

NOVEL APPROACH TO EVALUATE CENTRAL AUTONOMIC REGULATION IN ATTENTION DEFICIT/HYPERACTIVITY DISORDER (ADHD)

Running title: Autonomic regulation in ADHD

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SUMMARY

Attention deficit/hyperactivity disorder (ADHD) is one of the most commonly diagnosed developmental disorders in childhood characterized by hyperactivity, impulsivity and inattention. ADHD manifests in the child's development by deficits in cognitive, executive and perceptor-motor functions, emotional regulation and social adaptation. Although the exact cause has not yet been known, the crucial role in the development of this disease plays the interaction of genetic, neurobiological and epigenetic factors. According to current knowledge, ADHD is defined as a biological dysfunction of central nervous system with genetically or organically defined deficits in noradrenergic and dopaminergic neurotransmission associated with structural abnormalities, especially in prefronto-striatal regions. In this context, a significant part of the difficulties could be due to a faulty control of fronto-striato-thalamo-cortical circuits important for attention, arousal and executive functions. Moreover, ADHD is associated with abnormal autonomic regulation. Specifically, reduced cardiac-linked parasympathetic activity associated with relative sympathetic dominance indexed by low heart rate variability can represent a noninvasive marker for prefrontal hypoactivity. However, the mechanisms underlying altered autonomic regulation in ADHD are still unknown. In this aspect, the evaluation of central autonomic regulation by noninvasive methods, namely pupillometry and eye-tracking, may provide novel information for better understanding of the neurobiological pathomechanisms leading to ADHD.

Key words: ADHD, neurobiology, autonomic regulation, pupillometry, eye-tracking

INTRODUCTION

ADHD (Attention Deficit Hyperactivity Disorder according to American classification DSM-V) is a mental disease in the childhood with the highest prevalence, approximately 8-12% worldwide, with a more frequent occurrence among boys compared to girls in a ratio of 3-5:1 (Biederman and Faraone 2005, Skogli *et al.* 2013). ADHD is a common neurodevelopmental disorder characterized by pervasive and developmentally inappropriate levels of impulsivity, hyperactivity and inattention lasting at least 6 months with the beginning before the seventh year of age of the child and wide-ranging cognitive and behavioral impairments, including deficits in working memory, inhibitory control, and altered motivation (Sonuga-Barke 2002). According to current knowledge, ADHD is defined as a biological dysfunction of central nervous system (CNS) with genetically or organically defined deficits in noradrenergic and dopaminergic neurotransmission. It is suggested that part of the difficulties of ADHD is due to a faulty control of fronto-striato-thalamo-cortical circuits important for the management of behavioral responses such as attention, arousal and executive functions (Barkley 1997). Moreover, according to Neurovisceral and Polyvagal theory (Porges 1995, Thayer and Lane 2000, Porges 2007) these brain regions are part of a complex autonomic network described by Benarroch (1993) modulating the output to the sympathetic and parasympathetic neurons. Thus, the abnormalities in autonomic regulatory mechanisms could reflect pathomechanisms linked to ADHD.

This article is aimed to summarize the latest findings in the neurobiology of ADHD with focus on pupillometry and eye-tracking as novel methods suitable for quantifying of central autonomic regulation/dysregulation associated with ADHD.

NEUROBIOLOGY OF ADHD

In the neurobiology of ADHD, several major research areas, namely neuroimaging, neurophysiology and neurochemistry, and genetics show that specific brain abnormalities are involved in the ADHD pathophysiology leading to changes in biochemical regulation in the brain (Doggett 2004).

Neuroimaging studies

Relationships between brain morphology and ADHD can be identified by various neuroimaging methods, mainly magnetic resonance imaging (MRI) and its modalities. In MRI, the T1 imaging weighting is used for the assessment of brain macrostructure, such as volume and structure of white and gray matter, and the diffusion tensor imaging (DTI) for the assessment of brain microstructure, specifically to assess the integrity of the white matter pathways (Song *et al.* 2005). Initial brain macrostructural studies revealed that children suffering from ADHD show smaller brain volumes in all regions (Castellanos *et al.* 2002) however, some meta-analyses report differences mainly in basal ganglia with total brain or grey matter volume (Valera *et al.* 2007, Ellison-Wright *et al.* 2008, Nakao *et al.* 2011). Specifically, ADHD patients are characterized by reductions in total cerebral volume, prefrontal cortex, basal ganglia (striatum), dorsal anterior cingulate cortex, splenium of the corpus callosum, and cerebellum, in particular the posterior inferior vermis (Emond *et al.* 2009). These widespread macrostructural changes suggest that many areas of the brain are associated with ADHD diagnosis in childhood. In children and adolescents, ADHD symptoms such as distractibility, impulsivity, hyperactivity and forgetfulness were linked to decreased frontal and temporal grey matter and also caudate and cerebellar volumes (Arnsten 2006). Excluding changes in volume, studies of cortical development also showed a decrease in cortical thickness (Batty *et al.* 2010)

and a marked delay in brain maturation where the grey matter peaks were about 3 years later, most prominent in prefrontal regions important in the control of cognitive processes, than in healthy controls (Shaw *et al.* 2006, Shaw *et al.* 2007). In ADHD patients, DTI studies have revealed developmental changes in cortical white matter pathways in prefrontal regions and also in the pathways that surround the basal ganglia and cerebellum reflecting decreased myelination of axons (D'Agati *et al.* 2010). Growing evidence points to the fronto-striatal network involving lateral prefrontal cortex, dorsal anterior cingulate cortex, the caudate nucleus and putamen as well as fronto-parietal network as a likely contributor to the pathophysiology of ADHD (Bush *et al.* 2005). Neurobiological dysfunction of the circuits within the prefrontal cortex, namely dorsal fronto-striatal, orbito-fronto-striatal and fronto-cerebellar can lead to the symptoms of ADHD, such as impulsive and inattentive behavior. Functionally, dorsal fronto-striatal circuitry is linked to cognitive control, whereas circuits between striatum and orbitofrontal cortex is related to reward and motivation (Durstun *et al.* 2011). Prefrontal cortex dysfunction results in reduced ability to exercise control (Casey *et al.* 2007). Dysfunction of ventral striatum can lead to deficits in motivation and reward processing, whereas dysfunction in dorsal striatum can lead to problems with ability to predict what events will occur (Scheres *et al.* 2007, Plichta *et al.* 2009). The fronto-cerebellar circuits are implicated in timing and building temporal expectations and its dysfunction is related with problems of ability to predict when events will occur (Hoekzema *et al.* 2010). In addition, connections between the prefrontal cortex and amygdala have been suggested to play a role in ADHD, in particular fronto-amygdalar circuits could be involved in assignment of emotional valence to events and also inputs from amygdala can influence the recruitment of prefrontal control (Durstun *et al.* 2011). It is assumed that dysregulated functional communications in these networks are the cause of the behavioral dysfunction in ADHD diagnosis (Nagel *et al.* 2011).

It is suggested that hyperactivity-impulsive symptoms are associated with changes in the striatum and its connections (van Ewijk *et al.* 2012). The striatum includes the nucleus accumbens that forms a network with orbitofrontal cortex and anterior cingulate cortex associated with reward processing and motivational control, nucleus caudate facilitating cognitive control via connections with dorsolateral prefrontal cortex and the putamen regulating motor function as well as cognitive control (Oldehinkel *et al.* 2016). According to recent evidence, putamen and caudate may be functionally divided into smaller subregions (Choi *et al.* 2012). Investigation of putamen and caudate subregions functional connectivity might detect ADHD-related effects that cannot be revealed when the putamen and caudate are determined as homogenous regions. Specifically, the putamen is divided to an anterior region connected to pre-supplementary motor area, anterior cingulate cortex associated with learning new movements, and a posterior region linked to primary and secondary motor areas and this brain area is related to execution of skilled movements. The caudate is also divided into ventral region connected to prefrontal cortex, anterior cingulate cortex and amygdala to exert cognitive control over emotional loops, and dorsal region connected to prefrontal cortex, motor cortex and parietal cortex associated with cognitive control over action-based networks. In the latest study, Oldehinkel *et al.* (2016) observed that functional connectivity of the ventral caudate and posterior putamen networks increased with raised ADHD symptoms scores – inattention and hyperactivity/impulsivity. Furthermore, increased functional connectivity of posterior putamen with motor cortex and cerebellum results in decreased motor performance. They also found out increased functional connectivity of ventral caudate and posterior putamen with occipital cortex as a function of increased inattention that could be explained in the context of interactions between putamen, caudate, occipital cortex and attention networks which are aimed at sustaining attention to relevant stimuli while inhibiting attention to irrelevant stimuli (Shulman *et al.* 2009, Oldehinkel *et al.* 2016).

In ADHD, the present findings suggest that the superior longitudinal fasciculus (SLF) is dysfunctional (Tomasi and Volkow 2012, Franzen *et al.* 2013). The SLF is a large bundle of white matter in each cerebral hemisphere that connects the parietal, temporal and occipital lobes with ipsilateral frontal cortices. There are four subcomponents of the SLF (SLF I, SLF II, SLF III and SLF IV also called arcuate fasciculus) with different functional contributions to behavior. SLF I connects the medial and dorsal part of the frontal lobe with the cingulate and cingulum of the superior and medial parietal cortex. The SLF II connects the middle frontal gyrus and the postcentral gyrus of the parietal lobe and provides information on visual space and visuo-motor function contributing to spatial attention. The SLF III connects the rostral part of the inferior parietal lobule with the middle frontal gyrus, the inferior frontal gyrus and the frontal lobe and is associated with language articulation. The SLF IV connects the superior temporal gyrus and the ventrolateral prefrontal cortex that modulates language functions and audio-spatial information (Gehricke *et al.* 2017). The brain imaging study revealed a dysfunctional coupling in parts of the SLF II resulted in saccadic abnormalities that have been found in children with ADHD (Franzen *et al.* 2013, Matsuo *et al.* 2015).

In the context of other ADHD symptoms, the present findings suggest that the cortico-limbic circuitry connecting the amygdala with the thalamus and orbital frontal cortex responsible for emotional learning and behavioral regulation is dysfunctional resulting in dysfunctional motivation, emotionality and impulsivity - major contributors of the disruptive behaviors in ADHD individuals (Gehricke *et al.* 2017). The involvement of the SLF and cortico-limbic areas at the brain circuitry level may suggest audio-visual, motivational and emotional dysfunctions in ADHD. Just because, investigating the SLF and cortico-limbic networks may be a novel research direction for better understanding of the underlying mechanisms of ADHD (Gehricke *et al.* 2017). The major neuroimaging findings in ADHD are summarized in the Table 1.

Neurophysiology and neurochemistry of ADHD

From neurophysiological point of view, the specific abnormalities detectable in ADHD are closely related to the clinical manifestations of this disorder. Some authors, e.g. Sterman (2000) suggested that ADHD is formed as a consequence of biochemical disturbance in prefrontal cortex or associated areas resulting in an inadequate cognitive or behavioral response and reduced attention and control. More than 90% of children suffering from ADHD show changes in electroencephalography (EEG) activity (Sterman 2000). Due to EEG research, the frequency bands of the most interest in ADHD are theta, beta and alpha, alone or compared to each other (theta/beta ratio). Theta band activity reflects drowsiness or “cortical slowing” in a resting state. Beta band activity accompanies mental activity and concentration. Alpha band activity is observed during eyes-closed resting condition and is negatively associated with central nervous system arousal (Loo and Makeig 2012). In the past, EEG was used to study children with behavioral problems (many of whom could be diagnosed as ADHD today) with the first reports indicating fronto-central EEG slowing (Jasper *et al.* 1938). Previous research findings suggested that children diagnosed with ADHD exhibit increased fronto-central theta activity during resting state conditions indicating decreased cortical activity associated with underarousal (Chabot and Serfontein 1996, Di Lazzaro *et al.* 1998, Clarke *et al.* 2001, Hobbs *et al.* 2007). According to elevated theta levels and the general association of the beta band activity with attentional arousal, the ratio of theta to beta power during eyes-open or -closed resting conditions has also been reported to be higher in ADHD individuals (Monastra *et al.* 2001). Taken together, the increases in the theta band activity and also in the theta/beta ratio are the two most reliable EEG findings in ADHD (Loo and Makeig 2012). However, more recent studies failed to replicate these findings, showing no significant differences in the theta power in ADHD compared to healthy controls (Loo *et al.* 2009, Ogrim *et al.* 2012, Liechti *et al.* 2013, Poil *et al.* 2014, Giertuga *et al.* 2017). In addition, majority of ADHD studies reported

reduced activity in alpha and beta band activities linked to cortical hypoarousal (Di Lazzaro *et al.* 1998, Clarke *et al.* 2001, Loo *et al.* 2009). However, several independent studies identified a subgroup of ADHD children with increased frontal beta band power. This ADHD subgroup is mostly diagnosed as ADHD combined type exhibiting more delinquent behaviors and having a lower intelligence score compared to controls and ADHD children exhibiting decreased beta band activity (Clarke *et al.* 2002, Clarke *et al.* 2011). Regarding alpha band activity, recent study reported no significant differences for children suffering from ADHD, but adults diagnosed with ADHD combined type showed significantly reduced alpha activity in comparison with controls (Loo *et al.* 2010). Moreover, Prihodova *et al.* (2012) revealed no specific changes in ADHD sleep microstructure.

Furthermore, the ADHD is associated with several dysfunctional neurotransmitter systems. In particular, the noradrenergic and dopaminergic systems are involved in ADHD pathophysiology. The noradrenergic system has been associated with the modulation of higher cortical functions such as attention, vigilance and executive function. Moreover, noradrenergic activation affects the attention mainly by the maintenance of arousal (Biederman and Spencer 1999). Arnsten *et al.* (1999) supported a crucial role of noradrenergic functioning in higher cortical activity by suggestion that high levels of catecholamine release disrupt cognitive functions of the prefrontal cortex. In particular, the action of norepinephrine (NE) and dopamine (DA) is most studied in prefrontal cortex (PFC). PFC is essential for attention and behavioral regulation and requires an optimal catecholamine environment. Both catecholamines have an inverted U influence on prefrontal cortex function, whereby too much and also too little NE or DA impairs prefrontal cortex cognitive abilities. Modest levels of NE improve PFC function through its action at postsynaptic α_2A receptors, but high levels of NE impair working memory through actions at α_1 and β_1 receptors. Similar to NE, optimal DA D1-receptor stimulation is essential to PFC function, while high levels of DA release also impair working memory.

Genetic alterations in catecholamine pathways may contribute to dysregulation of PFC circuits in ADHD (Brennan and Arnsten 2008).

Moreover, in ADHD children, anticipatory dopamine cell firing is diminished, what is termed dopamine transfer deficit. According to this theory, children suffering from ADHD would have a delayed dopamine signal at the cellular level compared to the normal children with immediate dopamine signal and this can explain abnormal sensitivity to delay of reinforcement (Tripp and Wickens 2008). Recently, the dopamine transfer deficit theory has been also supported by fMRI studies that showed reduced responsiveness of the ventral striatum to rewards relative to controls (Scheres *et al.* 2007, Plichta *et al.* 2009). Although the mechanism of DTD is unknown, it could explain several ADHD symptoms. The several DSM-V symptoms of inattention such as „often fails to give close attention to details or makes careless mistakes in schoolwork, work or other activities“, „often has difficulty sustaining attention in tasks or play activities“, and „is often easily distracted by extraneous stimuli“ may be due to failure of anticipatory dopamine cell firing leading to control of behavior by actual instances of reinforcement rather than predicted reinforcement. Another inattention symptoms like „often does not follow through on instructions and fails to finish schoolwork, chores or duties in the workplace“ and „often avoids, dislikes or is reluctant to engage in tasks that require sustained mental effort“ can be explained by failure of conditioned reinforcers leading to increased sensitivity to delay of reinforcement. This theory also explains few DSM-V symptoms of hyperactivity and impulsivity e.g. „often leaves seat in classroom or in other situation in which remaining seated is expected“ explained by a lack of effective reinforcement due to smaller activity of dopamine neurons. The other symptoms such as „often has difficulty awaiting turn“, „often blurts out answers before questions have been completed“ and „often interrupts or intrudes on others“ may be interpreted by a failure of the transfer of dopamine cell firing activity in response to cues predicting reinforcement (Tripp and Wickens 2009). Beside

the dopaminergic and adrenergic system, serotonergic and cholinergic pathways may be involved in ADHD as well (Prince 2008).

Genetic basis for ADHD

ADHD is a disorder with high heritability ranging from 60 to 90%. Genetic factors play an important role in the pathophysiology of ADHD, but the mechanism of action is not completely understood (Wood and Neale 2010, Hawi *et al.* 2015). In recent review, Hawi *et al.* (2015) highlighted several candidate genes with significant association with ADHD. Products of these genes are involved in neurotransmission, with one half playing an important role in monoaminergic function, namely dopamine and serotonin transporters (*SLC6A3*, *SLC6A4*), D4, D5 and serotonin receptors (*DRD4*, *DRD5*, *5HT1B*) (Cook *et al.* 1995, LaHoste *et al.* 1996, Daly *et al.* 1999, Seeger *et al.* 2001, Hawi *et al.* 2002). Other risk loci were mapped in genes involved in synaptic transmission (*SNAP25*, *NOS1*, *LPHN3*, *GIT1*) (Brophy *et al.* 2002, Reif *et al.* 2009, Arcos-Burgos *et al.* 2010, Won *et al.* 2011). In addition, recent study indicated that *SLC2A3* variation, the gene encoding glucose transporter-3 (GLUT3), may result in neurocognitive deficits (in working memory and cognitive response control) known to contribute to ADHD risk. GLUT3 plays a crucial role in the cerebral glucose metabolism, providing energy for the activity of neurons, which, in turn, moderates the excitatory-inhibitory balance impacting both brain development and activity-dependent neural plasticity (Merker *et al.* 2017). Despite growing number of genome-wide association studies (GWAS) in child and adult ADHD, there has been limited success in showing significant genome-wide associations. Only two loci within the *CDH13* and the *GFOD1* genes, have exceeded the critical significance threshold for genome-wide associations with quantitative attributes of ADHD (Lasky-Su *et al.* 2008). *CDH13* encodes cadherin-13, a calcium-dependent protein important in cell-cell adhesion and neural cell growth, expressed in the cerebral cortex (Neale *et al.* 2010). Moreover,

single nucleotide polymorphisms within *CDH13* have been linked to working memory deficits and hyperactivity/impulsivity in ADHD individuals (Arias-Vásquez *et al.* 2011, Salatino-Oliveira *et al.* 2015). *GFOD1* is a gene expressed in the brain and involved in electron transport, but its implications for ADHD are less known (Franke *et al.* 2009). The selected candidate genes are summarized in the Table 2.

CENTRAL AUTONOMIC REGULATION

Autonomic nervous system (ANS) represents an important regulatory system involved in attention and self-regulation by influencing physiological arousal. Physiologically, this regulatory mechanism plays crucial role in adaptation to changing internal and external environment, however, after exposition to long-term, repetitive mental stress associated with altered sympathetic regulation, it may become maladaptive (Mestanik *et al.* 2015). In this context, interindividual differences in physiological measures (e.g. sympathovagal dynamic balance) and in the responsivity to various challenges of the stress response system may contribute to the risk for mental disorders (Tonhajzerova and Mestanik, 2017). Our previous studies revealed discrete ANS abnormalities in various psychiatric disorders (e.g. depression, autism) and also changes in ADHD-linked autonomic balance towards the cardiac vagal deficiency associated with sympathetic dominance using heart rate variability linear and nonlinear analysis (Tonhajzerova *et al.* 2009, Tonhajzerová *et al.* 2014, Bujnakova *et al.* 2016, Mestanikova *et al.* 2017). In this aspect, the inhibitory function of prefrontal cortex on subcortical structures including brainstem sympathoexcitatory circuits plays a crucial role in flexibility and adaptability (Thayer and Lane 2009). With regard to neurobiological basis of ADHD, delayed prefrontal cortical maturation combined with a lack of physiological autonomic development in ADHD children, as noted in recent studies (Buchhorn *et al.* 2012), could contribute to dominance of subcortical sympathoexcitatory regions resulting in ADHD

symptoms such as inattention (Tonhajzerova *et al.* 2016). However, the question related to noninvasive indices reflecting central autonomic regulatory network in ADHD is unresolved. Thus, the monitoring of the spontaneous eye movements using eye-tracking and studying pupillary light reflex by using pupillometry as potential noninvasive novel methods reflecting changes in central autonomic activity are discussed in the following paragraphs.

Visual attention indexed by eye-tracking

Eye-tracking is a method used to register eye movement to find the direction and targets of a person's gaze (figure 1). Information captured by eye-tracking methods are useful to reveal abnormalities in eye functioning but also to conduct cognitive studies focused on learning about people's emotions and intentions (Harezlak and Kasproski 2017). Despite eye-tracking intense research, it is still a very challenging task due to significant pupil reflectivity and variability of the shape and openness of the eye (Demjen *et al.* 2011). Eye movement, the most studied domain in cognitive neuroscience, as a result of interaction between various cognitive processes may provide knowledge about behavioral processes underlying mental disorders in children (Rommelse *et al.* 2008). Changes in saccadic eye movement appear to be involved in psychiatric disorders (Jagla 2016). Despite the fact that the basic brain mechanisms of saccadic eye movement paradigms are well documented in adults, this question is unclear in children and adolescents (Carlson 2000, Karatekin 2007). Emerging evidence shows that small involuntary eye movements, saccades and microsaccades, are a promising tool for investigation of cognitive processes impaired in ADHD individuals. Various paradigms have been used to investigate eye movements, the most commonly discussed types are saccadic paradigms, namely, antisaccades (AS), visually guided saccades (VGS), memory guided saccades (MGS) and smooth pursuit eye movements (SPEM). These paradigms are used to estimate basic oculomotor behavior and to investigate executive functions like inhibition and working memory

(Rommelse *et al.* 2008). In AS, visual onset is presented and the participants are required to make an eye movement away from the onset location to its mirror position (Everling and Fischer 1998). Successful performance of this task requires the top-down inhibition of a reflexive saccade to the onset location and the execution of a voluntary eye movement to the mirror position. Typical measures in AS are the number of directional errors reflecting a failure to suppress an inappropriate response due to problems with top-down regulation and saccade latency (Rommelse *et al.* 2008). AS activate the frontal eye fields, dorsolateral prefrontal cortex (DLPFC) and the supplementary eye fields (Everling and Munoz 2000). The majority of AS studies found an increased number of directional errors indicating a failure of top-down regulation in children with ADHD compared to controls (Karatekin and Asarnow 1998, Klein *et al.* 2003, O'Driscoll *et al.* 2005, Habeych *et al.* 2006). In MGS, the observers are required to look at a central fixation point. During this fixation, a target appears in the peripheral visual field and the participant is not allowed to make a saccade towards this target but has to remember its location. When the fixation point disappears, the observer is requested to make a saccade towards the memorized location. Typical measures are accuracy indicating the participant's ability to remember the location of the target, anticipatory errors reflecting inhibitory problems and saccade latency (Rommelse *et al.* 2008). MGS activate DLPFC, anterior cingulate cortex and supplementary eye field (Sawaguchi and Iba 2001, Ettinger *et al.* 2005). Studies reported more anticipatory errors in MGS indicating problems with inhibitory control in children with ADHD compared to controls (Ross *et al.* 1994, Castellanos *et al.* 2000, Mostofsky *et al.* 2001). Furthermore, more intrusive saccades, inappropriate eye movements, have been found in ADHD and are also reported in a variety of tasks, e.g. the go/nogo task (Castellanos *et al.* 2000). All these findings indicate a reduction in ability to suppress unwanted saccades and to control voluntary behavior and support the hypothesis of inhibition deficits in children with ADHD (Rommelse *et al.* 2008).

Pupillary light reflex indexed by pupillometry

Pupillometry as a simple, non-invasive method of pupil's diameter measuring provides important information about dynamic balance between sympathetic and parasympathetic nervous system. Changes of the pupil size are determined by the tone of two opposing smooth muscles, the dilatator and the constrictor. Pupillary constriction is mediated by the action of the sphincter muscle under parasympathetic control whereas pupillary dilation is mediated by the action of the dilatator under sympathetic control or by an inhibition of constrictor (Laeng *et al.* 2012). The balance of activity in sympathetic and parasympathetic branches depends on several factors, such as genetic influences, age, accommodative state and light conditions and after their standardization pupil's diameter measurement can be used for identifying deficits in both of the autonomic nervous system branches (Bremner 2009). Therefore, the pupillary light reflex provides an optimal model for investigating central autonomic regulation/dysregulation in mental disorders (Mestanikova *et al.* 2017).

With respect to ADHD, there are studies examining the autonomic regulation/dysregulation based on heart rate variability (Crowell *et al.* 2006, Tonhajzerova *et al.* 2009) and electrodermal activity (Shibagaki *et al.* 1993), but lack of those based on measurement of pupil diameter. Pupillary light reflex, the pupillary constriction in response to light, is regulated at the subcortical level. Light falling on the retina activates the pretectal olivary nuclei consequent activation of the Edinger-Westphal region, the parasympathetic center for pupillary constriction, resulting in generation of action potentials through the oculomotor nerves innervating the ciliary ganglion and leading to the activation of the constrictor muscle resulting in pupil constriction (Thompson 1992, Güler *et al.* 2008, McDougal and Gamlin 2015). Moreover, the Edinger-Westphal region receives inhibitory input from the subcortical brain structure locus coeruleus. Specifically, the activity of parasympathetic oculomotor center is

inhibited by the sympathetic-linked locus coeruleus. Reduction in this inhibitory input can lead to pupil constriction (Aston-Jones and Cohen 2005, Wilhelm *et al.* 2002). On the contrary, higher sympathetic nervous activity excites the neural inhibitory mechanism on Edinger-Westphal nucleus resulting in pupillary dilatation (Laeng *et al.* 2012). In addition, the Edinger-Westphal region is also modulated by inhibitory inputs from descending cortical pathways. Cognitive load associated with frontal and prefrontal cortical functioning mediated by direct cortical and indirect cortico-thalamo-hypothalamic pathways contributes to this inhibitory process (Steinhauer and Hakerem 1992, Steinhauer *et al.* 2004). Previously, smaller pupil dilation to visual stimulus was observed in the children with minimal brain dysfunction compared to controls (Zahn *et al.* 1978). Recently, Kara *et al.* (2013) found no significant differences between ADHD and healthy children in pupil diameters. Despite the fact that patients in our research showed altered PLR parameters - specifically smaller percentual change of constriction amplitude associated with slower average and maximum constriction velocity indicating a deficient parasympathetic activity (see figure 2), the further research is needed to elucidate this question.

CONCLUSION

The ADHD diagnosis is associated with widespread macrostructural and microstructural changes in central nervous system, affecting mainly the frontal, basal ganglia, anterior cingulate cortex, temporal, and occipito-parietal brain regions. Moreover, convergent data from neuroimaging, neuropsychology, neurochemical and genetics studies point to the involvement of the multiple circuits in the pathophysiology of ADHD. Functional neuroimaging has shown widespread dysfunction in neural systems involving the prefrontal, striatal and parietal regions. Dysfunction of various neurotransmitter systems involved in ADHD is supported by molecular genetic studies. The study of central autonomic regulation

using novel methods - PLR and eye-tracking can help us to elucidate neuropathophysiology of ADHD.

Conflict of interest

There is no conflict of interest.

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Table 1. Neuroimaging findings in ADHD

Study, year	Method	Main findings
Castellanos <i>et al.</i> 2002	MRI	Total cerebral and cerebellar volume reduction
Valera <i>et al.</i> 2007	MRI	Total cerebral volume reduction, volumetric differences in cerebellar regions, splenium of the corpus callosum and right caudate
Elison-Wright <i>et al.</i> 2008	MRI	Gray matter reduction in the right putamen/globus pallidus region
Nakao <i>et al.</i> 2011	MRI	Global reductions in gray matter volumes robustly localized in the basal ganglia
Emond <i>et al.</i> 2009	fMRI	Reduction in total cerebral volume, the prefrontal cortex, the basal ganglia, the dorsal anterior cingulate cortex, the splenium of corpus callosum and the cerebellum Hypoactivation of the dorsal anterior cingulate cortex, the frontal cortex and the basal ganglia
Arnsten 2006	fMRI	Decreased frontal and temporal gray matter Decreased caudate and cerebellar volume
Batty <i>et al.</i> 2010	fMRI	Decreased cortical thickness
Shaw <i>et al.</i> 2006, 2007	fMRI	Marked delay in brain maturation mainly in prefrontal regions

D'Agati <i>et al.</i> 2010	DTI	Developmental changes in cortical white matter pathways in prefrontal regions, the basal ganglia and cerebellum
Nagel <i>et al.</i> 2011	DTI	White matter microstructural abnormalities in bilateral temporo-occipital and corona radiate, left cerebellar and right fronto-parietal pathways
Durstun 2011	DTI	Dysregulated fronto-striatal, orbito-fronto-striatal and fronto-cerebellar circuits
Gehricke <i>et al.</i> 2017	DTI	Dysfunctional superior longitudinal fasciculus and cortico-limbic areas

MRI: magnetic resonance imaging, fMRI: functional magnetic resonance imaging, DTI: diffusion tensor imaging

Table 2. Selected candidate genes implicated in ADHD

	Gene product or function	Method(s) used to show association
<i>SLC6A3</i> <i>(DAT)</i>	Dopamine re-uptake, transporter	Candidate gene (Cook <i>et al.</i> 1995)
<i>DRD4</i>	Dopamine D4 receptor	Candidate gene (LaHoste <i>et al.</i> 1996)
<i>DRD5</i>	Dopamine D5 receptor	Candidate gene (Daly <i>et al.</i> 1999)
<i>SLC6A4</i>	Serotonin re-uptake, transporter	Candidate gene (Seeger <i>et al.</i> 2001)
<i>5HT1B</i>	Serotonin receptor	Candidate gene (Hawi <i>et al.</i> 2002)
<i>SNAP25</i>	Synaptosomal-associated protein 25, neurotransmission	Candidate gene (Brophy <i>et al.</i> 2002)
<i>NOS1</i>	Nitric oxid synthase, neurotransmission, neuroplasticity	Candidate gene (Reif <i>et al.</i> 2009)
<i>LPHN3</i>	Latrophilin 3, GPCR, cell adhesion, signal transduction	Linkage study (Arcos-Burgos <i>et al.</i> 2010)
<i>GIT1</i>	GPCR kinase, vesicle trafficking, cell adhesion, cell migration	Candidate gene (Won <i>et al.</i> 2011)
<i>SLC2A3</i>	Glucose-transporter 3	Candidate gene (Merker <i>et al.</i> 2017)

<i>CDH13</i>	Cell-cell adhesion and neural cell growth	GWAS (Lasky-Su <i>et al.</i> 2008) Candidate gene (Arias-Vásquez <i>et al.</i> 2011, Salatino-Oliveira <i>et al.</i> 2015)
<i>GFOD1</i>	Glucose-fructose oxidoreductase-domain containing 1, electron transport	GWAS (Lasky-Su <i>et al.</i> 2008)

GPCR: G protein-coupled receptor, GWAS: genome-wide association studies

FIGURE LEGENDS

Figure 1. Gaze polyline during 6s object tracking (Department of Physiology and Biomedical Center Martin; device: Mobile Eye-tracking Headset Pupil Labs, Germany).

Figure 2. Pupillary light reflex parameters in healthy control and patient with ADHD (Department of Physiology and Biomedical Center Martin; device: Pupillometer PLR-2000, NeurOptics, USA). INIT: diameter of the pupil before application of light stimulus (mm), END: diameter of the pupil after illumination at the peak of the constriction (mm), DELTA: percentual change of the pupil diameter during constriction, LAT: time of the onset of the constriction after application of light stimulus (latency), ACV: average constriction velocity (mm/s), MCV: maximum constriction velocity (mm/s), ADV: average dilatation velocity (mm/s).

Figure 1

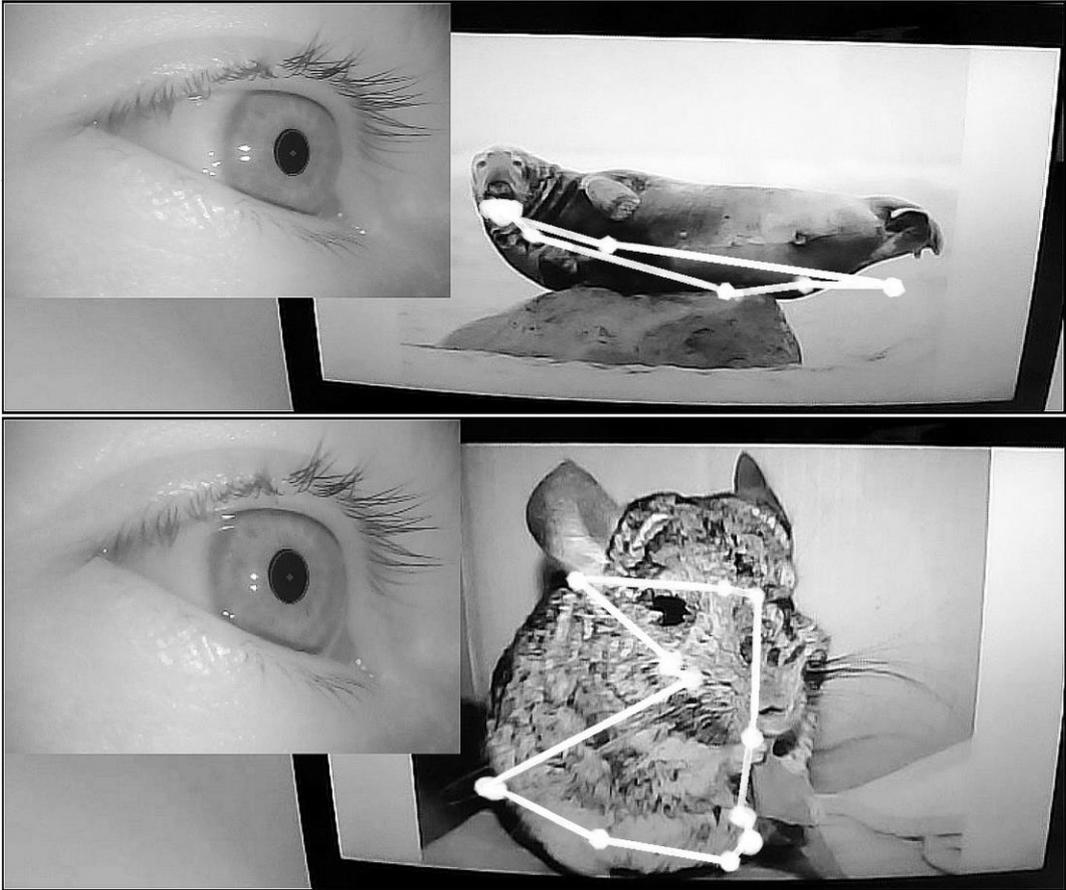
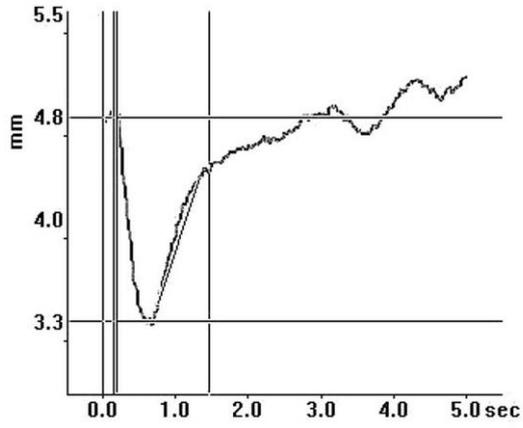


Figure 2

Control subject

INIT	END	DELTA	LAT
4.9	3.4	-30%	0.19
ACV	MCV	ADV	T75
-4.41	-5.41	1.60	0.83



ADHD

INIT	END	DELTA	LAT
5.7	4.5	-21%	0.22
ACV	MCV	ADV	T75
-3.75	-4.71	1.72	0.52

