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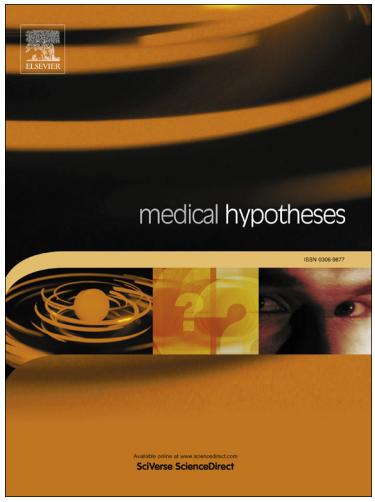

Infant Acetylcholine, Dopamine, and Melatonin Dysregulation: Neonatal Biomarkers and Causal Factors for ASD and ADHD Phenotypes

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Title: Infant Acetylcholine, Dopamine, and Melatonin Dysregulation: Neonatal Biomarkers and Causal Factors for ASD and ADHD Phenotypes

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ABSTRACT

Autism spectrum disorders (ASD) and ADHD are common neurodevelopmental disorders that benefit from early intervention but currently suffer from late detection and diagnosis: neurochemical dysregulations are extant already at birth but clinical phenotypes are not distinguishable until preschool age or later. The vast heterogeneity between subjects' phenotypes relates to interaction between multiple unknown factors, making research on factor causality insurmountable. To unlock this situation we pose the hypothesis that atypical pupillary light responses from rods, cones, and the recently discovered ipRGC system reflect early acetylcholine, melatonin, and dopamine dysregulation that are sufficient but not necessary factors for developing ASD and/or ADHD disorders. Current technology allows non-invasive cost-efficient assessment already from the first postnatal month. The benefits of the current proposal are: identification of clinical subgroups based on cause rather than phenotypes; facilitation of research on other causal factors; neonatal prediction of later diagnoses; and guidance for targeted therapeutical intervention.

BACKGROUND

Autism Spectrum Disorder (ASD) and Attention Deficit Hyperactivity Disorder (ADHD) are neurodevelopmental disorders with slowly emerging clinical profiles – diagnoses are rarely established before two years (ASD) or six years (ADHD) of age. This delay has impeded targeted and efficient research on neonatal biomarkers and causal relationships, because the underlying contributing factors are by this time elusive. The vast heterogeneity of phenotypes, primarily within the autism spectrum, together with high comorbidity, further complicates investigations; and common belief is that interacting factors account for the altered neurodevelopmental trajectories (1, 2). ASD and ADHD prevalence rates are at roughly 1 and 6-7 per cent, respectively, and their social and economic implications are enormous (3). To these ends, there is an immense need for neonatal biomarkers for ASD and ADHD that 1) focus research to causal relationships between neurological factors and phenotypes; 2) subcategorise diagnoses based on causal factors rather than phenotypes; and ultimately, 3) guide tailored therapeutical interventions that mitigate or prevent individual phenotypes by cancelling effects of specific causal factors using animal models. Here, we outline theory and methodology for assessing neonatal dysregulation of ASD- and ADHD-related neurotransmitters acetylcholine, dopamine, and melatonin using pupillometry and retroactive analyses based on follow-up clinical diagnoses. We suggest that the neurotransmitter dysregulations associated with ASD and ADHD are extant at birth and serve both as neonatal biomarkers and causal factors for clinical phenotypes.

Three prominent candidates, as contributing factors of ASD and ADHD, are dysregulation of neurotransmitter systems acetylcholine, dopamine, and melatonin. Acetylcholine has an important role in pre- and postnatal neurodevelopmental processes, including path finding and cell survival; whereas early etiological implications of dopamine and melatonin dysregulation are less known – but are later strongly associated with circadian rhythm deficiencies in children and adults with ASD and ADHD.

Firstly, assessing the cholinergic system is straightforward with the use of non-invasive pupillometry, which has been employed to register acetylcholine dysregulation to meet several ends across all age-groups, including infants (4, 5). Pupillometry as an instrument to tap acetylcholine regulation is facilitated by the pupillary light response (PLR) being predominantly driven by rods and cones in a highly acetylcholine-dependent four-neuron arc (*pretectal nuclei* → *Edinger-Westphal nucleus* → *ciliary ganglion* → *iris sphincter*). Atypical pupil responses in children with ASD have been shown in response to a brief flash of white light where ASD participants had greater response latencies and less pupil constriction than controls (6). Additionally, neuroimaging studies, post mortem brain tissue data (7, 8), rodent models (9), and molecular genetic studies also suggest that measures that tap the cholinergic system could be used to detect ASD.

Secondly, it has recently been shown that the PLR is also innervated by a new class of photoreceptors called intrinsically photosensitive retinal ganglion cells (ipRGC) that interplay and project via dopaminergic amacrine cells. ipRGCs have a singular spectral sensitivity peak at approximately 480nm (≈ cyan blue) (10) and projections mainly targets the olivary pretectal nucleus as part of the pupillary light reflex, and via the retinothalamic tract to the suprachiasmatic nucleus of the hypothalamus (which projects to e.g. the pineal gland to stimulate melatonin release) for circadian photo entrainment (11). Compared with rods and cones, ipRGCs react slower, do not habituate, and have a sustained response that usually decays gradually over several minutes. The discovery of ipRGCs is very recent, and it is perhaps not surprising that developmental or neuropsychiatric aspects of ipRGC pupil responses have hitherto not been studied at all. However, incitements to use the ipRGC response to classify ASD and ADHD come both from studies showing modulated dopamine and/or melatonin functioning (12) connected to circadian rhythm deficiencies in subpopulations of ASD and ADHD (13).

HYPOTHESIS

In summary, abnormal rod and cone induced PLR in subjects with ASD and/or ADHD compared to a population-based norm suggests dysregulation of acetylcholine, whereas abnormal ipRGC induced PLR compared to norm suggests dysregulation of dopamine through the mediating dopaminergic amacrine cells, as well as a compromised melatonin regulation. We accordingly introduce the hypothesis that assessment of rod/cone and ipRGC PLR during infancy can identify early dysregulation of neurotransmitters acetylcholine, dopamine, and melatonin leading to subsequent ASD or ADHD diagnoses; and consequently, that infants' cholinergic, dopaminergic and/or melatonin dysregulation is a sufficient but not required factor for developing the neuropsychiatric disorders ASD and ADHD.

METHODS AND CAVEATS

One advantageous feature of this hypothesis is that the methods are non-invasive and readily available using modern remote eye tracking. Simple procedures for screening the PLR longitudinally in infant populations would require less than 10 minutes per time point: a requisite three-minute dark adaptation (dim light) for pupil data acquisition followed by seven minutes of light stimulation selectively tapping the rod/cone and ipRGC systems.

[Figure 1 about here]

Eye-tracking and pupillometry have successfully been employed in numerous previous infant and child samples (4, 14). However, although methodologically well-established, two caveats should be noted.

Experimental stimuli that sustain infants' attention need to be developed. Such attention drawing stimuli should interfere minimally with pupil size, including pupillary dilation due to arousal of the sympathetic nervous system (14). Additionally, in both PLR (rod/cone and ipRGC) and PIPR (ipRGC) there are other neurotransmitters at play. Pupilloconstriction also depend on GABAergic transmissions, primarily in intraretinal connections; and glutamate in efferent retinal projections, both of which are implicated in ASD and ADHD etiology. We argue that this is not undermining the methodology but rather highlights prospective possibilities of assessing the integrities of additional neurotransmitter systems.

DISCUSSION OF IMPLICATIONS

We have here put forth a hypothesis that is testable through cost-effective, non-invasive, and procedurally straightforward means. It is motivated and supported by recent neurochemical and ophthalmological evidence in combination with a large body of research showing clinical adult and child neurotransmitter dysregulation (7, 9, 12, 15, 16). Furthermore, we argue that assessing the PLR is important while determining *other* underlying factors that contribute to pathological ASD and ADHD phenotypes by isolating the effects from neurotransmitter dysregulation. This will make it possible to identify subpopulations within disorders based on separate causal factors, which consequently open up for the engenderment of increasingly refined predictions of causal relationships in neurodevelopmental disorders. Moreover, but beyond the scope of this article, this also applies to conditions at the other end of the life span, such as Parkinson's and Alzheimer's disease (5).

The development of the human brain is a protracted process and, especially the neonate's brain, is characterised by plasticity. Environment and experience contribute to fundamental structural and functional reorganisation. Correspondingly, we hold that early neurochemical dysregulation, too, causes

developmental trajectories to go awry. The same underlying cause may diverge into separate clinical profiles, but we emphasise that delimiting the cause will invariably improve all subsequent conditions.

There are thus large incentives for the research community to start testing concrete hypotheses that pertain to early biomarkers and subsequent pathological phenotypes. Verification of causal relationships and, ultimately, effects of early and targeted therapeutical interventions, can be facilitated using rodent and non-human primate models which are readily available. Future large-scale efforts mapping multiple causal factors will require international commitment, and we therefore encourage all interested parties to form open science collaborations (17). However, already testing the presented hypothesis as a single/isolated factor can have profound socioeconomic implications and have the potential to substantially and directly improve future life-quality of millions.

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CAPTIONS TO ILLUSTRATIONS

Figure 1. (A) The cholinergic rod/cone stimuli should consist of brief flashes and analyses should comprise relative amplitude and latency of constriction, $(A_0^2 - A_m^2)/A_0^2$, and latency. (B) The ipRGC system can be measured by alternating between cyan (ipRGC) and red (rod/cone) continuous light or by 20 seconds of light followed by darkness. The transient pupil response (T) is mainly rods and cone activation that habituate over time. The sustained response (S) is larger for blue light compared to red light due to the spectral sensitivity of ipRGC, and T - S thus index ipRGC activation. Because the ipRGCs react slower than rods and cones their contribution to the PLR is distinguished in the post illumination pupil response (PIPR), where ipRGC pupilloconstriction continues much longer after stimulus offset. Our hypothesis states that infants later diagnosed with ASD or ADHD caused by dysregulation of acetylcholine and/or dopamine should display PLR respective PIPR alterations that are distinct from typically developing subjects.