First, we would like to thank the editors as well as the reviewers for their thoughtful comments. We feel strongly that their comments and suggestions have improved the revised manuscript’s quality and readability. Because we had significant alterations to the entire manuscript document, we did not highlight specific sections. Below, we have included detailed responses to each of the comments we received.

**Editorial Comments:**  
  
**Comment 1**: Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

**Response 1**: Thank-you, we proofread the document before submitting.

**Comment 2**: **Protocol Language:** The JoVE protocol should be almost entirely composed of numbered short steps (2-3 related actions each) written in the imperative voice/tense (as if you are telling someone how to do the technique, i.e. "Do this", "Measure that" etc.). Any text that cannot be written in the imperative tense may be added as a brief “Note” at the end of the step (please limit notes). Please re-write your ENTIRE protocol section accordingly. Descriptive sections of the protocol can be moved to Representative Results or Discussion. The JoVE protocol should be a set of instructions rather a report of a study. Any reporting should be moved into the representative results.

**Response 2**: We rewrote the protocol into short steps and reincorporate the extra information into the intro and discussion.  
  
**Comment 3**: Protocol Detail: Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. Please ensure you answer the “how” question, i.e., how is the step performed? (e.g. button clicks for software actions, numerical values for settings, etc). Alternatively, for steps that will not be filmed, add references to published material specifying how to perform the protocol action.   
**Response 3**: We included video with to show the many, many steps for analyzing video. There was not a voice over, but there is text instead within the video. Please let us know if the new uploaded supplementary files will work.

**Comment 4**: **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE’s instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.

**Response 4**: We have now altered the numbering system to comply with the suggestion above.

**Comment 5**: **Protocol Highlight:** After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

1) The highlighting must include all relevant details that are required to perform the step. 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 included in the highlighting.  
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.  
3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.  
4) Notes cannot be filmed and should be excluded from highlighting.  
5) Please bear in mind that software steps without a graphical user interface/calculations/ command line scripting cannot be filmed.  
6) Please match the videos provided (along with specific time stamps) to steps in the manuscript (the time stamps can be provided as a supplementary document). Please upload un-edited footage here -> <http://www.jove.com/files_upload.php?src=17872133>

**Response 5**: Completed just before submission and all other highlights removed The supplementary videos now have closed-captioning. The timestamps for behaviors are included in the sample video of the test.

**Comment 6**: **Results:** Please mention all statistical tests and sample sizes. It is unclear which test was used in Fig 4, 5

**Response 6**: We have now added this information into the figure legends.   
  
**Comment 7**: **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

**Response 7**: The discussion section has been edited to include all the subsections (1-5) listed above.

**Comment 8**: **References:**  
1) Missing citation on Line 115.

2) Please make sure that your references comply with JoVE instructions for authors. Citation formatting should appear as follows: (For 6 authors or less list all authors. For more than 6 authors, list only the first author then *et al.*): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. *Source*. **Volume** (Issue), FirstPage – LastPage, doi:DOI (YEAR).]

**Response 8**: Citation was added as # 14 and others following adjusted accordingly. In addition, we have now complied with the JoVE suggested format for citations.

**Comment 9**: **Commercial Language:** JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are Ellegaard, (CDL7233S Dome, Points North Surveillance, Manfrotto 244 friction arm kit; B&H photo, NVR-RACK64 Points North Surveillance, 3M 1522H, MBK Tape Solutions, Nasco’s, Microsoft Excel-, Dynasim, Tenderfoot, SAS (version 9.3; SAS Institute Inc, etc.

1) Please use MS Word’s find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names.

2) Please remove the registered trademark symbols TM/R from the table of reagents/materials.

**Response 9**:

1. We have now removed all commercial sounding language in the manuscript.
2. We have now removed the registered trademark symbols from the materials and reagent table.

**Comment 10**: Please define all abbreviations at first use.  
**Response 10**: We have now looked through the entire manuscript and have defined all abbreviations at first use.

**Comment 11**: Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.  
**Response 11**: We have edited the manuscript to comply with this comment.

**Comment 12**: If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

**Response 12**: The tables and figures are original and from pilot data collected specifically for testing the methodologies in the manuscript.

**Reviewer’s comments**

**Reviewer #1:**  
  
**Comment 1**: Manuscript Summary:  
The authors adapted a common, non-invasive behavioral test to detect changes in pigs with mTBI. The authors did not, but should, discuss other potential applications for HAT; such as using this tool to evaluate general welfare state of laboratory pigs without treatment (negative affective state given barren environment) when performing routine health checks. All the materials and equipment needed listed in the table and are described well in the main text.

**Response 1**: We would like to thank the reviewer for this really good point. We have added more information about other applications such as animal welfare and sickness detection.

**Comment 2**: There is no explanation given as to why this procedure begins with using operant conditioning to allow pigs to associate clicker with human/food reward.

**Response 2**: The conditioning discussion was added to the new section in the discussion “trouble-shooting”. The treats and clickers were incorporated when we were using a tripod and the test-person(s) would change their body posture and vocalize to call the pigs. By adding a treat, the pigs learned that the human was there for the treat, and not to provide feed, water, clean, etc. By adding the clicker, the human no longer needs to use a signal (i.e. vocal) that is human-dependent. The clicker signal can be used across laboratories. We learned that depending on the pig’s experience, some treats are less motivating than others, so we believe that this portion needs to be up to the laboratory to decide what is most motivating for their pigs.

**Comment 3**: Lines 97-103: The number of Yucatan minipigs for housing type B and Göttingen minipigs for housing type C are not given (boars and gilts).

**Response 3**: The age + SD were added or the 5 female Göttingen minipigs.

**Comment 4**: Lines 120-126: Item "D: Defecation area should be used as an indicator that pigs are acclimated to their environment". No reference is provided to defend this statement.

**Response 4**:Reference is now provided and explanation was moved to the discussion.

**Comment 5**: Lines 191-193: "If pigs never had any adverse interactions with people, they typically generalize and associate all people positively with food" Again, no reference is given to defend this statement.

**Response 5**: Reference is now provided and a better clarification was added in the discussion.

**Comment 6**: Lines 241-241: How inter-observer reliability is calculated is not described. This is an essential step in any animal behavior study.

**Response 6**: Thank you for pointing this out. We cited Martin and Bateson for various methods for measuring calculating both inter- and intra-observer reliability. In our results, we added a statistical description that specifies and cites previous calculations.   
  
**Comment 7**: Minor Concerns:  
Authors did not provide explanation of "Why" rodent models are not sufficient  
Authors did not define "Affective states"

**Response 7**: We modified these statements extensively in the introduction; this was the second reviewer’s concern as well.

**Comment 8**: Lines 114-115: This statement is not a general fact for swine behavior. It is a reference to one study that is not listed. Coffin et al., 2018 is cited in text, but not listed in references (also, other in-text citations are numerical).

**Response 8**: This section was moved into discussion and the citation is now provided.

**Comment 9**: Lines 293-295: This report of intra and inter-observer reliability has not been published.

**Response 9**: This was published as a master’s thesis report and is accessible online: Luo, Y. Swine Applied Ethology Methods for a Model of Mild Traumatic Brain Injury (Master’s Thesis). Retrieved from <http://hdl.handle.net/2097/35760> (2017).

**Comment 10**: Lines 282-291/Figure 3: This graph is not intuitive. There is no justification/rationale for color-coded pigs in the graph. Axis labels are needed on figure 3 (it is not clear why there is a secondary axis as well; it is assumed that both y-axis represent the approach %).

**Response 10**: The figure legend has been updated. This graph is a visual representation of how the formula and weighting was composed and will be explained in protocol-script. The colors are formulated similar to a heat map (red = most approach to black = least approach).

**Reviewer #2:**  
  
**Comment 1**: Manuscript Summary:  
The authors have created a behavioral task wherein the piglets behavior when approaching a human can be used to quantify the effects of mTBI and possibly other conditions (injury, sickness, or stress). First, the authors have embarked on a momentous task, that of creating and validating a new behavioral paradigm. However, significant revisions should be made.  
**Response 1**: We thank the reviewer for their thoughtful comments.

**Comment 2**: The authors state there is a need to develop and standardize functional tests specific to somatic, cognitive, and affective symptoms under study (lines 49-51), but continue to describe a behavioral test that has no known relation to any specific cognitive domain or brain region. Of course, some flexibility should be allowed as behavioral tests that have been in use for decades still see debate in academic literature on what these tests truly measure. However, it should be acknowledged that the underlying biology of the HAT test is unknown, and in its current state, it is more of a global rather than specific measure of approach behavior.

**Response 2**: The introduction was extensively changed to better reflect a general affective state of motivation vs. anhedonia and motivation rather than a focus on cognition and specific brain regions.   
  
**Comment 3**: The authors suggest that as there is no need to physically handle or train animals, thus reducing the subjectivity of the test. While this is true, the authors should also acknowledge the range of characteristics which make HAT impossible to standardize, e.g., the experimenter's height, gender, temperament, odor, relationship to the animal, etc. This limitation is addressed in table 2, however can the authors clarify the sample size? I see 7 pigs were used with two sessions each, but how many different familiar and unfamiliar humans were used, one each?). These are also limitations with other behavioral paradigms, but where other tasks make an object or food reward the stimulus and the human an auxiliary function of the task, here the human is the stimulus. Thus, it is impossible to standardize the stimulus of interest across labs, whereas the use of an object or food reward can easily be standardized. A comment on this limitation would be helpful.

**Response 4**: We agree with the reviewer’s comments and have now added more to the discussion, plus our additional studies reflecting familiar vs. unfamiliar humans. As previous studies in using hat for evaluating commercial pig production, we did not find differences between a familiar person and an unfamiliar person. Objects and food to be introduced early in life for standardization, and most biomedical researchers do not have direct access to developing pigs. In addition, we have developed another test that uses an object that moves rather than a human and compared the human approach behaviors to the object approach behaviors. The pigs behave very consistently to the human, but variable to the object, therefore, we think both tests (HAT followed by Object test) will be useful toolsets. We are currently preparing a manuscript detailing the protocol and findings from the object-approach test.

**Comment 4**: On line 61 it is stated that changes in the HAT measures should signify symptoms of mTBI rather than excess stress or fear, why? Is it not plausible that mTBI disrupts emotional processes (e.g., the amygdala) or the HPA axis, and the HAT is truly measuring the disruption of those specific processes? There have not been adequate controls to suggest that the severity of mTBI is the true measure. Proper habituation to a testing environment will still not remove the effect of stress during testing, although it will mitigate it. The human is most likely a source of stress, whether that be distress or eustress. Indeed, the authors go on to state on line 81 that HAT can be used to measure injury, sickness, and stress. These are competing hypotheses that should be reconciled or at least addressed prior to publishing.

**Response 4**: We thank the reviewer for this insightful comment. We have modified our introduction extensively to address these points. The stress response will indeed be activated whether the stressor causes distress or eustress, but pigs responses to leaving the pen and handling is variable, therefore, this test reduces that variation. There were sham-controls (received anesthesia-only) that were compared to Blast-treated pigs. Other than exposure to Blast, shams were treated identical to Blast-treated pigs. The wording in the manuscript now says “HAT was able to *distinguish* Sham-pigs from mTBI-pigs.”

**Comment 5**: Overall, there are questions that remain unanswered and claims put forth without relevant data for backing. However, it is unfair to judge new behavioral paradigms with those that have existed for decades. Based on the present data, the HAT appears to be a sensitive marker of mTBI, however it is recommended that the authors revise the manuscript with a more conservative attitude.

**Response 5**: The introduction was edited extensively and we have ensured that we did not over-reach. The main objective of this manuscript is to develop one new protocol for lab pigs, and it is not our intention to replace rodent models or replace HAT with other published behavioral tests.

**Comment 6**: On lines 90-91, it is stated that the approach index mitigates variation caused by confounding factors between labs. As the approach index is created by using the behaviors that are affected by confounds between labs, isn't it impossible for the AI to not also be subject to those confounds?

**Response 6**: We thank the reviewer for this comment and this sentence has been deleted.

**Comment 7**: Step 4: What is the pig acclimating to? The human? The reward? The use of a food reward turns this into an operant task that further complicates the behavior being tested. Is HAT supposed to be a measure of sociability? Of learning? Of exploratory behavior?

**Response 7**: The purpose of this step is further explained in the introduction and in the discussion. The food reward was added during our troubleshooting of the test. Pigs are exposed to humans daily for various purposes (feeding, cleaning, handling before treatments). The specific routine and treat allows the pig to anticipate what the test-human will do next so that it behaves similarly (when untreated or recovered) for every session.  
  
**Comment 8**: Close, mid, and far are arbitrary distinctions. Why not measure distance from the human using automated tracking software? This is more expensive, but less arbitrary and removes the possibility of binning errors. The author notes the use of such software in the manuscript (Step 6C).

**Response 8**: We wanted to make the first protocol using manual timestamping of the spatial positions of the pigs, so that all labs have access to this test. The new troubleshooting section and limitations to the test now include further explanation with citations.

**Comment 9**: There are two nose directions (away and towards human), what approach should be taken when the head is neutral to the human? Pigs may orient their head to the side, but still look at a human.  
**Response 9**: Figure 2A and the accompanying legend describes what was considered away and towards. The neutral positions were highly variable within- and between-observers, so the definition was reduced to two states rather than three.

**Comment 10**: Why is it suggested that pigs standing or walking without NNOB are in an agitated state (Line 232)? There is no data or reference for this behavior.

**Response 10**: We added more explanation and references in the introduction. NNOB are expressed in pigs ubiquitously, and were once considered stereotypies or even abnormal. Pig ethologists are now reaching a consensus that NNOB are an important functional behavior when expressed at normal time-budgets in a 24-h period. Pacing is often seen in captive exotic animals (i.e. zoo). Even among other species, the lack of NNOB during pacing is considered abnormal because the animal is expending energy without a purpose. NNOB has a clear purpose- it allows the animal to gather information about its environment, with the likely motivation to seek out a substrate to chew on.

**Comment 11**: Step 6: Why edit footage into three-minute sessions? Data can be binned post analysis.

**Response 11**: This is now better explained in the trouble-shooting section discussion.

**Comment 12**: Step 6D: The assertion that even trained observers do not need to pause, rewind, or time-stamp frame by frame is based on opinion and fosters an assumption that trained observers are not subject to their own internal biases. Pausing and re-winding is a valuable tool for accurately assessing behavior. It is recommended to omit or revise this section as is based on opinion.

**Response 12**: We have provided additional insights under the discussion as why we ended up with these methods. These methods were proposed based off of experience, but using guidelines for measuring behavior and our own experiences for improving the repeatability and reliability of the test.