nature portfolio

Peer Review File

A dual-pathway architecture for stress to disrupt agency and promote habit

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)
In this set of studies, Giovanniello and colleagues ask important and timely questions about the impact of prior stress on the amygdala-striatal mechanisms governing instrumental learning. Previous work has found that a period of stress promotes instrumental behavior that is "habitual," so called because the behavior does not seem to be governed by the current value of the outcome and is thus less flexible. This set of studies seeks to understand the underlying neural mechanisms involved and provides exciting data about one piece of the puzzle. This work builds upon the history of this lab in conducting innovative neuro-behavioral studies. The authors find that prior stress changes the reward response of amygdala neurons that project to the dorsomedial striatum (DMS) as measured during instrumental learning. Using the direction of change induced in each pathway by stress, they design chemogenetic and optogenetic experiments to counter these changes. Thus, they report that excitation of BLA-DMS neurons during learning "rescues" the stress-induced habitual behavior, as does inhibition of CeA-DMS neurons. They also can mimic the impact of stress by inhibiting BLA-DMS neurons during learning. Of note, the manipulations of pathway activity are made during instrumental learning, while the test for goal-directed vs habitual behavior is made later, when the pathway is not being manipulated. Taken together, these findings provide support for differential roles for the BLA vs CeA inputs to striatum in goal-directed vs habitual behavior, a finding that had some limited prior published support, and extend prior published work by recording activity during instrumental learning to understand how stress alters the impact of these two pathways. The calcium imaging data are especially exciting as the response to reward is so distinct in stressed vs unstressed mice. Overall, these findings represent an important step forward due to the elucidation of the specific alteration produced by prior stress that impacts the nature of what is learned. The applicability of these findings to many disorders impacted by stress, including PTSD, addiction, etc., is strong and these findings will be of broad interest. I am generally enthusiastic about this work but there are some shortcomings in the current instantiation, mostly revolving around the experimental design and statistical approaches for the pathway manipulation experiments, as detailed below. Importantly, mice are trained under two different regimes that varies from experiment to experiment, and implications of this are not presented well enough for most readers to follow. In addition, some of the claims lack strong statistical support.

1. The authors provide good evidence in Fig 1 that their 2-week stress treatment results in habitual behavior as compared to handled, unstressed controls. Importantly initial learning is similar in the two groups. As an aside, it is interesting to note that the average response rate between valued and devalued conditions are not different, thus indicating habit, although it is clear there is wide individual variation with most subjects showing either a large decrease in responding in the devalued condition or a large increase in responding in the devalued condition, in contrast to the control group in which there is really only 1 mouse showing an increase in the devalued condition. I bring this up to note that clearly normal goal-directed behavior is altered in many, but not all, of the mice, and the alteration is not a simple equal treatment of the lever under both conditions. This is not something I feel the authors need explore experimentally in this paper – it is out of scope – nor is this a point that needs addressing in review. This variability has been observed in many studies. But it does relate, in that the behavior is inherently "noisy" which makes multi-group, multi-condition statistical analysis difficult (see points 4 and 5 below).
2. Figure 2 depicts the exciting photometry data showing that, during learning, the response to reward delivery is different in controls vs stressed mice, in both the BLA-DMS pathway and the CeA-DMS pathway. One question I had was how long after reward receipt did it take for the signal in the CeA stressed group to return to baseline (session 4). I am curious if it stays up as long the mouse is consuming the pellet or if it is a longer-lasting change. The differences in the CeA signal in this group across the 4 training sessions are dramatic. It is also interesting that there is a big response to unpredicted reward

(supplement) in the CeA stressed group, but not to reward delivery in session 1. Can the authors comment on this?
3. The data depicted in Figs 1 and 2 were collected under the random ratio version of this task. The authors however chose to train mice under a random interval schedule for the the remainder of the experiments except two. The notion in the field is that the cognitive processes engaged during learning under the two regimes are at least somewhat distinct, including neurally. RI training tends to produce habitual behavior and RR training tends to produce goal-directed behavior. The authors' rationale for varying training schedules is that habitual behavior is more typical after training on RI than RR in many mouse studies, and in some of their manipulations they wish to bias against detecting goal-directed behavior. Yet the RI bias for habit is not apparent in the behavior of control mice in these studies, as they mostly remain sensitive to devaluation. More importantly, because the rationale for the manipulation experiments derives from the photometry experiments, which were conducted using the RR procedure, the tight link between the findings is missing. Ideally, the authors would run a second photometry experiment using the RI training procedure to show (or not) that the deficits in the reward signal in both pathways are also evident in the training procedure used for the majority of the remaining studies. This would be the most clean option. The other option is to provide much stronger rationale than they currently do, and to be very clear for the reader who is somewhat out of the field. I understand why the authors might have been reluctant to discuss these points in a paper aimed at broad readership, but I think the way it is laid out now is too superficial.
4. The authors use intersectional genetics to conduct pathway-specific chemogenetic manipulations. This is an excellent approach, but for these studies, it requires four groups of mice per experiment as there are two between-subject factors: Stress and Virus. The three-group ANOVA conducted in the main figures conflates these different factors. In addition, the authors show the data from a given experiment in two parts, leaving presentation of the comparison between the unstressed control virus group and the unstressed active virus group to the supplement. The issue is that these experiments by traditional approach should be analyzed in one omnibus ANOVA, not the ANOVAs currently used on the partial data. I do sympathize with the authors in that the individual variability, and in some cases very low sample sizes, provide obstacles to multi-group, multi-factor ANOVAs with both within- and between-subject factors, as is the case here. A perfectly sound approach is to conduct focused ANOVAs with between-subject factors to address the specific hypothesis. For example, for Figure 3 F, chemogenetic excitation of the BLA-DMS pathway, an ANOVA comparing mCherry Controls in both stressed and unstressed can confirm the baseline effect of stress in present; a second ANOVA between stressed controls and stressed hM3Dq can test the hypothesis that hM3Dq can rescue the stress-induced habit, etc. As presented now, the reader is given the impression that the supplemental data come from separate experiments although one can see the control group is the same.
5. A second issue with the statistical analyses even as currently conducted is that there is often not a significant interaction, yet the authors still conduct statistical comparisons across valued vs devalued conditions or across devaluation index groups. For example, there is not a significant group x value interaction in Fig 3f. And, even when the stats comparing across valued and devalued conditions don't seem to offer support, the authors conclude in some cases that the manipulation has had the desired impact. For example, for the CeA-DMS experiment in Fig 4d (hM4Di) the authors write that the manipulation returned the behavior to goal-directed in the stressed group, yet I do not see any statistical support for that. It is possible that reanalyzing the data using focused ANOVAs may provide a clearer route forward for the authors. It is also possible that the studies will require larger group sizes given the inherently variable nature of the behavior. I feel for the authors – I know these studies are not easy – but there may not be an alternative for moving forward with this interesting set of studies.

Minor:
The training schedules seem to have stretched out across more than 4 days. Can the authors clarify what is shown for the 4 days we see in the figures? For the chemogenetic studies, did mice received pre-session CNO every day of training or only when the schedule moved beyond CRF?

Referee #2

(Remarks to the Author)
Giovanniello and colleagues present a cleverly designed behavioral study that elegantly demonstrates the influence of mild stress on the tendency to engage in goal directed or habitual behaviors. First the authors find that chronic stress prior to engaging in an instrumental training protocol modifies the behavioral outcomes in that, stressed mice are more prone to engage in habitual behavior (less devaluation sensitive) relative to unstressed controls. Using in vivo fiber photometry to characterize amygdalar inputs from either BLA or CeA to dorsomedial striatum, the investigators see that BLA inputs show phasic increases at reward phase normally, but in mice that experienced chronic unpredictable varying mild stressors for 14 days prior to (but not during) the training, the BLA photometry signal in BLS shows decreases, instead. The necessity and sufficiency of this input is then tested in a series of experiments in which BLA is chemogenetically (Gq DREADD) activated prior to training (delivered by retrograde virus from DMS to include BLA-DMS circuitry) and BLA is optogenetically inhibited (Arch) by a brief pulse (2s) prior to reward delivery). The authors found that stimulating BLA is sufficient to prevent more habitual behavior associated with stressed mice. DREADD mice were more goal-directed than the stressed control group.

The second major finding of the study is the contribution of the CeA nucleus. Here the authors find that in normal (unstressed) mice, DMS photometry of retrogradely labeled CeA inputs shows that there is normally no significantly CeA modulation during the reward phase of the task. However, in stressed mice, during reward there is increasing magnitude of activity of CeA inputs over the 4 days of training.

Again, bidirectional manipulations are undertaken to understand the functional significance of this activity. Inhibiting CeA, either chemogenetically or optogenetically, restores goal-directedness more characteristic of the unstressed control group than the stressed control group, demonstrating the necessity of CeA activity. However, in contrast to BLA, optogenetic activation of CeA was not sufficient to promote habitual responding. However, it could potentiate a subthreshold /minimally

effective stress protocol (1 daily stressor instead of 2).

This study is important for a few reasons. First, it is an important advance to generate an animal model of stress that exerts effects on goal-directed behavior. Specifically,prior experiences of chronic mild stress differentially engage amygdalar circuitry to the striatum and that this influences behavior to promote habitual over goal-directed responding. This study also synthesizes a number of less specific manipulations and implications of amygdalo-striatal circuitry that had been made in the field to create a comprehensive model for amygdalar roles. Finally, these findings bear parallels to human behavioral studies of the relationship between stress and habitual/stimulus-response behavior.

There are many strengths to this study. The manuscript is clearly written, experiments are well described, methods use best practices (including documentation of exclusions, blinding during exclusion decisions with respect to viral /fiber targeting, and sample sizes), ancillary behavioral measures are thoughtfully used to establish which behavioral domains are and are not modified. In each experiment, the training protocol was rationally chosen to maximize a signal window to detect effects, i.e. if intervention was to promote goal directed behavior, the RI protocol was used to bias toward the null result (habitual behavior). Conversely, it the experiment was testing a manipulation predicted to promote habitual responding, the RR protocol was used to favor goal-directed responding. This is one example of the careful design and execution conveyed throughout the study.

1. It would seem that optogenetic activation of BLA inputs at the time of rewards should also be sufficient to restore goal directed responding, but this experiment is not reported only chemogenetic activation. Since only BLA chemogenetic activation and only opto at CeA, not at DMS terminal field, are shown to rescue behavior, it seems that for both nuclei circuits beyond the direct amgydalo-striatal projections may be involved. For BLA, while it is true that the nucleus was transduced with DREADD via retrograde DMS viral delivery, this does not necessarily mean that those soma don't project elsewhere and that that circuitry may mediate the behavioral effects. Do the authors have any BLA input opto DMS experiments to report that bear on these points?

2. For the CeA studies, I think the authors did a reasonable job at highlighting the caveat that the specific CeA circuitry mediating the behavioral effect is ambiguous. Stimulation is at cell body because "too few afferents" but interestingly the Arch approach seemed to work with local stim at DMS. Do the authors have any further thoughts on this?

Minor:
3. Figure 1 examples seem to convey a signal relationship that is not born out in the group averages below. Are the traces from one mouse or the entire group? For example, there appears to be opposing modulation between groups at lever press in both BLA and CeA, though no hint of this relationship in the averages. Please comment. I presume the sample mouse used for reward traces is the same as the press. Was it the case that mice with the biggest reward relationships also showed significant press relationships?

4. A statement was made that mice did not differ by weight because they were food restricted. However, the weight restriction says that it is 85-90% of free feeding body weight, so there still should be group differences.

Referee #3

(Remarks to the Author)
The manuscript by Giovanniello et al. describes an elegant set of experiments investigating the circuit-level mechanisms underlying the stress-induced emergence in habitual behaviors. The paper is extremely well written, describes a set of very interesting and novel experiments that would be of interest to the neuroscience community. The manuscript, however, does not provide the depth of investigation I would expect of a paper in Nature. Although I greatly enjoyed reading the paper, the results and conclusions felt premature and a bit superficial, particular because the paper seemed to raise more questions than I felt it addressed.

Strengths of the paper:
1) Provide new insights into the role of the CeA-->DMS circuit in habit learning: The role of the CeA-->DMS circuit has – to my knowledge – not been investigated and this is the first study to 1) identify the circuit and 2) propose a behavioral role of this circuit in habit learning.

2) Evidence preliminarily suggests a dynamic reorganization of circuitry in response to stress: The majority of studies investigating the neural circuits underlying maladaptive behaviors focus on pathological changes that occur within a single circuit. Giovanniello et al. provide intriguing evidence that two amygdala circuits are changing in opposite directions. This is quite a novel finding and could encourage others to think about circuit dynamics in future studies.

3) Converging evidence using different approaches and techniques: The paper is a tour de force of techniques and includes fiber photometry, optogenetics (both excitatory and inhibitory), chemogenetics, and tracing. Remarkably the findings are fairly consistent across all these approaches and provides a strong foundation for some of the arguments made by the authors.

Weaknesses of the paper:
4) Lack of insight into why circuits are being recruited differently after stress:
The paper describes an effect (amygdala circuits play dissociable roles in habit learning) and provides some causal support

for it. But it does not provide any insights into the mechanisms leading this change. For example, what about stress is causing this dynamic change in circuitry? What is the biology that mediates this change?

5) Unspecified effects of stress on goal-directed learning: The authors provide evidence that stress accelerates the transition from goal-directed to habitual behaviors in mice as assessed in a single devaluation test. They report that stress causes an attenuation in reward-related activity in the BLA-->DMS circuit (Figure 2-2), but no change in response to punishment. Stress, however, does not alter learning or rate of learning so exactly how it is leading to development of habitual behaviors remains a mystery (e.g., disruptions in credit assignment? Failure to update action-outcome representations appropriately?). This is not addressed by the authors and is a major weakness of the paper. Moreover, because all experiments were done using a devaluation test it is not known whether these effects are specific to the devaluation paradigm or would be observable in other tests of habitual behaviors.

6) Propose that there is dynamic shift in circuit recruitment following stress: The authors argue that stress causes a more rapid shift away from goal-directed behaviors to habitual behaviors and that this is driven by a reduction in BLA-->DMS activity and an increase in CeA-->DMS activity. Circuit recordings and manipulations, however, were all done between subjects and in a single circuit. I feel that additional evidence is needed to support this argument including 1) assessments of both circuits in the same mouse across training to show the change in circuit activity matches the change in behavior and 2) evidence that this time-dependent change occurs in unstressed mice but at a slower rate.

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)
The authors have put in considerable work to address the comments raised in the prior review, including new studies. I am especially appreciative of the contingency degradation study which adds to the evidence marshaled regarding the impact of stress on behavioral control in their model.
It would be interesting to have some indication of the function of the reward-related signals, in line with the comments from reviewer 3, in the associative process, but I find the current presentation of findings paints a compelling picture. I have no further major issues.

Minor:
Some sentences could use reworking. There are a few instances of sentences starting with ‘but’ or ‘though’ that are awlward or fragments, e.g. “Though behavior was variable in these groups with some marginal evidence of an influence on action-outcome learning, perhaps due to disruption of neurotypical activity.” And a few others missing words, e.g., “Controls showed evidence that they formed routine habits, insensitivity to devaluation.”

Referee #2

(Remarks to the Author)
The revised manuscript is very satisfying and furthers the impact and conceptual advances of the original manuscript substantially. I am further very pleased to see that the impact and rigor was appreciated by all 3 reviewers, however each contributing specific suggestions to clarify aspects. I find the responses satisfactory, especially with inclusion of comment on issues/limitations noting when the approach deviates from the "perfect/ideal" but not feasible.

Referee #3

(Remarks to the Author)
The revised manuscript by Giovanniello et al. includes three additional experiments that all provide additional support for their conclusion that chronic stress which leads to habitual behavior is due to a reorganization of amygdala-striatal connections. This continues to be a very strong, well written, and compelling manuscript. My concerns regarding the depth of the study, however, remain. For example, every experiment after the fiber photometry data presented in Figure 2 is confirmatory for their argument that stress causes a reduction in BLA-->DMS encoding and recruitment of the CeA DMS pathways using different techniques and approaches. While an impressive set of studies that all provide convergent support, no additional information about why or how stress does this is provided. The authors argue that this level of investigation is beyond the scope of the manuscript, but I respectfully disagree. Additionally, I remain concerned about the claim of circuit reorganization when only a single circuit is being assessed in an individual animal.

I have two additional comments for the authors:
1. For the sake of clarity and reproducibility, the authors should provide some insight into why the daily stressors had a range of hours – does 4-16 hrs for damp bedding mean that mice were kept in damp bedding for 4-16 hrs? How did that determine the length of the second stressor or did that not factor into this? Perhaps if the authors provide a few examples of the stress procedure for individual mice that would be helpful for people trying to replicate this procedure.
2. Argue that stress causes “..premature formation of inflexible habits” – Pg. 5. In order to make this conclusion the authors need to show that habitual behaviors eventually developed in control mice with additional training and that the transition to habitual behaviors occurs earlier in stressed mice. The data presented only show that stressed mice develop habits and

control mice do not under different schedules of reinforcement.

Minor comments:
1. Pg 5 - DMS-projecting BLA or CeA neurons – shouldn't this be BLA or CeA neurons that project to the DMS?

Author Rebuttal to Initial Comments:

Dear Dr. Gray and reviewers.

We thank you very much for the positive and constructive evaluation of our work. We are grateful for the energy, enthusiasm, and thoughtfulness the reviewers put into the evaluation of our report. Your feedback was very helpful in revising the manuscript. It helped us to produce a more clear and impactful report. As is detailed below in blue, we have addressed each concern. This includes new experiments, analyses, figures, and text edits. In particular, we have included 3 new experiments. To address Reviewer 3's point 5, we used contingency degradation to provide evidence that stress promotes habit formation by disrupting the action-outcome knowledge needed for goal-directed decision making (Fig. 1g-i). To address Reviewer 2's point 1, we used optogenetics to provide evidence that stimulating BLA→DMS projections at the time of earned reward during learning rescues action-outcome learning and prevents premature habit formation in stressed subjects (Fig. 3g-l). To address Reviewer 3's point 6, we used optogenetics to provide evidence that the CeA→DMS pathway is necessary for the natural habit formation that occurs for routine behaviors. This demonstrates that stress does, indeed, recruit the mechanisms required for normal habit formation (Fig. 4a-f). As requested by Reviewer 1, we have added N to increase the power and rigor of each optogenetic experiment. Further information is provided in response to the reviews below. Major changes to the manuscript are highlighted in blue in the related manuscript file with marked changes.

We also confirm that our manuscript complies with all formatting requirements. Our statistical reporting complies with the guidelines, see Figure legends, Supplemental Table 1, and the reporting summary. We have updated the Reporting summary and Editorial policy checklist. We include a data and code availability statement in the methods. Source data accompany the manuscript and code is available on Dryad. Extended data have been included.

Referee #1 (Remarks to the Author):

In this set of studies, Giovanniello and colleagues ask important and timely questions about the impact of prior stress on the amygdala-striatal mechanisms governing instrumental learning. Previous work has found that a period of stress promotes instrumental behavior that is "habitual," so called because the behavior does not seem to be governed by the current value of the outcome and is thus less flexible. This set of studies seeks to understand the underlying neural mechanisms involved and provides exciting data about one piece of the puzzle. This work builds upon the history of this lab in conducting innovative neuro-behavioral studies. The authors find that prior stress changes the reward response of amygdala neurons that project to the dorsomedial striatum (DMS) as measured during instrumental learning. Using the direction of change induced in each pathway by stress, they design chemogenetic and optogenetic experiments to counter these changes. Thus, they report that excitation of BLA-DMS neurons during learning "rescues" the stress-induced habitual behavior, as does inhibition of CeA-DMS neurons. They also can mimic the impact of stress by inhibiting BLA-DMS neurons during learning. Of note, the manipulations of pathway activity are made during instrumental learning, while the test for goal-directed vs habitual behavior is made later, when the pathway is not being manipulated. Taken together, these findings provide support for differential roles for the BLA vs CeA inputs to striatum in goal-directed vs habitual behavior, a finding that had some limited prior published support, and extend prior published work by recording activity during instrumental learning to understand how stress alters the impact of these two pathways. The calcium imaging data are especially exciting as the response to reward is so distinct in stressed vs unstressed mice. Overall, these findings represent an important step forward due to the elucidation of the specific alteration produced by prior stress that impacts the nature of what is learned. The applicability of these findings to many disorders impacted by stress, including PTSD, addiction, etc., is strong and these findings will be of broad interest.

We thank the reviewer very much for this kind and laudatory evaluation. We agree these are important and exciting results!

I am generally enthusiastic about this work but there are some shortcomings in the current instantiation, mostly revolving around the experimental design and statistical approaches for the pathway manipulation experiments, as detailed below. Importantly, mice are trained under two different regimes that varies from experiment to experiment, and implications of this are not presented well enough for most readers to follow. In addition, some of the claims lack strong statistical support.

1. The authors provide good evidence in Fig 1 that their 2-week stress treatment results in habitual behavior as compared to handled, unstressed controls. Importantly initial learning is similar in the two groups. As an aside, it is interesting to note that the average response rate between valued and devalued conditions are not different, thus indicating habit, although it is clear there is wide individual variation with most subjects showing either a large decrease in responding in the devalued condition or a large increase in responding in the devalued condition, in contrast to the control group in which there is really only 1 mouse showing an increase in the devalued condition. I bring this up to note that clearly normal goal-directed behavior is altered in many, but not all, of the mice, and the alteration is not a simple equal treatment of the lever under both conditions. This is not something I feel the authors need explore experimentally in this paper – it is out of scope – nor is this a point that needs addressing in review. This variability has been observed in many studies. But it does relate, in that the behavior is inherently "noisy" which makes multi-group, multi-condition statistical analysis difficult (see points 4 and 5 below).

The reviewer makes an observant and excellent point. We agree about the variability and, indeed, find it quite interesting. Of course, as the reviewer likely knows, there are many potential sources of variability in these tasks. But the variability in the stress group highlights the possibility of individual differences in susceptibility/resilience to stress-potentiated habit. This is something we hope to explore in future studies. We've made a brief mention of this in the discussion on Pg. 16 ¶1.

2. Figure 2 depicts the exciting photometry data showing that, during learning, the response to reward delivery is different in controls vs stressed mice, in both the BLA-DMS pathway and the CeA-DMS pathway. One question I had was how long after reward receipt did it take for the signal in the CeA stressed group to return to baseline (session 4). I am curious if it stays up as long the mouse is consuming the pellet or if it is a longer-lasting change.

This is a great question. We now include Extended Data Figure 2-3 (Pg. 41) showing the CeA→DMS fiber photometry calcium signal for 45 seconds following reward collection. As you can see from these data the CeA→DMS response to earned reward is long lasting, returning to baseline ~30 s after the reward collection. This is beyond the time of consumption. These data are discussed in the results on Pg. 6 ¶1.

The differences in the CeA signal in this group across the 4 training sessions are dramatic. It is also interesting that there is a big response to unpredicted reward (supplement) in the CeA stressed group, but not to reward delivery in session 1. Can the authors comment on this?

Thank you for pointing this out. This is interesting. It is also consistent with the evidence that the stress-induced engagement of the CeA→DMS pathway occurs with repeated reward experience during training, which may reflect engagement of this pathway with repeated reinforcement and/or opportunity to learn the value or salience of the reward. We speculate this CeA→DMS engagement could be a compensatory mechanism triggered in response to the lack of engagement of the BLA→DMS pathway. We now discuss this result with this Extended Data on Pg. 45. We also highlight that the CeA→DMS pathway is progressively recruited in the results on Pg. 6 and we discuss this progressive recruitment in the discussion on Pg. 15 ¶3.

3. The data depicted in Figs 1 and 2 were collected under the random ratio version of this task. The authors however chose to train mice under a random interval schedule for the the remainder of the experiments except two. The notion in the field is that the cognitive processes engaged during learning under the two regimes are at least somewhat distinct, including neurally. RI training tends to produce habitual behavior and RR training tends to produce goal-directed behavior. The authors' rationale for varying training schedules is that habitual behavior is more typical after training on RI than RR in many mouse studies, and in some of their manipulations they wish to bias against detecting goal-directed behavior. Yet the RI bias for habit is not apparent in the behavior of control mice in these studies, as they mostly remain sensitive to devaluation. More importantly, because the rationale for the manipulation experiments derives from the photometry experiments, which were conducted using the RR procedure, the tight link between the findings is missing. Ideally, the authors would run a second photometry experiment using the RI training procedure to show (or not) that the deficits in the reward signal in both pathways are also evident in the training procedure used for the majority of the remaining studies. This would be the most clean option. The other option is to provide much stronger rationale than they currently

do, and to be very clear for the reader who is somewhat out of the field. I understand why the authors might have been reluctant to discuss these points in a paper aimed at broad readership, but I think the way it is laid out now is too superficial.

Another great point. The literature and our data show that RI training does not automatically engage habit. Mice remain goal-directed on a RI schedule given more limited training. So, it is not surprising that control subjects don't show habits. We designed it so they wouldn't by using a short duration of training (except for one new experiment in which we overtrained subjects). We appreciate the suggestion to clarify and deepen the logic of our selection of reinforcement schedule. Our thinking was that if we were able to rescue action-outcome learning and goal-directed behavioral control in stressed mice using an RI schedule, because this schedule is more permissive to habits, the result would be more robust and convincing, than if we were to rescue such learning on a RR. Indeed, Reviewer 2 notes this as a careful component of the experimental design. We've now laid out this logic in the results on Pg. 9 ¶1. We have also updated the figures and figure legends to include the schedule of reinforcement on the procedure schematic and we have updated the results to describe and rationalize the schedule of reinforcement for each experiment, e.g., Pg. 4 ¶1. We've endeavored to make this understandable to those outside this field.

The question of how BLA→DMS and CeA→DMS pathway activity differs between schedules of reinforcement and whether the stress differentially impacts pathway activity based on schedule of reinforcement is an interesting and important question. It is also quite a substantial one that would likely require a year or more to address well. We now discuss this important future direction in the discussion on Pg. 15 ¶1.

4. The authors use intersectional genetics to conduct pathway-specific chemogenetic manipulations. This is an excellent approach, but for these studies, it requires four groups of mice per experiment as there are two between-subject factors: Stress and Virus. The three-group ANOVA conducted in the main figures conflates these different factors. In addition, the authors show the data from a given experiment in two parts, leaving presentation of the comparison between the unstressed control virus group and the unstressed active virus group to the supplement. The issue is that these experiments by traditional approach should be analyzed in one omnibus ANOVA, not the ANOVAs currently used on the partial data. I do sympathize with the authors in that the individual variability, and in some cases very low sample sizes, provide obstacles to multi-group, multi-factor ANOVAs with both within- and between-subject factors, as is the case here. A perfectly sound approach is to conduct focused ANOVAs with between-subject factors to address the specific hypothesis. For example, for Figure 3 F, chemogenetic excitation of the BLA-DMS pathway, an ANOVA comparing mCherry Controls in both stressed and unstressed can confirm the baseline effect of stress in present; a second ANOVA between stressed controls and stressed hM3Dq can test the hypothesis that hM3Dq can rescue the stress-induced habit, etc. As presented now, the reader is given the impression that the supplemental data come from separate experiments although one can see the control group is the same.

We appreciate the positive comments on our approach. Our effort to simplify the results, as the reviewer rightly points out, didn't enable the most rigorous statistical analysis. We've rectified this. We now show the full data sets in the main figures (e.g., Control-eYFP, Control-ChR2/Arch, Stress-eYFP, Stress-ChR2/Arch, See Figure 3 J-l and 3p-r, Figure 4j-l and p-r). Training data are, thus, analyzed with a 3-way ANOVA: Stress x Virus x Training session. Test press rate data are also analyzed with a 3-way ANOVA: Stress x Virus x Value. As suggested, we complement the 3-way ANOVA with focused 2-way Virus x Value ANOVAs for each stress group. The devaluation index is analyzed with a 2-way ANOVA: Stress x Virus. Thus, we isolate all variables. Because the chemogenetic experiments are replications of the optogenetic results and given the strong a priori hypothesis that devaluation should reduce pressing, for the test press rate data in these experiments we used a planned comparison approach evaluating valued v. devalued press rate for each group. For these experiments we still use 2-way Stress x Virus ANOVA for the devaluation index. Updated statistics are provided in the figure legends (statistic of most importance) and full statistical reporting is in Table 1.

5. A second issue with the statistical analyses even as currently conducted is that there is often not a significant interaction, yet the authors still conduct statistical comparisons across valued vs devalued conditions or across devaluation index groups. For example, there is not a significant group x value interaction in Fig 3f. And, even when the stats comparing across valued and devalued conditions don't seem to offer support, the authors conclude in some cases that the

manipulation has had the desired impact. For example, for the CeA-DMS experiment in Fig 4d (hM4Di) the authors write that the manipulation returned the behavior to goal-directed in the stressed group, yet I do not see any statistical support for that. It is possible that reanalyzing the data using focused ANOVAs may provide a clearer route forward for the authors. It is also possible that the studies will require larger group sizes given the inherently variable nature of the behavior. I feel for the authors – I know these studies are not easy – but there may not be an alternative for moving forward with this interesting set of studies.

As requested, we have added N to each optogenetic experiment to rectify this issue and provide, we think, quite convincing support for the conclusions. There is now a statistical interaction (Virus x Stress, Virus x Value x Stress, or Virus x Value) to support each conclusion from the manipulation results. These can be seen in the figure legends for each figure Pg. 10-11, 13-14, with full statistical reporting in Table 1 Pg. 54-55. The converging evidence provided by testing the main hypotheses in two ways (optogenetics and chemogenetics) further solidifies the conclusions.

Minor:

The training schedules seem to have stretched out across more than 4 days. Can the authors clarify what is shown for the 4 days we see in the figures?

This shows the last day of FR-1 (continuous reinforcement) training and each day of RR or RI training. We have clarified this in the figure legend for Figure 1 on Pg. 5.

For the chemogenetic studies, did mice received pre-session CNO every day of training or only when the schedule moved beyond CRF?

Every day of instrumental training. This is explained in the methods e.g., on Pg. 24 ¶3.

Referee #2 (Remarks to the Author):

Giovanniello and colleagues present a cleverly designed behavioral study that elegantly demonstrates the influence of mild stress on the tendency to engage in goal directed or habitual behaviors. First the authors find that chronic stress prior to engaging in an instrumental training protocol modifies the behavioral outcomes in that, stressed mice are more prone to engage in habitual behavior (less devaluation sensitive) relative to unstressed controls. Using in vivo fiber photometry to characterize amygdalar inputs from either BLA or CeA to dorsomedial striatum, the investigators see that BLA inputs show phasic increases at reward phase normally, but in mice that experienced chronic unpredictable varying mild stressors for 14 days prior to (but not during) the training, the BLA photometry signal in BLS shows decreases, instead. The necessity and sufficiency of this input is then tested in a series of experiments in which BLA is chemogenetically (Gq DREADD) activated prior to training (delivered by retrograde virus from DMS to include BLA-DMS circuitry) and BLA is optogenetically inhibited (Arch) by a brief pulse (2s) prior to reward delivery). The authors found that stimulating BLA is sufficient to prevent more habitual behavior associated with stressed mice. DREADD mice were more goal-directed than the stressed control group.

The second major finding of the study is the contribution of the CeA nucleus. Here the authors find that in normal (unstressed) mice, DMS photometry of retrogradely labeled CeA inputs shows that there is normally no significantly CeA modulation during the reward phase of the task. However, in stressed mice, during reward there is increasing magnitude of activity of CeA inputs over the 4 days of training.

Again, bidirectional manipulations are undertaken to understand the functional significance of this activity. Inhibiting CeA, either chemogenetically or optogenetically, restores goal-directedness more characteristic of the unstressed control group than the stressed control group, demonstrating the necessity of CeA activity. However, in contrast to BLA, optogenetic activation of CeA was not sufficient to promote habitual responding. However, it could potentiate a subthreshold /minimally effective stress protocol (1 daily stressor instead of 2).

This study is important for a few reasons. First, it is an important advance to generate an animal model of stress that exerts effects on goal-directed behavior. Specifically, prior experiences of chronic mild stress differentially engage amygdalar circuitry to the striatum and that this influences behavior to promote habitual over goal-directed responding. This study also synthesizes a number of less specific manipulations and implications of amygdalo-striatal circuitry that had

been made in the field to create a comprehensive model for amygdalar roles. Finally, these findings bear parallels to human behavioral studies of the relationship between stress and habitual/stimulus-response behavior.
We thank the reviewer very much for this kind and laudatory evaluation. We especially appreciate the reviewer identifying that our study creates a comprehensive model for amygdalar roles. We have added some experiments (in response to R3) and reframed the paper to highlight the basic function of each amygdala output, in addition to how stress works through these pathways.

There are many strengths to this study. The manuscript is clearly written, experiments are well described, methods use best practices (including documentation of exclusions, blinding during exclusion decisions with respect to viral /fiber targeting, and sample sizes), ancillary behavioral measures are thoughtfully used to establish which behavioral domains are and are not modified. In each experiment, the training protocol was rationally chosen to maximize a signal window to detect effects, i.e. if intervention was to promote goal directed behavior, the RI protocol was used to bias toward the null result (habitual behavior). Conversely, it the experiment was testing a manipulation predicted to promote habitual responding, the RR protocol was used to favor goal-directed responding. This is one example of the careful design and execution conveyed throughout the study.
We are grateful to the reviewer for highlighting the efforts we put into the rigor of this project.

1. It would seem that optogenetic activation of BLA inputs at the time of rewards should also be sufficient to restore goal directed responding, but this experiment is not reported only chemogenetic activation. Since only BLA chemogenetic activation and only opto at CeA, not at DMS terminal field, are shown to rescue behavior, it seems that for both nuclei circuits beyond the direct amgydalo-striatal projections may be involved. For BLA, while it is true that the nucleus was transduced with DREADD via retrograde DMS viral delivery, this does not necessarily mean that those soma don't project elsewhere and that that circuitry may mediate the behavioral effects. Do the authors have any BLA input opto DMS experiments to report that bear on these points?
Excellent suggestion! We did the experiment (Figure 3g-l; results on Pg. 8-9). We found that optogenetic stimulation of DMS-projecting BLA neurons at the time of reward during learning rescued action-outcome learning for goal-directed behavioral control in stressed subjects.

Unfortunately, there isn't a good way to prevent activation of collateral projections. We choose to activate neurons in the BLA that project to the DMS. But even if we had stimulated BLA terminals in the DMS this could have activated collaterals via backpropagation. Also, we found that activation of BLA terminals in the DMS produced seizures in a small pilot cohort and because this terminal stimulation method doesn't confer a major advantage, we chose to activate the soma to avoid animal welfare issues. That said, we did find that optogenetic inhibition of BLA terminals in the DMS, which does not affect any collaterals, disrupts action-outcome learning, supporting the involvement of the specific BLA→DMS pathway. We discuss the possibility that activation could have also recruited collaterals in the discussion on Pg. 15 ¶3.

2. For the CeA studies, I think the authors did a reasonable job at highlighting the caveat that the specific CeA circuitry mediating the behavioral effect is ambiguous. Stimulation is at cell body because "too few afferents" but interestingly the Arch approach seemed to work with local stim at DMS. Do the authors have any further thoughts on this?
Thanks for the opportunity to clarify this. We choose to stimulate at the cell bodies because stimulating at the terminals, as we note above, does not confer a ajor advantage and could have been less effective. This also paralleled the BLA→DMS stimulation effect, making the two more comparable. For the reasons noted above we couldn't stimulate BLA terminals in DMS. We chose to inhibit CeA terminals in the DMS to implicate specifically the CeA→DMS pathway without influencing collaterals.

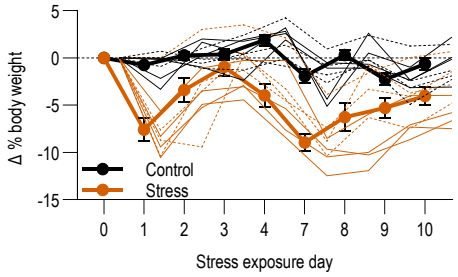
Minor:
3. Figure 1 examples seem to convey a signal relationship that is not born out in the group averages below. Are the traces from one mouse or the entire group? For example, there appears to be opposing modulation between groups at lever press in both BLA and CeA, though no hint of this relationship in the averages. Please comment. I presume the sample

mouse used for reward traces is the same as the press. Was it the case that mice with the biggest reward relationships also showed significant press relationships?

Thanks for pointing out that our description of the figures is unclear. The traces in Figure 2 are averaged across trials for each subject and then averaged across subjects with the shading reflecting the between-subject s.e.m. They are not representative traces. We have updated the figure legend to clarify this Pg. 7-8. The quantification focuses on the time before the press because this is when the subject would be initiating the action (rather than experiencing consequences of the action). There are not strong stress effects at this phase. Rather we see the strongest stress effects when subjects receive reward.

4. A statement was made that mice did not differ by weight because they were food restricted. However, the weight restriction says that it is 85-90% of free feeding body weight, so there still should be group differences.

This is a good point. For the reason the reviewer notes, body weights are slightly lower in the stress group. This was not significant because stress subjects initially drop weight due to stress and then regain some of it as stress progresses. See figure below.



Referee #3 (Remarks to the Author):

The manuscript by Giovanniello et al. describes an elegant set of experiments investigating the circuit-level mechanisms underlying the stress-induced emergence in habitual behaviors. The paper is extremely well written, describes a set of very interesting and novel experiments that would be of interest to the neuroscience community. The manuscript, however, does not provide the depth of investigation I would expect of a paper in Nature. Although I greatly enjoyed reading the paper, the results and conclusions felt premature and a bit superficial, particular because the paper seemed to raise more questions than I felt it addressed.

We thank the reviewer very much for this kind and laudatory evaluation. We agree this paper raises many questions. We think this highlights the importance and potential high impact of the findings. We are eager to spend the next many years answering those questions, including those raised below, and hope our results will spur others to engage in such research as well.

Strengths of the paper:

1) Provide new insights into the role of the CeA-->DMS circuit in habit learning: The role of the CeA-->DMS circuit has – to my knowledge – not been investigated and this is the first study to 1) identify the circuit and 2) propose a behavioral role of this circuit in habit learning.

2) Evidence preliminarily suggests a dynamic reorganization of circuitry in response to stress: The majority of studies investigating the neural circuits underlying maladaptive behaviors focus on pathological changes that occur within a single circuit. Giovanniello et al. provide intriguing evidence that two amygdala circuits are changing in opposite directions. This is quite a novel finding and could encourage others to think about circuit dynamics in future studies.

3) Converging evidence using different approaches and techniques: The paper is a tour de force of techniques and includes fiber photometry, optogenetics (both excitatory and inhibitory), chemogenetics, and tracing. Remarkably the findings are fairly consistent across all these approaches and provides a strong foundation for some of the arguments made by the authors.

We are very grateful for this recognition of the novelty and impact of our findings, as well as the converging evidence and rigor that provides strength behind them. Thank you.

Weaknesses of the paper:

4) Lack of insight into why circuits are being recruited differently after stress: The paper describes an effect (amygdala circuits play dissociable roles in habit learning) and provides some causal support for it. But it does not provide any insights into the mechanisms leading this change. For example, what about stress is causing this dynamic change in circuitry? What is the biology that mediates this change?

This is an excellent point that we very much agree is important. How is stress having these effects? What aspects of stress promote habits at a psychological level and differentially alter amgydala-striatal pathway activity? What are the neurobiological mechanisms? These are important questions with many possible answers. Addressing these questions thoroughly is well beyond the scope of a single paper, or even a handful of papers. This will require years of work. Work that we plan to do and hope our paper will spur others to do as well. We now note these important new directions of research and some related reasonable speculations in the discussion on Pg. 16 ¶1.

5) Unspecified effects of stress on goal-directed learning: The authors provide evidence that stress accelerates the transition from goal-directed to habitual behaviors in mice as assessed in a single devaluation test. They report that stress causes an attenuation in reward-related activity in the BLA-->DMS circuit (Figure 2-2), but no change in response to punishment. Stress, however, does not alter learning or rate of learning so exactly how it is leading to development of habitual behaviors remains a mystery (e.g., disruptions in credit assignment? Failure to update action-outcome representations appropriately?). This is not addressed by the authors and is a major weakness of the paper. Moreover, because all experiments were done using a devaluation test it is not known whether these effects are specific to the devaluation paradigm or would be observable in other tests of habitual behaviors.

This is an astute and important point. Thank you for raising it. In the first version, we did not sufficiently address how stress promotes habit. We appreciate the opportunity to better address in the revised manuscript. We also agree that it is important to demonstrate whether the ability of stress to promote habit is specific to the devaluation paradigm. Our main interpretation is that a history of chronic unpredictable stress disrupts encoding of the action-outcome association needed for goal-directed behavioral control and, thereby, biases learning towards a habit strategy. To provide additional evidence for this and demonstrate that stress potentiation of habit is not specific to the devaluation paradigm, we added a contingency degradation experiment. Indeed, contingency degradation is the other gold-standard assessment of goal-directed v. habitual behavioral control. It allows an assessment of the extent to which subjects have encoded and are using the action-outcome association to adapt behavior. Following stress and training to lever press to earn food pellets rewards, mice received contingency degradation in which presses continue to earn reward with the same probability as in training (P=0.1), but rewards were also delivered non-contingently with the same probability. Thus, reward delivery is no longer contingent lever pressing. If subjects have learned the action-outcome contingency and are using this update their behavior, their actions will be sensitive to a change in this contingent relationship such that they will reduce lever pressing when it is no longer needed to earn reward. Habits are marked by insensitivity to degradation of the contingent relationship between an action and its consequence. Whereas controls were sensitive to the contingency degradation, stressed mice were not. These data are shown in Figure 1g-l, Extended Data Figure 1-2 c-d, Extended Data Figure 1-3 and described in the results on Pg. 4-5 and in the discussion on Pg. 15 ¶2. We update the interpretation throughout to indicate that stress disrupts encoding of the action-outcome association needed for goal-directed decision making and promotes the formation of habits, which do not require such knowledge.

This interpretation is also supported by our neurobiological findings. Chronic stress attenuates activity in the BLA→DMS pathway we show to be necessary for the formation of the action-outcome associations and recruits activity in the CeA→DMS pathway that we now show (Figure 4a-f) is needed for natural habit formation (see more on this in response to your point 6 below). That manipulations (chemogenetic or optogenetic activation of BLA→DMS or chemogenetic or optogenetic inhibition of CeA→DMS) restricted to learning can rescue goal-directed behavioral control in stressed subjects indicates that stress is acting on the learning process to attenuate action-outcome learning and promote habit learning. We now make this point clearer in the results on Pg. 8 ¶1 and discussion on Pg. 15 ¶3.

6) Propose that there is dynamic shift in circuit recruitment following stress: The authors argue that stress causes a more rapid shift away from goal-directed behaviors to habitual behaviors and that this is driven by a reduction in BLA-->DMS activity and an increase in CeA-->DMS activity. Circuit recordings and manipulations, however, were all done between subjects and in a single circuit. I feel that additional evidence is needed to support this argument including 1) assessments of both circuits in the same mouse across training to show the change in circuit activity matches the change in behavior and 2) evidence that this time-dependent change occurs in unstressed mice but at a slower rate.

This is a very constructive and helpful point. Thank you! We agree it is important to show the natural function of the both pathways. In the first version, we had shown the normal function of the BLA→DMS pathway in supporting the action-outcome learning needed for goal-directed decision making. But we did not provide evidence of the normal function of the CeA→DMS pathway. We added the requested experiment showing that CeA→DMS projections mediate natural habit formation (Fig. 4a-f; results on Pg. 11 ¶1). Using optogenetic inhibition, we find that CeA→DMS projection activity is required at the time of reward for routine habits to form with overtraining. Thus, our data show that the CeA→DMS pathway is required for natural habit formation. This "habit" pathway is not typically engaged during early instrumental learning, but stress recruits this pathway progressively during learning and this is required for stress to promote habits. We interpret these data as evidence that stress attenuates activity in the BLA→DMS pathway needed for the action-outcome learning that supports goal-directed decision making and recruits CeA→DMS projections to promote habit formation. We really appreciate this suggestion and think it considerably strengthens both the basic and stress-related findings of the paper. We have reframed the paper throughout (e.g., Abstract, Introduction Pg. 3 ¶1, Results Pg. 8 ¶1, Pg. 11 ¶1, Discussion Pg. 14-15) to highlight the basic function of each pathway and how stress disrupts these functions to attenuate action-outcome-learning and promote habit formation.

Unfortunately, given the very close proximity of the BLA and CeA, it is not presently possible to record the activity of both BLA→DMS and CeA→DMS pathway at the same time. Should we try to record from the soma, we could not fit fibers in both BLA and CeA. Recordings of terminal activity with GCaMP and RCaMP are not sufficiently robust, especially with the RCaMP sensor. Soma recordings in contralateral hemispheres, could be plausible, but would provide largely similar information to recordings in separate subjects. Moreover, differences in the density of the pathways and brightness of the different sensors would make it difficult to rigorously directly compare their activity. Such recordings would also not allow us to provide evidence that the change in circuit activity matches the change in behavior because behavioral control strategy can only (at least at present in rodents) be assessed after learning. So, we could not perfectly map the change in pathway activity to the change in behavioral control strategy. We now note in the discussion on Pg. 15 ¶3 that dual pathway recordings and manipulations are needed to fully determine how activity in one pathway relates to activity in another.

Author Rebuttal to First Revision:

Dear Dr. Gray:
We again thank you very much for the positive and constructive evaluation of our work. We continue to be very grateful for the energy, enthusiasm, and thoughtfulness the reviewers put into the evaluation of our report. We are so pleased you find our work acceptable for your journal. We have now made all the requested modifications. This includes responding to the reviewer comments, the editorial revisions, and all the formatting requirements. We detail our responses to these, point-by-point below. In addition to the requested modifications, we have also very lighted edited the manuscript for precision and clarity.

Referees' comments:

Referee #1 (Remarks to the Author):
The authors have put in considerable work to address the comments raised in the prior review, including new studies. I am especially appreciative of the contingency degradation study which adds to the evidence marshaled regarding the impact of stress on behavioral control in their model.
It would be interesting to have some indication of the function of the reward-related signals, in line with the comments from reviewer 3, in the associative process, but I find the current presentation of findings paints a compelling picture. I have no further major issues.
We very much appreciate the laudatory remarks.

Minor:
Some sentences could use reworking. There are a few instances of sentences starting with 'but' or 'though' that are awlward or fragments, e.g. "Though behavior was variable in these groups with some marginal evidence of an influence on action-outcome learning, perhaps due to disruption of neurotypical activity." And a few others missing words, e.g., "Controls showed evidence that they formed routine habits, insensitivity to devaluation."
We have fixed these two statements and avoided starting sentences with but or though.

Referee #2 (Remarks to the Author):
The revised manuscript is very satisfying and furthers the impact and conceptual advances of the original manuscript substantially. I am further very pleased to see that the impact and rigor was appreciated by all 3 reviewers, however each contributing specific suggestions to clarify aspects. I find the responses satisfactory, especially with inclusion of comment on issues/limitations noting when the approach deviates from the "perfect/ideal" but not feasible.
We very much appreciate the laudatory remarks.

Referee #3 (Remarks to the Author):
The revised manuscript by Giovanniello et al. includes three additional experiments that all provide additional support for their conclusion that chronic stress which leads to habitual behavior is due to a reorganization of amygdala-striatal connections. This continues to be a very strong, well written, and compelling manuscript. My concerns regarding the depth of the study, however, remain. For example, every experiment after the fiber photometry data presented in Figure 2 is confirmatory for their argument that stress causes a reduction in BLA-->DMS encoding and recruitment of the CeA◊DMS pathways using different techniques and approaches. While an impressive set of studies that all provide convergent support, no additional information about why or how stress does this is provided. The authors argue that this level of investigation is beyond the scope of the manuscript, but I respectfully disagree. Additionally, I remain concerned about the claim of circuit reorganization when only a single circuit is being assessed in an individual animal.
We very much appreciate the laudatory remarks.
There are no claims about circuit reorganization. The terms "circuit reorganization", "reorganization", and "organization" do not exist in the manuscript. Perhaps the reviewer is referring to 3 statements about the "balance" of BLA and CeA input to the DMS. We have reworded such statements to be closer to the data.

I have two additional comments for the authors:

1. For the sake of clarity and reproducibility, the authors should provide some insight into why the daily stressors had a range of hours – does 4-16 hrs for damp bedding mean that mice were kept in damp bedding for 4-16 hrs? How did that determine the length of the second stressor or did that not factor into this? Perhaps if the authors provide a few examples of the stress procedure for individual mice that would be helpful for people trying to replicate this procedure.
We have clarified that the stress protocol was identical across mice within a cohort and have provided an example stress protocol in Supplemental Table 6.

2. Argue that stress causes "..premature formation of inflexible habits" – Pg. 5. In order to make this conclusion the authors need to show that habitual behaviors eventually developed in control mice with additional training and that the transition to habitual behaviors occurs earlier in stressed mice. The data presented only show that stressed mice develop habits and control mice do not under different schedules of reinforcement.
Indeed, with the excellent experiment suggested by this reviewer in the last review, in the revision we did show that habitual behaviors eventually develop in control mice with additional training on a random interval schedule and that this transition happens earlier in stressed mice. Figure 4e-f shows that control mice form habits with overtraining (8 sessions) on a random-interval schedule of reinforcement. Figure 4k-l (also 4w-x, 3k-l, 3q-r) shows that stress mice form habit with only 4 sessions of random interval training.
We add a statement to the discussion to clarify: Using two independent tests, we provide evidence in male and female mice that a recent history of chronic stress disrupts the action-outcome knowledge needed for agency and instead causes the formation of inflexible habits. "*Habit formation in stressed subjects was premature. Whereas we showed habits formed naturally with overtraining on a random-interval schedule, stressed mice form habits with only a limited amount of such training. Stress disrupts agency and promotes habit regardless of whether behavior is reinforced on the agency-promoting random-ratio schedule or the habit-promoting random-interval schedule.*"

Minor comments:
1. Pg 5 - DMS-projecting BLA or CeA neurons – shouldn't this be BLA or CeA neurons that project to the DMS?
We have made this change, though we think these mean the same thing.