Late chronotype is associated with enhanced amygdala reactivity and reduced fronto-limbic functional connectivity to fearful versus happy facial expressions

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Abstract

Increasing evidence suggests late chronotype individuals are at increased risk of developing depression. However, the underlying neural mechanisms that confer risk are not well understood. In the current report fifty healthy, right-handed individuals without a current or previous diagnosis of depression, family history of depression or sleep disorder underwent structural magnetic resonance imaging. Participants completed an implicit emotion processing task (gender discrimination) including happy and fearful facial expressions. Linear effects of chronotype on BOLD response in bilateral amygdala were tested for significance using nonparametric permutation tests. Functional connectivity between amygdala and prefrontal cortex was also investigated using psychophysiological interaction (PPI) analysis. A significant negative correlation between BOLD response and chronotype was observed in bilateral amygdala where later chronotype was associated with an enhanced amygdala response to fearful *vs.* happy faces. This response remained significant after sleep quality, age, gender, mood, and time of scan were included as covariates in the regression model. Later chronotype was also significantly associated with reduced functional connectivity between amygdala and dorsal anterior cingulate cortex (dACC). The current results appear consistent with theories of impaired emotion regulation of the limbic system (particularly the amygdala) associated with depression and may, in part, explain the increased vulnerability for depression in late chronotype individuals.

The vulnerability factors underpinning this link, however, are unclear. Here the relationship between two specific emotion regulation strategies, cognitive reappraisal and expressive suppression, and chronotype was investigated using multiple regression. Two hundred and fourty participants (age range 18- 80, 189 females) completed validated self-

report questionnaires assaying chronotype, neuroticism, depression symptomatology, sleep quality and emotion regulation. Eveningness was associated increased expressive suppression and morningness was associated with increased cognitive reappraisal after controlling for age, gender, depressive symptomatology, neuroticism and sleep quality. Trait expressive suppression and reduced cognitive reappraisal are known to increase depression risk. Our results suggest that eveningness is associated with impaired emotion regulation which may confer risk for future depression. These findings suggest modifiable markers that could be therapeutically targeted to prevent the onset of depression in late chronotype individuals.

Keywords: Chronotype, fMRI, Amygdala, Emotion, Depression, Regulation, PPI analysis

Introduction

Morningness-eveningness, or chronotype, refers to individual differences in diurnal preference (Horne & Östberg, 1976). Early chronotypes (colloquially referred to as larks), rise early and reach their peak early in the day (Schmidt, Collette, Cajochen, & Peigneux, 2007; Schmidt et al., 2012), whereas late chronotypes (night owls) prefer later bed and wake times (i.e. "synchronised" to an individual's optimal time of day according to their circadian profile). Chronotype is considered a relatively stable trait although patterns across the lifespan are recognised. Children tend to be early chronotypes, during adolescence there is a

marked shift towards late chronotype followed by a progressive swing to early chronotype during later adulthood (Randler, Freyth-Weber, Rahafar, Florez Jurado, & Kriegs, 2016).

A growing body of research suggests an association between late chronotype and psychological health – particularly major depression. For example, in a large cohort study (*n* = 1994), Antypa and colleagues reported that late chronotype was associated with current depression after controlling for socio-demographic, somatic health, and sleep-related factors (Antypa, Vogelzangs, Meesters, Schoevers, & Penninx, 2016). Late chronotype is also associated with increased likelihood of reporting depressive symptoms (Hidalgo et al., 2009; Levandovski et al., 2011), diagnosis of depression and use of antidepressant medication (Merikanto et al., 2015; Merikanto et al., 2013). Together, these findings clearly demonstrate an important link between late chronotype and depression but the observational nature of these data preclude investigation of the underlying vulnerability factors that confer increased risk for depression in late chronotype individuals.

Functional neuroimaging studies of depression have consistently reported increased amygdala reactivity to negative emotional stimuli (Disner, Beevers, Haigh, & Beck, 2011). In response to negative stimuli, depressed patients typically show a more intense and sustained amygdala response indicative of biased stimulus processing. This pattern of amygdala response is automatic (i.e. is present even when the stimuli are presented) and is reversed with successful pharmacotherapy (Anand, Li, Wang, Gardner, & Lowe, 2007; Fu et al., 2004; Sheline et al., 2001). Indeed, many previous studies report that amygdala, as well as parahippocampal gyrus, activity is an indicator of the emotional intensity experienced by an individual, and this activity can be up-regulated and down-regulated by higher cortical regions including the superior frontal gyrus, cingulate and premotor areas (see Frank et al., 2014, for meta-analysis). For example, increased activation in the left orbitofrontal, left superior frontal and anterior cingulate gyrus was observed during suppression of negative

emotions compared to maintaining negative emotions (Phan et al., 2005), and this enhanced activity was related to a reduction in self-reported negative effect (Mak, Hu, Zhang, Xiao, & Lee, 2009). In major depression, however, frontal cortical regions show abnormal activity; for example, hyperactivity in the subgenual anterior cingulate cortex (ACC) (Mayberg, 2003) generally considered the 'affect subdivision', but hypoactivity in the dorsal ACC (Davidson, Pizzagalli, Nitschke, & Putnam, 2002) the 'cognitive subdivision'. Taken together, these data suggest that current depression is associated with an elevated and sustained neural (amygdala) response to negative stimuli and impaired emotional regulation associated with an aberrant neural response in higher cortical regions including the ACC. This unregulated amygdala response generates a 'bottom-up' signal that biases emotional processing in higher cortical areas and results in maladaptive perceptions of the environment and social interactions (Disner et al., 2011).

In order to further explore the neural basis of emotional regulation, the functional connectivity between limbic regions and higher cortical areas has been investigated using psychophysiological interaction (PPI) analysis (Friston et al., 1997). The PPI term, which can be added to any linear model, represents the element by element multiplication (interaction) between an input variable (task time course) and a response variable (seed time course). A significant PPI indicates that the correlation in activity between two brain regions is different in different psychological contexts (O'Reilly, Woolrich, Behrens, Smith, & Johansen-Berg, 2012). In a meta-analysis of 49 PPI analysis studies, increased functional connectivity with the amygdala was observed in the inferior frontal gyrus, ACC and medial frontal gyrus in a reappraisal vs. maintain condition of an emotional regulation task (Di, Huang, & Biswal, 2017). This pattern of connectivity suggests the higher cognitive areas are effectively down-regulating, or inhibiting, the amygdala response to negative emotion although the direction of this effect cannot be determined. In depressed patients, however, this fronto-limbic

connectivity appears to be reduced (Dannlowski et al., 2009; Erk et al., 2010). Hence, there is evidence that neural emotional regulation processes are diminished in depression.

In order to investigate whether these aberrant neural responses are present before the onset of depression, it is necessary to examine neuroimaging data in never-depressed individuals at increased risk for developing depression. High neuroticism (a recognised risk factor for depression) is associated with elevated right amygdala response to fearful *vs.* happy faces (Chan, Norbury, Goodwin, & Harmer, 2009). Herringa and colleagues (2016) reported a positive correlation between right amygdala response to negative *vs.* neutral stimuli and childhood adversity (a recognised risk factor for depression) (Herringa et al., 2016). Monk *et al.*, reported increased activation in left and right amygdala to passive viewing of fearful faces in the offspring of depressed parents (Monk et al., 2008). More recently, Mannie and colleagues (2011) found no difference between offspring of depressed parents and matched controls in amygdala reactivity to negative facial expressions during an emotion matching task (Mannie, Taylor, Harmer, Cowen, & Norbury, 2011) but did report reduced activation of frontal regions which may reflect perturbed regulation of aversive stimuli. A direct comparison across these at-risk studies is challenging due to differences in task parameters (implicit gender discrimination, passive viewing or emotion matching) and participant characteristics. Nevertheless, current data suggest abnormal processing of emotional information in never-depressed at-risk individuals. In relation to emotion regulation, neuroimaging studies of never-depressed at-risk populations reveal similar functional connectivity impairments to depressed patients. For example, high neuroticism is associated with decreased functional connectivity between dACC and amygdala for sad compared to neutral faces (Cremers et al., 2010). Reduced functional connectivity between amygdala and rostral ACC has also been observed in carriers of the 5-HTTLPR polymorphism (Pezawas et al., 2005). Taken together, these data suggest that never-depressed at-risk individuals display

abnormal amygdala reactivity to negative stimuli and altered cognitive control processes responsible for emotional regulation, which may reflect a neural vulnerability marker present prior to the onset of depression.

In sum, previous evidence suggests that patients with depression have an enhanced amygdala response to negative facial expressions as compared to healthy individuals (Anand et al., 2007; Disner et al., 2011; Fu et al., 2004; Sheline et al., 2001). Other groups known to be at increased risk for developing depression also show a similar enhanced amygdala response to negative stimuli (Chan, Norbury, Goodwin, & Harmer, 2009; Herringa et al., 2015; Monk et al., 2008). To our best knowledge, however, no one has investigated amygdala reactivity to negative stimuli and related this to chronotype. Here we hypothesised that later chronotype would be associated with increased amygdala reactivity to negative (fearful) facial expressions, similar to the pattern of activity seen in depressed patients and other at-risk groups. A second objective was to explore amygdala-fronto connectivity as both depression, and risk for depression, have been associated with impaired emotional regulation of the amygdala by higher cortical regions (Mayberg, 2003). Here, we predicted that later chronotype would be associated with reduced connectivity between amygdala and brain regions implicated in emotion regulation.

Methods

Participants

The study was approved by the local ethics committee and written informed consent was obtained prior to any study procedures taking place. Participants were in good physical health and free of concurrent medication. Exclusion criteria were current or previous depression, presence of major depression in a biological parent, diagnosed sleep disorder (each assessed by self-report) and contraindication for MR examination. A total of 50 participants were recruited (38 females, age range 18-37 (M =21.24, SD = 3.77). Chronotype

was determined using the 5-item reduced Morningness-Eveningness Questionnaire (Adan & Almirall, 1991), based on the full version of the MEQ validated for a young adult population (18-32 years). Sleep quality was assayed using the Pittsburgh Sleep Quality Index (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). Depression and anxiety were measured using the Patient Health Questionniare-4 (Löwe et al., 2010). The time at which the MRI scan took place was agreed between the participant and investigator and recorded, but generally took place between 10am and 5pm.

Image data acquisition

All imaging data were acquired on a research dedicated 3T Magnetom Trio (Siemens, Erlangen, Germany) fitted with a 32-channel head coil and located at the Combined Universities Brain Imaging Centre (CUBIC). For each participant, we collected a T_1-weighted whole-brain scan (magnetization-prepared rapid acquisition with gradient echo (MPRAGE), inversion time (TI) = 1100 ms, repetition time (TR) = 1830 ms, echo time (TE) = 3.03 ms, flip angle (FA) = 11°, field of view (FOV) = 256 × 256 × 160 mm^3, voxel size = 1 × 1 × 1 mm^3). Functional MR data were acquired using a T2*-weighted echo planar imaging sequence (EPI, TR = 2000 ms, TE = 31 ms, FA = 85°, FOV = 192 × 192 × 87 mm^3 [29 slices, voxel size = 3 × 3 × 3 mm^3], number of measurements = 170, imaging bandwidth = 752 Hz/px, GRAPPA acceleration factor = 2). Gradient echo field mapping data were also acquired for EPI off-resonance distortion correction (TR = 400 ms, TE1 = 5.19 ms, TE2 = 7.65 ms, flip angle = 60°, FOV = 192× 192 × 126 mm^3, voxel size = 3 × 3 × 3 mm^3).

FMRI experimental task

During FMRI scanning, participants completed a well validated gender discrimination task involving the rapid presentation of greyscale fearful and happy faces taken from the NimStim database (Tottenham et al., 2009). Nine 20 second blocks of baseline (fixation

cross) were interleaved with 8 blocks of the emotional faces (again 20 seconds in duration, 4 blocks of fearful and four blocks of happy faces). Individual faces were presented for 100ms and the participant had to indicate, by button press, the gender of the face. Equal numbers of male and female faces were presented in each condition. Stimuli were presented on a personal computer using E-Prime (using version 2.10.242, Psychology Software Tools Inc., USA) and projected onto an opaque screen at the foot of the scanner bore, which subjects viewed using an angled mirror mounted above the head coil. Both accuracy and response time were recorded by E-Prime.

FMRI analysis pipeline

All image pre-processing and analyses were performed using FSL version 5.0.10 (FMRIB Software Library, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). The following pre-statistical processes were applied to all fMRI data: non-brain removal; rigid-body motion correction; high-pass temporal filtering (Gaussian-weighted least-squares fitting with frequency cut-off point = 60 s); correction of off-resonance geometric distortions in the EPI data using B0 field maps derived from the dual-echo gradient echo dataset; artifact removal based on probabilistic ICA (Independent Component Analysis – see below); spatial normalization to Montreal Neurological Institute (MNI152) 2 mm isotropic atlas space using boundary-based registration and non-linear registration (during registration, signal loss, resulting from through-slice field gradients, was calculated and used as a cost function mask to exclude voxels where signal loss was greatest); Gaussian filtering (full width at half maximum (FWHM) = 5 mm).

Controlling for structured noise

We conducted manual ICA-based artifact removal. The first author (CH) visually inspected all the independent component maps for each participant to identify noise

components based on both the spatial layout of the component maps and the power spectra of the associated time series (Griffanti et al., 2016). Variance uniquely associated to the components labelled as noise was subsequently regressed from each individual's data prior to statistical modelling (see below).

Analysis of functional imaging data

Analyses of data from individual subjects (first level analysis) were computed using the general linear model with local autocorrelation correction. Two regressors were defined (fearful and happy faces) and were convolved with a haemodynamic response function, using a variant of a γ function (i.e. a normalization of the probability density function of the γ function) with a standard deviation of 3 s and a mean lag of 6 s. In addition, temporal derivatives and estimated motion parameters (three translation and three rotation) were included in the model as regressors of no interest to increase statistical sensitivity.

At the group level, linear effects of chronotype on BOLD response in bilateral amygdala were tested for significance using non-parametric permutation tests (applying 5000 permutations). Control of the family-wise error rate was obtained using threshold-free cluster enhancement (Smith & Nichols, 2009). Left and right amygdala *a prior* regions of interest were taken from the Harvard-Oxford subcortical atlas distributed within FSL. This atlas is derived from T_1-weighted images of 37 subjects (21 male, age range 18-50) and combined to form population probability maps for 21 subcortical structures including the amygdalae.

Psychophysiological interaction analysis

In a complementary analysis, a generalised psychophysiological interaction analysis (PPI) (Friston et al., 1997; McLaren, Ries, Xu, & Johnson, 2012) was conducted across the whole brain in order to explore how functional connectivity between brain regions varied

with task. As a significant correlation between chronotype and BOLD signal was observed for both right and left amygdala (please see results), both regions were used separately as seed regions. At the individual level, the PPI GLM analyses included the original task conditions (fear and happy faces), the mean time course from each cluster identified in the analyses described above, and the two interaction terms (fear faces x seed, happy faces x seed). Temporal derivatives and six estimated motion parameters were also included in the model. This analysis identified regions that displayed stronger functional connectivity with the left/right amygdala for fearful facial expressions compared to happy facial expressions.

At the group level, the contrast images for the PPI effects were entered along with chronotype as a regressor in a whole-brain analysis. Brain regions that showed connectivity with the amygdala were identified, correlating positively or negatively with chronotype (rMEQ score). This was tested for significance using non-parametric permutation tests (applying 5000 permutations) and threshold-free cluster enhancement.

Results

Participants

Participant characteristics are presented in Table 1. Measures of anxiety ($r = 0.13$, $p > .05$), depression ($r = 0.17$, $p > .05$) and time of scan ($r = 0.013$, $p > .05$) were not significantly correlated with rMEQ. Chronotype scores were similar between male and female participants (independent samples *t*-test ($t(13.73) = -.68$, $p > .05$). However, there was a significant correlation between rMEQ and PSQI score ($r = 0.383$, $p < .01$) and age ($r = -0.334$, $p < .05$) such that late chronotype was associated with better sleep quality and older age.

Variable	*M* (SD)	Range (this sample)	Range (original scale)
Age	21.24 (3.77)	18-37	-
Gender	Female 38 (76%); Male 12 (24%)	-	-
Sleep quality	7.30 (3.42)	1 - 15	0 – 21
Chronotype	12.62(3.62)	6 - 20	4 – 25
PHQ-4 (Anxiety)	0.91 (1.09)	0 - 5	0 – 6
PHQ-4 (Mood)	1.61 (1.58)	0 - 6	0 -6

Table 1. Descriptive statistics: Basic demographics, sleep quality (PSQI), chronotype (rMEQ), mood and anxiety (PHQ-4). Please see Methods for questionnaire details. Values show mean (SD). Also included are the questionnaire range (minimum-maximum) for the study sample and original scale.

Behavioural results

Inspection of the behavioural data acquired during scanning indicated that participants were engaged with the task and were highly accurate to classify faces as male or female (> 85%) with discrimination accuracy for happy faces greater than fearful faces (fearful faces: M = 85.20%, SD = 9.84, happy faces: M = 88.70%, SD = 9.44, dependent samples t-test: $t(45) = -3.41$, $p = .001$). Response latencies to happy faces were similar to fearful faces (happy faces: M = 628.52 ms, SD = 120.96, fearful faces: M = 642.50 ms, SD = 132.52, dependent samples t-test: $t(45) = 1.84$, $p = .072$).

FMRI results

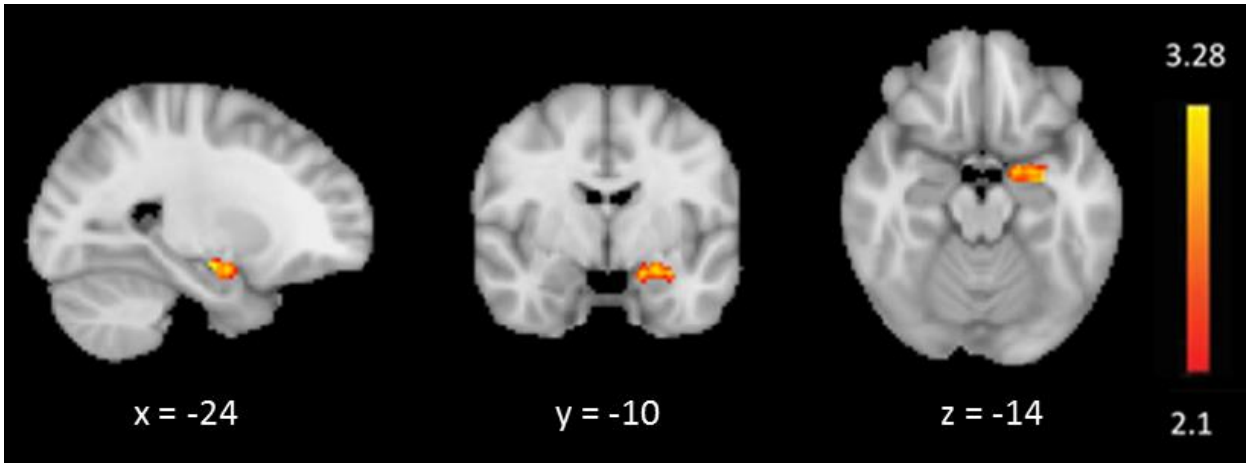
Using a region of interest approach, we observed a significant negative correlation between BOLD response and chronotype in bilateral amygdala (left amygdala x =-30, y = -6, z = -18, maximum t-value= 3.97; cluster size = 198 voxels, $p < .001$ [see Figure 1]; right amygdala x = 32, y = 2, z = -20, maximum t-value = 3.20; cluster size = 91 voxels, p = .012) such that participants with higher rMEQ scores (increased early chronotype) showed reduced activity to fearful vs. happy faces in left and right amygdalae (also see Supplementary material). There were no regions that exhibited a positive association between chronotype and BOLD response. In a further exploratory analysis, chronotype was regressed across the whole brain but no other regions were found to be significantly associated.

In a further analysis, restricted to the functional clusters observed above, we included PSQI score, gender, age, mood and anxiety levels (computed as the sum score for the mood and anxiety components of the PHQ-4) and time of scan (i.e. the start time of the experiment as recorded by the stimulus presentation software) as additional covariates. Bilateral amygdala activation remained significant although the amplitude and extent of activation was reduced (left amygdala x =-24, y = -10, z = -14, maximum t-value= 3.17; cluster size = 55 voxels, p = .018; right amygdala x =28, y = -4, z = -14, maximum t-value = 2.92; cluster size = 61 voxels, p = .014). This suggests the negative association observed between rMEQ and

297 BOLD response is robust, and largely independent of sleep quality, gender, age, mood,
298 anxiety levels and time of scan.

299

300 Figure 1. BOLD correlates with rMEQ in left and right amygdala (left amygdala displayed).
301 Early chronotype is associated with reduced BOLD response to fear vs. happy faces. Lower
302 numerals refer to coordinates in Montreal Neurological Institute (MNI) space. Colour bar and



303 numerals: *t* value range.

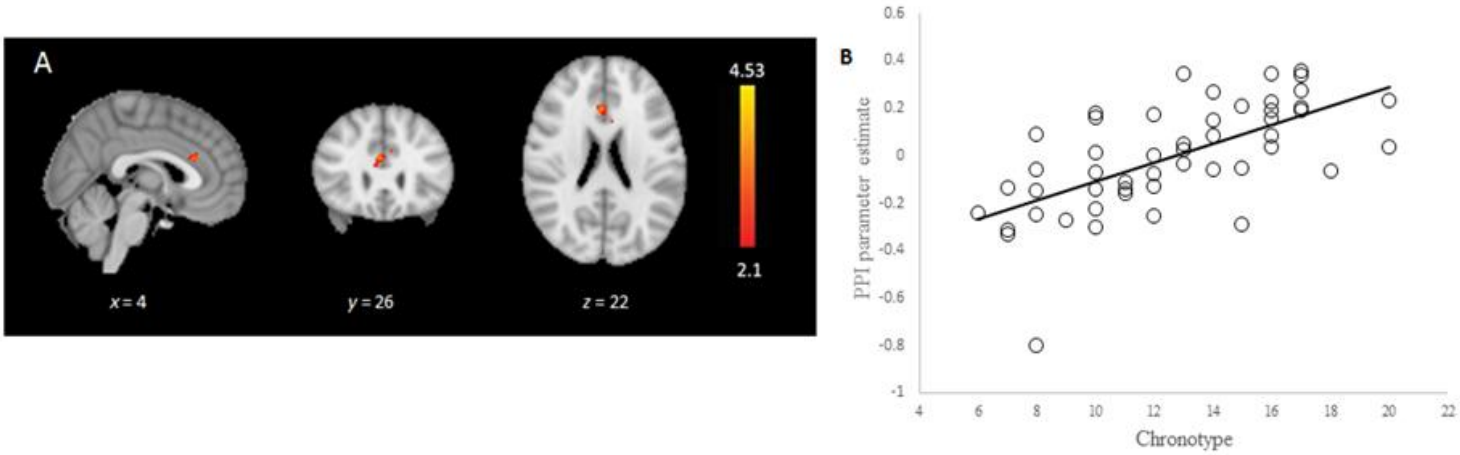
304

305 **PPI analysis results**

306 The PPI analysis revealed a positive association between chronotype (rMEQ) scores
307 and right amygdala – dACC coupling (x =4, y = 26, z = 22, maximum t-value = 4.53; cluster
308 size = 54 voxels, p = .026) and right frontal pole (x =34, y = 46, z = -2, maximum t-value =
309 5.16; cluster size = 40 voxels, p = .02) when viewing fearful faces compared to happy faces
310 [please see Figure 2]. As above (FMRI results) we included PSQI score, gender, age, mood
311 and anxiety levels and time of scan as additional covariates to assess the specificity of this
312 effect. Both the dACC cluster (x =4, y = 26, z = 22, maximum t-value = 3.77; cluster size =
313 33 voxels, p < .05) and frontal pole clusters (x =34, y = 48, z = -2, maximum t-value = 4.17;

cluster size = 40 voxels, $p < .05$) remained significant. This finding indicates that late chronotype (lower rMEQ scores) is associated with reduced functional coupling between right amygdala and dACC for fearful *vs*. happy facial expressions even after accounting for a number of possible confounds.

Figure 2. PPI analysis displaying A) brain regions identified as showing functional connectivity with the right amygdala that are positively correlated with chronotype (rMEQ) scores in response to fearful vs. happy facial expressions (colour bar and numerals as in Figure 1), and B) scatter plot showing positive association between right amygdala – ACC connectivity and chronotype scores.



Discussion

This is the first study, to our knowledge, to explore the neural basis of emotional processing biases towards negative facial expressions and related this to chronotype. A significant negative correlation was observed between BOLD response in bilateral amygdalae

and rMEQ score such that later chronotype (lower rMEQ score) was associated with increased BOLD response to fearful *vs.* happy faces. Moreover, a positive correlation between rMEQ score and functional connectivity between right amygdala and dACC was observed where earlier chronotype was associated with increased connectivity in response to fearful vs. happy faces. These effects were present independent of current or previous diagnosis of depression or sleep disorder, and were not driven by sleep quality, age, gender, measures of mood and anxiety or time of scan. Previous evidence has identified late chronotype to be associated with increased prevalence of depression (Antypa et al., 2016; Hidalgo et al., 2009; Levandovski et al., 2011; Merikanto et al., 2015; Merikanto et al., 2013) and hyperactivity of the amygdala to negative stimuli has been reported in depressed patients (Sheline et al., 2001) and individuals at increased risk for depression (Chan et al., 2009). Impaired emotional regulation has also been observed in depressed individuals (Erk et al., 2010) and never-depressed at-risk populations (Cremers et al., 2010; Pezawas et al., 2005). Here we observed that later chronotype was associated with hyperactive amygdalae and reduced amygdala-ACC connectivity to negative faces which may reflect the neural processes underlying vulnerability to depression in late chronotype individuals.

Biases in facial expression recognition have been reported in individuals with depression involving a discrimination bias towards negative emotions and/or away from positive emotions. For example, Gur and colleagues reported reduced sensitivity to recognise happy facial expressions and an increased likelihood to misclassify neutral faces as sad in a sample of depressed patients (Gur et al., 1992). Additionally, depressed patients have been shown to have reduced spatial attention towards positive facial expressions during a face-in-the-crowd task (Suslow, Junghanns, & Arolt, 2001). In a longitudinal study, patients were more likely to report ambiguous faces as being negative when they were first admitted as depressed than in a remitted state, and patients were more likely to relapse after six months if

they perceived faces as being more negative at admission or discharge (Bouhuys, Geerts, & Gordijn, 1999). This finding highlights the significance of negative biases in predicting relapse as theorists suggest that these negative biases play a key role in the aetiology and maintenance of depressed states. It is therefore important to develop prevention strategies that aim to identify and remove negative biases; for example, using psychological therapies (e.g. Cognitive Behavioural Therapy) to reverse these cognitions.

Similar biases have also been reported in remitted depressed patients (Anderson et al., 2011; Bhagwagar, Cowen, Goodwin, & Harmer, 2004) thereby suggesting that negative biases may either signal a 'scar' effect (arise as a consequence of previous depression) or a pre-existing cognitive vulnerability. However, negative biases have also been reported in never-depressed individuals at increased risk for developing depression; for example, Chan and colleagues (2007) reported an increased threshold to recognise happy facial expressions in highly neurotic individuals as compared to healthy controls (Chan, Goodwin, & Harmer, 2007). We recently reported increased recognition of sad facial expressions in late chronotypes (Berdynaj et al., 2016) and have replicated this finding in a larger independent sample (Horne, Marr-Phillips, Jawaid, Gibson, & Norbury, 2017). Taken together, these data suggest that negative biases are present in never-depressed at-risk individuals and may, therefore, reflect a vulnerability marker for depression in these groups.

The amygdala has been shown to play a key role in both facial expression recognition and depression. For example, increased activation in the left amygdala in response to masked emotional (both fearful and sad) facial expressions (Sheline et al., 2001) as well as facial expressions morphed between intensities of sadness (Fu et al., 2004) have been reported in depressed patients which normalised with antidepressant treatment. Surguladze and colleagues also reported a linear increase in activation in the left amygdala of depressed individuals in response to increasing expressions of sadness (Surguladze et al., 2005). Using a

meta-analytic approach to pool data across numerous functional neuroimaging studies (44 in total), Groenewold and colleagues concluded that depressed patients display hyper-activation to negative stimuli and reduced activation to positive stimuli in left and right amygdala; a pattern of activation consistent with the negative biases widely reported in depression (Groenewold, Opmeer, de Jonge, Aleman, & Costafreda, 2013). Aberrant amygdala responses to negative facial expressions have also been associated with never-depressed at-risk groups; including high neuroticism (Chan et al., 2009), childhood adversity (Herringa et al., 2015) and offspring of depressed biological parents (Monk et al., 2008). Here, we report similar findings associated with late chronotype in never-depressed individuals.

The nature of the current study i.e. an implicit facial recognition task, also suggests that the aberrant amygdala responses observed in the current study are related to maladaptive implicit processing of negative facial expressions. This is similar to other studies using implicit processing tasks e.g. an unconscious masked faces paradigm (Sheline et al., 2001) and implicit sad facial expressions (Fu et al., 2004) reporting enhanced amygdala reactivity in depressed patients. It has been suggested that hyperactivity of the limbic system (particularly the amygdala) generates a bottom-up signal which suppresses higher cortical areas responsible for processing emotional information resulting in maladaptive interpretations of the environment and social interactions (Disner et al., 2011). This may therefore explain the neural basis for the negative biases observed in depressed and at-risk populations. For example, Mannie and colleagues reported participants with a biologically depressed parent displayed no biases for personality descriptors but an overall increased reaction time, suggesting a fault in the initial coding of emotionally valenced words (Mannie, Bristow, Harmer, & Cowen, 2007). Together, these findings suggest that a heightened amygdala response to negative affective stimuli may explain an increased risk for major depressive disorder, including late chronotype individuals.

The heightened amygdala response we observed in individuals with a later chronotype was also associated with reduced functional connectivity with the dACC. This finding is in accordance with previous evidence that depressed patients show reduced dACC-amygdala functional connectivity in response to negative (angry and sad) vs. neutral facial expressions (Dannlowski et al., 2009). Moreover, reduced ACC-amygdala connectivity has also been observed in never-depressed at-risk populations including high neuroticism (Cremers et al., 2010) and individuals with a genetic risk of depression (Pezawas et al., 2005). As reviewed by Disner et al (2011), cognitive biases observed in depression appear to be influenced by: 1) neurobiological processes that initiate the cognitive bias, and 2) reduced cognitive control, which allows the bias to continue (Disner et al., 2011).

The dACC is part of a network of higher cortical areas including the prefrontal cortex (PFC); medial and lateral orbitofrontal cortex involved in the cognitive regulation of limbic regions associated with processing emotion. In healthy controls, the dACC, or 'cognitive subdivision' has been shown to be involved with down regulation of negative emotions and modulation of the neural activity of the amygdala (Mak et al., 2009; Phan et al., 2005). The dACC also plays a critical role in monitoring and adjusting emotional reactivity and cognitive control (Botvinick, Braver, Barch, Carter, & Cohen, 2001; Kerns et al., 2004; Pizzagalli, 2011), and has been shown to be hypoactive in major depression (Davidson et al., 2002). It has been suggested that higher cortical areas responsible for suppressing task-irrelevant information using a 'top-down' mechanism may be altered in depression. For example, Etkin and colleagues demonstrated top-down inhibition of amygdala activity by the rostral ACC during an emotional conflict task using dynamic causal modelling (Etkin, Egner, Peraza, Kandel, & Hirsch, 2006). Although the directionality of the effect cannot be determined in the current study, the reduced connectivity observed between the dACC and amygdala may therefore support the notion of impaired top-down regulation of the amygdala response by the

dACC in individuals with a later chronotype. Of note, Rosenberg and colleagues (Rosenberg, Maximov, Reske, Grinberg, & Shah, 2014) reported significantly lower fractional anisotropy (FA; a measure of microstructural integrity) in white matter underlying the left ACC in healthy males free of current or previous psychiatric disorder characterised as late chronotypes as compared to early and intermediate types. In depressed patients, cingulate FA predicts remission (Korgaonkar, Williams, Song, Usherwood, & Grieve, 2014) and ACC white matter abnormalities have been reported in elderly depressed patients which affected cognitive functions and emotion modulation (Alexopoulos, Kiosses, Choi, Murphy, & Lim, 2002; Ballmaier et al., 2004). By contrast, Olvet et al., (Olvet et al., 2016) found no difference in cingulate FA values between depressed patients and healthy controls. The lack of consensus in studies of depressed patients may reflect the heterogeneous nature of the disorder. Emerging evidence in late chronotypes (Rosenberg et al., 2014) indicates reduced microstructural integrity of the ACC which could relate to abnormal suppression of the amygdala response, although future studies are needed to directly investigate this.

Previous neuroimaging studies show altered emotional regulation in depressed patients. For example, Erk and colleagues reported reduced functional connectivity between DLPFC and amygdala when depressed participants down-regulated negative images compared to healthy controls (Erk et al., 2010). The ability to down-regulate the negative emotion was also negatively correlated with the participant's HAMD (Hamilton Rating Scale for Depression) score. Beauregard and colleagues reported enhanced activity in dACC, right anterior temporal pole, right amygdala and right insular when depressed participants were asked to down-regulate their emotions whilst viewing sad films (Beauregard, Paquette, & Levesque, 2006), and Johnstone and colleagues reported increased activity in right PFC and ventro-lateral prefrontal cortex (VLPFC) of depressed participants during reappraisal of negative images (Johnstone, van Reekum, Urry, Kalin, & Davidson, 2007). Both studies

reported enhanced activation of higher cortical regions involved in emotion regulation circuitry showing less efficient engagement of these regions.

Behaviourally, there is also evidence to suggest that major depression is associated with impaired emotion regulation. For example, Joormann and colleagues (2010) reported that depressed patients display a lack of inhibition of negative material during a negative affective priming task, which was associated with greater rumination i.e. a maladaptive process of 'recycling' thoughts (Joormann & Gotlib, 2010). In the same study, reduced inhibition of negative materials was also related to less use of cognitive reappraisal; a beneficial emotional regulation strategy involving re-interpreting the meaning of an emotional situation, and more use of expressive suppression; a maladaptive strategy involving inhibiting the expression of an emotion (Joormann & Gotlib, 2010). The misuse of these emotional regulation strategies, in particular rumination, has been shown to be important in the recurrence of depressive episodes and to some extent the chronicity of depressive disorders (for a review, see Nolen-Hoeksema, 2000). Similarly, at-risk populations display impaired emotional regulation processes. For example, decreased thought suppression (a strategy to inhibit unwanted and intrusive thoughts) and increased rumination have been reported to mediate the association between high neuroticism and depression (Lu, Yang, Zhang, & Qiu, 2017). In relation to chronotype, Antypa and colleagues found cognitive reactivity (the activation of negative thoughts in response to low mood) and rumination to be mediators of the association between late chronotype and depression, independent of insomnia and neuroticism (Antypa et al., 2017). Moreover, late chronotype was recently found to be associated with increased expressive suppression whilst early chronotype was associated with increased cognitive reappraisal after controlling for age, gender, depressive symptoms, neuroticism and sleep quality (Watts & Norbury, 2017). In addition, evening and intermediate types report reduced self-control of thoughts, emotions, impulses, performance

regulation and habit breaking (as measured by the Self-Control Scale) as compared to early chronotypes (Wang & Hu, 2016). Although our current data does not address the hypothesis directly, our data (increased amygdala reactivity and decreased dACC-amygdala functional connectivity) and earlier findings of reduced emotion regulation (Antypa et al., 2017; Wang & Hu, 2016; Watts & Norbury, 2017) appear consistent with this model of bottom-up suppression of higher cortical areas and top-down regulation of limbic regions and could, in part, explain the increased vulnerability for depression in late chronotype individuals. However, future studies designed to directly investigate this model of emotional regulation are needed.

In adulthood, late chronotypes are typically younger than early chronotypes showing peak lateness at ~ 19-20 years and shifting to a more early chronotype thereafter (Fischer, Lombardi, Marucci-Wellman, & Roenneberg, 2017; Randler et al., 2016). Also, compared to early chronotypes, late chronotypes are more likely to report poor sleep quality, daytime tiredness (Taillard, Philip, Coste, Sagaspe, & Bioulac, 2003), consumption of nicotine and alcohol (Adan, 1994; Taillard, Philip, & Bioulac, 1999). By contrast, here we report that later chronotype was moderately associated with older age and higher sleep quality. This may reflect our sample with a relatively limited age range and the fact we excluded participants with a current or previously diagnosed sleep disorder who are most likely to suffer from poor sleep quality. It is also suggested that late chronotypes often suffer from 'chronic social jet lag' due to the discrepancy between their endogenous sleep/wake rhythm and external constraints such as work schedules that typically start early in the day (Roenneberg, Wirz-Justice, & Merrow, 2003). We did not measure social jet lag so cannot exclude that a mismatch between internal rhythm and external demands impacted on the current findings. The underlying causes that lead to depression are likely to be multifactorial and there is a need for longitudinal studies to explore and determine effective strategies that promote

psychological well-being in this population. Interventions such as cognitive bias modification may be useful for the prevention of depression in late chronotype individuals. Also, experimental manipulations that allow late chronotype individuals to follow, or better match, their circadian rhythm may be effective in reducing depressive symptomatology. Indeed, Vetter and colleagues reported increased well-being on weekdays when factory workers had their most strenuous shifts abolished (late evening shifts for early chronotypes and early morning shifts for late chronotype) (Vetter, Fischer, Matera, & Roenneberg, 2015). Alternatively, correcting phase disturbance with bright morning light, melatonin or melatonin agonists may also be useful strategies to improve mood (Kasper et al., 2010).

Limitations

Interpretation of the current findings should take into consideration a number of limitations. Chronotype was determined using a single brief self-report metric (the rMEQ). Although widely used and ratings obtained using this tool correlate well with objective measurements future studies may benefit from using additional measures; for example, core body temperature, estimates of melatonin and cortisol levels, polysomnography, sleep diaries and actigraphy. In addition, we did not fix scan times relative to individual wake up times to ensure that participants were in similar circadian phase. This is of importance as previous neuroimaging studies have reported chronotype by time-of-day dependent effects on BOLD response to a number of cognitive tasks [i.e. synchrony effects] (Schmidt et al., 2015; Song et al., 2017). In an alternative approach designed to limit potential synchrony effects Reske *et al.,* (2015) scanned participants performing a variable load attention-to-motion task at a fixed interval (between 10 and 12 hours) post individual waking time. During high-attentional load early and late chronotype, as compared to intermediate types, showed reduced BOLD in right dorsolateral prefrontal cortex. At moderate attentional load a more complex pattern emerged, early chronotypes had greater BOLD response in bilateral insula whereas late chronotypes

showed reduced activation in right superior parietal cortex (Reske, Rosenberg, Plapp, Kellermann, & Shah, 2015). Using the same approach Rosenberg and colleagues (2015) explored chronotype effects on a semantic priming task. Across all contrasts reported late chronotypes, relative to early or intermediate types, showed increased activation in a number of anatomical locations previously implicated in semantic processing (Rosenberg, Reske, Warbrick, & Shah, 2015). The limited available evidence clearly points to both chronotype-specific and chronotype-by-time dependent effects on regional BOLD. However, whether these synchrony effects (Schmidt et al., 2015; Song et al., 2017) translate from cognitively demanding tasks to less demanding implicit emotional processing tasks as reported in current work is unknown. Here, including time of scan as a covariate did not impact on the pattern of results and confirmed that late chronotype is associated with increased amygdala response to negative stimuli and reduced fronto-limbic connectivity after controlling for a number of possible confounds. Future studies, however, would benefit from explicitly controlling scan time according to individual chronotype. Current, previous and family history of depression was determined using self-report. Future studies may benefit from reference to medical history or structured clinical interview to assess exclusion criteria. We also did not measure or exclude participants with high neuroticism trait which is another population proven to be at-risk of depression (Kendler, Gatz, Gardner, & Pedersen, 2006). There is some evidence that low neuroticism and early chronotype are correlated (Duggan, Friedman, McDevitt, & Mednick, 2014), however we have previously shown that biases in emotional processing are present in a similar sample of young adults despite no observable differences in neuroticism (Berdynaj et al., 2016). Also, there a number of hormones that show diurnal variation (e.g. cortisol). As we did not conduct a blood assay we cannot rule out neuroendocrine effects on the current results. Of particular note, repeated clinical observations have reported an association between acute depression and increased availability of cortisol (Cowen, 2010)

and elevated levels of cortisol is associated with hyperactivity of the amygdala (Tafet & Nemeroff, 2016). Against this, Kudielka et al, (Kudielka, Federenko, Hellhammer, & Wust, 2006) reported an increased cortisol awakening response in early vs. late chronotypes independent of sleep duration or awakening time. Similarly, Maierova and colleagues (Maierova et al., 2016) observed higher overall concentrations of cortisol in early chronotypes tested across a period of many hours. In this context, our observation of an association between late chronotype and increased activation in bilateral amygdala makes fluctuation in cortisol levels an unlikely explanation for our findings.

Conclusion

In conclusion, a clear association was found between late chronotype and increased sensitivity to negative emotional facial expressions in bilateral amygdala. Late chronotype was also associated with reduced dACC-amygdala functional connectivity suggesting impaired emotional regulation circuitry. These findings suggest that late chronotype is associated with an altered neural signature similar to that seen in depressed individuals and other at-risk groups and could be related to the 'chronic social jet lag' they often experience. The present findings highlight important clinical and theoretical implications for the prevention and treatment of depression in this at-risk group. Longitudinal studies are needed to investigate the predictive power of negative biases and impaired emotional regulation for the development of depression, as well as effective interventions to promote well-being in late chronotypes.

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Competing interests

The authors declare no issues of competing interests.

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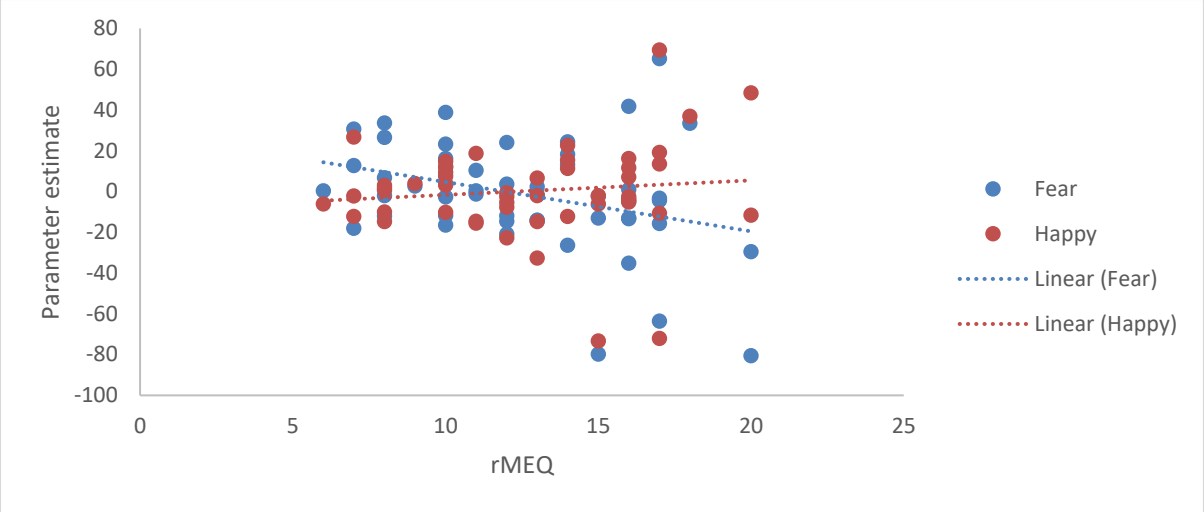
Supplementary Material

Inspection of the response in left amygdala to fear and happy faces separately (please see Supplemental Figure 1 below) as a function of chronotype indicates that later chronotype is associated with increased response to fear ($r(50) = -.32$, p .02) an opposite (non-significant) association was observed for happy facial expressions).



Supplemental Figure 1. Scatter plot showing a negative association between chronotype and BOLD response to fearful facial expressions (blue circles and dotted line). The relationship between chronotype and BOLD response to happy facial expressions is also shown (data in red).