**Editorial Comments:**

* All editorial comments have been addressed as requested.

**Reviewer #1:**

* *While the data is interesting and original, the protocol that the authors propose to calculate "temporal processing" doesn't make sense at all. They propose to integrate the area "above" the curve of absolute startle values as a measure (the indent) for temporal processing, however, all temporal information is in fact gone through this calculation. It is impossible to tell whether changes in this calculated value is due to a general change of PPI across all ISIs, through a shift in maximum PPI to a different ISI, or if PPI changes that are in any way ISI dependent. In fact, it would have been much more interesting to see the entire curves over ISIs across ages in order to assess potential changes in PPI that are ISI dependent (I guess there are none, see below, but even that is an interesting piece of information).*
  + We concur. The primary focus of the protocol is now exclusively on raw data, not any derived measures. The entire curves of ISIs across ages are presented to illustrate changes in PPI that are ISI dependent. The new Figure 4 presents these data.
* *Another huge problem is that the authors use absolute PPI values, not percent PPI. The increase in area "above" the curve across ages is most likely due to the increase in weight of the animals. Heavier animals result in larger baseline startle since they accelerate the startle platform more efficiently. Hence the increase in PPI reported here might in fact reflect a steady percent PPI, while baseline startle increases due to weight gain. This probably also accounts for the difference in males versus females. See Csomor PA, Yee BK, Quednow BB, Stadler RR, Feldon J, Vollenweider FX. Behav Brain Res. 2006 for more detail.*
  + As above, our focus is on raw data (i.e., absolute startle amplitude values). The focus on raw data rather than any derived measures, such as percent PPI, is, in our view, the solution, not the problem.
  + The difficulties with using percent PPI continue to be underappreciated, though well-established decades ago.
    - Despite the apparent simplicity and popularity of that approach, the frequent use of subjectively determined percentage data is not without potential consequence regarding validity of inferences made about meeting the assumptions of analysis of variance ([Bliss, 1938](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4937996/#R2)). Unfortunately, percentage data have error variances that are a function of the mean and are not normally distributed ([Bartlett, 1947](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4937996/#R1)); rather they are described by Poisson or bimodal distributions, depending on whether the data occur over a large portion of the percentage scale (bimodal) or are primarily grouped at either end (Poisson)([Cochran, 1940](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4937996/#R5)). The manipulation of ISI guided the present protocol, with the incorporation of a range of ISIs to determine the shape of the PPI response curves. The incorporation of a range of ISIs is fundamental to the establishment of a relatively precise and defined response function, and consequently, a more accurate assessment of response inhibition, as has been employed to examine alterations in the development of PPI as a function of developmental neurotoxin or drug exposure (e.g., [Fitting et al., 2006a](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4937996/#R17),[b](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4937996/#R18),[c](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4937996/#R19); [Ison, 1984](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4937996/#R28); [Mactutus, Harrod, Hord, Moran, & Booze, 2011](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4937996/#R39); [Moran et al., 2014](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4937996/#R42); McLaurin et al., 2017). The plotting of the raw startle amplitude scores, e.g., as in the log–log plot portrayed in [Figure 2](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4937996/figure/F3/), 3 and 4 graphically illustrates another advantage of the ISI function approach. Given that PPI is a model of temporal processing, the advantages of incorporating the temporal dimension, and presentation of absolute PPI values, in any assessment of PPI would appear undeniable.
  + The concern that “*heavier animals result in larger baseline startle since they accelerate the startle platform more efficiently”* is puzzling to us. The startle response is indexed by a piezoelectric accelerometer, not a load cell nor stabilimeter. The fact that acceleration is independent of mass was well-established centuries ago according to Newton’s second law.
  + The relevance of the Csomor et al. (2006) paper is unclear. Csomor et al. (2006) varied the level of pulse stimulus intensity and prepulse intensity, not ISI. Additionally, the paper is restricted to all male subjects.
* *In summary, many studies vary ISIs when measuring prepulses, and all it takes to do so is changing a small setting in the stimulus protocol (see also Valsamis B and Schmid S, JoVE, 2011). While the concept to use variable ISIs to probe for differences in temporal processing is very compelling, the proposed protocol falls short to do so, in fact, it removes the temporal information. I would love to see the data without this AUC calculations, but the full data set with temporal information present, published in a behavioural Neuroscience journal, with a discussion of its usefulness to probe for temporal processing changes/deficiencies. The use of visual prepulses preceding an acoustic startle pulse also probes for multisensory integration - dependent on the ISI on the level of the brainstem (at ISI <100 ms, or with potential cortical involvement at ISIs > 100ms). The comparisons of the temporal aspects between different prepulse modalities and with gap PPI is also very interesting.*
  + As above, the entire curves of ISIs across ages are presented to illustrate changes in PPI that are ISI dependent (Figure 4). Similarly, the entire curves of ISI are presented in all figures.
  + We appreciate the recognition of the compelling utility of using variable ISIs to probe for differences in temporal processing.
  + The Valsamis and Schmid (2011) paper illustrates and advocates the use of two ISIs (i.e., 30 and 100 ms) for prepulse inhibition. Additionally, Valsamis and Schmid propose the calculation of percent PPI, which, as discussed above, is fraught with consequences. In sharp contrast, our protocol takes that recommendation further into using a range of ISI values and describes the interpretation and analysis of the ISI function.
* *How were tactile prepulses separated from acoustic? The air passing through the tube cause normally a relatively easy to perceive hissing sound, therefore an acoustic as much as a tactile stimulus. If not omitted, this needs to the very least be acknowledged and maybe called an acoustic/tactile stimulus. The low overall startle response during tactile gap PPI at 0 and 4000 msec is probably caused by the constant hissing sound that masks the startle stimulus to some extent - hence the low baseline startle.*
  + We appreciate this comment and the configuration of the protocol recognized that concern. The sound of the air puff prepulse was measured, in the absence of background white noise, as 70 dB(A) inside the tube, 2.5 cm from the end of the test cylinder (at the position of the rodent’s ears). Thus, in the presence of 70 dB(A) background noise (again at the level of the rodent’s ears), the air puff was effectively a pure tactile stimulus.
  + This information has now been added to the protocol as step 1.5.2, in recognition that they are critical factors in the tactile stimulus parameter definition.
* *The "stimulus interval approach" should be called the "variable stimulus interval approach"*
  + The focus of the protocol is on manipulating ISI across a range of values. We have replaced the phrase “interstimulus interval approach” with “an approach varying interstimulus interval”.
* *All "auditory" has to be replaced by "acoustic", since the authors use sound, hence an acoustic stimulus. "Auditory" refers to the neurons processing acoustic information - the auditory neurons. Auditory stimulation would be an electrical stimulation of auditory neurons, not a stimulation by acoustic sound.*
  + As requested, we have replaced the term “auditory” with “acoustic.”
* *What animals were used in this study?*
  + Two sets of animals were included in the study. The assessment of sensory modality and psychostimulant exposure was conducted in ovariectomized female F344/N control animals (*n*=20) between 8 to 14 months of age. The longitudinal assessment of cross-modal PPI and gap-PPI was conducted in intact male and female F344/N control animals (male: *n*=20, female: *n*=17) between 1 and 5 months of age (i.e., PD 30 to PD 150).
  + Information regarding the animals used in the study has now been included in the protocol.
* *Reference to Fig. 2A (text page 8): "Auditory PPI is presented within the visual context" - what does this mean??*
  + Hardware and software limitations allow only two prestimulus modalities to be assessed at once. Cross-modal PPI was assessed using concurrently presented acoustic and visual prestimuli, as well as concurrently presented acoustic and tactile prestimuli. The data for acoustic PPI, therefore, is illustrated from the experimental paradigm including the concurrent presentation of acoustic and visual prestimuli.
  + The sentence has been changed to read “Data for acoustic PPI is presented from the experimental paradigm including the concurrent presentation of acoustic and visual prestimuli (i.e., visual context).” to improve clarity.
* *Instead of "punctuated" stimuli, it should maybe say "discrete”*
  + As requested, we have replaced the term “punctate” with “discrete.”
* *The ITI during habituation needs to be 10 sec, not msec.*
  + Thank you for catching this error. It has been corrected.

**Reviewer #2:**

* The authors of this manuscript describe a new approach to assess temporal processing using the classical PPI chambers used in the literature. As PPI approaches are also used in humans, this protocol can be very interesting for translational research between animals and humans. Although the manuscript is well-written, the authors should improve the way the protocol is explained in order to help the reproducibility of the protocol for all the readers.
  + We thank the reviewers for their kind comments regarding the translational importance of PPI. We have improved the way the protocol is explained to aid in the reproducibility of the protocol for all readers.
* In the introduction and discussion, the authors should compare this way of assessing temporal processing with other tasks assessing this function. The authors have focused the manuscript 100% in the PPI approach but if the protocol assess temporal processing it will be good to put it in the context of this function. What is known in the literature? Which are the tasks used?
  + Using prepulse inhibition as an exemplar paradigm, we present a protocol for manipulating interstimulus interval (ISI) to establish the shape of the ISI function to provide an assessment of temporal processing.
  + The focus of the present protocol is on the comparison of two methods for conducting and analyzing PPI (i.e., percent PPI vs. a range of ISI values). The discussion of the variety of different tasks that can be used to provide an assessment of temporal processing is beyond the scope of the protocol.
* When explaining the tasks, authors must show schemes/pictures to better define each protocol and apparatus used. Moreover, authors should better define the different modalities (cross-modal PPI and gap-PPI) from the beginning of the manuscript.
  + We concur and have included a schematic of the two experimental paradigms in the Introduction. Additionally, we have enhanced our definitions of the two experimental paradigms, including their similarities and inherent difference, in the introduction.
* In the literature can be good to explain if these modalities are also assessed in humans as the classical PPI is done.
  + Not to our knowledge.