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FACULDADE DE MEDICINA DE RIBEIRÃO PRETO

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Heritability in social anxiety disorder and its impact on executive functioning, facial expression recognition, and symptomatic profile.

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Doctoral Dissertation submitted to the Mental Health Graduate Program (PPGSM) of the Faculty of Medicine of Ribeirão Preto – University of São Paulo (FMRP-USP) as part of requirements to the Doctoral Degree

Advisor: Prof. Dr. José Alexandre de Souza Crippa

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Approved in:

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SUMÁRIO

ABSTRACT

SILVA, A.L.M. Heritability in social anxiety disorder and its impact on executive functioning, facial expression recognition, and symptomatic profile. 2018. Doctoral Dissertation. Faculdade de Medicina de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2018

Social Anxiety Disorder (SAD) is a mental disorder characterized by fear and anxiety in social situations that lead to functioning impairment and suffering. Although a very frequent disorder among the population, some aspects of development and maintenance of SAD are still unclear. This doctoral dissertation is divided into five articles, that aimed to explore some of these gaps in previous studies. The first one describes the Cross-cultural Adaptation and Psychometric Properties of the Brazilian Version of the Thought Control Questionnaire, a measure of Negative Repetitive Thoughts frequently reported as a variable related to SAD. The second one is a systematic review of different methods and perspectives in the estimative of heritability of SAD. The third one describes a twin study that aimed to estimate the heritability of SAD in Brazil. The fourth one describes an experimental cross-over study designed to evaluate cognitive deficits in executive functioning in an anxiety-induced condition and to estimate the heritability of these deficits. Lastly, the fifth describes an experimental cross-over study aimed to evaluate the accuracy and intensity of facial emotion recognition in an anxiety-induced condition and to estimate the impact of heritability in the recognition. Together, these five articles provide valuable insights that help understand the development and maintenance of SAD.

Keywords: Social Anxiety Disorder, Heritability, Executive Functioning, Negative Repetitive Thoughts, Facial Emotion Recognition

RESUMO

SILVA, A.L.M. Herdabilidade do Transtorno de Ansiedade Social e seu impacto nas funções executivas, reconhecimento de expressão facial e perfil sintomático. 2018. Tese (Doutorado) Faculdade de Medicina de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2018

O Transtorno de Ansiedade Social (TAS) é um transtorno mental caracterizado por medo e ansiedade em situações sociais que leva ao comprometimento e ao sofrimento do funcionamento. Embora seja um transtorno muito frequente entre a população, alguns aspectos do desenvolvimento e da manutenção do TAS ainda não são claros. Esta tese de doutoramento está dividida em cinco artigos, que visaram explorar algumas dessas lacunas em estudos anteriores. O primeiro descreve a Adaptação Transcultural e as Propriedades Psicométricas da Versão Brasileira do *Thought Control Questionnaire*, uma medida de Pensamentos Negativos Repetitivos frequentemente relatada como uma variável relacionada ao TAS. O segundo é uma revisão sistemática sobre diferentes métodos e perspectivas na estimativa da herdabilidade do TAS. O terceiro descreve um estudo com gêmeos que objetivou estimar a herdabilidade do TAS no Brasil. O quarto descreve um estudo experimental *cross-over* com o objetivo de avaliar déficits cognitivos no funcionamento executivo em uma condição induzida por ansiedade e estimar a herdabilidade desses déficits. Por fim, o quinto descreve um estudo experimental *cross-over* com o objetivo de avaliar efeitos da indução da ansiedade no reconhecimento de expressão facial e o efeito da herdabilidade no reconhecimento. Juntos, esses cinco artigos fornecem informações importantes que ajudam a entender o desenvolvimento e a manutenção do TAS.

Palavras-chave: Transtorno de Ansiedade Social, Herdabilidade, Funções Executivas, Pensamentos Negativos Repetitivos, Reconhecimento de Expressão Facial

– CHAPTER I –

“Cross-cultural Adaptation and Psychometric Properties of the Brazilian Version of the Thought Control Questionnaire (TCQ)”

ABSTRACT

Negative Repetitive Thoughts (NRT) are key aspects to diagnose some mental disorders, related to suffer and impairment. Identifying NRT is important due to its role in the development of mental disorders and possibilities of interventions in maladaptive coping styles. This study aimed to evaluate psychometrical properties of the Brazilian version of Thought Control Questionnaire (TCQ), a measure of NRT. TCQ and symptoms measures of anxiety disorders (Dimensional Anxiety Scales) were administered to 900 adults, aged 18 to 70 years (64.7% females). Psychometric properties investigated were: factor structure, internal consistency, test-retest reliability, and concurrent validity. The original five-factor structure of the TCQ did not fit well with the Brazilian data. Alternatively, a very similar six-factor structure based on the results of an Exploratory Factor Analysis is suggested. Also, the number of items in the Brazilian version presented some differences, considering that two items of the original version were excluded. All factors presented adequate internal consistency coefficients and test-retest reliability evidence. Two factors of the TCQ (worry and punishment) showed a significant correlation with anxiety symptoms measures. These results are discussed considering how the changes in factor structure may positively interfere in the assessment of NRT and the availability of a new measure of NRT in Portuguese.

KEYWORDS: Negative Repetitive Thoughts; Though Control Questionnaire; Anxiety; Anxiety Disorders; Psychometrics

INTRODUCTION

Negative Repetitive Thoughts (NRT) are vital aspects to diagnose some mental disorders, such as worries in Generalized Anxiety Disorder, obsessions in Obsessive-Compulsive Disorder, traumatic reminiscences in Post-Traumatic Stress Disorder and rumination in Depression (American Psychiatric Association – APA, 2013). Furthermore, NRT is related to worse health indexes (Segestrom, Roach, Evans, Schipper & Darville, 2010) and predictors of several mental disorders (Calmes & Roberts, 2007; Caselli, Ferretti, Leoni, Rebechi, Rovedo & Spada, 2010) and negative mood ((Segestrom, Tsao, Alden & Craske, 2000). However, NRT may play a significant role in adaptive tasks, such as problem-solving (Watkins, Moulds, & Mackintosh, 2005; Watkins & Moulds, 2005).

Thereby, it is essential to comprehend variables related to NRT that could help to understand the differences between the prediction of mental disorders and improvement in problem-solving tasks. The way that people cope with NRT may be one of these variables. For example, using suppression as a coping strategy to NRT is related to worse symptomatology and worse prognostic in mental disorders (Fox, Dutton, Yates, Geourgiou & Mouchilianitis, 2015; Shimizu & Shimizu, 2015). On the other hand, the reappraisal is a crucial coping strategy used in effective treatment to NRT (Kaplan et al., 2018), related to better outcome.

The Thought Control Questionnaire (TCQ) (Wells and Davies, 1994) is an instrument that assesses coping strategies related to NRT. In its original version, TCQ provides the assessment of five factors associated with coping in NRT: distraction, social control, worry, punishment, and reappraisal. TCQ has been used as outcome measure in several observational (Halvorsen, Hagen, Hjemdal, Eriksen, Sørli, Waterloo, Eisemann, & Wang, 2015; Tucker, Smith, Hollingsworth, Cole, & Wingate, 2017) and clinical (Allen, Krompinger, Matheus, Crosby & Elias, 2016) studies. Furthermore, TCQ has been adapted to use in many countries, such as Portugal (Ros & Orts, 2016), Turkey (Yorulmaz & Gençöz, 2008) and Germany (Fehm & Hoyer, 2004), maintaining its good psychometrical properties in transcultural contexts. However, despite the high prevalence of mental disorders related to NRT in Brazil (Rombaldi, Silva, Gazalle, Azevedo, & Hallak, 2010; Jansen et al., 2011), there is no instrument available to assess coping strategies to NRT adapted to Brazilian population.

Thus, this study aims to evaluate psychometrical properties of the Brazilian version of TCQ, especially considering its factor structure, internal consistency, and test-retest reliability. A secondary aim is to assess concurrent validity with other mental disorders, using

dimensional rating scales of anxiety disorders. It is expected that some factor of TCQ, such as worry and punishment, present a strong positive correlation with anxiety disorders measures, while factors distraction and reappraisal present negative correlation with anxiety disorders measures.

METHOD

Participants and procedures

Participants were 900 young adults and adults recruited by convenience sampling from universities and other educational institutions (e.g., schools for adults; post-graduation courses) in the Brazilian states of Rio Grande do Sul (RS, n = 447) and Minas Gerais (MG, n = 453). Students, teachers, professors, researchers and other employees in the institutions were invited to participate in the study. Classes were selected by convenience sampling within the institutions. The sample was 64.7% female (n = 582) and had a mean age of 22.27 years old (SD = 5.88; range = 18 – 70). The instruments were completed during class periods. After providing informed consent, participants completed the questionnaires individually in classrooms of 20 to 30 subjects.

In order to assess concurrent validity of the TCQ, participants also completed the DSM-5 Dimensional Anxiety Scales (DeSousa et al., 2017; LeBeau et al., 2012). To assess the test-retest reliability of the Thought Control Questionnaire, a subsample of 47 participants completed the scales again from seven to nine days later. This subsample was chosen by convenience sampling of one class from each of the two universities where researchers had the permission to conduct a retest data collection. The test-retest subsample was 74.5% female and had a mean age of 20.24 years old (SD = 2.34, range = 18 – 29). The study design was approved by the local Ethics Committee (project number 25.298)

Instruments

The Thought Control Questionnaire (Wells & Davies, 1994) is a 30-item self-report measure that assesses coping strategies to NRT. Participants rate how often they use the coping strategy related to the item in a 4 item Likert scale, ranging from 1 (never) to 4 (almost always). In the original study (Wells and Davies, 1994), internal consistency was adequate (Cronbach´s α ranging from .64 and .79 among the factors), and test-retest coefficients

ranging from r = .67 and r = .83 among the factors. TCQ was cross-culturally adapted to Brazil following recognized procedures based on standardized literature (Gjersing, Caplehorn, & Clausen, 2010). The Brazilian version of the TCQ is available upon request to the first author of this study.

The DSM-5 Dimensional Anxiety Scales (LeBeau et al., 2012) are a set of five anxiety-disorder-specific questionnaires, each composed of 10 items assessing the frequency of anxiety symptoms on a 5-point scale (0 = "never"; 1 = "occasionally"; 2 = "half of the time"; 3 = "most of the time"; 4 = "all of the time"). The scales were designed to measure the core symptoms of anxiety disorders in a concise and dimensional perspective, as a result of efforts from members and advisors from the Anxiety Disorders Subgroup of the DSM-5 Anxiety, OC Spectrum, Post-traumatic, and Dissociative Disorder Work Group, who developed a common template and disorder-specific items for the scales. The five anxiety disorders assessed by the scales include: Generalized Anxiety Disorder (GAD-D); Social Anxiety Disorder (SAD-D); Panic Disorder (PD-D); Specific Phobia (SP-D); and Agoraphobia (AG-D). The DSM-5 Dimensional Anxiety Scales were cross-culturally adapted to Brazil and presented adequate psychometric properties (DeSousa et al., 2017).

Data Analysis

Confirmatory factor analysis (CFA) was used to examine whether the original factor structure of the TCQ (Wells and Davies, 1994) would fit to the Brazilian data. CFA was conducted in Mplus using the Weighted Least Squares Means and Variance Adjusted (WLSMV) estimation method to account for the categorical ordinal nature of the scale items. For fit indices, we calculated the CFI, TLI, and RMSEA with 90% Confidence Interval. Values of the CFI and TLI equal to or higher than .95 represent a good fit. Values of the RMSEA equal to or lower than .05 represent a good fit (Brown, 2006; Hu & Bentler, 1999). As an alternative in case of poor CFA indices, an exploratory factor analysis (EFA) would be conducted using the same estimation method and fit indices to investigate the possibility of a new factor structure, specific to Brazilian context. In the EFA, values of the SRMR equal to or lower than .05 represent a good fit (Brown, 2006). Standardized regression weights as factor loadings of the items were calculated in both CFA and EFA. Loadings equal to or higher than .30 were deemed adequate.

Cronbach's alpha coefficients were calculated to evaluate the internal consistency of each of the TCQ factors. Alpha values above .70 were deemed adequate (Onwuegbuzie &

Daniel, 2002). Test-retest reliability was determined by calculating the Intraclass Correlational Coefficients (ICCs) between the factor scores of the TCQ at Time 1 and Time 2. ICCs were calculated in SPSS using Two-Way Mixed Effect Model and Absolute Agreement Type, with a confidence interval set to 95%. ICC estimates that exceeded .70 were deemed adequate (Murphy & Davidshofer, 1996).

Pearson correlations were calculated between the TCQ factor scores and the DSM-5 Dimensional Anxiety Scales scores to assess concurrent validity. We used *Z* tests to assess if the magnitude of the correlations were significantly higher for convergent measures than for divergent measures (Meng, Rosenthal, & Rubin, 1992).

RESULTS

1. Factor Structure

The CFA and EFA results are depicted in Table 1. The original five-correlated-factors model presented poor fit to the sample (i.e., RMSEA > .08, and CFI and TLI < .90). Since the original TCQ factor structure did not fit well to the Brazilian sample, an EFA was conducted. The EFA yielded a six-correlated-factors model with good fit indices. The model suggested in the EFA in the Brazilian sample maintained four of the five factors proposed in the original TCQ: Distraction; Worry; Punishment; and Reappraisal. Only the Social Control factor was split into two factors in the EFA (Table 2).

Tables 1 and 2 around here

The original Social Control factor was composed of six items, of which three were direct items that assessed social control (e.g., 'I don't talk about the thought to anyone') and three were items that assessed social openness that had their scores reversed before composing the factor score (e.g., 'I talk to a friend about the thought'). Indeed, the Social Control factor was the only factor of the TCQ in the original model that contained items that had to be reversed prior to calculating the factor scores. Nonetheless, in the EFA conducted in the Brazilian sample, the items that composed the Social Control factor loaded into two separated factors: Social Control (three items) and Social Support (three items) (Table 2).

Two other items presented differences in relation to the original TCQ structure in the EFA results. As can be seen in Table 2, the item 3 ('I focus on the thought') did not load higher than .30 in any of the six factors. Furthermore, the item 7 ('I dwell on other worries'), which was originally part of the Worry factor, presented a cross-loading in the Distraction and Worry factors, with an unexpectedly higher loading on the Distraction factor. Due to these results, items 3 and 7 were therefore excluded from the calculation of the TCQ factor scores.

Six factor scores for the TCQ were calculated based on the EFA results: Punishment; Social Control; Distraction; Reappraisal; Worry; and Social Support. These factor scores were used in the subsequent analysis of internal consistency, test-retest reliability, and concurrent validity with the DSM-5 Dimensional Anxiety Scales scores.

2. Internal Consistency and Test-Retest Reliability

Table 3 depicts the internal consistency coefficient values calculated for the TCQ factor scores. Cronbach's alpha values ranged from .672 to .853. All factors presented adequate internal consistency coefficients (i.e., between .70 and .90) except for the Worry factor, which present a marginally acceptable alpha (.672). Table 3 also depicts the test-retest reliability results. ICCs ranged from .714 to .869, therefore all factors presented adequate test-retest reliability evidence. The highest coefficients were found for the Social Support and the Distraction factors.

Table 3 around here

3. Concurrent Validity

Table 4 presents the Pearson correlation coefficients among the scores of the six TCQ factors, as well as the correlations between the TCQ factor scores and the DSM-5 Dimensional Anxiety Scales scores. Results of the *Z* tests demonstrated that the correlations between the Punishment and Worry factor scores and the DSM-5 Dimensional Anxiety Scales scores was stronger than the correlations between the remaining TCQ factor scores and the concurrent anxiety measures (all $p < .05$).

Table 4 around here

DISCUSSION

This study investigated the psychometric properties of the Brazilian version of the TCQ in a large non-clinical sample, including the relationship between coping strategies used in Negative Repetitive Thoughts and symptoms of anxiety disorders. The original five-factor structure of the TCQ did not fit well with the Brazilian data. Alternatively, a very similar six-factor structure based on the results of an Exploratory Factor Analysis is suggested. Also, the number of items in the Brazilian version presented some differences, considering that two items of the original version were excluded. Two factors of the TCQ (worry and punishment) showed a significant correlation with anxiety symptoms measures.

Concerning the original TCQ, the Brazilian version presented different factor structure. The Brazilian version of the TCQ presented a six-factor structure provided by the EFA. The only notable difference from the original study was that the original *Social Control* factor was divided into two factors, i.e., *Social Control* and *Social Support*. This seems to be a satisfactory solution to Brazilian version of TCQ considering that the highest alphas were found for the *Social Support* and the *Social Control* factors, despite them been composed of only three items each, suggesting that they may be used as separated strategies. Furthermore, a detailed analysis of the items indicates that this may improve the results of observational and clinical studies using TCQ. For instance, dividing social strategies in social support and social control could help clinicians to distingue diverse ways that patients may cope with negative repetitive thoughts from a social perspective. In a more specific situation, social support may be an adequate strategy (e.g., item 29) while social control may not contribute to an improvement in treatment (item 8). Additionally, the Brazilian version with six factors does not require scores to be reversed before calculating the factor scores of the questionnaire. This may reduce misunderstandings in the correction of TCQ and improve its use in large samples studies.

For concurrent validity, it was expected that the factors of TCQ presented different correlations between anxiety disorders, as they describe different of even opposite coping strategies (e.g., worry and distraction). This hypothesis was partially confirmed. The *Punishment* factor presented significant positive correlation with all anxiety disorders measures, suggesting that such an aggressive coping style to negative repetitive thoughts is related to development and maintenance of these disorders. The *Worry* factor presented significant positive correlation with measures of Generalized Anxiety Disorder (GAD), Social Anxiety Disorder (SAD) and Panic Disorder (PD) but did not with measures of Specific

Phobia and Agoraphobia. An alternative explanation to this observation is that GAD, SAD and PD are anxiety disorders related to continuous cognitive symptoms, while specific phobia and agoraphobia are better characterized by physiologic and behavioral symptoms in the presence of a feared stimulus (Clark & Beck, 2013). Therefore, it is somehow coherent that a cognitive strategy such as worry is related to disorders in which cognitive symptoms are important constructs to formulate the diagnosis.

On the other hand, factors that might be considered protectors for the development of mental disorders, such as reappraisal and social support, did not present negative significant correlation with anxiety disorders, as expected. Reappraisal and social support are essential therapeutic goals in some effective treatments for negative repetitive thoughts, including Cognitive Behavioral Therapy (Kaplan et al., 2018). However, in these treatments, reappraisal and social support are offered considering individual necessities and specific techniques. Consequently, although reappraisal and social support are consolidated coping strategies used in treatments to negative repetitive thoughts, they may not be effective when used the adequate support of a clinician.

The present study has some significant limitations. First, we used as participants a community sample in an ecological assessment. Therefore, it was impossible to determine some sample characteristics that may influence the results as medications in use, psychosocial treatments, previous mental disorder diagnosis, comorbid disorders and prior presence of negative repetitive thoughts. Additionally, we did not assess the validity through convergent measures that could provide a better understanding of how negative repetitive thoughts are related to other variables. However, the absence of instruments in Brazil in related field interferes in these purposes. Despite these limitations, the present study has shown that the Brazilian version of TCQ is a very important tool to research and clinical fields in mental health in Brazil, as it offers a measure of good validity and reliability to assess negative repetitive thoughts with an adequate factor structure specific to Brazilian population. Future studies with different people and in clinical settings are desirable and opportune.

REFERENCES

American Psychiatric Association (2013). *Diagnostic and statistical manual of mental disorders (5th ed.)*. Washington, DC: American Psychiatric Association

Brown, T. A. (2006). *Confirmatory factor analysis for applied research*. New York: The Guilford Press

DeSousa, D. A., Moreno, A. L., Osório, F. L., Crippa, J. A. S., LeBeau, R., Manfro, G. G., Salum, G. A., & Koller, S. H. (2017). Psychometric properties of the Dimensional Anxiety Scales for DSM-5 in a Brazilian community sample. I*nternational Journal of Methods in Psychiatric Research, 26*(3), e1531. doi:10.1002/mpr.1531

Fehm, L., & Hoyer, J. (2004). Measuring Thought Control Strategies: The Thought Control Questionnaire and a Look Beyond. *Cognitive Therapy and Research*, 28(1).

Gjersing, L., Caplehorn, J. R. M., & Clausen, T. (2010). Cross-cultural adaptation of research instruments: language, setting, time and statistical considerations. *BMC Medical Research Methodology, 10*, 13. doi:10.1186/1471-2288-10-13

Hu, L. & Bentler, P. M. (1999). Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. *Structural Equation Modeling: A Multidisciplinary Journal, 6*, 1-55. doi:10.1080/10705519909540118

Kaplan, D.M., Palitsky, R., Carey, A.L., et al. (2018). Maladaptive repetitive thought as a transdiagnostic phenomenon and treatment target: An integrative review. *Journal of Clinical Psychology*, 1-11. https://doi.org/10.1002/jclp.22585

LeBeau, R. T., Glenn, D. E., Hanover, L. N., Beesdo-Baum, K., Wittchen, H., & Craske, M. G. (2012). A dimensional approach to measuring anxiety for DSM-5. *International Journal of Methods in Psychiatric Research, 21*(4), 258-272. doi:10.1002/mpr.1369

Meng, X., Rosenthal, R., & Rubin, D. B. (1992). Comparing correlated correlation coefficients. *Psychological Bulletin, 111*, 172-175. doi:10.1037//0033-2909.111.1.172

Murphy, K.R., & Davidshofer, C.O. (1996). *Psychological Testing: Principles and Applications* (4th ed). Englewood Cliffs, NJ: Prentice Hall International Inc.

Onwuegbuzie, A. J., & Daniel, L. G. (2002). A framework for reporting and interpreting internal consistency reliability estimates. *Measurement and Evaluation in Counseling and Development, 35*, 89-103.

Ros, A.M.J., & Orts, L.M.P. (2016). El cuestionario de control del pensamiento (tcq): propiedades psicométricas de la versión portuguesa (Portugal). *Revista Argentina de Clinica Psicologica*, 25(1), 27-38.

Segerstrom, S. C., Roach, A. R., Evans, D. R., Schipper, L. J., & Darville, A. K. (2010). The structure and health correlates of trait repetitive thought in older adults. *Psychology and Aging*, 25(3), 505-515. http://dx.doi.org/10.1037/a0019456

Shimizu, K., & Shimizu, H. (2015). The Influence of Perfectionism and Thought Suppression on Negative Rumination. *Psychology Research*, 5(5), 292-299.

Watkins, E., Moulds, M., & Mackintosh, B. (2005). Comparisons between rumination and worry in a non-clinical population. *Behaviour research and therapy*, 43, 1577-1585. doi:10.1016/j.brat.2004.11.008

Watkins, E., & Moulds, M. (2005). Distinct modes of ruminative self-focus: Impact of abstract versus concrete rumination on problem solving in depression. *Emotion*, 5(3), 319-328. http://dx.doi.org/10.1037/1528-3542.5.3.319

Wells, A., & Davies, M.I. (1994). The Thought Control Questionnaire: A measure of individual differences in the control of unwanted thoughts. *Behavior Research and Therapy*, 32 (8), 871-878.

Yorulmaz, O., & Gençöz, T. (2008). Psychometric properties of three questionnaires that evaluate appraisal and control processes in OCD symptoms. *Turkish Psychological Articles*, 11, 1–13.

Table 1 - *Model fit indices for the factor structure of the Thought Control Questionnaire tested by means of Confirmatory and Exploratory Factor Analyses*

CFA	**χ^2 (*df*)**	**CFI**	**TLI**	**RMSEA [90% CI]**	
Original five-factor model	3750.26 (395)	.780	.758	.097 [.094 – .100]	
EFA	**χ^2 (*df*)**	**CFI**	**TLI**	**RMSEA [90% CI]**	**SRMR**
One-factor model	10195.50 (405)	.359	.311	.164 [.161 – .167]	.180
Two-factor model	5039.62 (376)	.695	.647	.117 [.115 – .120]	.126
Three-factor model	3084.79 (348)	.821	.776	.093 [.090 – .097]	.079
Four-factor model	1926.46 (321)	.895	.858	.075 [.071 – .078]	.057
Five-factor model	1315.64 (295)	.933	.901	.062 [.059 – .065]	**.044**
Six-factor model	770.61 (270)	**.967**	**.947**	**.045 [.042 – .049]**	**.031**

Note. CFI: Comparative Fit Index; TLI: Tucker–Lewis Index; RMSEA [90% CI]: Root Mean Square Error of Approximation with 90% Confidence Interval; SRMR: Standardized Root Mean Square Residual

Table 2. - *Standardized regression weights of the six-correlated-factor model of the Thought Control Questionnaire*

Brazilian questionnaire item	Factor (Standardized regression weight)					
	Punishment	Social Control	Distraction	Reappraisal	Worry	Social Support
11. Eu fico com raiva de mim mesmo(a) por ter...	**.827***	.060	.033	–.002	–.002	.039
13. Eu grito comigo mesmo(a) por ter o pensamento	**.770***	–.171*	–.028	.002	.128*	–.122*
6. Eu me dou uma punição por pensar tal pensamento	**.691***	.005	–.045	–.035	.112*	–.035
15. Eu dou um tapa ou um soco em mim mesmo(a)...	**.658***	–.072	.004	–.067	.006	–.017
28. Eu digo a mim mesmo(a) que alguma coisa ruim...	**.512***	.075	.102*	–.010	.140*	.061
2. Eu digo a mim mesmo(a) para não ser tão idiota	**.504***	.025	.185*	.091*	–.046	.012
8. Eu guardo o pensamento para mim mesmo(a)	–.042	**.873***	.006	.065*	.059*	–.098*
12. Eu evito falar sobre o pensamento	.168*	**.822***	.059*	–.045*	–.038	–.037
5. Eu não falo com ninguém sobre o pensamento	.004	**.776***	–.068*	–.008	.020	–.005
16. Eu busco um pensamento agradável	.045	.003	**.796***	.077*	–.215*	.057
21. Eu penso em alguma outra coisa	.006	.032	**.757***	.079*	–.035	–.059
30. Eu me mantenho ocupado(a) com outra coisa	–.035	.168*	**.652***	–.021	.059	.111*
1. Eu tento trazer imagens positivas à mente	.171*	–.088*	**.604***	.015	–.145*	.042
19. Eu faço alguma coisa que eu gosto	–.100*	.109*	**.584***	–.063	–.001	.274*
9. Eu me ocupo com trabalho	.039	.123*	**.453***	.148*	.097*	–.021
14. Eu analiso o pensamento racionalmente	–.154*	.076*	–.040	**.733***	.023	.012
20. Eu tento interpretar o pensamento de outra forma	–.016	–.061	.182*	**.683***	.007	.035
23. Eu tento pensar de uma maneira diferente	–.024	–.085*	.337*	**.644***	.050	–.041
10. Eu questiono se o pensamento é válido	.099*	.037	.051	**.611***	–.042	.000
27. Eu me pergunto o motivo de ter este pensamento	.371*	.000	–.018	**.564***	–.051	.056
22.Eu penso mais em um problema de menor...	–.002	–.120*	.440*	–.005	**.718***	–.033
26. Eu me concentro em pensamentos negativos...	.243*	.068	–.180*	.029	**.631***	.079
4. Eu troco o pensamento por outro pensamento ruim...	.138*	.042	.060	.014	**.613***	.013
18. Eu me preocupo com coisas menos importantes	–.047	–.096*	.383*	–.027	**.597***	.022
24. Eu penso sobre minhas preocupações do passado	.189*	.049	–.043	.174*	**.423***	.012
7. Eu me concentro em outras preocupações	.051	.070	**.509***	–.004	**.316***	–.073
25. Eu pergunto aos meus amigos se eles têm...	.049*	–.008	–.022	.000	–.013	**.919***
29. Eu converso com um amigo(a) sobre o pensamento	.008	–.193*	.002	.025	.006	**.798***
17. Eu procuro saber como meus amigos lidam...	–.029	–.056	.045	.048	.044	**.782***
3. Eu me concentro no pensamento	.079	.136*	–.166*	.154*	.274*	.084

Note. * $p < .05$

Table 3 - *Internal consistency and test-retest reliability coefficients of the Thought Control Questionnaire*

TCQ Factor	Reliability estimates	
	Internal consistency (Cronbach's α)	Test-retest reliability (Intraclass Correlation Coefficient)
Punishment	**.715**	**.794**
Social Control	**.849**	**.714**
Distraction	**.785**	**.867**
Reappraisal	**.770**	**.849**
Worry	.672	**.795**
Social Support	**.853**	**.869**

Table 4 - *Pearson correlations between the Thought Control Questionnaire factor scores and the DSM-5 Dimensional Anxiety Scales scores*

		Thought Control Questionnaire (TCQ)						DSM–5 Dimensional Anxiety Scales				
		Punishment	Social Control	Distraction	Reappraisal	Worry	Social Support	GAD–D	SAD–D	PD–D	SP–D	AG–D
TCQ Factor	Punishment	1	.177*	.121*	.171*	**.418***	.095*	**.449***	**.436***	**.439***	**.369***	**.420***
	Social Control		1	.062	.049	.187*	**–.329***	.129*	.168*	.134*	.122*	.111*
	Distraction			1	**.366***	.172*	.180*	.021	–.004	.048	.128*	.087*
	Reappraisal				1	.253*	**.315***	.050	.056	.009	.059	.038
	Worry					1	.148*	**.349***	**.347***	**.302***	.281*	.289*
	Social Support						1	.049	.014	–.012	.030	.055

Note. GAD: Generalized Anxiety Disorder; SAD: Social Anxiety Disorder; PD: Panic Disorder; SP: Specific Phobia; AG: Agoraphobia (AG-D); * $p < .001$. **Bold cells** refer to correlation coefficients > .30. Highlighted cells refer to correlation coefficients of the concurrent validity analysis.

– CHAPTER II –

Heritability of social anxiety disorder: a systematic review of methodological designs.

[Paper published at Archives of Clinical Psychiatry]

Reference: Moreno, A.L., Osório, F.L., Martin-Santos, R., & Crippa, J.A.S. (2016). Heritability of social anxiety disorder: a systematic review of methodological designs. Archives of Clinical Psychiatry, 43(4), 84-92.

– CHAPTER III –

“The impact of heritability on social anxiety disorder: a twin study”

ABSTRACT

Heritability of social anxiety disorder (SAD) have been estimated using different methods and populations but still unclear the relationship between genetic and environmental factors in development and maintenance of this disorder. Furthermore, heritability of SAD has never been studies in any development country using a twin-study design. This study aimed to estimate the heritability of SAD and other psychopathological in a twin-study in a developing country and the stability of these traits after 6 months. Participants were 110 twins (37 monozygotic twin dyads and 18 dizygotic same-sex twin dyads), aged from 18 to 51 years (76,4 % female). Participants were assessed through the following instruments: Patient Health Questionnaire, Social Anxiety Disorder – Dimensional Scale, Beck Anxiety Inventory, Self-Statements Public Speaking, Social Phobia Inventory and Thought Control Questionnaire. Correlations between monozygotic twins presented no difference when compared to correlations between dizygotic twins in all instruments. However, agreement of diagnosis was significant in monozygotic twins and not significant in dizygotic twins. Correlations between monozygotic twins maintained constant over time, suggesting that the heritability of SAD in adults is a stable variable. Results are discussed considering differences between clinical and populational studies and the importance of methodological refinement of designs to better understand heritability in SAD.

KEYWORDS: Heritability; Social anxiety disorder; twin, social phobia; genetics.

INTRODUCTION

Social Anxiety Disorder (SAD) is a prevalent condition, characterized by fear or anxiety in social situations that cause distress or avoidance in social situations and affects daily life activities (American Psychiatric Association – APA, 2013). Despite the existence of many models that explain the development and maintenance of SAD (Morán & Oláz, 2018), they all tend to agree that the disorder is a result of interactions between risk factors and stressful situations (Ollendick & Hirshfeld-Becker, 2002). Heritability refers to the degree of variation in a trait through populations that are due to genetic factors (Wray & Visscher, 2008), and one of the risk factors to the development of SAD (Stein et al., 2017).

A recent review of heritability of social anxiety disorder (Moreno, Osório, Martin-Santos, & Crippa, 2016) reported that heritability rates vary between 13% and 76%, suggesting that this variance may be the result of the large variety of methodological designs used to assess heritability. Additionally, the authors discussed that more studies should consider using dimensional measures to evaluate heritability based in recent changes in diagnostic criteria of mental disorders (Craske, 2012; Regier, Narrow, Kuhl, & Kupfer, 2009). Also, this review highlighted that heritability of SAD has never been studied in any developing country, a critical issue to consider establishing cultural and socio-economic differences in development and maintenance of social anxiety disorder.

Heritability may be estimated using different methodological designs. However, twin-studies are the most established design to study heritability (Moreno, Osório, & Crippa, 2018), in a scenario in which the genetic similarity can be explored. One of the possible approaches to estimate heritability in twins is using the correlation between their responses to a certain measure and considering the difference of the correlation between responses of monozygotic and dizygotic pairs.

This study aimed to estimate the heritability of SAD and other psychopathological traits using dimensional instruments that assess symptoms of these conditions in a twin-study in a developing country. We expect the correlation between scores of monozygotic twins to be higher than the correlation between dizygotic twins, considering that genetic variables seem to play a significant role in heritability in these traits. Furthermore, we hypothesized that correlation between scores of monozygotic twins would not differ when assessed between 6 months of an interval, considering that these traits are stable over time.

METHODS

Participants and Procedures

Participants were 110 twins (37 monozygotic twin dyads and 18 dizygotic same-sex twin dyads) recruited through announcements in regular media (local radios, TV programs, newspapers) and social media (fan page in Facebook exclusively designed to this study). Eligibility criteria for inclusion were: living in the metropolitan region of Ribeirão Preto (Inner State of São Paulo/Brazil); age over 18 years old; complete high school level of education. Eligibility criteria for exclusion was patients in course of any mental disorder. The exclusion criteria were fellfield based in a telephone application of SRQ-20 (Harding et al, 1980; Mari & Williams, 1986), a screening scale for mental disorders with adequate specificity and sensitivity to the purpose of the study. Patients that scored over the cutoff point in SRQ-20 (6 points) were submitted to a telephone application of SCID-IV (Modules of Mood Disorders, Psychotic Disorders, Anxiety Disorders and Substance-related Disorders) (First et al, 1997; Del-Ben, Vilela, Crippa, Hallak, Labate & Zuardi, 2001; Crippa, Osório, Del-Ben, Filho, Freitas & Loureiro, 2008). After the SCID-IV application, patients diagnosed with previous major depression disorder and anxiety disorders were maintained in the study, due the high comorbidity between these disorders and SAD (Stein & Stein, 2008) and the minimal impact of these disorders in the procedure. A subsample of 29 monozygotic twin dyads participated in a reassessment at least six months after the first assessment.

The study design was approved by the local Ethics Committee (project number 270.406). All participants that reported difficulties with symptoms described in one of the measures or pointed above the cutoff point of Patient Health Questionnaire (PHQ-9) (Kroenke, Spitzer & Williams, 2001) and Social Phobia Inventory (SPIN) (Connor, Davidson, Churchill, Sherwood, Foa, & Weisler, 2000) were referred to an outpatient service. The demographic characterization of the sample is depicted in Table 1.

Table 1 around here

Instruments

Self-Statements Public Speaking (SPSS) (Hofmann & DiBartolo, 2000). This scale consists of 10 items comprising self-perception of performance in public speaking. Each item is displayed as a likert scale and scored between 0 (full disagreement) and 5 (full agreement). The 10 items are divided in two subscales: positive self-evaluation (items 1, 3, 5, 6 and 9) and negative self-evaluation (items 2, 4, 7, 8 and 10). The Brazilian version of SPSS, used in this study, present good internal consistency ($.78 < \alpha > .90$) (Osório, Crippa, & Loureiro, 2008) and adequate discriminative validity between cases and controls of SAD (Osório, Crippa & Loureiro, 2012).

Social Phobia Inventory (SPIN) (Connor, Davidson, Churchill, Sherwood, Foa, & Weisler, 2000). The scale is a self-administered instrument that assesses physiological symptoms of fear and avoidance in SAD. It consists of 17 items evaluated on a five- point Likert likert scale, divided in 5 factors: talking to strangers and social situations; criticisms and embarrassment; physiological changes; authority figures; and avoiding being the center of attention, and public speaking. Several studies in Brazil (Osório, Crippa, & Loureiro, 2009; Osório, Crippa, & Loureiro, 2010c; Vilete, Coutinho, & Figueira, 2004; Vilete, Figueira, & Coutinho, 2006) were conducted to evaluate psychometric properties of SPIN, and demonstrate adequate internal consistency, acceptable concurrent validity with different instruments of auto- and heteroevaluation of SAD, and satisfatory discriminative validity between cases and controls of SAD, indicating that the Brazilian version of SPIN is adequate to use in clinical and research contexts.

Thought Control Questionnaire (TCQ) (Wells and Davies, 1994). TCQ is an instrument that assesses coping strategies to negative repetitive thoughts. The Brazilian version of TCQ (Moreno, DeSousa, Osório, & Crippa, in press), used in this study, consist of 28 items, scored in a 5-point likert, divided in six factors: punishment, social control, distraction, reappraisal, worry, and social support. The Brazilian version of TCQ present adequate internal consistency ($.67 < \alpha > .85$), acceptable Test-Retest Reliability (Intraclass correlation coefficients ranging from .71 to .87) and satisfatory concurrent validity with instruments used to assess anxiety symptoms (Moreno, DeSousa, Osório, & Crippa, in press)

Social Anxiety Disorder Dimensional Scale (SAD-D) (LeBeau, Glenn, Hanover, Bessdo-Baum, Wittchen & Craske, 2012). SAD-D is a scale that assesses symptoms of SAD according to DSM-5 diagnosis criteria. It consists of f 10 items displayed on a 5-point likert scale (0 = "never";1= "occasionally";2= "half of the time"; 3 = "most of the time";4= "all of

the time"), in one single factor. The Brazilian version of SAD-D (DeSousa, Moreno, Osório, Crippa, LeBeau, Manfro, Sallum & Koller, 2017), used in this study, present adequate internal consistency, test-retest reliability, convergent validity with SPIN and divergent validity with a measure of attention deficit hyperactivity disorder.

Patient Health Questionnaire (PHQ-9) (Kroenke, Spitzer & Williams, 2001). PHQ-9 is an instrument that assesses symptoms of major depression in the preceding two weeks. It consists of nine items scored on a four-point likert scale (0= "not at all"; 3 = "nearly every day"), in one single factor. The Brazilian version of PHQ-9 (Copyright © 2005 Pfizer Inc., New York, NY) presented adequate discriminative validity between major depressed patients and controls (Osório, Vilela-Mendes, Crippa, & Loureiro, 2009).

Beck Anxiety Inventory (BAI) (Steer & Beck, 1997). This is a 21-item scale that assesses anxiety symptoms. The items are displayed in a four-point likert scale, with total score ranging from zero to 63. The Brazilian version of BAI (Cunha, 2001), used in this study, presented good internal consistency (.71<α> .92) and adequate test-retest reliability.

Data analysis

The scores of the symptom measurement instruments (i.e., SAD-D, SPIN, SSPS, TCQ, PHQ-9, and BAI) were calculated and tested for normality. Skewness and kurtosis coefficients were evaluated along with the Kolmogorov-Smirnov and Shapiro-Wilk test results. Table 2 presents the detailed results of the normality analyses for each instrument. Five of the 12 instrument scores presented non-normal skewness or kurtosis coefficients (i.e., values above 1.00 or below –1.00). Furthermore, Kolmogorov-Smirnov test results indicated absence of normality for all scores and Shapiro-Wilk test results indicated absence of normality for all scores but the Re-appraisal factor of the Thought Control Questionnaire. Non-parametric tests were used for the investigation of the hypothesis for all scores in order to provide comparable results across instruments.

As suggested by the literature (e.g: López-Solà et al., 2014), cross-twin within-trait Spearman correlations were calculated to explore the contribution of genetic and environmental factors by comparing the correlations of each instrument score within the monozygotic dyads to the ones within the dizygotic dyads. Confidence intervals of 95% were calculated for each correlation using bias corrected accelerated (BCa) bootstrapping based on 1.000 random subsamples (Field, 2013).

To investigate the temporal stability of the symptom measurement instrument scores, two types of analyses were conducted. First, in line with the analysis comparing the correlations within monozygotic dyads to the ones within dizygotic dyads, we also calculated the correlations within the monozygotic dyads for the reassessment data (n = 29 dyads). Second, to investigate the temporal stability of the instruments in general, a test-retest reliability analysis was conducted for all monozygotic twins that completed both assessments (n = 58 twins). We calculated intraclass correlation coefficients (ICCs) between scores of the test and the retest assessments using Two-Way Mixed Effect Model and Absolute Agreement Type Analysis, considering a confidence interval of 95%. ICC values equal to or above .70 were deemed adequate (Murphy & Davidshofer, 1996).

RESULTS

Table 3 presents the results of the cross-twin within-trait Spearman correlations and the confidence intervals for the monozygotic dyads and the dizygotic dyads. Significative correlations were found between monozygotic twins in all the variables in the first assessment. In the dizygotic twins, correlation between the dyads presented significative values only in the following instruments: SPIN, Self-Statements Public Speaking – Negative Evaluation (SSPS_N); Thought Control Questionnaire – Punishment (TCQ_PU); Thought Control Questionnaire – Social Control (TCQ_SC); Thought Control Questionnaire – Worry (TCQ_WO); Patient Health Questionnaire (PHQ9); and Beck Anxiety Inventory (BAI). There were differences in the values of correlations when comparing monozygotic and dizygotic twins. However, when those values are compared considering confidence intervals (upper and lower bounds), no variables presented differences between the correlation in monozygotic and dizygotic twins.

To test a further hypothesis that heritability values are understated in populational samples and might be highlighted in clinical groups, we used the SPIN scores to divide individuals into ones that scored above and ones that scored below the 18-point cutoff score suggestive of clinical social anxiety (Osório, Crippa & Loureiro, 2010). After that, we compared the kappa coefficient as an agreement estimate within monozygotic twin dyads and within dizygotic twin dyads, i.e., percentage of dyads that presented the same classification for both twins (above/above or below/below cutoff) or opposite ones (above/below or below/above cutoff). For monozygotic twins, 22 dyads presented the same classifications and 13 dyads presented opposite ones, with a significant kappa coefficient (k = .240; p = .029). In

contrast, for dizygotic twins, 10 dyads presented identical classifications and 7 dyads presented opposite ones, with a non-significant kappa coefficient (k = .212; p = .156).

The temporal stability analyses were conducted in two ways. Table 3 depicts the comparison of the cross-twin within-trait Spearman correlations for the monozygotic dyads in the assessment and in the reassessment times. However, in the second assessment, the correlation between monozygotic twins was not significative in the SAD-DS and in the reappraisal factor of the TCQ. Considering confidence intervals to compare values of correlation between assessment and reassessment, no differences were found between the two occasions, indicating temporal stability of the assessed traits.

Furthermore, Table 4 presents the test-retest reliability results for all twins that completed both assessments. Considering the ICCs, we found adequate values of temporal stability to all instruments, except for the positive evaluation factor of SSPS and four factors of TCQ (Social Control, Social Support, Worry and Distraction).

DISCUSSION

This study aimed to estimate heritability of Social Anxiety Disorder in twins, using dimensional instruments. We expected that the correlation between scores of monozygotic twins would be higher than correlation between dizygotic ones. However, this hypothesis was not confirmed, considering that confidence intervals between correlations of monozygotic and dizygotic twins presented no difference. We also expected correlations of monozygotic twins to be constant over time, suggesting that the heritability of SAD in adults is a stable variable. We confirmed that second hypothesis using two separate times of assessment in monozygotic twins.

The hypothesis that monozygotic twins would present higher correlation of scores in instruments than the correlation of dizygotic twins was based in a variety of previous studies that presented these findings (Moreno, Osório, Martin-Santos & Crippa, 2016). In our sample, correlation values were different between monozygotic and dizygotic twins, but when confidence intervals were considered, this difference was not significative. One possible explanation to these conflicting results is the small sample used in the present study, considering that smaller samples lead to greater confidence intervals (Grieve, 1991). If this is the case, although it might have differences between monozygotic and dizygotic twins, it would not be clear due the large confidence interval of the correlations. It is important to highlight that some previous studies that estimated heritability based in differences of

correlations between monozygotic and dizygotic twins (eg: Elley, Rijsdijk, Perrin, O´Connor, & Bolton, 2003; Van Hulle, Schmidt, & Goldsmith, 2012) did not considered confidence intervals to assure differences between correlations, which may have impacted further analysis of heritability in SAD.

Other possible explanation to our negative finding is the fact that SAD is a complex disease, and by so, influenced by a wide variation of genetic, socio-ambiental and personal factors. In this scenario, familiar studies may tend to inflate rates of heritability, particularly in complex diseases that shared familiar environmental effects play a significant role in the development of the disease (Zuk, Hechter, Sunyaev, & Lander, 2012), which is the case of SAD (Morán & Orlaz, 2018). This expansion in heritability rates occurs because familiar studies often are based in a case-control perspective, and consequently, an overestimation of some characteristics in a sample that does not represent the role population on this anxiety disorder. This study used a populational approach, and participants were selected by convenience despite their previous diagnosis of SAD. This fact may have impacted the absence of difference in correlations between monozygotic and in between dizygotic twins, considering that they shared familiar environmental effects are also considered in dizygotic twins. Considering this, some authors discuss that the importance of case-control familiar studies in heritability should be seen in a critical perspective (Manolio, Collins, Cox, Goldstein, Hindorff, Hunter, & McCarthy, 2009; Joseph, 2012).

The hypothesis that heritability values are highlighted in clinical perspectives was also pointed in the results of the present study. When a cut-off point of a standard measure of SAD was used to divide participants in cases of SAD and healthy participants, agreement of diagnosis was significant in monozygotic twins and not significant in dizygotic twins. This result reinforces that might be a difference between heritability measures in populational based and clinical based studies, and more efforts should be dedicated to consolidating this hypothesis.

Previous studies reported that estimates of heritability tend to vary through time, when assessed by correlations between monozygotic twins (Moreno, Rocio, Osório & Crippa, 2016). However, all these studies have considered long periods of reassessment (at least two years) and were conducted in children and adolescents (Hallett, Ronald, Rijsdijk, & Happé, 2012; Kendler et al, 2008; Ogliari et al, 2010; Tzarskowski et al, 2012). As opposed, the present study was conducted with adults, with a brief period of reassessment (6 months) and no difference in correlation between pairs of monozygotic twins were found between times of assessment. This result suggests that heritability of SAD is a stable variable in adults, when considered through a brief period. Heritability variation in different stages of childhood and

adolescence is usually related to the idea that risk factors to development of SAD change through developmental stages (Kendler et al, 2008). Considering this idea, heritability in adults should be stable, since changes in risk factors in this stage are not considered to be related to development of the disorder.

This study has some limitations that should be considered. The sample in this study was selected by convenience, which have an important impact in the generalization of the findings. Most twin studies are based in birth and medical follow-up records selection method, providing previous information and perspective about the population that the sample will be selected. These records are still not in course in developing countries, and to encourage these records is essential to provide better heritability studies in these countries. Other characteristic in this study is the exclusion criteria, that did not include patients in course of another mental disorder. Bearing in mind that Social Anxiety Disorder is usually related to high comorbidity rates with other mental disorders (APA, 2013), this should be considered to comprehend the results of this study. Furthermore, it is important that future studies consider using healthy individuals to estimate SAD traits in a dimensional perspective in population to better understand how heritability impact the development of SAD.

REFERENCES

American Psychiatric Association (2013). *Diagnostic and statistical manual of mental disorders (5th ed.)*. Washington, DC: American Psychiatric Association

Arrais, K. C., et al (2010). Social anxiety disorder women easily recognize fearfull, sad and happy faces: The influence of gender. *Journal of Psychiatric Research*, 44, 535–540.

Connor, K.M.; Davidson, J.R.; Churchill, L.E.; Sherwood, A.; Foa, E.; Weisler, R.H. (2000). Psychometric properties of Social Phobia Inventory (SPIN). New self- rating scale. *British Journal of Psychiatry*, 176, 379- 86.

Crippa, J.A., Osório, F.L., Del-Ben, C.M., Filho, A.S., Freitas, M.C.S. & Loureiro, S.R. (2008). Comparability between telephone and face-to-face structured clinical interview for DSM-IV in assessing social anxiety disorder. *Perspectives in psychiatric care*, 44(4), 241-247.

Del-Ben, C.M.; Vilela, J.A.A.; Crippa, J.A.S.; Hallak, J.E.C.; Labate, C.M. & Zuardi, A.W. (2001). Confiabilidade da "Entrevista Clínica Estruturada para o DSM-IV – Versão Clínica" traduzida para o português. *Revista Brasileira de Psiquiatria*, 23 (3), 156-159.

Elley, T.C.; Rijsdijk, F.V.; Perrin, S.; O´Connor, T.G.; & Bolton, D. (2003). A multivariate genetic analysis of specific phobia, separation anxiety and social phobia in early childhood. *Journal of Abnormal Child Psychology*, 36(6), 839-848.

Field, A. P. (2013). *Discovering statistics using SPSS* (4th ed). London: Sage.

First, M.B.; Spitzer, R.L.; Gibbon, M.; Williams, J.B.W. (1997). *Structured clinical interview for DSM-IV axis I disorders – clinician version (SCID-CV)*. Washington (DC): American Psychiatric Press.

Grieve, A.P. (1991). Confidence intervals and sample sizes. *Biometrics*, 47(4), 1597-602.

Hallett, V.; Ronald, A.; Rijsdijk, F. & Happé, F. (2012). Disentangling the associations between autistic-like traits and internalizing traits: a community-based twin study. *Journal of Abnormal Childish Psychology*, 40(5), 815-827.

Hofmann, S.G.; DiBartolo, P.M. (2000). An instrument to assess self-statements during public speaking: scale development and preliminary psychometric properties. *Behavior Research and Therapy*, 31, 499-515.

Joseph, J. (2012). The "Missing Heritability" of Psychiatric Disorders: Elusive Genes or Non-Existent Genes? *Applied Developmental Science*, 16(2), 65-83. https://doi.org/10.1080/10888691.2012.667343

LeBeau, R. T., Glenn, D. E., Hanover, L. N., Beesdo-Baum, K., Wittchen, H., & Craske, M. G. (2012). A dimensional approach to measuring anxiety for DSM-5. International *Journal of Methods in Psychiatric Research*, 21(4), 258-272. Copyright © 2012 American Psychiatric Association.

Manolio, T.A.; Collins, F.S.; Cox, N.J.; Goldstein, D.B.; Hindorff, L.A.; Hunter, D.J.; McCarthy, M.I.; et al (2009). Finding the missing heritability of complex diseases. *Nature*, 461(8), 747-753. doi:10.1038/nature08494

Morán, V.E.; & Olaz, F.O. (2018). A review of the main explanatory models of social anxiety disorder. In: Osório, F.L.; & Donadon, M.F. (orgs) *Social Anxiety Disorder: Recognition, Diagnosis and Management.* New York: Nova Science Publishers.

Murphy, K. R., & Davidshofer, C. O. (1996). *Psychological Testing: Principles and Applications* (4th ed). Englewood Cliffs: Prentice Hall International Inc.

Osório, F.L.; Crippa, J.A. & Loureiro, S.R. (2008). Escala para Auto-Avaliação ao Falar em Público (SSPS): adaptação transcultural e consistência interna da versão brasileira. *Revista de Psiquiatria Clínica*, 35 (6), 207-211.

Osório, F.L.; Crippa, J.A. & Loureiro, S.R. (2009). Cross- cultural validation of the Brazilian Portuguese version of the Social Phobia Inventory (SPIN): study of the items and internal consistency. *Revista Brasileira de Psiquiatria*, 31 (1), 25-29.

Osório, F.L.; Crippa, J.A. & Loureiro, S.R. (2013). Validation of the state version of the Self-Statement during Public Speaking Scale. *Revista Brasileira de Psiquiatria*, 35 (1), 63-66.

Stein M.B.; Chen, C-Y.; Jain, S.; Jensen, K.P.; He, F.; Heeringa, S.G.; Kessler, R.C.; Maihofer, A.; Nock, M.K.; Ripke, S; Sun, X.; Thomas, M.L.; Ursano, R.J.; Smoller, J.W.; Gelernter, J; & On behalf of the Army STARRS Collaborators (2017). Genetic Risk Variants for Social Anxiety. *Am J Med Genet*, 174(B):120–131.

Van Hulle, C.A.; Schmidt, N.L.; & Goldsmith, H.H. (2012). Is sensory over-responsitivity distinguishable from childhood behavior problems? A phenotypic and genetic analysis. *Journal of Child Psychology and Psychiatry*, 53(1), 64-72.

Wells, A. & Davies, M.I. (2004). The thought control questionnaire: a measure of individual differences in the control of unwanted thoughts. *Behavior Research and Therapy*, 32 (8), 871-878.

Zuk, O.; Hechter, E.; Sunyaev, S.R.; & Lander, E.S. (2012). The mystery of missing heritability: Genetic interactions create phantom heritability. *PNAS*, 109(4), 1193-1198. https://doi.org/10.1073/pnas.1119675109

Table 1 - *Demographic characterization of the total sample and subsamples of monozygotic and dizygotic twins*

Variable	Total sample T1 (*n* = 110)	DZ T1 (*n* = 36)	MZ T1 (*n* = 74)	MZ T2 (*n* = 58)
Gender (female) *n* (%)	84 (76.4)	30 (83.3)	54 (73.0)	42 (72.4)
Age range (years)	18–51	18–35	18–51	18-52
Age *M* (*SD*) (years)	26.0 (7.6)	23.3 (5.0)	27.3 (8.4)	29.7 (8.8)
Educational level *n* (%)				
High school	26 (23.6)	8 (22.2)	18 (24.3)	12 (20.7)
College/University incomplete	29 (26.4)	15 (41.7)	14 (18.9)	8 (13.8)
College/University complete	55 (50.0)	13 (36.1)	42 (56.8)	38 (65.5)
Uses daily medication *n* (%)	40 (36.4)	16 (44.4)	24 (32.4)	27 (46.6)
Ever been in psychiatric or psychological treatment *n* (%)	41 (37.3)	14 (38.9)	27 (36.5)	26 (44.8)
Ever used psychiatric medication *n* (%)	17 (15.5)	6 (16.7)	11 (14.9)	16 (27.6)
Is currently in psychiatric or psychological treatment *n* (%)	14 (12.7)	5 (13.9)	9 (12.2)	7 (12.1)
Uses any psychoactive drug *n* (%)	7 (6.4)	1 (2.8)	6 (8.1)	2 (3.4)

Table 2 - Normality assumption analyses for the symptom measurement instrument scores (n = 110 twins)

	Skewness Coefficient	Kurtosis Coefficient	Kolmogorov-Smirnov Test Results	Shapiro-Wilk Test Results
SAD-D	1.346	1.390	.211; gl = 103; p < .001	.851; gl = 103; p < .001
SPIN	.849	–.134	.152; gl = 107; p < .001	.912; gl = 107; p < .001
SSPS_P	–.677	.314	.125; gl = 109; p < .001	.956; gl = 109; p = .001
SSPS_N	1.212	.629	.183; gl = 109; p < .001	.836; gl = 109; p < .001
TCQ_PU	1.040	.624	.150; gl = 110; p < .001	.901; gl = 110; p < .001
TCQ_SC	–.041	–1.084	.136; gl = 108; p < .001	.930; gl = 108; p < .001
TCQ_DI	–.531	.341	.116; gl = 109; p = .001	.962; gl = 109; p = .003
TCQ_RE	–.317	–.115	.100; gl = 107; p = .010	.978; gl = 107; p = .067
TCQ_WO	.475	.280	.125; gl = 108; p < .001	.970; gl = 108; p = .016
TCQ_SS	.399	–.753	.151; gl = 109; p < .001	.915; gl = 109; p < .001
PHQ9	.960	.633	.162; gl = 110; p < .001	.921; gl = 110; p < .001
BAI	1.357	1.802	.151; gl = 105; p < .001	.875; gl = 105; p < .001

Note. SAD-D: Social Anxiety Disorder Dimensional Scale ; SPIN: Social Phobia Inventory; SSPS_P: Self-Statements Public Speaking – Positive Evaluation; SSPS_N: Self-Statements Public Speaking – Negative Evaluation; TCQ_PU: Thought Control Questionnaire – Punishment; TCQ_SC: Thought Control Questionnaire – Social Control; TCQ_DI: Thought Control Questionnaire – Distraction; TCQ_RE: Thought Control Questionnaire – Reappraisal; TCQ_WO: Thought Control Questionnaire – Worry; TCQ_SS: Thought Control Questionnaire – Social Support; PHQ9: Patient Health Questionnaire; BAI: Beck Anxiety Inventory.

Table 3 - Cross-twin within-trait Spearman correlations of the symptom measurement instrument scores for the monozygotic dyads (assessment Time 1 and reassessment T2) and dizygotic dyads (assessment T1)

	DZ T1 (*n* = 18 dyads)			**MZ T1 (*n* = 37 dyads)**			**MZ T2 (*n* = 29 dyads)**		
	rho	**95% CI [LB; UB]**	***p***	**rho**	**95% CI [LB; UB]**	***P***	**rho**	**95% CI [LB; UB]**	***p***
SAD-D	.325	–.249; .775	.219	.667	.363; .879	< .001	.289	–.060; .570	.144
SPIN	.786	.467; .919	<.001	.558	.277; .781	<.001	.580	.200; .838	.001
SSPS_P	.313	–.298; .772	.221	.429	.057; .725	.008	.514	.176; .786	.004
SSPS_N	.704	.232; .980	.001	.635	.400; .809	< .001	.629	.364; .823	< .001
TCQ_PU	.889	.693; .971	<.001	.742	.522; .884	< .001	.625	.387; .801	< .001
TCQ_SC	.771	.617; .866	<.001	.548	.184; .793	.001	.606	.334; .780	< .001
TCQ_DI	.261	–.470; .806	.295	.661	.360; .845	< .001	.627	.327; .853	< .001
TCQ_RE	.500	–.124; .881	.041	.642	.425; .789	< .001	.442	.076; .731	.019
TCQ_WO	.781	.588; .865	<.001	.652	.398; .840	< .001	.656	.402; .831	< .001
TCQ_SS	.592	.126; .889	.012	.585	.323; .771	< .001	.596	.344; .763	.001
PHQ9	.676	.344; .875	.002	.635	.341; .842	< .001	.613	.312; .821	< .001
BAI	.733	.410; .882	.001	.618	.296; .832	< .001	.552	.175; .837	.003

Note. SAD-D: Social Anxiety Disorder Dimensional Scale ; SPIN: Social Phobia Inventory; SSPS_P: Self-Statements Public Speaking – Positive Evaluation; SSPS_N: Self-Statements Public Speaking – Negative Evaluation; TCQ_PU: Thought Control Questionnaire – Punishment; TCQ_SC: Thought Control Questionnaire – Social Control; TCQ_DI: Thought Control Questionnaire – Distraction; TCQ_RE: Thought Control Questionnaire – Reappraisal; TCQ_WO: Thought Control Questionnaire – Worry; TCQ_SS: Thought Control Questionnaire – Social Support; PHQ9: Patient Health Questionnaire; BAI: Beck Anxiety Inventory; LB: confidence interval lower bound; UB: confidence interval upper bound.

Table 4 - Intraclass correlation coefficients (ICCs) of the symptom measurement instrument scores in the assessment and reassessment for the monozygotic dyads (n = 29 dyads)

	ICC	95% CI [LB; UB]
SAD-D	.777	.612; .871
SPIN	.849	.741; .912
SSPS_P	.694	.482; .819
SSPS_N	.757	.586; .857
TCQ_PU	.832	.717; .901
TCQ_SC	.663	.428; .801
TCQ_DI	.556	.245; .738
TCQ_RE	.777	.621; .869
TCQ_WO	.691	.476; .818
TCQ_SS	.628	.370; .780
PHQ9	.834	.719; .902
BAI	.793	.645; .880

Note. SAD-D: Social Anxiety Disorder Dimensional Scale ; SPIN: Social Phobia Inventory; SSPS_P: Self-Statements Public Speaking – Positive Evaluation; SSPS_N: Self-Statements Public Speaking – Negative Evaluation; TCQ_PU: Thought Control Questionnaire – Punishment; TCQ_SC: Thought Control Questionnaire – Social Control; TCQ_DI: Thought Control Questionnaire – Distraction; TCQ_RE: Thought Control Questionnaire – Reappraisal; TCQ_WO: Thought Control Questionnaire – Worry; TCQ_SS: Thought Control Questionnaire – Social Support; PHQ9: Patient Health Questionnaire; BAI: Beck Anxiety Inventory; LB: confidence interval lower bound; UB: confidence interval upper bound.

– CHAPTER IV –

“Effect of Induced Social Anxiety and Heritability in Executive Functioning”

ABSTRACT

Social Anxiety disorder (SAD) is related to cognitive deficits which are partially explained by attentional control of threatening stimuli that leads to impairment in executive functioning. Assessing cognitive deficits in an ecological anxiety-related condition is important to understand their magnitude in daily patients' lives better. Furthermore, little is known about the heritability of cognitive deficits in SAD. This study aimed to evaluate cognitive deficits in executive functioning in an anxiety-related condition that simulates threatening stimuli in SAD. The second aim of this study was to estimate the heritability of executive functioning in an anxiety-related condition in a twin-study design. Participants were 110 twins (37 monozygotic twin dyads and 18 dizygotic same-sex twin dyads), aged from 18 to 51 years (76,4 % female). Participants were assessed through symptom measures (Patient Health Questionnaire, Social Anxiety Disorder – Dimensional Scale, Beck Anxiety Inventory, Self-Statements Public Speaking, Social Phobia Inventory and Thought Control Questionnaire) and have executive function evaluated using Wisconsin Card Sorting Test, Verbal Fluency Test and Victoria Stroop Test in a cross-over design, in which one condition Simulated Public Speaking Test was used to induce anxiety and other was neutral. Cognitive performance in anxiety-induced condition was no different than in the neutral condition. Furthermore, we observe that participants tend to improve their performances in the reassessment, independently of the order of the conditions, which characterizes either learning or habituation effect in the tests. There was no difference between correlations of monozygotic and dizygotic twins in WCST, supporting previous findings that strong genetics aspects do not influence WSCT. Results are discussed considering learning effects in cognitive performance and heritability of complex tasks.

KEYWORDS: Social Anxiety Disorder, Executive Functioning, Heritability, Twins.

INTRODUCTION

Social Anxiety Disorder (SAD) (APA, 2013) is a mental disorder characterized by fear and anxiety in social situations that lead to functioning impairment and suffering. The core of its phenomenology is related to the fear of being negatively evaluated by others, causing hypervigilance and avoidance. Several studies have also reported cognitive deficits in anxiety disorders (Stopa & Clark, 2000; Heinrichs & Hofman, 2001; Clark & McManus, 2002; Hirsch & Clark, 2004; Amir, Elias, Klumpp & Przeworski, 2003; Moreno, Ávila-Souza, Gomes & Gauer, 2015), a distinctive feature that may worse impairment in SAD.

Attentional Control Theory (ACT) (Eysenck & Derakshan, 2011; Eysenck, Derakshan, et al., 2007) is one of the approaches to understand cognitive deficits related to anxiety. The ACT proposes that cognitive deficits would be caused by impairments in executive functions of inhibition and alternation, leading to an increase of attentional control to the aversive stimuli in detriment of the attentional control to other stimuli. These attentional control to aversive stimuli would be, after that, responsible for performance and efficiency deficits. Some studies have offered some preliminary support to ACT in social anxiety, reporting cognitive deficits when emotional features are involved in executive function assessment (Mohnman & DeVito, 2017) or including an observer that leads to performance anxiety (Rezaei, Ramaghani & Fazio, 2017). Thus, it important to consider assessing cognitive performance during anxiety-related contexts, to explore attentional control deficits better.

Wisconsin Card Sorting Test (WCST) (Heaton, 1981) has been considered the gold standard instrument for assessing executive functions, due its high sensitivity that contribute to differential diagnosis (Van der Werf, Witter, Uylings, & Jolles, 2000, Brower & Price, 2001, Brosnan et al. 2002) and the variety of functions assessed (cognitive flexibility, inhibition, categorization, impulsivity and attention) (Heaton, Chelune, Talley, Kay & Curtiss, 2005). However, few studies had evaluated individual differences in the executive functions related to SAD using WCST (Topçuoğlu, Fistikci, Ekinc, Gimzal-Gönentür & Cömert-Agouridas, 2009; Fujii et al., 2013). In these studies, patients with SAD presented impairment in some WCST variables when compared to controls, and the magnitude of the impairment was related to the severity of SAD (Fujii et al., 2013). Nevertheless, such studies did not perform the evaluation of executive functions in anxiety-related scenarios, which may be an important aspect to understand if the magnitude of the deficits in the executive functions is related to the threatening situations. Also, former studies did not assess the efficiency of performance, factors that are hypothetically important for characterizing the

neuropsychological deficits in anxiety (Eysenck & Derakshan, 2011; Derakshan & Eysenck, 2009).

It is also essential that the assessment of the executive functioning includes more than one test, considering the variety of functions involved in this concept. Verbal fluency is a task frequently used in the assessment of executive functioning, mainly related to the semantic association, executive flow and shifting. Although been used to characterize cognitive deficits in anxiety disorders, the verbal fluency test does has not shown conclusive results regarding its sensitivity to detect these deficits specially in SAD (Castaneda, Tuulio-Henriksson, Marttunen, Suvisaari & Lönnqvist, 2008; Rosso, Young, Femia & Yurgelun-Todd, 2004; Airaksinen, Larsson & Forssel, 2005). The Victoria Stroop Test (Spreen & Strauss, 1998) is another widely used task in the assessment of executive functioning, exploring the Stroop effect to assess shifting and attentional control, although it has not been used to assess anxiety.

In addition to assessing the magnitude of anxiety-related cognitive deficits, it is also essential to determine the foundation of individual differences related to these deficits. In this scenario, heritability may be one of the sources of these differences, clarifying the relationship between attentional control as a function and the neurobiological basis for this function. Several authors support the hypothesis that a large part of the differences in healthy individuals is explained by genetic influences (Friedman, Miyake, Young, DeFries, Corley & Hewitt, 2008; Lessov-Schlaggar, Swan, Reed, Wolf & Carmelli, 2007; Carmelli, Swan, DeCarli & Reed, 2002). However, some experimental twin studies present controversies with this hypothesis, indicating that executive functioning present little evidence of genetic influence when WCST is used to assess individuals (Taylor, 2007; Kremen, Eisen, Tsuang & Lyons, 2007). It is also unclear how heritability may influence cognitive performance in anxiety-related tasks.

Considering these aspects, this study aimed to evaluate cognitive deficits in executive functioning in an anxiety-related condition that simulates threatening stimuli in SAD. The second aim of this study is to estimate the heritability of executive functioning in an anxiety-related condition in a twin-study design. We expect to observe cognitive deficits in the anxiety condition, compared to the neutral one. We also expect that the correlation between task-scores of monozygotic twins to be higher than the correlation between dizygotic twins, following the hypothesis that executive functioning is related to strong genetic influences.

METHODS

Participants and Recruitment Procedures

Participants were 110 twins recruited by convenience through announcements in regular (local radios, TV programs, newspapers) and social media (fan page in Facebook exclusively designed to this study). Of the 55 twin dyads, 37 were monozygotic twin dyads and 18 were dizygotic same-sex twin dyads. A subsample of 29 monozygotic twin dyads participated in a reassessment after at least 6 months from the first evaltuation. Eligibility criteria for inclusion were: living in the metropolitan region of Ribeirão Preto (Inner State of São Paulo/Brazil); age over 18 years old; complete high school level of education. Eligibility criteria for exclusion was patients in course of a mental disorder. The exclusion criteria were fellfield based in a telephone application of SRQ-20 (Harding et al, 1980; Mari & Williams, 1986), a screening scale for mental disorders with adequate sensitivity to the purpose of the study. Patients that scored over the cutoff point in SRQ-20 (6 points) were submitted to a telephone application of SCID-IV (Modules of Mood Disorders, Psychotic Disorders, Anxiety Disorders and Substance-related Disorders) (First et al, 1997; Del-Ben, Vilela, Crippa, Hallak, Labate & Zuardi, 2001). After the SCID-IV application, patients diagnosed with *previous* major depression disorder, dysthymia and anxiety disorders were maintained in the study, due the high comorbidity between these disorders and SAD (Stein & Stein, 2008) and the minimal impact of these disorders in the procedure. The study design was approved by the local Ethics Committee (project number 270.406). All participants that reported difficulties with symptoms described in one of the measures or pointed above the cutoff point of PHQ-9 and SPIN were referred to mental health outpatient service. The demographic characterization of the sample is depicted in Table 1.

[Table 1 around here]

Instruments and Tasks

Self-Statements Public Speaking (SPSS) (Hofmann & DiBartolo, 2000). This scale consists of 10 items comprising self-perception of performance in public speaking. Each item is displayed as a likert scale and scored between 0 (full disagreement) and 5 (full agreement).

The 10 items are divided in two subscales: positive self-evaluation (items 1, 3, 5, 6 and 9) and negative self-evaluation (items 2, 4, 7, 8 and 10). The Brazilian version of SPSS, used in this study, present good internal consistency ($.78 < \alpha > .90$) (Osório, Crippa, & Loureiro, 2008) and adequate discriminative validity between cases and controls of SAD (Osório, Crippa & Loureiro, 2012).

Social Phobia Inventory (SPIN) (Connor, Davidson, Churchill, Sherwood, Foa, & Weisler, 2000). The scale is a self-administered instrument that assesses physiological symptoms of fear and avoidance in SAD. It consists of 17 items evaluated on a five- point Likert likert scale, divided in 5 factors: talking to strangers and social situations; criticisms and embarrassment; physiological changes; authority figures; and avoiding being the center of attention, and public speaking. Several studies in Brazil (Osório, Crippa, & Loureiro, 2009; Osório, Crippa, & Loureiro, 2010c; Vilete, Coutinho, & Figueira, 2004; Vilete, Figueira, & Coutinho, 2006) were conducted to evaluate psychometric properties of SPIN, adequate internal consistency, adequate concurrent validity with different instruments of auto- and heteroevaluation of SAD, and adequate discriminative validity between cases and controls of SAD, indicating that the Brazilian version of SPIN is adequate to use in clinical and research contexts.

Thought Control Questionnaire (TCQ) (Wells and Davies, 1994). TCQ is an instrument that assesses coping strategies to negative repetitive thoughts. The Brazilian version of TCQ (Moreno, DeSousa, Osório, & Crippa, in press), used in this study, consist of 28 items, scored in a 5-point likert, divided in six factors: punishment, social control, distraction, reappraisal, worry, and social support. The Brazilian version of TCQ present adequate internal consistency ($.67 < \alpha > .85$), adequate Test-Retest Reliability (Intraclass correlation coefficients ranging from .71 to .87) and adequate concurrent validity with instruments used to assess anxiety symptoms (Moreno, DeSousa, Osório, & Crippa, in press)

Social Anxiety Disorder Dimensional Scale (SAD-D) (LeBeau, Glenn, Hanover, Bessdo-Baum, Wittchen & Craske, 2012). SAD-D is a scale that assesses symptoms of SAD according to DSM-5 diagnosis criteria. It consists of f 10 items displayed on a 5-point likert scale (0 = "never";1= "occasionally";2= "half of the time"; 3 = "most of the time";4= "all of the time"), in one single factor. The Brazilian version of SAD-D (DeSousa, Moreno, Osório, Crippa, LeBeau, Manfro, Sallum & Koller, 2017), used in this study, present adequate internal consistency, test-retest reliability, convergent validity with SPIN and divergent validity with a measure of attention deficit hyperactivity disorder.

Patient Health Questionnaire (PHQ-9) (Kroenke, Spitzer & Williams, 2001). PHQ-9 is an instrument that assesses symptoms of major depression in the preceding two weeks. It consists of nine items scored on a four-point likert scale (0= "not at all"; 3 = "nearly every day"), in one single factor. The Brazilian version of PHQ-9 (Pfizer (Copyright © 2005 Pfizer Inc., New York, NY)) presented adequate discriminative validity between major depressed patients and controls (Osório, Vilela-Mendes, Crippa, & Loureiro, 2009).

Beck Anxiety Inventory (BAI) (Steer & Beck, 1997). This is a 21-item scale that assesses anxiety symptoms. The items are displayed in a four-point likert scale, with total score ranging from zero to 63. The Brazilian version of BAI (Cunha, 2001), used in this study, presented good internal consistency (.71<α> .92) and adequate test-retest reliability.

Wisconsin Card-Sorting Test (WCST) (Heaton, Chelune, Talley, Kay & Curtiss, 2005). WCST is a neuropsychological test that measures cognitive flexibility, planning, abstraction and perseverative responding. WCST consists of four stimulus-cards and 128 response-cards, based in a combination of colors, shapes and quantities. In this test, participant is asked to match each of 128 cards to one of the four stimulus-cards according to a principle that must deduced from the pattern of the examiner's response to each placement. In this study, we also asked the patients to match the cards as soon as possible. The pattern changes after the participant completes a category (sequence of 10 correct responses). Perseverative errors are considered when participant sustain the answer pattern despite the feed-back provided by the examiner. The test is ended when participant completes 5 categories or when all 128 response-cards are used. The variables used in this study were: number of completed categories, number of perseverative errors, number of cards used in the test and time to complete the test (in seconds).

Victoria Stroop Test (VST) (Stroop, 1935; Spreen & Strauss, 1998). VCT (Spreen & Strauss, 1998) is a neuropsychological test, adapted from the classical Stroop experimental paradigma (Stroop, 1935) used to assess cognitive inhibition and selective attention. It consists of 3 cards, each one constituted of 24 stimuli. In the first card (Time I), the stimuli are colored rectangles (green, rose, blue and brown) and the participant should nominate the color that is printed as soon as possible. In the second card (Time II), stimuli are the names of the colors, printed in black ink, and the participant should read the words as soon as possible. In the third card (Time III), color-words are printed in an inconsistent color ink (i.e. the word "rose" is printed in green ink), and the participant should name the color of the ink instead of reading the word as soon as possible. The variables used in this study were: number of erros

in each card, time to complete each card, and interference score (time – in seconds- to complete the third card minus time to complete the first card).

Verbal Fluency Test (VFT). Verbal fluency is a variable extensively used in neuropsychology to assess executive function. Phonetic verbal fluency is measured by the one´s ability to generate words beginning with a specific letter while semantic verbal fluency is measured by the one´s ability to generate words related to a specific category. In this study, the letters F, A and S were used to assess phonetic verbal fluency and the category "animal" to assess semantic verbal fluency, due to its extensive use in neuropsychology literature (Tombaugh, Kozak & Rees, 1999). Participants were asked to generate the maximum of words with each letter or with the category in 60 seconds. The number of generated words were considered to analyses.

Experimental Procedures

Simulated Public Speaking Test (SPST) (McNair, Frankenthaler, Czerlinsky, White, Sasson, & Fisher, 1982) is an accurate procedure to induce anxiety, used in a series of experimental studies (e.g. Bergamaschi et al, 2011; Zuardi et al, 2017; Kamath, Urval, & Shenoy, 2017), based in the principle that anxiety increases as the individual performs a public speech without previous planning and under evaluation. In this study, we used the SPST as described in Hallak and colleagues (2010).

The procedure of the anxiety-inducing condition is summarized in Table 2. After a 15-min adaptation period, pretest measures (SPSS, SPIN, TCQ, SAD-D, PHQ9 and BAI) were taken in a quiet room. Immediately thereafter, the participant was taken to another room, equipped with a tv set and video camera, where he received the instructions provided by one of us and had 2 min to prepare a 4-min speech about 'the public transportation system of your city'. Participant was also told that the speech would be recorded on videotape and later analyzed by a psychologist. After the instructions, participants started the experimental procedure, following this order: Wisconsin Card-Sorting Test, Victoria Stroop Test and Verbal Fluency Task. Then, the subject started speaking in front of the camera while viewing his/her own image on the TV screen. Finally, participants were asked to remain calm in a comfortable chair for 15 minutes.

The procedure of the neutral condition is summarized in Table 2. In this condition, after a 15-min adaptation period, pretest measures (SPSS, SPIN, TCQ, SAD-D, PHQ9 and BAI) were taken in a quiet room. Immediately thereafter, the participant was taken to other

room. After the instructions, participants started the experimental procedure, following this order: Wisconsin Card-Sorting Test, Victoria Stroop Test and Verbal Fluency Task. Finally, participants were asked to remain calm in a comfortable chair for 15 minutes.

Monozygotic twins completed both conditions, with at least 6 months of interval between the two occasions. Half of monozygotic pairs started the procedure by the anxiety-inducing condition, while the other half stared the procedure by the neutral condition in a cross-over design. Dizygotic twins completed only the anxiety-inducing condition.

Data analysis

The scores of the symptom measurement instruments (i.e., SAD-D, SPIN, SSPS, TCQ, PHQ-9, and BAI) were calculated and tested for normality. Skewness and kurtosis coefficients were evaluated along with the Kolmogorov-Smirnov and Shapiro-Wilk test results. Table 3 presents the detailed results of the normality analyses for each instrument. Five of the 12 instrument scores presented non-normal skewness or kurtosis coefficients (i.e., values above 1.00 or below –1.00). Furthermore, Kolmogorov-Smirnov test results indicated absence of normality for all scores and Shapiro-Wilk test results indicated absence of normality for all scores but the Re-appraisal factor of the TCQ. Non-parametric tests were used for the investigation of the hypothesis for all scores in order to provide comparable results across instruments. The scores of the neuropsychological tasks were tested for normality following the same rationale. Table 4 presents the detailed results of the normality analyses for each task. Similarly, the results of the Komogorov-Smirnov and the Shapiro-Wilk tests indicated absence of normality for almost all variables. Therefore non-parametric tests were used for the investigation of the hypothesis for all scores in order to provide comparable results across tasks.

To investigate influences of the experimental manipulation on the neuropsychological variables, paired-sample t-tests were conducted comparing the scores of the monozygotic twins in the neutral and in the experimental conditions. Since the same participant was exposed to the same tasks in both neutral and experimental conditions, to investigate the possibility of a learning effect, paired-sample t-tests were also conducted comparing the scores of the twins on the neuropsychological tests in the assessment and in the reassessment regardless of the experimental or neutral conditions.

As previously suggested (López-Solà et al., 2014), cross-twin within-trait Spearman correlations were calculated to explore the contribution of genetic and environmental factors

by comparing the correlations of each instrument score within the monozygotic dyads to the ones within the dizygotic dyads. Confidence intervals of 95% were calculated for each correlation using bias corrected accelerated (BCa) bootstrapping based on 1.000 random subsamples (Field, 2013).

RESULTS

Table 5 presents the results of the cross-twin within-trait Spearman correlations and confidence intervals of the neuropsychological tasks scores for the dizygotic dyads and the monozygotic dyads that have undergone the anxiogenic experimental condition at the first assessment time. Significative correlations were found between monozygotic twins only in the following variables: cards used in WCST; perseverative errors in WCST; errors and time in Time III of VST. In the dizygotic twins, correlation between the dyads presented significative values in the following variables: number of words all the phonetic tasks (F, A and S); cards used in WCST; perseverative errors in WCST; errors in Time III of VST and interference score in VST. Considering this, only in two variables were found significative correlations in between monozygotic and dizygotic twins (Cards used in WCST and perseverative errors in WCST). There were found differences in the values of correlations when comparing monozygotic and dizygotic twins in these variables. However, when those values are compared considering confidence intervals (upper and lower bounds), there were no differences between the correlation in monozygotic and dizygotic twins in none of the variables.

The comparison of the performance of the monozygotic twins on the neuropsychological tests in the neutral and experimental conditions is depicted in Table 6. There were no significant differences in the variables investigated except for the number of cards used in WCST. Participants in the neutral condition used more cards than participants in the experimental condition.

To investigate the possibility of a learning effect on the tasks, the comparison of the performance of the twins on the assessment and the reassessment is depicted in Table 7. It can be observed that task time, number of cards used, and number of perseverative errors presented lower mean scores in the reassessment in WCST. Similarly, errors in the VST task were lower in the reassessment for task I and time in the VST was lower in the reassessment for tasks II and III.

DISCUSSION

This study aimed to evaluate the cognitive deficits in executive functioning in an anxiety-related condition that simulates threatening stimuli in SAD. We used the Simulated Public Speaking Test to create an anxiety-related condition and assessed executive function using three different tests. We expected to observe cognitive deficits in the anxiety condition, as compared to the neutral condition. However, we did not observe any substantial deficit between the two conditions that support this hypothesis. Furthermore, we observe that participants tend to improve their performances in the reassessment, independently of the order of the conditions, which characterizes either learning or habituation effect in the tests.

SPST have been used with success as a method to induce anxiety in experimental scenarios (Oliveira, Zuardi, Graeff, Queiroz & Crippa, 2011; Bergamashi et al., 2011; Linares, Zuardi, Pereira, Queiroz, Mechoulam, Guimarães & Crippa, 2016). In this study, we did not observe cognitive deficits when participants performed the SPST when compared to a neutral condition. In this scenario, it not possible to determine if cognitive deficits were not related to anxiety stimuli or if the simulated public speaking test did not correctly induce anxiety in this procedure. Further studies are needed to understand this question better.

Learning effect is observed when a participant improves his performance over time without any intervention that may justify it (Oliveira, Trezza, Busse, & Jacob-Filho, 2014). Learning effect in WCST was also reported in a study that attempts to identify an acute exercise effect on executive function (Wang, Shih, Pesce, Song, Hung, & Chang, 2015). A recent meta-analysis also supports the presence of learning effect in other executive function tasks, suggesting that this should be strongly considered in the assessment of executive functioning over time (Pasion, Gonçalves, Fernandes, Ferreira-Santos, Barbosa, & Marques-Teixeira, 2017). Many studies have suggested that learning through the task is improved when considered in a scenario where the tested person may observe errors and when the performance on the task is related to reward, in a motivated-based performance (Cyr & Anderson, 2017). This is may be the case of our study, considering that errors in WSCT and VST, but not in VFT, are observable by the tested person and the executing the test in a laboratory may be considered a threatening scenario in which the absence of errors is a reward.

Also, the present study aimed to estimate the heritability of executive functioning in an anxiety-related condition in a twin-study design. We compared correlations variables related to executive functioning in monozygotic twins with the correlation of the same variables in

dizygotic twins. We expect that one correlation between task-scores of monozygotic twins to be higher than the correlation between dizygotic twins, following the hypothesis that executive functioning is related to strong genetic influences. However, there was no difference between correlations of monozygotic and dizygotic twins in WCST, the only variable that presented significative values of correlations.

There is a strong agreement that executive functioning and more specifically flexibility are highly influenced by genetic features (Lee et al., 2012). However, these findings are not supported when the gold-standard measure to assess executive functioning is considered (Taylor, 2007; Kremen, Eisen, Tsuang & Lyons, 2007; Chou, Kuo, Lin, & Chen, 2010). Also, a genome-wide association study of cognitive flexibility assessed by the WCST demonstrates that both genetic and nongenetic factors impact cognitive flexibility (Zhang, Zhou, Lencz, Farrer, Kranzler, & Galernter, 2018). The results of the present study support the findings that strong genetics aspects do not influence WSCT. Further studies with larger samples are needed to explore the characteristics of WSCT that may be related to differences of heritability in this test when compared to other measures of executive functioning.

REFERENCES

American Psychiatric Association (2013). *Diagnostic and statistics manual of mental disorders: DSM-5*. Arlinton: American Psychiatric Association.

Bergamashi, M. et al. (2011). Cannabidiol Reduces the Anxiety Induced by Simulated Public Speaking in Treatment-Naïve Social Phobia Patients. *Neuropsychopharmacology*, 36, 1219–1226. https://doi.org/10.1038/npp.2011.6

Chou, L., Kuo, P., Lin, C., & Chen, W.J. (2010). Genetic and Environmental Influences on the Wisconsin Card Sorting Test Performance in Healthy Adolescents: A Twin/Sibling Study. *Behavior Genetics*, 40(22). https://doi.org/10.1007/s10519-009-9299-3.

Cyr, A. & Anderson, N.D. (2017). Learning from our mistakes: effects of learning errors on memory in healthy younger and older adults. In: Haslam, C. & Kessels, R. (orgs). *Errorless Learning in Neuropsychological Rehabilitation: Mechanisms, Efficacy and Application.* London: Routledge.

Derakshan, N. & Eysenck, M.W. (2009). Anxiety, Processing Efficiency, and Cognitive Performance: New Developments from Attentional Control Theory. *European Psychologist*, 14 (2), 168-176.

Eysenck, M.W., Derakshan, N., Santos, R., & Calvo, M. G. (2007). Anxiety and cognitive performance: attentional control theory. *Emotion*, 7, 336-353.

Eysenck, M.W., & Derakshan, N. (2011). New perspectives in attentional control theory. *Personality and individual differences*, 50(7), 955–960.

Field, A. P. (2013). *Discovering statistics using SPSS* (4th ed). London: Sage.

Friedman, N.P.; Miyake, A.; Young, S.E.; DeFries, J.C.; Corley, R.P. & Hewitt, J.K (2008). Individual Differences in Executive Functions Are Almost Entirely Genetic in Origin. *Journal of Experimental Psychology: General*, 137 (2), 201-225.

Fujii, Y. et al (2013). Severity of generalized social anxiety disorder correlates with low executive functioning. *Neuroscience Letters*, 543, 42-46.

Heaton, R. K. (1981). *Wisconsin card sorting test manual*. Odessa, FL.: Psychological Assessment Resources.

Heaton, R.K.; Chelune, G.J.; Talley, J.L.; Kay, G.G.; Curtiss, G. (2005). *Teste Wisconsin de Classificação de Cartas: manual revisado e ampliado*; adaptação e padronização brasileira Jurema Alcides Cunha et al. (2005). São Paulo: Casa do Psicólogo.

Lee, T., Mosing, M. A., Henry, J. D., Trollor, J. N., Ames, D., Martin, N. G., et al. (2012). Genetic influences on four measures of executive functions and their covariation with general cognitive ability: The Older Australian Twins Study. *Behavior Genetics*, 42(4), 528–538.

Linares, I., Zuardi, A.W., Pereira, L.C.G., Queiroz, R.H.C., Mechoulam, R., Guimarães, F.S., & Crippa, J.A.S. (2016). Cannabidiol presents an inverted U-shaped dose-response curve in the simulated public speaking test. *European Neuropsychopharmacology*, 26(2), S617. https://doi.org/10.1016/S0924-977X(16)31702-3

Kremen, W.S., Eisen, S.A., Tsuang, M.T., & Lyons, M.J. (2007). Is the Wisconsin Card Sorting Test a useful neurocognitive endophenotype? *Neuropsychiatr Genet*, 144(4), 403-406. DOI: 10.1002/ajmg.b.30527

Mohlman, J. & DeVito, A. (2017). The impact of social threat cues on a card sorting task with attentional-shifting demands. *Journal of Behavior Therapy and Experimental Psychiatry*, 57, 45-52. https://doi.org/10.1016/j.jbtep.2017.02.004

Moreno, A.L., Ávila-Souza, J., Gomes, W.B., & Gauer, G. (2015). Effects of Worry on Verbal and Visual Working Memory. *Psychology and Neuroscience*, 8(3), 341-349.

Moreno, A.L., DeSousa, D.A., Osório, F.L. & Crippa, J.A.S. (in press). Cross-cultural Adaptation and Psychometric Properties of the Brazilian Version of the Thought Control Questionnaire (TCQ).

Murphy, K. R., & Davidshofer, C. O. (1996). *Psychological Testing: Principles and Applications* (4th ed). Englewood Cliffs: Prentice Hall International Inc.

Oliveira, D.C.G., Zuardi, A.W., Graeff, F.G., Queiroz, R.H.C., & Crippa, J.A.S. (2011). Anxiolytic-like effect of oxytocin in the simulated public speaking test. *Journal of Psychopharmacology*, 26(4), 497-504. https://doi.org/10.1177/0269881111400642

Osório, F.L.; Crippa, J.A. & Loureiro, S.R. (2008). Escala para Auto-Avaliação ao Falar em Público (SSPS): adaptação transcultural e consistência interna da versão brasileira. *Revista de Psiquiatria Clínica*, 35 (6), 207-211.

Osório, F.L.; Crippa, J.A. & Loureiro, S.R. (2009). Cross- cultural validation of the Brazilian Portuguese version of the Social Phobia Inventory (SPIN): study of the items and internal consistency. *Revista Brasileira de Psiquiatria*, 31 (1), 25-29.

Osório, F.L.; Crippa, J.A. & Loureiro, S.R. (2013). Validation of the state version of the Self-Statement during Public Speaking Scale. Revista Brasileira de Psiquiatria, 35 (1), 63-66.

Pasion, R., Gonçalves, A.R., Fernandes, C., Ferreira-Santos, F., Barbosa, F., & Marques-Teixeira, J. (2017). Meta-Analytic Evidence for a Reversal Learning Effect on the Iowa Gambling Task in Older Adults. Frontiers in Psychology, 11(8), 1785.

Rezaei, F., Ramaghani, N.A.H., & Fazio, R.L. (2017). The effect of a third-party observer and trait anxiety on neuropsychological performance: the Attentional Control Theory (ACT) perspective. *The Clinical Neuropsychologist*, 31(3), 632-643. https://doi.org/10.1080/13854046.2016.1266031

Stopa, L. & Clark, D.M. (2000). Social phobia and interpretation of social events. *Behaviour Research and Therapy*, 38 (3), 273-283.

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology*, 18, 643– 662.

Taylor, J. (2007). Heritability of Wisconsin Card Sorting Test (WCST) and Stroop Color-Word Test Performance in Normal Individuals: Implications for the Search for Endophenotypes. *Twin Research and Human Genetics*, 10 (6), 829–834.

Topçuoğlu, V.; Fistikci, N.; Ekinc, Ö.; Gimzal-Gönentür, A. & Cömert-Agouridas, B. (2009). Assessment of Executive Functions in Social Phobia Patients Using the Wisconsin Card Sorting Test. *Turkish Journal of Psychiatry*, 20 (4), 322-331.

Wang, C., Shih, C., Pesce, C., Song, T., Hung, T., & Chang, Y. (2015). Failure to identify an acute exercise effect on executive function assessed by the Wisconsin Card Sorting Test. *Journal of Sport and Health Science*, 4(1), 64-72. https://doi.org/10.1016/j.jshs.2014.10.003

Zhang, H., Zhou, H., Lencz, T., Farrer, L.A., Kranzler, H., & Galernter, J. (2018). Genome wide association study of cognitive flexibility assessed by the Wisconsin Card Sorting Test. *American Journal of Medical Genetics*, 177(5), 511-519.

Table 1 - *Demographic characterization of the total sample and subsamples of monozygotic and dizygotic twins*

Variable	Total sample T1 (*n*=110)	DZ T1 (*n*=36)	MZ T1 (*n*=74)	MZ T2 (*n*=58)
Gender (female) *n* (%)	84 (76.4)	30 (83.3)	54 (73.0)	42 (72.4)
Age range (years)	18–51	18–35	18–51	18–52
Age *M* (*SD*) (years)	26.0 (7.6)	23.3 (5.0)	27.3 (8.4)	29.7 (8.8)
Educational level *n* (%)				
High school	26 (23.6)	8 (22.2)	18 (24.3)	12 (20.7)
College/University incomplete	29 (26.4)	15 (41.7)	14 (18.9)	8 (13.8)
College/University complete	55 (50.0)	13 (36.1)	42 (56.8)	38 (65.5)
Uses daily medication *n* (%)	40 (36.4)	16 (44.4)	24 (32.4)	27 (46.6)
Ever been in psychiatric or psychological treatment *n* (%)	41 (37.3)	14 (38.9)	27 (36.5)	26 (44.8)
Ever used psychiatric medication *n* (%)	17 (15.5)	6 (16.7)	11 (14.9)	16 (27.6)
Is currently in psychiatric or psychological treatment *n* (%)	14 (12.7)	5 (13.9)	9 (12.2)	7 (12.1)
Uses any psychoactive drug *n* (%)	7 (6.4)	1 (2.8)	6 (8.1)	2 (3.4)

Table 2 - Comparative between experimental and neutral condition.

Time	Experimental Condition	Neutral Condition
−0:30	Adaptation to the laboratory; instructions about the interview and measurements	Adaptation to the laboratory; instructions about the interview and measurements
−0:15	SPSS, SPIN, TCQ, SAD-D, PHQ-9, BAI	SPSS, SPIN, TCQ, SAD-D, PHQ-9, BAI
0	Room Change	Room Change
+ 0:01	Instructions about the SPST	WCST, VST, VFT
+ 0:03	WCST, VST, VFT	
+0:25	Speech preparation	Relaxation
+0:27	Start of speech	
+0:31	End of speech	
+0:32	Relaxation	
+ 0:40		End of procedure
+0:50	End of procedure	

Note. SAD-D: Social Anxiety Disorder Dimensional Scale ; SPIN: Social Phobia Inventory; SSPS: Self-Statements Public Speaking; TCQ: Thought Control Questionnaire; PHQ9: Patient Health Questionnaire; BAI: Beck Anxiety Inventory; WCST: Wisconsin Card Sorting Test; VST: Victoria Stroop Test; VFT: Verbal Fluency Task

Table 3 - Normality assumption analyses for the symptom measurement instrument scores

	Skewness	Kurtosis	Kolmogorov-Smirnov Test	Shapiro-Wilk Test
SAD-D	1.346	1.390	.211; *df*=103; *p*<.001	.851; *df*=103; *p*<.001
SPIN	**.849**	**–.134**	.152; *df*=107; *p*<.001	.912; *df*=107; *p*<.001
SSPS_P	**–.677**	**.314**	.125; *df*=109; *p*<.001	.956; *df*=109; *p*=.001
SSPS_N	1.212	**.629**	.183; *df*=109; *p*<.001	.836; *df*=109; *p*<.001
TCQ_PU	1.040	**.624**	.150; *df*=110; *p*<.001	.901; *df*=110; *p*<.001
TCQ_SC	**–.041**	–1.084	.136; *df*=108; *p*<.001	.930; *df*=108; *p*<.001
TCQ_DI	**–.531**	**.341**	.116; *df*=109; *p*=.001	.962; *df*=109; *p*=.003
TCQ_RE	**–.317**	**–.115**	.100; *df*=107; *p*=.010	**.978; *df*=107; *p*=.067**
TCQ_WO	**.475**	**.280**	.125; *df*=108; *p*<.001	.970; *df*=108; *p*=.016
TCQ_SS	**.399**	**–.753**	.151; *df*=109; *p*<.001	.915; *df*=109; *p*<.001
PHQ9	**.960**	**.633**	.162; *df*=110; *p*<.001	.921; *df*=110; *p*<.001
BAI	1.357	1.802	.151; *df*=105; *p*<.001	.875; *df*=105; *p*<.001

Note. SAD-D: Social Anxiety Disorder Dimensional Scale ; SPIN: Social Phobia Inventory; SSPS_P: Self-Statements Public Speaking – Positive Evaluation; SSPS_N: Self-Statements Public Speaking – Negative Evaluation; TCQ_PU: Thought Control Questionnaire – Punishment; TCQ_SC: Thought Control Questionnaire – Social Control; TCQ_DI: Thought Control Questionnaire – Distraction; TCQ_RE: Thought Control Questionnaire – Reappraisal; TCQ_WO: Thought Control Questionnaire – Worry; TCQ_SS: Thought Control Questionnaire – Social Support; PHQ9: Patient Health Questionnaire; BAI: Beck Anxiety Inventory.

Table 4 - Normality assumption analyses for the neuropsychological tasks scores

	Skewness	Kurtosis	Kolmogorov-Smirnov Test	Shapiro-Wilk Test
Verbal Fluency – F	**.223**	**–.042**	.122; *df*=109; *p*<.001	**.978; *df*=109; *p*=.075**
Verbal Fluency – A	**.526**	**.106**	.104; *df*=109; *p*=.004	.968; *df*=109; *p*=.010
Verbal Fluency – S	**.074**	**–.443**	.092; *df*=109; *p*=.022	**.983; *df*=109; *p*=.205**
Verbal Fluency – Animal Category	**.571**	1.605	.092 *df*=109; *p*=.023	.970; *df*=109; *p*=.014
Wincosin – TaskTime (in seconds)	**.452**	**–.278**	**.064; *df*=109; *p*=.200**	.974; *df*=109; *p*=.031
Wincosin – Cards Used	**–.263**	–1.555	.227; *df*=109; *p*<.001	.838; *df*=109; *p*<.001
Wincosin – Completed Categories	–1.313	**.469**	.390; *df*=109; *p*<.001	.679; *df*=109; *p*<.001
Wincosin – Perseverative Errors	1.598	3.040	.166; *df*=109; *p*<.001	.844; *df*=109; *p*<.001
Stroop– Time I	**.353**	**.954**	.091; *df*=109; *p*=.026	.972; *df*=109; *p*=.021
Stroop– Errors I	2.584	7.420	.477; *df*=109; *p*<.001	.493; *df*=109; *p*<.001
Stroop– Time II	10.434	108.908	.503; *df*=109; *p*<.001	.080; *df*=109; *p*<.001
Stroop– Errors II	10.440	109.000	.528; *df*=109; *p*<.001	.070; *df*=109; *p*<.001
Stroop– Time III	10.433	108.899	.503; *df*=109; *p*<.001	.081; *df*=109; *p*<.001
Stroop– Errors III	1.017	**.514**	.209; *df*=109; *p*<.001	.842; *df*=109; *p*<.001
Stroop– Interference Score (T3–T1)	10.398	108.393	.469; *df*=109; *p*<.001	.094; *df*=109; *p*<.001

Table 5 - Cross-twin within-trait Spearman correlations of the neuropsychological tasks scores for the monozygotic dyads and dizygotic dyads that have undergone anxiogenic condition

	DZ T1 (*n* = 18 dyads)				MZ T1 (*n* = 16 dyads)			
	rho	95 LB;	UB	*p*	rho	95 LB;	UB	*p*
Verbal Fluency – F	**.618**	**.157;**	**.847**	**.006**	.208	–.320;	.625	.439
Verbal Fluency – A	**.537**	**.124;**	**.766**	**.021**	.083	–.465;	.723	.759
Verbal Fluency – S	**.589**	**.177;**	**.828**	**.010**	.321	–.229;	.751	.226
Verbal Fluency – Animal Category	.335	–.105;	.711	.174	.409	–.195;	.854	.115
Wincosin – TaskTime (in seconds)	.412	–.092;	.789	.089	.406	–.117;	.818	.119
Wincosin – Cards Used	**.669**	**.272;**	**.910**	**.002**	**.530**	**–.018;**	**.843**	**.035**
Wincosin – Completed Categories	.394	–.148;	.828	.106	.386	–.189;	.895	.140
Wincosin – Perseverative Errors	**.533**	**.028;**	**.826**	**.023**	**.646**	**.118;**	**.957**	**.007**
Stroop – Time I	–.065	–.539;	.380	.798	.462	–.119;	.824	.072
Stroop – Errors I	–.059	–.174;	–.048	.817	.114	–.471;	.718	.675
Stroop – Time II	.282	–.387;	.766	.257	.441	–.198;	.920	.087
Stroop – Errors II *								
Stroop – Time III	**.725**	**.340;**	**.909**	**.001**	**.759**	**.369;**	**.943**	**.001**
Stroop – Errors III	.033	–.548;	.500	.896	**.530**	**.008;**	**.881**	**.035**
Stroop – Interference Score (T3-T1)	**.571**	**.076;**	**.854**	**.013**	.494	–.058;	.888	.052

Note. * No correlation was calculated for Errors II since only one individual from one twin dyad scored errors on this variable. LB: 95% confidence interval lower bound; UB: 95% confidence interval upper bound.

Table 6 - Neutral and Experimental Conditions Comparison

	Neutral		Experimental		Statistics		
	M	*(SD)*	*M*	*(SD)*	*t*	*df*	*p*
Verbal Fluency – F	13.45	(4.91)	14.40	(4.57)	–1.87	(57)	.066
Verbal Fluency – A	11.81	(4.08)	13.36	(1.66)	–1.03	(57)	.308
Verbal Fluency – S	11.59	(3.84)	12.33	(4.02)	–1.42	(57)	.160
Verbal Fluency – Animal Category	17.95	(5.32)	17.03	(4.87)	1.63	(57)	.108
Wincosin – TaskTime (in seconds)	349.81	(162.08)	304.35	(148.10)	1.66	(56)	.103
Wincosin – Cards Used	**95.23**	**(28.70)**	**86.11**	**(26.07)**	**2.78**	**(56)**	**.007**
Wincosin – Completed Categories	4.32	(1.25)	4.46	(1.20)	–1.05	(55)	.298
Wincosin – Perseverative Errors	8.12	(9.58)	6.63	(7.97)	1.19	(56)	.238
Stroop – Time I	15.86	(3.16)	16.34	(3.15)	–1.37	(57)	.177
Stroop – Errors I	.21	(.44)	.24	(.60)	–.35	(57)	.727
Stroop – Time II	1.66	(1.69)	1.55	(2.59)	.39	(57)	.701
Stroop – Errors II	.00	(0)	.02	(.13)	–1.00	(57)	.322
Stroop – Time III	23.18	(6.09)	23.02	(5.48)	.20	(57)	.845
Stroop – Errors III	1.22	(1.28)	1.00	(1.25)	1.03	(57)	.307
Stroop – Interference Score (T3-T1)	8.63	(8.64)	32.08	(192.)	–.93	(57)	.358

Table 7 - Assessment and Reassessment Comparison (Learning Effect)

	Assessment		Reassessment		Statistics		
	M	*(SD)*	*M*	*(SD)*	*t*	*df*	*p*
Verbal Fluency – F	13.62	(4.95)	14.22	(4.56)	–1.17	(57)	.246
Verbal Fluency – A	11.79	(4.04)	13.38	(1.67)	–1.05	(57)	.297
Verbal Fluency – S	11.88	(3.70)	12.03	(4.19)	–.29	(57)	.770
Verbal Fluency – Animal Category	17.59	(4.83)	17.40	(5.39)	.33	(57)	.741
Wincosin – TaskTime (in seconds)	**407.96**	**(155.45)**	**246.19**	**(108.50)**	**9.02**	**(56)**	**<.001**
Wincosin – Cards Used	**97.95**	**(26.86)**	**83.39**	**(26.77)**	**4.99**	**(56)**	**<.001**
Wincosin – Completed Categories	4.29	(1.27)	4.50	(1.17)	–1.60	(55)	.116
Wincosin – Perseverative Errors	**9.51**	**(9.66)**	**5.25**	**(7.33)**	**3.77**	**(56)**	**<.001**
Stroop – Time I	16.07	(3.22)	16.14	(3.11)	–.19	(57)	.852
Stroop – Errors I	**.33**	**(.63)**	**.12**	**(.37)**	**2.19**	**(57)**	**.033**
Stroop – Time II	**1.96**	**(1.73)**	**1.25**	**(2.51)**	**2.87**	**(57)**	**.006**
Stroop – Errors II	.00	(.00)	.02	(.13)	–1.00	(57)	.322
Stroop – Time III	**24.16**	**(5.28)**	**22.03**	**(6.07)**	**2.84**	**(57)**	**.006**
Stroop – Errors III	1.17	(1.33)	1.05	(1.20)	.55	(57)	.583
Stroop – Interference Score (T3-T1)	33.90	(192.21)	6.81	(4.36)	1.07	(57)	.288

– CHAPTER V –

“Social Anxiety Disorder and Facial Emotion Recognition: effects of induced anxiety in a twin study.”

ABSTRACT

Previous studies suggest significant differences in facial emotion recognition between Social Anxiety Disorder patients and healthy controls. This variable, among others, may be important to understand dimensional perspectives in this disorder better when anxiety-induced situations are used as part of the design. Furthermore, little is known about the impact of heritability in facial emotion recognition. This study aimed to investigate differences in facial emotion recognition related to an anxiety-inducing procedure and to explore how the heritability affects facial processing of facial emotion recognition, using a monozygotic and dizygotic twin sample. Participants were 110 twins (37 monozygotic twin dyads and 18 dizygotic same-sex twin dyads), aged from 18 to 51 years (76,4 % female). Participants were assessed through symptom measures (Patient Health Questionnaire, Social Anxiety Disorder – Dimensional Scale, Beck Anxiety Inventory, Self-Statements Public Speaking, Social Phobia Inventory and Thought Control Questionnaire) and through a facial emotion recognition tasks that assess recognition and intensity in a cross-over design, in which one condition Simulated Public Speaking Test was used to induce anxiety and other was neutral. Results suggest that participants needed less emotional intensity to respond to facial emotional stimuli in the anxiety-induced condition, although no difference was observed in the accuracy of answers. Further, significative correlations were only observed in dizygotic twins, making not possible to estimate heritability in this sample. Results are discussed considering attentional control in anxiety and the necessity of further studies in the field of heritability of facial emotion recognition.

KEYWORDS: Facial Emotion Recognition, Heritability, Social Anxiety Disorder, Social Phobia, Twins.

INTRODUCTION

Social Anxiety Disorder is an anxiety disorder characterized by fear and anxiety in social situations, with further avoidance or burden related to these situations (American Psychiatric Association, 2013). Another primary symptom of SAD is the fear of humiliation or disapproval from others, which may increase the impairment of this condition. Consequently, some cognitive theories have suggested that SAD patients may have differences in facial emotion recognition that may improve their capacity to judge humiliation and disapproval from others (Clark & Beck, 2011).

Currently, the bulk of the literature suggests that there are significant differences in facial emotion recognition between SAD patients and healthy controls (Machado-de-Souza et al., 2010). For example, the study of Arrais and colleagues (2010) reported that women with SAD needed less emotional intensity to recognize fear, happiness, and sadness. In another hand, other studies have used the facial emotion recognition an arousing procedure to determine specific biomarkers of SAD and depression (Luo et al., 2017).

The well-established dichotomic paradigm of differences between SAD patients and healthy controls have now started to be explored from a dimensional perspective, to better discriminate these differences (Filho et al., 2010). In this sense, studies with non-clinical participants are essential to fulfilling these aspects. For example, Qiu, Han, Zhai & Jia (2018) found that higher SAD scores in a non-clinical population are related to increased sensitivity in the facial emotion recognition.

However, to our knowledge, no research has explored differences in facial emotion recognition related to SAD in an anxiety-inducing procedure, which may help to understand specific differences related to anxiety state situations. Additionally, there is still a significant gap in the literature regarding the origins of differences in facial emotion recognition, since there are no data related to the impact of the heritability in these variables. Considering this, the main aim of this study is to investigate differences of facial emotion recognition associated with an anxiety-inducing procedure and to explore how the heritability affects facial processing of facial emotion recognition, using a monozygotic and dizygotic twin sample.

METHODS

Participants and Recruitment Procedures

Participants were 110 twins recruited by convenience through announcements in regular media (local radios, TV programs, newspapers) and social media (fan page in Facebook exclusively designed to this study). Of the 55 twin dyads, 37 were monozygotic twin dyads and 18 were dizygotic same-sex twin dyads. A subsample of 29 monozygotic twin dyads participated in a reassessment after at least 6 months from the first evaluation. Eligibility criteria for inclusion were: living in the metropolitan region of Ribeirão Preto (Inner State of São Paulo/Brazil); age over 18 years old; complete high school level of education. Subjects in the course of a mental disorder were exclused, based in a telephone application of SRQ-20 (Harding et al, 1980; Mari & Williams, 1986), a screening scale for mental disorders with adequate sensitivity to the purpose of the study. Patients that scored over the cutoff point in SRQ-20 were submitted to a telephone application of SCID-IV (Modules of Mood Disorders, Psychotic Disorders, Anxiety Disorders and Substance-related Disorders) (First et al, 1997; Del-Ben, Vilela, Crippa, Hallak, Labate & Zuardi, 2001). After the SCID-IV application, patients diagnosed with *previous* major depression disorder, dysthymia and anxiety disorders were maintained in the study, due the high comorbidity between these disorders and SAD (Stein & Stein, 2008) and the minimal impact of these disorders in the procedure. The study design was approved by the local Ethics Committee (project number 270.406). All participants that reported difficulties with symptoms described in one of the measures or pointed above the cutoff point of PHQ-9 and SPIN were referred to an specialized outpatient service. The demographic characterization of the sample is depicted in Table 1.

[Table 1 around here]

Instruments and Tasks

Self-Statements Public Speaking (SPSS) (Hofmann & DiBartolo, 2000). This scale consists of 10 items comprising self-perception of performance in public speaking. Each item is displayed as a likert scale and scored between 0 (full disagreement) and 5 (full agreement).

The 10 items are divided in two subscales: positive self-evaluation (items 1, 3, 5, 6 and 9) and negative self-evaluation (items 2, 4, 7, 8 and 10). The Brazilian version of SPSS, used in this study, present good internal consistency ($.78 < \alpha > .90$) (Osório, Crippa, & Loureiro, 2008) and adequate discriminative validity between cases and controls of SAD (Osório, Crippa & Loureiro, 2012).

Social Phobia Inventory (SPIN) (Connor, Davidson, Churchill, Sherwood, Foa, & Weisler, 2000). The scale is a self-administered instrument that assesses physiological symptoms of fear and avoidance in SAD. It consists of 17 items evaluated on a five- point Likert likert scale, divided in 5 factors: talking to strangers and social situations; criticisms and embarrassment; physiological changes; authority figures; and avoiding being the center of attention, and public speaking. Several studies in Brazil (Osório, Crippa, & Loureiro, 2009; Osório, Crippa, & Loureiro, 2010c; Vilete, Coutinho, & Figueira, 2004; Vilete, Figueira, & Coutinho, 2006) were conducted to evaluate psychometric properties of SPIN, adequate internal consistency, adequate concurrent validity with different instruments of auto- and heteroevaluation of SAD, and adequate discriminative validity between cases and controls of SAD, indicating that the Brazilian version of SPIN is adequate to use in clinical and research contexts.

Thought Control Questionnaire (TCQ) (Wells and Davies, 1994). TCQ is an instrument that assesses coping strategies to negative repetitive thoughts. The Brazilian version of TCQ (Moreno, DeSousa, Osório, & Crippa, in press), used in this study, consist of 28 items, scored in a 5-point likert, divided in six factors: punishment, social control, distraction, reappraisal, worry, and social support. The Brazilian version of TCQ present adequate internal consistency ($.67 < \alpha > .85$), adequate Test-Retest Reliability (Intraclass correlation coefficients ranging from .71 to .87) and adequate concurrent validity with instruments used to assess anxiety symptoms (Moreno, DeSousa, Osório, & Crippa, in press)

Social Anxiety Disorder Dimensional Scale (SAD-D) (LeBeau, Glenn, Hanover, Bessdo-Baum, Wittchen & Craske, 2012). SAD-D is a scale that assesses symptoms of SAD according to DSM-5 diagnosis criteria. It consists of f 10 items displayed on a 5-point likert scale (0 = "never";1= "occasionally";2= "half of the time"; 3 = "most of the time";4= "all of the time"), in one single factor. The Brazilian version of SAD-D (DeSousa, Moreno, Osório, Crippa, LeBeau, Manfro, Sallum & Koller, 2017), used in this study, present adequate internal consistency, test-retest reliability, convergent validity with SPIN and divergent validity with a measure of attention deficit hyperactivity disorder.

Patient Health Questionnaire (PHQ-9) (Kroenke, Spitzer & Williams, 2001). PHQ-9 is an instrument that assesses symptoms of major depression in the preceding two weeks. It consists of nine items scored on a four-point likert scale (0= "not at all"; 3 = "nearly every day"), in one single factor. The Brazilian version of PHQ-9 (Pfizer (Copyright © 2005 Pfizer Inc., New York, NY)) presented adequate discriminative validity between major depressed patients and controls (Osório, Vilela-Mendes, Crippa, & Loureiro, 2009).

Beck Anxiety Inventory (BAI) (Steer & Beck, 1997). This is a 21-item scale that assesses anxiety symptoms. The items are displayed in a four-point likert scale, with total score ranging from zero to 63. The Brazilian version of BAI (Cunha, 2001), used in this study, presented good internal consistency (.71<α> .92) and adequate test-retest reliability.

Facial Emotion Recognition Task (FERT) (Arrais, et al 2010). This is a computerized task comprising stimuli from the series Pictures of Facial Affect (Ekman and Friesen, 1976) depicting six basic emotions (happiness, sadness, fear, disgust, anger, surprise) and neutral faces. The stimuli were displayed in 24 blocks (4 actors. 6 emotions), in short videos that emotion intensity gradually increases from 0% (neutral face) to 100% (full emotion displayed). In this task, the participant is asked to touch the screen to stop the video as soon as he could recognize the emotion displayed in the computer screen. Later, the participant is asked to label the emotion previously recognized, touching the name of the emotion in the screen. The blocks were randomized in each session and a two-block version was used for practice before the actual trial. The variables used in this study were: emotional intensity (time spent to recognize each stimulus – in seconds) and accuracy of recognition.

Experimental Procedures

Simulated Public Speaking Test (SPST) (McNair, Frankenthaler, Czerlinsky, White, Sasson, & Fisher, 1982) is an accurate procedure to induce anxiety, used in a series of experimental studies (e.g. Bergamaschi et al, 2011; Zuardi et al, 2017; Kamath, Urval, & Shenoy, 2017), based in the principle that anxiety increases as the individual performs a public speech without previous planning and under evaluation. In this study, we used the SPST as described in Hallak and colleagues (2010), with some modifications.

The procedure of the anxiety-inducing condition is summarized in Table 2. After a 15-min adaptation period, pretest measures (SPSS, SPIN, TCQ, SAD-D, PHQ9 and BAI) were taken in a quiet room. Immediately thereafter, the participant was taken to other room, equipped with a tv set and video camera, where he/she received the instructions and had 2 min

to prepare a 4-min speech about 'the public transportation system of your city'. Participant was also told that the speech would be recorded on videotape and later analyzed by a psychologist. After the instructions, participants were asked to engage in neuropsychological tasks described elsewhere (Moreno, DeSousa, Osório & Crippa, in press). Thus, the subject started speaking in front of the camera while viewing his/her own image on the TV screen. Finally, participants were asked to complete the Facial Emotion Recognition Task and then remain calm in a comfortable position for 15 minutes.

The procedure of the neutral condition is summarized in Table 2. In this condition, after a 15-min adaptation period, pretest measures (SPSS, SPIN, TCQ, SAD-D, PHQ9 and BAI) were taken in a quiet room. Immediately thereafter, the participant was taken to other room. After the instructions, participants were asked to engage in neuropsychological tasks described elsewhere (Moreno, DeSousa, Osório & Crippa, in press). Finally, participants were asked to complete the Facial Emotion Recognition Task and then remain calm in a comfortable position for 15 minutes.

Monozygotic twins completed both conditions, with at least 6 months interval between the two occasions. Half of monozygotic pairs started the procedure by the anxiety-inducing condition, while the other half stared the procedure by the neutral condition, in a cross-over design. Dizygotic twins completed only the anxiety-inducing condition.

Data analysis

To investigate influences of the experimental manipulation on the facial emotional recognition task, paired-sample t-tests were conducted comparing the scores of the monozygotic twins in the neutral and in the experimental conditions. Since the same participant was exposed to the same tasks in both neutral and experimental conditions, to account for a possible learning effect, participants were randomly assigned to the neutral or the experimental condition in the first assessment and completed the other condition on the reassessment.

As suggested by the literature (López-Solà et al., 2014), cross-twin within-trait Spearman correlations were calculated to explore the contribution of genetic and environmental factors by comparing the correlations of each instrument score within the monozygotic dyads to the ones within the dizygotic dyads. Confidence intervals of 95% were calculated for each correlation using bias corrected accelerated (BCa) bootstrapping based on 1.000 random subsamples (Field, 2013).

RESULTS

The comparison of the performance of the monozygotic twins on the facial emotional recognition task between the neutral and experimental conditions is depicted in Table 3. There were significant differences on the emotional intensity used for completing the task for all emotions except for disgust. Participants needed less emotional intensity to give the answers in the experimental condition than in the neutral one.

In contrast, there were no significant differences on the number of correct answers in the trials between the neutral and experimental conditions (Table 3). Since the number of correct answers was not normally distributed, we also tested the differences using Wilcoxon signed ranked tests and also tested chi-square tests after dichotomizing the data in 'presented at least one correct trial'/'presented no correct trials'. Still no significant differences were found (data available upon request).

Table 4 presents the results of the cross-twin within-trait Spearman correlations and confidence intervals of the facial emotional recognition task variables for the dizygotic dyads and the monozygotic dyads that have undergone the anxiogenic experimental condition at the first assessment time. Significative correlations were found only between dizygotic dyads in variables related to emotional intensity. However, since significative correlations were not found in any variable in monozygotic twins, it is not possible to determine differences related to these variables in this sample of twins.

DISCUSSION

This study aimed to investigate the effects of anxiety situations in facial emotional recognition using an anxiety-induced procedure. Our data show that participants needed less emotional intensity to respond to facial emotional stimuli in the anxiety-induced condition, although no difference was observed in the accuracy of answers. A secondary aim of this study was to estimate the heritability of facial emotional recognition using a sample of monozygotic and dizygotic twins. However, significative correlations were only observed in dizygotic twins, making not possible to estimate heritability in this sample.

Previous studies have reported differences in emotional intensity between SAD patients and controls (Arrais et al., 2010). However, this difference was observed only in women, and only related to some emotions (fear, sadness, and happiness). Our data suggest that this difference may be extended to men and other emotions, except disgust. Furthermore,

according to the results, emotional intensity seems to vary not only related to the diagnosis of SAD but also in situations that are related to social anxiety, even in healthy individuals.

In a broad perspective, it is possible to affirm that anxiety is related to the higher attentional focus in challenging tasks (Eysenck & Derakshan, 2011). The fact that this is also described in this study in healthy participants, in an anxiety-induced situation, allows understanding that attentional resources are influenced not only by higher traits of anxiety (Clark & Beck, 2011), such as in anxiety disorders but also in state anxiety, caused by threatening situations. Among other further studies, this result may support the idea that hypervigilance is an anxiety-related variable that is modified not only in anxiety disorders, but also in anxiety induced situations, an essential data to support recent efforts to understand dimensional aspects of anxiety disorders (Craske, 2012).

Although a hot topic in the field of psychopathology, estimates of heritability in SAD have presented very controversial results, mainly related to the diversity of methods used (Moreno, Martin-Santos, Osório & Crippa, 2016). Regardless, estimate the heritability of variables associated with SAD, such as facial emotion recognition, is essential to understand better the complex puzzle that leads to the development and maintenance of SAD. Our attempt to estimate heritability was frustrated since the correlations between monozygotic twins were not significative. However, since this is the first study using this method, further studies are needed to understand this finding better.

REFERENCES

American Psychiatric Association (2013). *Diagnostic and statistics manual of mental disorders: DSM-5*. Arlinton: American Psychiatric Association.

Arrais, K. C., et al (2010). Social anxiety disorder women easily recognize fearful, sad and happy faces: The influence of gender. *Journal of Psychiatric Research*, 44, 535–540.

Bergamashi, M. et al. (2011). Cannabidiol Reduces the Anxiety Induced by Simulated Public Speaking in Treatment-Naïve Social Phobia Patients. Neuropsychopharmacology, 36, 1219–1226. https://doi.org/10.1038/npp.2011.6

Clark, D.A., & Beck, A.T. (2011). The Cognitive Model of Anxiety. In: Cognitive Therapy of Anxiety Disorders. New York: Guilford Press.

Connor, K.M.; Davidson, J.R.; Churchill, L.E.; Sherwood, A.; Foa, E.; Weisler, R.H. (2000). Psychometric properties of Social Phobia Inventory (SPIN). New self- rating scale. British Journal of Psychiatry, 176, 379- 86.

Craske, M.G. (2012). The R-DOC initiative: science and practice. Depression and Anxiety, 29(4), 253-256.

Del-Ben, C.M.; Vilela, J.A.A.; Crippa, J.A.S.; Hallak, J.E.C.; Labate, C.M. & Zuardi, A.W. (2001). Confiabilidade da "Entrevista Clínica Estruturada para o DSM-IV – Versão Clínica" traduzida para o português. Revista Brasileira de Psiquiatria, 23 (3), 156-159.

Ekman P, Friesen WV (1976). Pictures of facial affect. Palo Alto: Consulting Psychologists.

Eysenck, M.W., & Derakshan, N. (2011). New perspectives in attentional control theory. *Personality and individual differences*, 50(7), 955–960.

Field, A. P. (2013). *Discovering statistics using SPSS* (4th ed). London: Sage.

Filho, A.S., Hetem, L.A., Ferrari, M.C. et al (2010). Social anxiety disorder: what are we losing with the current diagnostic criteria? Acta Psychiatrica Scandinavia, 121(3), 216-226.

First, M.B.; Spitzer, R.L.; Gibbon, M.; Williams, J.B.W. (1997). Structured clinical interview for DSM-IV axis I disorders – clinician version (SCID-CV). Washington (DC): American Psychiatric Press.

Hofmann, S.G.; DiBartolo, P.M. (2000). An instrument to assess self-statements during public speaking: scale development and preliminary psychometric properties. Behavior Research and Therapy, 31, 499-515.

LeBeau, R. T., Glenn, D. E., Hanover, L. N., Beesdo-Baum, K., Wittchen, H., & Craske, M. G. (2012). A dimensional approach to measuring anxiety for DSM-5. International Journal of Methods in Psychiatric Research, 21(4), 258-272. Copyright © 2012 American Psychiatric Association.

Luo, L., et al. (2017). A dimensional approach to determine common and specific neurofunctional markers for depression and social anxiety during emotional face processing. Hum Brain Mapp, 00:000–000. https://doi.org/10.1002/hbm.23880

Machado-de-Souza, J.P, et al. (2010). Facial affect processing in social anxiety: Tasks and stimuli. Journal of Neuroscience Methods, 193, 1-6.

Moreno, A.L., Osório, F.L. Martin-Santos, R., & Crippa, J.A.S. (2016). Heritability of social anxiety disorder: a systematic review of methodological designs. Archives of Clinical Psychiatry, 43(4), 83-92.

Moreno, A.L., DeSousa, D.A., Osório, F.L. & Crippa, J.A.S. (in press). Cross-cultural Adaptation and Psychometric Properties of the Brazilian Version of the Thought Control Questionnaire (TCQ).

Osório, F.L.; Crippa, J.A. & Loureiro, S.R. (2008). Escala para Auto-Avaliação ao Falar em Público (SSPS): adaptação transcultural e consistência interna da versão brasileira. Revista de Psiquiatria Clínica, 35 (6), 207-211.

Osório, F.L.; Crippa, J.A. & Loureiro, S.R. (2009). Cross- cultural validation of the Brazilian Portuguese version of the Social Phobia Inventory (SPIN): study of the items and internal consistency. Revista Brasileira de Psiquiatria, 31 (1), 25-29.

Osório, F.L.; Crippa, J.A. & Loureiro, S.R. (2013). Validation of the state version of the Self-

Qiu, F., Han, M., Zhai, Y., & Jia, S. (2018). Categorical perception of facial expressions in individuals with nonclinical social anxiety. Journal of Behavior Therapy and Experimental Psychiatry, 58, 78-85.

Table 1 - *Demographic characterization of the total sample and subsamples of monozygotic and dizygotic twins*

Variable	Total sample T1 (*n*=110)	DZ T1 (*n*=36)	MZ T1 (*n*=74)	MZ T2 (*n*=58)
Gender (female) *n* (%)	84 (76.4)	30 (83.3)	54 (73.0)	42 (72.4)
Age range (years)	18–51	18–35	18–51	18–52
Age *M* (*SD*) (years)	26.0 (7.6)	23.3 (5.0)	27.3 (8.4)	29.7 (8.8)
Educational level *n* (%)				
High school	26 (23.6)	8 (22.2)	18 (24.3)	12 (20.7)
College/University incomplete	29 (26.4)	15 (41.7)	14 (18.9)	8 (13.8)
College/University complete	55 (50.0)	13 (36.1)	42 (56.8)	38 (65.5)
Uses daily medication *n* (%)	40 (36.4)	16 (44.4)	24 (32.4)	27 (46.6)
Ever been in psychiatric or psychological treatment *n* (%)	41 (37.3)	14 (38.9)	27 (36.5)	26 (44.8)
Ever used psychiatric medication *n* (%)	17 (15.5)	6 (16.7)	11 (14.9)	16 (27.6)
Is currently in psychiatric or psychological treatment *n* (%)	14 (12.7)	5 (13.9)	9 (12.2)	7 (12.1)
Uses any psychoactive drug *n* (%)	7 (6.4)	1 (2.8)	6 (8.1)	2 (3.4)

Table 2 - Comparative between experimental and neutral condition.

Time	Experimental Condition	Neutral Condition
−0:30	Adaptation to the laboratory; instructions about the interview and measurements	Adaptation to the laboratory; instructions about the interview and measurements
−0:15	SPSS, SPIN, TCQ, SAD-D, PHQ-9, BAI	SPSS, SPIN, TCQ, SAD-D, PHQ-9, BAI
0	Room Change	Room Change
+ 0:01	Instructions about the SPST	Neuropsychological tasks
+ 0:03	Neuropsychological tasks	
+0:25	Speech preparation	FERT
+0:27	Start of speech	
+0:31	End of speech	Relaxation
+0:32	FERT	
+ 0:40	Relaxation	End of procedure
+0:50	End of procedure	

Note. SAD-D: Social Anxiety Disorder Dimensional Scale; SPIN: Social Phobia Inventory; SSPS: Self-Statements Public Speaking; TCQ: Thought Control Questionnaire; PHQ9: Patient Health Questionnaire; BAI: Beck Anxiety Inventory; Neuropsychological tasks: Described elsewhere (Moreno, DeSousa, Osório & Crippa, in press); FERT: Facial Emotional Recognition Task.

Table 3 - Neutral and Experimental Conditions Comparison of the Faces Task

	Neutral		Experimental		Statistics		
	M	*(SD)*	*M*	*(SD)*	*t*	*df*	*p*
Geral_Intensidade	**84.03**	**18.44**	**76.60**	**16.37**	**3.07**	**(48)**	**.004**
Alegria_Intensidade	**70.75**	**20.81**	**63.98**	**18.48**	**2.23**	**(48)**	**.030**
Tristeza_Intensidade	**89.27**	**20.52**	**81.89**	**17.40**	**2.36**	**(48)**	**.023**
Medo_Intensidade	**85.74**	**19.13**	**77.81**	**21.22**	**2.62**	**(48)**	**.012**
Nojo_Intensidade	83.76	23.05	77.97	21.97	1.69	(48)	.098
Raiva_Intensidade	**96.89**	**23.33**	**86.21**	**17.23**	**3.43**	**(48)**	**.001**
Surpresa_Intensidade	**85.94**	**23.06**	**77.40**	**21.83**	**2.26**	**(48)**	**.028**
Est.Feminino_Intensidade	**81.81**	**18.19**	**75.45**	**16.37**	**2.46**	**(48)**	**.018**
Est.Masculino_Intensidade	**79.40**	**18.45**	**71.46**	**16.51**	**3.18**	**(48)**	**.003**
Geral_Acertos	.92	(2.60)	1.86	(5.09)	–1.14	(48)	.262
Alegria_Acertos	.08	(.57)	.33	(1.10)	–1.35	(48)	.182
Tristeza_Acertos	.06	(.42)	.31	(1.04)	–1.50	(48)	.141
Medo_Acertos	.12	(.38)	.22	(.58)	–1.04	(48)	.302
Nojo_Acertos	.12	(.38)	.27	(.75)	–1.15	(48)	.254
Raiva_Acertos	.45	(.70)	.41	(.86)	.27	(48)	.789
Surpresa_Acertos	.08	(.57)	.33	(1.04)	–1.41	(48)	.165
Est.Feminino_Acertos	.33	(1.04)	.82	(2.42)	–1.28	(48)	.205
Est.Masculino_Acertos	.59	(1.61)	1.04	(2.69)	–.99	(48)	.325

Table 4 - Cross-twin within-trait Pearson correlations of the faces tasks scores for the monozygotic dyads and dizygotic dyads that have undergone anxiogenic condition

	DZ T1 (n = 16 dyads)		MZ T1 (n = 14 dyads)	
	r	*p*	*r*	*p*
Geral_Intensidade	**.510**	**.044**	–.275	.341
Alegria_Intensidade	.166	.540	–.101	.732
Tristeza_Intensidade	**.582**	**.018**	–.174	.551
Medo_Intensidade	.358	.174	–.379	.182
Nojo_Intensidade	**.574**	**.020**	–.413	.142
Raiva_Intensidade	.413	.112	–.172	.556
Surpresa_Intensidade	.398	.127	–.020	.945
Est.Feminino_Intensidade	**.545**	**.029**	–.146	.619
Est.Masculino_Intensidade	.455	.077	–.365	.200