Response to Reviewer 2

In this paper the authors report results of a study in which three compounds that differentially effect monoamine and opioid systems interact when co-administered in two behavioral tests that have been thought to model depression as well as for molecular modeling to evaluate whether concurrent administration might alter binding sites of these compounds. The authors find a surprising and profound differences between one compound in the two behavioral tests but do find that co-administration of all three compounds does anti-depressant like effects comparable but not greater than a well-accepted control compound. The protocols and analysis are of good quality.

We thank the reviewer for the careful evaluation of our manuscript and for highlighting the importance of the topic. Your observations were highly valuable and helped us substantially improve the clarity, methodological rigor, and interpretability of our work. All changes have been clearly highlighted in the revised manuscript, in red. Our point-by-point responses are provided below in blue.

Comment 1. *A major limitation, for this reviewer, was the extent to which current results would help direct clinically relevant future studies.*

Response: Thank you for the comment. We have added a paragraph that states what these data do and do not support for clinical direction, and it is deliberately modest. Two consequences are drawn. First, the pairing that carries most of the observed effect is kappa-opioid blockade combined with an SNRI rather than the addition of a mu-opioid agonist, so the next preclinical step is a complete factorial design in a single cohort and not a further dose escalation of codeine. Second, because the effect was obtained in acute assays in naive animals, the clinical question these data inform is narrow, namely whether kappa antagonism added to an SNRI improves the stress-coping and reward-related domains that such assays engage. We also name the regimen that would be of translational interest, an SNRI combined with a kappa antagonist usable in humans such as aticaprant, and the conditions under which it would have to be tested.

Change and location: Section 3.5, new paragraph.

Comment 2. *The authors, to their credit, appropriate cite numerous limitations of many aspects of the current study but based on their own comments, it is unclear whether, for example, extending a similar approach to more currently acceptable behavioral models of depression as well as to both male and female mice of different strains would provide additional insight.*

Response: Thank you for the comment and for the suggestion. Section 3.7 has been rewritten so that each proposed experiment is tied to the question it would answer. The complete factorial design in a single cohort would establish whether codeine adds anything to kappa blockade combined with an SNRI and would remove the cross-cohort comparisons that limit the present study; isobolographic or response-surface designs would determine whether the interaction is supra-additive; concurrent locomotor controls and separate scoring of active behaviours would separate motor from affective components; chronic stress models with anhedonia and effort-based outcomes would test whether the effect extends to the domains that matter clinically; and both sexes and more than one strain would test generalisability. We say explicitly that these steps are necessary before the strategy can be considered translationally relevant.

Change and location: Section 3.7, rewritten; Section 3.5, new paragraph.

Comment 3. *The dramatic and unexpected difference between tail suspension and forced swim were not accompanied, in this reviewer's opinion, by a reasonable, testable hypothesis.*

Response: Thank you for the insightful comment. We have added one. The new passage proposes that the forced swim test imposes a thermoregulatory and energetic load that the tail suspension test does not, and that immobility is scored over a shorter window after a stabilisation period, so that a serotonergic-preferring drug may redistribute active behaviours without reducing total immobility. Three predictions follow and are stated as such: that venlafaxine increases active behaviours in the forced swim test without changing total immobility when swimming and climbing are scored separately; that reducing the thermoregulatory load by raising water temperature increases the sensitivity of the forced swim test to venlafaxine; and that the dissociation persists

when a concurrent locomotor control is included. We also state that until these experiments are done, the conclusion is restricted to the sensitivity observed under the conditions of this study.
Change and location: Section 3.1, final sentences of the last paragraph.

Comment 4. *The interactions between the three systems targeted by the authors are of interest but the best response only mimiced the behavioral effects of one well-accepted control compound.*
Response: Thank you for the comment. The revised manuscript no longer leaves it implicit. The triple combination did not exceed amitriptyline; it reached an effect of similar magnitude, with no significant difference detected, and the text now says so in those words wherever the comparison appears. We do not claim superiority over an established antidepressant. The interest of the regimen, as we now state in Section 3.5, lies in the mechanism rather than in the size of the acute effect: a combination that matches a tricyclic in an acute assay is worth pursuing only if it acts through a route that remains available when monoaminergic treatment fails, and that is a question these acute assays in naive animals cannot answer. Chronic stress models are needed, and are proposed.
Change and location: Abstract; Section 2.6; Section 3, opening paragraph; Section 3.5; Section 5, third paragraph.

Comment 5. *The significance of the current study and future studies based on these data needs to be better developed.*
Response: Thank you for the comment. Addressed by the two additions described above, taken together: the new paragraph in Section 3.5 on what the data do and do not support clinically, and the rewritten Section 3.7, in which each proposed experiment is linked to the specific question it would resolve. The Conclusions were also rewritten so that the claims they make match the evidence, and they end by stating what the structural data can and cannot establish.
Change and location: Sections 3.5, 3.7 and 5.