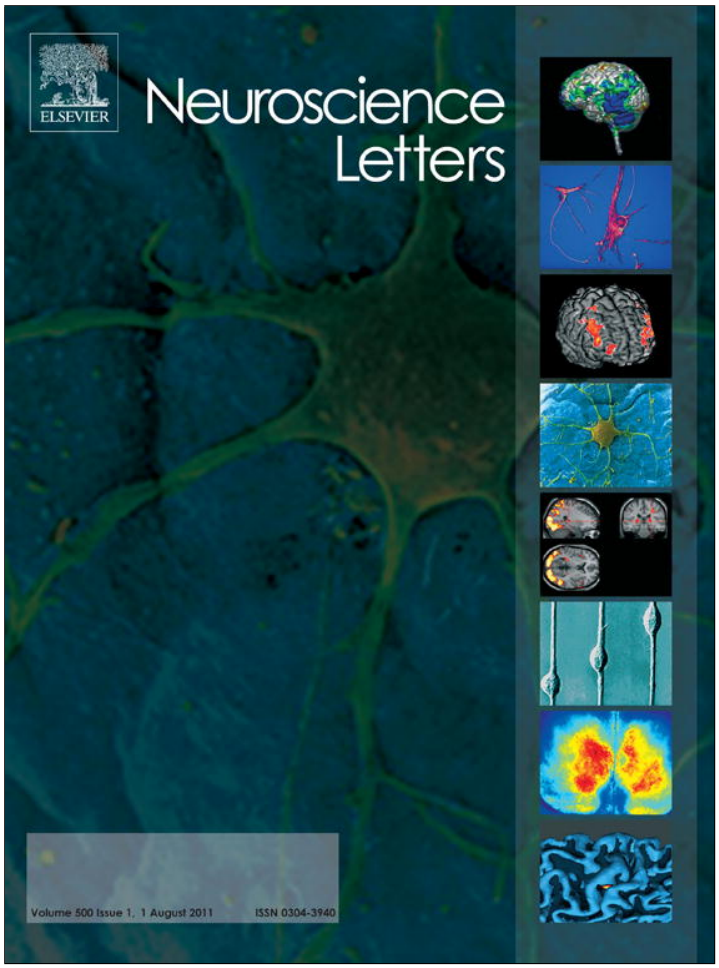

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Plenary article

The relationship between the neuromodulator adenosine and behavioral symptoms of autism

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ABSTRACT

The neuromodulator adenosine is an endogenous sleep promoter, neuroprotector and anticonvulsant, and people with autism often suffer from sleep disruption and/or seizures. We hypothesized that increasing adenosine can decrease behavioral symptoms of autism spectrum disorders, and, based on published research, specific physiological stimuli are expected to increase brain adenosine. To test the relationship between adenosine and autism, we developed a customized parent-based questionnaire to assess child participation in activities expected to influence adenosine and quantify behavioral changes following these experiences. Parents were naive to study hypotheses and all conditions were pre-assigned. Results demonstrate significantly better behavior associated with events pre-established as predicted to increase rather than decrease or have no influence on adenosine. Understanding the physiological relationship between adenosine and autism could open new therapeutic strategies – potentially preventing seizures, improving sleep, and reducing social and behavioral dysfunction.

Autism spectrum disorders (ASDs) affect as many as 1 in 110 individuals, and educational and developmental interventions demand significant public and private resources. Genes play a role [50], but, in the majority of cases, the underlying genetics are complex and involve at least 10 different genes [35], most likely interact with environmental factors [23], and are unlikely to be addressed via gene therapy in the foreseeable future. Early detection offers the potential to improve long-term outcomes, and in some cases genetic disorders – e.g., metabolic disorders such as phenylketonuria (PKU) – can be managed symptomatically with excellent results.

Neurotransmitters commonly linked to ASD include serotonin, dopamine, GABA, glutamate and acetylcholine [39], and interventions include antidepressant, antianxiety, antipsychotic and antiepileptic drugs [38]. Typically drugs target symptoms (e.g., inattention or internalizing disorders) rather than core impairments, often with significant side effects. New and better approaches are needed.

Here we explored the relationship between adenosine, a sleep-promoting, seizure-reducing neuromodulator [10], and behavioral symptoms of ASD (e.g., poor eye contact, repetitive movements). ASDs are associated with increased incidence of epilepsy and sleep disorders [30] – serious comorbidities in terms of medical management and quality of life. Adenosine is the core molecule of adenosine triphosphate (ATP), and decreased activation of the widely distributed adenosine A_1 receptor (A_1R) increases anxiety and seizure susceptibility in rodents; conversely, A_1R activation has well-established anticonvulsant and neuroprotective properties [10]. Adenosine A_{2A} receptor ($A_{2A}R$) expression is low in most areas, but high in dopamine-containing regions such as the basal ganglia; $A_{2A}R$ activation has been associated with reduced perseverative behaviors [45]. Caffeine, the most widely used psychoactive drug, is an adenosine receptor antagonist with potential for multiple neurological and psychiatric disorders [27,41]. Based on behavioral and physiological characteristics of ASD, including impaired sleep, increased seizures [5,30] and perseverative behaviors [29], an insufficient influence of adenosine may underlie some symptoms. Alternatively, even if levels are normal, increased adenosine may still be beneficial. We predict it could offer short term improve-

Abbreviations: ASD, autism spectrum disorders; A_1R, adenosine A_1 receptor; $A_{2A}R$, adenosine A_{2A} receptors.

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ments [33] with the potential to facilitate more long-term changes (e.g., decreased motor stereotypies might facilitate pro-social interactions).

It is impractical to measure CNS adenosine in humans, and peripheral adenosine levels (e.g., in plasma) are not informative. Nevertheless, basic and translational research has demonstrated that mechanical pressure or sudden physical impact [18], seizures [48], intense exercise [12], increased temperature (by 2 °C) [32], decreased pH (by 0.3 units) [8,9] and reduced glucose [26] all serve to increase brain adenosine directly or indirectly (via ATP dephosphorylation) within minutes, and lasting up to hours. Recent publications suggest that a ketogenic diet, an effective treatment for pediatric epilepsy and reported to improve autism [15] can act via A_1Rs [34].

In parallel, many repetitive and self-stimulatory activities characteristic of autism would be predicted to release ATP and/or adenosine. For example, rocking, spinning and Grandin's "hug machine" [13] exert mechanical pressure or induce sudden changes in acceleration, and thus may exert their effects through increased extracellular adenosine, similar to local increases via impact or acupuncture [19,21]. Conversely, basic research indicates that hyperglycemia and increased pH each could decrease adenosine acutely [8,9,40]. Because adenosine is uniquely poised to link metabolism and brain activity, we speculate that some behaviors which increase adenosine could represent metabolic reinforcement – a form of self-medication by attempting to self-regulate neuronal excitability through increased adenosine. Engaging in adenosine-increasing activities could thus lead immediately to decreased symptoms of autism.

To explore the possibility of a beneficial relationship between autism and adenosine – as suggested by animal models – we administered a customized behavioral questionnaire to parents of children with a confirmed ASD diagnosis via a validated national autism database. Questions were designed to examine behavioral changes following activities identified (based on research described above) as expected to increase, decrease or have no effect on adenosine. The present report reveals a significant relationship between engaging in stimuli preassigned as expected to increase adenosine and parent report of decreased severity of ASD symptoms.

Subjects were recruited with the assistance of the Interactive Autism Network (IAN) Research Database at the Kennedy Krieger Institute and Johns Hopkins Medicine – Baltimore, sponsored by the Autism Speaks Foundation. Autism diagnostic status was confirmed by IAN staff; prior work has shown that fully 98% of participants ascertained as on the spectrum according to IAN standard phenotyping procedures were ASD-positive according to clinicians' best estimate [28]. Participants had received a formal autism diagnosis according to DSM-IV criteria made by a qualified professional (e.g., psychologist, psychiatrist) and were above a threshold score of 12 on the Social Communication Questionnaire (SCQ) parent-report measure [42].

2000 parents of a child with a diagnosed ASD were invited to complete an internet-based questionnaire. 201 parents began and 191 (83%) completed it; after excluding participants over age 18, there were data for 166 children. Overall responses on 166 completed questionnaires contained information sufficient to generate scores for adenosine-increasing or non-increasing activities. Comparisons of available data indicated no significant group differences between those who did and did not complete it.

Enrollees ranged from 2.4 to 18 years, with a mean age of 9.5 years (SD = 3.8); there was a typical gender distribution of 138 (83%) males and 28 (17%) females. The autism diagnoses as identified by parents included Autistic Disorder ($n = 101$), Asperger Syndrome ($n = 32$), and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS; $n = 33$). Parents described their child's verbal level as nonverbal ($n = 18$), using single words ($n = 12$), using phrases ($n = 46$), or fully fluent ($n = 84$).

The customized questionnaire included categories with analogous adenosine-increasing or -neutral/decreasing conditions based on published basic and translational research; examples of probed activities are listed in Table 1. Adenosine-stimulating activities included high-intensity physical conditions, raising body temperature, and adhering to a low-carbohydrate (ketogenic) diet. Adenosine-neutral or -decreasing activities included consuming foods likely to lead to a state of hyperglycemia, gentle exercise, breaking a bone, or watching television. Caffeine is an adenosine receptor antagonist, thus blocking partially the effects of endogenous adenosine; chronic caffeine exposure upregulates receptor levels. Control activities were chosen to be similarly engaging and/or pleasurable as adenosine-stimulating activities.

After an episode of participation (10 min minimum, for intense (exhaustive) activity like the trampoline), parents reported whether they observed changes in functioning (within 1 h; 12 h for medical events; not specified for ongoing diet). Overall, categories were based on adenosine research with rodent models, coupled where possible with an established metabolic equivalent (MET; defined as work metabolic rate/basal metabolic rate) in humans [1]. Adenosine levels can change within minutes, and relevant timelines/intensities were established to avoid borderline changes with criteria for physical activities of >6.0 MET for adenosine-increasing activities and <3.0 MET for adenosine-decreasing or neutral activities. Ideally we would test the impact of behavioral activities on adenosine regulation in humans; however direct physiological assessments of adenosine are not feasible – blood and urine do not reflect central adenosine, and central nervous system measurements or manipulations are overly invasive at this point. Thus, data from this parent-report questionnaire provide an initial indirect and non-invasive test of this novel hypothesis.

Using a 5-point Likert scale ("lots of decline," "some decline," "no noticeable change," "some improvement," "lots of improvement"), parents reported whether they saw changes in each of nine domains (Table 2): social and communicative skills; repetitive or perseverative activities; eye contact; social interest; sound sensitivity; sleep; anxiety; adapting to transitions; and aggressive behaviors. Parents were also invited to provide qualitative descriptive comments. Note that the questionnaire probed for changes in behavior following engagement in the probed activities; thus, results do not simply reflect the fact that children were unable to engage in symptomatic behaviors such as hand-flapping during the activity. The sets of behavioral changes probed were drawn from relatively broad domains, as the goal in the present study was to sample from a wide set of possible symptom domains relevant for ASDs. While the data are thus an initial test of this relationship, they may be helpful in elucidating symptoms and activities of interest for subsequent research.

Responses were checked to ensure consistency between quantitative data and qualitative comments. For each activity, ratings were averaged across the nine domains to give a summary score; a score of 3 would indicate no change in behavior subsequent to the activity, and scores of 1 and 5 would indicate significant decline or improvement, respectively. These average behavioral change scores were transformed into a grand mean for adenosine-increasing or non-increasing activities (Table 1). Not all participants provided information in order to generate both scores (e.g., they may not have had more than one adenosine-stimulating activity). All available data are included in analyses as indicated.

A grand mean was available for both the increasing and non-increasing categories for 155 participants. A repeated-measures ANOVA on adenosine-increasing and non-increasing activities indicated a significant effect of activity, $F_{1,158} = 108.7$, $p < 0.001$, $\eta_p^2 = 0.41$. Adenosine-increasing activities received a mean score of

Table 1
Adenosine-stimulating or -neutral/decreasing activities probed for behavioral change, and the grand average of autism-specific behavior changes, on a scale of 1 (lots of decline) to 5 (lots of improvement).

Type of event	Adenosine-stimulating	M (SD)	Adenosine-neutral/decreasing	M (SD)
Physical stimulation	Run for 15 min	3.40 (0.69)	Leisurely walk for 30 min	3.22 (0.57)
	Swim for 30 min	3.64 (0.73)		
	Swing on a swing set for 30 min	3.49 (0.62)	Watch television for 30 min	2.74 (0.56)
	Jump on a trampoline for 10 min	3.51 (0.58)		
	Ride a roller coaster	3.03 (0.74)	Ride a carousel	3.19 (0.51)
	Deep pressure massage	3.62 (0.50)		
Medical experience	Head injury	2.64 (0.60)	Break a bone	2.59 (0.67)
	Experience general anesthesia	2.49 (0.93)	Experience local anesthesia	2.59 (0.90)
Temperature change	Fever for at least 1 h	2.87 (0.93)		
	Hot tub or bath for 30 min	3.54 (0.59)		
Dietary intake	Low-carbohydrate (ketogenic) diet	3.20 (0.47)	Drink a large glass of milk	2.84 (0.55)
			Eat a very sugary dessert or candy	2.71 (0.60)
Grand mean		3.27 (0.48)		2.87 (0.34)

Table 2
Functional domains and behavioral activities probed in the questionnaire.

Nine functional domains were probed for possible behavioral change
1. Social skills/communication (including language)
2. Anxiety
3. Ability to sleep
4. Repetitive or perseverative activities (such as rocking, lining up cars, etc.)
5. Transitions
6. Sensitivity to sound
7. Eye contact
8. Interest in other people
9. Aggressive behaviors (such as hitting or kicking) towards others

3.27 (SD = 0.49), indicating significantly better behaviors relative to those observed following non-increasing behaviors, which were associated with a mean score of 2.87 (SD = 0.34).

For adenosine-stimulating behaviors comparable directly to control behaviors, similar repeated-measures ANOVA analyses were conducted. For example, a comparison of running versus walking showed a significantly different change in autistic symptomatology, $F_{1,112} = 8.98$, $p = 0.003$, $\eta_p^2 = 0.07$. Experiencing a head injury compared to a broken bone led to no significant differences in symptomatology, $F_{1,9} = 0.54$, $p = 0.48$, $\eta_p^2 = 0.05$, but few participants contributed data to this comparison, limiting the possibility of finding an effect. For children who consume caffeine occasionally, consumption of a caffeinated beverage led to a significantly greater reduction in autistic symptomatology compared to consumption by children who consume caffeine regularly, $F_{1,23} = 4.94$, $p = 0.04$, $\eta_p^2 = 0.18$.

Results did not change when chronological age was entered as a covariate, suggesting that adenosine-related functional effects were unrelated to age. Furthermore, age in months was uncorrelated with behavior change as a function of adenosine-increasing stimuli, $r_{156} = -0.11$, $p = 0.17$. Parent-reported verbal level was used as an index of severity, categorized as "low-verbal" (nonverbal/single words), "verbal" (phrase speech), or "high-verbal" (fluent speech). A repeated-measures ANOVA on adenosine-affected behaviors, grouped by verbal level, indicated that there was no adenosine-by-group interaction, $F_{3,155} = 0.44$, $p = 0.73$, though there was a main effect of verbal level, $F_{3,156} = 3.20$, $p = 0.03$. This effect was driven by the nonverbal group, which showed less improvement with adenosine-increasing stimuli than the group with fluent speech, $p = 0.008$. There were no significant group differences for boys versus girls, $F_{1,153} = 0.68$, $p = 0.41$.

Although we had no a priori assumptions about diagnosis, a between-subjects comparison indicated a significant diagnosis (autism, PDD-NOS, Asperger) by adenosine interaction, $F_{2,156} = 3.40$, $p = 0.04$, $\eta_p^2 = 0.04$. The magnitude of the change in symptomatology following adenosine-stimulating versus -neutral/decreasing behaviors differed significantly for the autistic disorder versus Asperger syndrome groups, with the latter showing a significantly larger change (0.47) than the former (0.40). The overall difference, however, was small, as was the effect size.

Data obtained from an internet-based parent questionnaire designed to explore the relationship between adenosine and autism symptomatology indicate a significant relationship between events predicted to increase adenosine and parental observations of decreased behavioral symptoms of autism. Adenosine is a neuromodulator and the core of ATP; together these purine molecules form a unique link between cell energy and neuronal excitability. While abnormalities in purine metabolism have been reported in ASD [37], dysfunctional purine metabolism or inadequate adenosine has not been linked directly to core symptoms or functional differences in brain activity.

Adenosine promotes sleep, decreases seizures and decreases anxiety – all positive physiological effects [10,25], and A_1R-based therapies have long been pursued, primarily for brain injury and epilepsy [3]. Unfortunately, targeting adenosine receptors directly can result in unacceptable peripheral side effects. Thus, strategies for behavioral or metabolic upregulation of adenosine may provide a less disruptive approach [4,33]. A recent hypothesis proposed that caffeine, an adenosine receptor antagonist with differential effects depending on acute or chronic administration, could have beneficial effects in ASD [20]. Interestingly, we revealed a significant interaction between reported caffeine use and behaviors associated with autism, supporting a link between adenosine and autism.

To our knowledge, there are neither published clinical trials of adenosine in ASD nor direct evidence regarding changes in brain adenosine associated with ASD. However, isolated reports describe changes in ASD symptoms subsequent to activities analogous to those tested in this study. Watters and Watters [47] reported decreased stereotypical self-stimulatory behaviors in boys with autism subsequent to jogging, but not after academic work or watching television, and Escalona et al. found that children who received a massage at bedtime exhibited decreased stereotypical behaviors relative to children who heard a story [14]. A recent study found decreased ASD symptoms with a fever of 100.4 °C [7], possibly reflecting the sensitive relationship between temperature and adenosine [32].

A preliminary report tested the effects of a (non-standard) ketogenic diet in children with autism and found positive results: 60% of diet-compliant children showed improvements [15]. Maintenance on a ketogenic diet suppresses seizures via A_1Rs in mice [34], and a recent retrospective study of pediatric epilepsy treated with a ketogenic diet – a subset of the children displayed autistic characteristics – provides additional evidence: a ketogenic diet decreased clinically significant behavior [43], independent of reduced seizures [44]. A ketogenic diet also improved sociability and behavior in girls with Rett syndrome [22] and in an animal model of Rett syn-

drome [31]. While we are not proposing that adenosine explains all of these findings, further research may illuminate the relationship between adenosine physiology and symptoms of autism [33]. Along these lines, recent findings that acupuncture increases local adenosine [21] and improves multiple aspects of ASD [49] also deserve further investigation.

Significant effects of adenosine-increasing activities were unrelated to age or gender, and related somewhat to functional level based on verbal level and diagnostic group. Individuals with Asperger's had more significant behavioral effects of adenosine-increasing activities, as did individuals with phrase speech (although the effect size was very small in both cases); we plan to follow up on this finding. Here, epilepsy diagnosis, sleep patterns, medication status, and other details relevant to our hypothesis were not available, and a potential general limitation was reliance on parent report. However, all parents were naïve to study hypotheses, and thus these data are more likely to reflect actual behavioral changes. Notably, our results are consistent with previously published animal and human research related to a subset of stimuli targeted herein [14,15,32,33,47].

While the difference between adenosine-stimulating and control activities was relatively small (<0.5 on a 5-point Likert scale), the effect size was large, accounting for a full 55% of variance in symptomatology, and encouraging for an initial study. The response "no change" corresponded to a score of 3, and adenosine-increasing and non-increasing activities received overall scores of 3.27 and 2.89, respectively. These scores may reflect the particular sample of parents who completed the survey, the wording of the anchor points on the scale, or a more general developmental phenomenon. The current methodology limits our ability to distinguish among these and other possibilities.

The present study relied on a within-subjects design. Because of the focus on a cohort of autism-specific behaviors it was not possible to include a group of unaffected children. Future research will require direct manipulation of confirmed adenosine-regulating behaviors; the current research is critical in establishing the potential relevance of this approach to children with autism. A positive aspect of the present approach is that some adenosine-stimulating activities, such as vigorous exercise and massage, may reduce ASD symptoms and can impact positively on physical health via other pathways. That said, our findings are unlikely to reflect very general health-related changes because behavioral changes were demonstrated across a range of categories, including less clearly health-related activities.

These findings provide initial support for the possibility that adenosine-modulating strategies may be effective for a broad range of individuals with ASD. Coupled with previous research establishing adenosine as a sleep-promoter and seizure reducer, an important cohort of specific ASD symptoms may be lessened by increasing adenosine. Several neurochemical systems are targeted pharmacologically in ASD to alleviate depression, anxiety and attentional dysregulation and seizures [38]. Reduced GABAergic inhibition is thought to be a factor [24], and decreased expression of GABA receptors [16,17] has been reported in autism, but the interaction between GABA and adenosine in autism has not been established. Nevertheless, activation of adenosine receptors can suppress neuronal hyperexcitability caused by inhibition of GABAergic systems in vitro [46] and in vivo [2,11].

Interventions that increase adenosine may be a practical and effective strategy [34,36], could alleviate symptoms impacting learning and memory (sleep disruption, inadequate REM sleep, seizures [5,6]), and could combine with existing therapies for even greater benefits. In parallel with work in animal models of autism, it is critical to explore initially the link between adenosine and autism in humans. To date there are no evidence-driven biomedical treatments for the core symptoms of ASD; the need for new therapeutic targets is great. Consistent with recent reports and predictions [33], these data support the potential therapeutic benefits of adenosine, a neuromodulator with profound effects on neuronal activity, in ASD. Presently, recent advances in our understanding of adenosine regulation, and emerging adenosine-based therapies, offer broad clinical promise and deserve serious consideration for alleviating multiple behavioral and physiological aspects of ASD.

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