**Working Rebuttal Letter**

**BLUE = Stats/James**

**Green = Experimental Sonia and Kasey**

**Yellow = Writing Dennis, Sonia, and Kasey**

Editor

1. Introduction: I suggest deleting the text regarding the relevance of the study to alcoholism treatment. It is unclear how the current neuronal recording data will lead to better treatments. And Heilig and colleagues attempted to translate a CRF mechanism to human alcoholism and this attempt was unsuccessful.
2. Delete 'for the first time.' Such statement may or may not be correct and unnecessary. Also, change 'paradigm' to 'procedure' when describing the DID procedure.
3. Please report formal statistical results in the Results section, not the figure legend. Also report in this section and in Figure 1 alcohol intake in the DID procedure.

Reviewer 1

Major

1. It is unclear how the spiking data are aligned to the licking behavior. Presumably the data were aligned to the start of a lick bout rather than to each lick. Bout definitions and methods for data alignment should be clearly stated.
2. While it would be highly informative to have observed the activity of CRF neurons during water consumption to determine if these responses are related to licking or to alcohol itself, I appreciate that adding these experiments would essentially double the experiments/animals included in this submission. Are there any other behavioral events in the current data set that could be used to understand how these neurons are responding to other stimuli? If video of the behavior was recorded, it would be informative to determine neural activity aligned around interactions with food, or around initiation of movement. At the very least, care should be taken not to overstate these findings in terms of attributing neural activity to the consumption of alcohol.
3. It is unclear what is being correlated to produce the r values reported in figure 2B, or what the significance of these finding are. Please clarify in the methods, and include an interpretation of these findings in the discussion. Further, the late CRF group displays a striking bimodal distribution. Were the 5 cells with the high positive correlations during the late sessions from the same animal? Was there any behavioral characteristics of the animals that might explain the bimodal effect?

* ANSWER: WHAT CORRELATIONS ARE SHOWN AND WHY IMPORTANT
  + These are the correlations of the cumulative # of licks and the unit's normalized firing rate, analyzed in 5 min bins.
  + The major take-away is that relationship between CRF units firing rates and licking behavior changes after repeated binge drinking cycles, where non-CRF had no significantly shift in their distribution.
* ANSWER: RE THE BI-MODAL DISTRIBUTION
  + Regarding the bimodal distribution of fig 2B, late CRF:
    - primarily due to 1 mouse, which contained 4 out of the 5 positively correlated units.
    - There was nothing distinct in his behavior to explain the effect

1. Using a baseline period of -100 to -50ms prior to the lick may be biasing the cell classification due to the fact that it is likely to still contain significant stimulus-evoked activity. This is very close to the pre-lick period on the timescale of behavioral event. Given that there is significant pre-lick activity using the current definition, it seems more than likely that some of that activity would also be present more 50ms prior to the start of the bout. The authors should either use a longer baseline window or use the average firing rate of each cell outside of all the behavioral event windows throughout the entire session as a baseline.
2. More methodological description for phototagging is needed. What light power was used? How many trials of stimulation were performed?
3. What was the rationale for using r values of >0.9 for spike waveforms for classifying photo-identified units? This seems arbitrary, an empirically derived cutoff may be more appropriate. There are several approaches that could be taken which would improve confidence in the cell identification. For example using a cutoff of >3 Z scores from the mean of the spike waveform distribution of all of the naturally occurring spikes, or < plus or minus 0.05 r from the cross-corr of the waveforms during the baseline periods of the same cell. Please also report how many units were excluded based on this criteria.
4. The manuscript has quite a few grammatical mistakes and typos, and is difficult to read in several places. There are a very high number of non-canonical abbreviations, particularly the abbreviations of the unit types. Further, when describing the findings in the abstract and introduction it would be easier to follow if it was clearly distinguished when the authors are referring to stimulus evoked activity (e.g. “activity during licking for alcohol”) as opposed to general excitability or basal firing. In general, the manuscript would benefit greatly from being proofed for clarity and readability.

Minor

1. Why was the behavioral data presented in figure 2A from a different cohort of mice? Please be sure to indicate the number of animals in addition to the number of cells for all of the experiments.
2. The original DID paradigm papers should be referenced in the methods section.
3. The authors refer to the population of units that were not light responsive as “non CRF neurons”, however it cannot be determined whether these cells are truly CRF-. The lack of photoresponse may be due to a number of factors including viral penetrance and detection rates of the wire recordings. While light responsiveness can be used as a definitive classification of CRF+ neurons, the opposite is not true.
4. A photoresponse latency threshold of 10ms is stated in the methods, but 5ms is stated in the discussion.
5. Please be consistent with using either alcohol or ethanol throughout.

Reviewer 2

1. My first point is related to the division of CRF-positive neurons into their four populations. My first concern with this is terminology. The authors refer to one population of neurons as "CRF-predictive" neurons, which show increases in firing rates both during the baseline and licking periods. It is the term "predictive" that is problematic for me. Actually, the analyses and the accompanying figures (e.g. figure 3A) suggest that these neurons show a general increase later in a session, which is correlated with increases in drinking. To me, this is not necessarily "predictive" of the increase in licking behavior or binging, it is a correlate. That's not to say it is not important, but to term it predictive without more sophisticated analyses that show (on a trial by trial basis, for instance) that this signal is predictive of individual licking responses, the term in not justified.
   1. Come to think of it- Figure 2A shows increases in cumulative licks across a session, but can the authors show the number of licks across time and analyse these data statistically rather than the cumulative data?
   2. Do mice increase consumption within the session in the same temporal scale as the firing rate of these neurons increase?
   3. The authors could better justify why they ignore the other two population types in later analyses (i.e. the CRF-excited and CRF-inhibited groups), and focus only on CRF-predictive and CRF- non-responsive populations.
   4. What are the profiles of the other neuronal subtypes in relation to the later analyses?
   5. Could the authors show the distribution of CRF-predictive neurons by mouse? Are we seeing a reasonable distribution of neurons across their test subjects, or do these neurons come from a few mice?
2. My second point is related to the behavior to measure binge drinking. Is this a model that has been used before or is based on the wider literature? I didn't see any citations to suggest why the authors chose this behavior.
3. The discussion is way beyond the scope of the paper. Talking about interactions with dopamine neurons in the VTA in particular is very speculative. And I think this works against the authors goal of enhancing the potential interest in the experimental results.
   1. Why are these changes in firing rates important?
   2. How are they specifically related to the behavioral analyses?
   3. Why is CRF in particular likely to be involved and what do these data suggest in terms of treatment for addiction?
4. Can the statistical analyses be put in the results section of the MS as well as the figures and the specificity of the analyses better described?
   1. For example, how was it determined that the mice increase drinking across and within sessions. What measures are used?
5. The results section is at times hard going and could be streamlined throughout. Consistent terminology and greater description of what the results mean to non-ephys types would be helpful.

Reviewer 3

1. One important control experiment “To ascertain if CRF units encoded ethanol consumption” as well as to support “a relationship between prolonged/repeated ethanol consumption and the firing/burst properties of CRF neurons” would be to examine the selectivity of CRF neural activity in response to water, natural reward, and different doses of ethanol. Related, is CRF-P plasticity upon repeated binge drinking selective for ethanol?
2. How did CRF-P neurons behave during abstinence following repeated binge drinking sessions? Did they show cumulative increase in the basal firing/bursts (with no ethanol onboard) over the 5-week period? The authors discussed that CRF levels remain elevated days after binge drinking of alcohol, thus it will be important to know of CRF-P activity both during and after repeated sessions of binge drinking to learn about the nature of the plasticity.
3. Is there any correlation between CRF-P neuron firing and 1) on-going and 2) subsequent alcohol intake? The authors argue that binge drinking may induce neural plasticity that impacts subsequent drinking, thus it is important to know whether there is potential correlation between CRF-P activity and alcohol drinking behavior.
4. “CRF cell bodies are present in the extended amygdala including the CeA and bed nucleus of the stria terminalis (BNST) and release CRF through 2 Gs-coupled postsynaptic receptors, CRF1 and CRF2”: It is hard to understand how CRF may be released through CRF1 and CRF2, please clarify (e.g. positive feed-back?).
5. Fig. 1: May illustrate additional detail of the recording configuration to reflect the descriptive text (i.e. “the MEA electrodes were arranged in a U-shape around a central pore for optical fibers. The MEA connector was offset from the center in a chair-like configuration to allow for the optical fiber”. “Fiber implants were attached to the MEA at a slight angle toward the electrodes").
6. “CRF neurons were more excitable than CRF (NR) and non CRF neurons”: “excitable” should be “active” to be accurate.