

**The Impact of Neighborhood Disadvantage on Amygdala Reactivity:  
Pathways Through Neighborhood Social Processes**

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**Abstract:** Youth growing up in disadvantaged neighborhoods are more likely than their advantaged peers to face negative developmental outcomes, including lower academic achievement and greater mental health and behavioral problems. Although studies have shown that adversity can undermine positive development via its impact on the developing brain, few studies have examined the association between neighborhood disadvantage and neural function, and no study has investigated potential social mechanisms within the neighborhood that might link neighborhood disadvantage to altered neural function. The current study will evaluate the association between neighborhood disadvantage and amygdala reactivity during socioemotional face processing. We will also assess whether and which neighborhood-level social processes are related to amygdala reactivity, and whether these social processes mediate the association between neighborhood disadvantage and altered amygdala reactivity.

To examine these aims in a registered report, we will use a sample of twins aged 7-19 years ( $N=354$  families, 708 twins) recruited from birth records with enrichment for neighborhood disadvantage. Twins completed a socioemotional face processing fMRI task and a sample of unrelated participants from the twins' neighborhoods were also recruited to serve as informants on neighborhood social processes. We hypothesize that neighborhood disadvantage and *low* levels of neighborhood social processes will be associated with *greater* amygdala reactivity to socioemotional faces, and that *low* levels of positive neighborhood social processes will mediate the pathway from neighborhood disadvantage to sensitized amygdala reactivity. The findings from this research will provide foundational knowledge regarding mechanisms of influence of neighborhood disadvantage on amygdala function.

**Keywords:** Neighborhood, amygdala, social cohesion, ecological neuroscience

## 1. Introduction

Today, more than 8.5 million children in the U.S. live in high poverty neighborhoods, where at least 30% of residents live below the poverty line (The Annie E. Casey Foundation, 2019). Growing up in these high poverty neighborhood contexts negatively impacts developmental outcomes, including school readiness and academic achievement, as well as behavioral and emotional problems (Aneshensel & Sucoff, 1996; Kohen et al., 2008; Leventhal & Brooks-Gunn, 2000; Sastry, 2012; Xue et al., 2005). Despite the significant costs of growing up in neighborhood poverty for youth's development, there is limited research to elucidate the underlying mechanisms by which exposures within the neighborhood are instantiated in the brains and bodies of young children to undermine positive development.

A growing body of literature suggests that exposure to early adversity may undermine child development via structural and functional changes in the brain, particularly within the amygdala – a key node of the stress response system – which is responsible for determining the emotional significance of stimuli, threat processing, and fear conditioning (Davis & Whalen, 2001; LeDoux, 2003; LeDoux, 2000; Tottenham & Sheridan, 2010). The amygdala is sensitive to emotional facial expressions, especially those that signal threat or uncertainty (Fusar-Poli et al., 2009; LeDoux, 2003; Ochsner et al., 2012; Shi et al., 2013; Whalen & Phelps, 2009). Many forms of adversity, including childhood poverty, maltreatment, and extreme social deprivation, are associated with heightened amygdala reactivity during socioemotional processing in late childhood, adolescence, and adulthood (Hein & Monk, 2017; Javanbakht et al., 2015; Tottenham et al., 2011). Moreover, individual variability in amygdala reactivity during socioemotional processing has been associated with major psychiatric disorders (Etkin et al., 2004; Hyde et al., 2016; Monk, 2008). Thus, examination of the amygdala as a key node within the limbic system as it responds to socially relevant threat can help to elucidate how early life stress is linked to adverse outcomes in youth.

Although studies are beginning to delineate the potential impacts of adversity on amygdala function, most have focused on family processes (e.g., parenting, maltreatment) or distal factors (e.g., income), often with small convenience samples and little attention paid to the neighborhood context. This omission is surprising given the robust literature demonstrating a strong association between concentrated neighborhood disadvantage and negative behavioral, cognitive, and socioemotional outcomes (Aneshensel & Sucoff, 1996; Burt et al., 2016; Kohen et al., 2008; Leventhal & Brooks-Gunn, 2000; Sastry, 2012). The few studies that have examined neighborhood effects on the brain have found that greater neighborhood disadvantage is associated with greater increases in left and right amygdala volume longitudinally during adolescence (Whittle et al., 2017) and greater amygdala reactivity to emotional faces in adolescence and adulthood (Gard et al., 2017, 2020).

Much of the research examining adversity and amygdala reactivity to emotional faces has purposefully focused on faces indicating threat and distress (i.e., anger and fear) (e.g., Gard et al., 2017; Hein & Monk, 2017). However, amygdala reactivity to neutral facial expressions may also be impacted by neighborhood-level adversities (Gard et al., 2017, 2020). Due to the ambiguity of neutral faces, they may be interpreted as hostile or threatening, especially for those exposed to adversity (Gard et al., 2017; Marusak et al., 2017; Pollak et al., 2000). Moreover, given the role of the amygdala in processing the emotional significance of stimuli and prompting physiological and behavioral responses to perceived threats, the unpredictability of ambiguous neutral faces may be especially salient for youth growing up in neighborhood poverty (Davis & Whalen, 2001; LeDoux, 2003). Thus, the first aim of the current study will be to examine the association between neighborhood disadvantage and amygdala reactivity to facial expressions of threat and distress (i.e., anger and fear), as well as ambiguity (i.e., neutral faces).

Although important, studies of neighborhood disadvantage alone cannot explain *how* neighborhood disadvantage predicts child outcomes at the neural level. In more disadvantaged

neighborhoods, structural characteristics, such as poverty and residential instability, are associated with the breakdown of social ties and norms, which undermines residents' abilities to maintain community-level mechanisms of control that curb violent crime and promote the safety and wellbeing of residents (Morenoff et al., 2001; Sampson et al., 1997; Sampson & Groves, 1989). In particular, a wealth of developmental and sociological literature points to three important neighborhood-level social processes that are associated with neighborhood disadvantage and mediate associations between neighborhood disadvantage and youth behavioral outcomes: (1) neighborhood norms, (2) social cohesion, and (3) informal social control (Aneshensel & Sucoff, 1996; Elliott et al., 1996; Henry et al., 2014; Leventhal & Brooks-Gunn, 2000; Sampson et al., 2002; Xue et al., 2005).

Neighborhood norms are the shared beliefs regarding appropriate or expected behaviors and attitudes of neighborhood residents, especially regarding youth management, protection, and behavior, as well as neighborhood safety and management (Henry et al., 2014). In general, youth in the neighborhood are more likely to engage in deviant behaviors (e.g., smoking, violent behavior) when neighbors approve of, or fail to disapprove of, such behavior (Musick et al., 2008; Reed et al., 2011; Wright & Fagan, 2013). Social cohesion refers to the degree of mutual support, help, and trust amongst neighbors, and informal social control refers to the shared expectation and willingness of neighbors to intervene for the common good in accordance with socially prescribed neighborhood norms (Henry et al., 2014; Sampson et al., 1997). The degree of social cohesion and informal social control within a neighborhood is often combined to form the construct of collective efficacy (Sampson et al., 1997). Neighborhood disadvantage is associated with lower levels of neighborhood collective efficacy, which directly and indirectly impacts youths mental health and subjective well-being, academic achievement, and antisocial behaviors (Aneshensel & Sucoff, 1996; Brody et al., 2001; Dawson et al., 2019; Jackson et al., 2016; Odgers et al., 2009; Wang & Fowler, 2019; Woolley et al., 2008). Thus, a wealth of studies demonstrate that these

neighborhood social processes are important for youth *behavioral* outcomes. However, no study has addressed whether these social processes influence brain development broadly or amygdala reactivity to socioemotional faces specifically. Thus, our second aim is to investigate whether and which social processes within the neighborhood, including neighborhood norms and collective efficacy (i.e., the combination of social cohesion and informal social control), are related to amygdala reactivity to threat and ambiguity, and whether these social processes are a potential mechanism through which neighborhood disadvantage predicts amygdala reactivity during socioemotional processing.

Finally, in examining these questions there are several methodological gaps in the existing literature. First, much of the research focused on linking early adversity to brain function has focused on samples of convenience or on samples of extreme groups/clinical cases (e.g., families reported to child protective services). To best understand neighborhood effects, studies are needed that use representative sampling, but also are enriched for exposure to neighborhood poverty to increase representation of those in the most adverse contexts (Falk et al., 2013). Second, one potential weakness of some research on neighborhood effects is the sole reliance on parent-report of neighborhood conditions, which may include shared method variance with outcomes or bias that may reflect person-specific views of the neighborhood or gene-environment correlation between the reporting of neighborhood processes and youth brain function. Cutting-edge approaches can leverage objective, census-level data and the views of independent raters who live in the same context (i.e., multiple neighbors who are not related to the child) (Burt et al., 2019, 2020). The current study will utilize both approaches.

### **1.1. Specific Aims and Hypotheses**

In the current study, we will examine the association between neighborhood disadvantage and amygdala reactivity during socioemotional face processing of threat (i.e., anger and fear faces) and ambiguity (i.e., neutral faces) in a relatively large sample of youth (age 7-19 years,  $N = 708$ ). Second, we

will assess whether and which social processes within the neighborhood are associated with sensitized amygdala reactivity to threat and ambiguity, and whether these social processes mediate the effects of neighborhood disadvantage on amygdala reactivity during socioemotional processing. Given the wide age range of our sample (age 7 – 19) and the rapid development of the brain during this period, we will assess age as a moderator in the associations between neighborhood disadvantage, neighborhood social processes, and amygdala reactivity. Participants were recruited via birth records to be representative of families with twins living in southcentral Michigan with a high oversampling for exposure to neighborhood impoverishment. Thus, the study boasts a representative sampling frame, but with substantial (over)representation of families living in high poverty neighborhoods, which increases representation of youth facing substantial adversity; often the exact youth missing from other neuroimaging studies. In addition, we supplement this important sampling approach with census-reported data on neighborhood disadvantage and a novel assessment of neighborhood social processes by collecting reports of neighborhood social processes from sets of randomly selected individuals residing in the families' neighborhoods (i.e., neighbors). The current project is thus well-positioned, both in its design and analytic approach, to explore whether and how neighborhood social processes mediate the association between neighborhood disadvantage and amygdala reactivity. Specifically, we hypothesize that 1) neighborhood disadvantage will be associated with *greater* amygdala reactivity to both threat and ambiguity; 2) low levels of collective efficacy and permissive neighborhood norms will be associated with *greater* amygdala reactivity to threat and ambiguity; and 3) these social processes will mediate the pathway between neighborhood disadvantage and amygdala reactivity to socioemotional faces. Additionally, beyond mediation, literature has shown that high levels of collective efficacy often *moderate* associations between neighborhood factors and youth outcomes (e.g., Browning et al., 2014; Dawson et al., 2019; Fagan et al., 2014; Kingsbury et al., 2019) – that is, these social processes can be

protective in high poverty environments. Thus, 4) we will conduct an exploratory analysis examining whether positive neighborhood social processes (e.g., high collective efficacy) buffer (i.e., moderate) the effects of neighborhood impoverishment on amygdala reactivity to threat and ambiguity.

## **2. Materials and Methods**

### **2.1. Participants**

#### **2.1.1. Twin Families**

Participants are part of an on-going longitudinal twin study, the Michigan Twins Neurogenetics Study (MTwiNS). Twins were recruited from the Twin Study of Behavioral and Emotional Development – Child (TBED-C), a project within the Michigan State University Twin Registry (MSUTR) (Burt & Klump, 2013). TBED-C identified twins via birth records, a strong epidemiologic sampling frame, and included both a population-based sample of 528 twin families (1,056 twins) and “at-risk” sample of 502 twin families (1,004 twins). In collaboration with the Department of Vital Records in the Michigan Department of Health and Human Services (MDHHS), primary recruitment was carried out via anonymous mailings to twin families within a 120-mile radius of Michigan State University, an area including Detroit, Flint, Lansing, and other urban areas, as well as substantial parts of suburban and rural Michigan. Recruitment procedures for the population-based and the at-risk samples were identical except that for the latter, mailings were restricted to families residing in modestly-to-severely impoverished neighborhoods that contained over 10.5% of families living below the poverty line (the mean for the state of Michigan at the time, e.g., Burt et al., 2016). This recruitment strategy yielded overall response rates of 62% for the population-based sample and 57% for the at-risk sample. To be eligible for participation in the TBED-C, neither twin could have a cognitive or physical condition that would preclude completion of the study protocol, such as a significant developmental delay or deficit (as assessed via parental screen). Families participating in the population-based sample reported racial group memberships at rates approximating

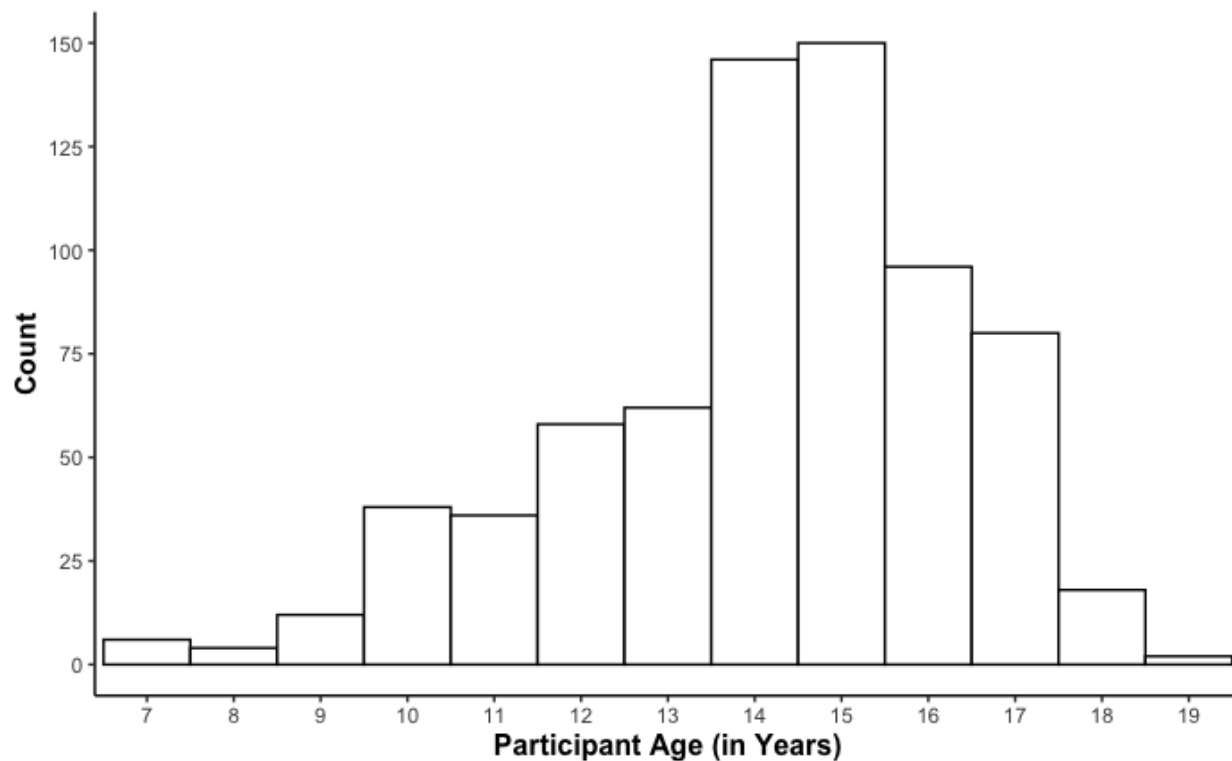


those of area inhabitants (e.g., 86.4% White, 5.4% Black; Burt & Klump, 2013). Compared to the population-based sample, the at-risk sample was significantly more racially diverse (76.3% White, 14.2% Black) and less advantaged, reporting lower family incomes (means of \$72,027 versus \$57,281; Cohen's  $d$  effect size = -0.38), and higher paternal felony convictions ( $d = 0.30$ ) (Burt et al., 2016, 2018). For more details on recruitment procedures, see (Burt & Klump, 2019).

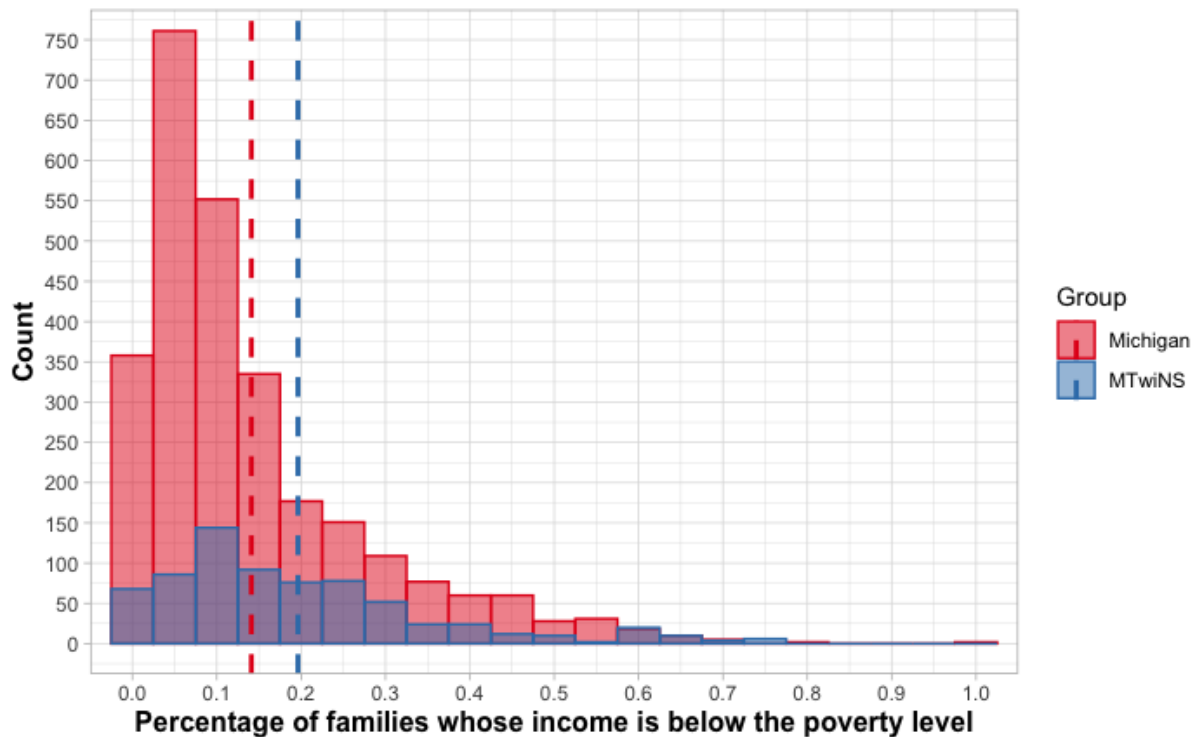
The current sample draws from the families originally eligible for the “at-risk” sample, including all at-risk sample families and those in the population-based sample that would have met criteria for the at-risk sample by living in a neighborhood with above average levels of neighborhood poverty. The sample includes 708 twins (354 families) (54.5% boys; 78.5% White, 13.0% Black, 8.5% other racial/ethnic group membership) aged 7 to 19 years ( $M_{age} = 14.14$ ,  $SD = 2.24$ ; 94.2% of the sample is between 10-17 years old, with only 11 twin pairs < 10 years old and 10 twin pairs > 17 years old; see Figure 1), resulting in a sample that represents families living in south-central Michigan with substantial oversampling for families living in impoverished neighborhoods. In the current MTwiNS sample, 64.1% of twin families live in neighborhoods with >10.5% of families living below the poverty line (mean percentage of families below the poverty line in the neighborhood is 19.6% and ranges as high as 77.0%) (see Figure 2). Note that, though families were originally recruited based on living in neighborhoods with above average levels of poverty, this recruitment occurred when children were 6 – 10 years old and since then some families no longer live in neighborhoods with above average poverty because they moved or because neighborhoods shifted (e.g., gentrified). Participants to be included in the present analyses meet basic fMRI eligibility criteria, such as the absence of metal in their body and willingness to participate in the scanning session (i.e., 557 of 708 twins were eligible for scanning and agreed to scan; see Table 1). This is an ongoing study and we will wait until we have accrued the full sample (>2 years in the future) to test core aims of the grant. However, to ask important, but non-central grant aim questions, we have used successive

“freezes” of the data to make sure that papers use the same groups of families and there is no concern about “stopping rules” (e.g., only included enough data to find significant results). In this case, we will use the current freeze of 354 families (708 twins) for this report. It is important to note that we have not analyzed any data yet at this freeze and the stopping time of this freeze is based on the pause of in-person research due to the COVID-19 pandemic (and thus is fairly random and could not reflect investigator-motivated stopping rules).

**Figure 1.** *Histogram of Participant Age*



**Figure 2.** *American Community Survey 5-Year Neighborhood Poverty Ratings for the MTwiNS sample and the State of Michigan*



*Note.* Dashed lines mark the mean percentage of families below the poverty line for each group (Michigan:  $N = 2,736$ ,  $M = 0.14$ ,  $SD = 0.13$  ; MTwiNS:  $N = 708$ ,  $M = 0.20$ ,  $SD = 0.16$ ).

**Table 1.** *Summary of Data Included in MTwiNS Analysis*

	Number Lost	Participants with Data
<b>Original Sample</b>		708
Declined MRI scan (including declining to remove jewelry/piercings)	27	
Uncomfortable with MRI scan	14	
Dental (e.g., braces, retainer)	17	
Metal in/on the body** (including recent surgery)	12	
Exceeding scanner size restrictions (e.g., overweight, broad shoulders)	5	
Major medical/neurological disorder (e.g., Autism, TBI, tumor)	17	
Incomplete Scan	17	
<b>Total Lost</b>	109*	
<b>Sample with imaging data</b>		557

\**Note.* An additional 21 families (42 twins) received an earlier/pilot version of the task that was not comparable to the current version; these participants were excluded from all analyses.

\*\*includes non-MRI safe implanted medical devices, having BBs/pellets or other non-removable metal inside of body, recent surgery, metallic tattoos, unremovable jewelry.

### **2.1.2. Neighborhood Informants**

A randomly selected sample of unrelated participants (i.e., neighbors of study families) from the same neighborhoods were recruited to serve as neighborhood informants on social processes. Mailing packets were sent to 10 randomly chosen addresses in each twin family's Census tract, inviting one adult resident per household to complete a survey. When an address was no longer inhabited (i.e., the letter was undeliverable), one attempt was made to find a replacement address. All participants provided informed consent. At a previous timepoint, this approach resulted in a sample of 1,804 neighbors (63.2% women; 80.4% White, 11.7% Black, 7.9% other racial/ethnic group membership;  $M_{age} = 52.4$ , with a range of 18 – 95 years) (Burt et al., 2016). The response rate for this previous timepoint was 70%, of which 70% agreed to participate (for a final participation rate of 49%). There was at least one neighborhood informant report available for all but one family, with an average number of 4.39 (SD = 1.64) informant reports per neighborhood. For the current timepoint, data collection from neighborhood informants is ongoing. We have collected data from 966 neighbors so far (61.2% female, 34.1% male, 4.7% missing/prefer not to answer; 86.5% White; 6.8% Black; 4.6% other; 2.1% missing/prefer not to answer). The response rate for the current timepoint is 51%, of which 62% have agreed to participate to date. Upon acceptance of the initial submission, we will freeze all neighborhood informant data, which has continued during the pandemic. We anticipate that for this report, we will, again, have approximately 4 informants or more, on average, per family neighborhood.

### **2.2. Procedure**

Youth and their primary caregivers took part in a day-long visit to the University of Michigan (UM) which included a one-hour fMRI scan for each youth at the UM fMRI lab. Twins provided assent

and parents provided informed consent for themselves and their children. Twins were then introduced to the scanning environment using a mock scanner and completed practice versions of several fMRI tasks. Youth were then scanned using blood-oxygen-level-dependent (BOLD) fMRI while completing several tasks, including the socioemotional face processing task described below. Families also completed a battery of questionnaires and were provided lunch. Additionally, primary caregivers completed a demographic interview with an examiner. Approval was obtained from the UM Institutional Review Board. Participants were compensated for their time.

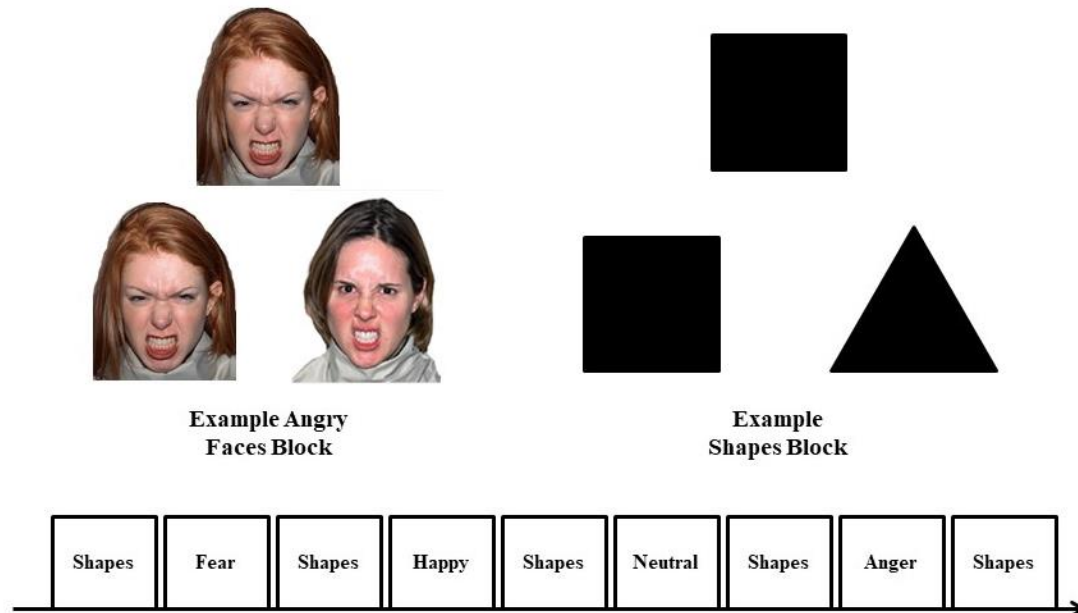
## **2.3. Measures**

### **2.3.1. Socioemotional Face Processing fMRI Task**

Participants performed an implicit emotional face processing task, which consisted of four blocks of perceptual face processing interleaved with five blocks of sensorimotor control (see also Gard et al., 2018; Hariri et al., 2002; Manuck et al., 2007). Participants viewed a trio of faces and selected one of two faces (bottom) identical to a target face (top; Figure 3). Each face processing block consisted of 18 images, balanced for sex, all derived from the NimStim standard set of pictures of facial affect (Tottenham et al., 2009). Each of the four face processing blocks consisted of a different emotional facial expression (i.e., anger, fear, happy, neutral), and participants were randomly assigned to one of four different orders of block presentation. During the sensorimotor control blocks, participants viewed 12 trios of simple geometric shapes (circles, squares, triangles) and selected one of two shapes (bottom) identical to a target shape (top; Figure 3). In the face processing blocks, each of the 18 face trios were presented for 2s with a variable interstimulus interval (ISI) of 2 – 6s for a total block length of 98s. A variable ISI was used to minimize expectancy effects and resulting habituation, as well as to maximize amygdala reactivity throughout the paradigm. In the sensorimotor control blocks, each of the 12 shape trios was presented for

2s followed by a fixation cross for 0.5s, for a total block length of 30s. An additional 4s of crosshair presentation followed each block. Total task time was 578s.

**Figure 3.** *Socioemotional Face Processing fMRI Task*



### 2.3.2. Neighborhood Characteristics

*Neighborhood disadvantage* was measured using the Area Deprivation Index (ADI) at the Census block group level (Kind et al., 2014). ADI scores measure indices of concentrated disadvantaged in the neighborhood via indicators of neighbors' education, employment, income, and poverty (e.g., home ownership rates, percentage of single-parent households, percentage of families living below the poverty line, percentage of those 16 years or older unemployed). ADI scores were calculated using the Singh method, which entails summing Singh's 17 census indicator weights by Singh's factor score coefficients for each indicator (Singh, 2003). The ADI uses the American Community Survey Five Year Estimates in its construction (e.g., the 2015 ADI uses the ACS data from 2015, which is a 5-year average of data obtained from 2011-2015). Importantly, this five-year period overlaps with the timing of data collection for the twin sample. The ADI provides a national percentile ranking at the block group level from 1 to

100. These percentiles are constructed by ranking the ADI from low-to-high for the nation and grouping the block groups into bins corresponding to each 1% range of the ADI. Group 1 is the lowest ADI, indicating the lowest level of “disadvantage” within the nation; whereas the group 100 is the highest ADI, indicating the highest level of “disadvantage.”

*Neighborhood Social Processes* were assessed from neighborhood informants using the Neighborhood Matters questionnaire, which includes three subscales (Henry et al., 2014). Within the Neighborhood Matters questionnaire, the *30-item Social Cohesion scale* assesses perceptions of support, help, and trust within the neighborhood. Each item begins with the stem, “In general, people in this neighborhood...” (e.g., In general, people in this neighborhood...are willing to help their neighbors). Responses are on a 5-point Likert-type scale on which 1 represented “Strongly Agree” and 5 represented “Strongly Disagree.” The *29-item Informal Social Control scale* assesses perceptions that community residents will undertake activities to maintain social order. Each item starts with the stem, “In general, what would someone in this neighborhood most likely do if...” (e.g., In general, what would someone in this neighborhood most likely do if...a child is left at home alone during the evening?). Participants selected 1 of 4 responses: (1) Do nothing; (2) Complain to or discuss with other neighbors; (3) Talk to someone who can do something about it, for example the police, a landlord, or a parent; (4) Do something directly, for example, step in and/or talk to the person or people involved. The *22-item Norms scale* assesses perceptions of behavioral norms in the neighborhood, with a focus on norms regarding child welfare and neighborhood safety. Each item starts with the stem, “In general, people in this neighborhood think...” (e.g., In general, people in this neighborhood think...adults should do something if a child is doing something dangerous, even if it is not their child). Responses are on a 5-point Likert-type scale on which 1 represented “Strongly Agree” and 5 represented “Strongly Disagree.” The social cohesion and

informal social control scales will be standardized and combined into a single measure of collective efficacy.

### 2.3.3. Covariates

**Demographics.** Primary caregivers completed a demographic interview with an examiner. To control for racial group in analyses, race will be coded as: 0 = White, 1 = Non-White. We are controlling for race, a socially constructed category, to control for differences in exposure to systemic racism and the various unequal exposures to poverty, stress, trauma, and opportunity for people of color and those not identifying as White in our country (Pager & Shepherd, 2008; Roberts & Rizzo, 2020; Sellers et al., 2006). Twin's chronological age and gender will also be included as covariates. In addition, we will control for other potential confounding variables related to socioeconomic context, including family income, as defined via primary caregiver reported monthly household gross income and any outside additional sources of income (e.g., government assistance or child support), as well as maternal education, defined via the primary caregiver's highest completed level of education.

**Parenting.** Twin's perceptions of parenting were assessed using the Parental Environment Questionnaire (PEQ). The PEQ is a 42-item inventory that assesses five factorially derived aspects of the parent-child relationship: Conflict, Parental Involvement, Child Regard for Parent, Parent Regard for Child, and Structure (Elkins et al., 1997). For the present study, we will use twin self-reports of the Conflict scale (12 items;  $\alpha = 0.75$ ) to index harsh parenting. Each item is rated on a 4-point Likert scale ranging from "definitely false" to "definitely true." Sample items from the Conflict scale include, "My parent often criticizes me" and "My parent sometimes hits me in anger." Possible scores for the Conflict scale range from 12 to 48 with higher scores indicating greater levels of harsh, conflictual parenting.



## 2.4. fMRI Acquisition and Processing

Each participant was scanned with one of two research-dedicated GE Discovery MR750 3T scanners located at the University of Michigan Functional MRI Laboratory. To take advantage of improvements in MRI data acquisition and harmonize our protocol with the Adolescent Brain Development Cognitive Development Study (Casey et al., 2018), we altered our acquisition protocol after the first 140 families (i.e., 280 twins). For the first 140 families (i.e., 280 twins), one run of 298 volumes was collected for each participant with blood oxygenation level-dependent (BOLD) functional images acquired via a 8 channel head coil and a reverse spiral sequence (TR/TE=2000/30 milliseconds, flip angle = 90°, FOV = 22cm), which covered 43 interleaved oblique slices of 3-mm thickness. High-resolution T1-weighted SPGR images (156, 1mm-thick slices) were aligned with the AC-PC plane, and later used during normalization of the functional images. For the remaining 214 families (i.e., 428 twins), one run of 730 volumes was collected for each participant in which BOLD functional images were acquired with a 32 channel head coil and a gradient-echo sequence with multiband acquisition (TR/TE=800/30 milliseconds, flip angle = 52°, FOV = 21.6cm), which covered 742 interleaved axial slices of 2.4-mm thickness. High-resolution T1-weighted SPGR images (208, 1mm-thick slices) were aligned with the AC-PC plane and used during normalization of the functional images. For both acquisition sequences, BOLD functional images encompassed the entire cerebrum and most of the cerebellum to maximize coverage of limbic structures.

Preprocessing for both acquisition sequences was identical, unless otherwise specified. Functional data were preprocessed and analyzed using Statistical Parametric Mapping version 12 (SPM12; Wellcome Trust Centre, London, United Kingdom). Raw k-space data from reverse-spiral sequence acquisition were de-spiked before reconstruction to image space. For multiband data, task-specific field maps were constructed from volumes of both anterior-to-posterior and posterior-to-anterior phase encoding; field

maps were applied after image construction to reduce spatial distortions and minimize movement artifacts. Slice timing correction was performed using the 23rd slice as the reference slice (reverse-spiral data) or the 2<sup>nd</sup> slice of each 10-slice band (gradient-echo data with multiband acquisition). Data from both acquisition sequences were then spatially realigned to the 10th slice of the volume. These spatially realigned data were coregistered to the high-resolution T1-weighted image, and segmented and spatially normalized into standard stereotactic space (MNI template). Finally, functional data were smoothed to minimize noise and residual difference in gyral anatomy with a Gaussian filter set at 6mm FWHM. After preprocessing, the Artifact detection Tools (ART) software package ([http://www.nitrc.org/projects/artifact\\_detect/](http://www.nitrc.org/projects/artifact_detect/)) is used to detect global mean intensity and translation or rotational motion outliers ( $> 4.5$  SD from the mean global brain activation,  $>2$ mm movement or  $2^\circ$  translation in any direction). For each participant with outliers, individual nuisance covariates are created for each outlier volume and included in the individual-level model (i.e., via spike regression). Any participant with  $>20\%$  motion outliers identified using ART will be excluded from analyses.

Additionally, because of the relatively extensive signal loss typically observed in the amygdala, single-subject BOLD fMRI data will only be included in subsequent analyses if there is a minimum of 90% signal coverage in the bilateral amygdala, defined using the Automated Anatomical Labeling (AAL) atlas in the WFU PickAtlas Tool, version 1.04 (Maldjian et al., 2003). Lastly, participants will be excluded if accuracy performance on the task is less than 70%. Youth with valid imaging data will be compared to youth without valid imaging data to ensure that they do not differ (all  $ps > .05$ ) on youth characteristics (i.e., chronological age, gender, and self-reported race) or primary caregiver characteristics (i.e., education or annual income). If included versus missing participants differ on any of these variables, these variables will be included as covariates in all models (Graham, 2009).

### 3. Experimental Design and Statistical Analyses

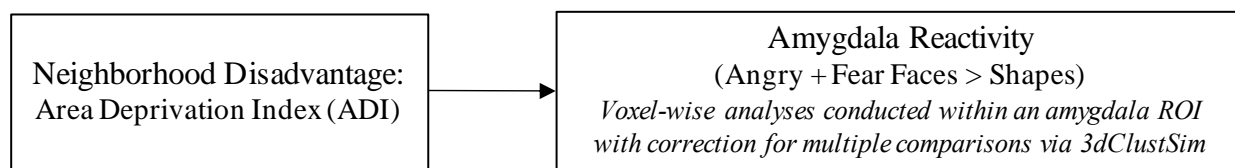
#### 3.1. Functional Data Analysis

The general linear model (GLM) will be used to conduct fMRI data analyses in SPM 12. Linear contrasts employing canonical hemodynamic response functions are used to estimate condition specific BOLD activation for each individual scan. These individual contrast images (i.e., weighted sum of the beta images) are then used in second-level random effects models that account for both scan-to-scan and participant-to-participant variability to determine mean emotion-specific reactivity using one-sample t-tests. The main goal of this study is to examine amygdala reactivity to emotional faces relative to a non-faces condition (shapes), with a focus on threat and distress (i.e., anger and fear faces) and ambiguity (i.e., neutral faces). Thus, we will examine amygdala reactivity within two planned contrasts: (1) *anger and fear > shapes* and (2) *neutral > shapes*. If we have significant findings for *anger and fear > shapes* contrasts, we will provide supplementary, exploratory analyses to examine whether these results are more highly related to anger versus fear by examining *anger > shapes* and *fear > shapes* separately. Consistent with past publications from our lab (Gard et al., 2018), a bilateral amygdala region of interest (ROI) will be defined structurally using the AAL Atlas definition in the WFU PickAtlas Tool, version 1.04 (Maldjian et al., 2003). We will then examine activation within this anatomically defined ROI. For completeness, in supplementary materials, a whole-brain analysis will also be conducted and reported for the two planned contrasts: *anger and fear > shapes* and *neutral > shapes*. We will conduct all amygdala and whole brain analyses using a cluster correction method via the most updated version of the 3dClustSim program using Analysis of Functional NeuroImages (AFNI) software version 16.1.14 (Cox, 1996; Cox et al., 2017) (within the amygdala ROI for the main analyses and across the entire brain for whole-brain supplementary analyses). Consistent with recommendations by Cox and colleagues (2017), we will implement the spatial autocorrelation function (i.e., the -acf option) to model the spatial smoothness of noise volumes. Group-

level smoothing values will be estimated from participants' individual-model residuals using the program 3dFWHMx, and then averaged across those subjects. 3dClustSim uses a Monte Carlo simulation to provide thresholds that will achieve a family-wise error (FWE) correction for multiple comparisons of  $p < .05$  within each ROI. We will use a voxel-wise threshold of  $p < 0.001$  for cluster sizes. Our cluster thresholds will be based on 2-sided tests and use the nearest neighbor definition of "face and edge" (i.e., 3dClustSim command: NN=2).

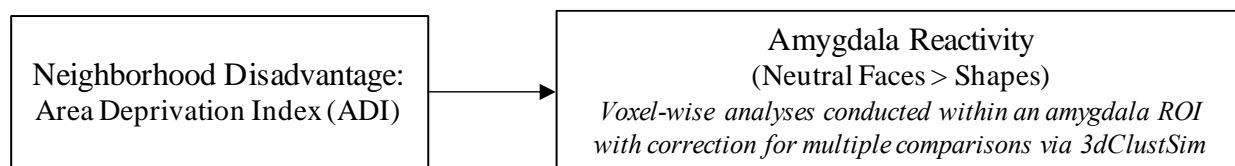
### 3.2. Aim 1: Is neighborhood disadvantage related to amygdala reactivity?

**Figure 4. Main Model 1 (Aim 1)**



\*Note. Covariates: age, sex, race, and scan type

**Figure 5. Main Model 2 (Aim 1)**



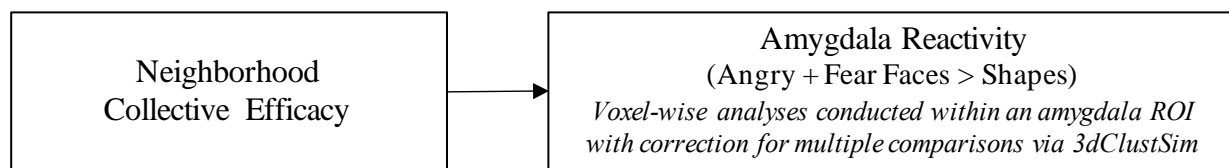
\*Note. Covariates: age, sex, race, and scan type

To determine the association between neighborhood disadvantage and amygdala reactivity, we will run multilevel multiple regression models using *Neuropointillist* (<https://github.com/IBIC/neuropointillist>) in conjunction with the *lavaan* package in R (at the group level, across all participants), with census-derived ADI scores predicting amygdala activation for the contrasts *anger and fear faces > shapes* and *neutral faces > shapes*. Group level activation will be analyzed within an anatomically defined bilateral amygdala ROI from WFU PickAtlas. Our main models will control for twin's age, sex, and race, as well as the scan type (i.e., multiband or spiral acquisition) and utilize a multilevel model to account for twin nesting within

family. Given the wide age range of our sample, we will also examine age as an exploratory moderator in the association between neighborhood disadvantage and amygdala reactivity. If we find significant associations between neighborhood disadvantage and amygdala reactivity controlling for twin's age, sex, and race, and scan type, we will run additional sensitivity models controlling for family income, maternal education, and harsh parenting in order to determine if neighborhood disadvantage predicts amygdala reactivity uniquely over and above these family-level adversities. Although these stringent models have rarely been used in other adversity-brain studies, controlling for the effects of harsh parenting and family SES (income and education), allows us to better attribute effects directly to neighborhood disadvantage, rather than potentially confounded family-level adversities (e.g., Gard et al., 2017). Lastly, all analyses will use full information Maximum Likelihood estimation with bootstrapped standard errors to accommodate for missing data. This approach via Neuropointillist and lavaan provides efficient and robust estimates even in the face of substantial missingness and is robust to skewed data.

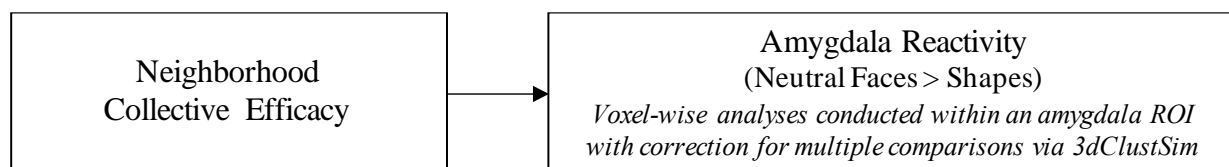
### 3.3. Aim 2: Are neighborhood social processes related to amygdala reactivity?

**Figure 6.** *Main Model 1 (Collective Efficacy) (Aim 2)*

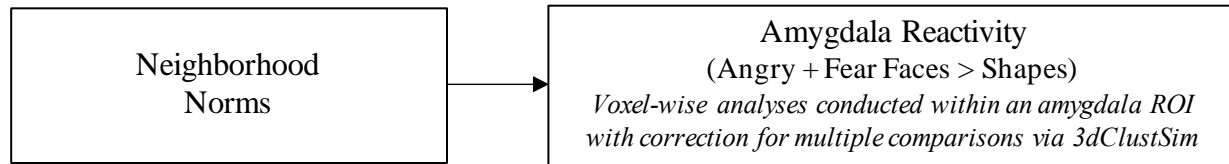


*\*Note.* Covariates: age, sex, race, and scan type

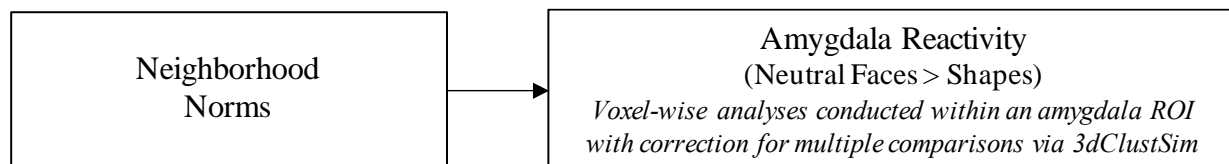
**Figure 7.** *Main Model 2 (Collective Efficacy) (Aim 2)*



*\*Note.* Covariates: age, sex, race, and scan type

**Figure 8.** *Main Model 3 (Neighborhood Norms) (Aim 2)*

*\*Note.* Covariates: age, sex, race, and scan type

**Figure 9.** *Main Model 4 (Neighborhood Norms) (Aim 2)*

*Covariates:* age, sex, race, and scan type

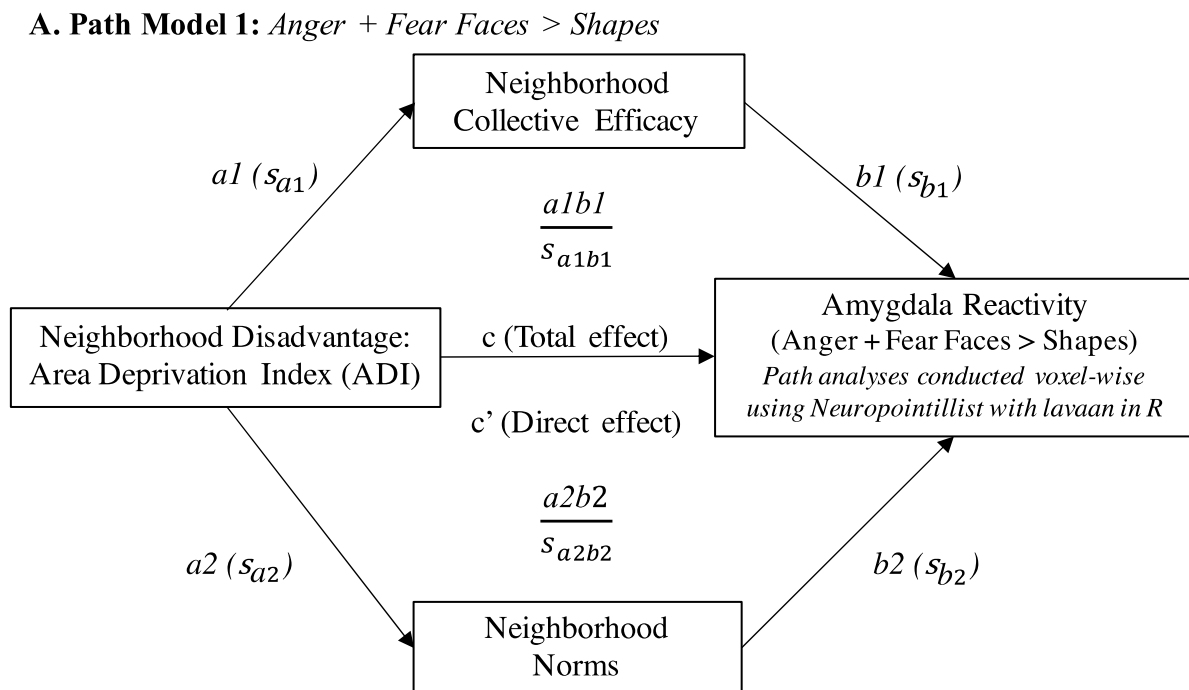
To determine whether or not neighborhood social processes (i.e., neighborhood norms and collective efficacy) are associated with amygdala reactivity, we will run multilevel multiple regression models using *Neuropsychologist* (<https://github.com/IBIC/neuropsychologist>) in conjunction with the *lavaan* package in R (at the group level, across all participants) to test whether each neighborhood social process predicts amygdala activation for the contrasts *anger and fear faces > shapes* and *neutral faces > shapes*. Group level activation will be analyzed within an anatomically defined bilateral amygdala ROI from WFU PickAtlas. Again, in our main models we will control for twin's age, sex, and race, as well as the scan type. Given the wide age range of our sample, we will also examine age as an exploratory moderator in the association between neighborhood social processes and amygdala reactivity.

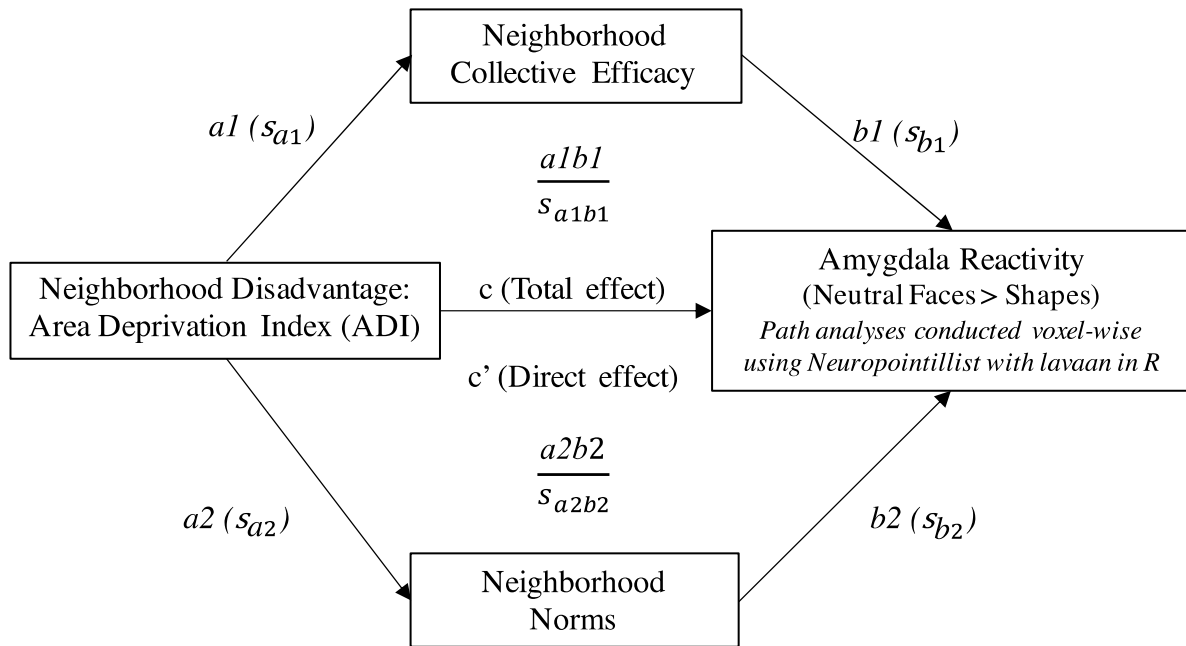
Furthermore, if we find significant associations between neighborhood social processes and amygdala reactivity while controlling for twin's age, sex, and race, and scan type, we will run additional sensitivity models controlling for the other neighborhood social process, in addition to other potential confounders: family income, maternal education, and parenting. Though these stringent models have rarely been used in other adversity-brain studies, we believe it is important to examine the extent to which

associations are due specifically to neighborhood processes, rather than other confounding variables associated with neighborhood disadvantage (i.e., family poverty, lower maternal education, harsh parenting). Finally, given that neighborhood crime, in particular, has been found to be associated with child mental health and individual differences in adolescents' emotional regulatory processing (McCoy et al., 2016; Ramey & Harrington, 2019), if we do find significant associations between neighborhood norms and amygdala reactivity, we will also conduct "specificity" models including items on the Norms scale that are and are not related to neighborhood crime in order to determine if norms related to crime are associated with amygdala reactivity over and above other neighborhood norms.

### 3.4. Aim 3: Do neighborhood social processes mediate the relation between neighborhood disadvantage and amygdala reactivity?

**Figure 10.** *Path Models (Aim 3)*



**B. Path Model 2: *Neutral Faces > Shapes***

Path analyses will be conducted voxel-wise (with correction for multiple comparisons using 3DClustSim) using *Neuropointillist* in conjunction with the *lavaan* package. We will only proceed with testing our path models if two conditions are met: First, amygdala activation from either the *anger and fear faces > shapes* or the *neutral faces > shapes* contrast must be correlated with either neighborhood social process, neighborhood norms and/or neighborhood collective efficacy (i.e., 1 or more of these 4 correlations must be statistically significant). Second, neighborhood disadvantage must also be significantly correlated with the same neighborhood social process that was associated with amygdala reactivity. That is, if we have significant a and b paths for the same neighborhood social process, we will proceed with testing our overall path model (which is likely to include some paths that are and are not significant). In order to limit analytic flexibility and control for the overlap of neighborhood social processes, we will include all neighborhood-level social processes (i.e., collective efficacy and neighborhood norms) in the same path model on the full sample (N=708). We will use full information Maximum Likelihood estimation with bootstrapping to accommodate missing data (and bootstrapping



protects against distortion of effects from violations of distributional assumptions), and we will apply multilevel modeling in *lavaan* to account for nesting within families. Our conservative model will control for covariates, including twin age, sex, and race, and scan type. If any paths or indirect effects are significant, we will also add in potential confounders (i.e., family income, maternal education, parenting) to the model to examine the specificity of effects to neighborhood processes. We will report covariance coverage for all variables.

Lastly, given that previous research has also found high levels of collective efficacy to be protective in high poverty environments (e.g., Browning et al., 2014; Dawson et al., 2019; Fagan et al., 2014; Kingsbury et al., 2019), we will examine neighborhood social processes as a potential moderator in the association between neighborhood disadvantage and amygdala reactivity to threat and ambiguity. In the moderation models, predictors will be mean-centered, and the interaction term will be created as the product of the centered predictors and the models will be run in Neuropointillist with *lavaan* as described above.

#### **4. Timeline**

This is an ongoing study that is fully funded by the National Institute of Mental Health (UG3/UH3 MH114249). All necessary support and approvals are currently in place for the proposed research. We will wait until we have accrued the full sample (>2 years in the future) to test the core aims of the grant. However, to ask important, but non-central grant aim questions, we have used successive “freezes” of the data to make sure that papers use the same groups of families and there is no concern about “stopping rules” (e.g., only included enough data to find significant results). In this case, we will use the data from the current freeze for this registered report, which results is a sample of 354 families (708 twins). Note that we have not analyzed any data yet at this freeze and the stopping time of this freeze is based on the restriction of in-person research due to the COVID-19 pandemic (and thus is fairly random and does not

reflect investigator-motivated stopping rules). Upon acceptance of the initial submission, we will freeze all neighborhood informant data (which has continued during the pandemic) and we expect to complete statistical analyses and a full manuscript within 3 months.

For the purpose of transparency, we include a description of all ongoing papers/analyses within this sample, the variables that were used, and at which freeze the data was examined to outline our knowledge of the data so far.

- Tomlinson, R. C., Burt, S. A., Waller, R., Jonides, J., Miller, A. L., Gearhardt, A. N., ... & Hyde, L. W. (2020). Neighborhood poverty predicts altered neural and behavioral response inhibition. *NeuroImage*, 209, 116536. This study examines the association between neighborhood poverty and inhibitory control using a Go/No-Go fMRI task at the N=140 families freeze. This study uses a single item from the census regarding the number of families in the census tract living below the poverty line and focused on neural activity from a Go/No-Go task. We have not used, nor examined the ADI measure proposed here, nor have we used any data from the current data freeze. Moreover, we have not examined whether the Go/No-Go behavioral nor neural data is related to any of the main study variables proposed in this report.
- *Gard et al. (In prep)* – uses the socioemotional faces task to examine amygdala activity and amygdala-PFC functional connectivity as they change across age and pubertal development using a previous N=240 families freeze. This paper does examine the task proposed in the current study, but does not examine the association between brain activity and any neighborhood predictors, nor outcomes. Findings from this study will inform our understanding of the task and whether we should control for pubertal status in these analyses (we will if related to amygdala reactivity to the contrasts described here). This paper lays the foundation for the current paper as it will show the task effects and delineate their association with key covariates in this study (e.g., age, gender).

- *Peckins et al (In prep)* – examines reward-related neural activity (using a reward task) and its association with age and puberty using the current data freeze. This paper does not examine neighborhood variables, nor does it utilize the emotional faces fMRI task data, as it focuses only on neural reactivity to our (separate) reward task.

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