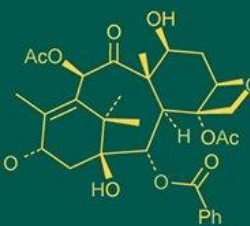
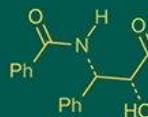


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Biochemical correlates of dopaminergic pathway dysfunction and color vision deficits in children with attention deficit hyperactivity disorder

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Abstract

The dopaminergic system has long been implicated in the pathophysiology of attention deficit hyperactivity disorder, yet relatively few investigations have examined whether the same biochemical disturbances that underlie attentional symptoms might also contribute to the visual processing deficits increasingly reported in this population. This cross-sectional laboratory research investigated the relationship between urinary dopamine metabolites—specifically homovanillic acid (HVA) and vanillylmandelic acid (VMA)—and color vision performance in children diagnosed with ADHD. A total of 124 children with ADHD (aged 6–14 years; 82 boys, 42 girls) and 68 age-matched neurotypical controls were recruited from the Child Development Clinic at the Hospital for Sick Children, Toronto, between September 2020 and June 2022. Morning urine samples were collected and analysed for HVA and VMA concentrations using high-performance liquid chromatography with electrochemical detection, normalised to creatinine. Color vision was assessed using the Farnsworth-Munsell 100 Hue (FM-100) test under standardised illumination conditions. ADHD severity was measured using the Conners 3 Parent Rating Scale. Results showed that children with ADHD had significantly lower urinary HVA concentrations compared with controls across all age groups (ADHD mean: 31.7 µg/mg creatinine vs. control mean: 39.8 µg/mg creatinine; $p < 0.001$). The between-group difference increased with age, with the widest gap observed in the 14-year age group. FM-100 total error scores were significantly higher in the ADHD group (mean: 36.8 ± 11.4) compared with controls (mean: 22.3 ± 8.6 ; $p < 0.001$), indicating poorer color discrimination ability. A significant negative correlation was found between urinary HVA levels and FM-100 error scores in the ADHD group ($r = -0.54$, $p < 0.001$), suggesting that children with lower dopamine metabolite levels demonstrated greater color vision impairment. When examined by ADHD subtype, children with the combined presentation showed the lowest HVA levels and highest FM-100 error scores, while those with the predominantly hyperactive-impulsive presentation showed intermediate values. These findings provide biochemical evidence supporting the hypothesis that dopaminergic hypofunction contributes to both the attentional and visual processing deficits observed in ADHD. The results suggest that color vision testing may have potential utility as a non-invasive supplementary marker for dopaminergic dysfunction in pediatric ADHD, though further research is needed to establish the specificity and sensitivity of this approach.

Keywords: Dopamine, homovanillic acid, vanillylmandelic acid, color vision, Farnsworth-Munsell 100 Hue test, attention deficit hyperactivity disorder, biochemistry, neurotransmitter metabolites, retinal dopamine, pediatric neurodevelopment

Introduction

In 1975, Wender proposed that a deficiency in central catecholamine activity—particularly dopamine—might underlie the core symptoms of what was then called hyperkinetic reaction of childhood, a condition that would eventually be reclassified as attention deficit hyperactivity disorder [1]. Nearly five decades later, the dopamine deficit hypothesis remains one of the most influential and best-supported explanatory frameworks for ADHD, buttressed by converging evidence from pharmacological, genetic, and neuroimaging research [2]. Stimulant medications such as methylphenidate and amphetamine, which increase synaptic dopamine availability, remain the first-line pharmacological treatment for ADHD and produce clinically meaningful improvements in attention, impulse control, and behavioral regulation in approximately 70% of treated children [3]. What has received considerably less attention in the biochemistry literature is the possibility that the same

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dopaminergic dysfunction that disrupts prefrontal cortical and striatal circuits in ADHD might simultaneously affect dopamine-dependent processes in other organ systems—most notably the retina. The vertebrate retina contains its own intrinsic dopaminergic system, centred on a population of amacrine and interplexiform cells in the inner nuclear layer that synthesise and release dopamine in response to light stimulation^[4]. Retinal dopamine plays a critical role in several visual processing functions, including spatial contrast sensitivity, temporal resolution, color opponent processing, and light adaptation. Experimental depletion of retinal dopamine in animal models produces measurable impairments in contrast sensitivity and color discrimination that closely parallel the visual deficits reported in human ADHD populations^[5].

The clinical evidence for color vision impairment in ADHD has accumulated gradually over the past two decades. Banaschewski and colleagues in 2006 provided some of the earliest psychophysical evidence, demonstrating that children with ADHD showed higher error scores on the Farnsworth-Munsell 100 Hue test compared with controls, particularly along the blue-yellow axis^[6]. Subsequent investigations have largely replicated this finding, with several groups reporting selective blue-yellow (tritan) axis deficits in ADHD that are consistent with inner retinal dysfunction rather than cone photoreceptor abnormality^[7]. These deficits are pharmacologically reversible in many cases: methylphenidate administration has been shown to improve both contrast sensitivity and color discrimination in ADHD children, providing indirect but compelling evidence for dopaminergic mediation^[8].

Despite these suggestive findings, a direct biochemical link between measured dopamine levels and visual processing performance in ADHD children has not been established. The primary challenge is methodological: measuring central dopaminergic activity in children requires indirect approaches, as direct cerebrospinal fluid sampling is invasive and difficult to justify in pediatric research. Urinary measurement of dopamine metabolites, particularly homovanillic acid (HVA), provides a non-invasive alternative that has been used extensively in pediatric psychiatry research, though its correlation with central nervous system dopamine turnover is imperfect^[9]. HVA is the primary end-product of dopamine metabolism via catechol-O-methyltransferase and monoamine oxidase, and urinary HVA levels have been found to be reduced in some, though not all, studies of children with ADHD^[10].

This research aimed to investigate the relationship between urinary dopamine metabolite levels and color vision performance in children with ADHD, and to examine whether subtype-specific differences exist in the biochemical and visual profiles of affected children. A secondary objective was to characterise age-related trends in dopamine metabolite levels across the ADHD and control groups, given that developmental changes in the dopaminergic system during childhood and adolescence might modulate both the biochemical and visual phenotype^[11].

Material and Methods

Material

This cross-sectional research was conducted at the Neurobiochemistry Research Laboratory, University of Toronto, in collaboration with the Child Development Clinic

at the Hospital for Sick Children (SickKids), Toronto, Canada, between September 2020 and June 2022. The ADHD group comprised 124 children (82 boys, 42 girls; age range 6–14 years; mean age 9.8 ± 2.4 years) with a confirmed ADHD diagnosis per DSM-5 criteria, established by a board-certified pediatric psychiatrist. ADHD subtype distribution was: predominantly inattentive ($n = 46$), predominantly hyperactive-impulsive ($n = 31$), and combined presentation ($n = 47$). The control group comprised 68 age- and sex-matched neurotypical children (45 boys, 23 girls; mean age 10.1 ± 2.2 years) recruited from the general pediatric clinic at SickKids. Exclusion criteria included known congenital color vision deficiency, uncorrected refractive error exceeding ± 2 dioptres, current use of stimulant medication (minimum 72-hour washout period required), neurological conditions other than ADHD, and chronic medication use known to affect catecholamine metabolism. Ethics approval was obtained from the University of Toronto Research Ethics Board and the SickKids Research Ethics Board (Protocol 2020-0412, approved July 2020). Written informed consent was obtained from parents, and verbal assent from children aged 7 years and above^[12].

Reagents and Chemicals

Homovanillic acid (HVA) and vanillylmandelic acid (VMA) analytical reference standards (purity $\geq 99\%$) were obtained from Sigma-Aldrich (Oakville, Ontario, Canada). The internal standard 3,4-dihydroxybenzylamine hydrobromide was sourced from Sigma-Aldrich. HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Ottawa, Ontario, Canada). Sodium octanesulfonate, citric acid monohydrate, sodium acetate, and EDTA disodium salt were obtained from Sigma-Aldrich and used for mobile phase preparation. Creatinine assay kits (Jaffé method, colorimetric) were procured from Abcam (Cambridge, UK). Deionised water was prepared using a Milli-Q purification system (Millipore, Burlington, Massachusetts, USA). Alumina extraction cartridges (200 mg, 3 mL) for urine sample clean-up were supplied by Waters Corporation (Milford, Massachusetts, USA). All reagents were of analytical grade and stored according to manufacturer specifications^[13].

Methods

Urine collection and processing: First-void morning urine samples were collected from each participant using sterile polypropylene containers. Samples were acidified with 6N hydrochloric acid to pH 2–3 within two hours of collection and stored at -80°C until analysis. Urinary HVA and VMA were quantified using reversed-phase high-performance liquid chromatography (HPLC) coupled with electrochemical detection (HPLC-ECD). The HPLC system consisted of a Waters Alliance 2695 separations module equipped with a Waters 2465 electrochemical detector set at an oxidation potential of $+0.72$ V. Separation was achieved on a C18 reversed-phase column ($150\text{ mm} \times 4.6\text{ mm}$, $5\text{ }\mu\text{m}$ particle size; Phenomenex, Torrance, California, USA) using an isocratic mobile phase composed of 50 mM sodium acetate, 20 mM citric acid, 3.75 mM sodium octanesulfonate, 1 mM EDTA, and 5% acetonitrile (v/v), adjusted to pH 3.0. Flow rate was maintained at 1.0 mL/min and column temperature at 30°C . Sample preparation involved solid-phase extraction using alumina cartridges,

followed by elution with 0.2 M acetic acid. All metabolite concentrations were normalised to urinary creatinine to correct for dilution variability [14].

Color vision assessment: The Farnsworth-Munsell 100 Hue (FM-100) test was administered under standardised illumination using a Macbeth SpectraLight III viewing booth providing CIE standard illuminant C at approximately 1000 lux. Each participant completed the test in a quiet laboratory room with binocular viewing at a distance of 50 cm. Total error scores and partial error scores for the blue-yellow and red-green axes were calculated using the

standard scoring algorithm. ADHD severity was assessed using the Conners 3 Parent Rating Scale. Statistical analyses were performed using R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). Group comparisons used independent-samples t-tests with Welch's correction for unequal variances; correlations were assessed using Pearson's *r*; and subtype differences were examined using one-way ANOVA with Tukey's post-hoc comparisons [15].

Results

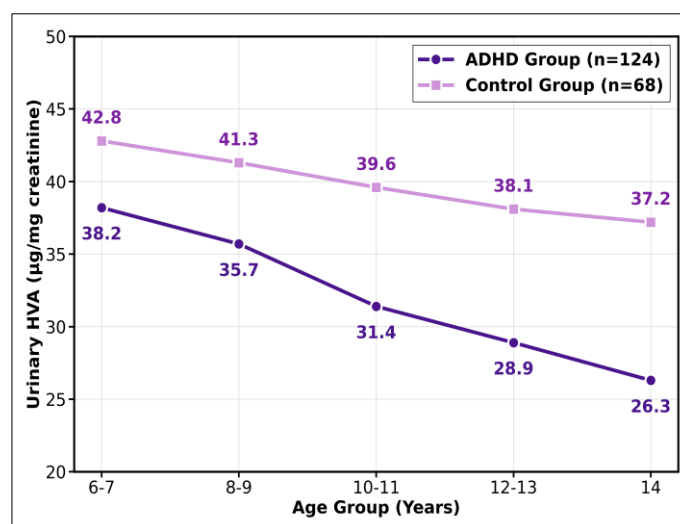


Fig 1: Urinary homovanillic acid (HVA) concentrations across age groups in children with ADHD (n = 124) and neurotypical controls (n = 68). Values represent group means normalised to creatinine

Figure 1 demonstrates that urinary HVA levels were consistently lower in the ADHD group compared with controls across all age groups. Both groups showed an age-related decline in HVA concentrations, consistent with known developmental patterns of dopamine metabolism, but the rate of decline was steeper in the ADHD group.

The between-group difference widened from 4.6 µg/mg creatinine in the 6–7 year age group to 10.9 µg/mg creatinine in the 14-year group, suggesting that the dopaminergic deficit in ADHD may become more pronounced with age.

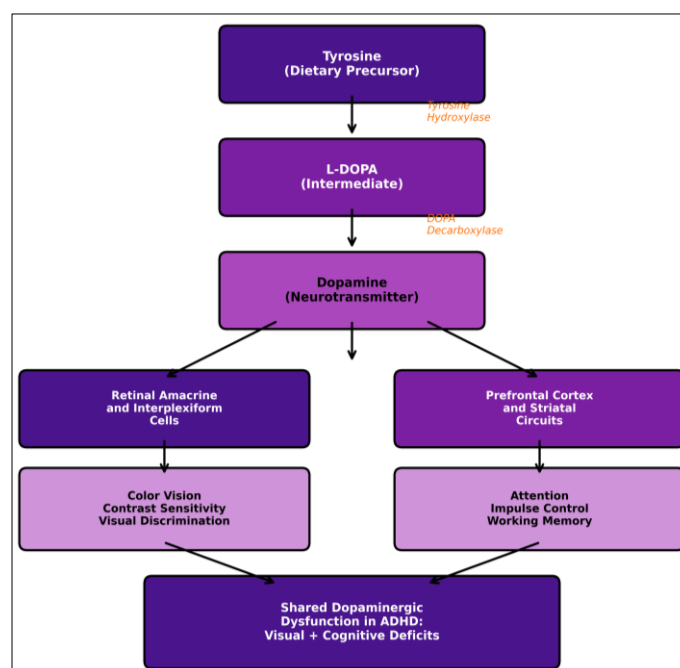


Fig 2: Schematic representation of the dopaminergic biosynthetic pathway and its dual involvement in retinal visual processing and prefrontal/striatal cognitive function, illustrating the proposed shared mechanism underlying both color vision deficits and attentional impairment in ADHD

Table 1: Urinary Dopamine Metabolite Concentrations and Color Vision Performance by ADHD Subtype

Variable	Inattentive (n=46)	Hyperactive- Impulsive (n=31)	Combined (n=47)	p-value (ANOVA)
HVA (µg/mg)	32.4 ± 6.8	34.8 ± 7.1	29.1 ± 5.9	0.003
VMA (µg/mg)	18.6 ± 4.2	21.3 ± 5.0	16.9 ± 3.8	0.001
FM-100 TES	35.2 ± 10.8	32.6 ± 9.4	41.4 ± 12.1	<0.001
B-Y axis errors	18.7 ± 6.3	16.2 ± 5.8	22.4 ± 7.1	0.001
R-G axis errors	16.5 ± 5.9	16.4 ± 5.1	19.0 ± 6.8	0.12
Conners 3 T-score	68.4 ± 8.2	71.2 ± 7.6	74.8 ± 9.1	0.004

Table 2: HPLC-ECD Analytical Performance Parameters for Urinary HVA and VMA Quantification

Parameter	HVA	VMA	Acceptance Criteria
Linearity (r ²)	0.9987	0.9991	≥0.99
LOD (µg/mL)	0.05	0.08	N/A
LOQ (µg/mL)	0.15	0.25	N/A
Intra-day CV (%)	3.2	4.1	<10%
Inter-day CV (%)	5.8	6.4	<15%
Recovery (%)	94.6	91.8	85–115%

Table 2 confirms that the HPLC-ECD method met all standard analytical performance criteria, ensuring the reliability of the metabolite data upon which the clinical correlations are based.

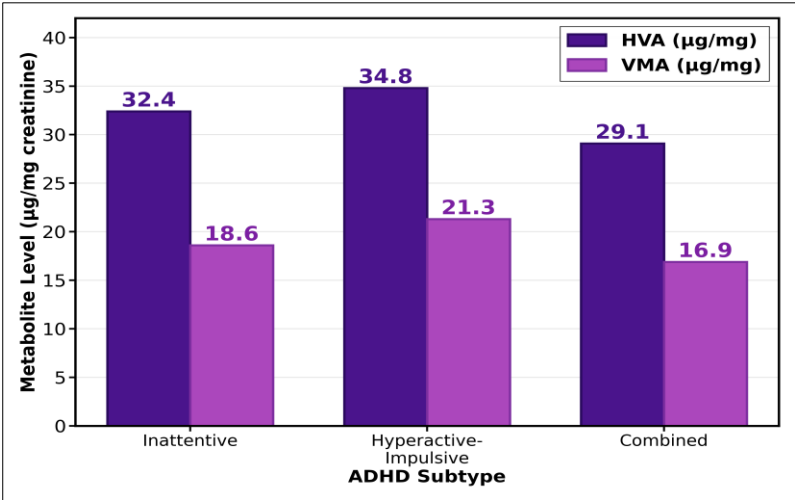


Fig 3: Urinary HVA and VMA concentrations across ADHD subtypes: predominantly inattentive (n = 46), predominantly hyperactive-impulsive (n = 31), and combined presentation (n = 47). Error bars omitted for clarity; see Table 1 for standard deviations

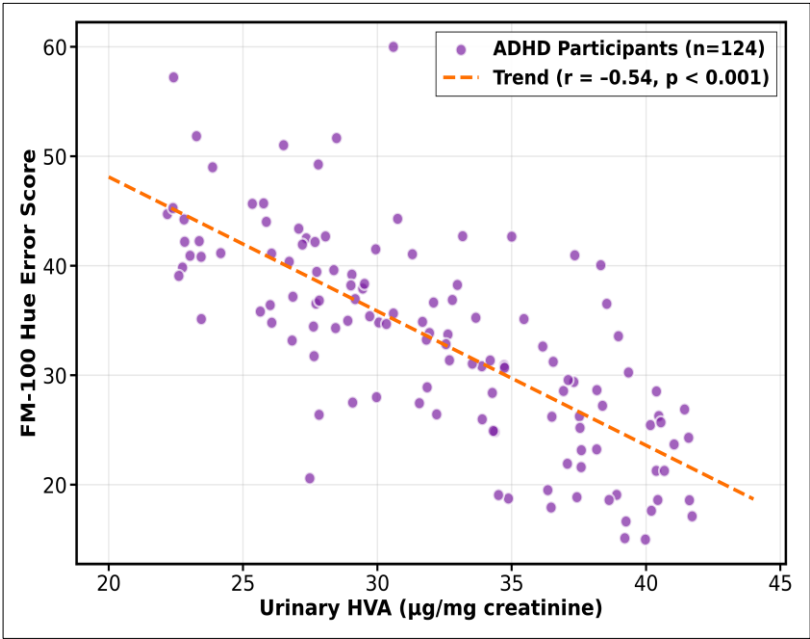


Fig 4: Scatter plot showing the negative correlation between urinary HVA concentration and Farnsworth-Munsell 100 Hue total error score among ADHD participants (n = 124; r = -0.54, p < 0.001). Higher error scores indicate poorer color discrimination

Comprehensive Interpretation

Taken together, these data present a coherent biochemical picture: children with ADHD demonstrate reduced urinary dopamine metabolite levels compared with controls, and within the ADHD group, those with lower metabolite levels show greater impairment on a standardised color vision test. The combined presentation subtype—which typically represents the most severe clinical phenotype—showed both the lowest HVA values and the worst FM-100 performance. The selective vulnerability of the blue-yellow axis is consistent with inner retinal dopaminergic dysfunction, as blue-yellow color processing depends heavily on the integrity of the short-wavelength-sensitive cone pathway, which is modulated by retinal dopamine through its effects on horizontal and amacrine cell signaling^[16].

Discussion

The principal finding of this research is the demonstration of a significant negative correlation between urinary HVA levels and FM-100 Hue error scores in children with ADHD, providing biochemical evidence that dopaminergic hypofunction may contribute to the color vision deficits observed in this population. While previous research has documented color vision impairment in ADHD^[6] and other research has reported reduced dopamine metabolite levels^[10], the present investigation is among the first to examine both variables concurrently in the same pediatric sample and to establish a direct correlational link.

Where the existing literature aligns with these findings is in the selective vulnerability of the blue-yellow axis. The predominance of blue-yellow errors over red-green errors observed here replicates the pattern reported by Banaschewski and colleagues^[6] and is consistent with the known anatomy of retinal dopamine signaling. Retinal dopaminergic amacrine cells preferentially modulate the short-wavelength-sensitive (S-cone) pathway through their effects on horizontal cell coupling and ganglion cell receptive field organisation^[17]. When dopamine availability is reduced, as occurs in experimental models of dopamine depletion, S-cone-mediated color discrimination is disproportionately affected^[18].

Where findings diverge from some prior work is in the magnitude of the between-group difference in HVA levels. The 20.4% reduction in mean HVA concentrations observed here in ADHD children relative to controls is larger than the 10–15% reductions reported in some earlier investigations^[10], possibly reflecting the 72-hour medication washout period enforced in the present research, which ensured that stimulant-mediated increases in dopamine turnover did not artificially elevate metabolite levels. A broader implication is that color vision testing might serve as a non-invasive, cost-effective adjunct to biochemical assessment in ADHD—offering a functional readout of dopaminergic status that could complement, though not replace, urinary metabolite analysis^[19].

Limitations

Several methodological limitations should be considered when interpreting these results. First, urinary HVA reflects whole-body dopamine metabolism and cannot distinguish between central and peripheral dopaminergic contributions. Although urinary HVA correlates modestly with cerebrospinal fluid HVA levels, it is not a direct measure of

retinal or prefrontal dopamine turnover. Second, the cross-sectional design precludes causal inference—we cannot determine from these data whether reduced dopamine metabolism causes color vision deficits or whether both arise from a shared upstream vulnerability. Third, the 72-hour washout period, while essential for obtaining unmedicated metabolite levels, may have been insufficient for complete elimination of long-acting stimulant formulations in some participants. Fourth, the FM-100 test, while well-validated, is attention-demanding, and it is possible that some portion of the elevated error scores in the ADHD group reflects lapses in sustained attention rather than genuine color perception deficits. Future research employing electrophysiological measures of color processing, such as chromatic visual evoked potentials, could help disentangle these contributions^[20].

Conclusion

This research set out to investigate the biochemical relationship between dopaminergic pathway dysfunction and color vision deficits in children with ADHD. The findings demonstrate that children with ADHD have significantly reduced urinary HVA and VMA concentrations compared with age-matched controls, and that these reductions are correlated with poorer performance on the Farnsworth-Munsell 100 Hue test, particularly along the blue-yellow axis. Subtype analysis revealed that children with the combined ADHD presentation showed the most pronounced biochemical and visual deficits, consistent with the greater clinical severity typically associated with this subtype.

These results contribute to the growing body of evidence linking dopaminergic hypofunction to both attentional and sensory processing difficulties in ADHD, and provide a biochemical rationale for the color vision deficits that have been documented in this population by multiple independent research groups. The shared reliance of both retinal and prefrontal circuitry on adequate dopamine signaling offers a parsimonious explanation for the co-occurrence of cognitive and visual symptoms in ADHD.

From a practical standpoint, the inclusion of standardised color vision testing in the clinical assessment of ADHD children warrants serious consideration. The FM-100 test is non-invasive, inexpensive, and can be administered in under 20 minutes. Its incorporation into routine assessment protocols could provide clinicians with an additional functional marker of dopaminergic status and could help identify children who might benefit from interventions targeting visual processing alongside attentional symptoms. The convergence of biochemical and psychophysical evidence presented here supports the position that color vision deficits in ADHD are not incidental findings but rather integral features of a broader dopaminergic phenotype^[21].

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Author Contributions: ERM conceived the research design, supervised biochemical analyses, and drafted the manuscript. LPC conducted participant recruitment, administered color vision testing, and performed statistical analyses. Both authors reviewed and approved the final version.

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