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Brain-derived neurotrophic factor DNA methylation mediates the association between neighborhood disadvantage and adolescent brain structure

Jo Wrigglesworth[a,b], Joanne Ryan[b,c], Nandita Vijayakumar[d], Sarah Whittle[a,e,*]

[a] Melbourne Neuropsychiatry Center, Department of Psychiatry, The University of Melbourne and Melbourne Health, Parkville, Victoria, Australia
[b] Disease Epigenetics, Murdoch Children's Research Institute, The University of Melbourne, Parkville, Victoria, Australia
[c] Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia
[d] Department of Psychology, University of Oregon, Eugene, OR, USA
[e] Melbourne School of Psychological Sciences, The University of Melbourne, Melbourne, Victoria, Australia

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ABSTRACT

Prior research indicates that socioeconomic disadvantage is associated with prefrontal cortical (PFC) development in childhood and adolescence, however the mechanisms of this link are unclear. This study investigated whether DNA methylation of the brain-derived neurotrophic factor (BDNF, which plays a key role in synaptic plasticity), mediated the association between neighborhood disadvantage and thickness of the PFC in adolescents. Neighborhood disadvantage was measured in 33 adolescents aged 12–13 years using the Socio-Economic Indexes for Areas. Buccal swabs, collected during mid-adolescence (aged 16–18 years), enabled *BDNF* DNA methylation of the widely studied *exon IV* promoter region to be measured. Cortical thickness was assessed during late-adolescence (aged 18–20 years) via T1-weighted magnetic resonance imaging (MRI). A significant negative association between disadvantage and *BDNF* DNA methylation at a specific site of the *exon IV* promoter was identified. Lower levels of methylation were also significantly associated with greater thickness of the lateral orbitofrontal cortex (lOFC), and right medial OFC. Lower levels of DNA methylation at this site also mediated associations between higher disadvantage and thinner bilateral lOFC thickness. These novel findings give insight into a potential biological mechanism that could further our understanding as to why brain development is affected by varying environmental exposures.

1. Introduction

There is much evidence linking exposure to adverse environments with poor adolescent behavior and mental health outcomes (Jaffee, 2017; Moylan et al., 2010; Rogosch et al., 2010). For example, children exposed to socioeconomic disadvantage are more likely to experience poor psychological wellbeing and educational attainment (Bradley and Corwyn, 2002; Brooks-Gunn and Duncan, 1997; McLoyd, 1998), in comparison to their more advantaged peers. Increasing evidence suggests a shift in brain development may partly underlie these associations (Hackman et al., 2010; Hair et al., 2015; Jednorog et al., 2012).

The human brain is a complex organ undergoing both structural and functional changes across the lifespan (Tamnes et al., 2013). This includes a linear increase in white matter volume, and a non-linear decrease in grey matter volume and cortical thickness that peaks in childhood and declines thereafter (Giedd et al., 2015; Mills et al., 2016). One of the last structures to mature during adolescence is the prefrontal cortex (PFC) (Bourgeois et al., 1994; Casey et al., 2008; Huttenlocher, 1979), a highly interconnected structure known for its role in executive functions (McEwen and Morrison, 2013; Miller and Cohen, 2001), emotion regulation (Ahmed et al., 2015) and social behavior (Blakemore and Choudhury, 2006).

Studies suggest that the brain is particularly sensitive to the environment during times of rapid brain development (Johnson et al., 2016). As such, the PFC may be likely to be impacted by adverse environmental exposures, including socioeconomic disadvantage, during adolescence. In support of this, studies measuring relations between socioeconomic disadvantage and brain structure in children and adolescents have observed thinner PFC thickness (Lawson et al., 2013), lower frontal volume (Hanson et al., 2011), and surface area (Noble et al., 2015) in disadvantaged children, and altered developmental trajectories of frontal brain volume during childhood and

* Corresponding author at: Melbourne Neuropsychiatry Center, Department of Psychiatry, The University of Melbourne, Parkville, Victoria, Australia.
E-mail address: swhittle@unimelb.edu.au (S. Whittle).

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adolescence in those with greater disadvantage (defined by either family income, or parental education) (Hair et al., 2015; Noble et al., 2012).

While the majority of findings to date have focused on individual or family-based measures of socioeconomic disadvantage, less work has examined how neighborhood disadvantage might influence adolescent brain structures. Neighborhood disadvantage, which may capture increased exposure to crime, violence and toxins (Evans, 2004; Meijer et al., 2012), in addition to a limited access to recreational areas, transportation systems (Meijer et al., 2012), and health care options, has, so far, been shown to adversely affect mental and biological health in children and adolescents (Kim, 2008). Notably, in a previous analysis of adolescents from the sample investigated in this manuscript, it was observed that higher levels of neighborhood disadvantage were associated with reduced frontal cortical thinning, particularly among adolescents with low exposure to positive parenting (Whittle et al., 2017). Changes in dendritic pruning, myelination and synaptic connections may be responsible. While the underlying mechanisms remain elusive (Casey et al., 2005; Tamnes et al., 2010), there is evidence to suggest that epigenetic mechanisms may play a role.

Epigenetics refers to the reversible regulation of various genomic functions, occurring independently of the underlying DNA code (Jaenisch and Bird, 2003; Rutten and Mill, 2009). The most widely studied of these epigenetic mechanisms is DNA methylation, which commonly involves the addition of a methyl group to cytosine nucleotide followed by guanine (CpG). Such CpG sites are distributed varyingly throughout the genome (Szyf, 2011), but are often found in high density in gene promoter regions (called 'CpG islands') (Rutten and Mill, 2009). DNA methylation of CpG sites in these promoter regions has the potential to silence gene expression (Rutten and Mill, 2009). Changes in DNA methylation patterns have been observed following environmental exposures (e.g. smoking and stress) and in association with a range of phenotypes and disease states, (Henikoff and Matzke, 1997; Rutten and Mill, 2009; Szyf, 2011). Although patterns of DNA methylation are largely tissue and cell specific, differences in peripheral DNA methylation have been observed in various disorders with neurobiological origins, including depression (Januar et al., 2015b; Jovanova et al., 2018), post-traumatic stress disorder (Ryan et al., 2016; Youssef et al., 2018) and dementia (Fransquet et al., 2018). However, so far, it remains unclear to what extent DNA methylation may mediate the association between socioeconomic disadvantage and brain structures, including cortical thickness (Swartz et al., 2017).

Brain Derived Neurotrophic Factor (BDNF*)* is a neurotrophin that plays an important role in regulating neuronal development, plasticity and survival (Lu and Figurov, 1997; Stenz et al., 2015). Much research has found links between adverse environmental exposures and *BDNF* DNA methylation (Kertes, 2017; Moser et al., 2015; Weder et al., 2014). Recent work has also found support for a link between worse neighborhood social environment and increased *BDNF* methylation in adults (Smith et al., 2017). Although rodent work suggests that early adversity leads to persistent changes in *BDNF* DNA methylation, causing altered *BDNF* gene expression in the PFC (Roth et al., 2009), no such research has been conducted in humans. In the only study to examine the association between *BDNF* DNA methylation (*exon IV*) and cortical thickness, a significant inverse correlation was observed (Na et al, 2016). However, as this was only observed in adult patients with major depressive disorder, further studies are required to investigate this association in a healthy adolescent population.

The aims of this study were to, in a longitudinal study of adolescent development, i) investigate the association between neighborhood disadvantage and *BDNF* DNA methylation in the promoter region of *exon IV* (where differential DNA methylation in response to environmental exposures has been observed); ii) examine the association between *BDNF IV* DNA methylation and regional PFC thickness during adolescence; and iii) determine whether *BDNF IV* methylation mediates the relationship between neighborhood disadvantage and PFC thickness. Based on the limited literature to date, we expected a direct positive correlation between neighborhood disadvantage and *BDNF* methylation, and a negative correlation between DNA methylation and cortical thickness. We also hypothesized a mediatory effect of *BDNF* DNA methylation between neighborhood disadvantage and cortical thickness, such that neighborhood disadvantage would be negatively correlated with cortical thickness via an increase in *BDNF* DNA methylation.

2. Methods

2.1. Study population

Participants originated from a larger study, The Orygen Adolescent Development Study (ADS). The ADS is a longitudinal study that aims to investigate the biological and psychosocial factors associated with the risk of emotional and behavioral problems commonly experienced by adolescents (Whittle et al., 2013). Using a self-report scale, The Early Adolescent Temperament Questionnaire – Revised (EATQ-R) (Ellis and Rothbart, 2001), adolescents from the Melbourne Metropolitan area were screened on key temperamental factors thought to promote risk or resilience for psychopathology. Those scoring high or low in effortful control and negative affectivity were oversampled to maximise intra-individual differences in psychological risk. Of the established cohort, 245 adolescents consented to take part in further research. In order to address the aims of the current study, socioeconomic disadvantage data from the ADS baseline assessment (12.8 ± 0.3 years), buccal derived DNA during mid-adolescence (16.7 ± 0.4 years), and magnetic resonance imaging (MRI) data collected during late adolescence (19.1 ± 0.4 years) were analysed. Although MRI data was available from earlier time points, given the aim of assessing *BDNF IV* DNA methylation as a potential mechanism linking SES and brain structure, only MRI data succeeding buccal swab collection was utilized. As outlined below, 33 adolescents had complete data on all variables of interest. Informed written consent was obtained from each adolescent and at least one parent/guardian at each time point. The University of Melbourne Human Research Ethics Committee, Australia, approved this research project.

2.2. Socio-economic disadvantage

Data on socioeconomic disadvantage was collected during early adolescence using the 2006 Socio-Economic Indexes for Areas (SEIFA). Four SEIFA indexes provide measures of neighborhood advantage and disadvantage by using Australian consensus data that takes into account financial burden, crime rates, and health outcomes (Pink, 2006). Based on a prior observation linking the Index of Relative Socio-economic Disadvantage (IRSD) scale with brain development in this sample (Whittle et al.,2017*)*, only this SEIFA index was utilized. Low scores for this index indicate that the individual lives within a neighborhood (small geographical area of approximately 250 homes) containing a higher proportion of disadvantaged people, relative to the wider community, at a given point in time (Pink et al., 2006). Higher scores indicate that a given neighborhood has a relatively low incidence of disadvantage (Pink et al., 2006). Scores are expressed as percentages (lowest score 0, highest score 100).

2.3. BDNF IV methylation assay

BDNF is a complex gene consisting of 11 exons and 9 promoters that differentially regulate their expression (Pruunsild et al., 2007). Areas of interest have been the CpG islands located at promoters I and IV of the *BDNF* gene, where hyper methylation has been associated with psychological disorders/mental health problems including depression (Kang et al., 2015), post-traumatic stress disorder (Kim et al., 2017), bipolar disorder (Carlberg et al., 2014), suicide (Keller et al., 2010), and

schizophrenia (Ikegame et al., 2013). The only study to measure an association between *BDNF* DNA methylation and cortical thickness in a clinical, adult population (Na et al (2016), focused on the *exon IV* region; in the current study this region was also targeted.

One hundred and eighty adolescents provided buccal swab samples when aged 16.7 ± 0.4 years (Epicenter - Catch-All™). Samples were stored in a sterilized container and kept at −80° temperatures before being processed. Following DNA extraction by standard procedures, participant samples with at least 100 ng of DNA underwent bisulphite conversion using the MethylEasy™Xceed Rapid DNA Bisulphite Modification Kit. The converted DNA was amplified, in triplicate, using forward and reverse primers that were previously designed via the SEQUENOM EpiDesigner [http://http://www.epidesigner.com], (Januar et al., 2015a). Primers covered a 372bp region of the *BDNF IV* promoter, located on chr11: 27 723 096-27 723 467 on the UCSC h19 assembly (Supplementary Fig. 1). Within this region, 7 CpG units (1.2, 3, 5.6, 8, 9.10, 11.12, & 15), consisting of 11 CpG sites, were predicted to provide measure of DNA methylation. Primers contained either an adaptor (F: 5'-aggaagagag), or a T7 tag (R: 5'cagtaatacgactcactatagggagaaggct) to facilitate the following *in vitro* transcription (Coolen et al., 2007). For 83 of the samples, sufficient DNA could be extracted at a good quality, enabling successful amplification. Using the Sequenom MassARRAY EpiTYPER platform, DNA methylation was quantified, in triplicate.

2.3.1. Data cleaning

Following quantification, standard quality control processing was performed. Specifically, replicates deviating 0.10 standard deviations above or below the median were treated as missing values. Participants with only one successful replicate were removed. Remaining replicates were averaged, thus providing a mean Beta-value ranging from 0 to 1, with 0 indicating no methylation and 1 equalling complete methylation. Following cleaning, raw methylation data for at least one of the seven predicted CpG units (1.2, 3, 5.6, 8, 9.10, 11.12 & 15) was obtained for 54 participants.

2.4. Measurement of adolescent brain structure

2.4.1. Image acquisition

Participants in late adolescence (19.1 ± 0.4 years) attended a session at the Royal Children's Hospital (RCH), Melbourne, Australia, where MRI was performed to assess brain structure. Using a 3 T Siemens scanner, T1-weighted images were attained using the following parameters: repetition time = 1900 ms; echo time = 2.24 ms; flip angle = 9; field of view = 23 cm; and 176 T1-weighted contiguous slices (voxel dimensions = 0.9 m^3).

2.4.2. Image processing

T1-weighted images were processed using the FreeSurfer 5.3 pipeline. FreeSurfer computes, on a vertex-by-vertex basis, cortical thickness as the shortest distance between the white matter boundary and the pial surface (Fischl and Dale, 2000). The thickness (mm) of 22 a priori regions (11 per hemisphere, **see** Table 2), were then determined using the Desikan atlas (Desikan et al., 2006). A trained group of raters visually inspected surface boundaries, and manually corrected and reprocessed errors resulting from segmentation miss-classification (Whittle et al., 2017).

2.5. Statistical analysis

Using the Stata 15.0 statistical software package, the distributions of brain, SES and epigenetic data were evaluated. DNA methylation data was positively skewed and thus a log transformation was applied, following the rescaling of zero methylated values (i.e. +0.001). Similarly, IRSD data was skewed, however, as this was owing to the design of the index no log transformation was applied (Pink, 2006). Brain regional thickness distributions were relatively normal and thus no transformations were applied.

A group of univariate regression models examined whether neighborhood disadvantage (i.e., IRSD score) was associated with *BDNF IV* methylation (Model 1); *BDNF IV* DNA methylation was associated with measures of PFC thickness (Model 2) and whether IRSD was directly associated with measures of PFC thickness (Model 3). For all models involving PFC ROIs, False Discovery Rate (FDR) correction (Benjamini and Hochberg, 1995), was applied to account for multiple testing (i.e., 22 PFC ROIs = 22 comparisons), thus reducing the risk of false positives. The potential confounding effects of age (i.e. at time of MRI) and sex were tested by including these in additional models. Where indicated, a boostrapping approach tested the mediatory effects of *BDNF IV* DNA methylation, in the association between IRSD and PFC thickness. Five thousand samples were taken and 95% confidence intervals (CIs) were used. Significant mediation was determined when CIs did not contain 0. Of note, mediation analyses were performed when there were significant associations between the independent variable and mediator, and mediator and dependent variable. A significant association between independent variable (IRSD) and dependent variable (PFC thickness) was not required; indirect effects in such situations are common (Preacher and Kelley, 2011).

3. Results

3.1. Characteristics of study population

Thirty-three participants (15 males) had complete data on neighborhood disadvantage (IRSD), *BDNF IV* DNA methylation, and PFC thickness. The characteristics of this cohort and mean levels of *BDNF IV* DNA methylation are summarized in Table 1. The regional prefrontal cortical thickness in the sample are given in Table 2.

3.2. Model 1 – socioeconomic disadvantage (IRSD) and BDNF IV methylation

Of the 7 CpG units measured in the *BDNF IV* promoter region, higher IRSD (i.e., lower neighborhood disadvantage) was positively associated with CpG unit 11.12 DNA methylation ($\beta = 0.011 \pm 0.003$, $p = 0.0001$). See Supplementary Table 1 for statistics. This association remained significant ($p = 0.016$) after controlling for age and sex.

Table 1
Participant Characteristics

Characteristic ($n = 33$)	Mean (SD)
Age, years:	
Early adolescence (T1)	12.8 (0.3)
Mid-adolescence (T2)	16.7 (0.4)
Late adolescence (T3)	19.1 (0.4)
Females, %	54.5
[a]IRSD	57.2 (30.1)
[b]*BDNF* DNA methylation:	
CpG 1.2 ($n = 31$)	4.4 (5.7)
CpG 3 ($n = 21$)	15.02 (10.8)
CpG 5.6 ($n = 31$)	4.3 (3.01)
CpG 8 ($n = 32$)	3.05 (4.1)
CpG 9.10 ($n = 32$)	1.9 (2.7)
CpG 11.12 ($n = 31$)	5.3 (4.2)
CpG 15 ($n = 31$)	6.2 (9.8)

SD = standard deviation.
[a] IRSD: Socio-economic Indexes for Areas (SEIFA) the index of relative socio-economic disadvantage.
[b] Beta values representing the percentage of Brain-Derived Neurotrophic Factor (*BDNF*) DNA methylation.

Table 2
Participant regional prefrontal cortical thickness (n = 33)

Prefrontal cortical region (mm):	Left hemisphere Mean (SD)	Right hemisphere Mean (SD)
Lateral orbitofrontal cortex	2.85 (0.15)	2.86 (0.16)
Medial orbitofrontal cortex	2.63 (0.15)	2.65 (0.14)
Superior frontal	3.07 (0.13)	3.02 (0.13)
Rostral middle frontal	2.65 (0.15)	2.57 (0.12)
Caudal middle frontal	2.85 (0.19)	2.80 (0.14)
Parsopercularis	2.86 (0.17)	2.82 (0.16)
Parsorbitalis	3.01 (0.27)	2.99 (0.20)
Parstriangularis	2.69 (0.17)	2.67 (0.16)
Frontal pole	3.10 (0.27)	3.12 (0.33)
Caudal anterior cingulate cortex	2.92 (0.24)	2.73 (0.20)
Rostral anterior cingulate cortex	3.18 (0.18)	3.05 (0.24)

SD = standard deviation.

3.3. Model 2 - BDNF IV methylation with PFC thickness

BDNF IV CpG 11.12 methylation was associated with regional PFC thickness and remained significant following correction for multiple testing (see Supplementary Table 2). Specifically, DNA methylation was negatively associated with bilateral lOFC (left lOFC: $ß = -0.15 \pm 0.038$, $p = 0.0005$; right lOFC: $ß = -0.14 \pm 0.045$, $p = 0.004$), and right medial orbitofrontal cortex (mOFC) thickness ($ß = -0.11 \pm 0.036$, $p = 0.005$). All associations remained significant ($p < 0.05$) when correcting for age and sex (left lOFC: $ß = -0.14 \pm 0.042$; right lOFC: $ß = -0.14 \pm 0.052$; right mOFC: ß = -0.095 ± 0.041). See Fig. 1 for an illustration of the significant associations.

3.4. Model 3 – neighborhood disadvantage (IRSD) and PFC thickness

IRSD score was not significantly associated with thickness in any PFC ROI (see Supplementary Table 2).

3.5. Mediation

The role of CpG 11.12 DNA methylation in mediating the association between IRSD and bilateral lOFC thickness and right mOFC thickness, was investigated. Significant negative indirect effects were observed between IRSD and bilateral lOFC, via CpG 11.12 methylation (**refer to** Fig. 2). That is, increased IRSD score (i.e., lower neighborhood disadvantage) was associated with lower bilateral lOFC thickness indirectly through increased CpG 11.12 DNA methylation (left lOFC: $CIs = -0.0037, -0.0006$; right lOFC: $CIs = -0.0032, -0.0004$).

4. Discussion

In our cohort of adolescents, a significant association was observed between lower neighborhood disadvantage and increased *BDNF* DNA methylation at 1 of 7 CpG units in the *exon IV* promoter region of the gene. DNA methylation of this gene region has been shown to influence *BDNF* expression (Pruunsild et al., 2007; Tadic et al., 2014), suggesting the potential functional significance of these findings. We also found that increasing DNA methylation of this same CpG unit during mid-adolescence was associated with thinner bilateral lOFC and right mOFC in late adolescence, and that *BDNF IV* DNA methylation mediated the association between neighborhood disadvantage and thickness of the bilateral lOFC, thus partly supporting our hypotheses.

To our knowledge no other study has examined the mediatory role of *BDNF IV* DNA methylation between socioeconomic disadvantage and cortical thickness in adolescents, however there have been individual studies investigating associations between *BDNF* methylation and either measures of socioeconomic disadvantage or brain structure. In a large sample of U.S. adults (aged 55–95 years) from the Multi-Ethnic Study of Atherosclerosis (MESA) project, a worse social environment (i.e. poor neighborhood aesthetic quality, safety and social cohesion) was significantly associated with higher levels of *BDNF* DNA methylation (i.e. of a non-promoter region of this gene), in blood (Smith et al., 2017). This contrasts with our findings indicating that living in a more disadvantaged neighborhood was associated with lower levels of *BDNF IV* DNA methylation. Interestingly, when the MESA study examined neighborhood disadvantage directly, rather than social environment, no significant association with *BDNF* methylation was observed. This discrepancy could be accounted for by the tissue-specific differences in DNA methylation patterns (blood vs. buccal samples), as well as differences in sample populations (adult vs. adolescent) and measures of neighborhood disadvantage (US vs. Australian census data). Furthermore, as our study had no measure of neighborhood social environment, we were unable to explore this difference further.

We found that increased CpG 11.12 methylation was associated with thinner bilateral lOFC and right mOFC thickness. While a number of previous studies have investigated the association between peripheral DNA methylation and brain structure and function, only one other study to date has focused on *BDNF* methylation and cortical thickness (Na et al., 2016). Na et al (2016) observed an inverse correlation between blood *BDNF IV* DNA methylation and cortical thickness in adults diagnosed with major depressive disorder. This supports the results of our study in healthy adolescents. Interestingly, there was also a significant inverse correlation between DNA methylation and serum *BDNF* levels in the previous study, which would support a functional role of DNA methylation in this region of the gene, although the exact CpG sites investigated differed from those in our study (D'Addario et al., 2012; Keller et al., 2010).

A number of previous studies have found associations between socioeconomic disadvantage and prefrontal cortical structure in children and adolescents (Hair et al., 2015; Hanson et al., 2011; Jednorog et al., 2012; Lawson et al., 2013; Noble et al., 2015). Results have been relatively consistent in linking higher socioeconomic disadvantage with a smaller/thinner cortex. Although we found no direct associations in our analysis, higher disadvantage was indirectly associated with thinner bilateral lOFC thickness. In our previous work, disadvantage was associated with relatively reduced thinning over time, albeit in different regions than implicated here (Whittle et al., 2017).

When considering the mediatory role of epigenetic mechanisms in linking associations between socioeconomic disadvantage and brain structure, no studies have examined the effects of *BDNF* DNA methylation. However, other work has investigated DNA methylation as a mechanism linking socioeconomic disadvantage, brain function and mental health. For example, one prior study considered the indirect effects of socioeconomic disadvantage (based on parental education and family income) on depressive symptoms via changes in methylation of the serotonin transporter gene (*SLC6A4*) and amygdala reactivity (Swartz et al., 2017). Specifically, a significant indirect effect was found whereby higher socioeconomic disadvantage was associated with greater increases in depressive symptoms in adolescents, via an increase in *SLC6A4* DNA methylation (measured via bisulphate pyrosequencing over two to three years), and amygdala reactivity (Swartz et al., 2017). Despite the clear discrepancies in the design of this study versus the current study (i.e. measure and source of methylation, along with associations with brain structure vs. function), and statistical methodology, it is interesting to note that both *BDNF* and *SLC6A4* are key genes in interacting signalling pathways, which play a major role in cortical neuronal network connectivity and plasticity (Pezawas et al., 2008). Our novel findings build upon this prior evidence and suggest that DNA methylation may play an important mediatory role in the association between SES and brain development.

4.1. Limitations and future directions

This study has both a number of strengths and weakness, which should be taken into account when interpreting these results. In

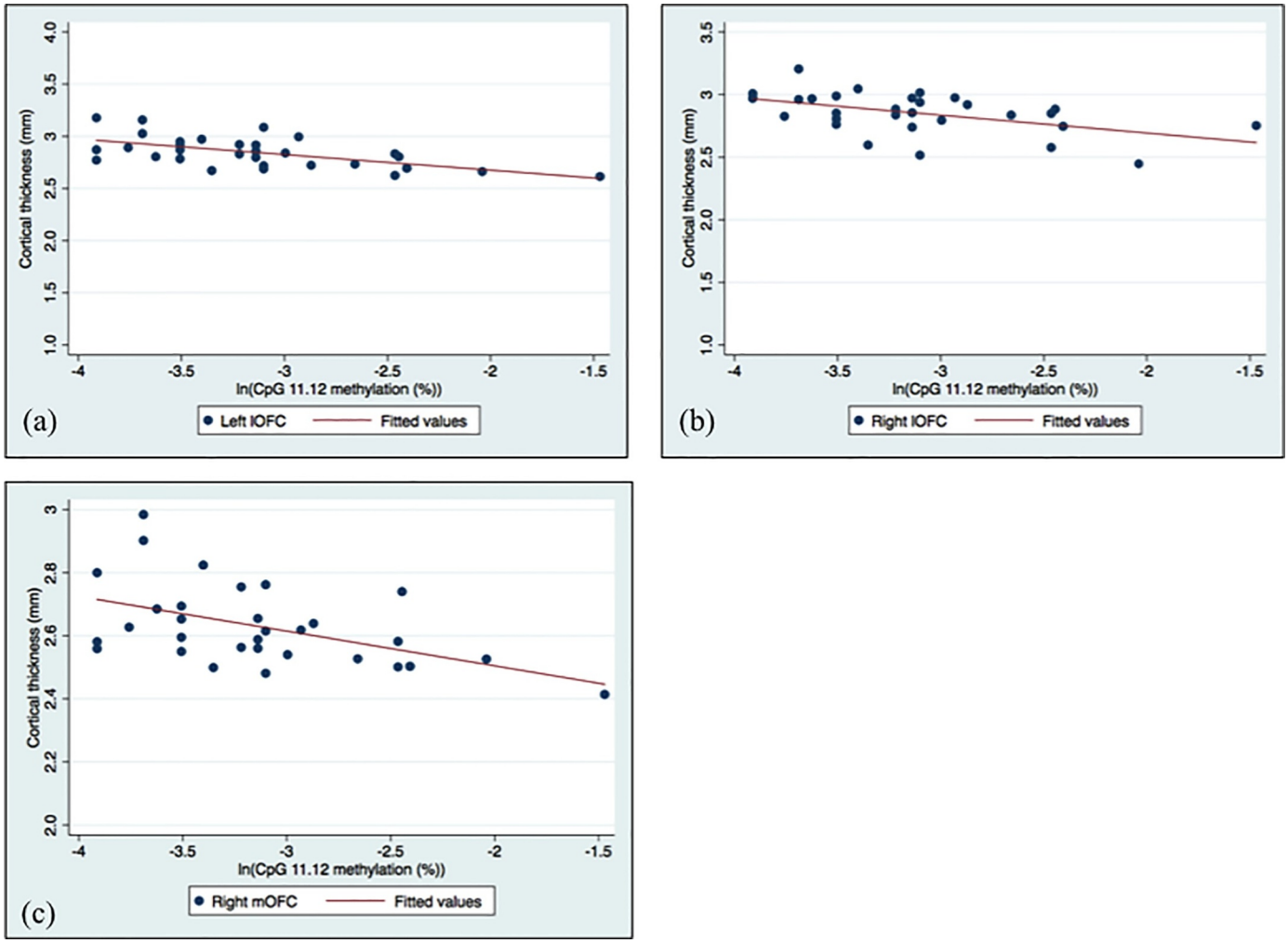

Fig. 1. Significant negative linear associations between *BDNF IV* DNA methylation at CpG 11.12 and (a) left lOFC thickness ($ß = -0.15 \pm 0.038$, $p = 0.0005$), and (b) right lOFC thickness ($ß = -0.14 \pm 0.045$, $p = 0.004$). (c) right mOFC thickness ($ß = -0.11 \pm 0.036$, $p = 0.005$). Note that methylation values are log transformed. lOFC = lateral orbitofrontal cortex. mOFC = medial orbitofrontal cortex.

comparison to other tissue types, buccal tissue is a less invasive approach, particularly for an adolescent population, to measuring DNA methylation, and the least likely to be confounded by the presence of other blood cell types (for example lymphocytes) (Lowe et al., 2013). The relevance of using peripheral tissues when relaying information about the brain has been debatable, however, inter-individual variability between multiple tissue types is minimal, and thus supports the potential of using peripheral tissue to measure changes occurring within the brain (Stenz et al., 2015). Furthermore, a similar embryonic origin to the brain suggests that measures in buccal tissue may be more likely to reflect those occurring within the brain.

Limiting our study was a small sample size, which was mostly related to the impact of a low quality or quantity DNA on PCR amplification, and thus lowered the power of this study. Poor buccal swab

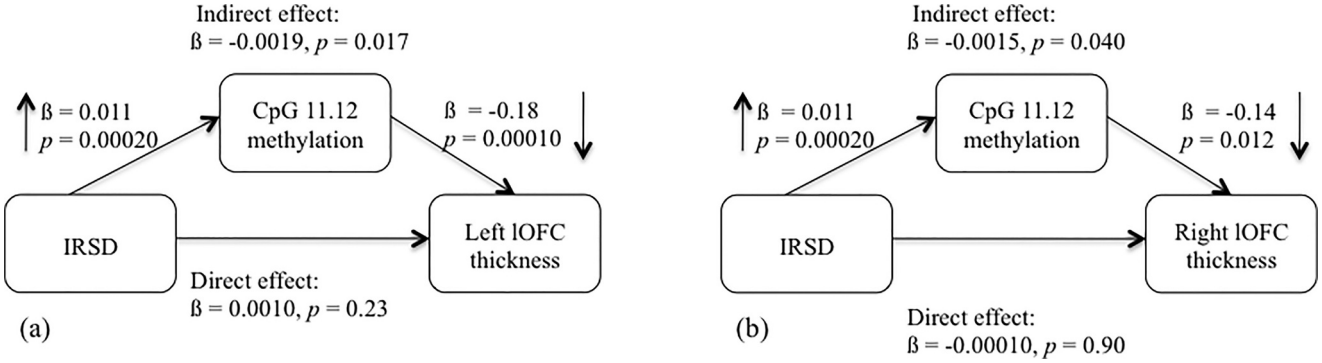

Fig. 2. Significant mediation models for (a) left and (b) right lOFC examining whether *BDNF IV* DNA methylation mediates the association between IRSD and lateral orbitofrontal cortical thickness.
For both models, higher IRSD (i.e. lower neighborhood disadvantage) was associated with lower lOFC thickness via increased CpG 11.12 methylation. lOFC = lateral orbitofrontal cortex; IRSD = Index of Relative Socio-economic Disadvanage. ($n = 31$).

collection techniques, such as insufficient brushing/scrapping, are the likely cause for the low yields observed in our samples. Secondly, as we have no measure of *BDNF* expression we cannot correlate measures of DNA methylation with changes in gene expression.

Neighborhood disadvantage is a census-based measure that takes into account a number of factors that define a detrimental neighborhood, including crime, and financial burden. However, we do not know what might be the specific aspects of neighborhood disadvantage (e.g., family stress, cognitive stimulation, environmental toxins or nutrition) driving associations with *BDNF IV* DNA methylation. Future studies should consider other measures of SES, including family-level indicators (e.g., parental education and occupation), in addition to parental emotional and cognitive function, as these factors may be important in influencing adolescent brain development via epigenetic mechanisms.

Lastly, although data was collected with temporal separation (i.e., neighborhood disadvantage in early adolescence, DNA methylation in mid-adolescence and brain structure in late adolescence), we did not have repeat measures of DNA methylation or brain structure and hence our interpretation of the direction of effects is speculative. Future studies with larger sample sizes and longitudinal designs would improve the power of the analysis and the ability to infer directionality of effects.

5. Conclusion

The aim of this study was to examine epigenetic associations, specifically *BDNF IV* DNA methylation, with neighborhood disadvantage, and regional PFC thickness. Using a combination of neuroimaging and DNA methylation data, our findings suggest that neighborhood disadvantage may be associated with a change in *BDNF IV* DNA methylation, which in turn may influence cortical development. While this is the first study to measure these associations in adolescents, future longitudinal research, with a larger sample size, is needed to corroborate our findings.

Conflict of interest

None to be reported.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.pscychresns.2018.12.012.

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