

# Psychosomatic Medicine

## Omega-3 supplementation and the neural correlates of negative affect and impulsivity: A double-blind, randomized, placebo-controlled trial in midlife adults --Manuscript Draft--

<b>Manuscript Number:</b>	PSY16-245R1
<b>Full Title:</b>	Omega-3 supplementation and the neural correlates of negative affect and impulsivity: A double-blind, randomized, placebo-controlled trial in midlife adults
<b>Short Title:</b>	Omega-3 neural correlates affect impulsivity
<b>Article Type:</b>	Original Article
<b>Section/Category:</b>	
<b>Keywords:</b>	omega-3 fatty acids; clinical trial; Negative affect; fMRI; impulsivity
<b>Abstract:</b>	<p>Objective: In clinical trials, omega-3 fatty acid supplementation improves symptoms in psychiatric disorders involving dysregulated mood and impulse control, yet it is unclear whether in healthy adults omega-3 fatty acid supplementation affects mood, impulse control and the brain systems supporting these processes. Accordingly, this study tested the hypotheses that eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid supplementation reduces negative affect and impulsive behaviors in healthy adults and that these changes correspond to alterations in corticolimbic and corticostratal brain systems which support affective and impulsive processes.</p> <p>Methods: Healthy volunteers (N = 272) consuming 300 mg/day or less of EPA and DHA were enrolled in a double-blind, randomized, placebo controlled clinical trial. Participants received either capsules providing 1000 mg of EPA and 400 mg of DHA versus identical appearing soybean oil capsules per day for 18 weeks. Negative affect and impulsivity were measured by questionnaire and ecological momentary assessment (EMA), as well as functional alterations in corticolimbic and corticostratal brain systems evoked by standardized fMRI tasks.</p> <p>Results: There were no group-by-time interactions for any questionnaire or EMA measures of mood and impulsivity. Likewise, no group-by-time interactions were observed for fMRI responses evoked within corticolimbic and corticostratal systems.</p> <p>Conclusions: In healthy adults with low intake of omega-3 fatty acids, moderate-dose supplementation for 18 weeks did not alter affect or impulsive behaviors, nor alter corticolimbic and corticostratal brain functionality.</p> <p>Trial Registration: Trial number RCT00663871, URL: <a href="https://www.clinicaltrials.gov/ct2/show/NCT00663871?term=NCT00663871&amp;rank=1">https://www.clinicaltrials.gov/ct2/show/NCT00663871?term=NCT00663871&amp;rank=1</a></p>

Willem J. Kop, PhD  
Tilburg University  
Editor, *Psychosomatic Medicine*

Dear Professor Kop,

Thank you for the opportunity to revise our manuscript, 'Omega-3 supplementation and the neural correlates of negative affect and impulsivity: A double-blind, randomized, placebo-controlled trial in midlife adults, which we submitted to *Psychosomatic Medicine*. We have indicated below on a point-by-point basis how we dealt with each Reviewer's comments.

**Reviewer #1:**

1. This is a well-written manuscript of a well-designed clinical trial of the effects of an omega-3 fatty acid supplement on a variety of outcomes in a sample of healthy adults. The authors should be congratulated on their careful attention to detail and their execution of this complicated study. I have a few comments and suggestions for the authors to consider.

**Response:** We thank the Reviewer for his/her positive comments regarding the execution of our study.

2. Although the effects of the omega-3 fatty acids EPA and DHA on disease or normal physical functioning are usually studied together, they can have different effects on targeted variables. For example, there is evidence that EPA and DHA differentially affect lipids, lipoproteins, and blood pressure (Mori Food & Function 2014). Also, EPA seems to be more effective at decreasing inflammation than DHA. Meta-analyses of trials of omega-3 on depression have suggested that EPA and not DHA may have therapeutic benefits for depression (Appleton et al. J Clin Nutrition 2011). Furthermore, not only the dose but the ratio of EPA to DHA may determine whether the supplement improves depression. In short, a study designed to assess the effects of a fixed combination of EPA and DHA on such a wide range of outcomes as described here may be very likely to run into problems. I would be interested in the authors' response to this point. If they agree they should acknowledge this in the discussion.

**Response:** We agree with the Reviewer that EPA and DHA differently affect outcome measures. The dosage of EPA and DHA chosen was based off of previous research (Blonk et al., 1990; Am J Clin Nutr). In our trial, levels of both EPA and DHA were significantly increased in participants in the treatment group. The dosage used was higher in EPA than DHA. The current trial and the previously published paper from the trial (Muldoon et al., 2016) focus on outcomes where EPA is known to have positive effects (inflammation and negative affect). For example, meta-analyses have suggested that EPA may have therapeutic benefits for depression (Hallahan et al., 2016). We have expanded our limitations section of the Discussion to acknowledge the Reviewer's points on this issue.

3. While the effect of increasing DHA intake on brain uptake in humans has not been investigated, a study of radio-labeled DHA in rats found that brain uptake of DHA may occur too slowly to affect the outcomes of most clinical trials. Is it possible that the duration or dosing of the omega-3 was not sufficient to observe differences in the functional MRI data which may depend in part on the uptake of omega-3 in the brain? The possibility that higher doses or longer duration may have provided a different outcome is made on page 16, but this should be considered in more detail.

**Response:** We appreciate the issue of slow brain uptake relative to blood and other organs. In rodents, brain DHA turnover is estimated to be just 2-8% per day (Rapoport et al., J Lipid Research, 2001; 42:678:-85) and half-life for recovery from depletion is about 3 weeks (Moriguchi et al., J Lipid Res,2001; 42:1-9). Still, our 18-week intervention period may be adequate, and several similar or shorter duration studies have shown effects on fMRI (e.g., McNamara RK, et al., Am J Clin Nutr, 2010; 91:1060-1067; Boesplug et al., J Nutri. Health Aging, 2016; 20:161-169). In line with the Reviewer's comments, we modified the limitations section of the Discussion to highlight these points.

4. It has also been shown that different individuals may require different amounts of omega-3 supplements to show comparable biological effects (Superko et al., Circulation 2013), possibly due to genetic variants that affect omega-3 metabolism. A secondary analysis of an omega-3 trial for depression that initially reported no effect for an omega-3 supplement found that patients with higher baseline RBC levels of omega-3 showed a greater improvement in response to the omega-3 compared to those with lower baseline levels (Carney et al., J Clin Psychiatry, 2016). The authors of this manuscript found no significant correlations between change in EPA or DHA and the questionnaire results, but did they investigate a possible relationship between baseline or post treatment RBC levels and the outcomes?

**Response:** We were unaware of the new paper by Carney and colleagues and appreciate the Reviewer bringing this to our attention. As the Reviewer noted, our original draft included supplementary analyses testing whether changes in blood EPA or DHA correlate with change in psychological questionnaire scores or EMA measures. No significant correlations were found. To directly address the Reviewer's question, we explored the relationship between baseline and post treatment RBC levels and outcomes. We found that there were no associations between RBC levels (EPA, DHA) and any of the main outcome variables at baseline or follow-up. Stimulated by the subgroup findings of Carney et al and as suggested by the Reviewer, we also tested for moderation of intervention effect by baseline (and post-supplementation) EPA and DHA. We observed no significant moderation. Because the study was designed as an omega-3, double-blind, placebo controlled trial with specific a priori hypotheses and planned tests, we respectfully wish to avoid over-reporting post-hoc analyses that were not part of our analytical plan or part of the main trial. In addition, we feel running additional correlations and moderation analyses such as these without correction or consistent results engenders multiple comparison problems. However, if the Editor and Reviewer feel reporting these results would

enhance the quality of the manuscript, we are happy to include them.

5. The trial registration on ClinicalTrials.gov lists a number of "primary" outcomes, including autonomic control of the heart, which is mentioned on page 6 of the manuscript. It is also suggested on page 6 that the results of the effects of omega-3 on ANS are presented in the manuscript cited, but they are not. This needs to be clarified. Do the authors intend to publish these results in another manuscript?

**Response:** Thank you for pointing out this issue. To clarify, the current manuscript concerns fMRI and affect measures, whereas we are working to report findings related to cardiac autonomic control and cognitive functioning elsewhere. We have revised the study design section of the Methods to avoid any misunderstanding.

6. It should be made clear that it is the original version of the Beck Depression Inventory that was used in this study. Although it is that version that is cited, the BDI-II is now so widely used that version used in this study should be identified in the Methods and Table as being the original version.

**Response:** We thank the Reviewer for drawing our attention to this issue. We now indicate that the original version of the Beck Depression Inventory was used in the Methods and Table.

7. On page 11, do the authors see any value in investigating the variability or intensity of single events of the affect/impulsivity ratings rather than just comparing the mean levels for the day? Is it possible that these moods may show greater variability or intensity when they occur which may not be captured by daily mean scores?

**Response:** We agree that looking at the variability and intensity of single events of the affect/impulsivity ratings rather than comparing the mean levels of each day would be interesting. However, our a priori hypotheses involved looking at more stable measures of mood. We are reluctant to perform such post-hoc analyses at the present time as we had no hypotheses about these metrics prior to the study or manuscript preparation. Further, data to compute these metrics and conduct these analyses are not readily available and they would fundamentally change the nature of our manuscript.

8. Although the authors note that conducting analyses using linear mixed effects modeling will handle missing data better than other possible models, did they also consider using multiple imputation? They used it in their earlier publication of inflammatory markers for this study. Regardless, they should report how much data were missing for each of these variables. The Consort flow diagram reports the number of trial completers, but it is still important to know how much data were missing for each of the primary outcome variables.

**Response:** We have now added a footnote in the results section showing how many participants were missing for each of the primary outcome measures. We did consider multiple imputation and last value carried forward. We chose mixed effects modeling for a number of

reasons. First, the proportion of missing data in this particular manuscript is relatively small, only about 6% dropped out. Even taking into account the missing data for EMA measures of those who completed the full trial ( $n = 10$ ) and those who dropped out of the study, the amount of missing data is still less than 10%. Secondly, it is not possible to account for missing data in voxel-wise neuroimaging analyses without violating statistical assumptions in random field theory. Accordingly, we avoided accounting for (imputing) missing data in the questionnaire/EMA measures since we could not do it for the fMRI. However, a recent fMRI and omega-3 paper (Bos et al, 2015) addressed this problem quite nicely. They reported their main affect/behavioral outcome measures not accounting for missing data and then used linear mixed effect modeling as a secondary analysis to confirm their results. We chose to follow the precedent set by this paper. We have now referenced the Bos paper in our statistical analyses section of the Methods. Another Reviewer suggested we run last value carried forward analyses and we have included these as a further sensitivity analysis.

9. In the sentence describing the model, "likewise" should be "listwise".

**Response:** We have changed the word "likewise" to "listwise."

10. In clinical trials it is usually customary to report the ITT analysis first, and then the completers' analysis. If there is a reason for not following this convention in this manuscript (page 13) it should be made clear.

**Response:** We thank the Reviewer for their comments. Please see response to comment 8 for further details. Briefly, we chose to report the non-ITT analyses first for two reasons. The first reason was to follow the precedent set in a recent fMRI omega-3 clinical trial (Bos et al., 2015; Neuropsychopharmacology). The second was to keep the manuscript standard since we cannot do ITT analyses in neuroimaging.

**Reviewer #2:**

1. This is a well-written report of a rigorous clinical trial. While the findings are largely non-significant, this is important evidence that should be published. However, there are a few relatively minor issues that should be addressed to improve the manuscript.

**Response:** We thank the Reviewer for his/her positive comments regarding our manuscript.

2. A summary of recruitment and screening criteria should be provided in the text, even if full information is in the Supplemental Material.

**Response:** We have now updated the Methods and Materials section to include the recruitment and screening criteria for this specific portion of the AHAB-II trial.

3. A more conservative analysis than linear mixed models, which does not use listwise

deletion in cases of missing data, would be to carry forward the last observation. In a supplemental clinical trial, this analysis would be useful for readers, even though drop-out was quite low. Please include this, perhaps as a sensitivity analysis.

**Response:** We have included this as an additional sensitivity analyses and have altered the Methods and Results Section accordingly.

4. According to the CONSORT flow diagram, less than 10% of phone screenings were enrolled and randomized. Elaboration of this point, and the potential impact it has on both the results and generalizability of the findings, should be included in the discussion.

**Response:** Thank you for making this point. We enrolled a subset of potentially eligible participants due to enrollment criteria and general willingness to attend the many study visits. Here, our selection was constrained by that fact that trial recruitment was limited to participants completing a preceding parent study (AHAB-2) with its own unique selection and eligibility criteria (e.g., working at least 25 hours/week, no cardiovascular or psychotropic medications). To address this point, we revised our Discussion to highlight the issue that our trial findings may not generalize to an unselected healthy adult population.

Minor points:

5. Introduction, third sentence: remove comma after "clinical-trials"

**Response:** We have removed the comma after clinical-trials.

6. The clinicaltrials.gov identifier is NCT00663871, not RCT00663871.

**Response:** We thank the Reviewer for bringing this error to our attention and have altered the manuscript to say NCT0063871.

**Reviewer #3:**

1. Comment: I commend the authors for choosing a supplementation duration longer than the usual 12 weeks and for selecting a relevant measure such as impulsivity as it is a multifactorial dimension, a facet of personality and a major component of various psychiatric disorders including ADHD and other attentional disorders. Neurobiological findings suggest that there are specific brain regions involved in impulsive behavior, although different brain networks may contribute to different manifestations of impulsivity, and that genetics may play a role. Thus I think this is a novel and promising way to investigate the mechanisms underlying the benefits of omega-3s on mood. I have very few comments that I am sure the authors will find easy to address.

**Response:** We thank the Reviewer for his/her positive comments regarding our manuscript.

2. Please provide inclusion/exclusion criteria for psychiatric disorders such as anxiety

**Response:** We have now added additional information to the Methods and Materials section to

describe the inclusion/exclusion criteria for psychiatric disorders.

3. The concept of impulsivity, specifically the dimension(s) measured by the BIS, is seen as a trait rather than a state. Some could therefore argue that one cannot directly reduce impulsivity. Could the authors comment on this and address this point in their conclusions.

**Response:** We agree that impulsivity as measured in this manner can be seen as trait rather than state. However, a number of recent studies have shown that impulsivity, measured by the BIS, can be altered through various interventions (e.g., Gruber et al., 2015; Int J Neurol Neurother; Tarrega, et al., 2015, Front. Psychol). We have now addressed this in the limitations portion of the Discussion section of the manuscript.

4. Choice of the supplementation. Why an EPA-rich supplementation? Could the authors provide additional information on absorption of omega-3 supplementation? Why not krill supplementation for instance.

**Response:** The large majority of fish and fish oil products are rich in both EPA and DHA but with more EPA than DHA. We had insufficient rationale to find and use a special product with unusual composition. Krill oil has not been widely tested and is much more expensive. There is some debate in the literature about whether certain formulations provide for better absorption (as triglycerides versus cholesterol esters), but measurement of levels in blood post supplementation remains an acceptable method of verifying and quantifying absorption.

5. Choice of placebo. Could they explain why they selected soybean supplementation?

**Response:** We sought a placebo that was least likely to have its own biological effects when increased by 2 grams per day. We chose soybean oil since it is the oil consumed in largest quantities in the US diet, and therefore the impact of a 2 gram increase should be inconsequential.

6. Could the authors explain why the EMA measures were done at week 16 and not week 18?

**Response:** Yes, the intervention included several end-of-intervention assessments. Not all could be carried out on the last day of the supplementation. We have added this information to the Methods section.

7. In terms of clinical measures. Why did the authors select the BDI? It appears to me that it may not assess negative affect well enough in a non-clinical population, specifically since it assesses mood state in the past 2 weeks? Have the authors considered using the PANAS?

**Response:** The BDI was selected because of its wide use and strong test-retest reliability. Participants were asked to assess mood “in the past week, including today.” We have now

altered the manuscript to include this information. We did consider using the PANAS, however, data were only available for the PANAS at the baseline visit.

8. BIS: Why did the authors select the total score and did not explore the subtests?

**Response:** We chose to select total score since it gives an overall measure of impulsivity. Since total score was non-significant, we did not assess the subtests. Had total score been significant, we would have assessed the subtests to see what specific factor(s) were driving the change in impulsivity.

9. In terms of EMA. Could they provide the exact instructions re timeframe? E.g. based on the last 2 weeks?

**Response:** Ecological Momentary Assessment (EMA) measures were taken in the moment. Participants were asked to record on 4-days (3 working days and 1 nonworking day). Participants were asked the specific question regarding the extent they were feeling the emotion at the time of the questionnaire. They were asked questions throughout these days and averages were calculated. There were an average of 54.4 (8.64) observations pre-supplementation and 55.53 (6.49) post-supplementation.

10. Could the authors provide the behavioral results of the fMRI tasks (reaction times, accuracy?).

**Response:** There are no accuracy data or meaningful variabilities in behavioral responding to report. The reason for this is that the task outcomes were fixed, regardless of the subject's responses. For example, in positive feedback blocks of the reward task, 75% of the subject's guesses were "correct" and rewarded with money, whereas in the three predominantly negative feedback blocks, only 25% of the guesses were correct. In the other task, accuracy in matching-to-sample was near ceiling across all subjects. By design, we thus minimized or controlled for individual differences in behavioral performance to more directly probe for inter-individual variability in neural responding.

11. Did the authors explore results at baseline and can they provide statistical maps/results to show that functional activation was as predicted?

**Response:** We did report the baseline results and confirmed expected patterns of activation, as were also seen at follow up. We apologize if this was not clear. Please see Supplementary Table 1 and Supplemental Figure 1.

12. Did the authors correlate fMRI activations, behavioral measures and clinical measures?

**Response:** Respectfully, the correlation analyses that the Reviewer is asking about are beyond

the scope of the present clinical trial. We are cautious to execute such analyses given issues pertaining to our lack of a priori hypotheses, our sample size for examining replicable individual difference effects, and multiple statistical testing.

13. Could the authors comment on the EPA/DHA ratio and how a different ratio could have been more beneficial for mood or emotion-related neural processing?

**Response:** Please see our response Reviewer 1, Comment 2. Briefly, we have updated the limitations section of the Discussion to address the possibility that the exact dosage and ratio of the study could influence the results.

14. Table 1. Please provide additional information on education, IQ

**Response:** We have now provided additional information in Table 1 to include means and standard deviations for education (years of school) and WASI IQ for each group. We have also included the difference statistics for these measures.

15. Why were participants included only if they worked for more than 25 hours?

**Response:** AHAB-2, the parent study from which participants for the trial were recruited, had aims testing work versus home life affect and BP (Joseph, Muldoon, Manuck, Matthews, MacDonald, Grosch, & Kamarck, 2016, *Psychosom. Med.*). So, employment was an enrollment criterion.

16. Could the authors clarify if participants were included if they had a lifetime history of anxiety, substance use, depression? What about neurological disorders?

**Response:** We apologize for the confusion. We have updated the Participants section of the Methods to explain that participants were excluded if they had a current diagnosis of DSM-IV Axis-I disorders. Major neurologic disorders were exclusionary where past history of either mood disorders or substance abuse were not.

17. Why did the placebo group involve more participants than the supplementation group? please address this either in methods or discussion.

**Response:** Whereas computerized randomization with minimization generally provides for 50:50 balance in number of participants per treatment condition, in this instance it generated slightly different group sizes (i.e., 49% versus 51%).

18. Was baseline dietary omega-3 estimated via FFQ only or via blood tests too?

**Response:** Eligibility was based upon omega-3 intake estimated from the FFQ. Blood level measurements were obtained, but results were not available on a time scale conducive to use in eligibility determinations. This has been clarified on p6 of the Methods.

**Reviewer #4:**

1. This is albeit largely negative, a very interesting intervention study in a large sample of adults investigating the effects of omega-3 fatty acids on several measures of affect and impulsivity, including the underlying neural circuitry. Just a few things before I would recommend this manuscript suitable for publication:

**Response:** We thank the Reviewer for his/her positive comments regarding the manuscript.

Methods:

2. What was the rationale behind the +/- 45 years age stratification?

**Response:** We wanted to protect against unequal groups based on age, and, therefore, used age as one the factors to be “minimized” in the computerized randomization process.

3. The placebo capsules contained 1% fish oil, this was a very very low dose EPA/DHA then? Or something else? Please clarify.

**Response:** 1% fish oil is used in placebo capsules to maintain some fishy smell and taste as a protection against loss of participant blinding.

4. Have the authors investigated the relation between the effects of omega-3 FAs and for instance age or BMI?

**Response:** We have conducted and are reporting several post-hoc supplementary analyses, looking for some indication of a treatment effect. Many others could be conducted. We want to be wary of going too far as we will encounter diminishing scientific and statistical rationale. Here, we do not have strong justification for examining moderating effects of BMI or age in this sample testing putative effects on affect and affect-related fMRI.

Results:

5. It seems there is quite some variance in the increase of both EPA and DHA in the intervention group post-intervention. Have the authors looked at dose-dependent effects on the affect and impulsivity measures, and in the fMRI-task? Could it be that there is a subsample of individuals in which these measures do improve (those with high pre-intervention ratings on negative affect or impulsivity), which is obscured due to large variance in the whole group? It would be very plausible, for a variety of biological reasons, that behavior and the underlying neural circuitry is not affected in the same way in everyone.

**Response:** We thank the Reviewer for this comment. The design of the study was to examine the effects of a set supplementation of omega-3 fatty acids on our outcome variables. Respectfully, we believe that if we were to implement these additional post-hoc analyses we would be capitalizing on chance variation. We felt it most defensible to test for moderation by gender and whether change in dependent measures correlate with change in fatty acid content of RBCs. The later may approximate the Reviewer's request for “dose-dependent effects.” As

noted above, we remain hesitant to report additional post-hoc analyses without compelling rationale.

**Discussion:**

6. Page 15: "Several sources have linked fatty acid deficiency to negative affect". Which sources? Please add references.

**Response:** We have updated this accordingly.

7. Page 16: "The current randomized and placebo-controlled ω -3 supplementation assessing negative affect impulsivity in a healthy population is the largest to date, and the first trial to assess both negative affect and impulsive behavior throughout the course of daily living pre- and post-intervention by EMA." Somewhat of a repetition of what is stated earlier in the discussion. And even though I appreciate the novelty of this study, I am much more interested in the reason why no effects were found in such a large group. As power could hardly have been the issue, what do the authors think drives this negative result.. What are the mechanisms by which omega-3 fatty acids affect impulsivity and affect? Or don't they do that at all? Following other recent findings it may be plausible that omega-3 fatty acids do not target dopaminergic systems very effectively.

**Response:** We have updated the limitations section of the Discussion and indicate that the null findings may reflect absence of effect or alternatively could be due to a variety of factors (e.g., constrained baseline symptomatology, dose and duration of supplementation).

**Minor things:**

8. Typo in abstract: "]Negative affect and impulsivity - was - measured.."

**Response:** We have now added the abstract accordingly.

9. Page 4, strange sentence: "The types and amounts of dietary fatty acids affect [...] active transport proteins"

**Response:** We have now altered the sentence.

10. Page 7, strange sentence: "Participants completed Ecological Momentary Assessment (EMA) recording days on average two weeks before beginning supplementation"

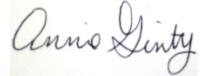
**Response:** We have altered the sentence to make it clearer.

11. Page 14, awkward phrasing: "There were no differences between groups on portion who guessed correctly their treatment assignment"

**Response:** We have re-wrote the sentence to make it sound less awkward.

Again, we thank the Editor and the Reviewers for their feedback regarding our manuscript and for their thoughtful suggestions. We feel the manuscript has been substantially improved and hope it is now suitable for publication in *Psychosomatic Medicine*.

Yours Sincerely,

A handwritten signature in black ink, appearing to read "Annie T. Ginty".

Annie T. Ginty, PhD on behalf of the authors

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## Omega-3 neural correlates affect impulsivity 1

Omega-3 supplementation and the neural correlates of negative affect and impulsivity: A double-blind, randomized, placebo-controlled trial in midlife adults

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**Number of supplementary material:** 2 (text document with 1 table)

**Conflicts of interest and source funding:** The study was funded by US Public Health Service Awards P01 HL40962, R01 HL101421, R21 HL081282, and T32 HL07560. The US Public Health Service had no role in the study design or implementation, data collection, statistical analysis, interpretation or manuscript composition.

## Abstract

**Objective:** In clinical trials, omega-3 fatty acid supplementation improves symptoms in psychiatric disorders involving dysregulated mood and impulse control, yet it is unclear whether in healthy adults omega-3 fatty acid supplementation affects mood, impulse control and the brain systems supporting these processes. Accordingly, this study tested the hypotheses that eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid supplementation reduces negative affect and impulsive behaviors in healthy adults and that these changes correspond to alterations in corticolimbic and corticostriatal brain systems which support affective and impulsive processes. **Methods:** Healthy volunteers (N = 272) consuming 300 mg/day or less of EPA and DHA were enrolled in a double-blind, randomized, placebo controlled clinical trial. Participants received either capsules providing 1000 mg of EPA and 400 mg of DHA versus identical appearing soybean oil capsules per day for 18 weeks. Negative affect and impulsivity were measured by questionnaire and ecological momentary assessment (EMA), as well as functional alterations in corticolimbic and corticostriatal brain systems evoked by standardized fMRI tasks. **Results:** There were no group-by-time interactions for any questionnaire or EMA measures of mood and impulsivity. Likewise, no group-by-time interactions were observed for fMRI responses evoked within corticolimbic and corticostriatal systems. **Conclusions:** In healthy adults with low intake of omega-3 fatty acids, moderate-dose supplementation for 18 weeks did not alter affect or impulsive behaviors, nor alter corticolimbic and corticostriatal brain functionality. **Trial Registration:** Trial number RCT00663871, URL: <https://www.clinicaltrials.gov/ct2/show/NCT00663871?term=NCT00663871&rank=1>

**Key words:** omega-3 fatty acids; clinical trial; negative affect; fMRI; impulsivity

**Acronyms used in text:** Eicosapentaenoic acid (EPA)  
Docosahexaenoic acid (DHA)

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Ecological momentary assessment (EMA)  
Omega-3 ( $\omega$ -3)  
functional Magnetic Resonance Imaging (fMRI)  
Beck Depression Inventory (BDI)  
Barratt Impulsiveness Scale (BIS)  
Buss and Perry Aggression Questionnaire (BPAQ)  
Blood oxygenation level-dependent (BOLD)  
Family-wise error rate (FWER)  
General linear model (GLM)  
Hemodynamic response function (HRF)

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## Introduction

Although omega-3 ( $\omega$ -3) polyunsaturated fatty acids are essential in the human diet, Americans consume these nutrients in minimal quantities<sup>1</sup>. There is cross-sectional evidence that deficiency of  $\omega$ -3 fatty acids associates with depression, hostility, aggressive behavior, and impulsivity in both psychiatric and non-psychiatric populations<sup>2-13</sup>. Clinical-trials also show  $\omega$ -3 fatty acids to have efficacy in some psychiatric disorders involving dysregulated mood and impulse control, including bipolar disorder<sup>14</sup>, borderline personality disorder<sup>15</sup>, major depressive disorder<sup>16-17</sup>, and attention deficit hyperactivity disorder<sup>18-19</sup>. However, meta-analyses of  $\omega$ -3 fatty acid clinical trials in psychiatric populations report the behavioral effects to be relatively small<sup>20-22</sup>, and  $\omega$ -3 fatty acid clinical trials assessing affect changes in non-psychiatric populations have produced mixed results. In some studies, for example,  $\omega$ -3 supplementation has been related to decreases in negative affect<sup>23-24</sup>, whereas other studies have found no effect of  $\omega$ -3 supplementation on negative affect or impulsivity<sup>25-28</sup>.

In view of existing evidence, it appears possible that  $\omega$ -3 fatty acids may alter brain functionality prior to emergent or reliably detectable behavioral changes. In support of this possibility, eicosapentaenoic (EPA) or docosahexaenoic (DHA) acids constitute 14-30% of fatty acids in the phospholipids of brain tissue, particularly neuronal gray matter, compared to less than 4% of fatty acids in plasma<sup>29-30</sup>. The types and amounts of dietary fatty acids affect neural phospholipid concentration, with exchange between serum and brain phospholipids in humans occurring by diffusion or active transport proteins<sup>31-34</sup>. Further, placebo-controlled human neuroimaging studies show that  $\omega$ -3 fatty acid supplementation alters neural activity during cognitively demanding tasks in children and adults<sup>35-37</sup>. In these studies,  $\omega$ -3 fatty acid

supplementation also improved cognitive performance, indicating that alterations in neural functionality could link ω-3 fatty acids with behavioral change<sup>35-37</sup>.

Accordingly, ω-3 fatty acids may relate to mood, impulsivity, and related behavioral processes, in part, by affecting brain systems supporting these processes; namely, corticolimbic and corticostriatal systems. Dysfunction of corticolimbic brain regions is associated with mood disorders, such as major depressive disorder and bipolar disorder<sup>38</sup>. Corticostriatal regions are also associated with appetitive, reward-dependent behavior and are strongly modulated by dopaminergic input<sup>39-40</sup>. Moreover, corticostriatal dysregulation is associated with disorders such as attention deficit hyperactivity disorder<sup>38</sup>. Animal studies have shown that fatty acid supplementation enhances production of neurotransmitters (e.g., serotonin and dopamine) that modulate corticolimbic and corticostriatal circuit function<sup>41-42</sup>. Additionally, cross-sectional human research has shown higher ω-3 fatty acid intake to associate with greater corticolimbic gray matter volume in healthy adults<sup>43-46</sup>.

Despite long-chain ω-3 being concentrated in the brain and appearing to have treatment efficacy for several psychiatric disorders, no human placebo-controlled study has yet examined whether the effects of EPA and DHA on negative affect and impulsive decision-making are accompanied, and potentially accounted for, by longitudinal alterations in corticolimbic and corticostriatal functionality. Nor have any placebo-controlled studies examined whether EPA and DHA affect corticolimbic and corticostriatal functionality in the absence of observable effects on affective and impulsive processes. The present study tested for these effects in a double-blind, randomized placebo-controlled design in a healthy adult sample by measuring pre- and post-intervention corticolimbic and corticostriatal circuit activity changes that were evoked by standardized affect and reward-based functional magnetic resonance imaging (fMRI)

tasks in conjunction with questionnaire and ecological momentary assessment of negative affect and impulsivity.

## Methods and Materials

### Participants

Participants were 134 men and 138 women between 30 and 54 years of age (see Figure 1 for Consort flow chart). A subset of participants ( $n = 121$ ) participated in a neuroimaging protocol at baseline and post-supplementation. All participants were drawn from the Adult Health and Behavior Project – Phase 2 (AHAB-II) project. AHAB-II recruited volunteers through mass mailings of recruitment letters to individuals selected from voter registration and other public domain lists from the greater Pittsburgh metropolitan area. Participants were free of major chronic medical disorders and consumed  $\leq 300$  mg/day of EPA+DHA, as estimated from a food frequency questionnaire, had no seafood allergies, and were not currently taking fish oil supplementation. Participants were also screened to exclude those with current diagnoses of DSM-IV Axis-I disorders using the structured Mini International Neuropsychiatric Interview<sup>47</sup>. Further details regarding screening criteria for the AHAB-II study are provided in the Supplemental Digital Content text.

### Study Design

Data were generated from an exploratory randomized, double-blind and placebo-controlled trial at a single site using supplemented dietary intake of long-chain  $\omega$ -3 polyunsaturated fatty acids in healthy mid-life adults. The trial was designed to test several putative primary prevention mechanisms, each linked to low dietary intake in published research: a) chronic systemic inflammation, b) low cardiac autonomic control, c) subtle

cognitive functioning, and d) behavioral measures of reward-related impulsivity and negative affect. The trial's first published report presented findings related to chronic inflammation, along with details of the protocol and study design, adverse events and study blinding<sup>48</sup>. The current report describes affect outcomes and substudy functional MRI results. The trial is registered on ClinicalTrials.gov (RCT00663871). The investigation was approved by the Institutional Review Board of the University of Pittsburgh, and was conducted between June 2008 and December 2011. All participants provided written informed consent and were paid for their participation.

## Intervention

Enrolled participants were randomized to one of two treatment conditions using R-Track, a secure web based program-wide clinical trial management system. Through minimization the marginal treatment distribution was balanced within levels of stratification factors of race (white vs. nonwhite), age (< 45 years, ≥ 45 years), and sex (male, female). Participants in the fish oil condition (n = 134) received a daily dose of two 1000 mg fish oil capsules, together providing 1000 mg EPA and 400 mg DHA. This dose was chosen because it has been shown to substantially increase EPA and DHA levels in serum or plasma<sup>49</sup>. Participants in the placebo condition (n = 138) received a daily dose of two identical appearing 1000 mg soybean oil capsules. The placebo capsules contained 1% fish oil, and both supplements contained mint flavor to help maintain participant blinding. Capsules were distributed in weekly blister packs to assist with adherence, each labeled with the week number and a code for treatment assignment. The assigned supplements were distributed by a study nurse blinded to condition immediately after randomization. Through this standardized

procedure, treatment allocation concealment was maintained. The treatment period was 18 weeks. Participants completed Ecological Momentary Assessment (EMA) recording days approximately two weeks before beginning supplementation. Due to scheduling reasons, follow-up EMA measures were completed approximately 16 weeks into supplementation, two weeks before the trial ended. Participants in the fMRI subsample began the fish oil trial on average two weeks after their baseline fMRI scan. Follow-up fMRI scans took place 16 weeks after beginning supplementation, two weeks before the trial ended.

### Fatty Acid Composition of Red Blood Cells (RBCs)

Fatty acid composition of red blood cells (RBCs) was determined pre- and post-supplementation by first preparing hemoglobin-free RBC ghost membranes as previously described and stored at -70°C until further analysis<sup>50</sup>. Methods for lipid extraction and quantitative determination of fatty acid distribution have been reported elsewhere<sup>47</sup>. The intra- and inter-assay coefficients were 1.98 % and 3.88 %, respectively, for fatty acid at mean concentration of >300 nmol/mL, and 3.57% and 8.62%, respectively, for fatty acid at mean concentration of <150 nmol/mL.

### Affect Measures

**Negative affect.** Negative affect was assessed using measures of depressive symptomatology and hostility. The original Beck Depression Inventory (BDI)<sup>51</sup> is a 21-item self-report measure having adequate discriminant validity<sup>52</sup>, test-retest reliability (test-retest reliability = .73 to .90)<sup>53</sup>, and internal consistency (Chronbach's alpha = .86) in clinical and non-clinical populations. Participants were asked to fill out the BDI reflective of their mood in the past week, including the day of testing. The cognitive, affective, and behavioral components of hostility were assessed using the 39-item version of the Cook-Medley Hostility

scale<sup>54-56</sup>. This measure has reasonable test-retest reliability (10-year test-retest reliability 0.74), internal consistency (Chronbach's alpha = .83) and construct validity<sup>56-57</sup>.

**Impulsivity and aggression.** The Barratt Impulsiveness Scale (BIS) is scored on a 4-point likert scale and consists of 30-items designed to assess control of thoughts and behavior (e.g., acts without thinking)<sup>58-60</sup>. The BIS has high internal consistency (alpha coefficients 0.79-0.83) and high reproducibility (reliability coefficient 0.85)<sup>59-60</sup>. Aggression was measured using the total score of the Buss and Perry Aggression Questionnaire (BPAQ)<sup>61</sup>, which demonstrates good test-retest reliability (test-retest reliability = 0.80) and internal consistency (Chronbach's alphas = .72-.85)<sup>61</sup>.

**Average Ecological Momentary Assessments.** Ecological momentary assessments (EMA) allowed for measurements of negative affect and impulsive behavior, throughout the daily course of living; providing a more comprehensive measure of the intensities of different emotions, rather than relying on a summary (i.e., by questionnaires)<sup>62</sup>. EMA assessments were completed using a 4-day monitoring protocol (3 working days and 1 nonworking day). The monitoring protocol consisted of two 2-day monitoring periods, usually one period at the beginning of the workweek and another at the end of the workweek, with at least one non-monitoring day in between. The mean (standard deviation) number of observations for each item pre- and post- supplementation were 54.4 (8.64) and 55.53 (6.49), respectively.

Participants carried a PDA (palm Z22, software: Satellite Forms) and answered a 43-item EMA questionnaire hourly on the PDA<sup>63-64</sup>. Each item asked participants to rate to what extent they were feeling the emotion. Answers corresponded with a 6-point Likert scale, 1 = strong "no" and 6 = strong "yes". Participants received extensive training and practice on the use of the PDA as well as feedback on compliance after completing a practice day. For this study, the

negative affect (consisting of upset, hostile, nervous, afraid, angry, lonely, sad) and anger expression (consisting of annoyed, yelled) scores were used.

### **Neuroimaging Tasks and Measures**

Participants engaged in two standardized affective and reward-based fMRI tasks designed to elicit activity changes in corticolimbic and corticostriatal brain systems<sup>65-67</sup> (for task details see Supplementary Text). In brief, the affective processing task required participants to match emotional facial expressions or simple geometric shapes to a target presented on a screen. The reward-based task required participants to make guesses that were rewarded or not rewarded with money.

**Image Acquisition and Preprocessing.** Functional blood oxygenation level-dependent (BOLD) images were collected on a 3T Trio TIM whole-body scanner (Siemens, Erlangen, Germany) using a 12 –channel phased-arrayed head coil. A small mirror was attached to the head coil to allow the participants to see the projector placed behind her or him while in the scanner. The functional BOLD image acquisition parameters can be found in the Supplementary Text.

The functional BOLD images were processed using Statistical Parametric Mapping software (SPM8; Wellcome Trust Centre for Neuroimaging, London, UK). Before analyses, BOLD images were realigned to the first image of the series by a 6-parameter rigid-body transformation, with the unwarped procedure in SPM being applied to adjust for geometric distortion due to movement. Realigned images were co-registered to each participant's T2-weighted structural image. Co-registered images were normalized by a 12-parameter nonlinear and affine transformation to the International Consortium for Brain Mapping 152 template

(Montreal Neurological Institute; MNI). Normalized images were smoothed by a 6mm full-width-at-half-maximum (FWHM) Gaussian kernel.

## Data Analysis

**Sample Size.** This study had a target sample size of 250 subjects (125 per treatment group) in order to achieve at least 0.80 power to detect differences in mean changes in negative affect and impulsivity from baseline to post-supplementation in terms of the standardized mean difference ( $d$ ) as small as  $d = 0.4$  between treatment groups. Sample size determination assumed a test-wise significance level of .01 when conducting two-sided hypothesis testing using linear contrasts within a repeated measures framework.  $\omega$ -3 supplementation clinical trials using fMRI are limited and have mainly been focused on cognitive function, however, positive effects in these studies were seen with relatively small sample sizes (largest sample, total  $N = 36$ )<sup>67</sup>.

**Demographics.** Baseline characteristics between groups were compared using one-way ANOVA for continuous data and chi-squared for categorical variables. Adherence to treatment was quantified as counts of returned pills and change in blood levels of EPA and DHA. Group differences in EPA and DHA over time were assessed using group ( $\omega$ -3 fatty acid, placebo) by time (baseline, follow-up) ANOVAs. An earlier paper from this trial demonstrated no group differences in adherence<sup>48</sup> between the two groups.

**Affect and impulsivity analyses.** Total scores for each of the questionnaires was used. Averages were created for EMA by creating an average for each day of assessment. Treatment differences between groups for all affect measures were examined using a series of group ( $\omega$ -3 fatty acid, placebo) by time (baseline, follow-up) ANOVAs. Intention-to-treat (ITT) analyses, including all participants randomized into the trial, were conducted using linear mixed effects

modeling where condition was included as a fixed factor<sup>19</sup>. These methods handle missing data points for the dependent variable without the use of listwise deletion.

**Post-hoc sensitivity analyses.** Mixed design ANOVAs were conducted examining if there were gender x treatment group x time interactions. Additionally, a series of bivariate regressions were run between change in DHA and EPA blood levels (level at follow-up minus baseline level) and affect variables (level at follow-up minus baseline level). Lastly, additional sensitivity analyses using the last observation carried forward method examining the effects of the trial on the main outcome variables were conducted. All affect related statistical analyses were performed using SPSS 21 (IBM Corp., Armonk, NY).

**fMRI analysis.** One-way ANOVAs were conducted to examine differences between the participants who had both fMRI and affect measures ( $n = 121$ ) compared to those who just had affect measures ( $n = 146$ ). After preprocessing, linear contrast images reflecting relative BOLD signal changes (i.e. Faces vs. Shapes; Reward vs. No Reward) were estimated for each participant. To this end, task conditions were modeled with rectangular waveforms convolved with the default SPM hemodynamic response function (HRF). Contrast images were then generated by general linear model (GLM) estimation using an explicit brain mask and incorporating outlier weighting using the Robust Weighted Least Squares toolbox (v3.1; <http://www.icn.ucl.ac.uk/motorcontrol/imaging/robustWLS.html>). Before estimation, low-frequency BOLD signal noise was removed by high-pass filtering (128sec cut-off). Finally, regression vectors derived from the realignment step were included in the GLMs to account for BOLD signal variance attributable to head movement. Individual contrast images were then submitted to group-level, one-sample t-tests. Main effects for task at each visit were conducted using one-sample T-tests comparing the conditions (Faces vs. Shapes; Reward vs. No Reward).

Group ( $\omega$ -3 fatty acid; placebo) x time (visit 1; visit 2) interactions were examined using a mixed design ANOVA for both tasks. We first performed *a priori* region of interest analyses focusing on the amygdala for the affective task and the ventral striatum for the reward-based task. The amygdala and ventral striatum are core components of the corticolimbic and corticostriatal circuits, respectively, and are most reliably engaged by these tasks<sup>65-66</sup>. These *a priori* analyses were supplemented with whole brain voxel wise analyses to examine corticolimbic and corticostriatal responses outside of our ROIs. See supplemental digital content for description of ROIs. Family-wise error rate (FWER) threshold of 0.05 and cluster threshold of  $k \geq 20$  were employed in all imaging analyses.

**Participant blinding and side effects.** Study staff assessed side effects by calling participants during Week 2 and Week 12, and at a brief appointment during week 7. At the end of the trial participants were asked to guess what group they were assigned to. Differences between groups in reported side-effects and correctly guessing their assigned group were analyzed using chi-square tests.

## Results

Of the original 272 participants, 255 completed the full trial<sup>1</sup>. There were no significant differences between groups in demographic characteristics (Table 1). There were significant group ( $\omega$ -3 fatty acid, placebo) by time (pre-, post-) interaction for red blood cell content of DHA and EPA. Specifically, supplementation increased EPA by 322% and DHA by 41% while levels did not change in the placebo group (Table 2).

## Measures of affect, impulsivity, and aggression

<sup>a</sup> Of the 255 participants who completed the full trial there was missing data on the following outcome measures: BDI (n = 1), CMH (n = 6), BIS (n = 3), BPAQ (n = 6), all EMA measures (n = 10).

Relative to placebo, the  $\omega$ -3 fatty acid group did not show any significant changes in questionnaire or EMA measures of negative affect or impulsivity/aggression,  $p$ 's  $> .20$  (Table 3). There were main effects of time for BDI and EMA hostility scores, wherein participants in both groups reported less hostility and depressive symptomology in post-test compared to pre-test values. The ITT, utilizing the entire sample who enrolled in the study ( $N = 272$ ), produced similar results for all dependent variables.

### Sensitivity Analyses

Additional post-hoc analyses examining potential interactions with gender for each of the measures of affect and impulsivity/aggression were conducted. There were no significant interactions. Change scores were calculated for both EPA and DHA blood levels and all questionnaire and EMA measures. Bivariate correlations between EPA and DHA change scores and change scores of all questionnaire and EMA measures were run. There were no significant correlations between EPA and DHA blood levels and any of the affective measurements. All results remained the same for main trial outcomes when using the last observation carried forward method.

### Functional measures

There were no differences on any demographic variables or affective measures at either pre- or post- intervention between the sub-sample participating in the fMRI protocol and those with only affective measures ( $p$ 's  $> .20$ ). Both fMRI tasks evoked the expected main effects (groups combined) on functional activity changes pre- and post-intervention (see Figure 2, Supplement Table 1). Relative to placebo,  $\omega$ -3 fatty acid supplementation did not change corticolimbic activity in response to the affective task in either ROI analyses or supplemental whole brain analyses. Similarly, there were no group-by-time differences in corticostriatal

activity changes in response to the reward-based task in either ROI analyses or supplemental whole brain analyses.

### Participant blinding and side effects

Overall, 33% in the fish oil group and 28% in the placebo group guessed their treatment assignment correctly ( $p = 0.58$ ). Compared to those on placebo participants receiving fish oil reported somewhat more “fishy belch or aftertaste” and “loose stool, bloating or gas pains,” but less “minty belch or aftertaste.” No serious adverse events were reported by any participants enrolled in the trial (completed or not). Additional detail on these analyses have been reported previously<sup>48</sup>.

### Discussion

Low dietary intake and low blood levels of ω -3 fatty acid consumption are associated with negative affect and impulsivity in both psychiatric and non-psychiatric populations<sup>2-13</sup>. Placebo controlled clinical trials demonstrate that fish oil supplementation can reduce symptoms in a number of psychiatric illnesses (e.g., depressive symptomology, aggression in borderline personality disorder)<sup>14-18</sup>. However, no study has yet examined if such supplementation reduces negative affect and impulsive decision-making in parallel with changes in the functionality of brain systems implicated in affect regulation and appetitive or reward motivation. This study aimed to address this possibility in a non-psychiatric sample of adult community volunteers not currently taking medication that could alter neural activity. The present study further used comprehensive measures, both questionnaire based and EMA, to assess affect and impulsivity before and after the intervention. While the ω -3 fatty acid group had significant increases in levels of both EPA and DHA as part of the trial, there were no significant changes in negative affect or impulsivity. Additionally, there were no intervention-

related alterations in task-evoked corticolimbic or corticostriatal activity in the ω-3 fatty acid group compared to placebo group.

To date, the current study is the largest ω -3 fatty acid supplementation trial to assess neural functionality pre- and post-intervention and the only such trial to our knowledge to use fMRI with affective-related changes in a non-psychiatric, adult population<sup>67</sup>. In a randomized, placebo-controlled study, children (N = 33) who received low (400 mg) or high (1200 mg) DHA supplementation increased functional activity in the frontal and occipital lobes during a cognitive task relative to children in the placebo group<sup>35</sup>. ω -3 fatty acid supplementation increased bold signal activity during a cognitively demanding task in older adults (N = 21) with memory impairment<sup>36</sup>. In a double-blind, counterbalanced, cross-over study of 13 young adults EPA-rich supplementation was associated with faster reaction times on a Stroop task and less functional activation in the anterior cingulate cortex and increased activation in the precentral gyrus during the task<sup>37</sup>. These initial studies suggest ω -3 supplementation can alter neural function during cognitively demanding tasks. However, a recent study demonstrated that in children with ADHD and age-matched controls ω -3 supplementation improved parent-rated attention, but did not alter measures of brain activity or cognitive control<sup>19</sup>. None of these studies assessed measures of affect or function of the corticolimbic and corticostriatal circuits.

It is somewhat surprising that previous studies have not sought to examine the neural correlates of ω -3 fatty acid related changes in affect. Several sources have linked fatty acid deficiency to negative affect<sup>2-7</sup>. Accordingly, the current study attempted to extend these observational and preclinical studies. Cross-nationally, low seafood consumption is associated with a high lifetime risk of depression<sup>2</sup>. Within populations, low fish consumption is associated with heightened odds of both depression<sup>3-4</sup> and hostility<sup>5</sup>. Low concentrations of specific ω -3

fatty acids in plasma and red blood cell membranes are found in those with clinically significant depression or depressive symptoms<sup>6-7</sup> and violent or aggressive behavioral tendencies<sup>8, 68-69</sup>. In two different cohorts of approximately 100 generally healthy volunteers, serum EPA and DHA concentrations co-varied with normative variability in depressive symptoms and self-reported impulsivity<sup>13, 70</sup>. Clinical trials assessing affect changes in non-psychiatric participants have produced mixed results. In some studies, ω -3 supplementation has reduced anger<sup>23</sup>, anxiety<sup>23-24</sup>, and depression<sup>23</sup>, while other studies have found no effect of on negative affect or impulsivity<sup>25-28</sup>. The current randomized and placebo-controlled ω -3 supplementation assessing negative affect impulsivity in a healthy population is the largest to date, and the first trial to assess both negative affect and impulsive behavior throughout the course of daily living pre- and post-intervention by EMA.

The trial is not without limitations. Participants consumed some ω -3 fatty acids before the trial began. They had a mean EPA+DHA intake near the US adult average of about 100 mg/day<sup>45</sup>. However, this amount is well below the recommended minimum consumption of 250 or 500 mg/day and did not differ between placebo and intervention group. It is possible that our observations were influenced by the exact dosage of EPA and DHA chosen here, as well as the duration of supplementation. There is some evidence that EPA and DHA have differential effects on different biological and behavioral outcomes<sup>71</sup>. Meta-analyses of trials of ω -3 on depression, for example, have suggested that EPA may specifically have therapeutic benefits for depression<sup>72</sup>. The current trial provided 1000mg/d of EPA and 400 mg/d DHA. The quantity was chosen to approximate the upper limit of what might be consumed through dietary measures (with greater amounts constituting “pharmacologic” doses), and the ratio reflecting that of fish oil capsules widely used internationally. We also note that ω -3 acid

supplementation has been reported to have measureable mood effects in patient populations<sup>72</sup>

(Hallahan et al., 2016). It is possible that due to slow brain turnover ω -3 acid supplementation may also result in similar outcomes among healthy adults if they given in larger doses for longer periods of time. In addition, mean scores for depressive symptomology were low and it could be argued that a “floor effect” had been reached. However, there was a range in total BDI scores at baseline (Range: 0-24) and follow-up (Range: 0-19) and in BDI change from baseline to follow-up (Range: -14 – 9). Similar ranges were seen for other main variable outcomes. Additionally, the concept of impulsivity could be seen as trait rather than state. Therefore, it could be argued that one cannot directly reduce impulsivity. However, recent short-term interventions have shown that certain interventions are able to reduce impulsivity. Finally, as study participants had to meet a large number of enrollment criteria, the findings may not be completely generalizable to the average adult population.

In summary, this randomized and placebo-controlled trial in healthy adults found no effect of 1400 mg per day of EPA+DHA supplementation for 18 weeks on corticolimbic or corticostriatal brain activity in healthy adults. Additionally, in line with some recent literature, there was no effect of supplementation on negative affect or impulsivity<sup>25-28</sup>. Future research may be needed to examine higher doses of supplementation and possibly psychiatric populations exhibiting clinical alterations in neural activity linked to dysregulated mood and impulsivity.

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Table 1. Baseline characteristics of randomized participants (N = 255)

<b>Characteristic</b>	<b>Fish Oil</b>	<b>Placebo</b>	<b>Difference Statistic</b>
Age	43.09 (7.54)	42.47 (7.03)	F (1,270) = 0.49, $p = .48$
Sex			$\chi^2 (1) = 0.001, p = .97$
Male	58	76	
Female	60	78	
Ethnicity			$\chi^2 (4) = 4.18, p = .38$
White	109	116	
Black	21	22	
Asian	1	0	
Multi-racial	1	0	
Other	2	0	
BMI	27.53 (5.92)	26.71 (4.60)	F (1,270) = 1.61, $p = .21$
Years of school	16.54 (2.77)	16.80 (2.68)	F (1,270) = .653, $p = .42$
completed			
WASI IQ	112.50 (12.61)	112.89 (12.55)	F (1,270) = .066, $p = .80$

Table 2. Blood levels of EPA and DHA pre- and post-intervention

	Placebo		Omega-3		Main group	Main time	Group x time
	Pre	Post	Pre	Post	effect, F	effect, F	interaction, F
EPA	0.44 (0.24)	0.41 (0.18)	0.45 (0.25)	1.45 (0.75)	169.59*	173.49*	199.44*
DHA	2.42 (0.89)	2.43 (0.98)	2.48 (0.87)	3.49 (1.19)	26.75*	57.62*	55.01*

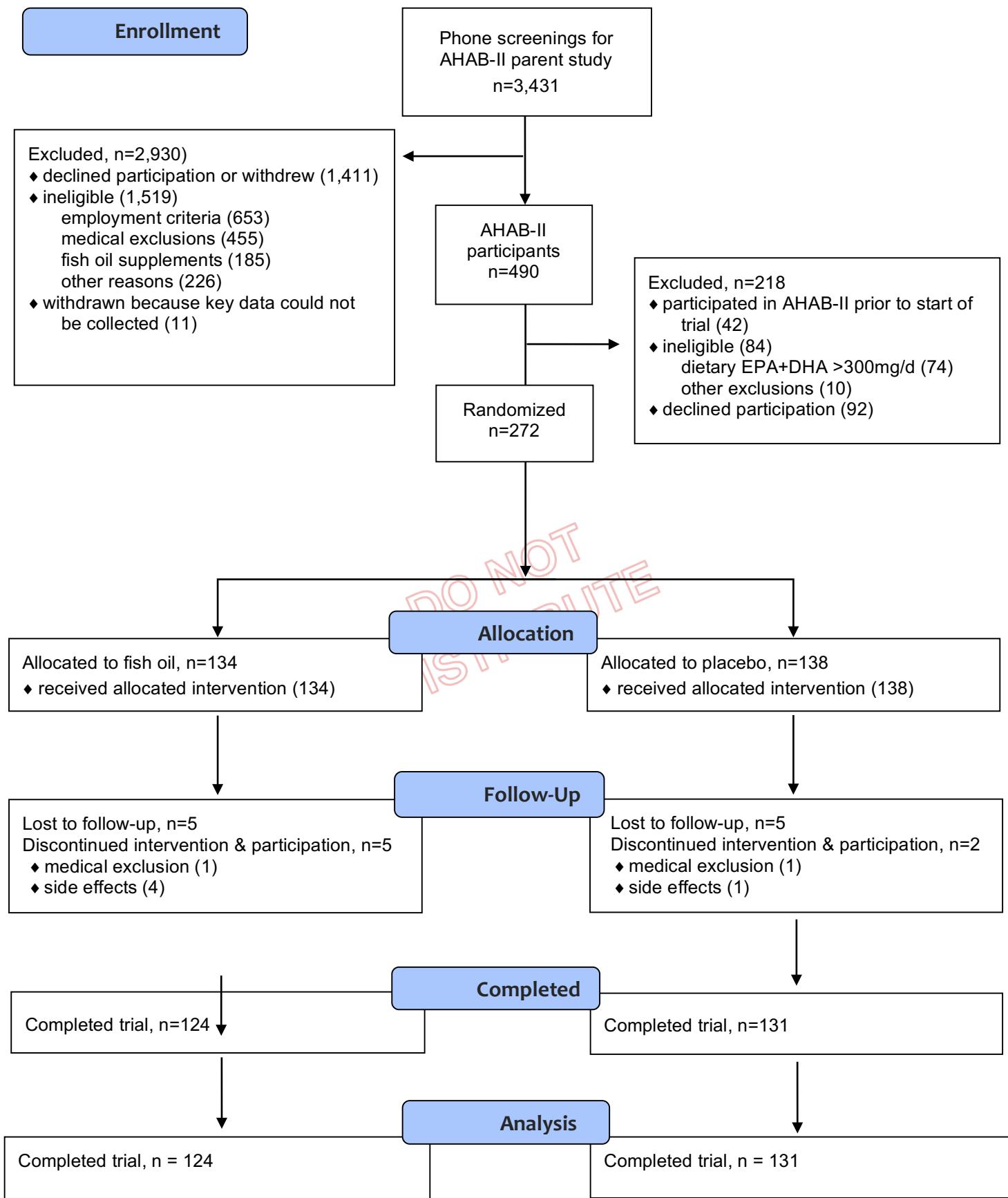
\*  $p < .05$



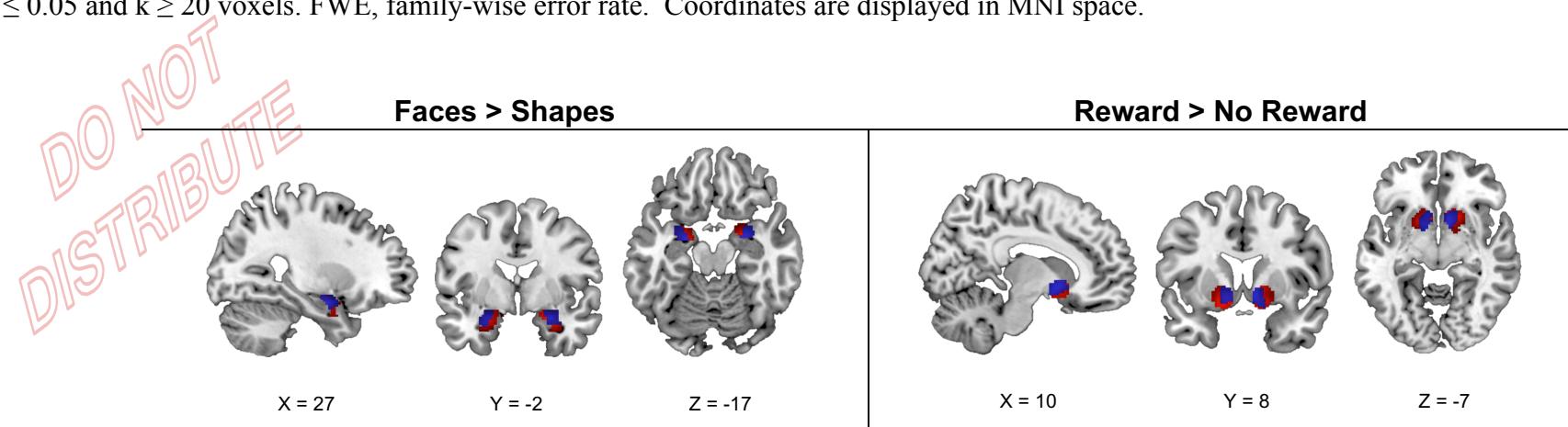
Table 3. Measures of negative affect and impulsivity/aggression pre- and post-intervention.

	Placebo		Omega-3		Main group	Main time	Group x time
	<u>Pre</u>	<u>Post</u>	<u>Pre</u>	<u>Post</u>	effect, F	effect, F	interaction, F
<b>Negative Affect</b>							
BDI <sup>a</sup> score	4.47 (3.86)	3.79 (3.43)	5.01 (4.87)	4.21 (3.81)	1.10	13.40*	0.07
CHM score	16.47 (7.34)	16.54 (7.21)	17.77 (7.92)	17.44 (7.59)	1.47	0.21	0.51
EMA negative affect	1.88 (0.70)	1.82 (0.73)	1.86 (0.71)	1.80 (0.68)	0.03	3.49	0.01
<b>Impulsivity/Aggression</b>							
BIS total score	59.51 (8.44)	59.38 (8.86)	59.23 (8.50)	60.10 (9.39)	0.29	0.004	0.20
BPAQ total score	56.07 (14.21)	55.51 (14.17)	58.33 (16.27)	57.63 (17.31)	1.35	1.23	0.02
EMA anger expression	1.50 (0.64)	1.53 (0.69)	1.52 (0.66)	1.49 (0.66)	0.90	0.02	1.13

<sup>a</sup> $p > .05$ <sup>a</sup>Original version of BDI used

**Figure 1.** Consort flow diagram of trial.

**Figure 2.** Clusters of relative BOLD activation at time 1 (in red) and time 2 (in blue) within the amygdala and ventral striatum regions-of-interest, as revealed by the Faces vs. Shapes (affective task) contrast and Reward > No Reward (reward task) contrast at FWE  $\leq 0.05$  and  $k \geq 20$  voxels. FWE, family-wise error rate. Coordinates are displayed in MNI space.





# CONSORT 2010 checklist of information to include when reporting a randomised trial\*

Section/Topic	Item No	Checklist item	Reported on page No
<b>Title and abstract</b>			
	1a	Identification as a randomised trial in the title	2
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
<b>Introduction</b>			
Background and objectives	2a	Scientific background and explanation of rationale	3, 4
	2b	Specific objectives or hypotheses	4, 5
<b>Methods</b>			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	6, Supplement
	4b	Settings and locations where the data were collected	6
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6, 7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6-10
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	10
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	6
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	6-7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	6-7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6-7

Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	6-7
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	10-13
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	11
<b>Results</b>			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	10, Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	6
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	31-33
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Figure 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	14, 15, 31-33
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	14
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	15
<b>Discussion</b>			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	18
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	18
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	15-18
<b>Other information</b>			
Registration	23	Registration number and name of trial registry	6
Protocol	24	Where the full trial protocol can be accessed, if available	6
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	20

\*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see [www.consort-statement.org](http://www.consort-statement.org).

sTable 1

(a) Brain areas exhibiting relative BOLD activation at time 1 identified by the 'Reward > No Reward' condition contrast in a random-effect model at FWER p < 0.05 and extent k>20 voxels.

Side	Region	BA	MNI Coordinates (peak)			t-value	Voxels
			X	Y	Z		
L	Superior frontal gyrus, orbital	11	-12	8	-8	7.99	818
R	Inferior parietal gyrus	40	46	-36	42	7.62	464
R	Caudate		12	14	0	7.32	523
R	Superior frontal gyrus, orbital	11	20	38	-18	7.3	502
R	Inferior temporal gyrus	37	50	-76	-8	6.98	111
L	Inferior occipital gyrus	19	-44	-78	-6	6.66	68
R	Middle frontal gyrus	9	32	44	40	6.59	85
R	Middle occipital lobe	19	32	-92	2	6.02	38
R	Inferior temporal gyrus	20	52	-50	-14	5.98	34
L	Superior frontal gyrus	6	-32	0	66	5.7	20
	Thalamus		0	-6	14	5.55	24
R	Middle frontal gyrus	46	46	42	16	5.4	29

(b) Brain areas exhibiting relative BOLD activation at time 1 identified by the 'Faces > Shapes' condition contrast in a random-effect model at FWER p < 0.05 and extent k>20 voxels.

Side	Region	BA	MNI Coordinates (peak)			t-value	Voxels
			X	Y	Z		
R	Middle temporal gyrus	18	22	-96	0	29.75	34560
L	Gyrus rectus	11	-2	52	-22	10.68	1142
R	Thalamus		6	-10	6	6.52	55
L	Postcentral gyrus	6	-62	-8	38	6.41	70
L	Middle temporal gyrus	39	-50	-70	16	6.31	99
R	Precuneus	30	4	-54	22	6.03	71

(c) Brain areas exhibiting relative BOLD activation at time 2 identified by the 'Reward > No Reward' condition contrast in a random-effect model at FWER p < 0.05 and extent k>20 voxels.

Side	Region	BA	MNI Coordinates (peak)			t-value	Voxels
			X	Y	Z		
L	Inferior parietal gyrus	40	-34	-52	48	7.99	2220
R	Inferior parietal gyrus	7	34	-60	36	7.53	2095
L	Precentral gyrus	9	-48	2	46	6.07	211
L	Temporal_Inf_L	37	-46	-64	-6	5.97	36
R	Middle frontal gyrus	46	48	44	14	5.85	37
R	Middle frontal gyrus	6	40	2	54	5.67	68
R	Precentral gyrus	6	36	0	40	5.62	38
R	Caudate		8	6	-8	5.6	30
L	Middle frontal gyrus	46	-44	38	26	5.48	26

(d) Brain areas exhibiting relative BOLD activation at time 2 identified by the 'Faces > Shapes' condition contrast in a random-effect model at FWER p < 0.05 and extent k>20 voxels.

Side	Region	BA	MNI Coordinates (peak)			t-value	Voxels
			X	Y	Z		
R	Inferior frontal gyrus, triangular	47	24	-98	8	15.72	14848
R	Superior frontal gyrus, medial	8	4	36	50	6.96	317
R	Gyrus rectus	11	2	50	-16	6.42	34
R	Superior frontal gyrus, medial	9	6	58	36	6.26	124
L	Middle temporal gyrus	21	-50	-4	-18	6	69
R	Middle frontal gyrus	6	36	4	60	5.69	42
R	Angular gyrus	7	34	-64	44	5.56	81
L	Middle occipital lobe	39	-38	-76	20	5.49	64

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## Supplemental Digital Content

**Participant recruitment and exclusion/inclusion criteria.** Participants had to: work at least 25 hours per week outside of the home (requirements for a sub-study examining association between occupational stress and CHD) and have above an 8<sup>th</sup> grade reading level. Additionally, participants could not: have a history of cardiovascular disease, schizophrenia or bipolar disorder (assessed via the M.I.N.I. International Neuropsychiatric Interview<sup>1</sup>), chronic hepatitis, renal failure, neurological disorder, lung disease requiring treatment, or stage 2 hypertension (SBP/DBP  $\geq$  160/100); have excessively consumed alcohol ( $\geq$  5 portions 3-4 times per week); be prescribed medications with autonomic effects or have used insulin, glucocorticoid, antiarrhythmic, antihypertensive, lipid-lowering, psychotropic, or prescription weight-loss medications; be pregnant; or be shift workers. Participants for the current trial met the following additional criteria: dietary EPA+DHA  $\leq$  300 mg/d, based upon the modified 2005 Block Food Frequency Questionnaire (NutritionQuest, Berkeley, CA, USA); no seafood allergies, not taking fish oil supplementation, and fasting serum triglyceride  $<$  400 mg/dl.

**Affective Task.** To elicit corticolimbic reactivity during one functional magnetic resonance imaging (fMRI) run of 390 sec, participants completed a standardized protocol comprised of four blocks of a facial expression matching task, two with angry faces and two of fearful faces, interleaved with five blocks of a shape-matching control task. This protocol evokes reliable individual differences in corticolimbic reactivity, especially in the amygdala. During the facial expression matching trials, participants viewed an array of three faces per trial (all of one target expression) and selected via button press on a fiber optic response glove one of the two faces (at bottom) that matched a center target face (at top). Each facial expression matching block consisted of six trials (4 sec presentation times; variable 2–6 sec intertrial interval; three trials of each gender). All expressions were from a standard stimulus set. In five blocks of shape-matching control trials, participants viewed three shapes (vertical and horizontal ellipses and circles) and selected one of two (at bottom) that matched a center target (at top). Each shape-

matching block consisted of six trials presented sequentially for 4 sec. All blocks were preceded by an instruction: “Match Faces” or “Match Shapes.”

**Reward Task.** To elicit corticostriatal activation during a single functional magnetic resonance imaging (fMRI) run of 342 s, participants completed a standardized protocol comprised of receiving positive (Reward) and negative feedback (No Reward) in the context of gaining or losing a monetary reward. In this blocked-design fMRI protocol, participants performed a variant of a card-guessing game developed by Delgado et al.<sup>2</sup>, which we have detailed previously<sup>3</sup>. For this guessing task, participants were pseudo-randomly presented with trials that signaled winning (Reward) or losing (No Reward) a monetary reward to be received at the end of the game. On each trial, participants had 3 s to guess (via button press) whether the value of a forthcoming visually presented playing card would be higher or lower than the value 5, with guesses made by index and middle finger button presses, respectively. After a guess, the value of the card was presented for 500 ms. The card value was then followed by Reward (a green upward arrow for a “correct” guess, signaling reward) or No Reward (a red downward arrow for an “incorrect” guess, signaling loss) for an additional 500 ms. After receiving feedback, participants viewed a crosshair fixation for 3 s, yielding a total trial length of 7 s. In all, participants completed 9 blocks of 5 trials as follows: 3 blocks of predominantly Reward (75% correct guesses), 3 blocks of predominantly No Reward (25% correct guesses), and 3 interleaved blocks of control (C) trials. For C trials, participants made alternating button presses during the presentation of an “X” (3 s), followed by an asterisks (500 ms) and a yellow circle (500 ms). As explained previously<sup>3</sup>, an incongruent trial was randomly presented within each feedback block (e.g., 1 of 4 trials during Reward blocks was incorrect, resulting in No Reward) to prevent participants from anticipating specific feedback for each trial and to maintain engagement, motivation, stimulus saliency, and unpredictability—thus decreasing the likelihood of habituation. All feedback and C blocks were preceded by a 2 s instruction (“guess number” or “press button,” respectively), resulting in total block lengths of 38 s each. All participants were unaware of the fixed outcome probabilities associated with feedback blocks, and they were falsely

led to believe that their performance would determine net monetary gains. Instead, all participants received US \$10.

**Image Acquisition parameters.** (FOV) = 200×200 mm, matrix = 64×64, repetition time (TR) = 2000 ms, echo time (TE) = 29 ms, and flip angle (FA) = 90°. Thirty-four slices were collected each 3 mm thick with no gap in an inferior to superior direction. A total of 172 BOLD volumes were collected throughout the duration of the task. For spatial co-registration of the BOLD images, T2 weighted neuroanatomical images were acquired over 2 min 6 sec by these parameters: FOV = 200×200 mm, matrix = 256×256, TR = 3000 ms, inversion time (TI) = 100 ms, TE = 11/101 ms, and FA = 150° (36 slices, 3mm thick, no gap).

### Description of Region of Interests (ROIs) masks

The amygdala ROI included the left and right amygdala regions labeled in the AAL atlas, as implemented in the WFU\_PickAtlas Toolbox<sup>4</sup>. It had volume of 1984 mm<sup>3</sup> for the right amygdala, with a center of mass at MNI x = 27.1 mm, y = - 0.6 mm, z = -18.8 mm. The volume of the left amygdala ROI was 1760 mm<sup>3</sup>, with a center of mass at MNI x = -23.5 mm, y = -2 mm, z = -18.5. For ROI analyses of the amygdala, the left and right masks were combined, creating a total ROI volume of 3744 mm<sup>3</sup>.

The ventral striatum has been defined to encompass the nucleus accumbens and adjacent portions of the putamen and ventral caudate<sup>5-8</sup>. Here, we employed a ventral striatal (VS) mask based on the anatomical probability map accessed from the THOR Center for Neuroinformatics (<http://neuro.imm.dtu.dk/services/jerne/ninf/neuroinformatics.html>)<sup>9</sup>. This particular VS probability map was constructed by a kernel-density meta-analysis<sup>10</sup> of publications included in the BrainMap database (<http://brainmap.org>). We refined our VS mask to include only gray matter voxels that had probability of being within the VS at a threshold of at least 50%. Anatomically, our resulting VS mask included the anterior portion of the ventral putamen and caudate, as well as some of the anterior globus pallidus. Dimensionally, the VS mask had center of mass of x: ±16mm, y: ±9mm, z: -6mm, with widths of 14mm, 18mm, and 14mm along the x-, y-, z-axes respectively. Note

that these boundary widths reflect totals for the mask along each axis, centered at the MNI coordinate anchoring each axis. The volume of our VS mask was 3744mm<sup>3</sup>.

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