EJN European Journal of Neuroscience

FENS Federation of European Neuroscience Societies

Striatal alcohol cue-reactivity is stronger in male than female problem drinkers

Anne Marije Kaag, Reinout W. Wiers, Taco de Vries, Tommy Pattij and Anna E. Goudriaan

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Review timeline:

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Editor: Rita Goldstein
Reviewer 1: Anna B. Konova (New York University, USA)
Reviewer 2: Reagan Wetherill (University of Pennsylvania, USA)

1st Editorial Decision 25-Mar-2018

Dear Dr. Kaag,

Your manuscript has been reviewed by external reviewers as well as by the Section Editor, Dr. Rita Goldstein, and ourselves.

As you can see the reviewers indicate that your experiments generated new and important information. However, in their very careful and detailed reviews, they raise a series of issues that need to be clarified/resolved before we can consider your manuscript further for publication in EJN. Most of the points needs simply need clarification of the text and/or inclusion of additional material. Particularly confusing is the fact that Figs 1 and 2 are identical plots! Please carefully address each of the points that they have raised in your revised version of the paper.

We also noted the following points that need to be addressed:

- Ensure that the reporting of statistical data adheres to EJN guidelines, notable report precise P values (e.g. in Table 1).
- Author qualifications/titles should be removed from author list
- References are not quite in EJN style (too many authors named in in-text citations), the reference list needs checking for missing information and all authors of cited papers should be included in the reference list
- Please carefully proof-read the manuscript, there are some typos and grammar errors that need fixing
- Compliance with DoH?
- Table 1: the *s and the numbers in brackets need to be explained in the caption
- In accordance with EJN guidelines, replace the bar charts with more informative scatter plots, hybrid plots or similar.
- we probably need larger figures for publication.
- Fig 3 is not cited in the text (apart from the place holder)
- Fig 2 should not be cited in a sub-heading
- The figure legends do not fully describe the figures. They should be completely understandable without reference to the text and include the statistical data.

When revising the manuscript, please embolden or underline major changes to the text so they are easily identifiable and DO NOT leave 'track change' formatting marks in your paper. Please ensure that you provide a text and a figure file for the Graphical Abstract (as detailed in the instructions below). When carrying out your revisions please refer to the checklist below and visit the EJN author guidelines at www.ejneuroscience.org

When finalized, please upload your complete revised manuscript onto the website, as a Word file (.doc, or .docx). Please also ensure that a complete set of tables and figures is included as separate files, even if these have not changed from the originals. At this stage it is necessary to provide high resolution figures. Please see important instructions below.

Please go into https://mc.manuscriptcentral.com/ejn - Author Centre - manuscripts with decisions where you will find a 'create a revision' link under 'actions'. We ask that you please indicate the way in which you have responded to the points raised by the Editors and Reviewers in a letter. Please upload this response letter as a separate Word (.doc or PDF) file using the file designation "Authors' Response to Reviewers" when uploading your manuscript files. Please DO NOT submit your revised manuscript as a new one. Also, please note that only the Author who submitted the original version of the manuscript should submit a revised version.

If you are able to respond fully to the points raised, we would be pleased to receive a revision of your paper within 12 weeks.

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Thank you for submitting your work to EJN.

Kind regards,

Paul Bolam & John Foxe
co-Editors in Chief, EJN

Reviews:

Reviewer: 1

Comments to the Author
Kaag et al. examined ventral and dorsal striatum response to alcohol cues (relative to control cues) in a moderate sized sample of men and women with problem alcohol use. The main findings of the study were that, as previously shown, these two regions showed differential response to alcohol picture cues that were more pronounced in more severe drinkers; additionally, the authors provide novel evidence that these response are also independently enhanced in problem male drinkers relative to female drinkers despite the two being fairly well matched on alcohol use severity and task-related subjective craving. Overall the study has several methodological strengths including a sufficient sample size to examine sex differences. The question the authors set out to answer may also have important implications for alcohol cue reactivity research, which often fails to account for participants' sex.

Several limitations are also noted, however, as described in detail below that the authors may need to address before the full significance of their findings can be assessed:

(1) Very little information is provided about the content of the task stimuli although this is a major point of the study. Do these stimuli include social cues (e.g., people)? Equal number of beer and wine images? This information is also important for understanding the exploratory preferred drink type findings.

(2) Understanding the stimuli better might also speak to the observed sex differences: Is it an issue of the magnitude of alcohol cue effects in neural reward systems or differences in the content or type of cues that would trigger these effects for men versus women? The latter could also perhaps be addressed by data on subjective experience of the cues (e.g., perceived pleasantness and arousal ratings). Were such data collected?

(3) Also related, please report on participant task engagement/accuracy on the target picture trials.

(4) Please report statistical information for significant effects as well as non-significant effects throughout the results section. It is not clear from the text alone how small the non-significant effects were (e.g., the interaction effects).

(5) I was unable to find in the references the behavioral results paper cited as "Kaag et al., 2017" that reports on current sample. Please add this information.

(6) Figures 1 and 2 look identical. Figure 2 looks to be the incorrect one as the figure caption refers to AUDIT effects but the legend refers to sex effects. Please provide the correct plots for both figures.

(7) Were there sex differences in response to the soft drink control pictures (assuming these are appetizing for participants)? In general, it is not clear from the data presented if the observed sex differences are specific to alcohol cue-triggered changes in striatum activation, or represent a more generalized difference in reward sensitivity between men and women (or between men and women with alcohol use disorder).

(8) One factor that has been shown to affect reward sensitivity (and drug use behavior) in women is menstrual cycle phase. Were these data collected and did they contribute to the observed effects?

(9) How recent was alcohol use in this sample? Were participants breathalyzed?

(10) Does alcohol intake differ if adjusted by body size or BMI? While men might drink higher amounts the anticipated impact of alcohol may not differ if adjusted by differences in size and body composition between men and women (Table 1).

(11) Overall the sample is very young and not very severe. Did I understand correctly that more severe alcohol users were excluded from the study? "Participants were excluded if they had a DUDIT score of 12 or higher" (p. 5). Please provide a justification for this exclusion criterion.

(12) In the analyses of drink type, it is not surprising that effects were only significant within the beer preferring subgroup as this was the largest subgroup. However, it is surprising that the effects were so distinctly different (opposite) for beer + wine preferring participants, as this group should presumably be sensitive to all picture types not only the beer and wine specific pictures. Overall, the preferred drink type effects feel too exploratory and the authors might consider instead showing the sex effects remain (or do not remain) when the analyses control for preferred drink type (condensed into three types: beer only, wine only, or more than one drink type).

(13) A deeper discussion is needed on the lack of any regions showing reactivity to alcohol relative to control cues in women. The authors focused on the ventral and dorsal striatum and the anterior cingulate cortex. But were there any brain regions, outside these ROIs, that showed

higher activity to alcohol relative to control cues in women (i.e., in whole-brain analyses)? As men and women did not differ in task-related subjective craving, what then underlied these subjective experiences in women?

(14) It is also interesting that sex effects and alcohol severity effects were co-localized in the ventral and dorsal striatum. How might this relate to what is known about the clinical course of alcohol addiction in men relative to women?

(15) While the ventral and dorsal striatum were investigated as separate ROIs, no distinction was made in the manuscript regarding their differential role in alcohol cue reactivity or anticipated sex-specific effects. The introduction and discussion could better justify the focus on these ROIs and the authors' specific hypotheses about them.

Reviewer: 2

Comments to the Author
This manuscript describes a study that examined neural cue-reactivity in male and female problem drinkers who were matched on age and alcohol use severity. The manuscript contributes to the literature by focusing on sex differences in alcohol cue reactivity, which has strong parallels to the existing cigarette smoking literature. Although informative, there are aspects of the manuscript that, if addressed, would yield a more useful, more readable article:

1. The abstract should include additional information. Specifically, please include sample size and the number of males/females. Also, what was the analytic approach? Statistical thresholds? Further, describing the subjects as "problem drinkers" is not helpful… please clarify.
2. The introduction should include a rationale for why the authors focused on the ventral/dorsal striatum and ventral/dorsal anterior cingulate cortex as regions of interest.
3. In the final paragraph of the introduction describes subjects as "severe drinkers". Again, please clarify.
4. Based on the participant description, subjects were treatment-seekers. As such, this should be noted in the introduction and discussion.
5. Several assessments were obtained, however, additional information on other substance use is needed. For example, how many subjects per group used cannabis? Used tobacco? How much and how often? These factors could influence neural responses. Most importantly, when was time of last use? Were any of the subjects in Nicotine withdrawal during scanning? Had any participant used cannabis in the 20-30 hours prior to scanning? These factors also influence neural responses. Please add to Table 1.
6. Along these lines, were there differences between those who were in the contemplation vs action stages? It is possible that those in the action stage could have altered neural responses due to trying to regulate craving, etc.
7. For the imaging analyses, what was the voxel-wise and cluster-wise threshold for each ROI? I'm assuming that the "mean activation" was based on a contrast? Please clarify.
8. The discussion would benefit from a discussion of the existing literature on sex differences in neural responses to cigarette cues. Please see (Wetherill et al., 2013, Biology of Sex Differences; Franklin et al., 2015 Nicotine Tobacco Research; Dumais et al., 2017 Drug and Alcohol Dependence).
9. The discussion should include a paragraph on limitations.

Authors' Response 18-Apr-2018

European Journal of Neuroscience, editorial office
April 2018

Anne Marije Kaag, PhD
University of Amsterdam
Department of developmental psychology
The Netherlands
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Dear Professor Bolam, Professor Foxe and Professor Goldstein,

Thank you for considering our manuscript: "Striatal cue-reactivity in male, but not in female problem drinkers" for publication in European Journal of Neuroscience. We like to thank the reviewers for their positive remarks about our study and their constructive comments. We have adapted our manuscript according to the suggestions of both reviewers and the editorial comments. We think the manuscript has improved substantially, and hope that we have addressed all suggestions for improvement and concerns in a satisfactory manner.

We hope that with these alterations our revised manuscript is ready for publication in European Journal of Neuroscience. Please find our point-by-point response to the reviewers below and our revised manuscript with all changes to the manuscript marked in yellow.

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On behalf of all authors,

Sincerely yours,
Anne Marije Kaag, PhD
Professor Anna E. Goudriaan
Professor Taco J. de Vries
Tommy Pattij, PhD
Professor Reinout Wiers

Point-by-point list of editorial and reviewers' comments and responses

Editorial comment

1. Comment: Ensure that the reporting of statistical data adheres to EJN guidelines, noTable report precise P values (e.g. in Table 1).
Reply: We have now reported all precise p-values in Table 1. To maintain readability, the precise statistics of the tests that are reported in Table 1, have been removed from the main manuscript so that they are reported in Table 1 only. While looking up the precise test statistics, we noted that two of the reported test statistics were incorrectly reported in the previous version of the manuscript (that is the correlation between AUDIT-scores and state and trait anxiety). We have added the correct p-values in Table 1 and adjusted the text in the manuscript accordingly. In addition, we have carefully checked all test-statistics reported in the manuscript and no other errors were found. Because the correlation between AUDIT-scores and state/trait anxiety were originally reported as being non-significant, while actually being significant, some of the exploratory analyses were redone, to correct for the potential confounding relation between AUDIT-scores and state and trait anxiety. In summary, when including state and trait anxiety in the analyses, none of the analyses changed regarding significances previously reported in the earlier version of the manuscript.

Revised text (results-clinical measures, page 12): Correlation analyses between alcohol use severity and the other clinical and demographic variables, demonstrated that alcohol use severity was positively correlated with age, alcohol intake, craving related to loss of control, craving related to negative reinforcement, depressive symptoms, impulsivity and state and trait anxiety (see Table 1 for exact test statistics).

Revised text (results: self-reported cue-induced craving, page 13): demonstrated that there was no significant time by sex by AUDIT interaction effect (F1,52¬=2.524, p=0.118, ηp2=0.047), therefore the sex by AUDIT-interaction term was removed from the model.

Revised text (results: self-reported cue-induced craving, page 13): There was no main effect of time or a significant time by sex-interaction effect on craving (F1,52=0.985, p=0.326, ηp2¬=0.019).

Revised text (results: self-reported cue-induced craving, page 13): demonstrated that the cue-reactivity task only

significantly increased craving in participants with an AUDIT-score ≥ 19 (F1, 26=42.63, p<0.001, ηp2=0.62) but not in participants with an AUDIT–score < 19 (F1,52=1.654, p=0.210, ηp2=0.062).

Revised text (results: self-reported cue-induced craving, page 13): The relation between alcohol use severity and cue-induced craving remained significant after including age, alcohol intake, craving related to loss of control and negative reinforcement, depressive symptoms, impulsivity and state/trait anxiety as covariates of non-interest in the model (F1,45=4.549, p=0.039, ηp2=0.096).

Revised text (results: self-reported cue-induced craving, page 13): In addition, we explored if there was a significant effect of "preferred drink type" on craving, but this was not the case (F1,51=0.360, p=0.782, , ηp2=0.021)

Revised text (results: ROI analyses on cue-reactivity, page 14): Repeated measures analyses on the ROIs, with sex as factor and AUDIT as covariate, demonstrated that there was no significant sex by AUDIT by cue-type interaction effect (DS: F1,51<0.001, p=0.996, ηp2<0.001; VS: F1,51=<0.597, p=0.443, ηp2=0.012; dACC: F1,51<0.001, p=1.00, ηp2<0.001; vACC: F1,52=0.311, p=0.580, ηp2=0.006).

Revised text (results: ROI analyses on cue-reactivity, page 14): There was no main effect of stimulus type in the dorsal or ventral anterior cingulate cortex (dACC: F1,52=1.197, p=0.279, ηp2=0.023; vACC: F1,52=1.270, p=0.265, ηp2=0.024)

Revised text (results: ROI analyses on cue-reactivity, page 14): This effect, however, did not remain significant after including alcohol intake, cannabis use severity, craving related to negative reinforcement, depressive symptoms and state and trait anxiety as covariates of non-interest in the model (F1,45=2.818, p=0.100, ηp2=0.059).

Revised text (results: ROI analyses on cue-reactivity, page 15): None of these variables were, however, significantly correlated to cue-reactivity in the ventral striatum (BDI: r=-0.138, p=0.318; CUDIT: r=0.040, p=0.776; alcohol intake: r=0.035, p0.804; DAQreinforcement: r=-0.113, p=0.417; STAI-State: r=0.018, p=0.896; STAI-trait:r=-0.097, p=0.486).

Revised text (results: ROI analyses on cue-reactivity, page 15): None of these variables were, however, significantly correlated to cue-reactivity in the DS (BDI: r=-0.99, p=0.477; CUDIT: r=0.128, p=0.355; alcohol intake: r=0.017, p=0.902; DAQreinforcement: r=0.018, p=0.896; STAI-state: r=0.065, p=0.642; STAI-trait:r=-0.025, p=0.855).

Revised text (results: ROI analyses on cue-reactivity, page 16): There was no significant stimulus type by sex interaction effect on cue-reactivity in the ventral anterior cingulate cortex (F1,45=0.604, p=0.441, ηp2=0.013) or dorsal anterior cingulate cortex (F1,45=0.128, p=0.722, ηp2=0.003).

Revised text (results: ROI analyses on cue-reactivity, page 16): Follow-up analyses based on the median split of the AUDIT-scores, demonstrated that the ventral striatum in participants with an AUDIT score < 19 responded stronger, but non-significantly, to control-pictures (F1,25=1.268, p=0.271, ηp2=0.048) whereas the ventral striatum in participants with an AUDIT-score ≥ 19 responded stronger, but non-significantly, to alcohol pictures compared to control pictures (F1,26=2.695, p=0.113, ηp2=0.094).

Revised text (results: ROI analyses on cue-reactivity, page 17): The interaction between AUDIT-scores and cue-induced activity in the ventral striatum remained significant after including age, alcohol intake, craving related to loss of control, craving related to negative reinforcement, depressive mood, impulsivity and state/trait anxiety as covariates of non-interest in the model (F1,43=9.803, p=0.003, ηp2=0.186).

Revised text (results: ROI analyses on cue-reactivity, page 17): Follow-up analyses based on the median split of the AUDIT-scores demonstrated that participants with an AUDIT-score < 19 responded stronger , but non-significantly, to control-pictures (F1,25=0.327, p=0.572, ηp2=0.013) whereas participants with an AUDIT-score ≥ 19 responded significantly stronger to alcohol pictures (F1,26=4.42, p=0.045, ηp2=0.15).

Revised text (results: ROI analyses on cue-reactivity, page 17): The interaction between AUDIT-scores and cue-induced activity in the dorsal striatum remained significant after including age, alcohol intake, craving related to loss of control and negative reinforcement, depressive mood, impulsivity and state/trait anxiety as covariates of non-interest in the model (F1,46=9.407, p=0.004, ηp2=0.180).

Revised text (results: ROI analyses on cue-reactivity, page 17): There was no significant stimulus type by AUDIT interaction effect on cue-reactivity in the ventral anterior cingulate cortex (F1,52=1.943, p=0.169, ηp2=0.036) or dorsal anterior cingulate cortex (F1,52=2.069, p=0.157, ηp2=0.038).

Revised text (results: ROI analyses on cue-reactivity, page 17): However, when correcting for age, alcohol intake, craving related to loss of control and negative reinforcement, depressive mood, impulsivity and state/trait anxiety, there was a

significant stimulus-type by AUDIT interaction effect in the ventral cingulate cortex (F1,45=5.089, p=0.029, ηp2=0.106) and dorsal anterior cingulate cortex (F1,45=6.513, p=0.014, ηp2=0.132).

Revised text (results: ROI analyses on cue-reactivity, page 16): These effects remained insignificant after including alcohol intake, cannabis use severity, craving related to negative reinforcement, depressive symptoms and state and trait anxiety as covariates of non-interest in the model (vACC: F1,45=0.604, p=0.441, ηp2=0.013; dACC: F1,45=0.128, p=0.722, ηp2=0.003)

Revised text (results: ROI analyses on cue-reactivity, page 18): A repeated measures analyses with stimulus type as repeated measure, sex as independent factor and cue-induced craving as covariate did not demonstrated a significant stimulus type by sex by craving interaction effect on neural cue-reactivity (VS: F1,51=0.067, p=0.796, ηp2=0.001; DS: F1,51=0.181, p=0.672, ηp2=0.004; vACC: F1,51=0.137, p=0.713, ηp2=0.003; dACC:F¬1,51=0.343, p=0.561, ηp2=0.007) or a significant stimulus type by craving interaction effect on neural cue-reactivity (VS: F1,52=1.312, p=0.257, ηp2=0.025; DS: F1,52=1.257, p=0.267, ηp2=0.024; vACC: F1,52=2.244, p=0.140, ηp2=0.041; dACC: F1,52=3.322, p=0.074, ηp2=0.060).

2. Comment: Author qualifications/titles should be removed from author list

Reply: these have now been removed from the author list

3. Comment: References are not quite in EJN style (too many authors named in in-text citations), the reference list needs checking for missing information and all authors of cited papers should be included in the reference list

Reply: We apologize for the use of an incorrect citation style. We have now adjusted all citations according to the EJN citation style.

4. Comment: Please carefully proof-read the manuscript, there are some typos and grammar errors that need fixing

Reply: We have carefully re-read the manuscript, and removed all remaining typos and grammar errors.

5. Comment: Compliance with DoH?

Reply: The study was indeed in compliance with the Declaration of Helsinki and this has now been added to the manuscript.

Revised text (methods-participants, page 5): . The study was carried out in accordance with the Declaration of Helsinki and approved by the Psychology ethics committee of the University of Amsterdam.

6. Comment: Table 1: the *s and the numbers in brackets need to be explained in the caption

Reply: An explanation has now been added in the last row of Table 1.

7. Comment: In accordance with EJN guidelines, replace the bar charts with more informative scatter plots, hybrid plots or similar.

Reply: We have now adjusted the Figures accordingly. The data is now presented by a scatterplot including an indication of the mean values.

8. Comment: We probably need larger Figures for publication.

Reply: We have now added a high resolution version of the Figures as separate files.

9. Comment: Fig 3 is not cited in the text (apart from the place holder)

Reply: We had indeed forgotten to refer to Figure 3 in the main text of the manuscript. As suggested by reviewer #1, we have removed the analyses related to Figure 3 (the relation between cue-reactivity and drink of preference) entirely from the manuscript and therefore Figure 3 is no longer included in the revised version of the manuscript.

10. Comment: Fig 2 should not be cited in a sub-heading

Reply: We have removed the reference to Figure 2 from the sub-heading and added it to the main text of that paragraph (“Stimulus type by alcohol use severity interaction on cue-reactivity in the VS, DS, vACC and dACC”).

11. Comment: The Figure legends do not fully describe the Figures. They should be completely understandable without reference to the text and include the statistical data.

Reply: Thank you for pointing this out, we have added some additional information to the description of the Figures, including statistical data.

Revised text (Figure 1): There was a significant sex by stimulus type interaction effect in the dorsal and ventral striatum, but not in the dorsal or ventral ACC. More specifically, reactivity in the ventral and dorsal striatum in response to alcohol stimuli versus soda stimuli was significant stronger in male compared to female problem drinkers. Values indicated with an asterisk (*) represent a significant sex by stimulus-type interaction effect at p<0.05

Revised text (Figure 2): There was a significant AUDIT (alcohol use severity) by stimulus type interaction effect in the ventral and dorsal striatum. More specifically, in individuals with the highest scores on alcohol use severity, the ventral and dorsal striatum responded stronger to alcohol stimuli compared to soda stimuli, whereas in individuals with the lowest scores on alcohol use severity, the ventral and dorsal striatum responded stronger to soda stimuli compared to alcohol stimuli. For visualization purposes the data is plotted based on the median split of the AUDIT-scores. Values indicated with an asterisk (*) represent a significant AUDIT by stimulus-type interaction effect at p<0.05

Comments reviewer #1

1. Comment: Very little information is provided about the content of the task stimuli although this is a major point of the study. Do these stimuli include social cues (e.g., people)? Equal number of beer and wine images? This information is also important for understanding the exploratory preferred drink type findings.

Reply: We thank the reviewer for this relevant suggestion. We have now added this information:

Revised text (material and methods – cue reactivity task, page 8): More specifically, the passive stimuli consisted of beer (n=15) and wine (n=15) pictures without social context whereas the active stimuli consisted of males or females drinking beer (n=15) or wine (n=15). Importantly, the stimuli with males drinking beer or wine were only shown to the male participants, whereas the stimuli with females drinking beer or wine were only shown to the female participants.

2. Comment: Understanding the stimuli better might also speak to the observed sex differences: Is it an issue of the magnitude of alcohol cue effects in neural reward systems or differences in the content or type of cues that would trigger these effects for men versus women? The latter could also perhaps be addressed by data on subjective experience of the cues (e.g., perceived pleasantness and arousal ratings). Were such data collected?

Reply: The stimuli used have previously been validated in 193 female and 86 male social drinkers on “urge to drink”, “valence”, “arousal” and “having control” (Pronk et al.,2015). The picture set has therefore not been validated in the current study. Pronk et al., (2015), however, demonstrated that alcohol pictures induced a stronger urge to drink in males compared to females, whereas females reported a stronger positive valence towards the nonalcoholic pictures, which could partly explain the reported sex-differences in neural cue reactivity. No sex differences were reported on arousal or control. We have now included these sex-differences in rating of the pictures, in the discussion.

Revised text (discussion, page 20): An alternative explanation could be that substance-related cues may not be optimal to induce cue-reactivity in females, and therefore could result in a paradigm that may not provide neurobiological markers for substance use severity or relapse in females. The stimuli used in the current study, have been validated previously in 193 female and 86 male social drinkers, with a mean AUDIT-score of 9.2 and 12.0, respectively (Pronk et al., 2015). In this study it was demonstrated that the alcohol pictures induced a stronger urge to drink in males compared to females, whereas females reported a stronger positive valence towards the nonalcoholic pictures, which could partly explain the reported sex-differences in neural cue reactivity.

3. Comment: Also related, please report on participant task engagement/accuracy on the target picture trials.

Reply: We agree with the reviewer that this information is relevant. We have therefore added the following paragraphs to the methods and results sections:

Revised text (methods – statistical analyses, page 10): To test if performance of the cue-reactivity task (the correct identification of target pictures) was affected by sex and AUDIT-scores, a 2-way ANOVA was performed with the number of correctly identified target trials as dependent variable, sex as independent factor and AUDIT-scores as covariate.

Revised text (results-performance on the cue-reactivity task, page 12): Performance on the cue-reactivity task. A 2-way ANOVA with the number of correctly identified target trials as dependent variable, sex as independent factor and AUDIT-scores as covariate was performed to test if the performance on the cue-reactivity task was affected by sex and alcohol use severity. Because there was no significant sex by AUDIT interaction effect ($F_{1,54}=0.083$, $p=0.775$, $\eta p^2 0.002$) this interaction term was removed from the model. This analyses did not reveal a significant main effect of sex ($F_{2,52}=1.147$, $p=0.289$, $\eta p^2=0.002$) or AUDIT-scores ($F_{2,52}=3.136$, $p=0.082$, $\eta p^2=0.057$) on correctly identified target trials.

4. Comment: Please report statistical information for significant effects as well as non-significant effects throughout the results section. It is not clear from the text alone how small the non-significant effects were (e.g., the interaction effects).

Reply: This relevant statistical information was indeed missing from the manuscript. We have now added all non-significant statistical values as well. Also see our reply to the first comment of the editor.

5. Comment: I was unable to find in the references the behavioral results paper cited as "Kaag et al., 2017" that reports on current sample. Please add this information.

Reply: Thank you for pointing out this omission, we have now added this reference to the reference list.

6. Comment: Figures 1 and 2 look identical. Figure 2 looks to be the incorrect one as the Figure caption refers to AUDIT effects but the legend refers to sex effects. Please provide the correct plots for both Figures.

Reply: Thank you for pointing out this mistake. We have indeed included the same Figure for Figure 1 and Figure 2. We have now added the correct Figure to the revised version of the manuscript.

7. Comment: Were there sex differences in response to the soft drink control pictures (assuming these are appetizing for participants)? In general, it is not clear from the data presented if the observed sex differences are specific to alcohol cue-triggered changes in striatum activation, or represent a more generalized difference in reward sensitivity between men and women (or between men and women with alcohol use disorder).

Reply: These important follow-up tests were indeed missing from the results section. We have now added these, demonstrating that despite of a significant sex by cue-type interaction effect, males and females did not differ in their response to the soft drink control, neither was there a significant effect of cue-type in either males or females.

Revised text (results: ROI analyses on cue-reactivity, page 14): Within group follow-up tests demonstrated that in both males and females there was no significant effect of cue-type (males: $F_{1,27}=2.853$, $p=0.103$, $\eta p2=0.096$; females: $F_{1,26}=1.195$, $p=0.284$, $\eta p2=0.044$), and there was no significant effect of sex on the response of the VS to either neutral or alcohol stimuli (neutral: $F_{1,55}=0.331$, $p=0.569$, $\eta p2=0.006$; alcohol: $F_{1,55}=1.020$, $p=0.317$, $\eta p2=0.019$).

Revised text (results: ROI analyses on cue-reactivity, page 15): Within group follow-up analyses demonstrated that there was a (trend) significant effect of cue-type in males ($F_{1,27}=4.088$, $p=0.053$, $\eta p2=0.132$), but not in females ($F_{1,26}=0.673$, $p=0.419$, $\eta p2=0.025$), neither was there a significant effect of sex in response to either neutral ($F_{1,53}=0.916$, $p=0.343$, $\eta p2=0.017$) or alcohol stimuli ($F_{1,53}=0.595$, $p=0.444$, $\eta p2=0.011$).

8. Comment: One factor that has been shown to affect reward sensitivity (and drug use behavior) in women is menstrual cycle phase. Were these data collected and did they contribute to the observed effects?

Reply: Unfortunately, we did not collect these data. We have added this to the discussion section as a limitation of our study.

Revised text (discussion, page 23): Another limitation of the current study is that we did not control for menstrual cycle of the included women, while there are important indications that the phase of the menstrual cycle affects the sensitivity to reward- and substance related stimuli (Dreher et al., 2007; Ossewaarde et al., 2010; Sakaki & Mather, 2013; Franklin et al., 2014; Banis & Lorist, 2017). Hence, future studies could either include women that are in the same phase of the menstrual cycle or should at least assess in which phase of the menstrual cycle the women are, in order to properly control for differences in menstrual cycle.

9. Comment: How recent was alcohol use in this sample? Were participants breathalyzed?

Reply: We want to thank the reviewer for noticing that this information was indeed missing from the original manuscript. As we have now added to the methods and results section, participants were all instructed not to consume alcohol in the 12 hours prior to the experiment, which was validated using a breathalyzer test (tests were all negative). Moreover, the time line follow-back procedure was used to assess days since last drink (2.18 days and 2.59 days in males and females, respectively) and possible sex differences in days since last drink (no significant difference, $F_{1,53}=0.768$, $p=0.385$).

Revised text (methods: demographic and clinical assessment, page 7): Participants were instructed not to consume alcohol in the 12 hours preceding the study, which was validated using a breathalyzer test (which was negative for all participants). Alcohol intake in the 14 days prior to the experiment was assessed using the Time Line Follow-Back procedure (Sobell & Sobell, 1992).

Revised text (results: clinical measures, page 11): Moreover, there were no differences between males and females in their drink of preference, motivation to change drinking or days since last drink (see Table 1 for precise test statistics).

10. Comment: Does alcohol intake differ if adjusted by body size or BMI? While men might drink higher amounts the anticipated impact of alcohol may not differ if adjusted by differences in size and body composition between men and women (Table 1).

Reply: We agree with the reviewer that it is indeed interesting to see whether alcohol intake also differs between males and females

when correcting for differences in body size or BMI. Unfortunately, we only have body weight of the participants to include in the analyses. Doing so, we see that alcohol intake between males and females does not differ after including weight as a covariate of non-interest ($F_{1,47}=2.469$, $p=0.123$). However, sex-related differences in the pharmacokinetics of alcohol are suggested to be more strongly related to differences in gastric metabolism instead of weight (e.g., Erol & Karpyak, 2015 in Alcoholism: Clinical and Experimental Research). Therefore, we do not see enough relevance of including weight-corrected sex-differences in alcohol intake in the revised version of the manuscript.

11. Comment: Overall the sample is very young and not very severe. Did I understand correctly that more severe alcohol users were excluded from the study? "Participants were excluded if they had a DUDIT score of 12 or higher" (p. 5). Please provide a justification for this exclusion criterion.

Reply: We agree with the reviewer that the overall sample size was relatively young. However, alcohol use severity was on average (an AUDIT-score of 19) very high and strongly indicative of an alcohol use disorder. The DUDIT, the drug use disorder identification test, was included to exclude participants with a likely substance use disorder other than alcohol. We have now further specified this in the methods section of the revised manuscript.

Revised text (methods: participants, page 6): Participants were excluded if they had a DUDIT score of 12 or higher (to exclude participants with a likely substance use disorder other than alcohol).

12. Comment: In the analyses of drink type, it is not surprising that effects were only significant within the beer preferring subgroup as this was the largest subgroup. However, it is surprising that the effects were so distinctly different (opposite) for beer + wine preferring participants, as this group should presumably be sensitive to all picture types not only the beer and wine specific pictures. Overall, the preferred drink type effects feel too exploratory and the authors might consider instead showing the sex effects remain (or do not remain) when the analyses control for preferred drink type (condensed into three types: beer only, wine only, or more than one drink type).

Reply: We agree with the reviewer that these analyses may be too exploratory to draw meaningful conclusions on the relation between cue-reactivity and "drink of preference". We have therefore adopted the useful suggestion by the reviewer and removed these analyses from the manuscript and instead reported that the significant sex by stimulus type interaction effects in the VS and DS remained after correcting for drink of preference.

Revised text (methods: statistical analyses, page 11): To explore if significant effects remained after controlling for "alcoholic drink of preference", the analyses were repeated with "alcoholic drink of preference" (beer only, wine only or more than one drink type) included as covariate of non-interest.

Revised text (results: ROI analyses on cue-reactivity, page 15): An exploratory analysis furthermore demonstrated that the significant sex by cue-reactivity interaction effect remained after including "drink of preference" as covariate of non-interest in the model ($F_{1,51}=4.963$, $p=0.030$, $\eta p2=0.089$). With other words, sex differences in neural cue-reactivity in the VS were not explained by differences in alcoholic drink of preference.

Revised text(results: ROI analyses on cue-reactivity, page 16): An exploratory analysis furthermore demonstrated that the significant sex by cue-reactivity interaction effect remained after including "drink of preference" as covariate of non-interest in the model ($F_{1,51}=5.005$, $p=0.030$, $\eta p2=0.089$). With other words, sex differences in neural cue-reactivity in the DS were not explained by differences in alcoholic drink of preference.

Revised text (results: ROI analyses on cue-reactivity, page 16): Moreover, these effects remained insignificant after including "drink of preference" as covariate of non-interest in the model (vACC: $F_{1,51}=1.353$, $p=0.250$, $\eta p2=0.026$; dACC: $F_{1,51}=0.326$, $p=0.570$, $\eta p2=0.006$).

13. Comment: A deeper discussion is needed on the lack of any regions showing reactivity to alcohol relative to control cues in women. The authors focused on the ventral and dorsal striatum and the anterior cingulate cortex. But were there any brain regions, outside these ROIs, that showed higher activity to alcohol relative to control cues in women (i.e., in whole-brain analyses)? As men and women did not differ in task-related subjective craving, what then underlied these subjective experiences in women?

Reply: We agree with the reviewer that it is surprising that we did not find any regions that showed reactivity to alcohol cues relative to control cues in women. As we have, however, already argued in the discussion, we believe that this is due to the fact that cue-reactivity in women is likely to be more pronounced when using alcohol cues with a negative emotional valence (page 20: "If cue-reactivity in females is, indeed, more related to negative emotional states, it could be expected that sex differences in anterior cingulate activation are (more) pronounced when using alcohol pictures with a negative emotional valence."). We do, however, agree with the reviewer that a whole-brain analysis could provide additional information on the neural pathways that underlie subjective experiences in women. The whole brain analysis, however, did not reveal any significant effects outside the predefined ROIs and hence is not very informative on this matter. We believe that the self-reported increases in craving may be the result of a reporting bias which could explain why we did not find any significant correlation between self-reported craving and cue-reactivity. As suggested by the reviewer we have now added the null-results of the whole brain analyses to the revised version of the manuscript and we have provided a more thorough

discussion on the lack of any regions showing cue-reactivity in women.

Revised text (methods: statistical analyses, page 11): To explore if there were any significant effects outside the predefined ROIs, a whole brain analysis was performed, with sex as independent factor and AUDIT-scores included as regressor. These second level analyses were family-wise error (FWE) rate corrected on cluster level ($p<0.05$), with an initial height threshold on voxel level of $p<0.001$.

Revised text (results: whole brain analysis on cue-reactivity, page 18): The whole brain analysis did not show a significant sex by stimulus type or AUDIT by stimulus type interaction effect outside the predefined regions of interest.

Revised text (discussion, page 22): Alternatively, the small increase in self-reported craving that we did demonstrate could have been the result from a report bias (for example: the participants could report an increase in craving because they assume this is expected from them). This could explain why we did not find any significant relation between self-reported craving and neural cue-reactivity, in addition to the finding that we did not demonstrate any neural cue-reactivity in females, despite the fact that they did report a significant increase in self-reported craving.

14. Comment: It is also interesting that sex effects and alcohol severity effects were co-localized in the ventral and dorsal striatum. How might this relate to what is known about the clinical course of alcohol addiction in men relative to women?

Reply: Based on the current study we feel that it is too speculative to expand on the implications of these findings on the clinical course of AUD in men relative to women. The reason for this is that we expect that stronger striatal cue-reactivity in men relative to women, is mainly related to the type of task employed (reward related alcohol cues instead of stress-related alcohol cues). As we have already emphasized in the discussion (page 20) future cue-reactivity studies should include both men and women, as well as both reward and stress-related stimuli, in order to draw a clearer picture on how sex-differences in neural cue-reactivity are related to the course of addictive disorders. (See discussion, page 20:"This not only emphasizes the importance of including both men and women in cue-reactivity studies (Goel et al., 2014), but also underlines that different cues should be used to induce cue-reactivity (e.g. stressors and imagery in addition to substance-related pictures), as including these will result in a clearer picture on what constitutes cue-reactivity and how this multi-faceted construct is related, for instance, to the course of addictive disorders.")

15. Comment: While the ventral and dorsal striatum were investigated as separate ROIs, no distinction was made in the manuscript regarding their differential role in alcohol cue reactivity or anticipated sex-specific effects. The introduction and discussion could better justify the focus on these ROIs and the authors' specific hypotheses about them.

Reply: The reviewer is right that a rationale for this distinction was not made in the original version of the manuscript. We have now added this rationale to the introduction of the revised version of the manuscript:

Revised text (introduction, page 5): Although sex-differences have been reported in both the ventral and dorsal parts of the striatum and ACC during alcohol, nicotine and cocaine cue-reactivity, the current study focused on these regions separately, because these regions are suggested to be related to specific processes in addiction. That is, the VS is thought to be more related to hedonic and initial alcohol use, whereas the DS is more related to habitual and compulsive alcohol use (Vollstädt-Klein et al., 2010; Everitt, 2014). Moreover, the vACC is believed to be specifically involved in relapse and craving (Goldstein, Alia-Klein, et al., 2009; Li et al., 2013; Seo et al., 2013; Courtney et al., 2015), whereas the dACC is suggested to be specifically related to control (Brody et al., 2007; Goldstein, Alia-klein, et al., 2009; Courtney et al., 2015).

Comments reviewer #2

1. Comment: The abstract should include additional information. Specifically, please include sample size and the number of males/females. Also, what was the analytic approach? Statistical thresholds? Further, describing the subjects as "problem drinkers" is not helpful… please clarify.

Reply: We agree with the reviewer that some relevant information was missing from the abstract. We have now included this in the revised version of the abstract.

Revised text (abstract): In this study we therefore investigated neural cue-reactivity in a sample of male (n=28) and female (n=27) problem drinkers (matched on age and alcohol use severity) with an average alcohol use disorder identification test score of 12 which is indicative of a likely alcohol use disorder. Neural cue-reactivity data were extracted from four regions of interest: the ventral and dorsal striatum and the ventral and dorsal anterior cingulate cortex with a significance level set at $p<0.05$.

2. Comment: The introduction should include a rationale for why the authors focused on the ventral/dorsal striatum and ventral/dorsal anterior cingulate cortex as regions of interest.

Reply: A rationale for making a distinction between the ventral and dorsal parts of the striatum and ACC was indeed missing. We have now further elaborated on this in the introduction. Also see our reply to comment 15 by reviewer #1.

Although sex-differences have been reported in both the ventral and dorsal parts of the striatum and ACC during alcohol, nicotine and cocaine cue-reactivity, the current study focused on these regions separately, because these regions are suggested to be related to specific processes in addiction. That is, the VS is thought to be more related to hedonic and initial alcohol use, whereas the DS is more related to habitual and compulsive alcohol use (Vollstädt-Klein et al., 2010; Everitt, 2014) . Moreover, the vACC is believed to be specifically involved in relapse and craving (Goldstein, Alia-Klein, et al., 2009; Li et al., 2013; Seo et al., 2013; Courtney et al., 2015) whereas the dACC is suggested to be specifically related to control (Brody et al., 2007; Goldstein, Alia-klein, et al., 2009; Courtney et al., 2015).

3. Comment: In the final paragraph of the introduction describes subjects as “severe drinkers”. Again, please clarify.

Reply: We have now replaced the term “severe” drinker with “problem” drinker throughout the revised version of the manuscript and we have explained the term problem drinker in the last paragraph of the introduction (as well as in the abstract):

Revised text (introduction, page 4): Therefore, the aim of this study is to further explore sex differences in visual alcohol cue-reactivity in a sample of male and female problem drinkers, with a minimum alcohol use disorder identification test (AUDIT) score of 12, which is indicative of a likely AUD.

4. Comment: Based on the participant description, subjects were treatment-seekers. As such, this should be noted in the introduction and discussion.

Reply: While the participants were motivated to change their drinking behavior, they were recruited through (online) advertising and not through official treatment institutions. Hence, we do not feel that the term ‘treatment seeking” is suited for the current population. We have tried to clarify this in the revised version of the manuscript

Revised text (methods: participants, page 6): 55 problem drinkers (27 females) were included in the study. Participants were recruited through internet and poster advertisements in the local community of Amsterdam and the Psychology faculty of the University of Amsterdam, asking for individuals who wanted to reduce their alcohol intake.

5. Comment: Several assessments were obtained, however, additional information on other substance use is needed. For example, how many subjects per group used cannabis? Used tobacco? How much and how often? These factors could influence neural responses. Most importantly, when was time of last use? Were any of the subjects in Nicotine withdrawal during scanning? Had any participant used cannabis in the 20-30 hours prior to scanning? These factors also influence neural responses. Please add to Table 1.

Reply: We agree with the reviewer that this is very relevant information, unfortunately we only have data on how often in the last 12 months participants used drugs, but we do not have information on time of last use. We did ask participants to abstain from any drug (other than tobacco) in the 12 hours preceding the experiment, but we did not include an objective measure to validate abstinence from substance other than alcohol. Because participants were not instructed to abstain from tobacco prior to the experiment, we do not expect them to have experienced any nicotine withdrawal during scanning. We have now clarified this in the revised version of the manuscript and added all information that we do have on substance use.

Revised text (methods: demographic and clinical assessment, page 7): Participants were also instructed to abstain from any drug (other than nicotine) in the 12 hours preceding the study, but we did not include an objective measure to verify this.

Revised text (methods: demographic and clinical assessment, page 7): Drug use (GHB, LSD, methamphetamine, heroin, ketamine, cannabis, cocaine, amphetamine, opiates) in the past 12 months was assessed using an ordinal scale with the categories: never, 1-2 times, 3-5 times, 6-10 times, 11-20 times, 21-40 times or more than 40 times.

Revised text (methods: statistical analyses, page 10): Chi square tests were used to assess sex-differences in substance use. Table 1 shows the number of participants that indicated to have used tobacco, cannabis and drugs other than cannabis at least once in the 12 months preceding the study.

6. Comment: Along these lines, were there differences between those who were in the contemplation vs action stages? It is possible that those in the action stage could have altered neural responses due to trying to regulate craving, etc.

Reply: We agree with the reviewer that this is indeed an interesting question, in general. Exploratory analyses, however, did not reveal any significant effect of stage on neural cue reactivity in the ROIs (VS: $F_{1,52}=0.744$, $p=0.392$, DS: $F_{1,52}=0.197$,$p=0.659$, vACC: $F_{1,52}=3.348$, $p=0.073$, dACC: $F_{1,52}=0.574$, $p=0.452$). Because there were no differences between males and females in their self-reported readiness to change drinking behavior, we do not feel that adding these analyses to the result section is relevant for the understanding of the demonstrated sex by stimulus type interaction effects on cue-reactivity. We have therefore chosen not to include this analysis in the revised version of the manuscript.

7. Comment: For the imaging analyses, what was the voxel-wise and cluster-wise threshold for each ROI? I'm assuming that the "mean activation" was based on a contrast? Please clarify.

Reply: As we have described in the methods section of the manuscript, Marsbar was used to extract the mean activation during alcohol and control pictures. Because it was not clear that this referred to first level contrasts, we have rewritten this sentence. In addition, because the raw beta estimates were extracted, no voxel-wise or cluster-wise thresholds are needed. As suggested by reviewer #1 (comment 13) we have now also included a whole brain analyses, that was family-wise error (FWE) rate corrected on cluster level ($p<0.05$), with an initial height threshold on voxel level of $p<0.001$. We hope that we have now clarified these issues sufficiently.

Revised text (methods: Functional magnetic resonance imaging data acquisition and analyses, page 9): For the region of interest (ROI) analyses, the first level contrasts for alcohol pictures and control pictures were entered in a second level full factorial design with stimulus type as factor. Subsequently, the Marsbar toolbox (http:// marsbar.sourceforge.net) was used to extract the mean activity for the alcohol contrast and control contrast, for each ROI.

8. Comment: The discussion would benefit from a discussion of the existing literature on sex differences in neural responses to cigarette cues. Please see (Wetherill et al., 2013, Biology of Sex Differences; Franklin et al., 2015 Nicotine Tobacco Research; Dumais et al., 2017 Drug and Alcohol Dependence).

Reply: There is indeed relevant research on sex differences on neural cue-reactivity to substance cues other than alcohol. Because these studies are, however, largely inconsistent, we decided not to include these in the original version of the manuscript. We have, however, now included a reference to the study by Franklin in our discussion on the influence of menstruation cycle on cue-reactivity (see our reply to comment 8 by reviewer #1) as well as references to several other studies demonstrating sex differences in ACC cue-reactivity in substance use disorders, other than AUD (including tobacco use disorder studies) in both the introduction as well as the discussion.

Revised text (introduction, page 4): Moreover, while sex differences in anterior cingulate cortex (ACC) cue-reactivity have not been reported in alcohol drinkers, cue-reactivity studies in other substance use disorders have reported less ACC cue-reactivity in female, compared to male, cocaine users (Volkow et al., 2011) and smokers (Dumais et al., 2017). However, also greater ACC cue reactivity has been found in female, compared to male, cocaine users (Kilts et al., 2004) and smokers (Zanchi et al., 2016), whereas some studies did not find any sex differences in ACC cue reactivity (Wetherill et al., 2013).

Revised text (discussion, page 19): Moreover, the current findings are also in line with several neural cue-reactivity studies in nicotine dependence (Cosgrove et al., 2014; Dumais et al., 2017)

9. Comment: The discussion should include a paragraph on limitations.

Reply: We agree with the reviewer that limitations should always be included. While we have already included suggestions for future research, based on the limitations of the current study, we have now more specifically stated what the limitations of the study are.

Revised text (discussion, page 22): To our knowledge this is one of the first studies that has extensively studied sex differences in neural-cue reactivity in problem drinkers. Although we included a non-clinical population of problem drinkers, the average AUDIT-score in this population (19, ranging from 12 to 29) is strongly indicative of an AUD (Johnson et al., 2013). Nonetheless, the current results warrant replication in a clinical population. Another limitation is that, while males and females in the current study did not significantly differ in their preference of alcohol-containing beverages, we did not match males and females on their preference. Despite the fact that drink of preference did not significantly affect the sex by cue-type interaction effect in the VS and DS, future cue-reactivity studies should match males and females on drink of preference. Another limitation of the current study is that we did not control for menstrual cycle of the included women, while there are important indications that the phase of the menstrual cycle affects the sensitivity to reward- and substance related stimuli (Dreher et al., 2007; Ossewaarde et al., 2010; Sakaki & Mather, 2013; Franklin et al., 2014; Banis & Lorist, 2017). Hence, future studies should either include women that are in the same phase of the menstrual cycle or at least assess in which phase of the menstrual cycle the women are, in order to properly control for differences in menstrual cycle. Lastly, future studies addressing sex differences in alcohol cue reactivity should employ alcohol cues that are relevant for both males and females, for instance by using cues that contain an emotional context.

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2nd Editorial Decision 21-May-2018

Dear Dr. Kaag,

Your revised manuscript was re-evaluated by external reviewers as well as by the Section Editor, Dr. Rita Goldstein and ourselves. We are pleased to inform you that that it will be accepted for publication in EJN pending a few minor revisions indicated by the Reviewer. In addition to these points please supply a graphical abstract.

When finalised, please upload your complete revised manuscript onto the website as a Word (.doc or .docx), or .rtf file. Please also ensure that a complete set of tables and figures is included as separate files, even if these have not changed from the originals. At this stage it is necessary to provide high resolution figures. Please see important instructions below.

Please go into https://mc.manuscriptcentral.com/ejn - Author Centre - manuscripts with decisions where you will find a 'create a revision' link under 'actions'. We ask that you please indicate the way in which you have responded to the points raised by the Editors and Reviewers in a letter. Please upload this response letter as a separate Word (.doc) file using the file designation "Authors' Response to Reviewers" when uploading your manuscript files. Please DO NOT submit your revised manuscript as a new one. Also, please note that only the Author who submitted the original version of the manuscript should submit a revised version.

If you are able to respond fully to the points raised, we shall be pleased to receive a revision of your paper within 30 days.

Thank you for your support of this Special Issue of EJN.

Best wishes,

Paul Bolam & John Foxe
co-Editors in Chief, EJN

Reviews:

Reviewer: 2

Comments to the Author
The authors responded adequately to my concerns.

Reviewer: 1

Comments to the Author
I thank the authors for their detailed replies to the reviewer comments, and agree that the manuscript is improved.

I have only a couple of last points below:

• I would suggest modifying the title of the manuscript slightly, for example to “Striatal cue-reactivity is stronger in male than female problem drinkers” as the stimulus main effect in males was nonsignificant (VS) or marginal (DS).

• Related, please modify statement that visual drug cue paradigms only work in males. This statement is not fully supported by the data. Concluding instead that they might work better in males for interrogating addiction mechanisms may be more accurate.

• p. 14 The loss of the interaction effect in the VS when accounting for covariates, despite these covariates not being correlated with cue-reactivity in the VS: I think a sentence is needed that speaks to how the reader should think about these set of result. This likely means that the effect did not remain significant (dropped to P=0.1) because of a loss of degrees of freedom, rather than these variables accounting for significant variance.

• I am also not sure what to make of the nonsignificant AUDIT by stimulus type interactions in the ventral and dorsal ACC that become significant after accounting for several covariates. There might be a collinearity issue with these analyses. I would suggest performing the control for covariates only when the main, hypothesized effect of interest is significant.

• A few of the new text sections in the results should be edited for clarity, e.g., p. 15 “With other words, sex differences in neural cue-reactivity in the VS was not explained by differences in alcoholic drink of preference.”

• Please replace “insignificant” with “nonsignificant” (Results section on the ventral and dorsal ACC).

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Authors' Response 23-May-2018

Authors' Responses to Reviewer #1

1. Comment: I would suggest modifying the title of the manuscript slightly, for example to "Striatal cue-reactivity is stronger in male than female problem drinkers" as the stimulus main effect in males was nonsignificant (VS) or marginal (DS).

Reply: We have now adjusted the title accordingly

Revised title: Striatal alcohol cue-reactivity is stronger in male than female problem drinkers

2. Related, please modify statement that visual drug cue paradigms only work in males. This statement is not fully supported by the data. Concluding instead that they might work better in males for interrogating addiction mechanisms may be more accurate.

Reply: We agree with the reviewer that this conclusion was not fully appropriate. We have adjusted this in the abstract and the rest of the manuscript accordingly.

Revised text (abstract): While the cue-reactivity paradigm induced similar levels of self-reported craving in males and females, visual alcohol-cues induced significantly stronger striatal activation in male compared to drinkers.

3. Comment: p. 14 The loss of the interaction effect in the VS when accounting for covariates, despite these covariates not being correlated with cue-reactivity in the VS: I think a sentence is needed that speaks to how the reader should think about these set of result. This likely means that the effect did not remain significant (dropped to P=0.1) because of a loss of degrees of freedom, rather than these variables accounting for significant variance.

Reply: We have now added this explanation to the results section of the manuscript

Revised text (results, page 14): None of these variables were, however, significantly correlated to cue-reactivity in the VS (BDI: r=-0.138, p=0.318; CUDIT: r=0.040, p=0.776; alcohol intake: r=0.035, p0.804; DAQreinforcement: r=-0.113, p=0.417; STAI-State: r=0.018, p=0.896; STAI-trait:r=-0.097, p=0.486), suggesting that the changes in significance level are likely to be related to a loss of degrees of freedom, instead of these variables accounting for significant variance.

4. Comment: I am also not sure what to make of the nonsignificant AUDIT by stimulus type interactions in the ventral and dorsal ACC that become significant after accounting for several covariates. There might be a collinearity issue with these analyses. I would suggest performing the control for covariates only when the main, hypothesized effect of interest is significant.

Reply: We have now removed these exploratory analyses if the effect of interest (sex by stimulus type interaction effect and audit by stimulus type interaction effect) was not significant.

5. Comment: A few of the new text sections in the results should be edited for clarity, e.g., p. 15 "With other words, sex differences in neural cue-reactivity in the VS was not explained by differences in alcoholic drink of preference."

Reply: We have carefully read all the new text sections, and adjusted the text to improve clarity of needed.

Revised text (page 10): To test if performance on the cue-reactivity task (the correct identification of target pictures) was affected by sex and AUDIT-scores

Revised text (page 12): This analyses did not reveal a significant main effect of sex ($F_{2,52}=1.147$, $p=0.289$, $\eta p2=0.002$) or AUDIT-scores ($F_{2,52}=3.136$, $p=0.082$, $\eta p2=0.057$) on the number of correctly identified target trials.

Revised text (page 14): Within group follow-up tests demonstrated that in both males and females there was no significant effect of cue-type (males: $F_{1,27}=2.853$, $p=0.103$, $\eta p2=0.096$; females: $F_{1,26}=1.195$, $p=0.284$, $\eta p2=0.044$) and no significant effect of sex on the response of the VS to either neutral or alcohol stimuli

Revised text (page 15): With other words, sex differences in neural cue-reactivity in the VS were not explained by sex differences in alcoholic drink of preference

Revised text (page 15): With other words, sex differences in neural cue-reactivity in the DS were not explained by sex differences in alcoholic drink of preference

6. Comment: Please replace "insignificant" with "nonsignificant" (Results section on the ventral and dorsal ACC).

Reply: The word insignificant is no longer to be found in the revised version of the manuscript.

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