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Myo-Inositol Mediates the Effects of Traffic-Related Air Pollution on Generalized Anxiety Symptoms at Age 12 Years

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Abstract

Background—Exposure to traffic-related air pollution (TRAP) has been linked to childhood anxiety symptoms. Neuroimaging in patients with anxiety disorders indicate altered neurochemistry.

Objectives—Evaluate the impact of TRAP on brain metabolism and its relation to childhood anxiety symptoms in the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS).

Methods—Adolescents (n=145) underwent magnetic resonance spectroscopy. Brain metabolites, including myo-inositol, N-acetylaspartate, creatine, choline, glutamate, glutamate plus glutamine, and glutathione were measured in the anterior cingulate cortex. Anxiety symptoms were assessed using the Spence Children's Anxiety Scale. TRAP exposure in early-life, averaged over childhood, and during the 12 months prior to imaging was estimated using a validated land use regression model. Associations between TRAP exposure, brain metabolism, and anxiety symptoms were estimated using linear regression and a bootstrapping approach for testing mediation by brain metabolite levels.

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Results—Recent exposure to high levels of TRAP was associated with significant increases in myo-inositol (β = 0.26; 95%CI 0.01, 0.51) compared to low TRAP exposure. Recent elevated TRAP exposure (β = 4.71; 95% CI 0.95, 8.45) and increased myo-inositol levels (β = 2.98; 95% CI 0.43, 5.52) were also significantly associated with increased generalized anxiety symptoms with 12% of the total effect between TRAP and generalized anxiety symptoms being mediated by myo-inositol levels.

Conclusions—This is the first study of children to utilize neuroimaging to link TRAP exposure, metabolite dysregulation in the brain, and generalized anxiety symptoms among otherwise healthy children. TRAP may elicit atypical excitatory neurotransmission and glial inflammatory responses leading to increased metabolite levels and subsequent anxiety symptoms.

Keywords

Air pollution; neuroimaging; adolescents; mental health; metabolites

1. Introduction

Exposure to air pollution is a well-recognized global health problem associated with more than 3.3 million premature deaths annually.(1) Evidence from toxicological and epidemiologic studies suggest the central nervous system is particularly vulnerable to air pollution suggesting they might have a role also in the etiology of mental disorders. (2–5) Anxiety disorders are particularly relevant as these are among the most commonly occurring class of psychiatric disorders with lifetime prevalence of 29% (6) and a pre-adolescent prevalence ranging from 3 to 41%.(7) Childhood anxiety also carries increased risk of later-life anxiety, deviant conduct, substance abuse, and suicide, the second leading cause of death among adolescents and young adults. (8–12) Previous reports in adults and the elderly have demonstrated more recent exposure to ambient fine particulate matter (PM2.5) is associated with increased anxiety symptoms. (13, 14) While cognitive deficits and externalizing behaviors have been observed among children exposed to air pollution,(15–17) its impact on mental health outcomes, and to a lesser extent anxiety, is less clear and inconsistent. Prenatal exposure to polycyclic aromatic hydrocarbons (PAHs), a component of air pollution due to the combustion of fossil fuels, has been associated with increased anxiety in children at age 6–7 years. (18) A London-based twin study did not find evidence of an association between PM2.5 and anxiety symptoms. (19) Among 344 children participating in the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS), our group has observed significant associations between early-life, childhood, and recent exposure to traffic-related air pollution (TRAP) and symptoms of anxiety at age 12 years.(20)

Potential mechanisms by which air pollution exerts neurotoxic effects have been posited and include indirect pathways via systemic inflammation, production of reactive oxygen species, and direct exposure of pollutants to the brain resulting from translocation of particles to extrapulmonary sites, including the brain via the blood stream and directly via the olfactory nerve.(21–24) Utilizing proton magnetic resonance spectroscopy (MRS), we characterized brain metabolism within the anterior cingulate cortex (ACC), a key region implicated in the neurobiology of mental health disorders and important for executive functioning and regulating emotional processing, among otherwise healthy pre-adolescents. (25–28) Given

the recently published findings by our group reporting the main effect of TRAP on anxiety symptoms (20), the primary objective of this study was to assess the mediating role of brain metabolism on the association between TRAP exposure and anxiety symptoms.

2. Methods

2.1 Study Population

Participants were enrolled in the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS), a previously described prospective birth cohort of infants born (35+ weeks of gestation) between 2001 and 2003 in the Greater Cincinnati Region.(29, 30) Briefly, children residing less than 400 meters or greater than 1500 meters from a major roadway, defined as having greater than 1,000 trucks per day, were eligible for enrollment. Enrolled children and caregivers completed demographic and clinical evaluations at ages 1, 2, 3, 4, 7, and 12 years to obtain information regarding the participant's health and general wellbeing, housing characteristics, residential history, and secondhand smoke (SHS) exposure. Parents of children enrolled provided informed consent and informed assent was obtained from all children at the age 12 year visit. The Institutional Review Boards at the University of Cincinnati and Cincinnati Children's Hospital Medical Center approved the study.

2.2 Nested Imaging Study

A nested magnetic resonance imaging (MRI) study was conducted at the age 12 year visit with the objective to examine differences in imaging outcomes among children exposed to high and low TRAP. Of the 762 participants enrolled in the CCAAPS cohort study at birth, 452 met the following eligibility criteria to participate in the nested imaging study: 1) children whose exposures to TRAP (estimated as described below) from birth through age one were in the highest and lowest quartiles, and 2) children whose birth record address was less than 400 m from a major road (as defined above). The nested study was oversampled for high TRAP exposure (n=262). Attempts were made to recruit all 452 eligible children; however, only 344 children were seen at the age 12 year visit and only 147 were eligible and interested in participating in the nested imaging study ($n_{\text{high trap}} = 81$, $n_{\text{low trap}} = 66$).

2.3 Traffic-Related Air Pollution (TRAP) Exposure Assessment

Estimates of TRAP from birth through age 12 years were derived using a previously developed and validated land-use regression (LUR) model based on 5 years of ambient air sampling for PM_{25} (PM with an aerodynamic diameter <2.5 microns) and elemental carbon (EC) conducted at 27 sampling sites from 2001–2006.(31–33) The fraction of elemental carbon attributable to traffic (ECAT), a marker of traffic-related particulate matter specifically related to diesel combustion, was determined for each sampling site based on UNMIX and positive matrix factorization (PMF) models and used to develop a land use regression (LUR) model. (31, 32, 34) For this study, we applied the LUR model to estimate time weighted ECAT exposures for each participant based on all reported home addresses where the child resided 1) at birth (early-life), 2) in the 12 months prior to the age 12 year visit (recent), and 3) an average childhood exposure from birth to the age 12 year clinical visit. Further, given the eligibility criteria for the nested MRI study, exposure to ECAT at each time point was modeled as a categorical variable (high vs. low) dichotomized at the

75th percentile of exposures estimated at each time period (early-life, recent, and average childhood EC AT).

2.4 Child-Reported Anxiety Assessment

The Spence Children's Anxiety Scale (SCAS), a 45-item measure, was provided to the children with standard instructions for completion at the age 12 year visit. Trained staff offered to read the items aloud and provide assistance when needed. (35) Responses are given on a 4-point Likert scale indicative of the frequency of a given symptom- 0= never, 1= sometimes, 2= often, 3=always. The SCAS assesses total anxiety as well as six subdomains, including panic/agoraphobia, social phobia, separation anxiety, obsessive-compulsive disorder, physical injury fears, and generalized anxiety. T-scores, with a mean of 50 and a standard deviation of 10, were generated for each subscale. Higher T-scores represent a greater degree of reported anxiety symptoms.

2.5 Magnetic Resonance Spectroscopy

During the age 12 year visit, 147 children underwent quantitative MRS of the brain using a Philips Achieva MR scanner equipped with a 32-channel head coil operating at 3 Tesla (3T). A three-dimensional (3D), high-resolution, isotropic, T1-weighted fast Fourier echo (FFE) imaging sequence was performed using 8.2 milliseconds (ms) repetition time (TR), 3.7 ms echo time (TE), 1057 ms inversion time (TI), 8 degree flip angle, sensitivity encoding factor (SENSE) of 2, contiguous slices with a 1 mm thickness, 1 mm x 1 mm voxel sizes. A single voxel point resolved spectroscopy (PRESS) sequence was conducted using a 3000 ms TR, 30 ms TE, and 128 averages with water suppression along with an embedded unsuppressed water reference series of 16 averages. A 2 × 2 × 2 cubic centimeter single voxel was prescribed midline bilaterally about the perigenual ACC within the medial frontal lobe localized from the 3D T1-FFE anatomical imaging sequence (Figure 1). The primary neurochemicals routinely detected include N-acetyl aspartate (NAA), creatine (Cr), choline (Cho), myo-inositol (mI), glutamate (Glu), glutamate and glutamine (GLX) with the addition of glutathione (GSH).

The raw spectra were imported for quantitative spectral processing into LCModel commercial software.(36). The raw metabolite levels were adjusted for the tissue contributions from gray matter, white matter and cerebrospinal fluid (CSF) using FSL (37, 38), adjusted to the T1 and T2 relaxation decay rate corrected water concentration, and corrected for literature reported T1 and T2 relaxation decay rates of the primary metabolites (NAA, Cr, mI).(39–41) However, secondary metabolite concentrations (Glu, GLX, and GSH) were unadjusted for T1 and T2 relaxation decay. (42) MRS data from two participants was not incorporated into this analysis due to an incomplete MRI examination and poor spectral quality.

2.6 Statistical Analyses

Descriptive statistics were used to examine child, maternal, and household characteristics at enrollment, age 12, and the subset of participants who completed the MRI study. Differences between those in the nested MRI study with adequate MRS data (n=145) and those not

partaking in the nested MRI study at age 12 years (n=199) were compared using chi-square and T-tests, as appropriate.

The statistical approach consisted of four planned (a *priori)* phases and a series of linear regression models. First, linear regression models were used to confirm the relationship between ECAT exposure at three time points (early-life, average childhood, and recent) and each child-reported anxiety scale, modeled separately (Table S1). We focused on the following anxiety subscales significantly associated with ECAT exposure in our larger cohort analysis of 344 CCAAPS participants: generalized anxiety, total anxiety, social phobia, and panic/agoraphobia ($Y=\alpha_1 + cE+e_1$).(20) Second, we assessed the association between brain metabolite levels (modeled individually) and child-reported anxiety symptoms focusing on anxiety scales shown to be associated with ECAT in the preceding linear regression models ($Y= \alpha_3 + c'E+ bM+e_3$). In this phase, we adjusted for multiple comparisons using the false discovery rate of 0.05. Third, focusing on the ECAT exposures and metabolites found to be significantly associated with the previously listed anxiety subscales, we determined the effect of ECAT exposure (early-life, recent, and/or average childhood exposures) on brain metabolite levels (i.e. potential mediators) ($M= \alpha 2 + aE + e2$). Lastly, we formally tested the potential mediation effect on only those brain metabolites shown to be associated with both ECAT and anxiety scales. The indirect effect of the metabolites was estimated with the product of the coefficients a and b (a*b). Bootstrapping was used to estimate the 95% confidence intervals for the indirect effects through repeated sampling of the data (n=1000); 95% confidence intervals not containing zero were considered significant. (43)

Covariates included in all models were selected based on prior literature demonstrating their relationship with neurobehavioral outcomes or their potential role as a confounder in the relationship between TRAP exposure and neurobehavior. Covariates included child's race (black/non-black), household income, maternal age at study enrollment, caregiver depression assessed by the Beck Depression Inventory, relational frustration pertaining to the parent-child relationship (assessed by the Parent Relationship Questionnaire), and serum cotinine levels. (44–51) All covariates were assessed at the age 12 year visit with the exception of maternal age and child's race. Covariates included in the final adjusted models were either significantly ($p < 0.05$) associated with the outcome or their inclusion resulted in a > 10% change in the ECAT parameter estimate. Current use of anxiety and/or depression medication was considered as a potential covariate given that it might impact brain metabolite levels; however, less than 4% (3.4 % for anxiety medications, 1.36% for depression medications) of the CCAAPS nested MRI participants were currently (at the age 12 year visit) taking medication and thus we did not include current medication use in the statistical models. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

3. Results

3.1 Characteristics of Participants

Comparisons between demographic, exposure, and other characteristics between CCAAPS participants at enrollment (n = 762), those completing the age 12 year visit (n = 344), and

those with MRS (n = 145) are provided in Table 1. Children included in this analysis were, on average, 12.2 years old when completing the visit, the majority were white (73.8%, n = 107), and 41.4% (n=60) were female. On average, mothers were 29.7 years old at the time of birth and 33% (n=48) of families had a total household income of greater than $90,000 (Table 1).

The average (SD) ECAT concentrations during early-life [0.43 (0.18) μg/m^3], at the time of the age 12 year visit [0.38 (0.13) μg/m^3], and throughout childhood [0.39 (0.13) μg/m^3] can be found in Table 1. Participants in the nested MRI study had higher early-life exposure to ECAT likely due to the eligibility criteria to participate in the MRI sub-study. The majority of participants (n=102, 70%) did not change exposure categories over the 12 years of follow-up with half (n=22) of the remaining participants changing from high (early-life) to low (average childhood or recent) ECAT exposure over the 12 year follow-up. Child-reported anxiety symptoms demonstrate typical distribution patterns with mean T-scores ranging from 48.8 of 50.4 (Table 1). The analytic sample size for adjusted models was 127 due to missing covariate data.

3.2 Exposure to TRAP and Anxiety Symptoms

Children exposed to high ECAT levels at the time of the age 12 year visit (recent exposure) reported significantly more generalized anxiety symptoms (β = 4.71; 95% CI 0.95, 8.45) compared to children exposed to low ECAT. Early-life, childhood average, and recent ECAT exposures were not significantly associated with the remaining anxiety subscales in our nested population (Table S1); however, the direction of effect for other ECAT exposure periods and anxiety subscales was similar to that observed in our larger (n=344) cohort analysis. (20) Further, we conducted a sensitivity analysis on just those participants that did not change exposure categories over the 12 year follow-up (n=93). This resulted in a similar magnitude and direction of effect but only a marginally significant result (p=0.15). Given that the objective of this manuscript is to investigate mediation, the remainder of our analyses focuses solely on recent ECAT exposure and generalized anxiety symptoms using our full analytic sample (n=127).

3.3 Relationship between Brain Metabolites and Generalized Anxiety

Using FDR correction (Benjamini-Hochberg) for 7 tests, we observed statistically significant (FDR = 05) associations between myo-inositol and generalized anxiety symptoms. Specifically, children reporting a greater degree of generalized anxiety symptoms exhibited elevated ml levels (β = 2.98; 95% CI 0.43, 5.52) (Figure 2). The remaining metabolites were not significantly associated with generalized anxiety symptoms (Table 2) nor were myo-inositol levels associated with other anxiety outcomes (Table S2). Thus, the remainder of our analyses focuses solely on recent ECAT exposure, myo-inositol levels, and generalized anxiety symptoms.

3.4 Exposure to TRAP and Brain Metabolites and the Mediating Role of Myo-Inositol

In the ACC, elevated recent ECAT exposure was significantly associated with increased mI (β = 0.26; 95% CI 0.01, 0.51) compared to children with low ECAT exposure (Table S3). In combination with a series of linear regression models outline above and bootstrapping, we

identified significant indirect effects operating through mI levels (β = 0.57; 95% CI 0.55, 0.60). Specifically, 12% of the total effect (β = 4.78; 95% CI 4.66, 4.89) between recent ECAT exposure and generalized anxiety symptoms can be explained by mI levels (Figure 3).

4. Discussion

Adolescents with elevated TRAP exposures in the previous 12 months demonstrate altered brain metabolism with increased mI concentrations measured within the ACC. They also demonstrate greater generalized anxiety symptoms accompanied by elevated mI. The mediation analysis suggests that 12% of the total effect between TRAP and generalized anxiety symptoms can be explained by mI levels. These observed metabolic alterations associated with TRAP may behaviorally manifest in adolescence with increased anxiety symptoms arising as a result of altered neurochemistry.

Neuroinflammation has been posited as a potential mechanism for TRAP-induced neurotoxicity.(52) Systemic inflammatory responses and/or ultrafine particles that reach the brain and result in neurodevelopmental delays are suspected to trigger neuroinflammation with brain damage exerted through many possible mechanisms including oxidative stress, mitochondrial dysfunction and glutamatergic excitotoxicity. (53–55) Specifically, the metals and hydrocarbons that contribute to TRAP's composition have been shown to exert neuroinflammatory effects with diffuse vascular changes, increased pro-inflammatory cytokine levels [interleukin-lbeta (IL-1β), IL-6, tumor necrosis factor alpha (TNFa)], activation of astrocytes and microglia, and neurite atrophy within the brain.(53, 56–63)

Our study reports three main findings in support of the neuroinflammatory hypothesis. First, we observed increased myo-inositol levels to be associated with elevated recent TRAP exposure. This is an important finding as myo-inositol has links to neuroinflammation and is an important metabolite for many brain processes. Inositol is a natural derivative of glucose found in cellular systems and the most abundant, stable and biologically active stereoisomer is mI.(64) A sustained supply of ml is required for the synthesis of membrane phospholipids. In humans, ml is synthesized in the kidney and brain; however, in neurons the primary source is from recycling of the phosphatidyl inositol-cycle.(65) Increases in mI levels have been described in diseases with marked astrocytic gliosis, microglial activation and brain inflammation.(66) Interestingly, mI accumulates preferentially in astrocytes, which, together with endothelial tight junctions, provide selective permeability of the blood brain barrier (BBB) ultimately increasing the presence of inflammatory markers leading to toxic elevations of intracellular calcium.(67) Further, the transient nature of mI reflects active processes, as opposed to the metabolites (such as NAA, Cr, and Cho) that reflect structural nature of neural systems, supporting our finding with recent rather than early-life and average childhood TRAP exposures.

Second, mI was associated with increased generalized anxiety symptoms (Figure 2). Although the literature is limited, human studies among adults with and without psychiatric disease provide additional support of our findings that altered neurochemistry plays an important role in sub-clinical anxiety symptoms. Modi et al. evaluated 24 healthy, educated young (23.2 + 2.2 years) adults with proton MRS of the ACC and hippocampus.(68) Within

the scanner, state anxiety was assessed; outside the scanning environment, trait anxiety levels were assessed using the State-Trait Anxiety Inventory (STAI). Within the ACC, significantly higher mI/Cr and GLX/Cr levels were found among the high anxiety group (STAI total trait score above 70) compared to the low anxiety group, without any group differences for Cr concentrations. Further, many *in vivo* studies of psychiatric disease states and cognitively normal adults report associations between brain mI levels and markers of inflammation such as plasma, CSF and serum C-reactive protein (CRP) levels.(69, 70) These findings suggest that mI may be related to the severity of anxiety symptoms and raise the possibility that dysregulation of mI within the ACC may be linked to neuroinflammation and ultimately the pathophysiology of childhood anxiety disorders even at a sub-clinical level.

Third, we observed that the effect of TRAP on generalized anxiety was partially mediated through mI levels. Independently, TRAP exposure has been associated with adverse effects on cognitive, behavior, and psychomotor development in children and changes in brain structure and function.(71) Guxens et al. observed that prenatal fine particle exposure was related to alterations in the cerebral cortex of children, and these changes partially mediated the association between fine particle exposure and impaired cognitive function.(72) It is important to keep in mind that the majority of children in our study report normal levels of anxiety symptoms for this age group (i.e. otherwise healthy children). However, it is possible that the small increases in generalized anxiety symptoms paired with ongoing physical, biological, and/or psychological factors might eventually lead to more clinically relevant anxiety symptomology. Given that generalized anxiety disorders are one of the most frequent of all psychiatric disorders seen in primary care, (73) the findings of our study are noteworthy and warrant further investigation as to whether the detected neurochemistry changes resulting from TRAP exposure are causative or the result of distinct anxiety endophenotypes. Nonetheless, to our knowledge, this is the first study to demonstrate through mediation a potential pathway linking TRAP exposure, mI dysregulation in the brain, and generalized anxiety symptoms.

The strengths of our study include a lifetime collection of address history for the estimation of TRAP exposures, a comprehensive assessment of covariates and potential confounders (i.e., SES factors, biomarkers of tobacco smoke exposure, maternal psychological functioning, and details on the parent-child relationship), a thorough assessment of anxiety, and the implementation of MRS. However, we also note limitations. We sampled only one location with proton MRS, the perigenual ACC. This approach allowed for more rigorous quantification, however it reduces comparisons of our results with others along with the functional outcomes localized to other brain regions. For instance, a study of children ages 8 to 12 years in Barcelona did not reveal any association between measures of air pollution, as reflected by elemental carbon and NO_2, and brain metabolite levels in the frontal white matter (74). This may reflect the different nature of air pollutants evaluated as well as differences in MRS technique and brain region sampled. In the current study, we selected the ACC due to the significance in cognition and behavioral regulation and previous reports of deposition of TRAP within cortical regions.(53, 75) Further, while we recognize their importance, we were unable to control for other potential residual confounders such as noise from traffic or psychosocial stress resulting from neighborhood conditions. Lastly, the main effect of recent TRAP on generalized anxiety was the only significant finding we were able

to replicate among the participants in the nested MRI study and thus the only relationship we could formally test for mediation by brain metabolite levels. The reasons for this are likely multifactorial including reduced power to detect significant results due to a smaller sample size, differences observed in early-life TRAP exposures between the parent and nested study (Table 1), and the nature of myo-inositol and its role in responding to recent exposures and regulating active processes likely associated with current anxiety symptoms.

5. Conclusions

High levels of TRAP exposure are associated with increased mI in the ACC, which in turn is associated with a greater degree of generalized anxiety symptoms. However, we are unable to determine if the finding is a direct result of TRAP constituents depositing within the brain, specifically the ACC. Thus, further studies are needed to determine the mechanism of action for TRAP neurotoxicity (direct/indirect) and whether a change in myo-inositol levels can behaviorally manifest as symptoms of anxiety disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

TRAP	traffic-related air pollution
CCAAPS	Cincinnati Childhood Allergy and Air Pollution Study
MRS	magnetic resonance spectroscopy
PM	particulate matter
UFPs	ultrafine particles
MRI	magnetic resonance imaging
ACC	anterior cingulate cortex
SHS	secondhand smoke
LUR	land-use regression
ECAT	Elemental carbon attributable to traffic

SCAS	Spence Children's Anxiety Scale
3T	3 Tesla
3D	three-dimensional
FFE	weighted fast Fourier echo
ms	milliseconds
TR	repetition time
TE	echo time
TI	inversion time
SENSE	sensitivity encoding factor
PRESS	point resolved spectroscopy
NAA	N-acetyl aspartate
Cr	creatine
Cho	choline
ml	myo-inositol
Glu	glutamate
GLX	glutamate and glutamine
GSH	glutathione
CSF	cerebrospinal fluid
PRQ	Parenting Relationship Questionnaire
PI	phosphatidyl inositol
nPM	nanoscale particulate matter
CRP	C-reactive protein
BBB	blood brain barrier
IL-1β	interleukin-lbeta
TNFa	tumor necrosis factor alpha
GAD	generalized anxiety disorders
IFN	interferon
STAI	State-Trait Anxiety Inventory

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Highlights

- Magnetic resonance spectroscopy (MRS) was used to investigate the effects of traffic-related air pollution on brain metabolism and generalized anxiety.
- Recent exposure to traffic-related air pollution was associated with increases in myo-inositol; increases in myo-inositol were also associated with increased generalized anxiety symptoms.
- These findings suggest traffic pollution may elicit a change in neurochemistry, consistent with neuroinflammation, resulting in increases in anxiety symptoms.

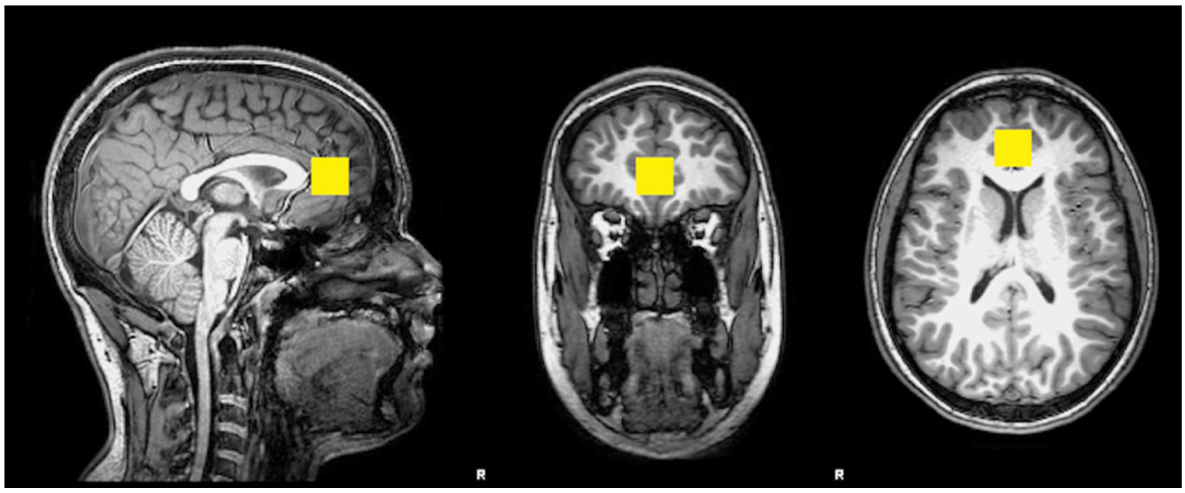

Figure 1.
Representative location within the perigenual anterior cingulate cortex for the 8 cubic centimeter (2 cm per side) spectroscopic voxel positioned on T1 weighted imaging slices centered in the sagittal, coronal and axial plane orientations.

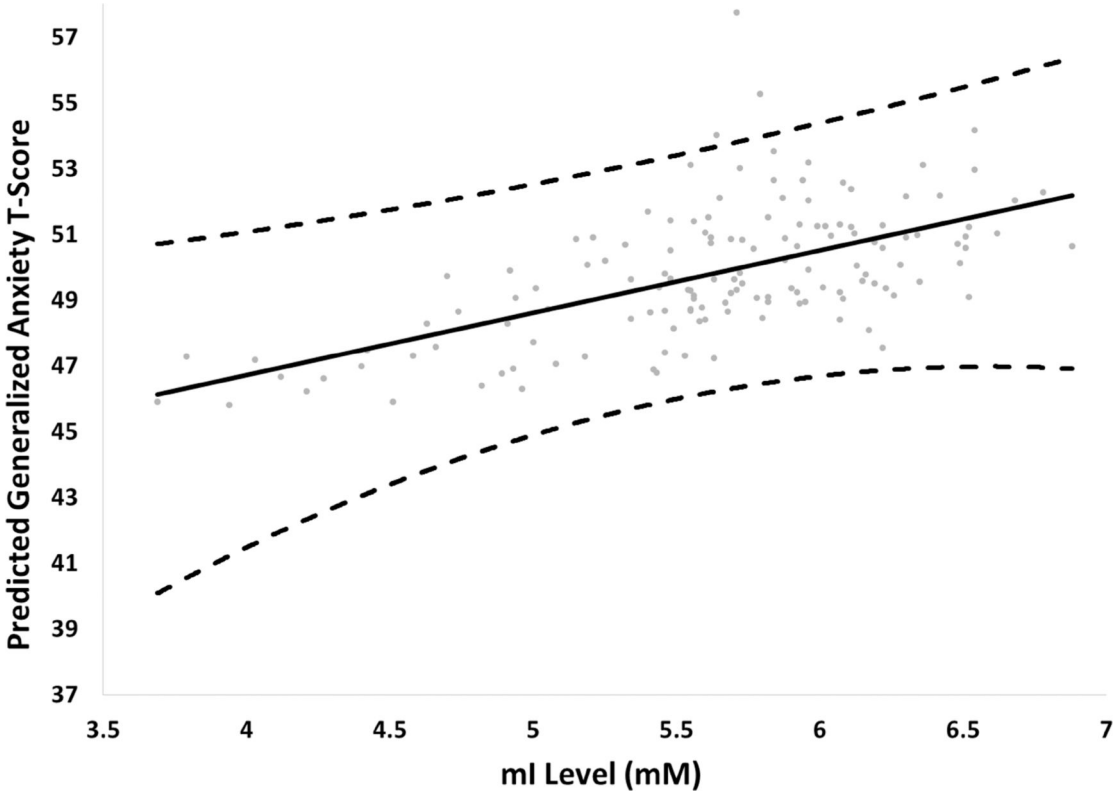

Figure 2. Relationship between myo-inositol (mI) levels and child-reported generalized anxiety T-scores.

Analysis is adjusted for child's race, total family income (yr. 12), maternal age at enrollment, maternal depression (yr. 12), serum cotinine (yr. 12), and PRQ relational frustration (yr. 12). Dashed lines represent the 95% confidence intervals.

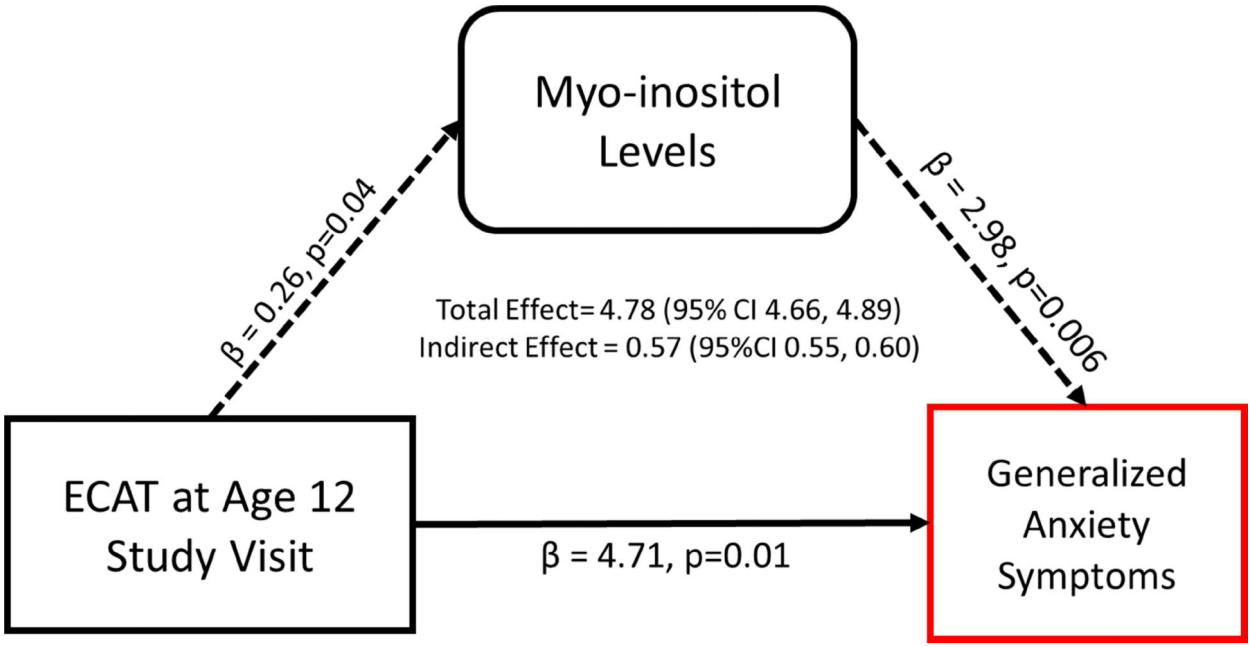

Figure 3. Pathway linking recent ECAT exposure to generalized anxiety symptoms.

All models are adjusted for child's race, total family income (yr. 12), maternal age at enrollment, maternal depression (yr. 12), serum cotinine (yr. 12), and PRQ relational frustration (yr. 12). Coefficients for each path are based on individual linear regression models. Bootstrapping was used to provide confidence intervals for testing indirect effects.

Table 1.

Comparison of characteristics in the CCAAPS cohort at enrollment, year 12 visit, and the nested MRI study

Child Characteristics	**Enrollment (n = 762)** *n (%) or Mean (SD)*	**Year 12 Study Visit (n=344)**[a] *n (%) or Mean (SD)*	**Nested CCAAPS MRI study (n=145)** *n (%) or Mean (SD)*	*p-value*[c]
Sex				
Male	415 (54.5%)	191 (55.5%)	85 (58.6%)	0.33
Female	347 (45.5%)	153 (44.5%)	60 (41.4%)	
Race/Ethnicity[b]				
White	587 (77.0%)	261 (75.9%)	107 (73.8%)	0.44
Black/More than One Race	175 (23.0%)	83 (24.1%)	38 (26.2%)	
Birth Weight (lbs)	7.5 (1.2)	7.6 (1.2)	7.5 (1.2)	0.55
Duration of Breastfeeding (months)	5.7 (65)	6.3 (6.9)	5.7 (7.0)	0.13
Serum Cotinine (age 12 yr.) in ng/ml	--	0.32 (1.33)	0.45 (1.86)	0.41
Child-Reported SCAS T-scores				
Generalized Anxiety T-Score	--	48.8 (8.4)	49.6 (9.1)	0.57
Total Anxiety T-Score	--	48.4 (9.7)	48.9 (9.8)	0.79
Obsessive Compulsive T-Score	--	50.3 (9.5)	50.4 (9.9)	0.82
Panic/Agoraphobia T-Score	--	48.8 (9.1)	48.8 (8.9)	0.98
Social Phobia T-Score	--	49.3 (9.1)	50.2 (9.0)	0.65
Caregiver Characteristics				
Caregiver Depression using BDI-II	--	6.4 (4.0)	6.9 (6.5)	0.31
Age at Enrollment in Years	30.0 (5.7)	30.7 (5.9)	30.3 (5.6)	0.22
Education at Age 1				
≤ High School	185 (24.9%)	72 (21.6%)	36 (25.7%)	
Some College or Trade School	196 (26.4%)	94 (28.1%)	35 (25.0%)	0.24
≥ College Degree	361 (48.7%)	168 (50.3%)	63 (49.3%)	
Relational Frustration T-Score		48.2 (9.5)	48.8 (9.7)	0.53
Household Characteristics				
Household Income (Baseline Study Visit)				0.28
< $20,000	129 (17.5%)	58 (17.4%)	30 (21.4%)	
$20,000 to < $40,000	129 (17.5%)	54 (16.2%)	22 (15.7%)	
$40,000 to < $70,000	210 (28.5%)	96 (28.8%)	38 (27.1%)	
$70,000 to < $90,000	196 (26.6%)	89 (26.7%)	38 (27.1%)	
> $90,000	73 (9.9%)	36 (10.8%)	12 (8.6%)	0.46
ECAT at Age 12 y Visit in μg / m^3		0.37 (0.12)	0.38 (0.13)	0.41
Early-Life ECAT in μg / m^3	0.39 (0.13)	0.39 (0.14)	0.43 (0.18)	< 0.01
Childhood Average ECAT in μg / m^3		0.38 (0.10)	0.39 (0.13)	0.36
Brain Metabolite Levels in millimolar (mM)				
mI	--	--	5.63 (0.63)	
NAA	--	--	8.38 (0.92)	
Cr	--	--	7.72 (0.57)	

Child Characteristics	**Enrollment (n = 762)** *n (%) or Mean (SD)*	**Year 12 Study Visit (n=344)**[a] *n (%) or Mean (SD)*	**Nested CCAAPS MRI study (n=145)** *n (%) or Mean (SD)*	*p-value*[c]
Cho	--	--	1.61 (0.18)	
Glu	--	--	8.78 (0.73)	
GLX	--	--	11.18 (1.41)	
GSH	--	--	2.44 (0.35)	

[a]Due to missing data, the number of children with serum cotinine at age 12 yr. visit (n=300) and nested sample (n=130), Generalized Anxiety T-scores (n=339), and caregivers with Depression BDI scores at age 12 yr. visit and the nested study (n=334 and 142, respectively) are decreased

[b]Reported at study enrollment

[c]Differences in means and proportions were tested using T-tests and Chi-square statistics, respectively, to test for differences in 145 MRI participants at age 12 compared to 199 non-participants. Pearson correlations between early-life, childhood average, and recent ECAT levels were 0.54 (early-life and recent), 0.82 (childhood average and recent), and 0.83 (early-life and childhood average).

Abbreviations: N-acetyl aspartate (NAA), creatine (Cr), choline (Cho), myo-inositol (ml), glutamate (Glu), glutamate and glutamine (GLX) with the addition of glutathione (GSH); standard deviation (SD); elemental carbon attributable to traffic (ECAT); Spence Children's Anxiety Scale (SCAS).

Table 2.

Effect of Brain Metabolite Levels on Child-Reported Generalized Anxiety Symptoms[a]

Metabolite (Predictor)	ß	95% CI	P-value
mI	2.98	0.43, 5.52	**0.006**
NAA	0.56	−1.21, 2.32	0.54
Cr	0.67	−2.13, 3.47	0.64
Cho	1.91	−7.86, 11.67	0.71
Glu	1.54	−0.65, 3.74	0.17
Glx	−0.31	−1.42, 0.79	0.58
GSH	0.47	−4.02, 4.97	0.83

[a]Models are adjusted for child's race, total family income (yr. 12), maternal age at enrollment, maternal depression (yr. 12), serum cotinine (yr. 12), and PRQ relational frustration (yr. 12); β represents the estimate for each metabolite (n=127)