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J Camosun Psyc Res. (2022). Vol. 4(1), pp. 191-222.
Submitted Fall 2021; accepted June 2022.

What Are the Biological Effects of Anxiety?

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ABSTRACT

In this paper, we sought to understand the biological effects of anxiety, especially in the return of students to in-person classes. Previous research has shown that general anxiety is associated with irritable bowel syndrome (IBS), increases in ambulation (movement), elevations in the stress hormone cortisol, increased risk of inflammatory disease, hyperhidrosis (excessive sweating), and increased amygdala size. In our correlational study, we tested the strength of these relationships by examining naturalistic daily changes in their variables longitudinally over a period of one week. We measured amygdala activity by increases of heart rate after watching frightening videos, inflammation by measuring the circumference of the wrist, cortisol level by a daily symptom questionnaire, IBS symptoms by an IBS symptoms questionnaire, locomotor activity by step count tracking, hyperhidrosis symptoms by partially filling out the Hyperhidrosis Quality of Life Index, and anxiety by the Hamilton Anxiety Rating Scale (HAM-A). Data pooled across participants in our correlational study showed significant correlations only between IBS and anxiety. Knowing that there is a correlation between IBS and anxiety can be helpful in treatments of IBS patients by using anti-anxiety medications in some cases, especially for patients with emotional distress.

1. Introduction

1.1 Research Problem

Anxiety can be debilitating for many, and after a year of socially-distanced learning, we would like to know how students are being affected biologically. For example, several of this paper's authors suffer from Generalized Anxiety Disorder and have noticed a worsening of anxiety symptoms with returning to campus after a long time without socializing. We would like to gain a more in-depth understanding of the biological effects of anxiety, such as digestion problems, stomach pain, excessive sweating, and feelings of a racing heart. Perhaps finding a solution to control the biological effects resulting from anxiety can help many people who are struggling with this issue, as well as helping those who are experiencing this type of anxiety for the first time after returning to school.

1.2 Literature Review

One biological effect of anxiety previously found was changes in the medial temporal lobe of Generalized Anxiety Disorder patients through neuroimaging

using scans from an MRI. These scans found significant grey matter and amygdala size increase in patients suffering from anxiety. For example, in an experimental study by Suor et al. (2020), researchers asked participants both youth and adult, to complete a self-reported anxiety questionnaire (Liebowitz Social Anxiety Scale) followed by a resting MRI scan. Based on these results, the researchers suggested that there is a significant volumetric increase in the amygdala in patients suffering from Generalized Anxiety Disorder. An increase in the amygdala can potentially be used as a biomarker that predicts anxiety, as well as creating a different course of future treatment plans based on these biomarkers to decrease remission rates of anxiety.

Another biological effect of anxiety is elevated inflammatory activity, which can increase the risk of inflammatory disease. For example, in a correlational study by O'Donovan et al. (2010), 27 participants were taken from a sample of those who scored in the clinical range on the Hospital Anxiety and Depression Scale, and 29 participants who scored low on the scale. Blood was then collected in clot activator tubes, to measure the levels of cytokine interleukin-6. Based on their results, the researchers suggested that those with higher levels of anxiety had higher levels of the proinflammatory hormone cytokine interleukin-6 and were at a higher risk of developing an inflammatory disease.

A third effect of anxiety is abnormalities in stress hormones and inflammatory markers. For example, in an experimental study by Hoge et al. (2018) adults with generalized anxiety disorder were put into two different groups. One was a mindfulness-based stress reduction group and the other was an attention control class group. The participants were put in either group and took the trier social stress test before and afterwards. It was shown that adrenocorticotropic hormone (ACTH), which produces the stress hormone cortisol, decreased in the participants who were in the mindfulness-based stress reduction group. Based on these results, the researchers suggested that meditation-based interventions lower the stress hormone cortisol in people with generalized anxiety disorder. This shows that anxiety can have biological effects and people with anxiety tend to have higher stress hormones which can lead to health issues. Using methods such as mediation-based interventions can help ease anxiety and lower the chances of health issues due to anxiety.

Additionally, another effect of anxiety is Irritable Bowel Syndrome (IBS). For example, in a non-experimental study by Drews & Hazlett-Stevens (2008), researchers asked participants to complete a series of questionnaires forms such as the Generalized Anxiety Disorder Questionnaire (GAD-Q-IV) for GAD diagnosis and ROME II Diagnostic Criteria for Irritable Bowel Syndrome (IBS). Based on the completed forms, researchers found that 4.3% of participants met the criteria for both IBS and GAD, the majority of whom were women. In fact, participants meeting the criteria for IBS were more likely the ones who were also diagnosed with GAD. Therefore, researchers suggested that there was an association between IBS and GAD since the majority of people diagnosed with IBS were also suffering from high trait anxiety.

A fifth biological effect of anxiety is the change in ambulation in response to early exposure to life's adversity, such as isolation. For example, in the experimental study by Novoa et al. (2021), adolescent male rats were either housed together or housed separately and then were put in an Open Field Test. An Open Field Test

consists of activity chambers where the rat was placed for 10 minutes and could roam freely. Their activity was monitored by infrared beams. When the beam was broken the position of the rat was determined. Rats that spent more time in the middle of the cage were considered to be less anxious. The socially isolated rats spent less time in the middle and moved around the activity chambers much more than the rats that were group-housed. The rats that ambulated more showed more anxiety. Based on these results, the researcher suggested that ambulation is increased in rats with anxiety that have been socially isolated.

Lastly, a biological effect of anxiety is hyperhidrosis, i.e., excessive sweating. For example, in a correlational study by Davidson et al. (2002), 375 participants, who met the criteria of either the DSM IIIR or the DSM IV for Social Anxiety Disorder (SAD) filled-out the Brief Social Phobia Scale (BSPS) and the Social Phobia Inventory (SPI). On the BSPS, 93 participants reported having hyperhidrosis, and on the SPI, only 88 did. When the 93 individuals with hyperhidrosis were compared to the 166 who reported experiencing either no or mild sweating in the BSPS, the first group was shown to score higher on the Sheehan Disability Scale. When the 88 participants with hyperhidrosis were compared with the 122 that reported no or minimal sweating in the SPI, the first group scored higher rates of social anxiety in the Liebowitz Social Anxiety Scale. Based on the 25% of the individuals in the sample that experienced excessive sweating, the researchers found that hyperhidrosis "was associated with more severe social anxiety symptoms and disability" (Davidson et al., 2002, p. 1331).

1.3 Hypotheses

Based on the above literature review, we predicted the following hypotheses:
Hypothesis #1: If anxiety increases then amygdala activity will increase.
Hypothesis #2: If anxiety increases then IBS symptoms will increase.
Hypothesis #3: If anxiety increases then locomotor activity will increase.
Hypothesis #4: If anxiety increases then the stress hormones cortisol will increase.
Hypothesis #5: If anxiety increases then the levels of cytokine interleukin-6 (pro-inflammatory hormone) will increase.
Hypothesis #6: If anxiety increases then hyperhidrosis (excessive sweating) will increase.

2. Methods

2.1 Participants

The six authors of this paper served as the participants in its studies. The participants ranged in age from 21-28 years old, with an average age of 23.7 years, and included all participants that identified as females. The participants were all undergraduate students at Camosun College who completed the current studies as an assignment for Psyc 215 Biological Psychology and were grouped together due to their mutual interest in anxiety. All participants experience different forms of social anxiety, and three of them were clinically diagnosed with Generalized Anxiety Disorder (GAD) and one suffers from Irritable Bowel Syndrome (IBS).

2.2 Correlational Study Methods

We first performed a correlational study to test concurrently all of our hypotheses by examining naturalistic daily changes in their variables longitudinally. Each participant kept a study journal with them at all times over this study's one-week period in order to

record self-observations of the following seven variables: (1) amygdala activity, (2) levels of cytokine interleukin 6, (3) levels of the stress hormone cortisol, (4) IBS symptoms, (5) locomotor activity, (6) hyperhidrosis symptoms, and (7) anxiety.

2.2.1 Levels of the Stress Hormone Cortisol

To measure high levels of the stress hormone cortisol, each participant filled out a cortisol questionnaire based on symptoms that correlate with high levels of cortisol (see Appendix D). The participants checked which symptoms apply to them each morning at the same time for seven consecutive days. This questionnaire is scored out of six because there are six main symptoms and the higher the score the participants have, the more likely it is correlated with high levels of cortisol.

2.2.2 Levels of Cytokine Interleukin 6

To measure levels of cytokine interleukin 6, since it is known to produce inflammation, participants measured it by the circumference of the wrist. The circumference was measured at 7 am every day for 7 consecutive days. Each day participants used a measuring tape to measure wrist circumference and then completed a table that includes the date, time and measurement in cm (see Appendix F).

2.2.3 Amygdala Activity

To measure amygdala activity, participants monitored changes in their heart rate levels in response to frightening events. The participants recorded their heart rate before and after watching a frightening event using the finger and wrist test method. There were 7 different frightening event videos that were taken from youtube, each one with a duration between 2 mins to 7 mins. Each video had a unique fear factor of jump scares for each of the participants to watch over the course of 7 days.

2.2.4 IBS Symptoms

To measure IBS symptoms, participants completed the IBS Symptoms Evaluation questionnaire (see Appendix B). This questionnaire consists of 10 IBS symptoms that each item scores on a scale of 0 (none) to 3 (severe) with the total score of 0-30, where <15 indicates the participant may not be suffering from IBS, 15-24 indicates the participant may be suffering from IBS and 25-30 indicates the participant is likely to be suffering from IBS. Participants completed this form daily (preferably before going to bed) for a week.

2.2.5 Locomotor Activity

Locomotor activity was measured by a fitness tracker or an application on their phone. The fitness trackers used were Fitbits. The phone applications used were Apple Health and Samsung Health. The participants recorded their results at the end of the day for 7 consecutive days on the forms provided by the experimenter (see Appendix E).

2.2.6 Hyperhidrosis

To measure hyperhidrosis, 5 items from the HidroQoL (Hyperhidrosis Quality of Life Index) were presented to the participants. They were: 1) “My choice of clothing is affected [by my excessive sweating]”; “I feel uncomfortable physically expressing affection (e.g. hugging others)”; 3) “I worry about leaving sweat marks on things”; 4) “My appearance is affected [e.g.: face looking oily]”; and 5) “I think about sweating” (see Appendix G). These sentences were slightly altered in the questionnaire, in order to better fit this particular research. Instead of the verbs being in the present, they were all conjugated in the past tense. Also, at the beginning of every sentence, the word “today” was added, as participants were supposed to reflect only on their experiences throughout a given day. To answer each of those questions, participants had three

options, which were: "very much", "a little", or "No, not at all". The total possible score in the full version of the questionnaire (with 18 items) is 36, and in this case, with only 5 items, the total possible score is 10.

2.2.7 Anxiety

To measure anxiety, all the participants filled out the Hamilton Anxiety Rating Scale (HAM-A) on a daily basis (see Appendix A). The scale includes 14 broad items, for instance, one item is called "fears", which includes "of dark, of strangers, of being left alone, of animals, of traffic, of crowds". For each item, participants had to pick one out of five options: 0 was equivalent to "not present", 1 to "mild", 2 to "moderate", 3 to "severe", and lastly, 4 to "very severe". The total possible score range is 0-56, where <17 indicates mild severity, 18-24 is mild-moderate severity, and 25-30 is moderate severity. Based on the sum of each of these numerical answers, one's level of anxiety was assessed.

3. Results

As shown in Table 1, IBS syndrome was significantly correlated with anxiety severity. In fact, it was statistically significant for two of the participants (r = .84, p = .015 and r = .88, p = .006) as well as significantly correlated using pooled standardized data (r = .46, p = .001; see Figure 2), but IBS syndrome was not significantly correlated with anxiety severity using pooled raw data (r = .15, p = .333; see Figure 1). In contrast, the correlation between hyperhidrosis and anxiety severity was only statistically significant for one participant (r = -.84, p = .01). Another participant data did not show any variation in hyperhidrosis; therefore their data was not considered in the final results. As for the rest of the participants (all $r \leq .72$, all $p \geq .07$), the pooled raw data (r = .24, p = 35; see Figure 5) and the pooled standardized data (r = .01, p = 0.96; see Figure 6), all showed no significant correlation. Moreover, locomotor activity and anxiety severity were not significantly correlated in the pooled raw data (r = -0.01, p = 0.93; see Figure 9) and the pooled standardized data (r = 0.22, p = 0.16; see Figure 10) but there was one participant that showed a significant correlation (r = 0.89, p = 0.0039). Furthermore, high cortisol levels and anxiety severity was significantly correlated in the pooled raw data (r = 0.32, p = 0.03; see Figure 11), but not correlated in the pooled standardized data (r = 0.10, p = 0.51; see Figure 12), and one participant showed a significant correlation (r = 0.86, p = 0.00). Additionally, high levels of cytokine interleukin 6 and anxiety severity were not correlated in both the pooled raw data (r = 0.23, p = 0.25203; see Figure7) and the pooled standardized data (r = 0.19, p = 0.2315; see Figure 8). Similarly, increased amygdala activity and anxiety severity were not correlated in both the pooled raw data (r = 0.25, p = 0.106905; see Figure 3) and the pooled standardized data (r = -0.22, p = 0.165128; see Figure 4). Therefore, based on the comparison of the correlation coefficients using pooled standardized data, IBS symptoms showed the strongest correlation with anxiety severity.

4. Discussion

4.1 Summary of Results

Based on previous research, we hypothesized that increases in six biological variables would follow an increase in anxiety: amygdala activity (Hypothesis #1), levels of cytokine interleukin 6 (Hypothesis #2), levels of the stress hormone cortisol (Hypothesis #3), irritable bowel syndrome symptoms (Hypothesis #4), locomotor

activity (Hypothesis #5), and hyperhidrosis symptoms (Hypothesis #6). Data pooled across participants in our correlational study supported the predicted relationship of irritable bowel syndrome symptoms with anxiety (Hypothesis #4) but not with any of the other 5 hypotheses listed.

4.2 Relation of Results to Past Research

Our correlational study indicated that high cortisol levels and anxiety severity were not correlated, which is not consistent with previous research. Based on the study done by Hoge et al. (2018), participants in the mindfulness-based stress reduction had a decrease in ACTH which produces the stress hormone cortisol. This showed that participants had high-stress hormone cortisol due to anxiety levels and it decreased when using mindfulness. The methodology of our correlational study differed from that of Hoge et al., (2018) study and this might be why there was a difference in results. In Hoge et al. (2018), Area-Under-the-Curve (AUC) concentrations were calculated for adrenocorticotropic hormone (ACTH) whereas in our research the participants had to fill out a questionnaire that consisted of symptoms of high cortisol. Blood work is a more accurate way of measuring cortisol levels rather than a questionnaire. This could be the reason that high cortisol levels and anxiety severity were not correlated in our research.

Our correlational study found that high levels of cytokine interleukin 6 were not correlated, which is not consistent with previous research. According to the study conducted by O'Donovan et al. (2010), participants showed a correlation between high levels of anxiety and higher levels of cytokine interleukin 6. The methodology used in this study differed from the methods used in O'Donovan et al. (2010) and this is potentially the cause of the differing results. In O'Donovan et al. (2010) levels of cytokine, interleukin 6 were measured by collecting blood in clot activator vacutainer tubes. In our research, participants recorded the circumference of their wrist to measure levels of cytokine interleukin 6 in participants. In comparison with measuring wrist circumference, blood tests are a more precise and reliable way of collecting levels of cytokine interleukin 6.

Our correlational study indicated that amygdala and anxiety did not have a significant correlation, which is not consistent with previous research. According to the study conducted by Suor et al. (2020), Generalized Anxiety Disorder and amygdala activity were significantly correlated. The methodology used in this study differed from the methods used in Suor et al., (2020), as we did not have the proper technology (fMRI) to accurately measure differences in grey matter and amygdala sizes. In our research, we measured amygdala activity by heart rate. Participants measured their heart rate before and after watching a frightening scene and then it was measured alongside the Hamilton Anxiety Scale. One of the reasons this may not have been accurate is due to the scenes provided, as everyone's fear tolerance can be different. Another reason this may not have been accurate is that we did not have proper technology for measuring heart rates and it may have been calculated incorrectly due to participant error. Further studies should attempt to have proper technology available to provide a better chance at a more accurate correlation between anxiety and amygdala activity.

Our correlational study indicates that irritable bowel syndrome symptoms are correlated with anxiety severity which is consistent with the previous research. According to the study conducted by Drews and Hazlett-Stevens (2008), participants

meeting criteria for IBS were more likely to meet diagnostic criteria for generalized anxiety disorder. Although in the previous study sample, there were participants who were diagnosed with IBS, in our study only one participant was clinically diagnosed with IBS and the rest only showed some of the symptoms of IBS. However, both studies despite having differences in terms of study sample concluded that there is an association between IBS and anxiety.

Our correlational study indicated that hyperhidrosis and anxiety did not have a significant correlation, which is not consistent with previous research. According to the study conducted by Davidson et al. (2002), SAD and excessive sweating were significantly correlated. Reasons, why no correlation was found in the present study, could be due to the different methodology used, as in this paper, the HydroQoL Questionnaire was the only hyperhidrosis was measured, and in the study mentioned, the participants answered four questionnaires, and none of them was the HydroQoL. Another fact is that not all participants were diagnosed with SAD, as a matter of fact, only one was, meanwhile in Davidson's study, all 375 participants suffered from SAD. One last reason for the discrepancy in our results could be due to the transition of seasons (from Fall to Winter during the time of our study), seeing that with the lowering of temperatures, it could be argued that participants noticed less of their own sweating. Further studies should attempt to understand how hyperhidrosis is experienced at different times of the year, and how its symptoms manifest in colder temperatures.

Our correlation study indicated that locomotor activity was not correlated with anxiety severity, which is not consistent with the previous research. Based on the study conducted by Novoa et al. (2021), the rats that ambulated more showed more severity in their anxiety. The methodology of our experimental study differed from that of Novoa et al. (2021) study and this might account for the discrepant results. First, a difference between the studies is the type of participants used. In Novoa et al. (2021), they observed male rats as their subjects while in our experimental study we used 6 female human subjects. Furthermore, Novoa et al. (2021) used an Open Field Test to observe the locomotor activity while we used step count to track locomotor activity. In our experiment, an anxiety questionnaire was used to help participants assess their level of anxiety each day, whereas in Novoa et al. (2021) they concluded just by activity level of the rats the severity of anxiety. Further studies should examine whether locomotor activity needs aerobic or anaerobic activity to better predict the severity of anxiety.

4.3 Implications of Results

Since the result indicates that there is an association between IBS symptoms and anxiety, this can be helpful in terms of treatments and medication for IBS patients who are struggling with anxiety. We hypothesized that amygdala activity, IBS symptoms, locomotor activity, stress hormones cortisol and levels of cytokine-6 will increase if anxiety increases as well. However, our finding only indicated that there is a correlation between IBS symptoms and anxiety. This study sought to understand the biological effect of anxiety and found that increasing IBS symptoms is one of the many biological ways that anxiety affects the body.

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Table 1

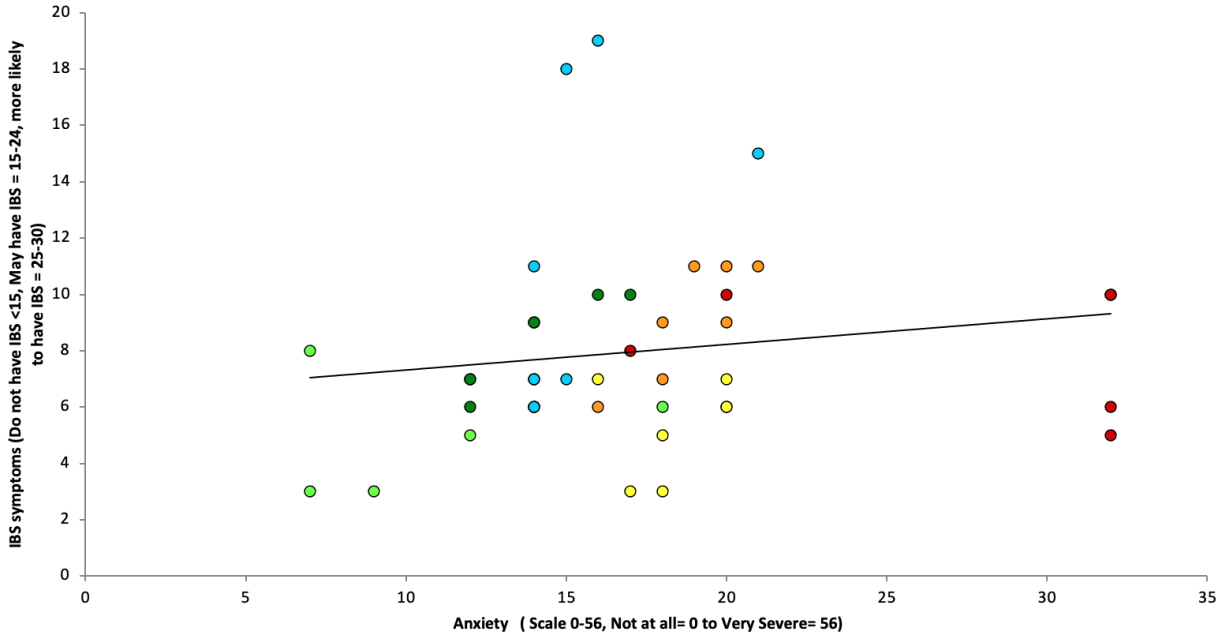
Correlation for Study Variables

Variables	Participant #1		Participant #2		Participant #3		Participant #4		Participant #5		Participant #6		Pooled raw data		Pooled standard-ized data	
	r	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*
Amyg-dala Activity & Anxiety severity	-.51	7	-.30	7	.24	7	.19	7	-.51	7	-.43	7	0.25	7	-0.22	7
Cytokine Interleuk-in 6 & Anxiety severity	0.00	7	-.07	7	0.36	7	-0.05	7	0.44	7	0.45	7	0.23	7	.0.19	7
Cortisol levels and Anxiety Severity	-0.09	7	0.05	7	-0.26	7	*0.86	7	0.12	7	-0.06	7	0.32*	7	0.10	7
IBS & Anxiety Severity	.05	7	.84*	7	.31	7	.24	7	.88*	7	.45	7	.15	7	.46*	7
Loco-motive Activity & Anxiety Severity	0.05	7	0.49	7	0.12	7	*0.89	7	-0.02	7	-0.23	7	-0.01	7	0.22	7
Hyperhi-drosis & Anxiety Severity	-.84*	7	.22	7			.72	7	.30	7	-.35	7	-.20	7	.01	7

* $p < .05$.

Figure 1

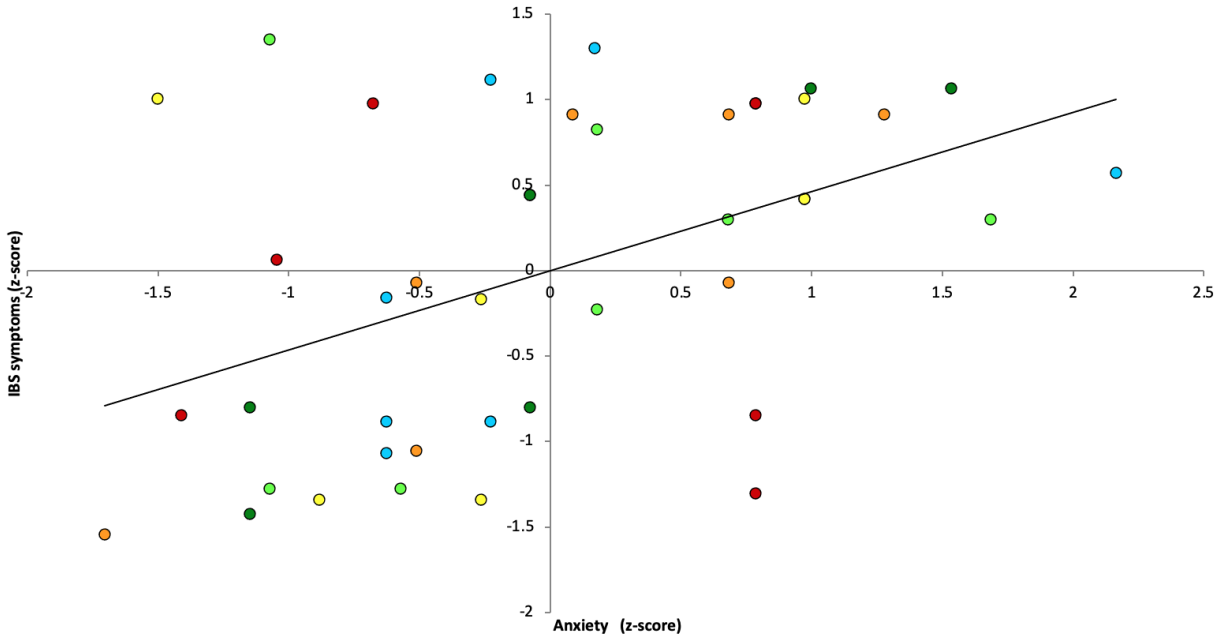
Association Between IBS Symptoms and Anxiety Severity Using Pooled Raw Data



Notes. Marker colour differentiates participants: red = participants #1, orange = participants #2, yellow = participant #3, light green = participants #4, dark green = participants #5, light blue = #6. Some data might not be visible in the figure due to overlapping markers.

Figure 2

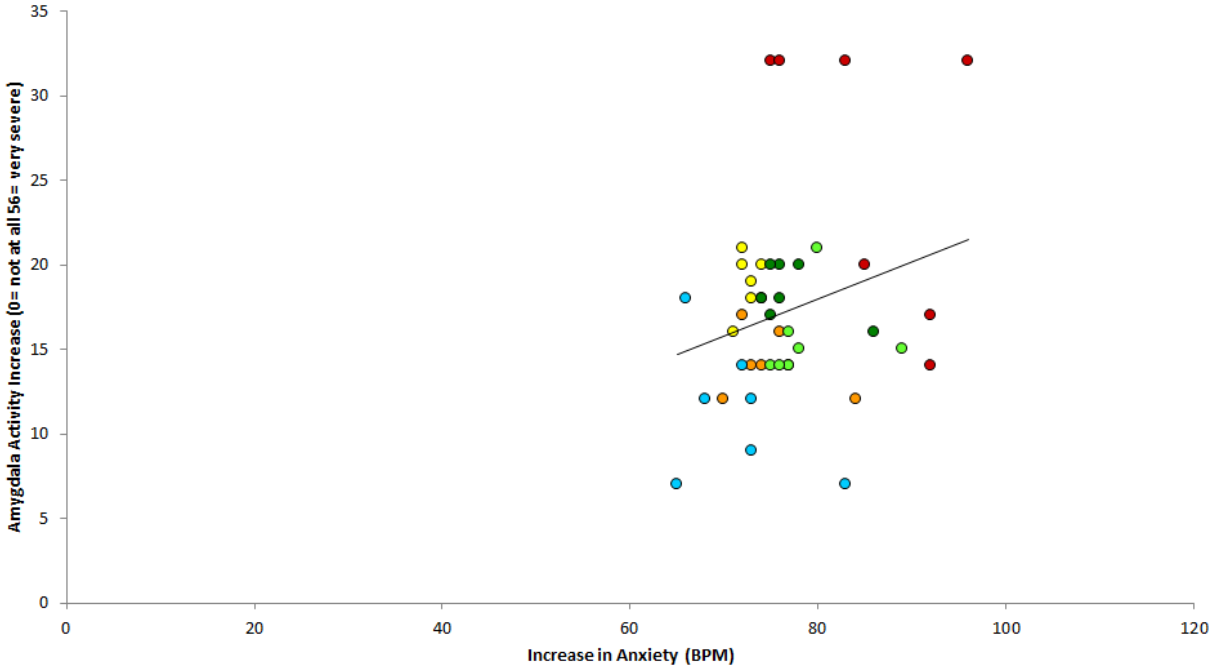
Association Between IBS Symptoms and Anxiety Severity Using Pooled Standardized Data



Notes. Marker colour differentiates participants: red = participants #1, orange = participants #2, yellow = participant #3, light green = participants #4, dark green = participants #5, light blue = #6. Some data might not be visible in the figure due to overlapping markers.

Figure 3

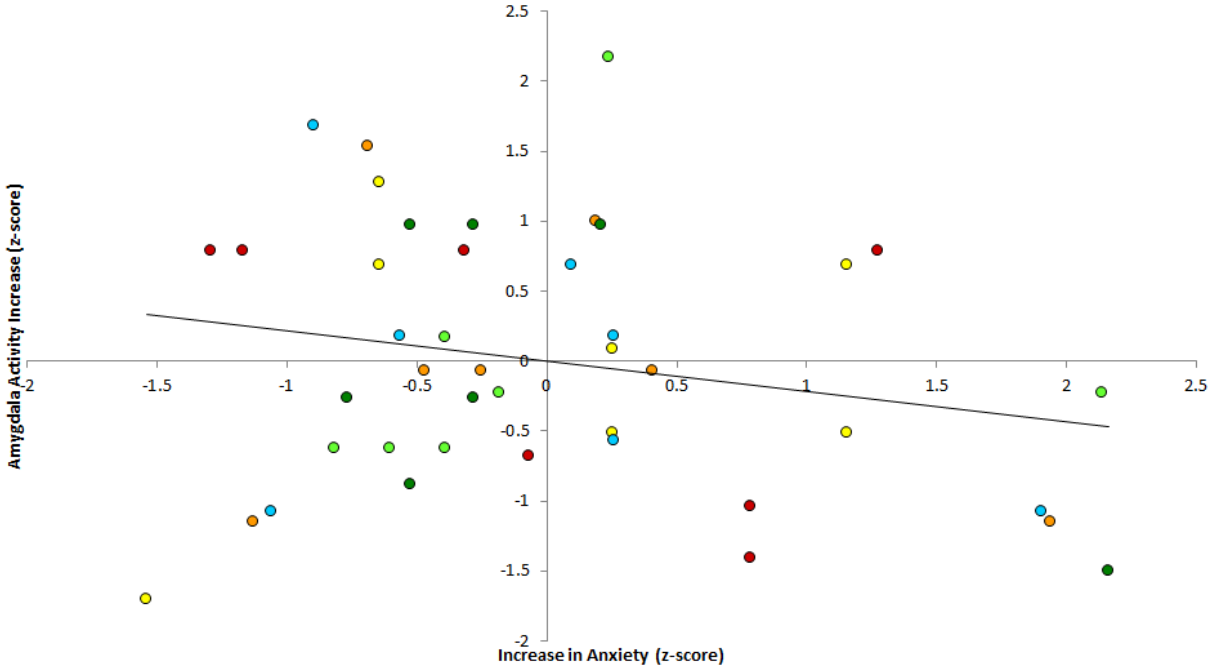
Association Between Amygdala Increase and Anxiety Increase Using Pooled Raw Data



Notes. Marker colour differentiates participants: red = participants #1, orange = participants #2, yellow = participant #3, light green = participants #4, dark green = participants #5, light blue = #6. Some data might not be visible in the figure due to overlapping markers.

Figure 4

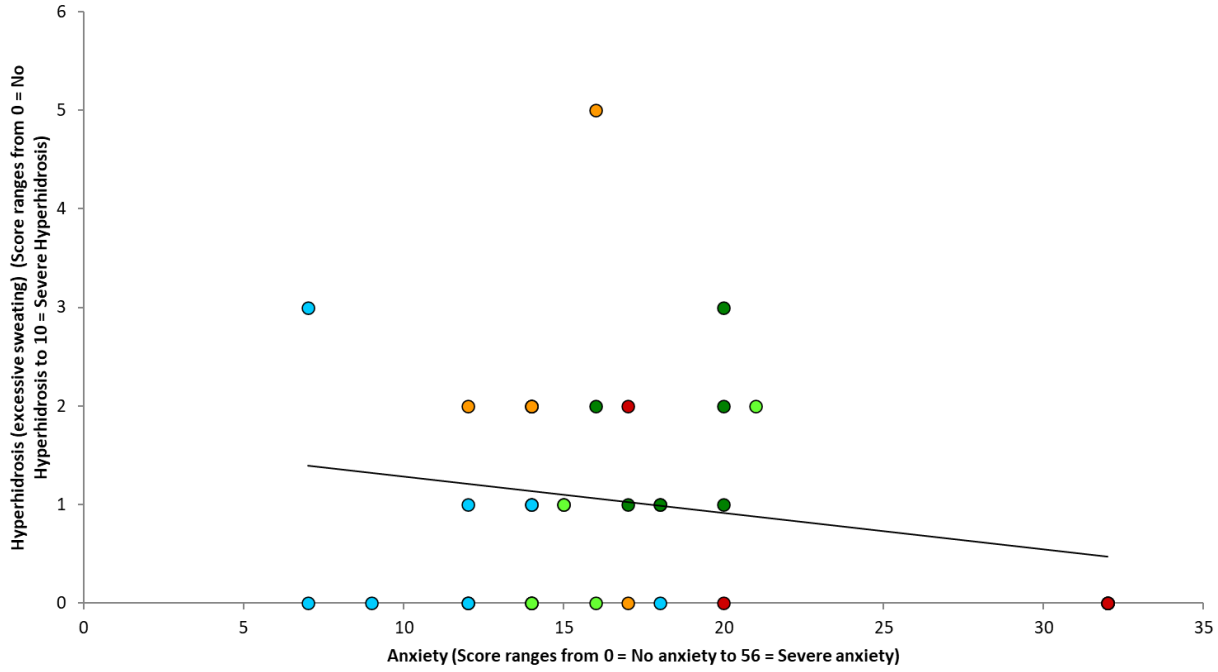
Association Between Amygdala Increase and Anxiety Increase Using Pooled Standardized Data



Notes. Marker colour differentiates participants: red = participants #1, orange = participants #2, yellow = participant #3, light green = participants #4, dark green = participants #5, light blue = #6. Some data might not be visible in the figure due to overlapping markers.

Figure 5

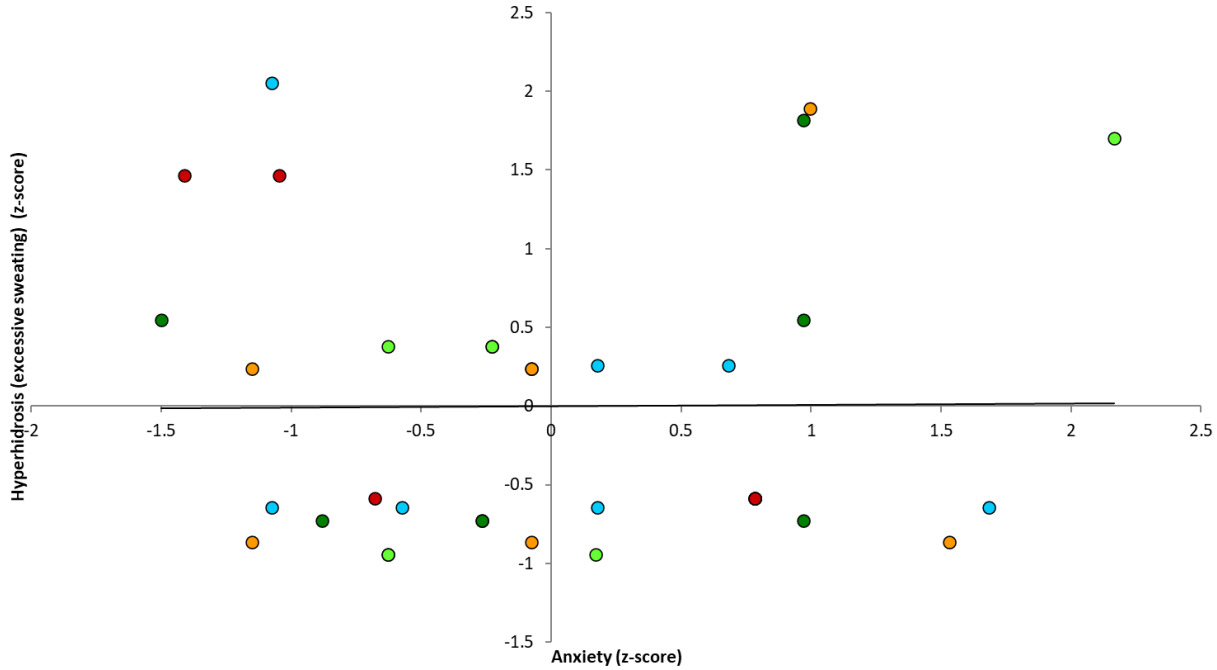
Association Between Hyperhidrosis Symptoms and Anxiety Severity Using Pooled Raw Data



Notes. Marker colour differentiates participants: red = participants #1, orange = participants #2, yellow = participant #3, light green = participants #4, dark green = participants #5, light blue = #6. Some data might not be visible in the figure due to overlapping markers.

Figure 6

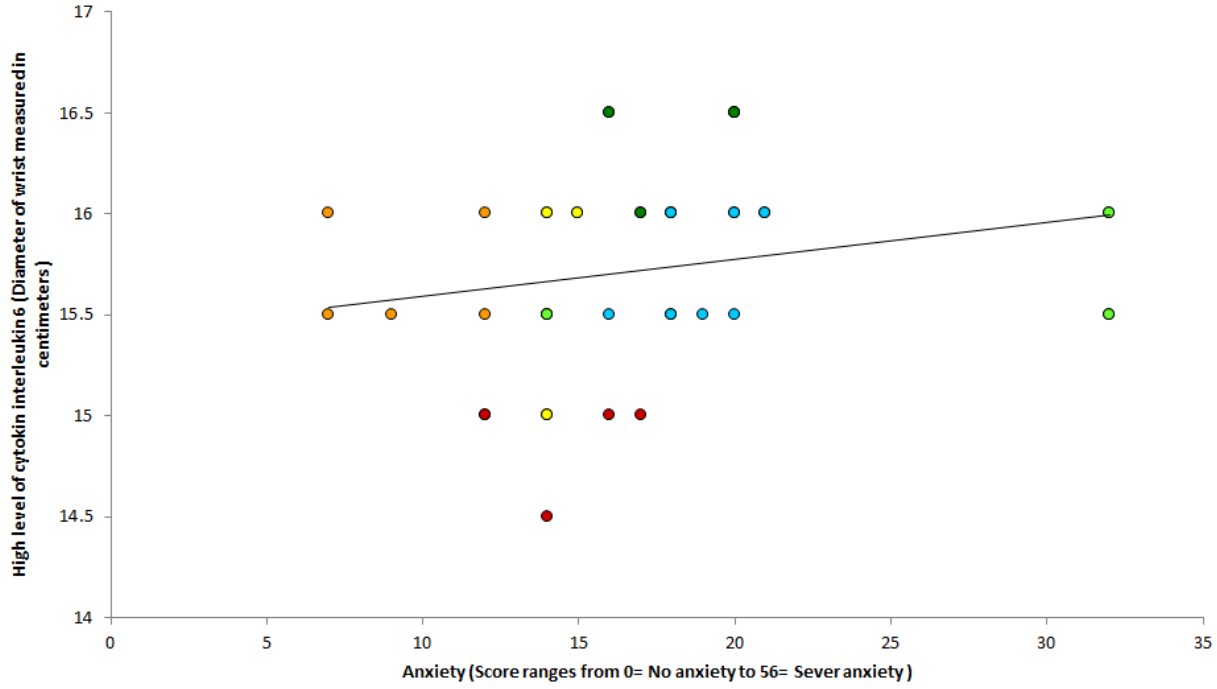
Association Between Hyperhidrosis Symptoms and Anxiety Severity Using Pooled Standardized Data



Notes. Marker colour differentiates participants: red = participants #1, orange = participants #2, yellow = participant #3, light green = participants #4, dark green = participants #5, light blue = #6. Some data might not be visible in the figure due to overlapping markers.

Figure 7

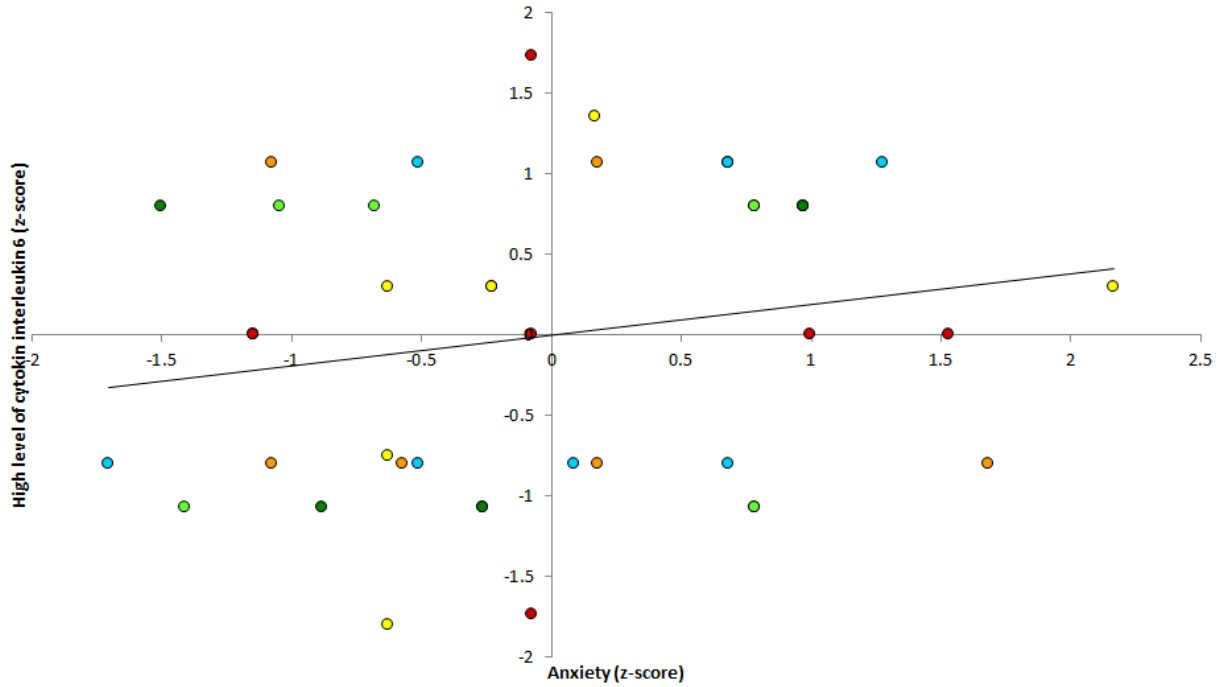
Association Between High Cytokine Interleukin 6 and Anxiety Severity Using Pooled Raw Data



Notes. Marker colour differentiates participants: red = participants #1, orange = participants #2, yellow = participant #3, light green = participants #4, dark green = participants #5, light blue = #6. Some data might not be visible in the figure due to overlapping markers.

Figure 8

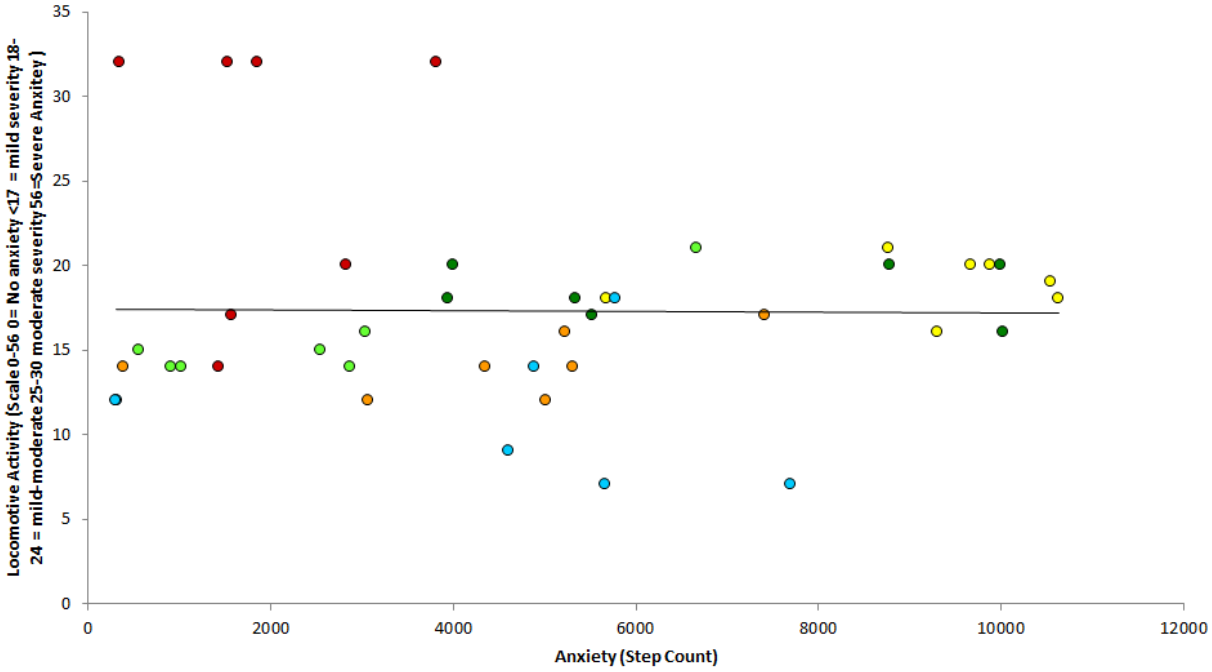
Association Between High Cytokine Interleukin 6 and Anxiety Severity Using Pooled Standardized Data



Notes. Marker colour differentiates participants: red = participants #1, orange = participants #2, yellow = participant #3, light green = participants #4, dark green = participants #5, light blue = #6. Some data might not be visible in the figure due to overlapping markers.

Figure 9

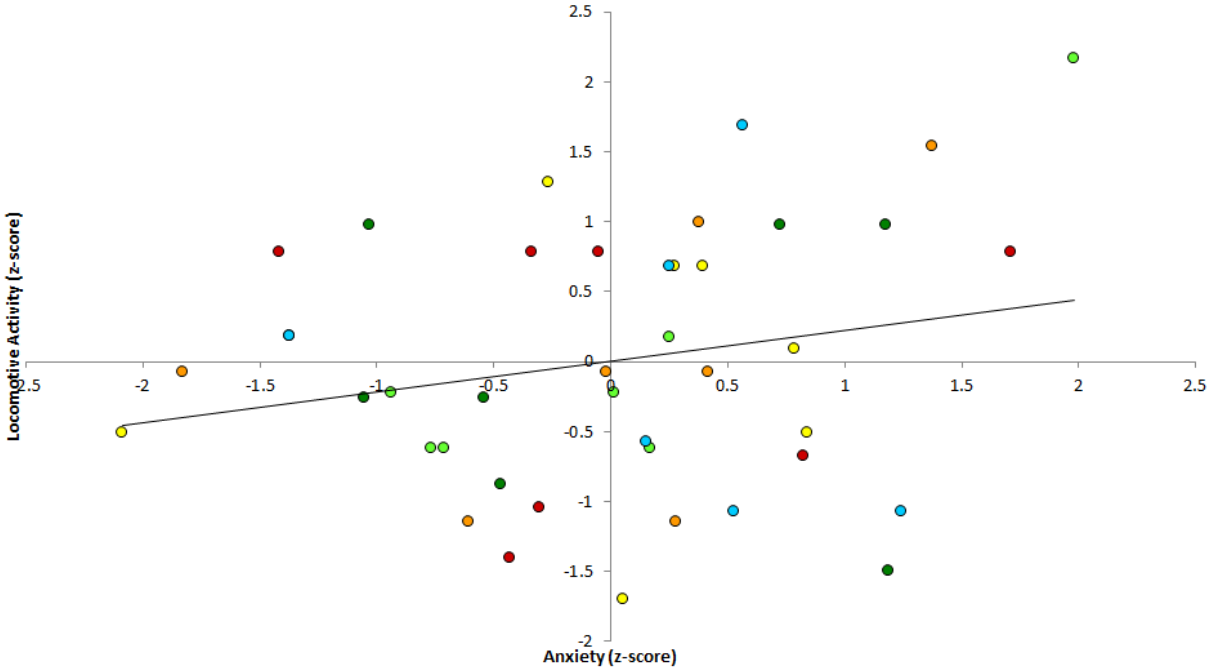
Association Between Locomotive Activity and Anxiety Severity Using Pooled Raw Data



Notes. Marker colour differentiates participants: red = participants #1, orange = participants #2, yellow = participant #3, light green = participants #4, dark green = participants #5, light blue = #6. Some data might not be visible in the figure due to overlapping markers.

Figure 10

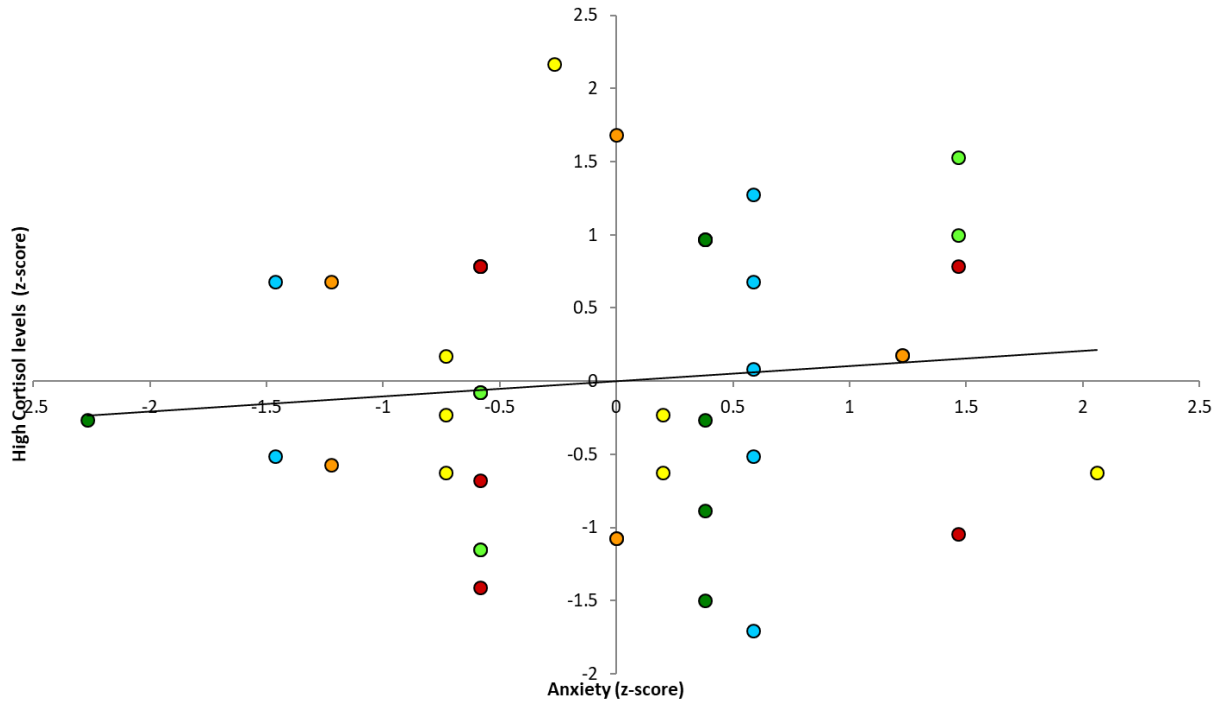
Association Between Locomotive Activity and Anxiety Severity Using Pooled Standardized Data



Notes. Marker colour differentiates participants: red = participants #1, orange = participants #2, yellow = participant #3, light green = participants #4, dark green = participants #5, light blue = #6. Some data might not be visible in the figure due to overlapping markers.

Figure 11

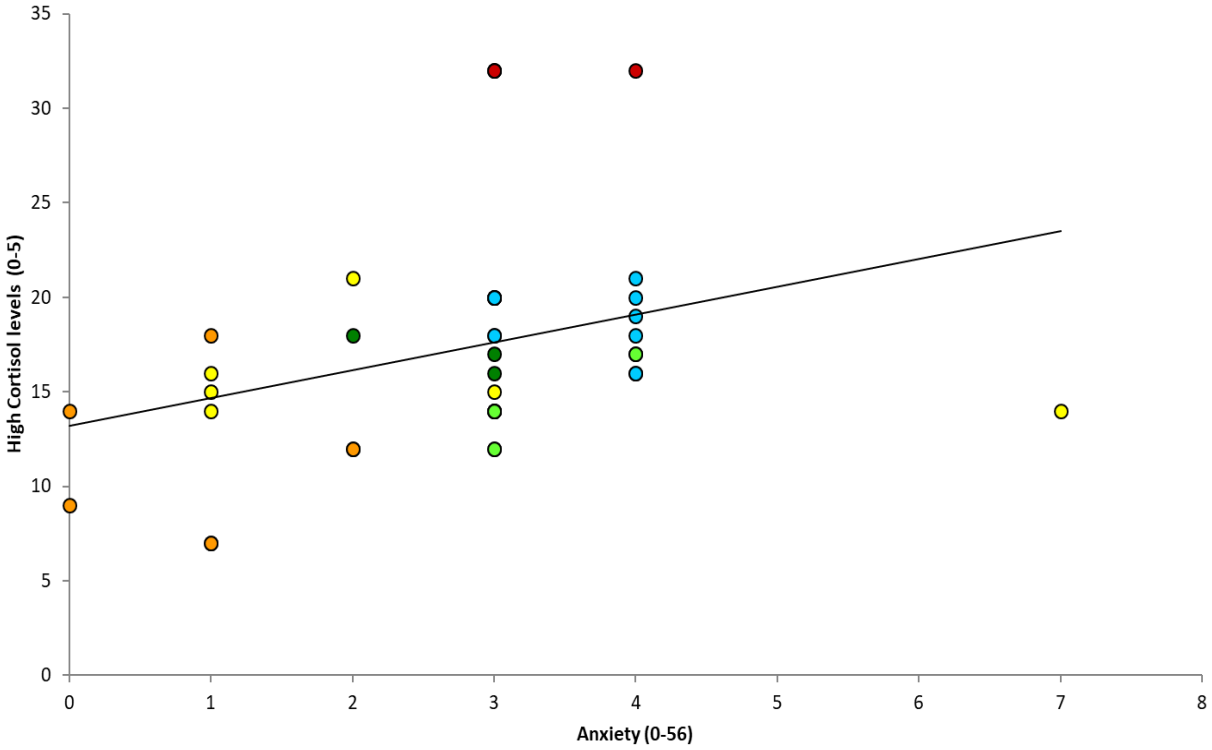
Association Between High Cortisol Symptoms and Anxiety Severity Using Pooled Standardized Data



Notes. Marker colour differentiates participants: red = participants #1, orange = participants #2, yellow = participant #3, light green = participants #4, dark green = participants #5, light blue = #6. Some data might not be visible in the figure due to overlapping markers.

Figure 12

Association Between High Cortisol Symptoms and Anxiety Severity Using Pooled Raw Data



Notes. Marker colour differentiates participants: red = participants #1, orange = participants #2, yellow = participant #3, light green = participants #4, dark green = participants #5, light blue = #6. Some data might not be visible in the figure due to overlapping markers.

Appendix A

Hamilton Anxiety Rating Scale (HAM-A)

Below is a list of phrases that describe certain feeling that people have. Rate the patients by finding the answer which best describes the extent to which he/she has these conditions. Select one of the five responses for each of the fourteen questions.

0 = Not present, 1 = Mild, 2 = Moderate, 3 = Severe, 4 = Very severe.

1 Anxious mood [0] [1] [2] [3] [4]

Worries, anticipation of the worst, fearful anticipation, irritability.

2 Tension [0] [1] [2] [3] [4]

Feelings of tension, fatigability, startle response, moved to tears easily, trembling, feelings of restlessness, inability to relax.

3 Fears [0] [1] [2] [3] [4]

Of dark, of strangers, of being left alone, of animals, of traffic, of crowds.

4 Insomnia [0] [1] [2] [3] [4]

Difficulty in falling asleep, broken sleep, unsatisfying sleep and fatigue on waking, dreams, nightmares, night terrors.

5 Intellectual [0] [1] [2] [3] [4]

Difficulty in concentration, poor memory.

6 Depressed mood [0] [1] [2] [3] [4]

Loss of interest, lack of pleasure in hobbies, depression, early waking, diurnal swing.

7 Somatic (muscular) [0] [1] [2] [3] [4]

Pains and aches, twitching, stiffness, myoclonic jerks, grinding of teeth, unsteady voice, increased muscular tone.

8 Somatic (sensory) [0] [1] [2] [3] [4]

Tinnitus, blurring of vision, hot and cold flushes, feelings of weakness, pricking sensation.

9 Cardiovascular symptoms [0] [1] [2] [3] [4]

Tachycardia, palpitations, pain in chest, throbbing of vessels, fainting feelings, missing beat.

10 Respiratory symptoms [0] [1] [2] [3] [4]

Pressure or constriction in chest, choking feelings, sighing, dyspnea.

11 Gastrointestinal symptoms [0] [1] [2] [3] [4]

Difficulty in swallowing, wind abdominal pain, burning sensations, abdominal fullness, nausea, vomiting, borborygmi, looseness of bowels, loss of weight, constipation.

12 Genitourinary symptoms [0] [1] [2] [3] [4]

Frequency of micturition, urgency of micturition, amenorrhea, menorrhagia, development of frigidity, premature ejaculation, loss of libido, impotence.

13 Autonomic symptoms [0] [1] [2] [3] [4]

Dry mouth, flushing, pallor, tendency to sweat, giddiness, tension headache, raising of hair.

14 Behavior at interview [0] [1] [2] [3] [4]

Fidgeting, restlessness or pacing, tremor of hands, furrowed brow, strained face, sighing or rapid respiration, facial pallor, swallowing, etc.

Appendix B

IBS Symptoms Evaluation

Name:

Age:

Date:

- Please rate your symptoms everyday by placing a tick in the box that best describes each symptom (Please tick none if you do not have this symptom).

	No symptoms or very rarely **None**	Occasional or mild symptoms **Mild**	Frequent symptoms **Moderate**	Continuous symptoms **Severe**
Abdominal pain/discomfort				
Abdominal bloating/distension				
Increased flatulence/wind				
Belching or burping				
Stomach/abdominal gurgling				
Incomplete evacuation (feeling of inability to pass all stool)				
Nausea				
Heartburn				
Acid regurgitation				
Tiredness/lethargy				

Appendix C

Heart rate vs Frightening Scene

Heart rate vs Frightening Scene:

Day 1:

- Heart rate before=

https://youtu.be/mBYGUn6Q7tQ

- Heart rate after=

Day 2:

- Heart rate before=

https://youtu.be/S51jIrunYuY

- Heart rate after=

Day 3:

- Heart rate before=

https://youtu.be/OxRIWBoluzs

- Heart rate after=

Day 4:

- Heart rate before=

https://youtu.be/lTb-DIU9vj8

- Heart rate after=

Day 5:

- Heart rate before=

https://youtu.be/pfGEdmZyVyY

- Heart rate after=

Day 6:

- Heart rate before=

https://youtu.be/ck1NO9MyQsM

- Heart rate after=

Day 7:

- Heart rate before=

https://youtu.be/nt9D55oZCnE

- Heart rate after=

Appendix D

High levels of cortisol questionnaire

Name:

Age:

Date:

Please check which symptoms you experienced on each day.

Symptoms:	MON	TUES	WED	THURS	FRI	SAT	SUN
Rounding of the face (Weight gain)							
Acne							
Fatigue							
Headache							
Flushed face							
Score:	/5	/5	/5	/5	/5	/5	/5

-The higher the score, the higher the chance that you are experiencing high levels of cortisol.

Appendix E

Daily Step Count

Name: **Age:** **Sex:**

Date	Time	Step Count

Appendix F

Daily Inflammation Measurement

Name:

Age:

Sex:

Date	Time	Measurement in cm

Appendix G

	Very much (2)	**A little (1)**	**No, not at all (0)**
Today, my choice of clothing was affected [by my excessive sweating].			
Today, I felt uncomfortable physically expressing affection (e.g. hugging others).			
Today, I worried about leaving sweat marks on things.			
Today, my appearance was affected [by my excessive sweating].			
Today, I thought about sweating.			