocol (line 271 - 272).**

What happens with the negative values that the discrimination index can throw?  
**The negative discrimination index indicates a prone to the old object, which could be neophobia that comes with anxiety.**

In line 191: what means before and after treatment?

**We apologize for the obscure expression. In our study published on Particle and Fibre Toxicology, we arranged the training of the rotarod test before the treatment of silica nanoparticles so that the baseline of the mice in the test was the same. If our reader wanted to test motor function with this battery, we suggest to arrange the rotarod training before the treatment/onset of disease/modeling as shown in the protocol.**

In Figure 1 rotarod test was made before OF Is it correct? How the authors discard any possible bias in the open field if the rotarod test is done before?  
**Rotarod test contains motor learning and motor function test. Naïve mouse learns how to focus on the test, balance itself on the accelerating rod and stay on as long as possible. In the current battery, we aim to study motor function but not motor learning. Therefore, we suggested in Figure 1 to do the rotarod test training in the naïve mice before treatment / the onset of phenotype to ensure that all the mice have learnt the skill.**

**In our study about silica nanoparticles that published in *Particles and Fibre Toxicology*, we trained the mice in the rotarod test, start the exposure and then do the open field test. Therefore, the interval between the rotarod test and the open field test was 3 weeks. We studied the performance of mice with or without experience in the rotarod test in the open field test. The results shown below indicated that the locomotor activity in the open field test was not affected by the rotarod test.**



In line 200, can the authors explain better why the third day of training is taken as a baseline of motor function?  
**The first trial of rotarod test, which was conducted before treatment or onset of disease, trains the mice to acquire motor learning. Mice learnt the skill of staying on the rod for longer time in the first two days and the performance on the third day normally reach the plateau. Therefore, we used the third day as the baseline of the motor function.**

Line 207: What kind of cues the authors used. Please explain  
**The cue was visual cues, which were circle, square, pentagon, and triangle (line 281).**

Line 218: how long is the room habituation before begging the test?  
**As stated in 1.3.4., the room habituation is 15 to 30 min. We have specified in the revised manuscript (line 292).**

How long after the other tests the Morris water maze is performed  
**Morris water maze test was performed 24 hours after other tests.**

Line 233: how much time the animal rests between sessions in the same day. Only one trial per animal during probe phase for Morris water maze  
**The mice usually gets at least 30 min rest between sessions.**

Authors proposed rotarod test only for Parkinson disease or also for AD, What is the logic to consider this test under a scenario of Alzheimer's disease?  
**When the animal model is known to manifest AD symptom, which is featured as cognitive impairment, cognitive tests such as Morris water maze test and novel object recognition test should be used in priority. However, when developing a new mice model or studying the neurotoxicity of a toxin that may potentially induce neurodegeneration, comprehensive behavioral analysis would provide more information. In this scenario, studying motor function with rotarod test can help the interpretation of tests that requires motor function.**

Reviewer #2:  
  
Manuscript Summary:  
The manuscript describes a battery of tests to assess motor function, cognitive function and 'mood' in rodents. The investigators have put forth a behavioral assessment battery that has three key features to positively impact progress:  
1. Well Supported. The tests used and methods detailed are well supported across a range of disciplines and their incorporation into a visualized experiment will be an important contribution to several scientific fields (neuroscience, neurobehavior, drug development and preclinical treatment screening, etc).  
2. Translational / Clinically Meaningful. The selected metrics have face validity as comparative assays of several clinically relevant functional subdomains essential to maintain independence and quality of life in humans (see NIH Toolbox).  
3. Economy of time and resources. These tests are not overly stressful, non-invasive, easily repeatable. This supports the feasibility and cost of time/effort for both animals and investigators.  
  
Major Concerns:  
1. Introduction implies test battery designed to detect impairments that accompany AD or PD and yet all example data and discussion context center only on short term treatment with nanoparticles in young C57BL6 mice. Test performance changes with age and neurological conditions. This is acknowledged in discussion (pg 8 of 10, lines 384-390) with statement that "optimization is required for behavioral assessment in these models," but the introduction is misleading. Please revise.

**Thank you very much for the instruction. We have made the correction accordingly in the Introduction part.**

2. Statistics: the only statistical test mentioned are for unpaired t-tests. This a a major limitation. Repeat analysis requires use of 2-way Repeated Measures ANOVAs at minimum. The battery proposed multiple tests with multiple outcomes and hypotheses being tested simultaneously. This would involve multiple comparisons, and a correction factor should be applied, or multivariate analysis models (strongly suggested).

**We used repeated measures 2-way ANOVA when analyzing the training of Morris water maze test and the rotarod test, as demonstrated in the figure legend. Utilizing ANOVA would be of great value when studying the time course of the behavioral changes in mice. Although we highlighted points that improves the stability of the performance of control mice in the tests, there are variants that cannot be controlled in each trial. Firstly, the status of the mice cannot be the same. Hence the data in the open field test and elevated plus maze test, both requires spontaneous locomotion, could only be compared with the control group tested at that time. Secondly, the outcome of other tests also depends on the experimental condition. For example, in social interaction test, each trial requires a novel helper. It would be difficult to control the individual difference of the helpers used in different trial. Based on these factors, we did not compare changes in different time point in these tests.**

3. Please include statement about animal handlers and training. Animal behavior is sensitive to investigator. When possible, the same animal handler or investigator should perform longitudinal behavioral assessments. At minimum, attention to intra- and inter-rater reliability during protocol training should be adhered to (Intraclass correlation coefficient, ICC, >0.80 between raters when possible)

**Thank you for the reminding. We were aware that proper handling is critical for the outcome of behavioral tests. Hence the mice were handled by the same experimenter during the entire study. We have added this point in the protocol as advised (line 112).**

4. Assessment battery designed for repeat testing, but proposed frequency of testing should be stated, and interpretation of change (or no change) over time warrants discussion. Authors acknowledge this in results section (lines 289-290: "Other tests cannot be repeatedly tested in the same group of mice, as the experience greatly interfere the performance."). Please provide a recommended testing frequency or limits (basement/ceiling effects) for each test in the assessment battery. Provide evidence when available.  
**Thanks for the instruction to improve our manuscript. We have made the suggestion as required in the Discussion section.**

Minor Concerns:  
1. Introduction: paragraph 1, line 41. "…largely unknown and associated with environmental factors or toxins." Recommend to strike extraneous text after "unknown."

**Thanks for the advice. We have revised this sentence accordingly (line 51).**

2. Introduction: paragraph 2, line 51-52 "Results of these two tests…" Sentence unclear and unnecessary introduction (but appropriately addressed in discussion). Please omit sentence from introduction.

**Thanks for the suggestion. We have deleted this sentence from introduction in the revised manuscript.**

3. Introduction: paragraph 2. Include references for open statements about symptoms and specific impairments in etiology and progression of disease (e.g. references needed for: "Cognitive domains including short-term memory and episodic memory are most susceptible to neurodegeneration.")

**Thanks for the constructive advice. We have included the references as required.**

4. Introduction, paragraph 2, lines 64-65. Last sentence can be omitted.

**Thanks for the instruction. We have deleted this sentence as suggested.**

5. Methods, 1.3.1, line 103. Omit "Do not delete for no good reason."

**Thanks for the instruction. We have deleted this sentence as suggested.**

6. Methods, 1.3.1, line 104-105. Add references or specific example to statement: "Tail suspension test is more stressful than forced swimming test."

**Thanks for the instruction. We have made the correction in this sentence.**

7. Methods, 2.1, Open Field. Camera recording should also detect total distance traveled, speed of movement, and time spent not moving (freezing behavior). These measures may be more sensitive to detect change over time and with intervention. Please expand this section and provide data to support use of 'line crossing' vs. these other standard open field test outcomes.

**We appreciate your constructive advice. Open field test can be used to analyze locomotor function, anxiety, spontaneous activity, and even memory etc.. Observation in open field test is valuable when at the beginning of investigating a new treatment or mutation because the open field test can show whether behavior is within normal limits. A mouse that does not move for several minutes in the open field is unlikely to be worth testing in more complex behavioral tests. Therefore, locomotor is studied in the battery. Using ‘line crossing’ or ‘squares entering’ to indicate locomotor function is described in Deacon. Nature Protocols. 2006., which divided the open field arena into small squares and the number of square entering or line crossing reveals how much the mouse moves in the arena, i.e. the locomotor function. This method is important for some non-behavioral laboratory as analysis software is costly. Other parameters, such as freezing, is also important, hence is added in the revised protocol as suggested (line 156 – 168).**

8. Open field and Elevated plus maze are tested on the same day. Please state time interval between tests.

**The elevated plus maze test is tested after all the mice has been finished testing in the open field test (line 213 – 214). Depending on the number of the mice and arena, the interval between these two tests was 1 to 2 hours in my experience.**

**As the experimental set-up, the configuration of the apparatus, the lighting, and sometimes even behavioral room, is different among these two tests, it was unlikely that the elevated plus maze test could be interfered by the open field test. Moreover, exploration in the open field test has been suggested to increase the open arm exploration if elevated plus maze test is performed afterwards. (Walf AA, et al,. Nat Protoc. 2007)**

9. Results, paragraph 2, pg 6 of 10, lines 285-284. Please revise sentence "The data showed that normal mice consistently had 2 folds of the preference to the novel object comparing to the old object (Figure 3B)." Statement meaning unclear.

**Thanks for the instruction. We have revised this sentence (line 368 – 369).**