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CARDIOVASCULAR SYMPATHETIC AROUSAL IN RESPONSE TO DIFFERENT MENTAL STRESSORS

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Short title: Sympathetic arousal in response to mental stress

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Summary

The altered regulation of autonomic response to mental stress can result in increased cardiovascular risk. The laboratory tests used to simulate the autonomic responses to real-life stressors do not necessarily induce generalized sympathetic activation; therefore, the assessment of regulatory outputs to different effector organs could be important. We aimed to study the cardiovascular sympathetic arousal in response to different mental stressors (Stroop test, mental arithmetic test) in 20 healthy students. The conceivable sympathetic vascular index – spectral power of low frequency band of systolic arterial pressure variability (LF-SAP) and novel potential cardio-sympathetic index – symbolic dynamics heart rate variability index 0V% were evaluated. The heart and vessels responded differently to mental stress – while Stroop test induced increase of both 0V% and LF-SAP indices suggesting complex sympathetic arousal, mental arithmetic test evoked only 0V% increase compared to baseline ($p<0.01$, $p<0.001$, $p<0.01$, respectively). Significantly greater reactivity of LF-SAP, 0V%, heart rate (HR) and mean arterial pressure (MAP) were found in response to Stroop test compared to mental arithmetic test potentially indicating the effect of different central processing (0V%, LF-SAP: $p<0.001$; HR, MAP: $p<0.01$). The different effectors´ sympathetic responses to cognitive stressors could provide novel important information regarding potential pathomechanisms of stress-related diseases.

Keywords: sympathetic nervous system, mental stress, systolic arterial pressure variability, heart rate variability, symbolic dynamics.

Introduction

The sympathetic nervous system is one of the key components of a nonlinear network of the stress-related regulatory mechanisms. It mediates substantial part of allostatic processes regulating the perturbations of homeostasis and maintaining the stability of the organism through the changes of physiologic functions (McEwen 2007). This adaptive regulation affects multiple organ systems including cardiovascular system as a principal effector of the sympathetic central-peripheral pathway.

Sympathetic nervous system exerts direct effects on cardiovascular functions through cardiac nerves and vascular neural regulation and indirect effects by adrenal medullary influences and stimulation of renal juxtaglomerular cells (Charkoudian and Rabbitts 2009). Physiologically, these mechanisms play vital role in adaptation of hemodynamics to changing internal and external environment. However, they may become maladaptive after exposition to long-term, repetitive or excessive mental stress associated with altered sympathetic regulation, both exaggerated or diminished (Lovallo 2011). Detrimental effects on cardiovascular health result in increased risk of morbidity, *e.g.* elevated blood pressure (BP) or accelerated process of atherosclerosis (Chida and Steptoe 2010).

Formerly, the studies of the cardiovascular reactivity to stress were focused mainly on the changes of mean values of heart rate (HR) and BP (Falkner *et al.* 1979, Matthews *et al*. 1993). However, the computers´ development enables to study cardiovascular regulatory mechanisms contributing to the HR and BP „*beat-to-beat*“ variability, *i.e.* oscillations of HR/BP around their mean value. With regard to cardiac sympathetic activity, the low frequency component of heart rate variability (LF-HRV, 0.04-0.15 Hz) was used as a beta-adrenergic regulation marker and the ratio LF/HF (HF - high frequency band of HRV, 0.15-0.40 Hz) as a parameter reflecting the relative sympathetic contribution to autonomic HR control (Pagani *et al.* 1986, Malliani *et al.* 1991, Lucini *et al.* 2002a). However, these assumptions are still controversial and extensively discussed. In this context, recent studies noted that LF-HRV is mainly determined by the activity of parasympathetic nervous system, and LF/HF ratio should not be considered as an unambiguous index of cardiac sympatho-vagal balance (Goldstein *et al.* 2011, Rahman *et al.* 2011, Reyes Del Paso 2013). Importantly, the non-linear HRV analysis - symbolic dynamics - was found to be sensitive to the changes of the sympathovagal balance, and 0V% index could be associated with the conditions characterizing the sympathetic activation, such as orthostatic load (Porta *et al.* 2001, Guzzetti *et al.* 2005, Tonhajzerova *et al*. 2010). Nevertheless, our previous study firstly showed that 0V% index could reflect the beta-adrenergic activation during cognitive mental tests (Visnovcova *et al.* 2014). Therefore, this index might represent novel approach for noninvasive assessment of cardiac sympathetic regulation that is indepedent on the other effects, such as myocardial preload and afterload influencing frequently used beta-adrenergic index pre-ejection period (Newlin and Levenson 1979, Beauchaine *et al.* 2007). Notably, the studies related to the interaction „stress and 0V% index" are rare.

On the other hand, the alpha-adrenergic sympathetic vascular regulation is determined by spectral analysis of systolic arterial pressure variability in low frequency band (LF-SAP, 0.04-0.15 Hz) (Zhang *et al.* 2002). The LF-SAP was found to be higher in response to acute mental stress, such as Stroop task (*e.g.* Ginty *et al*. 2014). Additionally, the increased baseline values of LF-SAP and greater alpha-adrenergic response to orthostatic stress were previously found in hypertension or chronic psychosocial stress (Lucini *et al.* 2002b, 2005). It seems that BP analysis in low frequency band could represent a sensitive method for assessment of alpha-adrenergic sympathetic regulatory mechanisms, nevertheless, the myogenic vascular regulation could constitute other physiological mechanism contributing to LF-SAP (Zhang et al. 2002). However, detailed comparison of the effect of different cognitive tasks on vascular sympathetic regulation combined with simultaneous assessment of beta-adrenergic activity could offer novel complex view on cardiovascular response to stress.

Further, it was found that laboratory tests are able to simulate the patterns of reactions as found in real life and predict future cardiovascular risk status (Chida and Steptoe 2010, Zanstra and Johnston 2011). However, the stressors do not necessarily induce generalized response, and differences in activation degree can be found between distinct sympathetic outflows (Fechir *et al.* 2008). Therefore, the assessment of the sympathetic regulation of different effectors (*i.e.* heart and vessels) in response to different stressors may be of great importance.

Therefore, we aimed to study cardiovascular sympathetic reactivity in response to different mental stressors using linear and nonlinear analysis of blood pressure and heart rate variability in healthy young students. To the best of our knowledge, this is the first study to asses the complex potential cardio-sympathetic regulation indexed by symbolic dynamics (0V% index) combined with evaluation of conceivable sympathetic vascular regulation indexed by LF-SAP in response to stress.

Methods

The study was approved by the Ethics Committee of Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava in accordance with the Helsinki Declaration. All the participants gave informed written consent before participation in the study.

Subjects

The studied group consisted of 20 healthy nonobese medical students attending the 5^{th} year of Jessenius Faculty of Medicine (11 women, age 23.1±0.8 yr., BMI 22.3±2.4 kg/m^2). Following exclusion criteria were strictly applied during enrollment of the subjects: underweight, overweight or obesity, smoking, alcohol and drug abuse, acute illness, history of chronic cardiovascular, respiratory, endocrinological, neurological, infectious diseases or mental disorders. The subjects were instructed to refrain from using substances which could affect the autonomic nervous system activity (*e.g.* caffeine, alcohol) at least 12 hours before the examination.

Procedure

The examinations were peformed under standard conditions in a sound-attenuated room and minimalization of stimuli, standard temperature 23°C and humidity 45-55%, in the morning between 9.00 a.m. - 12.30 p.m. after normal breakfast 2 h prior to the examination. At the beginning, antropometric parameters were examined (InBody J10, Biospace, Korea) to exclude potential effect of weight abnormalities. The recording devices were set and calibrated and subjects were instructed to sit comfortably in a special armchair and not to speak or move unless necessary. In the context of the potential disturbance evoked by examining staff, the complete stress procedure was performed by the same one investigator-specialist, and only one participant was examined in the clinical laboratory. After 15 minutes required to avoid a potential stress effect of laboratory environment and examining person, the subjects remained in a sitting position. The continuous recording of RR-interval (VarCor PF8, Dimea, Czech republic) and continuous beat-to-beat BP monitoring (Finometer PRO, Finapres medical systems, Netherlands) were performed in the following order: baseline phase, Stroop test, rest period, and mental arithmetic test. The duration of each phase was 6 minutes. The participants were not informed about the order of the tests.

Mental tasks

Stroop color word test (Thought Technology, Ltd., Canada): The subjects were instructed to name the ink colors (green, yellow, orange, red, blue, purple) in which the words stimuli (name of the colors) were written and displayed on the screen. The meanings of the words were congruent or incongruent with the ink color. The metronome was used as a distracting element during the test.

Mental arithmetic test (Psychodiagnostic, Slovak Republic): During the test, three-digit numbers were displayed at the different random places on the screen. The task was based on the summation of three-digit numbers into one-digit numbers and making decision whether the final result is even or odd by pushing the keyboard arrow (left – odd, right – even). The metronome was applied during the test as a distracting sound.

Evaluated parameters

Two distinct components of sympathetic regulatory effects on cardiovascular system were studied – conceivable sympathetic vascular regulation indexed by LF-SAP, and complex potential cardio-sympathetic regulation indexed by symbolic dynamics index 0V%.

The *LF-SAP* comprises rhythmic fluctuations of systolic BP in frequency range 0.04-0.15 Hz, the so-called Mayer waves. The origin of this rhythmic variability has been explained mainly by two mechanisms: *the pacemaker effect* of the central nervous structures generating slow sympathetic nervous activity rhythms independently of peripheral afferent inputs and *the baroreflex theory* based on the studies with blockade of baroreflex loop resulting in marked reduction of Mayer waves (Julien 2006). The activity of alpha-adrenergic nerves is considered to be the major determinant of LF-SAP variations mediated by changes in peripheral vascular resistance, although intrinsic vasomotor rhythmicity affected by local mechanisms may play a significant role, as well (Zhang *et al.* 2002). Thus, spectral power of LF-SAP can be used as a conceivable marker of sympathetic vascular regulation.

Index 0V% is a parameter of symbolic dynamics method of HRV nonlinear analysis. It is based on the transformation of the time series into series of symbols which reflect the levels of the ranges of RR-intervals duration. Afterwards triplets of symbols (heartbeats) are classified into different paterns according to changes (variations) of the levels: 0V (zero variation), 1V (one variation), 2LV (two like variations) and 2UV (two unlike variations). Consequently, the rates of occurence of these patterns are evaluated. The 0V pattern is associated with conditions of sympathetic activation, thus it is considered to be a potential marker of beta-adrenergic sympathetic activity (Porta *et al.* 2001, Guzetti *et al.* 2005, Visnovcova *et al.* 2014). In addition, the average HR (beats per minute, bpm) and mean arterial BP (MAP, mmHg) were calculated for each phase.

The reactivity of the LF-SAP, 0V%, HR and MAP in response to mental stress tests compared to baseline was evaluated as a percentual change of the parameters using mathematical expression: [(value during test – baseline value) / baseline value] x 100 (%).

Statistical analysis

Statistical analysis was performed using the statistical software package SYSTAT 10 for Windows (SSI, Richmond, CA, USA). The non-gaussian/gaussian distribution was ascertained by Lilliefors test. The spectral analysis parameter LF-SAP was logarithmically transformed for the analysis. Then, the Wilcoxon test was used for between-periods comparison of data with non-gaussian distribution and paired Student's t-test for data with gaussian distribution. The probability p<0.05 was considered as significant. The data are expressed as mean ± SEM.

Results

The effect of the mental stress tests

The *Stroop test* evoked significant increase of mean values in both parameters - log LF-SAP and 0V% - compared to baseline (p<0.001, p<0.01, respectively; Fig. 1 and Fig. 2). Additionally, HR and MAP were significantly higher during the test compared to baseline (84.0±4.3 bpm vs. 72.1±2.4 bpm, 96.4±2.4 mmHg vs. 87.4±1.8 mmHg, respectively; p<0.001 for both).

The *mental arithmetic test* evoked significant increase in the cardiac sympathetic index 0V% compared to baseline value (p<0.01; Fig. 2). Additionally, HR and MAP were significantly higher during the test compared to baseline (79.4±3.7 bpm vs. 72.1±2.4 bpm, 92.6±2.5 mmHg vs. 87.4±1.8 mmHg; p<0.001, p<0.01 respectively). No significant change was found in log LF-SAP (Fig. 1).

Between-tests comparison

Both parameters log LF-SAP and 0V% were significantly higher in response to Stroop task compared to mental arithmetic test (p<0.001, p<0.01, respectively; Fig. 1 and Fig. 2). The reactivity in log LF-SAP and 0V% was significantly greater in response to Stroop test compared to mental arithmetic test (p<0.001 for both, Fig. 3). Additionaly, mean values of HR and MAP were significantly higher during Stroop test compared to mental arithmetic test (84.0±4.3 bpm vs. 79.4±3.7 bpm, 96.4±2.4 mmHg vs. 92.6±2.5 mmHg, respectively; p<0.01

for both). Reactivity in HR and MAP was significantly greater in response to Stroop test compared to mental arithmetic test (16.1±3.9 % vs. 9.9±3.0 %, 10.4±1.8 % vs. 6.0±1.9 %, respectively; $p<0.01$ for both).

Discussion

The main finding of this study was that heart and vessels responded differently to cognitive mental tasks. The conceivable sympathetic vascular activation indexed by LF-SAP was induced only by the Stroop task, and cardio-sympathetic response indexed by 0V% was evoked by both cognitive tasks with greater activation in response to Stroop test. Moreover, the cognitive tasks lead to significant increase in HR and MAP with greater reaction during the Stroop test.

The autonomic regulation of the effector organs is achieved by multiple structures at all levels of the central nervous system forming a complex interconnected system called central autonomic network (Benarroch 1993). Importantly, these structures are also involved in the neural circuits regulating the executive, attentional and affective functions, forming a system coordinating the actual psychophysiological demands of these functional units with the modulation of the autonomic response to environmental challenges as suggested by neurovisceral integration model (Thayer and Lane 2000). For example, at the cortical level insula integrates the interoceptive stimuli with emotional and cognitive processing, modulates the autonomic regulation through connection with hypothalamus, and it is connected with anterior cingulate cortex (ACC) involved in control of sympathetic activation in response to cognitive and emotional load (Craig 2003, Beissner *et al.* 2013). Therefore, the patients with ACC lesion are not able to induce sympathetic activation adequate to workload demands (Critchley *et al.* 2003). From this context, the activation of ACC could represent an important mechanism contributing to sympathetic arousal in stress. Importantly, the autonomic-cardiovascular regulation is directly affected by the medial prefrontal cortex activity, as well. In particular, the ventral region inhibits the activation of the hypothalamus-pituitary-adrenal axis as well as the sympathetic nervous system, the dorsal division enhances the cardiovascular reaction to stress, probably due to modulation of the parasympathetic activity (Wager *et al.* 2009). Taken together, the cardiovascular sympathetic arousal induced by mental stress should be viewed as a result of complex interaction of several central regulatory mechanisms.

Our findings revealed both alpha- and beta-adrenergic activation in response to Stroop test, but not in mental arithmetic test that was associated only with beta-adrenergic reactivity. These results are in agreement with other study that revealed greater sympathetic response indexed by electrodermal activity, trapezius muscle electromyography, skin vasoconstriction and reduction of pulse wave transit time in Stroop test compared to mental arithmetic test despite the fact that mental arithmetic was subjectively more stressful (Fechir *et al.* 2008). The differences in sympathetic responses to these tasks could be potentially influenced by distinct central processing patterns and psychophysiological demands. During the Stroop test, the conflict of two competitive stimuli requires discrimination of the sources of information, task-relevant control of selective attention and choosing the response regardless of the automatic response tendencies (MacLeod and MacDonald 2000). The ACC seems to play a key role in this process - it is maximally activated during the Stroop performance and disruption of its activity by transcranial magnetic stimulation reduces the Stroop effect (MacLeod and MacDonald 2000, Hayward *et al.* 2004). Importantly, the cingulate cortex is also involved in the regulation of cardiovascular responses, particularly in the initiation of descendent efferent commands for the autonomic and subsequent cardiovascular reactions to stressors. The BP reactivity during the Stroop test correlated with the activity in ACC and individuals with larger BP reactions showed greater activation of the posterior cingulate cortex reciprocally connected with anterior divisions (Gianaros *et al.* 2005a,b). Thus, the ACC was suggested to represent possible functional neural correlate of exaggerated BP reactivity in response to Stroop test. Based on these findings, we could expect that the complex cardiovascular reaction to Stroop test in this study was significantly influenced by the activity of ACC.

Another important cortical region involved in selective attention regulation during the Stroop task is dorsolateral prefrontal cortex, which seems to be involved in top-down attentional control – representing and maintaining the attentional demands of the task (MacDonald *et al*. 2000). This was confirmed by the application of the repetitive transcranial magnetic stimulation over the dorsolateral prefrontal cortex - significant decrease of the reaction time in the Stroop task was found, however, the interference effect remained unchanged (Vanderhasselt *et al.* 2006). We could assume that these central regulatory mechanisms could be considerably involved in the modulation of the cardiac beta-adrenergic and vascular alpha-adrenergic response to Stroop test in our study.

In contrast to Stroop test, we can suggest different neural mechanisms involved in mental arithmetic test that potentially influence cardiovascular sympathetic reactivity. Specifically, functional specialization of the left inferior parietal cortex associated with automatization of the simple arithmetic processes result in lower attentional demands exerted on the ACC and prefrontal cortex during the mathematic tasks (Rivera *et al*. 2005). Moreover, the role of dorsolateral prefrontal cortex in attention flexibility and inhibitory control is probably important only during difficult complex arithmetic processes and effortful controlled retrieval of the arithmetic facts (Rivera *et al.* 2005, Klein *et al.* 2013). On the contrary, the resolution of the simple addition tasks could be facilitated by an automatic retrieval of the arithmetic facts stored in long-term memory (Klein *et al.* 2013). Therefore, the higher cardiovascular reactivity in Stroop test could be induced by the effect of more attention-demanding task resulting in more complex central processing compared to automatic cognitive processing associated with mental arithmetic.

Secondly, we could speculate about the effect of verbalization on the cardiovascular reactivity during verbal (Stroop) and nonverbal (arithmetic) tasks. In Stroop test, Boutcher and Boutcher (2006) revealed significantly greater HR and MAP in verbalized form compared to nonverbal Stroop test. Moreover, participants perceived verbal and nonverbal versions to be similarly difficult and attention demanding, therefore, the higher autonomic reactivity could be influenced by the effect of vocalization motor response. In contrast, other studies found no differences in HR and MAP during verbal and nonverbal Stroop test and mental arithmetic test suggesting that physiological components of vocalization do not affect the cardiovascular response to task (Seraganian *et al*. 1997, Barbosa *et al.* 2010). From this context, the effect of verbalization is still unclear. We suggest that the possible influence of this mechanism should be taken into account in the interpretation of our findings.

Moreover, our findings of cardiovascular reactivity to cognitive mental stressors could be also influenced by individual characteristics and related psychological aspects, such as motivation, or other factors, e.g. task difficulty (Seraganian *et al.* 1997, Boutcher and Boutcher 2006). In addition, potential effect of cognitive and affective processes fixed to past stressor should be taken into account in the context of application of the two consecutive tests. Repeated or chronic activation of the cognitive representation of psychological stressor, *i.e.* perseverative cognition, as manifested in worry and rumination, can result in prolonged sympathetic activation (Brosschot *et al.* 2006, 2010). The neural pathway of this mechanism seems to involve the disruption of the inhibitory control of subcortical structures by the prefrontal cortex resulting in the breakdown of a common reciprocal inhibitory cortical-subcortical neural circuit (Thayer and Lane 2002, Brosschot *et al*. 2006).). The perseverative processes occur in the complex central autonomic network including the ACC and other structures which seem to play a key role in central processing of mental tasks, particularly the Stroop test (Benarroch 1993, MacLeod and MacDonald 2000, Hayward *et al.* 2004). On the other hand, the effect of the previous stressor can be diminished by various psychological factors, *e.g.* reduction of attention paid to the past stressor - cognitive distancing or changing the way the stressor is regarded - cognitive restructuring (Larsen and Christenfeld 2011). From this perspective, we could hypothesize that the onset of the second stress task could involve the effect of distraction with consequent minimization of reactive remnants of the Stroop test on the mental arithmetic task. Taken together, we assume complex neuro-psycho-physiological mechanisms influencing the cardiovascular sympathetic reactivity in response to different mental stressors. Further research is needed to elucidate these processes.

In terms of clinical application, differentiation of the effectors of sympathetic responses according to the nature of the stressor could reveal important novel findings about possible pathomechanisms of stress-related diseases. We may expect that similar differences presented in this study could be found in response to real-life stressors. However, this assumption need to be confirmed by independent study. Importantly, some pathological conditions, *e.g.* depressive or anxiety disorders with impaired attention and executive functioning, are associated with altered autonomic regulation at rest and in response to stress indicating potential increased cardiovascular risk (Castaneda *et al.* 2008, Tonhajzerova *et al.* 2010, 2012, Kemp *et al.* 2012). The detailed study of the cardiac and vascular responses to neuropsychological tests could help to elucidate the central-peripheral interaction within the stress and related health outcomes.

Study limitations

Our study included a relatively small homogeneous group of healthy young adults. The findings of this study need to be independently validated in larger sample including the control group undergoing the procedure without the mental tasks which could detect potential effect of environment and examining staff.

This study addressed the changes in cardiovascular sympathetic arousal to different stressors determined by the novel index of nonlinear analysis of short-term HRV - symbolic dynamics index (0V%), and by the linear analysis of short-term systolic BP variability in low-frequency band (LF-SAP, 0.04-0.15 Hz) influenced predominantly by sympathetic division of autonomic nervous system. Since our study did not comprise direct invasive assessment of sympathetic neural activity, conclusions regarding exact quantitative neural signals in alone sympathetic branch of autonomic nervous system could not be drawn from the short-term HR and BP variability analysis. Further research in this field is needed.

Conclusion

In conclusion, we found distinct responses of the heart and vessels to two cognitive mental tasks using noninvasive complex assessment of the conceivable marker of sympathetic vascular regulation and potential cardio-sympathetic index. Further studies are needed to characterize and elucidate the pathways linking mental stress and mechanisms of specific cardiovascular effectors´ response modulation.

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Legends to figures

Figure 1. Conceivable sympathetic vascular response to mental stressors indexed by spectral power of the low frequency band of systolic arterial pressure variability (LF-SAP).

Figure 2. Potential cardio-sympathetic response to mental stressors indexed by symbolic dynamics index 0V%.

Figure 3. Comparison of the reactivity of the conceivable sympathetic vascular index LF-SAP and potential cardio-sympathetic index 0V% to mental stress tests. LF-SAP – spectral power in low frequency band of systolic arterial pressure.

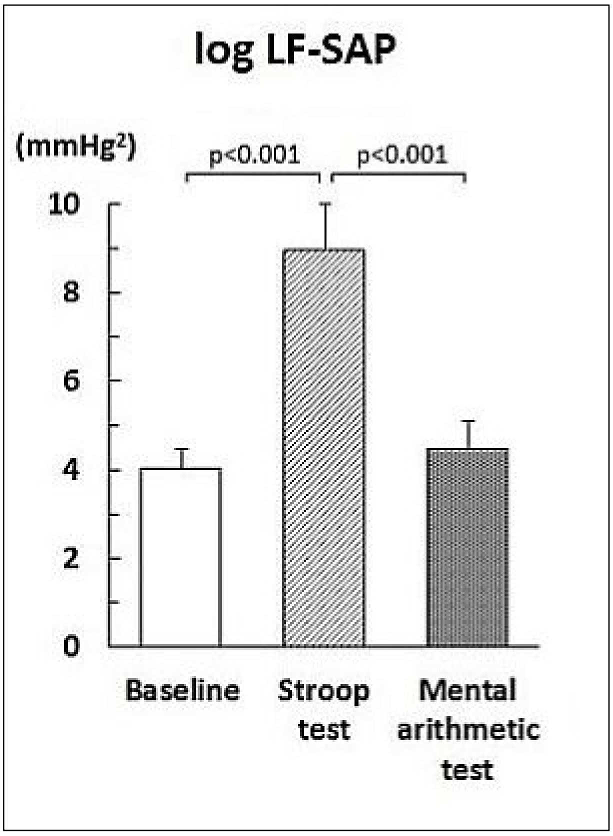

log LF-SAP
(mmHg2)
p<0.001
p<0.001
10
8
6
4
2
0
Baseline
Stroop test
Mental arithmetic test

Fig. 1

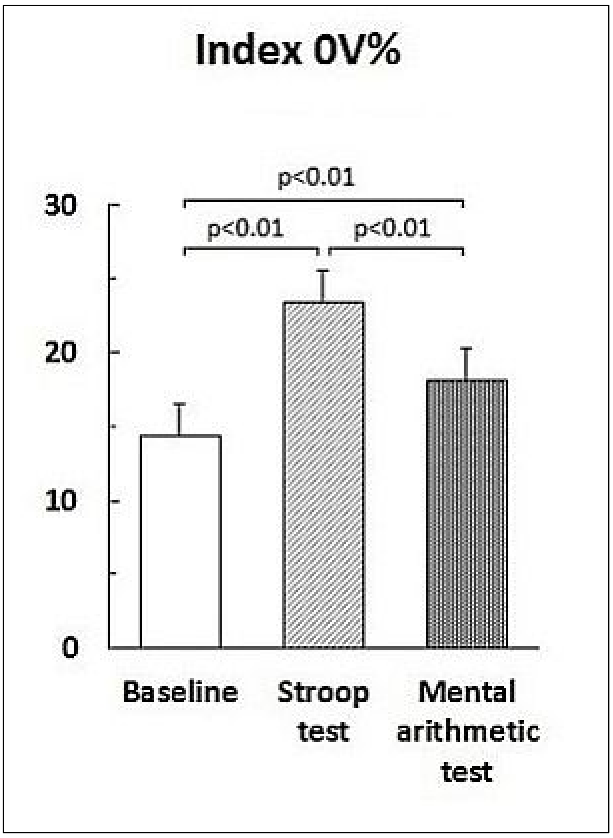
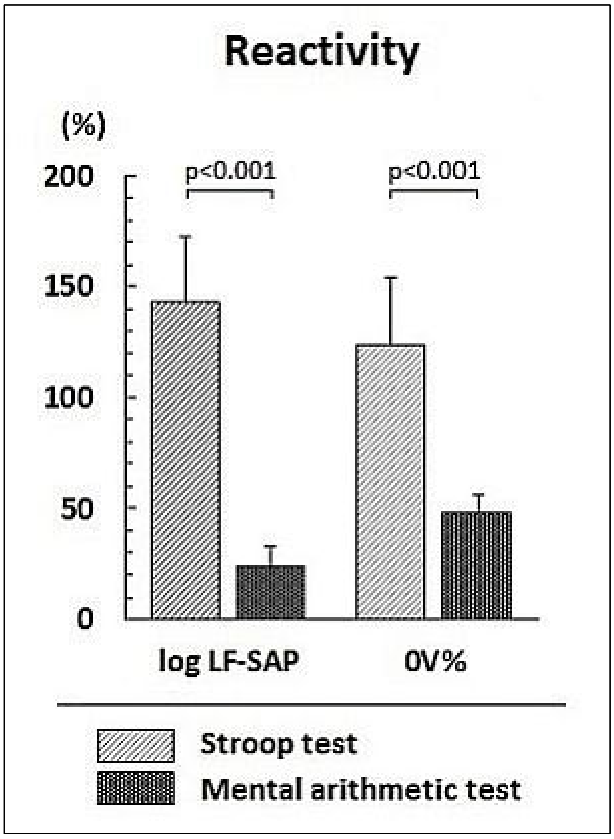

Index 0V%
p<0.01
p<0.01
p<0.01
30
20
10
0
Baseline
Stroop test
Mental arithmetic test

Fig. 2



Reactivity
(%)
200
150
100
50
0
p<0.001
p<0.001
log LF-SAP
OV%
Stroop test
Mental arithmetic test

Fig. 3