

**Dr. Courtney Lynd Daigle**  
Assistant Professor

October 1, 2019

Dr. Nam Nguyen  
Manager of Review  
Journal of Visualized Experiments

Dear Dr. Nguyen and anonymous reviewers,

We would like to extend our deepest gratitude to you and the anonymous reviewers for the feedback and genuine effort to enhance the quality of our Manuscript JoVE60641 "Fear tests don't always measure fear: the use of traditional fear tests to quantify different emotional circuits in the brain."

We are thankful for your input and recommendations as they have enhanced the quality of information provided and clarity of message to the reader. Should any part of this manuscript require further alteration, please let us know and we will make the necessary changes.

Below are our responses to the concerns raised by the editor anonymous reviewers.

Should you have any questions or concerns, please do not hesitate to contact us directly.

Sincerely,

Courtney Lynd Daigle, Ph.D.  
Assistant Professor, Animal Welfare  
Department of Animal Science  
Texas A&M University

P: 979-862-9171  
E: cdaigle@tamu.edu

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

2. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please sort the Materials Table alphabetically by the name of the material.

**AU: we have provided as much information regarding the products as is available to the authors**

3. Please submit each figure as a vector image file to ensure high resolution throughout production: (.psd, ai, .eps., .svg).

**AU: Corrected**

4. Please upload each Figure individually to your Editorial Manager account.

**AU: Corrected**

5. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file.

**AU: Corrected**

6. Please use SI abbreviations for time in the Tables: s instead of sec, etc.

**AU: Corrected**

7. Please revise the title to be more concise. Additionally, please do not use contractions.

**AU: the title has been revised to “The use of traditional fear tests to evaluate different emotional circuits in cattle”**

8. Please rephrase the Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to ...”

**AU: The revised summary is as follows: “Here, we present a protocol to conduct a variety of behavioral tests in cattle that have been designed to evaluate emotions. A battery of behavioral tests (Open Field Test, Startle Test, Bovine Zero Maze, Exit Velocity, Pen Score, and Chute Score) were conducted to evaluate different components of animal temperament.”**

9. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s).

**AU: Corrected**

10. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.  
For example: Farmtek, SILENCER, etc.

**AU: Corrected**

11. Please insert a one liner spacer between each protocol step.

**AU: Corrected**

12. 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. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

**AU: Corrected**

13. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. 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.

**AU: Corrected**

#### **Reviewers' comments:**

##### **Reviewer #1:**

Manuscript Summary:

The summary should be more specific to give a complete idea of the different tests covered in the article.

**AU: The summary now contains reference to all of the tests conducted.**

Minor Concerns:

In the introduction no reference should be made to the work tables. In this case it would be pertinent to make a state of the art on the main themes of the article.

**AU: New Lines: “The goal of this article is to visually document the different fear tests used for cattle, present the type of data that is generated from these different tests, evaluate the repeatability, validity and reliability of these tests, demonstrate how to evaluate the relationships among the behaviors captured from these tests, and suggest which emotional circuit could be evaluated with each test.”**

Line 173: should be test 6 and not test 3.

**AU: Corrected**

In the results chapter line 198 and following - in the body of the text, reference should be made to the tables for the respective results. And the table results should have a larger description in the body text.

The inclusion of the main conclusions of the work is recommended.

**AU: corrected**

**Reviewer #2:**

Manuscript Summary:

The manuscript describes the protocol for a set of tests for "fear" or temperament in cattle, with some general discussion of how these are analysed and can be interpreted. Some data from the tests is presented.

**AU: the format for this journal indicates that the data used here can be hypothetical in nature. In order to preserve the novelty of information that has been generated from this specific research trial, we are using hypothetical data to demonstrate how the tests could be conducted, decoded, and analyzed.**

The set of tests described is appropriate, and includes some less frequently used tests that may be of use for other researchers to consider. There is some good critical discussion of the interpretation of certain tests. The protocols described are fairly simple and easy to follow.

Major Concerns:

My biggest concerns with this manuscript are that there is little discussion of the rationale behind the detailed protocols, and the discussion of statistical analysis is so general that it provides little guidance. The clear benefit of describing all of these tests together would be to discuss how to use them in conjunction with one another, which is not really discussed. The scoring system referred to for some tests may not be the ideal scientific method.

**AU: Substantial effort has been made to increase the clarity of direction regarding statistical analysis. Because these tests are typically conducted in conjunction with physiological or other behavioral testing, the ultimate analysis of these results will depend on the hypothesis of the researcher using this test. That was the motivation for the author's choice to keep the description of the statistical analysis general. However, in lieu of the reviewer's comments, we have added information regarding the repeatability, validity, and reliability of some of the metrics in these tests. This particular article is not a hypothesis-driven manuscript as this is a methods paper that seeks to demonstrate and standardize methodology that is used within the field.**

**The scoring systems were developed in the early 1990s, and while we agree that they may be subjective, they have been widely used in the literature and are therefore important to include in this video documentation of methodology.**

Minor Concerns:

It is not entirely clear that these data are novel, and although data on repeatability are given, no information about the repeated testing is given so these results are not very informative.

**AU: As this is a methods paper, the results are not intended to make a novel contribution to the scientific literature. The purpose of this article is to demonstrate how to conduct the tests and what an analysis of the data could look like. However, the way in which the data is analyzed can vary greatly depending upon the design of the hypothesis-driven research in which these tests are used.**

Specific comments follow:

1. 113: Why was a group size of 5 chosen? The discussion could address whether group sizes are likely to have an impact on the results.

**AU: The group of 5 was chosen because that is what is standard in the literature and previous studies.**

l. 117-119 "Observe the behaviour" is not specific enough for others to follow this protocol. The scores described in Table 3 are about "aggression", not "fear", so their fit with this paper is unclear. However, the lower scores that are labelled as reflecting some level of aggression actually seem to describe behaviour more associated with fear. This scoring system may not be appropriate, and should be critically discussed.

**AU: this was addressed in the above comment.**

l. 121 Why is this cleaning not recommended for the chute tests?

**AU: this is impractical based upon the realities of cattle handling.**

l. 171 No such protocol for cattle seems to be described in the review paper cited. Where did this come from?

**AU: this paper describes the zero maze that is regularly used in biomedical research. We are the first group to conduct this test in cattle, therefore, there are no other references with which to cite. Further, we used the metrics that were collected in the rodent maze to guide the metrics we chose to evaluate in the maze designed for cattle.**

l. 210: Is there justification for stating that this will remain stable over time, based on one study?

**AU: Additional references have been added to support this statement.**

l. 226-8: The logic behind this statement is not clear.

**AU: The research that evaluated this test has recently been accepted for publication.**

l. 320: Justification for this claim is also unclear.

**AU: Explicit explanation of this phenomenon can be found in “The Archaeology of Mind” by Panksepp and Biven. A reference to this has been added to the text.**

l. 354-55: Is there a source for this statement about cattle's tendencies?

**AU: this section has been revised.**

Table 2, "restless": I think the word "management" is incorrect but am not sure what this is supposed to mean.

**AU: Thank you. This was a typo. “management” has been changed to “manageable”**

**Reviewer #3:**

#### Manuscript Summary:

I was not entirely sure how to review this. It is a great idea to visual present each of the different fear tests and to talk about their pros and cons. There have been a lot of questions raised about what emotions/motivations each of the test actually assessed, and using the Panksepp categories as a framework is a good idea. I am unsure what results should be presented. Is the purpose to present the different tests and their pros and cons? If so, do we need the stats on repeatability and relation to growth? Or is it a paper on the relationship between the tests? Then we need more correlations between the tests presented, and more comprehensive stats on repeatabilities etc. for the BZM, Pen Score, OFT or startle test.

I would be tempted to go for the 'what are the different tests, pros and cons and discussion of whether they all measure fear or other emotions' approach.

**AU: the results have been substantially revised to provide pros and cons, repeatability, validity (regarding ADG), and reliability for all tests and what emotional circuit we propose is being evaluated. A sample cluster analysis has been conducted on results from multiple tests to demonstrate how the data could be evaluated synergistically.**

#### Major Concerns:

As above, either go for a 'discussion of what these test actually tell us' approach or a 'what is the statistical relationship between these tests' approach

**AU: we took the approach of how repeatable, reliable, and valid indicators of growth are each of these tests, pros and cons of each test, and what these tests actually tell us. We also included sample analysis for evaluating the interrelationships among the different tests. However, as this is a methods paper, we did not seek to address any specific hypothesis but more demonstrate how you could use the data generated from these tests to address a specific hypothesis-based research study.**

#### Minor Concerns:

Authors: it says Amanda Hubbard in the opening piece and Amanda Mathias further down L54.

**AU: Thank you for this. During the period of manuscript preparation, Ms. Mathias became Mrs. Hubbard.**

I think you are following the Panksepp model here? I think it provides a good framework to consider temperament tests, ie separating fear from panic/grief (separation) is clearly important, but that model is not universally accepted. It would be good to acknowledge this here. Also acknowledge Panksepp in Table 1 ('after Panksepp' is how you do it I think??).

In the results, the Pankseppian emotional systems that each test might involve are linked. It would be good to formalise this in a hypothesis here in the introduction. What elements do you envisage are involved in each test? What will you do to test this?

**AU: This is not a hypothesis-driven research paper. We have added language at the beginning of the discussion explaining that these circuits are not mutually exclusive and will influence one another, so researchers should be aware that they may be evaluating more than one emotional circuit during a single test. A listing of what emotional circuits are most likely activated during each of these tests is included in the results section.**

L74: Description of animals and housing. Some more details of the testing procedure are needed here. What order were the tests done in? Did all animals experience all tests in the same order? Over how many days were the tests run? One per day on consecutive days or were there rest/wash-out days as well? ADG is mentioned in the results. How/when was this assessed?

**AU: A general description of how the animals were housed and the order in which tests were conducted has been included.**

A lot of other authors have used flight score and exit velocity. Flight speed/flight time was originally designed by Heather Burrow in Australia in 1988 and has been used extensively in that country. Are you just giving a few examples? If you want to be comprehensive, you could look wider. Also for the chute test. Outside of the US, it is generally called a 'crush score'. However, this could become a review in its own right! I think I would acknowledge Burrow, but then suggest that you are giving examples of where these tests have been used. However, acknowledging work outside the US would be diplomatic.

**AU: Our intention is to show that exit velocity is one of the stalwarts of cattle temperament evaluation. Because this metric is so easily captured, our hope, in future studies is to evaluate the efficacy of this behavioral metric in evaluating other components of cattle temperament beyond fear. We have added language to acknowledge that chute score and crush score are evaluating the same thing. We have also added references to highlight the wide use of exit velocity across the cattle temperament literature.**

What about the docility test as pioneered by Le Neindre et al. in France? This is used in France extensively in Limousin breed programmes. Why was this not included? Focus on procedures used in the US?

**AU: We attempted to avoid using any breed-specific evaluations**

L209. See comments above, but here the emotional systems are linked to the tests. However, it is not clear what the rationale for this was. Exit velocity is certainly 'fear', but as they are isolated, isn't there an element of 'panic' as well? I think it would be good to be clearer about how you assigned the tests to the elements.

**AU: Additional language has been added throughout the manuscript to address this and clarify the text to the reader.**

L225: 'seking'

**AU: Corrected**

Table 2. In definition of nervous - Typical temperament is management? Manageable?

**AU: Corrected**