Biological Psychology Manuscript Draft

Manuscript Number: BIOPSY-D-16-00081R1

Title: Yoga Practice Improves Executive Function by Attenuating Stress

Levels

Article Type: Research Paper

Keywords: HPA axis, task switching, Hatha yoga, working memory

Abstract: Background: Prolonged activation of the hypothalamus-pituitary-adrenal system is thought to have deleterious effects on brain function. Neuroendocrine studies suggest that brain exposure to higher cortisol concentrations contribute to cognitive deficits as we age. Mind-body techniques such as yoga have shown to improve stress levels by restoring the body's sympathetic-parasympathetic balance. The objective of this study was to determine whether yoga practice moderated the stress response resulting in improved executive function.

Methods: Sedentary community dwelling older adults (N=118, Mean age=62.02) were randomized to an 8-week yoga intervention or a stretching control group. At baseline and following 8 weeks, all participants completed measures of executive function, self-reported stress and anxiety and provided saliva samples before and after cognitive testing to assess cortisol.

Results: Yoga participants showed improved accuracy on executive function measures and an attenuated cortisol response compared to their stretching counterparts who showed increased cortisol levels and poor cognitive performance at follow up. The change in cortisol levels as well as self-reported stress and anxiety levels predicted performance on the running span task, n-back working memory and task switching paradigm (β 's=.27 to .38, p's \leq .05 for yoga and β 's=-.37 to -.47, p's \leq .01 for stretching control).

Conclusion: Eight weeks of regular yoga practice resulted in improved working memory performance that was mediated by an attenuated response to stress as measured by self-report stress and objective salivary cortisol measurements. This trial offers evidence for non-traditional physical activity interventions such as yoga that may be helpful in restoring HPA balance in older adults, thereby preventing cognitive decline.

*Highlights (for review)

Highlights

- An 8-week Hatha yoga intervention attenuated stress response in an older adult sample.
- The change in salivary cortisol predicted cognitive performance.
- Novel and preliminary evidence for the role of HPA axis in the yoga-cognition relationship.

BIOPSY-D-16-00081 - Revision 1

Response to Reviewers' comments:

Associate Editor Comments:

You will see that both reviewers point out the importance of your research and commend you for adding to the sparse literature in this field. They also both bring up some important concerns. If you choose to submit a revised manuscript, you will need to carefully address all of these concerns. Please pay particular attention to the first reviewer's major concerns about the use of broad terms, missing data, far-reaching conclusions and inconsistencies between your results and discussion, as well as the second reviewer's concerns about your statistical approach.

Thank you for the opportunity to revise the manuscript based on the reviewer comments and suggestions. We believe we have thoroughly addressed the reviewer concerns in the revision and it has substantially improved the quality and flow of the manuscript. We thank both reviewers for their insights and for recognizing the importance of this work, especially in the limited scientific research on yoga.

Reviewer #1: I enjoyed reading your manuscript and I believe it is an important addition to the scarce research on yoga. However, I have three major issues and a few minor issues that I suggest need to be addressed before the manuscript is ready.

The first major problem concerns the use of broad terms, missing data and far-reaching conclusions. Most importantly, it is not justified to state that yoga improves cognitive performance, while only working memory and task switching were your dependent variables and not even all of them changed significantly, not to mention the effect sizes. I believe that should be made clear, especially in the abstract, which is what will be read the most.

We agree with the reviewer about the use of "cognitive performance" which can mean a variety of cognitive functions. We have replaced cognitive performance with "executive function" which was the focus of the study. We have made this change throughout the manuscript as appropriate. Thank you!

Also, please make your hypotheses more explicit in the introduction (i.e. state which affective states and cognitive constructs are being focused on) and explain what makes your study a systematic investigation. In the revised manuscript, we have specified the two hypotheses and our measures in the Introduction Section as follows.

"We hypothesized that i) the yoga intervention would result in lowered cortisol levels for the yoga group at follow-up when compared to the stretching-strengthening control group, and ii) changes in salivary cortisol, perceived stress and state anxiety would predict improved accuracy on the executive function measures following the 8-week yoga intervention."

We had used the term systematic to emphasize the nature of the study – randomized trial - that allows a systematic investigation. However, since the RCT nature of the study has been stated earlier in the same paragraph, we have deleted the words 'systematic investigation' to avoid repetition – page 5.

Additionally, there are several inconsistencies between your results and the discussion section. For instance, cortisol predicted performance on only one of the two working memory measures that you used so you cannot state that it predicts working memory (pg.12, line 53). Similarly, cortisol predicted the

outcome not only on the task switching, but also on the n-back in the stretching group (p9.13, line 4). The results section claims that the anxiety score predicted the n-back results in the yoga group, while the discussion states that neither stress nor anxiety showed associations with cognitive measures. The beneficial effect of yoga on cognitive function is mentioned in the discussion section, but it is not reported in the results section. Please clarify that yoga reduced only post-stressor cortisol levels, while there is no change in cortisol levels if the stressful event is absent; yoga did not reduce stress nor anxiety better than stretching; yoga was better than stretching only in 1 out of 4 task switching aspects and only and in 2 out of 4 aspects of working memory; the strongest predictor of cognitive performance were cortisol levels in the stretching group for the task switching

The results have been revised to clearly address the two hypotheses stated in the above response. We agree with the reviewer that the yoga intervention did not lead to lowered cortisol (pre-stressor) levels (hypothesis 1 not supported). Yoga reduced post-stressor levels which predicted some improvements in executive function (hypothesis 2 only partially supported). In addition, for the stretching and strengthening group increased post stressor cortisol levels predicted poor performance on the executive function measures.

This has been revised in the manuscript on page 13 – discussion.

The second major issue is insufficient information about study procedures and instruments. It would be useful for future studies to know more about your yoga and stretching instructors (i.e. how many were there per group, their gender and experience). Also, add the ratios of asanas, breathing exercises and meditation in the yoga group or, if possible, add a table with a description of each session. Regarding data collection and analysis, it is unclear if all participants have undergone cognitive testing in one group or if they were in separate rooms because you say that it was at the same time of the day (pg.8, line 17). Clarify that the diluted cortisol samples were reanalysed (pg.11). Add more information about PSS because it is not clear that it measures stress during the past month, instead of life-time stressful events (Pg. 9). Explicitly write that you only used the state subscale of STAI.

We have included more details about the instructors and the nature of the groups in the revision – page 6. We interviewed and hired experienced certified instructors for the trial but do have an official record of their years of experience. The instructor: participant ratio and gender of the instructors are reported in the revision – page 6.

We have also referenced a previously published sample yoga and stretching exercise session – page 6.

Cognitive testing was conducted in groups of 5-7 participants in a computer lab. Each participant was seated at an independent cubicle as they completed the measures. This information has been included in the revision on page 7.

Re-analyses of the diluted cortisol samples is not stated in the revision – page 12

For both PSS and STAI-S – the measures paragraphs have been revised to explicitly state the time period stated as part of the instructions – page 9.

Minor issues are related to clarity, simplicity and punctuation. Firstly, I suggest to revise the "deleterious effects of HPA axis on brain function", which is also an opening line in your abstract and is repeated three times in the manuscript as a well-known fact. Although it certainly is an eye-catcher, it basically states that chronic stress is bad for the brain, thus I think you should be more specific regarding what aspects of brain function get damaged and support it with more references and expand the existing ones (e.g. Lupien and colleagues - which aspects of mental health and cognition get impaired and include effect sizes).

We have elaborated on the findings of Lupien and colleagues and cited studies that link cortisol and elevated glucocorticoid levels to specifically, executive function and memory. The references cited are reviews/ nature reports and do not examine effect sizes, but identify brain regions and functions that show impaired functioning. We have included these references in the revision as well – page 3 introduction, paragraph 2.

The potential mechanisms of the effect of yoga on cognition is too speculative and not supported with references (pg. 12, lines 33-49). Similarly, the absences of the significant betas is justified by the length of the intervention and participants' health, but I do not see the logic behind this. Also, the inconsistency between the informal feedback and the results is more likely to be due to the social desirability bias (Pg. 13, line 14).

We have revised this section of the discussion and stated the results in the context of the hypotheses as suggested by the reviewer (in your earlier comment).

We agree with the reviewer that other subjective factors such as social desirability bias or outcome expectations may have influenced the results. We were trying to communicate that the measure we used may not be the best or optimal for this sample (high functioning, healthy) and duration of the intervention (only 8 weeks). Perhaps objective physiological measurements of anxiety, stress or mood will serve as unbiased measures, and may more accurately and cleanly capture the true effects by overcoming bias or expectations that can influence participant responses on paper pencil measures. We have included this point in the discussion on page 14 – paragraph 2. In the conclusions, we have restated the need for future research with more sophisticated state of art measurements for both cognition and stress, anxiety – page 16.

The sentences that should be paraphrased for simplicity and clarity are: pg. 3, line 11 (also add hyphen "both short-term and long-term interventions"; pg.3, line 16 (working memory and attentional control are parts of executive function), pg.3, line 45 (mention the effect sizes as they are usually small to moderate). A few instances to change the punctuation: pg.3, line 23 ("downregulating") and line 26 (hypothalamic-pituitary-adrenal).

Thank you! We have made these edits to improve the clarity of the statements in the revision. We agree with the reviewer that (working memory and attentional control are parts of executive function) – in the review cited in the introduction, the effect sizes were calculated for i) executive function (working memory, inhibitory control and task switching), ii) attention and processing speed, and iii) memory. We have therefore reported those effect sizes and addressed the small to moderate nature of the effect as suggested by you.

Reviewer #2: Comment: I commend the authors for focusing on the potential and promising effects of yoga on stress and cognition. Yoga is indeed non-invasive, easy to apply and can be easily implemented in clinical and non-clinical settings. Given the sparse literature in this research field additional information about the biological mechanisms underlying the benefits of yoga is urgently needed. Considering the significance of this study, I would highly recommend that the authors provide a more robust rationale for their study, including a better structured outline of the study procedure, and consider or discussing additional/alternative analyses (for instance mediational analyses) in their discussion section. It's only by conducting this kind of analyses that one could conclude that "working memory was mediated by an attenuated response to stress as measured by self-report stress and objective salivary cortisol measurements".

Introduction

1. Please provide additional references related to fMRI and EEG findings following yoga interventions, with and without behavioral measures. This would provide evidence of the presence of neural mechanisms underlying the benefits of yoga.

We have included 4 studies that examine yoga/mindfulness and its association with gray matter volume in different regions of the brain. The studies are mostly cross sectional in nature that examine yoga practitioners or 'experts' with age-, sex- matched controls. Only one study (reference # 52) has examined changes in brain volume before and after a yoga intervention in older adults.

2. If possible, please provide some references linking physiological measures (e.g. heart rate, skin conductance, respiratory activity) and yoga. Stress and mood are strongly related to the autonomic system.

We have included studies examining yoga and autonomic system in the discussion on page 14 – paragraph 2. These include HRV, vagal tone and respiratory function.

3. Since we talk about stress in this paper some references to yoga interventions in individuals with mood disorders and anxiety could be helpful too to build an argument in support to studying the effects of yoga. The link between mood and yoga could be a potential future direction to be included in the discussion section.

Stress, anxiety and depression have been among the most commonly studied outcomes in the context of yoga. We have cited the reviews/meta-analyses that have summarized the effects of yoga on stress, anxiety and depression in the introduction of the manuscript (References 12-14). On the discussion we have re-stated the need to examine the effects of yoga in clinical samples as future directions - page 17, last paragraph.

4. There are several types of yoga and meditation techniques are closely related to yoga practice. Did the authors plan to differentiate the benefits of yoga from those of meditation? If so what was the rationale for choosing one type of yoga over another in this study.

For this study we chose a Hatha style of yoga since it is the most widely practiced form of yoga in the US. We agree that the different styles of yoga may have differential health benefits. There are also various

mindfulness and meditation practices (independent of yoga) which have shown improved stress outcomes. To address these points, we have included the following statements in the discussion:

Page 14: "Other awareness-based behavioral interventions such as mindfulness have shown to moderate the neural-cortisol association 53"

Page 15: "Future studies should replicate these outcomes across different age groups, among clinical populations and examine the dose-response relationship of varied durations and frequencies of yoga practice."

5. The authors should discuss the role of age, gender and hormones in the release of endorphins and response to yoga or stretching in general. This would make the introduction/rationale of the study more compelling and robust.

We have included a reference (#49) that Page 14-15,

6. Is there any longitudinal data related to the long-terms calming effects of yoga/exercise?

We did search the literature to examine this. However, to our knowledge, there are no longitudinal studies that have examined the long-term effects of yoga practice on physical or mental health. Most are intervention studies with clinical or non-clinical populations. The reviews about yoga for anxiety and stress cited in the introduction and discussion are the only scientific evidence for yoga effects on these outcomes. (References #8, 9, 10 in the manuscript).

7. Methods: I noticed that there were more females than males in both groups. Was this intentional and/or does it reflect the reality of a gender disparity in yoga classes for instance?

This was not intended. Both men and women were recruited for participation but the response rates from males were lower, it may perhaps reflect the gender disparity as you suggested! We have included this point in the discussion of the revised manuscript – page 16.

8. Could the authors provide additional information on exclusion/inclusion criteria, specifically in terms of the psychiatric history of the participants? If this was not exclusion criteria could the authors mention how many individuals suffered from mood disorders or anxiety.

We collected participants' personal health history information at baseline. The question specific to psychiatric history asks: "Have you been diagnosed with an emotional or mental health disorder?" – Yes/NO. "If yes, please list diagnosis and year". This was self-reported by the participants. Reviewing the raw data, we have no participants who reported diagnosed mental health problems. We did screen participants for depression using the Geriatric Depression Scale (as reported in the Methods on page 5). Although the question listed above was not part of the "exclusion" criteria, we have included this important point in the discussion section of the manuscript as we address the need for more studies with other clinical populations.

We understand the reviewer point of view for systematically recording any existing mood/anxiety/stress related psychiatric illnesses in the context of this study and its outcomes. Since our sample consisted of

healthy community dwelling older adults, we probably did not see any participants with self-reported diagnosed mental health conditions. But this certainly remains a population to explore – alongside other clinical populations that may benefits from yoga based interventions for improving not only cognitive health but also lower anxiety/stress and depression.

We have included these points in the revised discussion on page 15.

9. I would highly recommend that the authors provide a diagram showing the temporal sequence of administration for cognitive tasks and salivary measurements. This could be placed within the "Procedure" section.

Thank you for this feedback. The manuscript currently has 1 figure and 4 tables. Given the publication guidelines and word limits, we have included a brief statement that clearly states the sequence of measurements in text – page 7. In summary, participants provided a pre-stressor saliva sample – completed cognitive testing – provided a second post stressor saliva sample and completed the self-report questionnaires.

10. Could the authors please mention whether the description or general presentation given to participants at the beginning of classes was standardized? Did the instructors mention that yoga/stretching have benefits or encourage people to relax? Did the instructor read/memorize a script to read at the beginning/during each session? How were times/days of sessions selected?

Throughout the course of the study, all research staff emphasized the study goals as "examining the mental and physical health benefits of yoga and stretching-strengthening exercises". Within the structured group exercise sessions, instructors were not given scripts to read out/share with the participants. Cues for relaxation were given by instructors only if it was part of the exercise (e.g. savaasana – corpse pose in yoga and relax in between sets of exercises for stretching-strengthening group). Basically, instructors for both groups were blinded to the study outcomes and asked to lead the classes like they would in a community health center/gym/studio to maximize generalizability. Dates and times for the intervention were determined based on availability of exercise studios and rooms and instructors.

These points are addressed in the revision on page 6, under 'procedures'.

11. Statistics: please mention if distributions, outliers and missing data was checked for all the variables. Since the authors mention that they used an ANCOVA could they please clarify which covariates they included. Could the authors explain why they did not consider including demographic data to the regression models (e.g. IQ)? Also did they consider conducting ordinal regressions using the entire sample and including a group variable coded as 1=yoga, 2=stretching. Did the authors consider conducting mediation analyses to check direct and indirect effects of anxiety and cortisol on cognition? If not, did they consider doing this and could they mention in the discussion which alternative analyses they could have conducted. Could the authors mention what their statistical threshold was, and if multiple comparison correction methods were used?

We have elaborated on the distributions and raw processing of data in the data analysis section – page 11. The covariates used were reported as a footnote for table 2, but now have been incorporated in text on page 11.

We used the enter method for regressions to determine how much each predictor variable uniquely contributed to the variance in cognitive outcomes. This serves as a conservative estimate for regressions with multiple predictors. We have specified the regression type in the data analysis section in the revision – page

We agree with the reviewer that there are several methods to examine the data. Given the pilot nature of this study and the small sample size and the number of variables, we did not conduct mediation analyses or test of path models. We did not choose ordinal regression since our dependent variables are all continuous. Following discussions with all authors, we determined multiple regressions to be best suited for our limited sample size, characteristics of the variables and the pilot nature of the study. We hope that this study will serve as a platform for future research in this field to use state of the art measures and technology combined with larger RCTs that will allow for robust outcome measurement and definitive conclusions about mediation and model development.

We have included the need for larger samples and systematic path analyses or mediation analyses to develop and test models in future research – revised manuscript on page 17.

12. Were cognitive tasks age-appropriate or was there a ceiling effect? I noticed that accuracy was elevated (above 80%). I would address this point in the discussion.

The scores on the cognitive measures are comparable to other studies with similar populations. We do observe a relatively high performance since these individuals are healthy with no cognitive deficiencies. This in turn explains the smaller effect sizes we observe in this group. We have alluded to the healthy, high functioning characteristics of this study sample in discussion on page 13.

13. Did participants change their exercise and nutritional lifestyle during the yoga/stretching course? Did authors check for health or weight changes? I would discuss this in the discussion too.

At the start of the intervention, all participants were asked to continue with their everyday routine outside of the intervention. The research staff instructed all participants to self-report any significant changes to their lifestyles. No such changes were reported by participants in either groups. This maybe in part due to the short duration (8-weeks) of the intervention. We objectively assessed weight at baseline and post intervention – We observed no significant mean changes for either group. We have addressed this in the discussion on page 15. We agree with the reviewer, more specifically for longer RCTs, systematically documenting changes to diet, exercise and other health behaviors (smoking or drinking patterns, etc.) should be done periodically throughout the course of the intervention.

14. When presenting the regression results I would recommend that the authors provide R2 (in the table) and mention how independent variables were entered into the analysis (enter, stepwise, forward). We have included the R² in the revision. We used the enter method to determine how much each predictor variable uniquely contributed to the variance in cognitive outcomes. We have specified the

regression type in the data analysis section in the revision. R^2 values are reported in the revised tables 3 and 4.

Minor details

1. I am not sure if the use of the word "sedentary' refers here to individuals reporting low levels of physical activity or is it based on official Physical Activity Guidelines.

We recruited individuals who reported less than 2 days/week of structured physical activity over the past 6 months. In most cases, they reported no structured activity at all and hence volunteered for this physical activity trial. We believe that "sedentary community dwelling" best describes this population as these participants reported very low to no participation in physical activity prior to enrollment in the study.

2. In the abstract please replace "Mage" with "M age"

We have changed this to "Mean age" in the abstract.

Yoga Practice Improves Executive Function by Attenuating Stress Levels

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This study was registered at Clinical Trials.gov (NCT01650532) and conducted at University of Illinois, Urbana-Champaign.

Abstract

Background: Prolonged activation of the hypothalamus-pituitary-adrenal system is thought to have deleterious effects on brain function. Neuroendocrine studies suggest that brain exposure to higher cortisol concentrations contribute to cognitive deficits as we age. Mind-body techniques such as yoga have shown to improve stress levels by restoring the body's sympathetic-parasympathetic balance. The objective of this study was to determine whether yoga practice moderated the stress response resulting in improved executive function.

Methods: Sedentary community dwelling older adults (N=118, Mean age=62.02) were randomized to an 8-week yoga intervention or a stretching control group. At baseline and following 8 weeks, all participants completed measures of executive function, self-reported stress and anxiety and provided saliva samples before and after cognitive testing to assess cortisol.

Results: Yoga participants showed improved accuracy on executive function measures and an attenuated cortisol response compared to their stretching counterparts who showed increased cortisol levels and poor cognitive performance at follow up. The change in cortisol levels as well as self-reported stress and anxiety levels predicted performance on the running span task, n-back working memory and task switching paradigm (β 's=.27 to .38, p's \leq .05 for yoga and β 's=-.37 to -.47, p's \leq .01 for stretching control).

Conclusion: Eight weeks of regular yoga practice resulted in improved working memory performance that was mediated by an attenuated response to stress as measured by self-report stress and objective salivary cortisol measurements. This trial offers evidence for non-traditional physical activity interventions such as yoga that may be helpful in restoring HPA balance in older adults, thereby preventing cognitive decline.

Keywords: HPA axis, task switching, Hatha yoga, working memory

Introduction

Mind-body interventions such as yoga are promising approaches for improving cognitive function in older adults. In a recent meta-analysis of the yoga-cognition literature, Gothe & McAuley¹ found that both, acute (short-term) and chronic (long-term, interventions) practice of yoga was associated with small to moderate improvements in cognition, including executive function processes (g=.27), attention and processing speed (g=.29) and memory (g=.18). In spite of the growing interest and evidence for the cognitive effects of yoga, there have been no systematic investigations of potential mechanisms that may underlie this relationship.

Preliminary evidence suggests that yoga has a downregulating effect on both the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis in response to stress². Stress, in general, may lead to anxiety and depression, involving chronic sympathetic activation and activation of HPA³. Research has also shown that prolonged activation of the HPA system has detrimental effects on brain function^{4,5} and neuroendocrine studies suggest that brain exposure to higher cortisol concentrations contribute to cognitive deficits as we age^{6,7}. In a recent review, Lupien and colleagues⁸ concluded that chronic exposure to stress hormones, whether it occurs during the prenatal period, infancy, childhood, adolescence, adulthood or aging, has an impact on brain structures involved in cognition and mental health. They concluded that the frontal lobe, which has been repeatedly associated with executive functions^{9,10}, seems to be sensitive to glucocorticoid effects during human aging. A longitudinal study also found that elevated plasma glucocorticoid levels over years in older adults negatively correlated with hippocampal volume and memory¹¹.

Yoga has been shown to have immediate psychological effects including decreased anxiety¹², depression¹³, stress¹⁴, and improved QOL and well-being¹⁵. Cortisol, a stress hormone and an end-product of the HPA axis has been assessed in some yoga studies and lower cortisol

levels have been associated with improved affect^{16–18}. These studies suggest that yoga has an immediate quieting effect on the SNS/HPA axis response to stress. La Forge¹⁹ suggested that at least part of the power of yoga's effects is its ability to help participants cultivate better stress management skills. While the precise mechanism of action has not been determined, it has been hypothesized that some yoga exercises cause a shift toward parasympathetic nervous system dominance²⁰, which may impact cognitive performance.

Salivary cortisol is an excellent indicator of plasma-free cortisol, increasingly used to assess hypothalamic-pituitary-adrenal axis secretory activity and rhythm^{21,22}. A randomized controlled trial (RCT) comparing African dance and Hatha yoga showed that cortisol levels decreased after the Hatha yoga session in healthy college students and the changes in positive affect and change in cortisol were negatively correlated 16. Kamei and colleagues 17 also reported similar reduction in serum cortisol after a 50 minute yoga session in a sample of 8 yoga instructors. A 3-month Iyengar yoga program for middle aged women also showed decreased salivary cortisol and improvements in stress, anxiety and well-being²³. Similar results were observed in a 6-week RCT with breast cancer outpatients²⁴. Decreased anxiety, stress, depression and salivary cortisol were reported by the yoga group patients as compared to the usual care controls. Other analogous mind-body techniques, including mindfulness based stress reduction²⁵, transcendental meditation²⁶ and tai-chi²⁷ have been shown to elicit a similar relaxation response along the SNS and HPA axis activity. Anxiety remains a critical moderator within the field of cognition because it is associated with adverse effects on two central executive functions involving attentional control: inhibition and set shifting²⁸. It is plausible that changes in stressrelated physiological systems and affect may be among the mechanisms that lead to improved cognitive performance following yoga practice.

In a previously published RCT, we reported the beneficial effects of this 8-week Hatha yoga intervention on executive functions – specifically the domains of working memory and task switching among sedentary, but healthy older adults²⁹. In light of these primary outcomes and the literature suggesting changes in the SNS and HPA axis activity, herein we investigate cortisol and self-reported stress and anxiety as potential mediators of the yoga-cognition relationship. We hypothesized that i) the yoga intervention would result in lowered cortisol levels for the yoga group at follow-up when compared to the stretching-strengthening control group, and ii) changes in salivary cortisol, perceived stress and state anxiety would predict improved accuracy on the executive function measures following the 8-week yoga intervention.

Methods

Participants

Figure 1 shows the flow of participants through the trial. Detailed recruitment, screening and trial procedures have been previously reported 29,30 . Briefly, participants (N = 118, males = 26; mean age = 62.0 ± 5.6) were low active, healthy community dwelling older adults who volunteered to participate in an 8-week exercise based RCT. They were recruited between March 2012 and January 2013 through University of Illinois list serves, fliers, community groups and postings and screened to determine eligibility for the study. In order to participate in the trial, participants had to be between 55-79 years of age, English speaking, report being sedentary for at least the previous six months (<2 days per week of structured physical activity), have no ongoing regular yoga practice, be able to get up and down from the floor, have good or corrected vision (20/40), and be willing to be randomized into one of two exercise groups. Since the primary outcomes included neurocognitive variables, all participants were screened to rule out possible cognitive deficiency using the modified Telephone Inventory for Cognitive Screening 31

with a cut-off of 21, and for depressive symptomology using the shortened version of the Geriatric Depression Scale³² which has a cut-off point of ≥ 5 . Individuals who responded to advertisements completed a telephonic prescreening interview to determine whether they met these inclusion criteria and consented to have their physician be contacted for approval to participate in the exercise study. Individuals were excluded from participation if they did not meet the above criteria or their physician refused to provide approval for participation.

Procedures

After obtaining the physician's consent, participants were scheduled to complete baseline cognitive assessments and provided with a questionnaire to collect basic demographic information. After all baseline data were obtained, participants were stratified by age and sex and randomized into one of two groups: Hatha yoga or stretching-strengthening exercise. For both groups, classes met 3 times a week for an hour over the 8-week period. Dates and times for the intervention were determined based on availability of exercise studios and instructors. Different instructors led the two arms of the trial; yoga was supervised by two certified yoga instructors (1 male and 1 female) and the stretching-strengthening sessions were led by two certified fitness trainers (both female). Instructors in both groups were blinded to the specific study outcomes and were encouraged to lead the classes like they would in a community health center or gym. The instructors in both groups monitored participants' attendance over the course of the 8-week program and the instructor to participant ratio was on average 1:15 per class. A sample exercise session for both groups has been previously reported³⁰.

Hatha yoga group: The yoga intervention was designed as a beginner but progressive 8week program involving supervised group sessions led by certified yoga instructors. Hatha style of yoga was chosen for this intervention since it is the most widely practiced form in the United

States. Within the 3 sessions each week, new postures, breathing and meditative exercises were introduced on Mondays, Wednesday's class helped to further develop concepts while reviewing and adding some new postures. Friday's class was dedicated to reviewing the week's material to ensure the yoga practice was smooth and progression was gradual and steady through the 8-week intervention. Yoga practice was done barefoot and participants used yoga mats, yoga blocks and belts during the sessions. Some of the yoga exercises practiced by the participants included: warrior pose, easy lotus pose, sun salutations, deep breathing exercises such as alternate nostril breathing, and meditative exercises focusing on thoughts, breath and mantra repetitions.

Stretching and strengthening control group: This group served as an active exercise control group to the yoga intervention for the period of 8-weeks. Participants received the same level of interaction with the research staff and same opportunities for social interaction with others in their group and the certified trainer as the Hatha yoga group. No Hatha yoga postures, breathing and meditative exercises were practiced by this group ensuring that it served as a true attentional exercise control to the yoga intervention. Participants in this group also met on the same days and times at a separate exercise studio, to engage in stretching and strengthening exercises that met the Center for Disease Control (CDC) anaerobic recommendation³³. Each class consisted of a warm up and a cool down and the participants completed 10-12 repetitions of 8-10 different exercises led by a certified personal trainer. Similar to the yoga intervention, the exercises were progressive over the course of 8-weeks and resistance bands, blocks and chairs were used to perform the exercises which included bicep curls, tricep extensions, flutter kicks seated in the chair etc. These exercises targeted all major muscle groups but did not involve any yoga based postures, breathing or meditative elements.

Measures

Demographics and Testing Day Procedures

Participants completed a basic demographic questionnaire including age and date of birth, sex, marital status, occupation, income, education and past experience with yoga. On the day of testing, participants arrived in groups of 5-7 in a computer lab and completed the study measures at independent cubicles. The testing started at 2:00 pm and participants provided the pre-stressor saliva sample. They were then instructed to complete the cognitive measures on the computer and provided a second post-stressor saliva sample at 2:40 pm and complete the self-reported stress and anxiety questionnaires. The same sequence and time was maintained for all participants at baseline and post-intervention.

Salivary Cortisol

An extensive meta-analysis³⁴ suggests that significant cortisol responses were elicited by cognitive tasks (d=.20), effects were significantly greater for assessments obtained in the afternoon (d=.46) and 21-40 minutes after the onset of the stressor (d=.38-.41). Capitalizing on these findings, in the present study, two saliva samples were collected at each time-point during the cognitive testing appointments that were held in the afternoon. The cognitive assessments were used as psychological stressors to elicit a stress response, and saliva samples before beginning the session (pre-stressor) and 40 minutes into the session (post-stressor) were collected from the participants. This response to stressor (pre-stressor cortisol levels/post-stressor cortisol levels) at follow-up was used as a predictor for analyses. The passive drool procedure was employed which involved allowing the subject to pool saliva in his/her mouth (~1.5ml) and then send it through a short straw into a vial. Because cortisol production has a circadian rhythm^{22,35} the pre and post cognitive assessments and saliva sample collection were conducted at the same time of the day (2:00 pm pre-stressor and 2:40 pm post-stressor) for all participants at both time-

points. Samples were stored at -80° C upon collection. A high sensitivity cortisol enzyme immunoassay kit (Catalog No: 1-3002 (5PK 1-3002-5), Salimetrics Inc., State College, PA) was used to assess cortisol levels in the saliva samples. All saliva samples for a participant were assayed together, as per the kit manufacturer's instructions, in duplicates by a blinded analyst (RK). The end-point absorbance for the assay was measured at 450 nm on a Synergy-2 plate reader (Biotek, Winooski, VT). The detection range for the assay was 0.012- $3.000~\mu g/dL$. Samples exceeding $3.0~\mu g/dL$ (very high cortisol concentrations), were re-analyzed in duplicates after dilution (10x). A 4-parameter sigmoid minus curve fit was examined for each plate based on the standards and controls (R² range: .999 to 1). The intra- and inter-assay coefficients of variation (CVs) were < 15% as recommended by the manufacturer.

Self-reported Stress and Anxiety

Participants completed the following self-reported measures during the pre- and post- cognitive testing sessions immediately following the post-stressor saliva sample collection.

Perceived Stress Scale. Perceived Stress was measured using the 14-item Perceived Stress Scale³⁶. Each item is rated on a 5 point scale from never (0) to very often (4) as the participants respond to statements reflecting "how stressed you have been over the past month". The scale has showed adequate reliability and is designed to measure the degree to which situations in one's life are appraised as stressful. The scale score is computed by aggregating the item scores with higher scores indicating greater stress.

State Trait Anxiety Inventory – State subscale. The State subscale of the State Trait

Anxiety Inventory 37 was administered at the cognitive testing sessions. This 20 item subscale measures state anxiety – a temporary condition experienced in specific situation. Participants respond to statements that evaluate "how you feel right now, at this moment" and items are

scored on a four point likert scale (1=not at all to 4=very much so) resulting in a range of scores from 20-80. Higher scores are indicative of greater anxiety.

Cognitive Assessments

At baseline and following the 8-week intervention, all participants completed the same neuropsychological test battery. We employed three established computer based tests using E-prime 2.0 (Psychology Software Tools, Inc., Sharpsburg, PA) to assess the executive functions of working memory and task switching. The cognitive assessments employed in this study have been described in detail previously²⁹ and are briefly reported in this manuscript.

Working Memory Measures. Two measures of working memory capacity, the running memory span and n-back task were used. The letter version of the running memory span test³⁸ involved reporting the last n letters (n= 3, 4, 5 or 6) from a string of m+n letters presented one at a time, on a computer screen. Following the sequential presentation, participants made their recall responses by clicking the cells of a 3x4 grid displaying all letters from the set of possible letters. Partial recall score was calculated for trials when m>0 and total recall score presented performance on trials where m=0.

In addition to the running span task, a modified serial n-back task ^{39,40} was administered which involved two consecutive phases: 1-back, and 2-back. Each phase required the participants to discriminate between sequences of letters that served as stimuli. In the 1-back condition, the participant was instructed to respond as quickly and accurately as possible if the current letter was the same as the previous trial for the 1-back condition, and two trials previous for the 2-back condition. Accuracy on the 1 and 2 back conditions was examined.

Task Switching Measure. The odd or even, low or high version of the task switching paradigm was used in this study^{41 42}. Participants were asked to switch between judging whether

a number was odd or even and judging whether it was low or high (i.e., smaller or larger than 5). The task consisted of single block of trials (either rule) and a mixed block (both rules). Accuracy on single and mixed blocks as well as repeat (trials when the preceding trial involved the same task, e.g. blue followed by blue) and switch trials (when the preceding trial was of the other task, e.g. blue followed by pink) was examined.

Data Analysis

Data were analyzed using SPSS 20.0 (IBM Corp., Armonk, NY). Prior to all hypotheses testing, all raw data were checked for errors in data entry, normal distribution, and outliers. Except for cortisol, all behavioral measures were normally distributed. Independent sample *t*-tests were conducted to examine whether significant mean differences existed in demographic, cognitive, cortisol and self-reported stress and anxiety variables between the two groups at baseline. Analysis of covariance models, with age, sex and baseline scores as covariates were employed to determine group differences at follow-up for the study variables. Change scores were then calculated for each of the cognitive variables (e.g. accuracy at follow up – accuracy at baseline). In an attempt to explore the predictors of the yoga-cognition relationship, multiple linear regression analyses by group were conducted to determine whether change in the salivary cortisol level, anxiety and stress scores (controlling for age and sex) predicted improvements in each of the executive function measures. We used the enter method to determine how much each predictor variable uniquely contributed to the variance in cognitive outcomes.

Results

Table 1 shows the participant characteristics by group. Within both groups, the majority of the participants were female, married, working full time, well-educated and relatively affluent. Approximately 20% of the study participants represented minority groups. An independent

samples t-test showed no significant differences between conditions on any of these characteristics (all p's \geq .20).

The means and standard deviations of the variables measuring anxiety, stress and salivary cortisol are presented in Table 2. Again, no baseline differences were observed between the two groups (all p's \geq .15). Salivary Cortisol were measured in $\mu g/dl$ and were in the range of 0.1-2 $\mu g/dl$ as expected for normal healthy subjects⁴³ except for 3 samples which were diluted 10X and reanalyzed for cortisol. A distribution of cortisol data showed 8 outliers (n=5 for yoga, n=3 for control) that were more than 3 standard deviations from the mean levels at least one time-point including follow-up. Two of the 8 participants had reported life events (death in the family, laid off at work) to the research staff during the course of the trial, however no record was documented for the remaining 6 participants who exhibited extremely high cortisol levels. These data points were dropped from further analyses to avoid situational bias, resulting in a sample size of 100 at follow up for cortisol analyses.

Group differences at follow-up

At follow-up, the ANCOVA reported a significant group difference for post-stressor cortisol levels [F(1,97)=4.55, p=.04, partial η^2 =.05] where the yoga group showed lower values than the stretching control (.15 μ g/dL Vs. .18 μ g/dL respectively). No significant group differences were observed on the self-report stress and anxiety scales at follow up. However, significant time effects were observed for both perceived stress [F(1,106)=11.58, p=.001, partial η^2 =.10] and state anxiety scores [F(1,106)=9.05, p=.003, partial η^2 =.08]. The time effects were in the expected direction with both groups reporting lowered self-reported stress and anxiety after 8-weeks of exercise.

Predictors of the yoga-cognition relationship

Results from the multiple regression analyses conducted for each of the cognitive measures are presented in Tables 3 and 4. Multiple regression analysis on the running span scores indicated that post-stressor change in salivary cortisol predicted change in the total recall scores (β =.38, p=.01) and the partial recall score (β =.30, p=.04) for the yoga group. The result was in the expected direction such that an attenuated cortisol response to the stressor (i.e. cognitive tasks) predicted improved total and partial recall scores on the running span task. On the n-back task, reduced state anxiety predicted improved accuracy on the 1-back (β =.27, p=.04) for the yoga group, whereas increase in salivary cortisol was predictive of poor performance on the more cognitively demanding 2-back (β = -.37, p=.01) for the stretching strengthening group. On the task switching outcomes increased salivary cortisol consistently predicted poor accuracy on the mixed (β =-.46, p=.002), repeat (β =-43, p=.004) and switch (β =-.47, p=.002) trials for the stretching and strengthening group.

Discussion

To our knowledge this is the first RCT to examine cortisol and self-reported affect as mediators of the yoga-cognition relationship. Our first hypothesis that the yoga intervention would result in lowered pre-stressor cortisol levels at follow-up was not supported. However, in response to a stressful event such as cognitive assessments, the yoga group showed an attenuated cortisol response. This attenuated response, along with self-reported state anxiety and perceived stress predicted improved accuracy on the running span and 1-back tasks of working memory. The control group on the other hand showed an elevated cortisol response that predicted poor performance on the 2-back and task switching outcomes. Thus, our second hypothesis of changes in salivary cortisol, state anxiety and perceived stress predicting cognitive performance was only partially supported in this study. While cortisol was predictive of executive function

performance, an attenuated response in the yoga group was associated with improved accuracy on most working memory outcomes, whereas an elevated response was associated with poor accuracy on task switching outcomes within the control group.

The current study demonstrated small effect sizes as per Cohen's classification of the magnitude of the effect size ⁴⁴. The standardized beta values ranged from .27 to .38 and these small effect size maybe in part due to our sample of normal and healthy subjects in the trial who scored high on accuracy on most of the cognitive measures. Although the predictors did not consistently account for variance in the cognitive performance measures, the changes in cortisol following yoga practice and improving executive functions are important findings. Stress and anxiety may be particularly critical in affecting information processing. The ability to self-regulate emotions has been found to be a key component in enhancing cognition ⁴⁵. It is possible that the calming effects of yoga combined with the increased capacity to focus on the present improved cognitive performance after 8-weeks of regular practice. Yoga enhances present moment awareness by teaching participants to notice subtle distractions (feelings; thoughts) while repeatedly bringing attention back to the meditation object such as one's breath or specific parts of the body. This process can promote attentional stability.

Other than examining effects on cortisol, studies have also shown yoga to increase parasympathetic dominance including reduced heart rate and increased heart rate variability⁴⁶. In an 8-week Hatha yoga intervention, Papp et al⁴⁷ reported improved heart rate variability suggesting an increased vagal tone and reduced sympathetic activity in a sample of 50 year old adults. Similar results were reported by Santaella et al⁴⁸ following their 4-month RCT that showed improved respiratory function and sympathovagal balance. Cortisol, although a widely used measure of stress and the HPA axis, is influenced by age, gender, health status and

behaviors as well as stress perceptions⁴⁹. Self-report questionnaires are also prone to bias and participants may provide socially desirable responses on the stress and anxiety questionnaires such as those used in this trial. Responses may also be influenced by participant's outcome expectations. Future research needs to examine physiological markers of the autonomic nervous system, such as heart rate variability, neurotransmitters and hormone parameters of the HPA/SNS and their role in the yoga-cognition relationship.

Other than the mechanisms explored in this study, there are some neurobiological processes that may explain the relationship between yoga and executive function. Cross-sectional neuroimaging studies with yoga practitioners have reported greater gray matter volume in several brain regions including the frontal lobe, largely responsible for executive processes^{50,51}. In an RCT, Hariprasad et al.⁵² reported increases in bilateral hippocampal gray matter volume after a 6-month yoga intervention in older adults. In the physical activity-cognition literature, experiencing novel activities⁵³ and anaerobic interventions have been shown to have unique cognitive benefits on brain structure and function⁵⁴. From this neuroscientific perspective, tasks that involve mind wandering, memory consolidation, thought focused inwards often referred to as self-referential thinking, and taking the perspective of others into one's own view of the world have been associated with the default mode network. This is a network of brain regions that are active when the individual is at rest but not focused on the outside world^{55,56}. There is evidence that supports a relationship between the default mode network and executive function, where increased default mode network function has been associated with better working memory performance in young adults⁵⁷, and better performance on a range of executive function tasks in older adults^{54,58-60}. At the same time there is emerging evidence for the role of stress in the functional connectivity of the brain and its default mode network function ⁶¹. Awareness-based

behavioral interventions such as mindfulness have shown to moderate the neural-cortisol association ⁶². The holistic practice of yoga in this study was largely awareness-based and a self-referential mind-body practice which appears to have modulated the stress response and in turn, improved cognitive performance on the executive function measures for the yoga participants.

Although this study contributes greatly to the sparse yoga-cognition literature, it does have some limitations. Although the salivary cortisol analyses were blinded, the assessment protocol used in this trial could be refined in future work. Taking into account the duration of the study and participant burden to complete assessments, only two saliva samples were collected at each time-point in the present study. Collecting multiple saliva samples at each timepoint and at various times of the day to account for the circadian rhythm of cortisol may serve as more reliable markers. The perceived stress scale did not accurately capture situational stressors such as being laid off at work or death in the family. Such life events should be formally recorded in the future, to more accurately and reliably account for outliers in the data sets. Similarly, exercise or nutrition based lifestyle changes or adoption of other health behaviors should be systematically documented, particularly for longer interventions. Since the primary outcome of the study was cognition, we only screened for cognitive deficiencies at baseline. Although the participants were healthy and reported no mental or emotional disorders, future studies examining stress as a mediator should formally screen for psychiatric history and diagnosed mental health illnesses, particularly mood and anxiety disorders. Questions about the optimal dose of yoga to modulate stress and gain cognitive benefits cannot be determined in this trial. Although we advertised to recruit both men and women for this trial, a larger proportion of females volunteered for the study which may suggest gender disparites in adoption and practice

of mind-body therapies such as yoga. Finally, the lack of a follow-up timepoint limits us from determining the maintainence of cognitive benefits after the cessation of the intervention.

In conclusion, changes in salivary cortisol in response to a stressor were predicive of executive function performance in the present study. The yoga group showed an attenuated stress response, it predicted performance on the running span task. The control group on the other hand, showed an elevated stress response that predicted poor performance on some of the executive function measures. The precise neurobiological changes within the brain remain unknown, and other biomarkers including neurotransmitters and growth factors need to be inverstigated. Larger well-powered RCTs need to be conducted that will allow for statistical mediation analyses and formulation path models that explain the relationship between yoga and improved executive function and overall cognition. Future studies should adopt advanced neuroimaging techniques and replicate these outcomes across different age groups, among clinical populations with diagnosed mood or anxiety disorder as well as cognitive deficiencies, and examine the dose-response relationship of varied durations and frequencies of yoga practice.

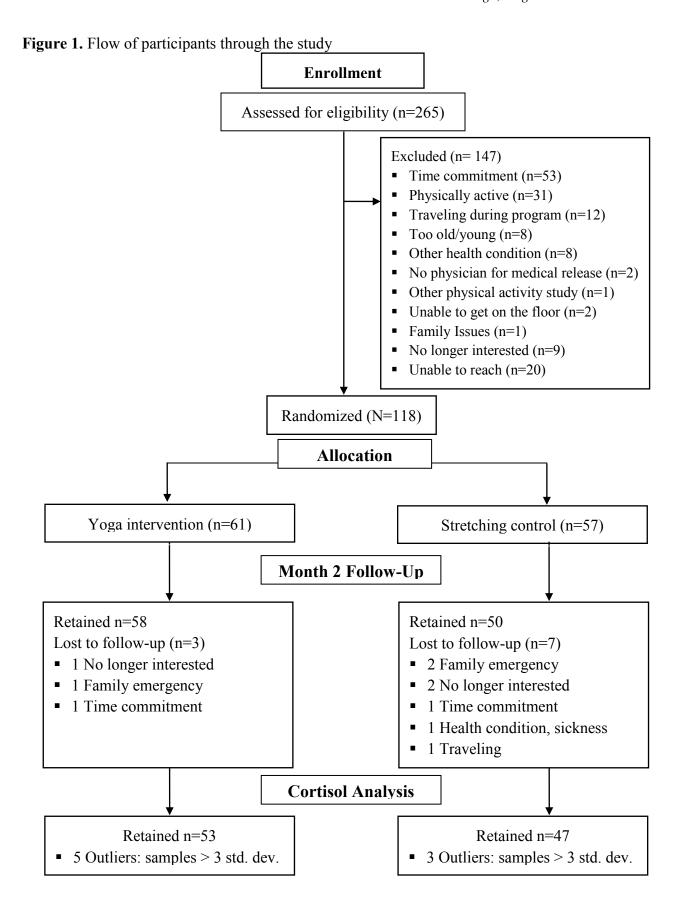


Table 1. Demographic characteristics of participants at baseline

	Yoga Contracteristics of participants at baseline							
	n=61	n=57						
Age (mean, sd)	62.1 (±5.82)	62.0 (±5.39)						
Sex (n, %)								
Females	49 (80.3%)	43 (75.4%)						
Males	12 (19.7%)	14 (24.6%)						
Race (n, %)								
African American	7 (11.5%)	4 (7.0%)						
Asian	2 (3.3%)	2 (3.5%)						
American Indian/Alaskan Native	0 (0%)	2 (3.5%)						
More than one race	1 (1.6%)	3 (5.3%)						
Caucasian	51 (83.6%)	46 (80.7%)						
Education (n, %)								
< College degree	14 (23%)	25 (43.8%)						
> College degree	47 (77%)	32 (56.2%)						
Income (n, %)								
< 40,000	11 (17.9%)	16 (28.2%)						
> 40,000	50 (82.1%)	41 (71.8%)						
Marital status (n, %)								
Married	34 (55.7%)	39 (68.4%)						
Separated/Divorced	15 (24.6%)	11 (19.3%)						
Widowed	7 (11.5%)	4 (7%)						
Single	3 (4.9%)	2 (3.5%)						
Partnered/Significant other	2 (3.3%)	1 (1.8%)						
Employment status (n, %)								
Retired	13 (21.3%)	13 (22.8%)						
Working part time	15 (24.6%)	13 (22.8%)						
Working full time	32 (52.5%)	30 (52.6%)						
Unemployed	1 (1.6%)	1 (1.8%)						
Yoga experience (n, %)								
Had tried it in the past	18 (29.5%)	20 (35.1%)						
Would probably try it	43 (70.5%)	37 (64.9%)						

Note: No significant group differences observed at baseline, all p's $\geq .19$

Table 2: Means and standard deviations at baseline and follow up for salivary cortisol, anxiety, stress and executive function variables

	Yo	oga	Co	ANCOVA	
	Pre	Post	Pre	Post	p value*
Salivary cortisol (µg/dl)					
Pre-stressor	$.15 \pm .07$	$.18 \pm .06$	$.16 \pm .09$	$.16 \pm .06$.11
Post-stressor	$.16 \pm .10$	$.15 \pm .07$	$.17 \pm .10$	$.18 \pm .08$.04
State Anxiety	33.10 ± 9.3	30.71 ± 9.7	32.77 ± 8.9	30.80 ± 9.7	.98
Perceived Stress	12.11 ± 6.4	10.67 ± 5.6	13.95 ± 7.2	12.18 ± 6.4	.77
Running Span					
Partial recall	15.61 ± 6.86	17.97 ± 6.68	16.89 ± 5.85	16.76 ± 6.81	.01
Total recall	29.48 ± 8.93	31.64 ± 8.71	29.3 ± 8.32	29.68 ± 9.24	.11
N-Back					
1back accuracy	$0.97 \pm .05$	$0.98 \pm .03$	$0.96 \pm .05$	$0.97 \pm .06$.18
2back accuracy	$0.8 \pm .18$	$0.87 \pm .11$	$0.84 \pm .13$	$0.82 \pm .17$.003
Task Switching					
Single accuracy	$0.94 \pm .10$	$0.98 \pm .05$	$0.92 \pm .09$	$0.94 \pm .11$.02
Mixed accuracy	$0.87 \pm .16$	$0.94 \pm .08$	$0.84 \pm .22$	$0.88 \pm .21$.09
Repeat accuracy	$0.89 \pm .14$	$0.95 \pm .07$	$0.86 \pm .22$	$0.88 \pm .21$.10
Switch accuracy	$0.85 \pm .18$	$0.93 \pm .09$	$0.83 \pm .23$	$0.87 \pm .21$.15

Note: *ANCOVAs examining group differences post intervention, controlling for age, sex and baseline scores

Table 3: Multiple regression results with predictors for the working memory change scores

	Yoga Group											
Measures Predictors	Running Span - partial score			Running Span – total score			N-back – 1back accuracy			N-back – 2back accuracy		
	β	t	р	β	t	р	β	t	р	β	t	р
Salivary Cortisol	.30	2.08	.04	.38	2.63	.01	23	-1.60	.11	.05	.35	.73
State Anxiety	.12	.85	.40	02	16	.87	.27	2.07	.04	.03	.21	.83
Perceived Stress	.06	.43	.67	.07	.46	.65	11	80	.43	19	-1.30	.20
	$R^2 = .14$ $R^2 = .16$					$R^2 = .20*$			$R^2 = .13$			
Stretching Group												
	β	t	р	β	t	p	β	t	p	β	t	p
Salivary Cortisol	10	62	.54	.06	.41	.69	.02	.16	.88	37	-2.67	.01
State Anxiety	15	91	.37	14	85	.40	.12	.69	.49	04	25	.80
Perceived Stress	10	64	.53	08	47	.64	.08	.47	.64	.25	1.75	.09
	$R^2 = .06$			$R^2 = .06$			$R^2 = .08$			$R^2 = .21*$		

Note: * p<.05

Table 4: Multiple regression results with predictors for the task switching change scores

	Yoga Group											
Measures Predictors	Single Trials Accuracy			Mixed Trials Accuracy			Repeat Trials Accuracy			Switch Trials Accuracy		
	β	t	p	β	t	p	β	t	p	β	t	p
Salivary Cortisol	12	83	.41	.07	.47	.64	.07	.46	.65	.08	.48	.63
State Anxiety	.03	.18	.86	.03	.17	.86	02	11	.91	.05	.35	.73
Perceived Stress	29	-1.99	.05	06	38	.70	06	40	.69	04	25	.80
	$R^2 = .13$			$R^2 = .04$			$R^2 = .08$			$R^2 = .03$		
Stretching Group												
	β	t	p	β	t	р	β	t	p	β	t	p
Salivary Cortisol	25	-1.63	.11	46	-3.24	.002	43	-3.29	.002	50	-3.46	.001
State Anxiety	10	63	.53	.19	1.28	.21	.25	1.65	.12	.13	.83	.41
Perceived Stress	22	-1.39	.17	07	50	.62	08	53	.53	08	51	.61
- N		$R^2 = .13$ $R^2 = .24*$			$R^2 = .24*$			$R^2 = .24*$				

Note: * p<.05

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