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The Worsening Trajectory Of Social Impairment In Preterm Born Young Adults And Its Association With Altered Amygdalar Functional Connectivity

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THE WORSENING TRAJECTORY OF SOCIAL IMPAIRMENT IN PRETERM BORN YOUNG ADULTS AND ITS ASSOCIATION WITH ALTERED AMYGDALAR FUNCTIONAL CONNECTIVITY

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Survivors of preterm birth experience long-lasting behavioral problems characterized by increased risk of depression, anxiety, and impaired social functioning. The amygdala is a key region for social functioning, and alterations in amygdalar structure and connectivity are thought to underlie social functioning deficits in many disorders, including preterm birth. However, the trajectory of social impairments in PT and their association with functional connectivity of the amygdala are not well-studied in former preterm born individuals (PTs).

It was hypothesized that PTs would show impaired social functioning compared to term controls beginning in early childhood and continuing to young adulthood. It was also hypothesized that amygdala resting state functional connectivity is altered in PT born young adults, and that alterations in amygdala functional connectivity would mediate increased internalizing behavior and socialization problems in PT born young adults.

In a group of former very PT infants (600 to 1250 grams birth weight) and matched term (T) controls, measures of social and emotional behavior were examined using the Child Behavior Checklist (CBCL) administered at ages 8, 12, and 16, the Youth Self Report administered at age 16, and the Vineland Adaptive Behavior Scales (VABS) administered at ages 8 and 18. Amygdalar functional connectivity was examined using resting-state functional magnetic resonance imaging at age 20.

By parent report, preterm-born children and adolescents exhibit behaviors demonstrating increased social impairment compared to their term-born peers, starting at school-age and becoming more prominent by young adulthood. PT demonstrate a worsening trajectory in CBCL Withdrawn scores from school-age to young adulthood compared to T (group*time interaction $p=0.03$), and maternal education has a protective effect on this trajectory in the PT population (withdrawn group*time interaction $p=0.01$). Furthermore, amygdalar connectivity is altered in the formerly prematurely-born, and markers of social impairment correlate negatively with altered amygdala-posterior cingulate cortex connectivity (Social competence $r=-0.37$, $p=0.03$; socialization $r=-0.42$, $p=0.01$).

As this cohort of PTs does not include individuals who suffered any form of neurologic injury, their parent-reported increase in behavioral markers of social impairment may be attributable to prematurity rather than to neurologic injury. Moreover, these data suggest that previously established social impairments in PT as compared to T worsen during the critical period of transition from school-age to adolescence and suggest a possible neural underpinning for these impairments experienced by prematurely-born individuals.

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I thank Dr. Laura Ment for her guidance and encouragement over the last four years. She has taught me much about preterm neurodevelopment, research design, and balancing a research and clinical career and I'm very grateful for her mentorship.

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Table of Frequently Used Abbreviations

PT	Preterm
T	Term
CBCL	Child Behavior Checklist
YSR	Youth Self Report
VABS	Vineland Adaptive Behavior Scales
rs-fMRI	Resting state functional magnetic resonance imaging
PCC	Posterior cingulate cortex
L-STG	Left superior temporal gyrus

Introduction[1]

Premature Birth: Overall Implications

Emerging data suggest that preterm-born children are at high risk for social impairment and emotional problems in addition to the well-established risk of neurodevelopmental handicap; however, the latter is much more well-described and remains largely the focus of counseling families about the longterm risks to prematurely born individuals.

Preterm birth is a significant global public health problem: in 2017, 9.93% of US births were preterm, with 2.76% born before 34 weeks (2). Globally, as many as 11% live births occur before 37 weeks of gestation (3, 4). In the US, the rate of PT birth increased from the 1980s through 2006 and has recently begun increasing again over the last few years (5). There are racial, ethnic, and socioeconomic disparities in rates of preterm birth, with non-Hispanic African Americans having the highest rates and even higher rates among mothers with low educational attainment (6).

The consequences of preterm birth are far-reaching and include acutely increased mortality as well as significant long-term morbidity and increased societal costs. Advances in obstetric and neonatal care have improved survival for preterm born neonates; however, these children are still at high risk for significant health problems, including physical as well as neurodevelopmental problems (6, 7). These include pulmonary and cardiovascular problems, major neurologic impairments such as cerebral palsy, cognitive impairment, and sensory impairments, and more subtle learning,

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[1] Portions of thesis text are taken from the author's published manuscript:
1. Johns CB, Lacadie C, Vohr B, Ment LR, and Scheinost D. Amygdala functional connectivity is associated with social impairments in preterm born young adults. *Neuroimage Clin.* 2018.

behavioral, and emotional problems (6, 8-10). In 2010, about 2.7% of PT survivors globally were estimated to have moderate or severe neurodevelopmental impairments, and the number of PT survivors with subtler emotional or behavioral problems is likely much higher though not well established (3).

Emotional and Social Problems in the Prematurely Born

Survivors of preterm birth experience long-lasting behavioral problems characterized by increased risk for depression, anxiety, and impairments in social functioning (8-13). Social difficulties in PT emerge in early childhood and persist into adolescence. In early childhood, PTs show increased internalizing behavior, impaired emotional regulation, and poorer peer play, and are reported by parents to have increased social problems (14-17). Specific domains in which PT commonly struggle compared to T include social withdrawal and difficulties with peers (18).

The transition to adolescence appears to be especially difficult for PTs. A recent prospective study of behavioral and emotional problems in extremely PT-born children from school-age to young adulthood showed consistent increase in emotional symptoms and peer problems in PT compared to T controls which was greater in young adulthood compared to school-age (19). This is concordant with an increased risk of bullying in PT in adolescence (20, 21). Furthermore, PTs show increased internalizing behaviors both by parent and teacher report in early adolescence (22) and fail to follow the age-related normal decline in these behaviors during the transition from adolescence to adulthood (23). It is theorized that decreased social skills in early childhood and a rise in internalizing behaviors may lead to difficult social relationships in adolescence and young adulthood in PT, which then manifests as social withdrawal (18).

Even in adulthood, PT are less extraverted, take fewer risks, and have lower self-esteem compared to their term-born peers (12, 24). Because of these impairments in social functioning, PT-born adults are less likely to maintain committed relationships or become parents (25). In addition, these symptoms have been linked to increased psychiatric morbidity in the PT population at young adulthood, including anxiety, depression, and social phobias (10, 11, 26-28). Interestingly, most of these reports are from parents or caregivers, and self-report data are rarer. However, in general, even when parents report social, emotional, and behavioral problems, PT-born adolescents do not report significant problems compared to term peers (29, 30).

Neurodevelopment in Prematurely Born Individuals

Preterm birth is associated with alterations in cortical and subcortical regional volume as well as with disruptions in neural connectivity networks that can persist into adolescence and adulthood (31-33). While some of these changes may be due to perinatal factors including procedures (34) during what would normally be a period of significant neurodevelopment while in utero (35), there is increasing evidence that pre-natal factors such as maternal stress may play a role (36, 37). While many cortical and subcortical areas may be affected by preterm birth, the limbic areas are of particular interest given their role in responding to stress and coordinating emotional responses.

The Amygdala: Function and Connectivity

A key brain region for social functioning is the amygdala (38). Lesion studies show that damage to the amygdala impairs individuals' abilities to recognize complex social emotions in facial expressions (39, 40). Amygdalar volume and functional connectivity with cortical regions correlates with social network size in young adults (41, 42), and alterations to amygdalar circuitry contribute to social processing deficits in many

disorders, such as autism spectrum and anxiety disorders (43-45). Similarly, reduced social functioning in PTs has been attributed to alterations in amygdalar structure and function (13, 46-48).

The amygdala develops early in life and exhibits some volume and connectivity changes from infancy to adulthood in typically developing individuals. The amygdala grows rapidly during infancy in healthy full-term born children and reaches its maximum volume by late school-age, with small volume changes during adolescence and adulthood (49, 50). Amygdalar functional connectivity develops similarly early in life: in healthy full-term infants, the amygdala is positively correlated with subcortical regions including the contralateral amygdala, hippocampus, insula, hypothalamus, and thalamus and negatively correlated with the prefrontal cortex, posterior cingulate cortex, and visual cortex (36, 46). In late infancy and early childhood, amygdalar-thalamic connectivity decreases and amygdalar-right ventral temporal lobe connectivity increases (51), but from early childhood to adulthood, amygdalar connectivity with subcortical regions remains largely unchanged with the exception of a few regions (52). Amygdalar connectivity with the medial prefrontal cortex increases with age beginning around age 10, whereas connectivity with a region including the insula and superior temporal sulcus as well as with the posterior cingulate cortex decreases with age after early adolescence (52). Additional subtle amygdalar connectivity changes are mediated by both post-natal factors such as parental interactions (53-55) and pre-natal factors including maternal stress (36, 37) with potential subsequent consequences for emotional and social development.

While alterations in functional connectivity for specific networks, such as language, are well characterized across development in those prematurely born (32),

functional connectivity of the amygdala in PT has been less well-studied. In PT neonates, amygdalar connectivity is decreased to frontal cortex and sub-cortical regions (36, 46) and correlates with internalizing symptoms at 2 years of age (46). In PT adults at 30 years of age, amygdalar connectivity is decreased to the right posterior cingulate cortex, left precuneus, and increased to the superior temporal sulcus (47). However, despite evidence that amygdalar connectivity in typically developing individuals exhibits changes during adolescence and young adulthood (52, 56), this age range has not been examined in previous studies of amygdalar connectivity in PTs. Together, these studies suggest the need to investigate the association between social functioning and amygdalar connectivity in PT young adults.

In this work, we examined social functioning from school age to young adulthood and amygdalar connectivity during young adulthood in a cohort of very PT and term control participants. Measures of social and emotional development were evaluated by both parent and self-report at ages 8, 12, 16 and 18. Neuropsychological scores were examined longitudinally for both PT and T. Assessment scores were then compared to amygdalar functional connectivity using resting-state functional magnetic resonance imaging between study groups at age 20, and finally, social behavior differences were correlated with alterations in the amygdala.

Specific Hypotheses and Aims

Hypotheses:

Hypothesis 1: Preterms without any history of perinatal brain injury will show significantly more internalizing behavior and social difficulties beginning at age 8 compared to term-born peers, and these difficulties will persist into young adulthood.

Hypothesis 2: Resting fMRI patterns of amygdala - cortical connectivity will differ between term and preterm born young adults.

Hypothesis 3: Alterations in functional connectivity will correlate with increased internalizing behavior and socialization problems seen in adolescents and young adults who were born preterm.

Specific Aims

Specific Aim 1: To further clarify the trajectory of internalizing behavior and social problems from school-age to young adulthood in preterms without any significant history of perinatal brain injury.

Specific Aim 2: To elucidate the development of amygdala - cortical functional connectivity in adolescents and young adults who were born preterm.

Specific Aim 3: To correlate those connectivity differences with differences in internalizing behaviors and socialization problems in children and adolescents born preterm vs. full-term.

Methods

This study was designed by Christina Johns, Laura Ment, MD, and Dustin Scheinost, PhD. The neuropsychological data and rs-fMRI data were collected as part of the follow-up MRI component of the Multicenter Randomized Indomethacin Intraventricular Hemorrhage Prevention Trial (NS27116), which was designed and led by Dr. Ment and performed at the Yale University School of Medicine in New Haven, CT, the Warren Alpert Medical School of Brown University in Providence, RI, and Maine Medical Center in Portland, ME (57, 58). The protocols for this study were reviewed and approved by institutional review boards at each study center. Children provided written assent; parent(s) or guardians provided written consent for the study. Brain scans were obtained and analyzed at the Yale University School of Medicine.

Statistical analyses of the neuropsychological data were designed by Christina Johns with the guidance of Drs. Ment and Scheinost. Analyses of the rs-fMRI data were designed by Dr. Scheinost as described in detail by him below (see *Image Parameters*, *Common Space Registration*, *Connectivity Processing, Amygdalar Seed Connectivity, and Motion Analysis* below). Rs-fMRI analysis was performed by Christina Johns, Dr. Scheinost, and Cheryl Lacadie. Connectivity and neuropsychological correlations were performed by Christina Johns.

Participants

The PT neuropsychological cohort consisted of the 437 surviving former PT participants enrolled in the follow-up MRI component of the Multicenter Randomized Indomethacin Intraventricular Hemorrhage Prevention Trial (57, 58). The PT participants all weighed between 600-1250 grams at birth. These participants were evaluated at ages 8, 12, 16 and 18 with neuropsychological testing. At each age point,

PTs were excluded from the neuropsychological analysis for any of three reasons: 1. Any evidence of perinatal brain injury, defined by intraventricular hemorrhage, low-pressure ventriculomegaly, and/or periventricular leukomalacia, 2. Incomplete demographic, WISC, or neuropsychological questionnaires, and 3. Outlier scores on any of the included neuropsychological measures. Outlier scores were defined as scores at least 3 interquartile range above the third quartile on any of the included measures.

A subset of participants recruited from the Yale site only was tested with the Youth Self Report (YSR) at age 16. Participants were excluded from analysis of this questionnaire for the same reasons as above.

Term (T) control participants were recruited at age 8 years from the local community or randomly selected from a telemarketing list and matched to the PT participants in terms of age, gender, and zip code, as a proxy for socio-economic status. Term controls participated in the 8, 12, 16, and 18-year visits.

A subset of participants from the neuropsychological cohort was recruited for MRI testing at age 20 years.

Neuropsychological Assessment

All participants were tested with the CBCL (59) at ages 8, 12, and 16 years and the VABS (60) at ages 8 and 18 years to assess social and emotional development and adaptive behavior. Participants also completed the Weschler Intelligence Scale for Children, Third Edition (WISC-III) (61) at ages 8, 12, and 16 years to assess intellectual ability, from which Full IQ (FIQ) scores were used in the analysis. A subset of participants was tested with the YSR (62) at age 16 years to assess social and emotional development from the participant's, rather than the parent's, point of view. T scores for each domain were used for the CBCL, YSR, and VABS.

The CBCL is a validated, parent/caregiver-completed questionnaire of child emotional and behavioral problems over the past 6 months. Measures of social development included in this study included scores in the following scales: Social Competence, Social Problems, Anxiety Problems, Anxious/Depressed, Withdrawn, and Affect Problems. At ages 8 and 12 years, only the Social Problems, Anxious/Depressed, and Withdrawn scales were assessed. In this questionnaire, higher scores for Social Problems, Anxiety Problems, Anxious/Depressed, Withdrawn, and Affect Problems reflect a worse level of functioning, whereas lower scores in Social Competence reflect a worse level of functioning. The Social Competence scale includes items such as participation in activities and frequency of contact with friends, and the Social Problems scale includes items such as a child's ability to get along with peers, amount of play time spent with peers of same age, and whether a child acts his/her age. The Withdrawn scale includes items such as avoiding eye contact and refusing activity, the Anxious/Depressed scale includes items such as frequency that the child's feelings are hurt, whether the child is upset by separation, and frequency of sadness. The Anxiety Problems scale assesses dependency, not sleeping alone, and number of fears. Clinical range scores for these scales are defined as being in the bottom two percentiles of T scores for Social Competence (T scores ≤ 37) and the top two percentiles for the remainder of the scales (T scores ≥ 70).

The YSR is similar to the CBCL, but is self-administered (62). Measures from this instrument included in this study include the following: Activities and Social (subscales) and Anxious/Depressed, Withdrawn, and Social Problems (syndrome scales). DSM Affective Problems and DSM Anxiety Problems scales were also included. These scales assess items similar to those assessed in the CBCL. These DSM-oriented scales are

comprised of measures consistent with DSM-5 categories (Affective Problems: dysthymia and major depressive disorder; Anxiety Problems: generalized anxiety disorder, separation anxiety, and specific phobia) as identified by experts (63). Clinical range scores on the YSR are defined as in the CBCL: for the Syndrome and DSM-oriented scales, scores ≥ 70 are in the clinical range, and for the subscales scores ≤ 31 are in the clinical range.

The VABS is a parent/caregiver-completed questionnaire that evaluates adaptive and maladaptive behavior in children. Measures of social development used from the VABS included scores in the following domains: Adaptive Behavior, Socialization, Interpersonal Relationships, Play and Leisure Time, and Coping Skills. The latter three scales are subsets of the "socialization" scale in the VABS. Items assessed in each domain include the following: Socialization – amount of time playing with peers, helping others, and sharing toys/possessions, Interpersonal Relationships – asking others to play and taking turns in activities, Play and Leisure Time – playing in games and playing with peers, and Coping Skills – controlling anger during unexpected events and cooperation with others. The Adaptive Behavior domain is a composite measure of the above domains. At age 8 years, only the Adaptive Behavior and Socialization domains were assessed. A higher score reflects a better level of function in that domain. Scores ≤70 for the Adaptive Behavior and Socialization domains and ≤10 for the Interpersonal, Play and Leisure, and Coping domains are designated as clinical range.

Image parameters

Participants were scanned in a Siemens 3T Tim Trio scanner as previously described at age 20. After a first localizing scan, a high-resolution 3D volume was collected using a magnetization prepared rapid gradient echo (MPRAGE) sequence (176

contiguous sagittal slices, slice thickness 1mm, matrix size 192×192, FoV = 256mm, TR = 2530 ms, TE = 2.77 ms, flip angle = 7°). Next, a T1-weighted anatomical scan (TR = 300 ms, TE = 2.55 ms, FoV = 220 mm, matrix size 256×256, thickness = 6 mm thick, gap = 1mm) was collected with 25 AC-PC aligned axial-oblique slices. After these structural images, acquisition of functional data began in the same slice locations as the axial-oblique T1-weighted 2D Flash image. Functional images were acquired using a T2* sensitive gradient-recalled single shot echo-planar pulse sequence (TR = 1550ms, TE = 30ms, flip angle = 80, Bandwidth = 2056 Hz/pixel, 64*64 matrix, field of view: 220mm x 220mm, interleaved acquisition). Two functional runs consisted of 190 volumes (5-minute scan length) with the first four volumes discarded to allow the magnetization to reach the steady-state.

Common Space Registration

First, anatomical images were skull stripped using FSL (https://fsl.fmrib.ox.ac.uk/fsl/) and any remaining non-brain tissue was manually removed. All further analyses were performed using BioImage Suite (64) unless otherwise specified. Anatomical images were linearly aligned to the MNI brain using a 12-parameter affine registration by maximizing the normalized mutual information between images. Next, anatomical images were non-linearly registered to an evolving group average template in an iterative fashion using a previously validated algorithm. This algorithm iterates between estimating a local transformation to align individual brains to a group average template and creating a new group average template based on the previous transformations. The local transformation was modeled using a free-form deformation parameterized by cubic B-splines. This transformation deforms an object by manipulating an underlying mesh of control points. The deformation for voxels in

between control points was interpolated using B-splines to form a continuous deformation field. Positions of control points were optimized using a conjugate gradient descent to maximize the normalized mutual information between the template and individual brains. After each iteration, the quality of the local transformation was improved by increasing the number of control points and decreasing the spacing between control points to capture a more precise alignment. A total of 5 iterations were performed with decreasing control point spacings of 15 mm, 10 mm, 5 mm, 2.5, and 1.25 mm. To help prevent local minimums during optimization, a multi-resolution approach was used with three resolution levels at each iteration. The functional data were linearly registered to the 2D Flash image. The 2D Flash image was linearly registered to the MPRAGE image. All transformation pairs were calculated independently and combined into a single transform, warping the single participant results into common space. This single transformation allows the individual participant images to be transformed to the common space with only one transformation, thereby reducing interpolation error.

Connectivity Processing

Images were slice time and motion corrected using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/). Several covariates of no interest were regressed from the data, including linear and quadratic drifts, mean cerebral-spinal-fluid (CSF) signal, mean white-matter signal, and mean gray matter signal. For additional control of possible motion-related confounds, a 24-parameter motion model (including six rigid-body motion parameters, six temporal derivatives, and these terms squared) was regressed from the data. The functional data were temporally smoothed with a Gaussian filter (approximate cutoff frequency=0.12Hz). A gray matter mask was applied to the data, so only voxels in the gray matter were used in further calculations.

Amygdalar Seed Connectivity

A seed comprised of the bilateral amygdala was defined for the connectivity analyses (shown in Figure 5) on the reference brain and transformed back (via the inverse of the transforms described above) into individual participant space. To account for possible drop-out effect and poor amygdala coverage in the fMRI scans, the overlap between the amygdala seed and individual participant space was calculated, and participants with less than 30% overlap were excluded (6 PT and 4 T were excluded from the analysis based on this). The time course of the reference region in a given participant was then computed as the average time course across all voxels in the reference region. This time course was correlated with the time course for every other voxel in gray matter to create a map of r-values, reflecting seed-to-whole-brain connectivity. These r-values were transformed to z-values using Fisher's transform, yielding a map representing the strength of correlation with the seed for each participant. Finally, the connectivity maps were smoothed with a 6 mm full width half maximum Gaussian kernel.

Motion Analysis

As group differences in motion have been shown to confound connectivity studies, we calculated the average frame-to-frame displacement for each participant's data. In line with current reports, one PT with an average frame-to-frame displacement >0.30 were removed from the analysis. We detected no significant difference between PTs and Ts (PTs: motion=-0.14±0.07; Ts: motion=0.11±0.04; $p>0.05$).

Statistical Analyses

We analyzed differences in demographic characteristics between PT and T using Fisher's exact test for categorical variables and *t* test for continuous variables. Demographic variables included gender (reported by the participant at each age point and

classified as male or female), maternal education, and race/ethnicity. Maternal education was classified in a binary fashion as less than a high school education or greater than or equal to a high school education, and race/ethnicity was classified as White or non-White.

Linear regression was used to compare neuropsychological outcomes between PTs and Ts at each age, with covariate adjustment for age at instrument administration, gender, race/ethnicity, maternal education status, instrument respondent, and full IQ. Significance was assessed at $p<0.05$.

Repeated measures ANOVA was used to analyze neuropsychological outcomes longitudinally. For these analyses, only subjects with complete testing at ages 8, 12, and 16 (for CBCL measures) and ages 8 and 18 (for VABS measures) were included. Repeated measures ANOVA was also used in a secondary, exploratory analysis to assess the effect of maternal education level on CBCL and VABS scores over time in PT individuals. For the purposes of this analysis, maternal education was classified in a binary fashion as less than a high school education or greater than or equal to a high school education. There were not enough T subjects with complete data to further stratify by maternal education. Pearson's correlation coefficients were used to assess associations of CBCL and VABS measures over time for PT participants with complete neuropsychological data at each age. There were not enough T subjects with complete neuropsychological data at all ages to perform a correlation analysis. Significance was assessed for each of these analyses at $p<0.05$.

Imaging data were analyzed using voxels *t*-tests. Significance was assessed at a cluster-level threshold of $p<0.01$ family-wise error correction for between group comparisons. All maps were corrected for multiple comparisons across gray matter using

cluster-level correction estimated via Monte Carlo simulations. AFNI's 3dClustSim (version 16.3.05 which fixed the 3dClustSim “bug”) was used to estimate a cluster size of 1701 mm^3 using 10,000 iterations, an initial p-value threshold of 0.01, the gray matter mask using in preprocessing, and smoothness values estimated from the residuals using 3dFWHMx.

Exploratory analyses were performed in the sub-cohort of imaged participants to assess the association between functional connectivity and behavior using Pearson’s correlation coefficients. This analysis was restricted to only brain regions and social behavior scores that differed significantly between PTs and Ts in the full behavioral cohort. Additionally, associations were tested within the PT and T groups separately in order to minimize bias. The significance level was $p<0.05$.

Results

Participants

The PT neuropsychological cohort consisted of the 437 surviving former PT participants enrolled in the follow-up MRI component of the Multicenter Randomized Indomethacin Intraventricular Hemorrhage Prevention Trial (57, 58). Figure 1 details the number of participants included in the analysis at each age point.

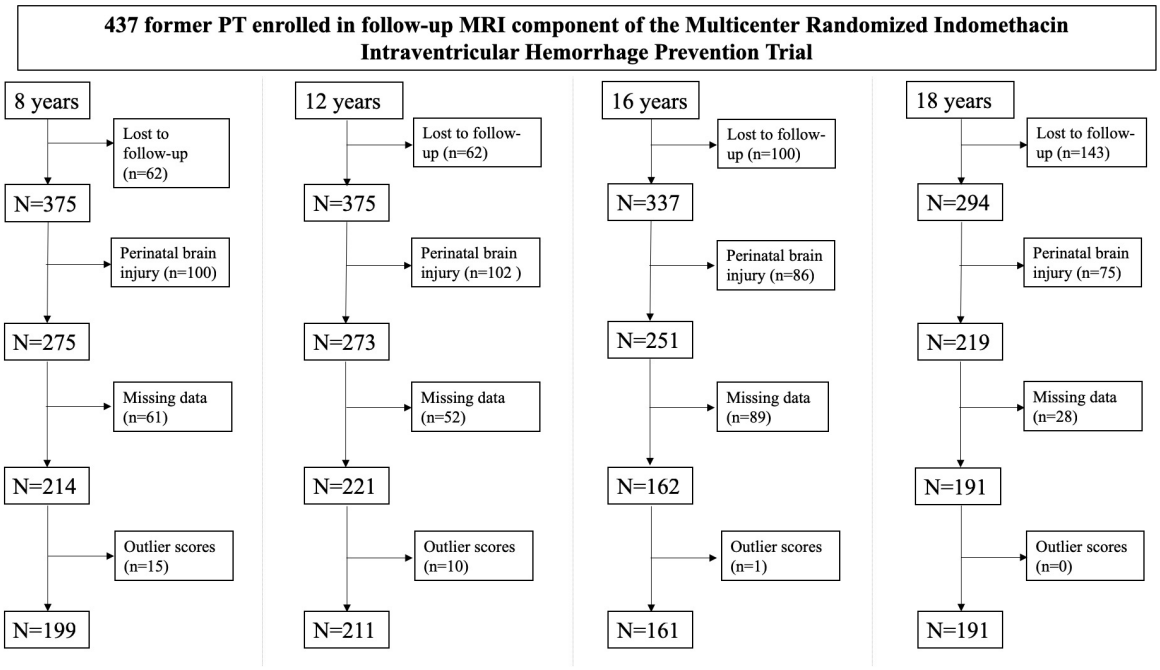

Figure 1A. Participants included in neurobehavioral analyses at each age. All participants were drawn from the 437 surviving former PT participants enrolled in the follow-up MRI component of the Multicenter Randomized Indomethacin Intraventricular Hemorrhage Prevention Trial. Questionnaire data required for inclusion were a demographic questionnaire and the WISC-III at all age points and the CBCL and VABS at age 8, CBCL at age 12, CBCL at age 16, and VABS at age 18. Outliers were defined as participants scoring at least 3 times the interquartile range above the third or below the first quartile for any of the neurobehavioral outcome measures assessed.

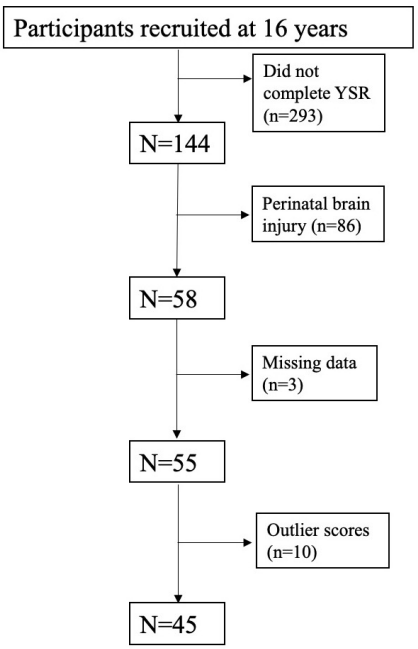

Figure 1B. Participants included in the YSR analysis at age 16 years. These participants were drawn from the 437 surviving former PT participants enrolled in the follow-up MRI component of the Multicenter Randomized Indomethacin Intraventricular Hemorrhage Prevention Trial, but were only recruited from the Yale site. Questionnaire data required for inclusion were a demographic questionnaire, the WISC-III, and the YSR. Outliers were defined as participants scoring at least 3 times the interquartile range above the third or below the first quartile for any of the neurobehavioral outcome measures assessed.

At age 8, 199 PTs were included in the analysis of Child Behavior Checklist (CBCL) and Vineland Adaptive Behavior Scales (VABS) testing. 238 participants were excluded from analysis: 62 were lost to follow-up, 100 had evidence of perinatal brain injury, and 61 were excluded due to incomplete testing on the Weschler Intelligence Scale for Children (WISC), CBCL, VABS, or demographic questionnaires. An additional 15 PTs with outlier scores on included measures in the CBCL and/or VABS were excluded from the analysis. The participants who were lost to follow-up at age 8 and who had available demographic data were similar to the included participants in gender makeup and race, but had significantly lower maternal education levels (percentage of participants with maternal education < high school: 11% in included group, 32% in lost to follow-up group, p=0.0002).

At age 12, 211 PTs were included in the CBCL analysis. 226 were excluded: 62 were lost to follow-up, 102 had evidence of perinatal brain injury, and 52 had incomplete testing on the WISC, CBCL, or demographic questionnaires. An additional 10 PTs with

outlier CBCL scores were excluded. Again, the participants who were lost to follow-up were similar in gender and race to the included participants but had significantly lower maternal education levels (percentage of participants with maternal education < high school: 9% in included group, 39% in lost to follow-up group, $p<0.0001$).

At age 16, 161 PTs were included in the analysis. 276 participants were excluded: 100 were lost to follow-up, 86 had evidence of perinatal brain injury, and 89 had incomplete testing on the WISC, CBCL or demographic questionnaires. One PT was labeled as an outlier based on CBCL scores and excluded from analysis. Participants who were lost to follow-up at the 16-year visit were similar in gender and race but had lower maternal education levels (percentage of participants with maternal education < high school: 8% in included group, 36% in lost to follow-up group, $p<0.0001$).

45 PTs (all recruited from the Yale site only) were included in the YSR analysis at age 16. From the full cohort of PT, 100 participants were excluded due to being lost to follow up, 86 had perinatal brain injury, and 193 were not tested with the YSR. An additional 3 subjects were excluded due to having incomplete WISC or demographic questionnaires. 10 subjects with outlier YSR scores were excluded from the analysis.

At age 18, 191 PTs were included in the analysis. Of the 245 participants who were excluded from analysis, 143 were lost to follow-up, 75 had evidence of perinatal brain injury, and 28 had incomplete testing on the WISC, VABS or demographic questionnaires. There were no PTs excluded due to outlier scores on the VABS. The PTs who were lost to follow-up at the 18-year visit were similar in gender makeup to the included PTs but had significantly higher proportions of minority participants (25% included, 42% lost to follow-up, $p=0.003$) and of participants with low maternal education levels (10% included, 33% lost to follow-up, $p<0.0001$).

At age 8, 25 Ts were included in the CBCL and the VABS analysis, after excluding 18 participants for incomplete questionnaires and 9 for outlier scores. At age 12, 90 Ts were included in the CBCL analysis after excluding 17 participants for incomplete questionnaires and 4 for outlier scores. At age 16, 66 Ts were included in the CBCL analysis, after excluding 27 participants for incomplete questionnaires and 9 for outlier scores. Also at age 16, 56 Ts were included in the YSR analysis, after excluding 41 participants for incomplete data and 5 for outlier scores. At age 18, 71 Ts were included in the VABS analysis, after excluding 10 participants for missing data and 14 participants for outlier scores.

PT and T participants (n=47) from the neuropsychological cohort were recruited for the MRI study at age 20 years. In total, 17 Ts and 19 PTs, all with complete neuropsychological data at ages 16 and 18, met data quality criteria (described in Methods above) and were included in the imaging portion of the study.

Demographic Characteristics

Demographic data for the PTs and Ts for the 8, 12, 16, 18 and 20-year visits are shown in Table 1. The PTs and Ts included in all age cohorts were similar in gender makeup, race, and maternal education level. At ages 8, 12, and 16, there was a statistically significant difference in age between PTs and Ts at time of neuropsychological testing, likely due to consistent recruitment efforts for PTs for each visit around the time of their birthday, whereas Ts were recruited at any point during that year and therefore demonstrated increased age spread. Although this age difference may be clinically significant at age 8, it likely becomes clinically insignificant as the participants aged. There was no significant difference in the age at scan for PTs and Ts included in the imaged sub-cohort.

TABLE 1. Demographic data for study participants in the neuropsychological cohorts

	Neuropsychological cohort					
	8 year - CBCL and VABS			**12 year - CBCL**		
	T (n=25)	**PT (n=199)**	**p**	**T (n=90)**	**PT (n=211)**	**p**
Female n (%)	12 (48%)	94 (47%)	0.94	49 (54%)	102 (48%)	0.33
White n (%)	20 (80%)	145 (73%)	0.45	63 (70%)	149 (71%)	0.91
Caregiver education <high school*, n (%)	3 (12%)	22 (11%)	0.89	7 (8%)	18 (9%)	0.83
IQ, M ± SD	111.4 ± 14.0	94.8 ±16.4	**<0.001**	105.2 ± 15.2	92.0 ± 16.0	**<0.001**
Gestational age (weeks), M ± SD	-	28.1 ± 2.0	-	-	28.1 ± 2.1	-
Birthweight (grams), M ± SD	-	958.2 ± 177.4	-	-	966.1 ± 173.2	-
Age at CBCL (years), M ± SD	9.1 ± 0.6	8.3 ± 0.2	**<0.001**	12.7 ± 0.8	12.2 ± 0.3	**<0.001**
Age at VABS, M ± SD	9.1 ± 0.6	8.3 ± 0.2	**<0.001**	-	-	-
	16 year - CBCL			**16 year - YSR**		
	T (n=66)	**PT (n=161)**	**p**	**T (n=56)**	**PT (n=45)**	**p**
Female n (%)	40 (53%)	77 (48%)	0.41	32 (57%)	21 (47%)	0.62
White n (%)	50 (67%)	106 (65%)	0.85	40 (63%)	26 (47%)	0.07
Caregiver education <high school*, n (%)	4 (5%)	13 (8%)	0.46	4 (6%)	6 (11%)	0.38
IQ, M ± SD	102.8 ± 16.5	89.5 ± 17	**<0.001**	102.8 ± 15	91.1 ±17	**<0.001**
Gestational age (weeks), M ± SD	-	28.3 ± 2	-	-	28.5 ± 2	-
Birthweight (grams), M ± SD	-	969.0 ± 172	-	-	952.9 ± 184	-
Age at CBCL/YSR (years), M ± SD	16.2 ± 0.3	16.1 ± 0.2	**0.01**	16.2 ± 0.3	16.0 ± 0.1	**<0.001**
	18 year - VABS			**20 year - Imaged**		
	T (n=71)	**PT (n=190)**	**p**	**T (n=17)**	**PT (n=19)**	**p**
Female n (%)	39 (55%)	87 (46%)	0.26	7 (41%)	9 (47%)	0.75
White n (%)	63 (74%)	143 (75%)	0.89	12 (71%)	16 (84%)	0.43
Caregiver education <high school*, n (%)	4 (5%)	20 (10%)	0.12	1 (6)	2 (11%)	1.0
IQ, M ± SD	103.8 ±16	91.6 ±16	**<0.001**	99.4 ± 17	93.1 ± 10	0.20
Gestational age (weeks), M ± SD	-	28.1 ± 2	-	-	28 ±1.8	-

Birthweight (grams), M ± SD	-	960.7 ± 181	-	-	957±171	-
Age at questionnaire/scan (years), M ± SD	18.1 ± 0.9	18.4 ± 1.4	0.09	19.9 ± 1	19.9 ± 1	0.93

*For 8, 12, and 16-year-olds, this reflects maternal education, whereas for 18-year-olds, this reflects the education of the subject's caregiver (mother or other caregiver)

Neuropsychological Testing Analysis – Parent Report

Neuropsychological instruments evaluating social and emotional behavior were administered to parents of both PTs and Ts at ages 8, 12, 16, and 18. Full data for each instrument are shown in Table 2.

TABLE 2. Social and emotional behavior scores (parent reported) of neurodevelopmental cohort, separated by age

Behavioral domains		**8T (n=25)**	**8PT (n=199)**	**p**
Respondent other than mother, n (%)		1 (4%)	11 (5.5%)	0.75
CBCL	Withdrawn	51.2 ± 2.81	52.53 ± 3.87	**0.03**
	Anxious/Depressed	52.04 ± 3.99	52.94 ± 5.13	0.13
	Social Problems	50.60 ± 1.53	53.55 ± 5.63	0.15
VABS	Socialization	102.16 ± 12.44	89.38 ± 12.13	**0.001***
	Adaptive Behavior	107.08 ± 12.66	88.36 ± 15.48	**0.0001***
	Maladaptive Behavior	5.4 ± 4.22	5.09±- 4.88	0.12
Behavioral Domains - CBCL		**12T (n=90)**	**12PT (n=211)**	**P**
Respondent other than mother, n (%)		5 (5.56%)	14 (6.64%)	0.72
Withdrawn		51.34 ± 2.88	52.56 ± 4.94	0.37
Anxious/Depressed		51.61 ± 3.10	53.42 ± 5.31	0.09
Social Problems		52.47 ± 4.75	55.21 ± 7.04	0.19
Behavioral domains - CBCL		**16T (n=66)**	**16PT (n=161)**	**P**
Respondent other than mother, n (%)		0 (0%)	0 (0%)	1.00
Social Competence		51.91 ± 8.49	45.57 ± 9.15	**0.002***
Social Problems		51.42 ± 2.87	54.59 ± 6.37	0.07
Anxious/Depressed		50.89 ± 1.64	53.81 ± 5.57	**0.001***
Anxiety Problems		50.86 ± 1.82	54.28 ± 5.91	**<0.001***
Withdrawn/Depressed		52.21 ± 3.29	56.27 ± 7.68	**0.003***
Affect Problems		51.83 ± 3.26	55.24 ± 7.16	**0.01**
Behavioral domains - VABS		**18T (n=71)**	**18PT (n=190)**	**p**
Respondent other than mother, n (%)		8 (11%)	31 (16%)	0.31
Adaptive Behavior		103.83 ± 13.18	95.28 ± 17.46	0.10
Socialization		108.08 ± 10.34	98.12 ± 15.44	**0.01**$^{+}$
Interpersonal		16.04 ± 2.40	14.32 ± 2.86	**0.03**
Play and Leisure		16.59 ± 1.09	14.61 ± 3.01	**<0.001**$^{+}$
Coping		16.30 ± 2.19	15.24 ± 2.90	0.45

Covariates include gender, race, caregiver education, age at time of response, respondent, and full IQ.

Scores are Mean±SD

$^{*, +}$:Bonferroni correction for multiple comparisons (*: corrected $p<0.008$, +: corrected $p<0.01$)

PTs showed impaired social and emotional behavior according to parent report beginning at age 8. At that time, PTs had significantly higher (worse) scores in the Withdrawn domain of the CBCL ($p=0.03$) and significantly lower (worse) scores in the Socialization ($p=0.001$) and Adaptive Behavior ($p=0.0001$) domains of the VABS when compared to T born peers. At age 12, after controlling for demographic variables and full IQ scores, PTs and Ts did not show any significant differences in any of the social and emotional behavior domains on the CBCL. At age 16, PTs had significantly lower (worse) scores in Social Competence ($p=0.001$) and significantly higher (worse) scores in the Anxious/Depressed ($p=0.001$), Anxiety Problems ($p<0.001$), Withdrawn/Depressed ($p=0.003$), and Affect Problems ($p=0.01$) domains on the CBCL. At age 18, again in the parent-reported analysis, PTs had significantly lower (worse) scores in the Socialization ($p=0.01$), Interpersonal ($p=0.03$), and Play and Leisure ($p<0.001$) domains of the VABS. Notably, the majority of differences seen at ages 8, 16, and 18 between PT and T survive a Bonferroni correction for multiple comparisons (see Table 2 for corrected p values).

In a gender-stratified regression analysis of the above neuropsychological testing, there emerged differences in social and emotional behavior scores between PTs and Ts. This analysis is presented in Table 3. At age 8, there were no significant differences between PT and T in either females or males in the CBCL. However, at age 8, female PTs scored significantly worse than female Ts in all domains on the VABS (Socialization ($p=0.005$), Adaptive Behavior ($p<0.001$), and Maladaptive Behavior ($p=0.008$)) whereas male PTs only scored worse in the Maladaptive Behavior domain ($p=0.04$). At age 12, there were no significant differences between PT and T on the CBCL domains in either females or males. At age 16, female PTs scored worse than female Ts on the Social Competence ($p=0.047$) and Anxiety Problems ($p=0.02$) domains of the CBCL, whereas

male PTs scored worse than male Ts on the Anxious/Depressed (0.045), Anxiety Problems (0.04), and Withdrawn/Depressed (p=0.04) domains on the CBCL. At age 18, there were no significant differences between female PTs and Ts on the VABS, but the male PTs scored worse on the Socialization (p=0.04) and Play and Leisure (p=0.001) domains when compared to male Ts.

TABLE 3. Social and emotional behavior scores (parent reported) of neuropsychological cohort, separated by gender

	Female			**Male**		
CBCL	**8T (n=12)**	**8PT (n=94)**	**p**	**8T (n=13)**	**8PT (n=105)**	**p**
Respondent other than mother, n (%)	0 (0%)	4 (4.2%)	0.47	1 (7.7%)	7 (6.7%)	0.89
Withdrawn	51.17 ± 3.21	52.66 ± 3.82	0.13	51.23 ± 2.52	52.42 ± 3.92	0.05
Anxious/Depressed	52.25 ± 3.84	52.39 ± 4.67	0.62	51.85 ± 4.28	53.44 ± 5.50	0.11
Social Problems	50.75 ± 2.05	52.71 ± 4.13	0.17	50.46 ± 0.88	54.30 ± 6.63	0.26
VABS	**8T (n=18)**	**8PT (n=102)**	**p**	**8T (n=16)**	**8PT (n=111)**	**p**
Socialization	101.39 ± 12.45	89.61 ± 12.58	**0.005***	96.88 ± 13.37	88.52 ± 12.40	0.37
Adaptive Behavior	107.78 ± 14.66	89.30 ± 16.56	**<0.001***	98.69 ± 12.93	86.67 ± 15.64	0.16
Maladaptive Behavior	7.33 ± 5.74	4.95 ± 4.82	**0.008***	8.19 ± 7.30	5.91 ± 5.50	**0.04**
CBCL	**12T (n=49)**	**12PT (n=102)**	**p**	**12T (n=41)**	**12PT (n=109)**	**p**
Respondent other than mother, n (%)	2 (4.08%)	6 (5.88%)	0.64	3 (7.32%)	8 (7.33%)	1.0
Withdrawn	51.10 ± 2.65	52.59 ± 5.15	0.33	51.63 ± 3.14	52.56 ± 4.75	0.83
Anxious/Depressed	51.76 ± 3.14	53.26 ± 5.16	0.23	51.44 ± 3.09	53.57 ± 5.46	0.28
Social Problems	51.92 ± 4.09	54.68 ± 6.45	0.28	53.12 ± 5.42	55.72 ± 7.54	0.45
CBCL	**16T (n=34)**	**16PT (n=76)**	**p**	**16T (n=32)**	**16PT (n=85)**	**p**
Respondent other than mother, n (%)	0 (0%)	0 (0%)	1	0 (0%)	1 (0%)	0.54
Social Competence	52.79 ± 8.43	46.75 ± 8.69	**0.047**	50.97 ± 8.58	44.52 ± 9.47	0.07
Social problems	51.44 ± 3.06	54.59 ± 6.50	0.10	51.41 ± 2.71	54.59 ± 6.28	0.52
Anxious/depressed	50.94 ± 1.65	53.71 ± 5.47	0.07	50.84 ± 1.65	53.91 ± 5.70	**0.045**
Anxiety problems	50.53 ± 1.21	53.96 ± 5.83	**0.02**	51.22 ± 2.27	54.56 ± 6.00	**0.043**
Withdrawn/depressed	51.32 ± 2.20	56.12 ± 8.07	0.20	53.16 ± 3.98	56.40 ± 7.36	**0.04**
Affect Problems	51.82 ± 3.12	55.86 ± 8.13	0.18	51.84 ± 3.45	54.69 ± 6.17	0.17
VABS	**18T (n=39)**	**18PT (n=87)**	**p**	**18T (n=32)**	**18PT (n=104)**	**p**
Respondent other than mother, n (%)	3 (7.7%)	13 (14.9%)	0.21	5 (15.6%)	18 (17.3%)	0.82
Adaptive Behavior	107.97 ± 11.08	96.45 ± 16.97	0.33	98.78 ± 13.92	94.13 ± 17.88	0.27
Socialization	109.28 ± 9.91	98.35 ± 14.45	0.20	106.63 ± 10.82	97.85 ± 16.25	**0.04**
Interpersonal	16.03 ± 2.35	14.25 ± 2.71	0.16	16.06 ± 2.50	14.35 ± 3.00	0.10
Play and Leisure	16.72 ± 0.83	14.76 ± 3.26	0.36	16.44 ± 1.34	14.35 ± 3.12	**0.001**$^{+}$
Coping	16.77 ± 1.94	15.24 ± 2.86	0.82	15.72 ± 2.37	15.25 ± 2.93	0.56

Controlling for gender, race, caregiver education, age at time of response, respondent, and full IQ.
Scores are Mean±SD
Bonferroni correction for multiple comparisons (: corrected p<0.02; +: corrected p<0.01)

Neuropsychological Testing Analysis – Child Report

At age 16, a subset of the PTs and Ts were evaluated with the YSR, which is a self-report instrument evaluating similar domains as the CBCL. Interestingly, in this self-report analysis, PTs had significantly lower (better) scores in the DSM Affective Problems scale (p=0.04, see Table 4). In all other domains, there was no significant difference between PTs and Ts. There were no significant differences between PT and T when stratified by gender for any of the measures on the YSR (data not shown).

TABLE 4. Social and emotional behavior scores (child reported) of YSR sub-cohort

Behavioral domains	16T (n=56)	16PT (n=45)	p
Respondent other than self, n (%)	0 (0%)	0 (0%)	1.00
Activities	48.28 ± 10.40	48.09 ± 11.05	0.42
Social	52.84 ± 7.82	49.38 ± 9.41	0.07
Anxious/Depressed	52.21 ± 4.15	52.27 ± 3.48	0.62
Withdrawn	52.91 ± 4.24	53.20 ± 5.07	0.86
Social Problems	53.43 ± 4.63	51.98 ± 2.94	0.13
DSM scale: affective problems	52.86 ± 4.26	51.47 ± 2.10	**0.04**
DSM scale: anxiety problems	52.43 ± 4.02	52.62 ± 3.87	0.54

Controlling for gender, race, caregiver education, age at time of response, respondent, and full IQ. Scores are Mean±SD

Longitudinal Analysis of Neuropsychological Testing

For this analysis, only PT and T with complete CBCL or VABS testing at each age point were included. For the CBCL analysis, 119 PT and 20 T were included, and for the VABS analysis, 154 PT and 21 T were included. The three CBCL domains that were measured at ages 8, 12, and 16 were Withdrawn, Anxious/Depressed, and Social Problems. Means for PT and T at each age are shown in Table 5 and depicted in Figure 2. In the Withdrawn domain, PT showed significantly worsening scores by age 16 when compared to Ts (group p=0.11; time p=0.03; group*time p=0.03). There were no

significant effects in the Anxious/Depressed domain, but in the Social Problems domain PT had consistently worse scores compared to Ts; there was also an effect of time (group p=0.01; time p=0.003; group*time p=0.63). The VABS domains measured at ages 8 and 18 were Socialization and Adaptive Behavior. In both domains, PT scored significantly more poorly than Ts at both ages (Socialization group p=0.0002, Adaptive Behavior group p<0.0001). Both PT and T Socialization scores significantly improved with time (time p=0.0003). There were no significant interactions of group and time for either of the VABS measures.

TABLE 5. Longitudinal analysis of neuropsychological scores in PT and T from school-age to young adulthood

CBCL		**T (n=20)**	**PT (n=119)**	**Analysis**	**p**
Withdrawn	8 years	51.1	51.98	Group	0.11
	12 years	52.4	52.24	Time	**0.03**
	16 years	51.85	55.50	Group*Time	**0.03**
Anxious/Depressed	8 years	51.6	52.77	Group	0.06
	12 years	52.25	52.97	Time	0.62
	16 years	50.45	53.78	Group*Time	0.06
Social Problems	8 years	50.65	53.01	Group	**0.01**
	12 years	51.9	54.85	Time	**0.003**
	16 years	50.45	53.91	Group*Time	0.74
VABS		**T (n=21)**	**PT (n=154)**	**Analysis**	**p**
Socialization	8 years	100.67	90.22	Group	**0.0002**
	18 years	106.9	98.34	Time	**0.0003**
				Group*Time	0.63
Adaptive Behavior	8 years	89.46	104.81	Group	**<0.0001**
	18 years	95.27	104.95	Time	0.13
				Group*Time	0.15

PT and T were included in this analysis only if they had complete data at each age point.

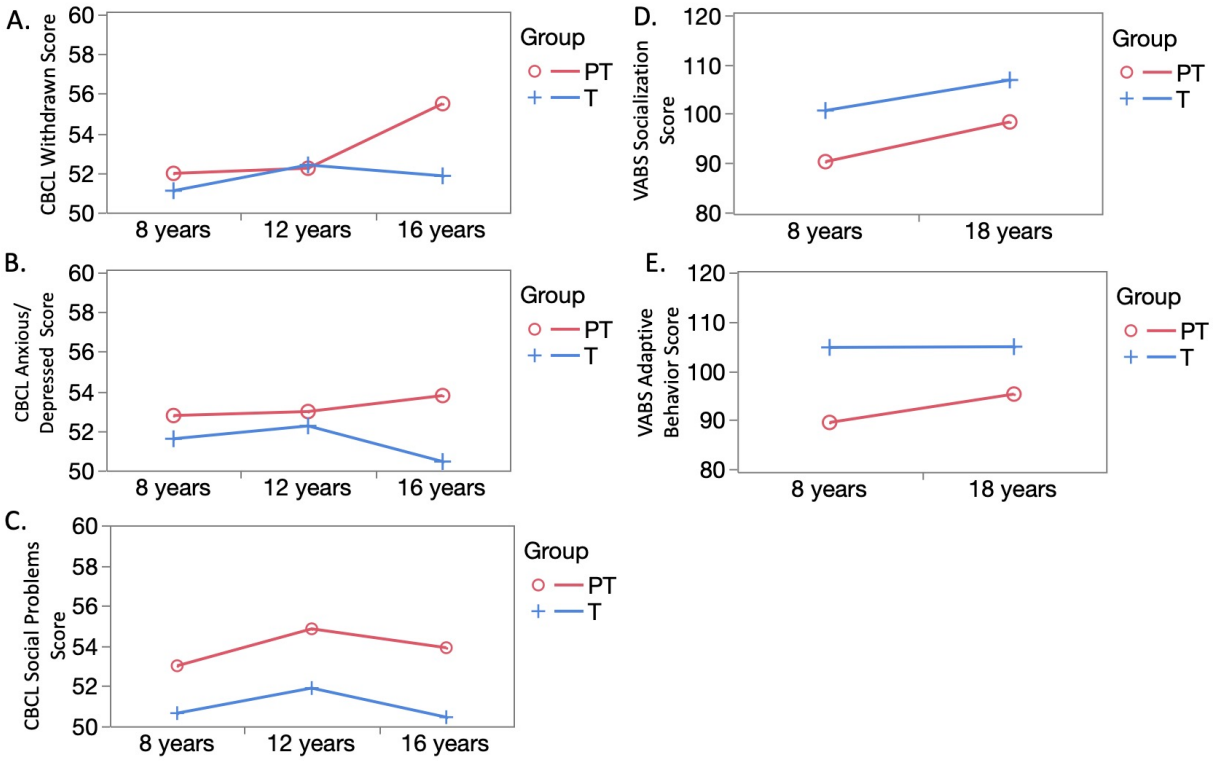

FIGURE 2. Mean scores for CBCL domains and VABS domains for PT and T over time. PTs demonstrate significantly worsening scores over time compared to Ts in the Withdrawn domain of the CBCL (A – group: p=0.11, time: p=0.03, group*time interaction: p=0.03). There were no significant differences between PT and T in the Anxious/Depressed domain of the CBCL (B – group: p=0.06, time: p=0.62, group*time interaction: p=0.06). In the Social Problems domain of the CBCL (C), PTs have significantly worse scores than Ts (group: p=0.01), and both groups demonstrate worsening over time (time: p=0.003), though there is no significant group*time interaction (p=0.75). In the Socialization (D) and Adaptive Behavior (E) domains of the VABS, PTs have significantly worse scores than Ts (Socialization – group p=0.0002, Adaptive Behavior - group p<0.0001). Both groups demonstrate improvement in Socialization scores over time (time: p=0.0003). There was no significant group*time interaction for either Socialization (p=0.63) or Adaptive Behavior (p=0.15).

For PTs with complete neuropsychological data at each age, 8-year scores in all domain were significantly positively correlated with scores in adolescence and young adulthood. The largest correlations were between Withdrawn scores at ages 8 and 12 (r=0.44, p<0.0001), Social Problems scores at ages 8 and 12 (r=0.54, p<0.0001), and Adaptive Behavior scores at ages 8 and 18 (r=0.38, r<0.0001). Correlation coefficients

for all domains and ages are shown in Table 6 and graphical representations are shown in Figure 3.

TABLE 6. Correlation of neuropsychological scores between school-age, early adolescence and young adulthood in PT participants

	8 years and 12 years		8 years and 16 years	
	Correlation Coefficient	P	Correlation Coefficient	P
Withdrawn	0.439	**<0.0001**	0.276	**0.002**
Anxious/Depressed	0.343	**0.0001**	0.313	**0.0005**
Social Problems	0.526	**<0.0001**	0.354	**<0.0001**
	8 years and 18 years			
Socialization	0.236	**0.003**		
Adaptive Behavior	0.382	**<0.0001**		

PT were included in this analysis only if they had complete data at each age point.

Exploratory Analysis of Maternal Education and Social Behavior

PT subjects with complete neuropsychological data at each age were further compared in a secondary exploratory analysis by maternal education level (see Table 7 and Figure 4). T subjects were not compared by maternal education level due to low subject numbers. On the CBCL, PT children of mothers with less than a high school education demonstrated worsening scores in the Withdrawn and Social Problems domains over time, as compared to PT children of mothers with greater than or equal to a high school education. There was a significant effect of both time and a significant interaction between group and time on the trajectory of Withdrawn (group p=0.24; time: p=0.0003, group*time: p=0.01) and Social Problems (group p=0.48; time: p=0.0002, group*time: p=0.009) scores in this cohort. There were no significant effects of group or time nor any interaction between group and time on Anxious/Depressed scores.

On the VABS, PT children of mothers with greater than or equal to a high school education scored higher on both the Socialization (group: p=0.0001, time: p=0.004; group*time p=0.63) and Adaptive Behavior (group: p<0.0001, time: p=0.02; group*time

p=0.15) domains at ages 8 and 18. There were no significant interactions between group and time in this model.

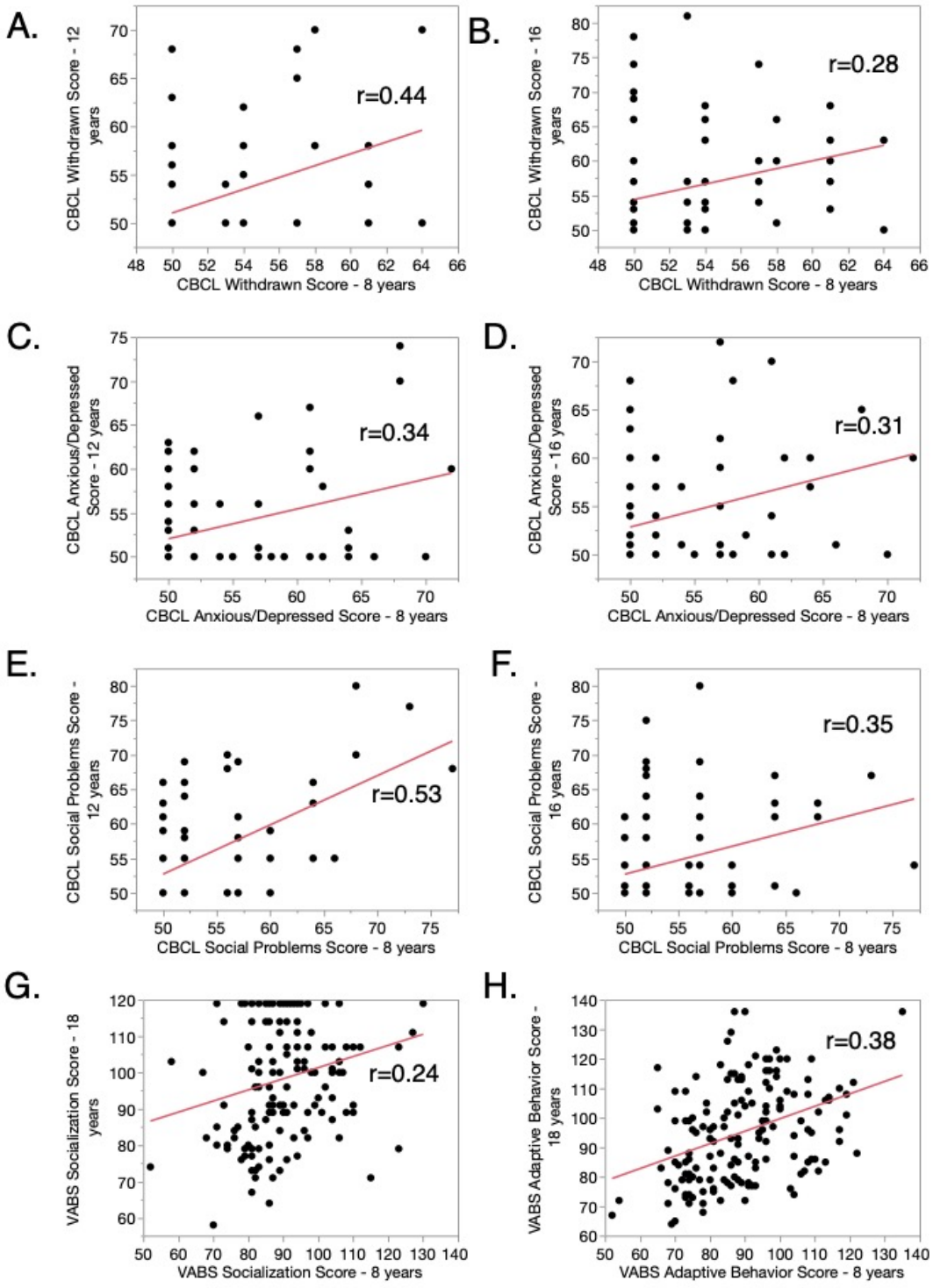

FIGURE 3. Scores in all CBCL (Withdrawn – A and B, Anxious/Depressed – C and D, and Social Problems – E and F) and all VABS (Socialization – G and Adaptive Behavior – H) domains are significantly positively correlated between ages 8 and 12 (CBCL), 8 and 16 (CBCL) and 8 and 18 (VABS) ($p<0.01$ for all).

TABLE 7. Longitudinal analysis of neuropsychological scores in PT from school-age to young adulthood, by maternal education level

		<High School Education (n=8)	**≥High School Education (n=111)**	**Analysis**	**p**
Withdrawn	8 years	51.00	52.05	Group	0.24
	12 years	55.75	51.98	Time	**0.0003**
	16 years	57.75	55.33	Group*Time	**0.01**
Anxious/Depressed	8 years	51.38	52.87	Group	0.95
	12 years	54.25	52.88	Time	0.33
	16 years	53.63	53.79	Group*Time	0.35
Social Problems	8 years	50.50	53.19	Group	0.48
	12 years	58.38	54.6o	Time	**0.0002**
	16 years	56.50	53.72	Group*Time	**0.009**
		<High School Education (n=15)	**≥High School Education (n=139)**	**Analysis**	**p**
Socialization	8 years	81.47	91.17	Group	**0.0001**
	18 years	86.67	99.6	Time	**0.004**
				Group*Time	0.63
Adaptive Behavior	8 years	76.13	90.90	Group	**<0.0001**
	18 years	81.00	96.81	Time	**0.02**
				Group*Time	0.15

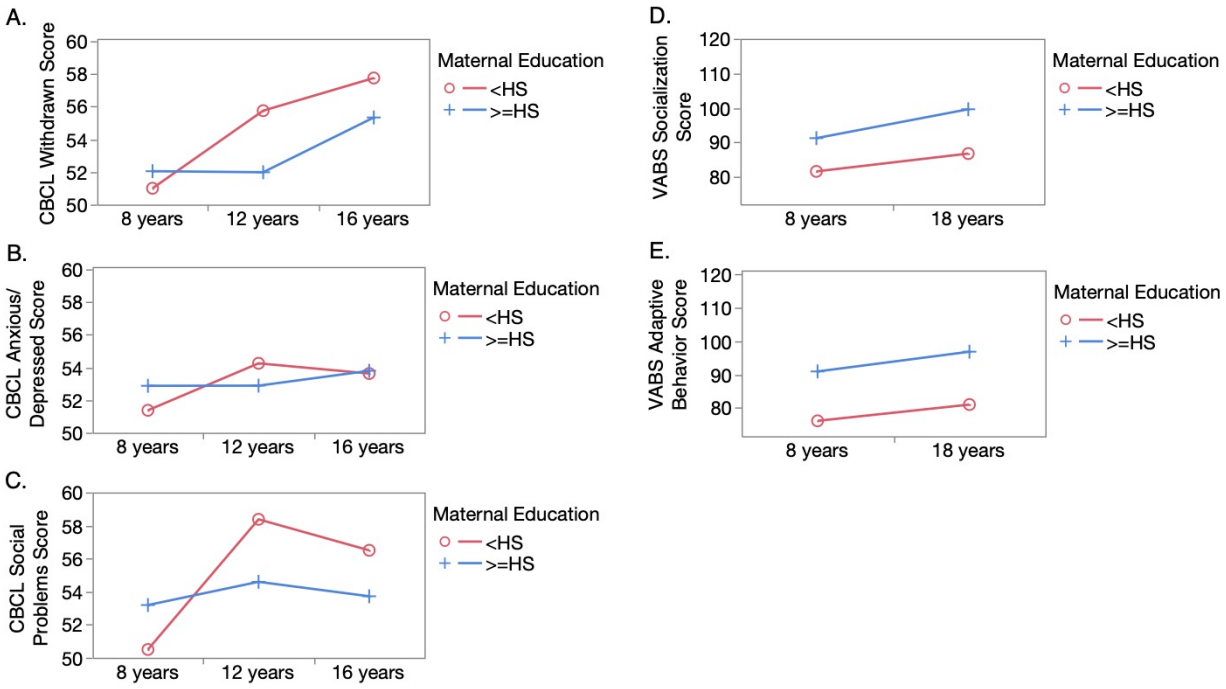

FIGURE 4. Mean scores for CBCL and VABS domains for PT over time, separated by maternal education level. Higher maternal education is associated with decreased worsening of Withdrawn (A – group p=0.24, group*time interaction p=0.01) and Social Problems (C – group p=0.48, group*time interaction p=0.009) scores in PT. There is a significant effect of time for both education groups in Withdrawn (A – time p= 0.0003), Social Problems (C – time p=0.0002), Socialization (D – time p=0.004), and Adaptive Behavior (E – time p=0.02) scores. Overall, PT scored significantly lower in the Socialization (D – group p=0.0001) and Adaptive Behavior (E – group p<0.0001) domains. There were no significant effects of group, time, or group*time interaction for the Anxious/Depressed domain (B – group p=0.95, time p=0.33, group*time p=0.35) and no significant group*time interactions for Socialization (D – p=0.63) or Adaptive Behavior (E – p=0.15).

Amygdalar Seed Connectivity Analysis

For both PTs (Figure 5B) and Ts (Figure 5C), regions positively connected to the amygdala include the insula, temporal region/left superior temporal gyrus, and hippocampus. Regions negatively connected to the amygdala include the posterior cingulate (PCC) for both study groups, and dorsal lateral prefrontal cortex, medial prefrontal cortex, and lateral parietal cortex for PTs. In PTs compared to Ts, the amygdala showed significantly increased connectivity to a region in the parietal lobe that

included the left precuneus and bilateral PCC (Figure 5D). Additionally, when total PT connectivity was compared to total T connectivity, the amygdala showed significantly increased positive connectivity to the left superior temporal gyrus (LSTG) (Figure 5E).

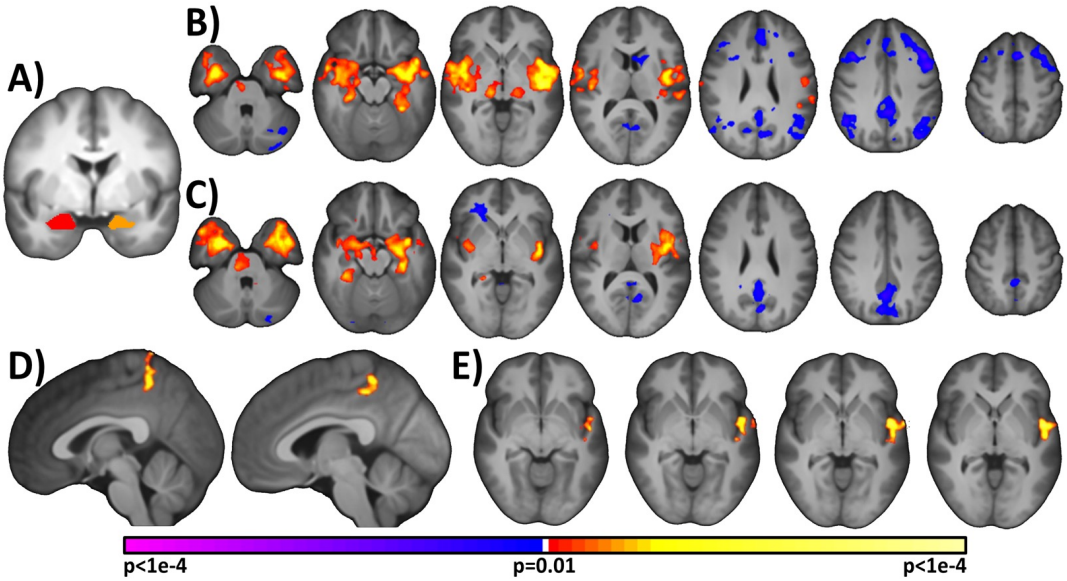

FIGURE 5: Amygdalar seed connectivity. A) The bilateral amygdala seed is shown in orange and red. The amygdalar connectivity based on the bilateral seed is shown **B)** for preterms and **C)** for terms. For both groups, the amygdala is connected positively to the insula, temporal region/left superior temporal gyrus, and hippocampus. Regions negatively connected to the amygdala include the posterior cingulate (PCC) for both study groups, and dorsal lateral prefrontal cortex, medical prefrontal cortex, and lateral parietal cortex for PTs. **D)** Total amygdala connectivity differences between PTs and Ts: for PTs compared to Ts, the amygdala showed significantly increased connectivity to a region in the parietal lobe that included the left precuneus and bilateral PCC. Two sequential slices are depicted for complete visualization of this region.
E) Total amygdala connectivity differences between PTs and Ts: For PTs compared to Ts, the amygdala showed significantly increased positive connectivity to the left superior temporal gyrus. Four sequential slices are depicted for complete visualization of this region. Images are thresholded at $p<0.01$, corrected. Slices are shown in radiological convention.

Amygdala Connectivity and Behavioral Correlation

In the sub-cohort consisting of only participants with imaging data at age 20, PT had worse scores in Social Competence at age 16 ($p=0.03$) and in Adaptive Behavior ($p=0.02$) and Coping ($p=0.01$) at age 18 (see Table 8). These analyses did not control for demographic variables or IQ due to the small sample sizes. Behavioral scores for the PTs

and Ts included in the imaging sub-cohort were not significantly different from behavioral scores in the full neuropsychological cohort.

TABLE 8. Social and emotional behavior scores in imaged sub-cohort

CBCL	**Imaged sub-cohort**		
Behavioral domains	**16T (n=17)**	**16PT (n=18)**	**p**
Respondent other than mother, n (%)	0 (0%)	0 (0%)	1.00
Social Competence	50.8 ± 10	43.3 ± 9.2	**0.03**
Social Problems	54.2 ± 6.6	54.7 ± 8	0.83
Anxious/Depressed	52.5 ± 4.6	55.6 ± 7.1	0.14
Anxiety Problems	52.5 ± 5.1	55.2 ± 6.0	0.17
Withdrawn/Depressed	54.3 ± 6.1	56.2 ± 6.8	0.38
Affect Problems	55.4 ± 6.8	56.6 ± 9.2	0.68
VABS	**18T (n=17)**	**18PT (n=19)**	**p**
Respondent other than mother, n (%)	1 (6%)	2 (11%)	1.00
Adaptive Behavior	102.7 ± 17	90.3 ± 14	**0.02**
Socialization	105.7 ± 14	97.0 ± 14	0.07
Interpersonal	15.7 ± 2.4	14.7 ± 2.8	0.25
Play and Leisure	15.4 ± 2.6	15.1 ± 2.7	0.69
Coping	16.6 ± 2.5	14.2 ± 2.9	**0.01**

Scores are Mean ± SD

Combined, PT and T amygdala-PCC connectivity was significantly negatively correlated with Social Competence on the CBCL (r=-0.37, p=0.03) and Socialization on the VABS (r=-0.42, p=0.01) (Figure 6 and Table 9). Independently, PTs and Ts showed negative fit lines between both measures and amygdala-PCC connectivity, suggesting that group differences in the measures or connectivity were not responsible for the observed correlation. However, these associations did not reach significance when PTs and Ts were analyzed separately. Additionally, while Anxious/Depressed, Withdrawn,

and Affect Problems were significantly correlated with amygdala-PCC connectivity, these correlations were driven by high leverage points. No significant correlations between social and emotional behavioral scores and amygdala-LSTG connectivity were observed (Table 9).

TABLE 9. Correlation between amygdala connectivity and social and emotional behavior scores

Amygdala-PCC			**Amygdala-L STG**		
Domains measured at 16 years by CBCL	**Rho**	**P**	**Domains measured at 16 years by CBCL**	**Rho**	**P**
Social Competence (n=35)	-0.37	**0.03**	Social Competence (n=35)	-0.31	0.07
Anxious/Depressed (n=32)	0.38	**0.02**	Anxious/Depressed (n=32)	0.23	0.20
Anxiety Problems (n=33)	0.27	0.13	Anxiety Problems (n=33)	0.29	0.10
Withdrawn (n=35)	0.35	**0.04**	Withdrawn (n=35)	0.02	0.92
Affect Problems (n=35)	0.34	**0.04**	Affect Problems (n=35)	0.07	0.70
Domains measured at 18 years by VABS	**Rho**	**P**	**Domains measured at 18 years by VABS**	**Rho**	**P**
Socialization (n=36)	-0.42	**0.01**	Socialization (n=36)	-0.19	0.26
Play and Leisure (n=36)	-0.22	0.20	Play and Leisure (n=36)	-0.03	0.86
Interpersonal (n=36)	-0.19	0.26	Interpersonal (n=36)	-0.09	0.62

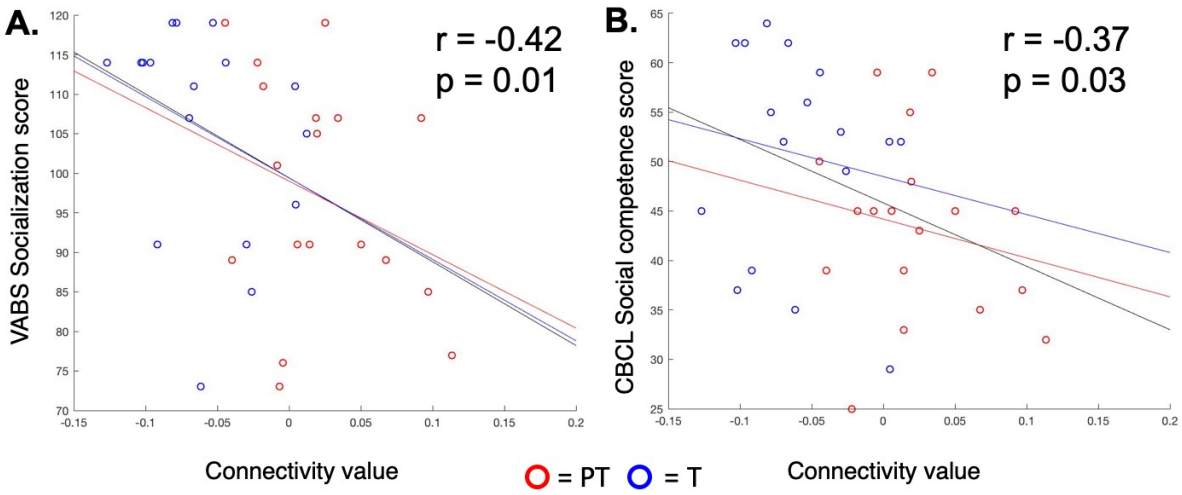

FIGURE 6: Association between amygdala-PCC connectivity and social vulnerability. Combined, PT and T amygdala-PCC connectivity was significantly negatively correlated with **A)** Socialization on the VABS and **B)** Social Competence on the CBCL. Independently, PTs and Ts showed negative fit lines between both measures (Socialization and Social Competence) and amygdala-PCC connectivity, suggesting that group differences in the measures of connectivity were not responsible for the observed correlation. Best fit line for PTs is shown in red, for Ts is shown in blue, and for both groups combine is shown in grey.

Discussion

Beginning at school age and continuing into young adulthood, very preterm-born individuals are more vulnerable to social impairments than their term-born peers. Employing longitudinal neurobehavioral testing and resting state fMRI, we demonstrate worsening trajectories in social and emotional domains critical for the successful transition to young adulthood in preterm subjects compared to term controls. In exploratory analyses, higher maternal education levels appear to be protective for the PT group, and PT social and emotional scores at school-age are significantly positively correlated with scores in early adolescence and young adulthood. Investigation of the neural pathways contributing to these findings demonstrates that that amygdalar connectivity is altered for those prematurely-born and behavioral markers of social functioning correlate with altered amygdala-PCC connectivity. The correlation between amygdala hyperconnectivity and measures of social functioning in these PT young adults suggests one possible neural underpinning for the PT social phenotype, a constellation of symptoms including increased social difficulties, heightened levels of anxiety and depression, decreased extroversion, and poor self-esteem that has been previously described (10, 12, 20, 24). Finally, our cohort of PTs does not include participants who suffered any form of perinatal neurological injury, suggesting that the findings we report may be attributable solely to prematurity.

Compared to term-born peers, PTs in this cohort show decreased parent-reported social competence and socialization beginning in school-age and lasting to young adulthood, which are composite measures of social skills including interpersonal relationships, involvement in activities, and coping skills in social situations. PTs in this cohort also show increased anxiety, depression, and affect problems, which is consistent

with previous work showing that individuals who are born preterm are at higher risk for psychiatric disorders such as anxiety, depression, and phobias beginning in early school age (65), and persisting into adolescence and young adulthood (10, 26, 28). These findings echo previous descriptions of social and emotional behavior in PTs (9) providing further evidence that social impairment seen in PT children without perinatal neurologic injury persists from school age into adolescence and young adulthood.

This social impairment appears to worsen from school-age to young adulthood when PT are compared to T. In the Withdrawn domain on the CBCL, which assesses behaviors and characteristics including shyness, preferring to be alone, and refusing to talk, the PT score trajectory demonstrates significant worsening compared to T. As described above, these characteristics align with previous descriptions of PT in cross-sectional studies; however, to our knowledge there are few studies assessing the specific trajectory of social and emotional problems in PT during the transition from school-age to adolescence and young adulthood, which is a tumultuous period in social and emotional development (66). Linsell et al described overall stable differences in emotional and behavioral problems between PT and T, with similar overall trajectories between the two groups (19); however, in the emotional problems sub-category, PT demonstrated a worsening in scores compared to T, similar to this study. It is possible that this worsening of social impairment in PT represents underlying subtle social impairments that, though present in PT from early childhood, become increasingly evident to parents and caregivers as typically developing peers undergo developmentally normal social growth in adolescence.

Among PT, maternal education appears to be a protective factor in the trajectory of social and emotional problems during adolescence. In both the Withdrawn and Social

Problems domain, PT children of mothers with a high school education or higher demonstrated slower worsening than PT children of mothers with lower education levels. Although the number of subjects with low maternal education among the cohort with complete neuropsychological data is small, this trend suggests that the trajectory of social development in PT may be modifiable. Similar effects of maternal education have been reported on the trajectories of PT language development (67), further supporting the importance of optimal external influences on PT neurodevelopment throughout childhood and adolescence.

Adolescence is an important time for social development and changing demands and expectations of PT adolescents may exacerbate subtle differences that began in early childhood. Indeed, in this study, PT social and emotional scores in all domains at school-age were significantly positively correlated with scores in early adolescence and young adulthood, suggesting that early impairments may be predictive of later problems. Prior studies have shown similar correlations of social-emotional behavior across ages in PT born children (68). This relationship should be leveraged to intervene on these patients during early childhood and school age, when problems first present, which may lessen the morbidity for PTs in adolescence and young adulthood. We are unaware of any existing interventions designed to increase social skills for PT-born children and adolescents, but there is promising literature supporting similar interventions for children and adolescents with other conditions, including social phobia, ADHD and autism spectrum disorders, who are at risk of social vulnerability (69-72). These interventions, which range from play therapy to structured social interaction simulations, may be adaptable to PT and should be further explored in order to optimize lifetime outcomes from a social and emotional standpoint.

In contrast to robust differences seen in PTs compared to Ts by parent report, PTs in this study did not show any difference in social competence or in anxiety and depression when measured by child self-report, and in fact scored significantly lower on the DSM: Affective Problems scale on the YSR, which consists of measures that are consistent with dysthymia and major depressive disorder (62). This discordance between parent and child report of characteristics of PT children and adolescents has been previously described (30, 73). These results support the notion that PT do not view themselves as impaired in social functioning or as having increased anxiety or depression compared to term born peers. It is possible that PTs do not value the same level of social interaction as Ts, and therefore don't perceive altered social functioning where their parents do. It is also possible that PTs view themselves as on par with T born peers in terms of social development, whereas parents perceive a difference. Further study, including more objective measures of social functioning, will be necessary to fully explore this difference between parent and child reports of PT social functioning.

When PTs and Ts are segregated by gender, some differences in social functioning appear from school age to young adulthood. At age 12, female PTs appear to be more impaired than male PTs when compared to their T peers, but these impairments shift towards male PTs by young adulthood. Previous studies examining gender differences in behavioral and mental health outcomes among PT are variable (74). Many report that PT males have increased internalizing symptoms and social problems compared to T males, but reports differ for PT females. Some studies report similar social and emotional phenotypes for PT and T females, whereas others report increased internalizing and social problems in PT females, both in adolescence and young adulthood (30, 75, 76). Although typically developing adolescent females have higher

rates of internalizing behaviors, depression and anxiety (77), in the PTs included in this study, males appear to exhibit increased internalizing behaviors and social problems compared to females.

Our data provide further evidence that alterations in PT amygdalar connectivity are observed in a continuum across the lifespan. The amygdala is among those regions that experience the earliest prenatal structural and functional growth (50) and is a major hub of the "social brain" (38, 40, 45). Amygdalar functional connectivity is altered in PT-born neonates (36, 46) as well as in PT-born adults (47). Although early adolescence represents a period of amygdala connectivity changes in typically developing controls (52), to the best of our knowledge, there are no published studies of amygdalar connectivity in PT during adolescence or young adulthood. The overall pattern of amygdala connectivity in PTs in this study was similar to previously described amygdala connectivity in healthy adults (78). Nevertheless, the PTs have decreased negative connectivity from the amygdala to the left precuneus and bilateral PCC and increased positive connectivity to the left STG when compared to term-born peers. Both are areas that have previously been implicated in social perception and social behavior in both typically developing adults and in adults with social anxiety disorder (79-81). These findings are similar to connectivity differences found in a separate cohort of PT-born adults at age 30 (47).

Connectivity between the amygdala and PCC negatively correlates with measures of social functioning in a cohort of combined PTs and Ts. Hyperconnectivity of the amygdala to the PCC has been associated with childhood anxiety disorders (82) and altered amygdala-PCC connectivity has been associated with social anxiety disorder in adults (79, 83). Together, these studies suggest that the association between behavior and

amygdala-PCC connectivity is not specific to PTs, and that hyper-connectivity in this circuit is related generally to social and emotional behavioral problems.

The posterior cingulate cortex is part of the default mode network (DMN) and is also thought to play a role in social cognition (84), in particular in evaluating others' mental states (85) and in emotion processing (86). As impaired emotion recognition contributes to decreased social competence (87), this may be a factor underlying the relationship between altered amygdala connectivity and social impairments.

Together, the persistence of social deficits in PT from school-age through young adulthood and the presence of correlations between these deficits and clear alterations in amygdalar connectivity add to the large body of knowledge that PT face substantial social difficulties during formative years and suggest that they may be due to underlying changes in functional connectivity. Though PT scores in this study do not reach the "clinical range," the consistent and significant differences between PT and T in social and emotional problems and large body of knowledge that social difficulties in PT may have serious and lasting developmental impact make these findings concerning. To our knowledge, it is not yet common practice to discuss these risks with parents of PT infants at the time of birth or NICU discharge; however, given the significant risks associated with these deficits including increased bullying in adolescence (20, 21), increased psychiatric illness in PT adults who were bullied as adolescents (88), and low educational attainment (25) it seems imperative that the full spectrum of PT social and psychological outcomes be discussed with families.

Our study has several strengths: we provide further evidence of increased social and emotional impairments in a large cohort of PTs from school-age to young adulthood, further elucidate a concerning trajectory of worsening PT social function during

adolescence, and demonstrate a negative correlation between social functioning and amygdala-PCC connectivity in participants with clinically unremarkable MRI studies at 18 years of age. These data provide an evaluation of the relationship between the PT social functioning and amygdala connectivity during adolescence, a time of significant change in both social demands and neural connectivity. Furthermore, the participants included in this analysis had no history of neurologic injury and normal clinical MRI scans at the time of study, suggesting that alterations in connectivity and function are due to prematurity rather than prior injury.

There are several limitations to this analysis. First, while we believe the longitudinal nature of this study and significant retainment of subjects a strength of this work, there were many subjects lost to follow up in the PT cohort. The participants who were lost to follow-up had significantly lower maternal education than those who were included in the analyses at ages 8, 12, 16, and 18. As we found that maternal education has a protective effect on the trajectory of social and emotional problems in PT adolescents, the participants who were lost to follow-up may have actually been more impaired than those who were included, potentially biasing our results. Additionally, our longitudinal study may have been underpowered due to significant increase in loss to follow-up at ages 16 and 18. By this time, many participants were no longer residing locally and could not continue to participate in the study. Nevertheless, we believe these findings to be important and hypothesis-generating, and the longitudinal trajectory of social impairment among PT deserves further study.

Second, we acknowledge that there are significant differences in IQ score between PTs and Ts in our large neurobehavioral cohort, consistent with existing literature (89). Lower IQ may predispose the PTs to increased social impairments

compared to typically developing term controls; likewise, it may alter the PTs' self-perception of this condition. While the differences in social behavior between PT and T in the large neurobehavioral cohort remained present after controlling for IQ, we were unable to control for IQ in our imaging analysis due to small sample size.

Third, while our imaging data is a strength of this study, the size of our imaging cohort was limited. As such, we were not able to adequately analyze the imaging-behavior correlations for PTs and Ts separately; nor were we able to control for demographic covariates. Furthermore, we did not have sufficient power to examine the relationship between all connections and social behavior to confirm that these correlations are specific to altered amygdala-PCC connectivity. We were also unable to correlate imaging findings with our longitudinal analysis of CBCL and VABS scores due to sample size. This analysis would be extremely valuable in parsing out the underlying neural cause of the PT social and emotional developmental trajectory, and in determining which, if any, interventions may alter underlying functional connectivity and thus behavior.

Fourth, while our findings demonstrate increased social impairment among PT, it is non-specific and could be due to impairments in different functional domains. Given the format of the instruments we used to evaluate social functioning, we are unable to parse out the specific mechanisms leading to this social impairment.

Finally, we do not have perinatal and longitudinal data about other factors that may impact long term cortical development and neurodevelopmental outcomes. For example, prenatal exposure to maternal stress impacts amygdala functional connectivity (37) and exposure to increased painful procedures in the neonatal period alters brain architecture and increases incidence of internalizing behaviors in PT born children (90-

92). Furthermore, there is evidence that differing parenting styles can impact neurodevelopment (53-55), but unfortunately, we are unable to assess these factors in our study population. Therefore, it will be important to re-examine the relationship between amygdala connectivity and social impairment in groups of PT with more detailed information about pre and perinatal exposures, parental stress and parenting styles in order to more accurately risk-stratify this population.

As survival continues to improve for prematurely born neonates, it is increasingly important to more accurately determine risk for adverse neurodevelopmental outcomes and develop interventions to mitigate these morbidities. Adolescence is a time of major social and emotional changes, including increased social pressure from peers, emerging independence from parents, and changing interpersonal relationships (66), and our results affirm that PTs continue to experience significant and worsening social and emotional difficulties during this stage of life. Future work should interrogate the relationship between developmental trajectories of altered amygdala connectivity and social impairment in PT to develop and test interventions that may be successful in decreasing this lifelong social vulnerability.

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