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Neural connectivity biotypes: associations with internalizing problems throughout adolescence

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Original Article

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Abstract

Background. Neurophysiological patterns may distinguish which youth are at risk for the well-documented increase in internalizing symptoms during adolescence. Adolescents with internalizing problems exhibit altered resting-state functional connectivity (RSFC) of brain regions involved in socio-affective processing. Whether connectivity-based biotypes differentiate adolescents' levels of internalizing problems remains unknown.

Method. Sixty-eight adolescents (37 females) reported on their internalizing problems at ages 14, 16, and 18 years. A resting-state functional neuroimaging scan was collected at age 16. Time-series data of 15 internalizing-relevant brain regions were entered into the Subgroup-Group Iterative Multi-Model Estimation program to identify subgroups based on RSFC maps. Associations between internalizing problems and connectivity-based biotypes were tested with regression analyses.

Results. Two connectivity-based biotypes were found: a *Diffusely-connected* biotype ($N = 46$), with long-range fronto-parietal paths, and a *Hyper-connected* biotype ($N = 22$), with paths between subcortical and medial frontal areas (e.g. affective and default-mode network regions). Higher levels of past (age 14) internalizing problems predicted a greater likelihood of belonging to the *Hyper-connected* biotype at age 16. The *Hyper-connected* biotype showed higher levels of concurrent problems (age 16) and future (age 18) internalizing problems.

Conclusions. Differential patterns of RSFC among socio-affective brain regions were predicted by earlier internalizing problems and predicted future internalizing problems in adolescence. Measuring connectivity-based biotypes in adolescence may offer insight into which youth face an elevated risk for internalizing disorders during this critical developmental period.

Symptoms of internalizing disorders (especially anxiety and depression) frequently co-occur (Garber & Weersing, 2010; Hankin et al., 2016) and tend to increase during adolescence (Petersen et al., 2018), particularly in girls (Altemus, Sarvaiya, & Epperson, 2014; Rutter, Caspi, & Moffitt, 2003). Importantly, difficulties associated with internalizing symptoms in adolescence, even those that are subclinical, can persist into adulthood (Petersen et al., 2018; Winefield, Hammarström, Nygren, & Hägglöf, 2013) and predict poorer physical health (Jamnik & DiLalla, 2019) and suicidal ideation (Betts et al., 2016; Fergusson, Horwood, Ridder, & Beautrais, 2005). Studies of the hierarchical structure of mental health problems have consistently identified internalizing problems as a fundamental dimension common across multiple mental health disorders (Kotov et al., 2017; Lahey et al., 2004, 2008), accounting for the comorbidity of depressive, anxiety and stress disorders in adolescents (Blanco et al., 2015) and adults (Carragher, Krueger, Eaton, & Slade, 2015). However, internalizing behaviors are inwardly focused and often go unnoticed and untreated (Stein & Fazel, 2015). Importantly, adolescence is a critical period in which to intervene and halt the progression of long-term mental health difficulties. Identifying biomarkers related to internalizing problems may provide clues about which adolescents show elevated levels of these symptoms or risk for future problems (Strimbu & Tavel, 2010).

Brain connectivity is a promising potential biomarker of internalizing symptoms. Resting-state functional connectivity (RSFC) provides a metric of coordinated activity across regions in the absence of task demands and offers insight into stable neural patterns of socio-emotional processing with individual specificity (Finn et al., 2015; Gratton et al., 2018). Studies of RSFC have implicated certain networks (i.e. collections of brain regions with intrinsically coordinated activity) as being particularly relevant to internalizing symptoms. Here, we examined the roles and overall functional neural architecture of the default mode (DMN), limbic ventral affective (VAN), and cognitive control (CCN) networks in relation to internalizing symptoms in adolescents using a biotyping approach. We examined these specific networks based on prior work documenting altered RSFC of the DMN, VAN, and CCN in adolescents

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with internalizing psychopathologies, such as depression and anxiety (Burkhouse et al., 2019; Cullen et al., 2014; Roy et al., 2013). Given existing evidence, we expected that internalizing symptoms would be related both to aberrant connectivity within the DMN, VAN, and CCN, as well as differential connectivity between the networks.

The DMN comprises a network of regions (e.g. medial prefrontal cortex, posterior cingulate cortex, posterior parietal cortex) involved in self-referential processing and autobiographical memory, such as introspection (Philippi, Tranel, Duff, & Rudrauf, 2015). Indeed, maladaptive self-referential processes, including rumination and repetitive negative thought (Berman et al., 2011) are present in internalizing psychopathology (McEvoy et al., 2019). Furthermore, elevated DMN RSFC has been found in adolescents with clinical depression (Ho et al., 2015) and youth with elevated levels of subclinical anxiety and depression symptoms (Coutinho et al., 2016).

The VAN encompasses areas involved in emotion processing and regulation (e.g. amygdala, hippocampus, insula) (Catani, Dell'acqua, & Thiebaut de Schotten, 2013). Heightened connectivity of this network may underlie the negative mood and aberrant affective processing that characterizes internalizing problems (Ladouceur et al., 2005). Higher VAN connectivity has been found in adolescents and adults diagnosed with depression (Connolly et al., 2017; Tang et al., 2018) and anxiety (Liu et al., 2015; Roy et al., 2013) compared to healthy controls. In healthy adolescents, higher RSFC within the salience network (which shares regions with the VAN) is associated with higher levels of rumination, suggesting that connectivity among regions involved in emotion processing may contribute to recurring inwardly-focused negative thoughts (Ordaz et al., 2016).

Finally, the CCN includes regions implicated in goal-directed processes implicated in emotion regulation (e.g. dorsolateral prefrontal cortex; dlPFC, dorsal anterior cingulate cortex; dACC). Lower connectivity of the CCN may contribute to ineffective cognitive regulation of negative thoughts (Goschke, 2014). Lower RSFC of the CCN is found in adolescents with clinical depression (Pannekoek et al., 2014), those with familial risk for depression (Clasen, Beevers, Mumford, & Schnyer, 2014), and those with anxiety (Yang et al., 2018). Additionally, decreased CCN connectivity has been associated with elevated levels of depression symptoms in healthy adults (Schultz et al., 2018) and adolescents (Strikwerda-Brown et al., 2015).

Aberrant between-network connectivity also relates to internalizing symptoms. For example, adolescents with lower RSFC between the amygdala (a region of the VAN) and regions of the CCN exhibited escalating symptoms of depression over time (Scheuer et al., 2017). Similarly, reduced RSFC between the amygdala and prefrontal areas has been shown in relation to greater trait anxiety (Greening & Mitchell, 2015). Additionally, lower RSFC connectivity of a 'salience and emotion network' (similar to the VAN) with the CCN from adolescence to adulthood has been found in adolescents with internalizing symptoms (Burkhouse et al., 2019). Given the role of the VAN in emotion processing, and the role of the CCN in cognitive control, lower coupling between these networks may indicate disrupted emotion regulation. Taken together, RSFC studies suggest that internalizing symptoms, at clinical and nonclinical levels, are associated with alterations in RSFC within and between brain networks.

Recent research suggests that RSFC connectivity patterns track with the emergence of internalizing symptoms. Among adolescents with familial risk for depression, those with lower RSFC within CCN regions (e.g. between the inferior parietal lobule and subgenual ACC, and between the left and right dorsolateral PFC) were more likely to develop depression 3–4 years later (Hirshfeld-Becker et al., 2019). Further, task-based functional connectivity among regions of the VAN and CCN (e.g. amygdala and prefrontal areas) while viewing emotional faces predicts changes in internalizing symptoms among nonclinical youth (Gard et al., 2018). Although previous work has identified patterns of RSFC that are associated with changes in internalizing problems, no work has evaluated whether RSFC connectivity profiles identified through data-driven approaches naïve to the distribution of internalizing symptoms can be used to prospectively *predict* internalizing symptoms.

Defining neural profiles, or 'biotypes' (Williams, 2017), based on patterns of coordinated activity in networks (e.g. within- and between-network RSFC) may provide insight into affective processes that are otherwise masked by the use of group-averaged approaches (e.g. comparing participants with a disorder to healthy volunteers) such as in the case of adolescent depression (Chahal, Gotlib, & Guyer, 2020) or by examining relations with a specific symptom or set of symptoms. For example, adults with depression have been differentiated from healthy adults based on the connectivity-based subgroup (i.e. biotype) to which they belong (Price et al., 2017). Subtypes of depression have also been identified based on individuals' connectivity-based biotype status (Price, Gates, Kraynak, Thase, & Siegle, 2017). Given these findings, parsing individuals into subgroups based on their RSFC patterns may facilitate identification of individuals with heightened affective dysregulation and shared psychosocial impairments.

It remains unknown, however, whether connectivity-based biotypes differentiate youth with elevated *v.* non-elevated subsyndromal internalizing problems. Additionally, it is unclear whether connectivity-based biotypes reflect only concurrent internalizing problems or a stable pattern of problems throughout adolescence. The novel application of connectivity biotyping to samples of adolescents in longitudinal studies may offer insights to these important inquiries.

The current study sought to test these questions in an undiagnosed adolescent sample whose internalizing problems were measured at age 14, 16, and 18 years and who completed a resting-state functional scan at age 16 years. The Subgroup Group Iterative Multiple Model Estimate (S-GIMME) (Gates, Lane, Varangis, Giovanello, & Guiskewicz, 2017) approach utilized in previous studies (Price, Gates, Kraynak, Thase, & Siegle., 2017; Price et al., 2017) was applied to adolescents' neuroimaging data to test for connectivity-based biotypes with distinct RSFC profiles and to examine resulting biotypes in relation to internalizing problems. We hypothesized that (H1) adolescents would be parsed into connectivity-based biotypes based on similarity in RSFC patterns of internalizing symptom-relevant networks (i.e. the DMN, VAN, and CCN) at age 16, (H2) with one biotype displaying RSFC patterns previously reported in relation to internalizing symptoms, e.g. heightened VAN-DMN and/or lower VAN-CCN (Burkhouse et al., 2019; Geng, Li, Chen, Li, & Gu, 2016; Price, Gates, Kraynak, Thase, & Siegle, 2017; Scheuer et al., 2017). We further hypothesized that connectivity-based biotype would be a biomarker of internalizing problems throughout adolescence, where (H3) prior symptoms (age 14) would predict biotype status at age 16, (H4) current (age 16) problems would be associated with biotypes, and (H5) biotypes would predict future (age 18) internalizing problems (Prenoveau et al., 2011).

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In exploratory analyses, we examined sex differences, building on a large literature documenting elevated internalizing problems in girls relative to boys during this period (Altemus et al., 2014).

Method and materials

Participants

The sample included 73 Mexican-origin adolescents (40 female, *M* Age = 16.26 years, S.D. = 0.50) enrolled in a neuroimaging sub-study who were recruited from a larger 10-year, longitudinal study of risk for and resilience to substance use problems. Participants in the parent study included 674 families with a child in fifth grade (*M* Age = 10.85 years, 50% female) who were drawn at random from school rosters during the 2006–2007 and 2007–2008 academic years. Participants were recruited into the neuroimaging sub-study based on data from two measures indicating they had either engaged in ($N = 37$) or abstained from ($N = 36$) substance use in the 9th grade (age 14–15). This enriched sample excluded any adolescents taking psychotropic medications. Four participants were excluded from analyses due to excessive movement in the scanner (i.e. 20% of the data were removed by censoring), and one was excluded due to incomplete self-reported data, resulting in a sample of 68 (54.4% female) youths included in the current analyses. Previous work published on this sample has reported on patterns of RSFC in association with years since initial substance use onset (Weissman et al., 2015) and with changes in family income across adolescence (Weissman, Conger, Robins, Hastings, & Guyer, 2018).

Measures

Internalizing problems

Participants completed the Mini Mood and Anxiety Symptom Questionnaire (MASQ) (Watson et al., 1995) at ages 14, 16, and 18. The MASQ provides a reliable and valid assessment of current levels of internalizing problems (Lin et al., 2014), and includes four subscales: General Distress (e.g. 'How much have you felt depressed?'), Anxious Arousal (e.g. 'Have you felt like you were having trouble swallowing?'), Anhedonic Depression (e.g. 'How much have you felt like you had a lot to look forward to?'), and Anxiety (e.g. 'How much have you felt keyed up or on edge?'). Alphas for the full-scale MASQ score ranged from 0.85–0.90 across the three measurement occasions. Following prior research in the larger study of the current sample (Parrish, Atherton, Quintana, Conger, & Robins, 2016), internalizing problems were modeled as latent variables at each measurement occasion. An internalizing problems factor (λs = 0.35–0.93, 0.35–0.96, 0.25–0.97; total $\omega = 0.92$, 0.89, and 0.93 at ages 14, 16, and 18, respectively) was created using indicators of General Distress, Anhedonic Depression, Anxiety, and Anxious Arousal at each wave. Internalizing factor scores were computed using regression; the factor model was created using maximum likelihood estimation in the Lavaan package in R (Rosseel, 2012).

To reduce the impact of extreme observations on data analyses, a 90% winsorization adjustment was used, such that internalizing problem scores below the 5th percentile were set to the 5th percentile, and scores above the 95th percentile were set to the 95th percentile (online Supplemental Fig. S1). The advantage of the winsorization technique in lessening extreme observations and the estimation of regression coefficients has been described in previous work (Chen, Welse, & Chan, 2001; Yale & Forsyth, 1976).

Other relevant measures

Participants self-reported their pubertal status using the Petersen Physical Development Scale (Petersen, Crockett, Richards, & Boxer, 1988) annually from ages 10–15 years. Pubertal timing (i.e. age of pubertal onset) was estimated using separate Gompertz growth models for males and females (Chahal et al., 2018). Additionally, at age 16, participants reported on their use of marijuana and alcohol in the past 3 months using the Alcohol, Tobacco, and Other Drugs survey (Elliot, Ageton, & Huizinga, 1982). Adolescents completed the Woodcock-Johnson III IQ test (Woodcock, McGrew, & Mather, 2001) at approximately age 10 when entering the parent study. Major Depressive Disorder and Generalized Anxiety Disorder diagnosis variables were based on the Computerized Diagnostic Interview Schedule for Children [C-DISC; (Blouin, Perez, & Blouin, 1988)] and collected at age 16. The C-DISC is a structured interview covering 36 mental health disorders for children and adolescents using the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria.

Resting-state scan

Functional magnetic resonance imaging (fMRI) data were collected on a Siemens 3 T TIM Trio MRI scanner with a 32-channel head coil. During a resting-state fMRI scan, participants were instructed to lie still with their eyes open and to focus on a white fixation cross on the screen. A series of 223-time points were acquired with an echo-planar imaging T2-weighted sequence: echo time (TE) = 27 ms; repetition time (TR) = 2000 ms, isometric voxel size = 3.5 mm^3, slices = 35 (interleaved acquisition), field of view (FOV) = 224 mm, flip angle = 80°. The first three volumes were discarded to ensure magnet stabilization. To register the functional images to anatomical space, a high-resolution T1-weighted scan was acquired using an MPRAGE sequence (TE = 4.33 ms; TR = 2500 ms; isometric voxel size = 0.9 mm^3; slice thickness = 0.95 mm; slices = 208; FOV = 243 mm; flip angle = 7°). fMRI data preprocessing procedures are described in the online Supplemental Material.

Data analysis

Regions of interest (ROIs)

The original GIMME algorithm is well-suited for connectivity analyses of 5–15 ROIs (Gates & Molenaar, 2012). We selected the same 15 ROIs examined by Price, Lane, et al. (2017), given that these regions have been implicated previously in neuroimaging studies of internalizing symptoms (Burkhouse et al., 2019; Geng et al., 2016; Scheuer et al., 2017). Similar to Price, Lane, et al. (2017), 8 mm radius spherical masks were applied around peak coordinates of (1) CCN regions including dACC, bilateral posterior parietal cortex (PPC), and left dlPFC; (2) VAN nodes including bilateral nucleus accumbens (Nacc), bilateral anterior insula, bilateral ventrolateral PFC (VLPFC), bilateral amygdala, and subgenual ACC (sgACC); and (3) DMN regions including posterior cingulate cortex (PCC) and perigenual ACC (pgACC) (coordinates for all ROIs are included in Supplemental Material). For each participant, the mean time series across voxels within each ROI was extracted and inputted to the S-GIMME algorithm.

Connectivity-based biotypes

Briefly, GIMME is an unsupervised approach (i.e. no *a priori* assignment of clustering threshold or grouping variables) that

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utilizes a structural equation framework (Kim, Zhu, Chang, Bentler, & Ernst, 2007) to arrive at the group and individual connectivity estimates among ROIs. In the structural equation framework, significant paths between ROIs are directed, indicating that one ROI statistically predicts another (i.e. ROI → ROI), rather than representing an undirected association. In validation tests, GIMME reliably estimates group- and individual-level connectivity maps, including specific region-to-region directional paths (Gates & Molenaar, 2012). S-GIMME (i.e. subgroup version of GIMME) uses a community detection algorithm to determine whether subgroups exist in individual connectivity maps. This approach has demonstrated strong performance in correctly separating subgroups in Monte Carlo simulations (Gates et al., 2017). Details about S-GIMME procedures are described in the Supplemental Material. S-GIMME (Gates et al., 2017) was used to detect connectivity-based biotypes (Hypotheses 1–2), which were then examined in relation to internalizing problems (Hypotheses 3–5).

Connectivity-based biotype associations with internalizing problems

For hypotheses 3–5, three regression models were used to test whether (1) past levels of internalizing problems (age 14) predicted connectivity-based biotype status (age 16), (2) current internalizing problems were associated with biotype, and (3) biotype predicted later internalizing problems (age 18). First, logistic regression was used to test the effect of age 14 internalizing problems and the interaction of internalizing problems and gender on biotype assignment. Second, multiple linear regression was used to test the association between current internalizing problems, biotype, and gender. Multiple linear regression was also used to test the main and interactive effects of biotype and gender on age 18 internalizing problems, controlling for age 16 internalizing problems. All models included covariates of IQ given documented associations between IQ and network connectivity (Suprano et al., 2019), substance use history used for recruitment (engaged in substance use as of age 14–15, yes/no) given our previous findings that substance use is associated with cognitive-reward connectivity (Weissman et al., 2015), and head motion given known effects of motion on connectivity estimates (mean framewise displacement) (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012).

Connectivity-based biotype associations with internalizing problems trajectories

In addition to testing associations between biotype and internalizing problems at each time-point, we used longitudinal multilevel modeling to derive per-person empirical Bayesian estimates of intercept and linear slope; we then used multiple linear regression to test the association between longitudinal model trajectory parameter estimates and biotype.

Network paths and internalizing problems

As an exploratory post-hoc analysis, all significant paths present in individual-level connectivity maps (which could have resulted from individual-, subgroup-, or group-level path presence) were tallied (i.e. binarized as present/absent) for each participant to compare between- and within-network functional connectivity across subgroups as in (Price et al., 2017). Path weights were not compared as they were only estimated if the region-to-region path was included in an individual's model.

Results

The average standardized internalizing problems factor scores were −0.02 (S.D. = 0.53, range = −0.33–1.63), −0.02 (S.D. = 0.18, range = −0.17–0.48), and −0.05 (S.D. = 0.1, range = −0.13–0.26) at ages 14, 16, and 18, respectively. At ages 14 and 16, females exhibited higher internalizing scores ($M = 0.11$ and 0.03) than males ($M = -0.17$ and −0.08), $t(45.60, 54.88) = 2.35$ and 2.74, $p = 0.023$ and 0.008. Adolescents who had engaged in substance use (based on age 14–15 study recruitment status) showed higher age 14 internalizing scores ($M = 0.12$) than those who had not engaged in substance use ($M = -0.17$), $t(43.09) = -2.39$, $p = 0.021$.

Hypotheses 1–2: connectivity-based biotypes

In addition to lagged autocorrelations at every ROI, several paths were significant and present at the group level (Fig. 1), comprising connections between bilateral regions (i.e. right to left amygdala; left to right Nacc; left to right VLPFC), and connections between regions of the VAN, DMN, and CCN networks. Two biotypes emerged from the community detection approach. Biotype 1 ($n = 46$, 60.86% female) exhibited paths between distal brain regions (i.e. paths were longer and connected regions further in anatomical space), such as between regions of the VAN and the CCN (e.g. right VLPFC and right PPC), and the VAN and the DMN (e.g. the left Nacc and pgACC). Biotype 2 ($n = 22$, 40.9% female) exhibited paths connecting regions closer in anatomical space, including ventromedial areas, particularly between ipsilateral subcortical areas (e.g. left amygdala and left Nacc; right amygdala and right Nacc) and among prefrontal regions (e.g. left VLPFC and pgACC; pgACC and sgACC). Overall, Biotype 2 showed a greater number of subgroup-specific paths, compared to Biotype 1. All significant contemporaneous paths were accompanied by significant lagged paths; however, we focused on contemporaneous paths as these are more directly interpretable.

Based on these RSFC patterns, the two biotypes were labeled *Diffusely-connected* (i.e. characterized by distal subgroup-specific paths) and *Hyper-connected Ventromedial* (i.e. characterized by a greater number of subgroup-specific paths overall, specifically short-range ventromedial prefrontal paths), respectively (Fig. 1). Mann–Whitney *U* tests of tallied subgroup-specific connections between networks (four in the *Diffusely-connected* and eight in the *Hyper-connected* group) revealed that individuals in the *Hyper-connected* group showed greater connectivity of pathways from the DMN to the VAN, from the DMN to the CCN, and from the VAN to the DMN ($W = 163$, 76, and 183.5 respectively, FDR $ps < 0.001$) (online Supplemental Table S1, Fig. S2). Biotypes did not differ on the average age at scan, recruitment group status (i.e. substance use at age 14–15), alcohol and/or marijuana use in the past 3 months, pubertal timing (i.e. a longitudinal model-derived estimate of pubertal onset via annual self-reported physical development at ages 10–15), verbal or fluid IQ (age 10), or head motion based on mean framewise displacement (all $ps > 0.10$; Table 1; online Supplemental Fig. S3).

Hypothesis 3: internalizing (age 14) rightarrow connectivity-based biotype (age 16)

Results of the binary logistic regression indicated that there was a significant association between past internalizing problems

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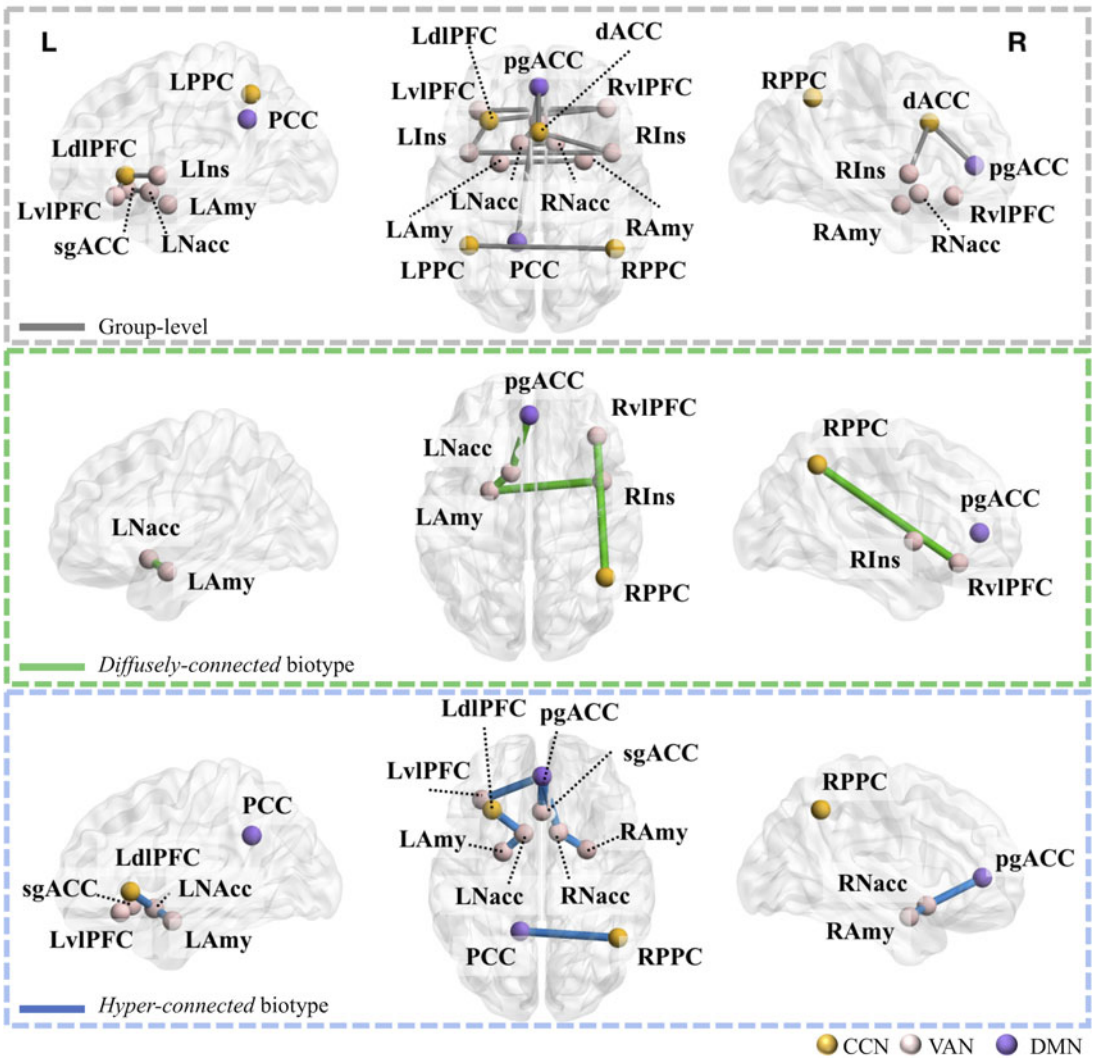

Fig. 1. Connectivity paths significant in the total sample, the *Diffusely-connected* biotype, and the *Hyper-connected* biotype. The Subgroup Group Iterative Multiple Model Estimation program was used to identify biotypes based on similarities in resting-state functional connectivity in adolescents. Two biotypes were found. Connectivity paths present in all participants are depicted in grey, paths unique to the *Diffusely-connected* biotype are in green, and paths unique to the *Hyper-connected* biotype are in blue. Nodes of the cognitive control network (CCN) are presented in gold; ventral affective network (VAN) in pink; default mode network (DMN) in purple. L = left; R = right; PPC = posterior parietal cortex; dlPFC = dorsolateral prefrontal cortex; vlPFC = ventrolateral PFC; PCC = posterior cingulate cortex; Ins = insula; Amy = amygdala; Nacc = nucleus accumbens; sgACC = subgenual anterior cingulate cortex (ACC); pgACC = perigenual ACC; dACC = dorsal ACC. (Monochrome): The Subgroup Group Iterative Multiple Model Estimation program was used to identify biotypes based on similarities in resting-state functional connectivity in adolescents. Two biotypes were found. Connectivity paths present in all participants are depicted in the top panel, paths unique to the *Diffusely-connected* biotype are in the middle panel, and paths unique to the *Hyper-connected* biotype are in bottom panel. L = left; R = right; PPC = posterior parietal cortex; dlPFC = dorsolateral prefrontal cortex; vlPFC = ventrolateral PFC; PCC = posterior cingulate cortex; Ins = insula; Amy = amygdala; Nacc = nucleus accumbens; sgACC = subgenual anterior cingulate cortex (ACC); pgACC = perigenual ACC; dACC = dorsal ACC.

(age 14) and connectivity-based biotype status at age 16 ($\chi^2(1) = 5.73$, $p = 0.017$), such that a higher internalizing score at age 14 was associated with a greater likelihood of belonging to the *Hyper-connected* than the *Diffusely-connected* biotype. A significant main effect of gender on biotype status was also found ($\chi^2(1) = 5.68$, $p = 0.017$); males were more likely than females to belong to the *Hyper-connected* biotype (Table 2, Fig. 2b).

Hypothesis 4: internalizing (age 16) leftrightarrow connectivity-based biotype (age 16)

The linear regression revealed that there was a significant association between connectivity-based biotype and concurrent internalizing problems (age 16), $F(1, 62) = 2.93$, $p = 0.004$. $R^2 = 0.42$. Specifically, adolescents in the *Hyper-connected* biotype showed greater internalizing problems at age 16, compared to those in the *Diffusely-connected* biotype. The main effect of gender and the interaction between connectivity-based biotype and gender on age 16 internalizing problems were not significant ($p = 0.092$ and 0.057) (Table 2, Fig. 2c). The interaction between biotype and gender was not significant, but the visualized pattern suggested that the *Hyper-connected,* relative to the *Diffusely-connected*, biotype had greater internalizing problems in females only; males showed similar levels of internalizing problems regardless of biotype (online Supplemental Fig. S4).

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Table 1. Sample characteristics

	Diffusely-connected (*N*=46)	Hyper-connected (*N*=22)	Statistic testing group differences	Statistical significance (*p*)
Age at scan (years)	16.20 (0.54)	16.36 (0.47)	$t(47.29) = -1.27$	0.21
Pubertal timing (age of onset)	11.22 (1.46)	10.97 (1.39)	$t(43.25) = 0.66$	0.51
Gender (% Female)	60.86% ($n = 28$)	40.90% ($n = 9$)	Fisher's exact	0.19
Substance use risk (% high)	50.00% ($n = 23$)	59.10% ($n = 13$)	Fisher's exact	0.44
Alcohol use (past 3 months)	1.09 (3.84)	0.23 (0.70)	$t(51.17) = 1.45$	0.15
Marijuana use (past 3 months)	4.93 (18.85)	0.90 (1.59)	$t(46.50) = 1.44$	0.16
Head motion (mean FD, mm)	0.16 (0.10)	0.18 (0.09)	$t(47.27) = -0.71$	0.48
Verbal IQ (age 10)	91.00 (13.45)	92.32 (10.31)	$t(52.96) = -0.44$	0.66
Fluid IQ (age 10)	99.16 (13.75)	95.50 (12.34)	$t(46.12) = 1.10$	0.28
MDD diagnosis (age 16-life)	0	0		
GAD diagnosis (age 16-life)	0	0		

Note: $N = 68$.
FD, framewise displacement; MDD, Major Depressive Disorder; GAD, Generalized Anxiety Disorder.
Means and standard deviations are presented for numeric variables. Sample proportions are presented for categorical variables. Substance use risk was based on recruitment at age 14-15 (0 = 'never used, don't plan to'; 1 = 'have used').

Table 2. Logistic and linear regression models testing associations between connectivity-based biotype, gender, and internalizing problems in adolescence

Model/Test	Outcome variable	Effects	Estimate	S.E.	*F* or *Z* value	*p*	FDR *p*	Effect size	Comparison of means	Tukey pairwise *p*'s
1: Logistic Regression	Biotype (16)	Int (14)	1.332	0.637	2.092	**0.036***	**0.035***	1.394		
		Gender	1.538	0.694	2.216	**0.027***	**0.033***	1.712	Males > Females	**0.025***
		Int (14) × Gender	0.387	1.764	0.220	0.826				
2: Linear Regression	Int (16)	Biotype	0.181	0.062	2.925	**0.004***	**0.010***	.420	HC > DC	**0.024***
		Gender	−0.084	0.049	−1.708	0.092				
		Biotype × Gender	−0.166	0.085	−1.934	0.057				
3: Linear Regression	Int (18)	Biotype	0.0856	0.035	2.397	**0.018***	**0.030***	.380	HC > DC	**0.035***
		Gender	−0.003	0.027	−0.123	0.903				
		Biotype × Gender	−0.067	−0.048	−1.392	0.169				
		Int (16)	0.217	0.069	3.149	**0.003***	**0.010***			

Note: $N = 68$. * and Bold font indicates $p < 0.05$.
Int (age), internalizing problems latent variable factor score at different ages of adolescence; DC, diffusely-connected biotype; HC, hyper-connected biotype.
A logistic regression was used to test the effect of internalizing problems at age 14 on connectivity-based biotype at age 16. Adolescents with greater internalizing problems at age 14 showed a higher probability of belonging to the *Hyper-connected* biotype at age 16. Boys were more likely than girls to belong to the *Hyper-connected* biotype. In model 2, a linear regression was used to test the association between connectivity-based biotype and concurrent internalizing problems (age 16). Adolescents in the *Hyper-connected* biotype showed higher internalizing problems at age 16. A linear regression model was also used to test the association between connectivity-based biotype and later internalizing problems (age 18), controlling for internalizing problems at age 16. Adolescents in the *Hyper-connected* biotype showed greater increases in internalizing problems from age 16 to 18. Cohen's *d* effect sizes are reported for models 2 and 3 and the odds ratios are reported for model. All analyses included covariates of substance use recruitment group and head motion (mean framewise displacement).

Hypothesis 5: connectivity-based biotype (age 16) rightarrow internalizing (age 18)

Linear regression showed that there was a significant main effect of biotype on age 18 internalizing problems, controlling for age 16 internalizing problems, $F(1, 60) = 2.40$, $p = 0.018$, $R^2 = 0.38$. Membership in the *Hyper-connected* biotype predicted more internalizing problems at age 18, even accounting for age 16 problems. The main effect of gender and the interaction effect

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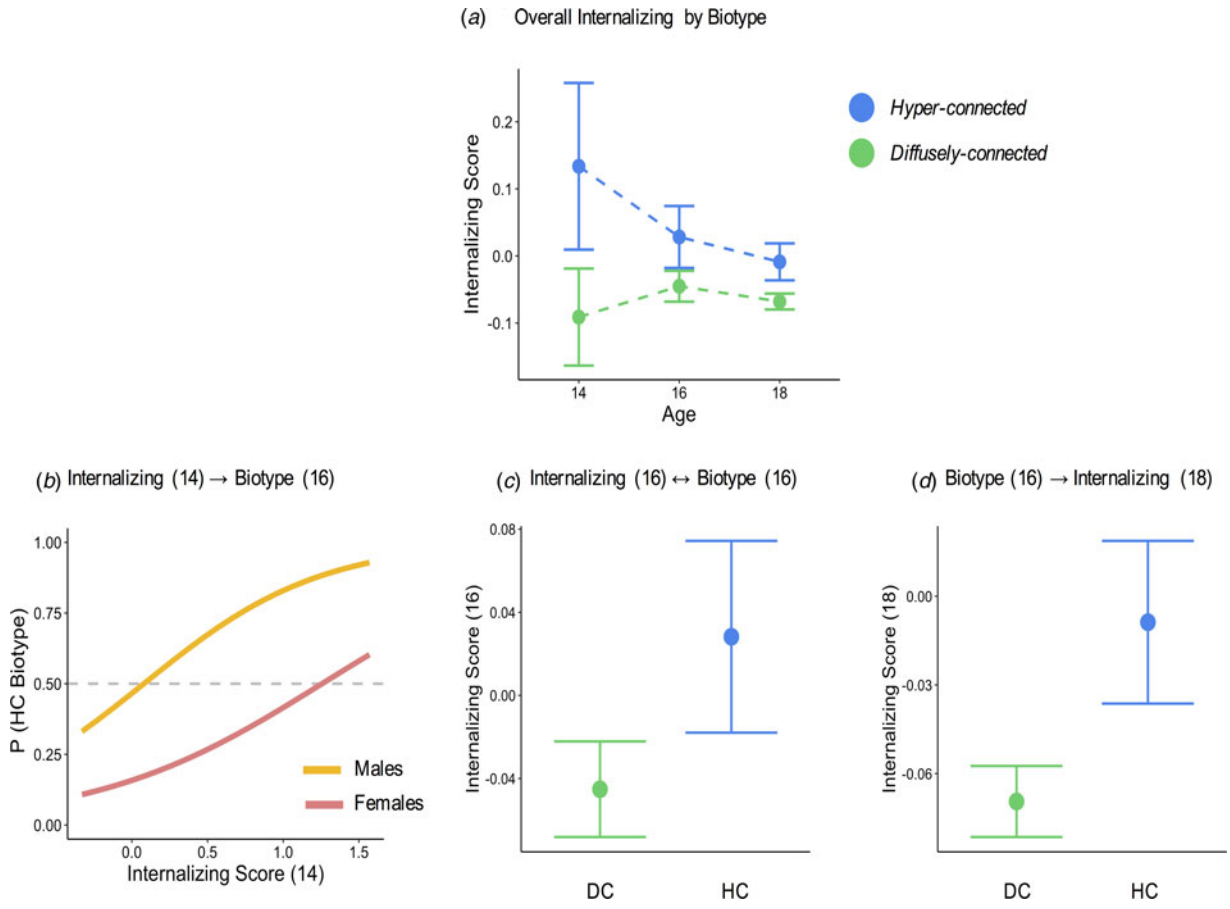

Fig. 2. Connectivity-based biotype associations with past, current, and future internalizing problems. (*a*) Connectivity-based biotype differences in internalizing problems throughout adolescence. At all time-points, adolescents in the *Hyper-connected* biotype exhibited higher internalizing problems than those in the *Diffusely-connected* biotype. (*b*) A logistic regression model revealed that adolescents with greater internalizing problems at age 14 showed a higher probability of belonging to the *Hyper-connected* biotype at age 16. (*c*) A linear regression model showed that adolescents in the *Hyper-connected* biotype showed higher internalizing current internalizing problems (age 16). (*d*) Connectivity-based biotype predicted later internalizing problems (age 18), controlling for current problems (age 16). Adolescents in the *Hyper-connected* biotype showed greater increases in internalizing problems from age 16 to 18. *p* = probability (%); Int (Age) = internalizing problems latent variable factor score at different ages of adolescence; Biotype = connectivity-based biotype; HC = *Hyper-connected* biotype; Green= *Diffusely-connected* biotype; Blue = *Hyper-connected* biotype; Circles = females; Triangles = males. All analyses included covariates of IQ, substance use recruitment group and head motion (mean framewise displacement). (Monochrome): (*a*) Connectivity-based biotype differences in internalizing problems throughout adolescence. At all time-points, adolescents in the *Hyper-connected* biotype exhibited higher internalizing problems than those in the *Diffusely-connected* biotype. (*b*) A logistic regression model revealed that adolescents with greater internalizing problems at age 14 showed a higher probability of belonging to the *Hyper-connected* biotype at age 16. Solid Line = females; Dashed Line = males; *p* = probability (%). (*c*) A linear regression model showed that adolescents in the *Hyper-connected* biotype showed higher internalizing current internalizing problems (age 16). (*d*) Connectivity-based biotype predicted later internalizing problems (age 18), controlling for current problems (age 16). Adolescents in the *Hyper-connected* biotype showed greater increases in internalizing problems from age 16 to 18. Int (Age) = internalizing problems latent variable factor score at different ages; Biotype = connectivity-based biotype; HC = *Hyper-connected* biotype; DC = *Diffusely-connected* biotype; Circle = *Diffusely-connected* biotype; Triangle = *Hyper-connected* biotype; All analyses included covariates of substance use recruitment group and head motion (mean framewise displacement).

of biotype and gender on age 18 internalizing problems were not significant ($p = 0.90$ and 0.17) (Table 2, Fig. 2d), however, the visualized pattern suggested that females had greater biotype-related differences in internalizing problems relative to males (online Supplemental Fig. S4).

Connectivity-based biotype associations with internalizing problems trajectories

Tests using linear regression showed a significant effect of biotype on the internalizing problems intercept such that the *Hyper-connected* biotype showed greater initial internalizing problems at Time 0 (age 14) compared to the *Diffusely-connected* biotype, $F(4, 63) = 2.27$, $p = 0.027$, $R^2 = 0.13$. Additionally, the *Hyper-connected* group exhibited a negative slope of change (decrease) in internalizing problems over time, whereas the *Diffusely-connected* group exhibited, on average, a positive slope (increase) in internalizing problems, $F(4, 63) = -2.27$, $p = 0.027$, $R^2 = 0.13$ (online Supplementary Fig. S5). As can be seen from the plot of average internalizing symptom scores per biotype at each time-point (Fig. 1a), the finding of decreasing internalizing symptoms in the *Hyper-connected* biotype was due to higher initial scores that, on average, lessened over time, yet remained higher than scores in the *Diffusely-connected* biotype.

Network paths and internalizing problems

Associations between directed path counts and internalizing scores across time points were measured. Path counts for the three connection types that differed across biotypes (i.e. DMN

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to the → VAN, DMN → CCN, VAN → DMN) were included in one multivariate analysis of variance model as main effects on internalizing scores (three dependent variables of age 14, 16, and 18), with gender interactions, and motion and recruitment group as covariates. The number of DMN → CCN paths was positively associated with internalizing problems, $F(1, 55) = 6.82–8.20$, $p = 0.01$, 0.02, and 0.001 at ages 14, 16, and 18 respectively. Females with higher DMN → VAN connectivity showed higher internalizing problems at age 16, and all participants with higher DMN → VAN connectivity showed higher internalizing problems at age 18, $F(1, 55) = 5.02$ and 4.17, $p = 0.03$ and 0.05, respectively. Greater VAN → DMN connections were associated with more internalizing problems at age 18 in females, but not males, $F(1, 55) = 8.31$, $p = 0.001$ (online Supplemental Table S2).

Discussion

The current study examined whether connectivity-based biotypes in mid-adolescence were linked with internalizing problems throughout adolescence. Adolescents were parsed into two biotypes based on similarities of RSFC at age 16. The *Diffusely-connected* biotype had more paths connecting distal regions and the *Hyper-connected Ventromedial* group had more paths connecting relatively proximal ventromedial and subcortical areas. Youth who reported greater internalizing problems at age 14 were more likely to belong to the *Hyper-connected* biotype. Youth in the *Hyper-connected* biotype also exhibited greater internalizing problems at age 16 and showed an increase in internalizing problems from age 16 to 18. The current study is the first to show that connectivity-based biotypes can distinguish levels of internalizing problems throughout adolescence in a community (i.e. nonclinical) sample of youth.

The study is novel in using a community detection approach to parse adolescents into biotypes based on their RSFC patterns involving regions relevant to internalizing problems, independent of self-reported data. The biotypes were associated with past and current internalizing problems and predicted later emotional difficulties. The findings suggest that, even in a nonclinical sample, youth with more *v.* fewer internalizing symptoms exhibit dissociable neural signatures. It is important to note that the two biotypes shared more similarities in functional neural architecture than differences, as evidenced by the group-level paths (Fig. 1). Individuals within each biotype had similar connectivity patterns that were differentially associated with internalizing symptom measures, yet the two biotypes may not be as 'categorically' different as a hard classification method would suggest. Rather, our findings suggest that youth on an internalizing spectrum generally share a number of similarities in connectivity patterns, and biotyping may reveal more detailed neural patterns underlying the presence of affective dysregulation as measured by symptom dimensions at different points in adolescence. Altogether, RSFC patterns in mid-adolescence may transcend diagnostic boundaries and serve as biomarkers of an individual proclivity for internalizing symptoms.

The patterns of RSFC found in the *Hyper-connected* biotype, including greater DMN-CCN, have also been found in prior studies of adolescents with higher, but subclinical elevated levels of internalizing symptoms (Alarcón, Cservenka, Rudolph, Fair, & Nagel, 2015; Greening & Mitchell, 2015). Given the role of the CCN in executive control and the DMN in self-referential introspective processing (Barrett & Satpute, 2013; Buckner, Andrews-Hanna, & Schacter, 2008), these two networks typically show negatively correlated activity (Goulden et al., 2014). Elevated DMN-CCN connectivity, therefore, may reflect an inability to disengage from internally focused thoughts in the presence of external task demands (Dwyer et al., 2014). Indeed, prior research shows that adolescents with higher connectivity between the DMN and CCN during a cognitive control task were more likely to engage in co-rumination with peers (Alarcón, Pfeifer, Fair, & Nagel, 2018). Although the connectivity biotypes differed on other between-network connectivity values in the present study, greater DMN-CCN connectivity was stably associated with internalizing problems throughout adolescence. Thus, this neural signature may play a crucial role in classifying biotypes that serve as markers of internalizing symptoms.

RSFC patterns in the *Hyper-connected* biotype were also similar to those found in studies of adolescents with diagnosed depression and/or anxiety (Cullen et al., 2014; Ho et al., 2015; Roy et al., 2013). Increased connectivity of emotion-processing regions, particularly medial prefrontal and subcortical areas, with other affective regions and with self-referential areas is also documented in relation to adolescent depression (Pannekoek et al., 2014; Song, Zhang, & Huang, 2016) and anxiety (Roy et al., 2013). Additionally, in the current study, the *Hyper-connected* biotype showed paths between regions related to reward, stress, and affective processing (RNacc-RAmygdala, LNacc-LAmygdala, LdlPFC-LNacc, and RNacc-pgACC). Aberrant connectivity of the frontal-striatal reward network has been found in adolescent depression (Morgan et al., 2016) and social anxiety (Jarcho et al., 2015), potentially contributing to altered processing of reward in both disorders (Zald & Treadway, 2017). Finally, elevated connectivity of DMN regions with the CCN has been shown to relate to rumination and severity of symptoms in adolescents with current and remitted MDD (Jacobs et al., 2014) and adults with high social anxiety as adolescents (Maresh, Allen, & Coan, 2014). The shared connectivity features found between the *Hyper-connected* biotype and prior studies of adolescent depression and anxiety suggest that the neural architecture related to internalizing symptoms is roughly similar to that of clinical internalizing psychopathologies, corroborating the utility of measuring these features dimensionally rather than categorically (Kotov et al., 2017).

Compared to females, males with higher internalizing problems at age 14 were more likely to belong to the *Hyper-connected* biotype at age 16. Probing between-network connectivity measures, however, provided evidence for female-specific neural patterns underlying internalizing problems although this could be due to girls endorsing these problems to a greater degree at ages 14 and 16. Our findings suggest that the biotyping approach was not generally gender-specific, as it allowed for identifying males and females with elevated internalizing problems. Although the *Hyper-connected* biotype identified in this study may represent both males and females with elevated internalizing problems at age 14, this brain-behavior association may become more female-specific in late adolescence, when females exhibit greater levels of internalizing problems compared to males (Salk, Hyde, & Abramson, 2017). It is also possible that the functional neural architecture related to internalizing problems becomes gender-differentiated in late adolescence, and the connectivity of the ROIs in the current study was more sensitive to females', rather than males', concurrent and future internalizing problems. Future work is needed to understand how connectivity-based biotypes may relate to symptoms of other types of psychopathology that have a sex-differentiated occurrence (e.g. externalizing disorders in males).

The current study has some limitations. The S-GIMME data-driven approach is used to characterize directional influences of brain regions and has been shown to outperform traditional

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subgrouping approaches (Gates et al., 2017). Although S-GIMME demonstrates robustness for sample sizes as small as 25 (Gates et al., 2017), subgrouping approaches are inherently affected by those who are included in a sample, and findings may be altered within a larger or more variable (e.g. in ethnicity or age) sample. The current study contained Mexican-origin adolescents with a wide range of internalizing symptoms, but they were not recruited based on having a clinical diagnosis of depression or anxiety. Understanding the generalizability of the current findings and robustness and replicability of the biotypes would be facilitated by future larger studies incorporating youth from various ethnicities and/or clinical profiles. Additionally, the three occasions of internalizing measures allowed for testing prospective predictions although resting-state fMRI data were collected only at one occasion. Multiple waves of neuroimaging data are necessary to (a) examine the stability over time of connectivity-based biotypes, (b) disentangle the direction of effects between network connectivity and internalizing symptoms, and (c) understand how associations with symptoms vary with age, particularly given documented developmental changes of functional connections in adolescence (Baker et al., 2015; Dwyer et al., 2014; Larsen & Luna, 2018).

In conclusion, we used a data-driven approach to characterize, for the first time, connectivity-based biotypes associated with past, current, and later internalizing problems in adolescents. Internalizing problems at age 14, particularly in males, predicted a higher probability of being classified in the *Hyper-connected* biotype at age 16. The *Hyper-connected* biotype also showed greater internalizing problems at age 16, concurrent with the fMRI scan. Finally, the *Hyper-connected* biotype showed greater internalizing problems at age 18, pointing to the predictive utility of the connectivity-based biotyping approach. Greater RSFC between self-referential and cognitive processing regions were found in adolescents with more internalizing problems throughout adolescence, suggesting that this RSFC signature may be informative for differentiating youth based on the dimension of internalizing difficulties. RSFC patterns may be useful biomarkers of affective dysregulation in relatively normative adolescents. Biotyping may aid in identification of affected youth and improve understanding of the functional neural architecture associated with internalizing symptoms.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S003329172000149X.

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