Brainstem volume mediates seasonal variation in depressive symptoms: A cross sectional study in the UK Biobank cohort

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Seasonal differences in mood and depressive symptoms affect a large percentage of the general population, with seasonal affective disorder (SAD) representing the most common presentation. SAD affects up to 3% of the world’s population, and it tends to be more predominant in females than males. The brainstem has been shown to be affected by photoperiodic changes, and that longer photoperiods are associated with higher neuronal density and decreased depressive-like behaviours. We predict that longer photoperiod days are associated with larger brainstem volumes and lower depressive scores, and that brainstem volume mediates the seasonality of depressive symptoms. Participants (N = 9289, 51.8% females and 48.1% males) ranging in age from 44 to 79 years were scanned by MRI at a single location. Photoperiod was found to be negatively correlated with low mood and anhedonia in females while photoperiod was found to be positively correlated with brainstem volumes. In females, whole brainstem, pons and medulla volumes individually mediated the relationship between photoperiod and both anhedonia and low mood, while midbrain volume mediated the relationship between photoperiod and anhedonia. No mediation effects were seen in males. Our study extends the understanding of the neurobiological factors that contribute to the pathophysiology of seasonal mood variations.

Seasonal fluctuations in mood and depressive symptoms affect a large number of the general population, and these depressive symptoms such as depressed mood and fatigue have been found to be greater in winter compared to summer seasons in higher latitude countries1–4. Populations with seasonal affective disorder (SAD), a type of recurring major depression with a seasonal pattern, represent the most common form of seasonal fluctuations in mood5–8. SAD is often characterized by depression and fatigue occurring in winter with full remission taking place in summer. It has been reported that SAD affects up to 3% of the world’s population, and it tends to be more predominant in females than males with a reported female-to-male ratio of 4:17–9. Females have been found to suffer from mood changes and depressive symptoms related to dark and cloudy weather at a greater rate compared to males4,9. Although seasonal variations in mood have been studied widely among sexes, little is known about the neurobiological factors linking light exposure and mood.

It has been suggested that changes in photoperiod (duration of sunlight) may be associated with seasonal mood variations10,11 by shifting the circadian phase with its associated disruptions in sleep and other health outcomes. However, photoperiodic changes have also been suggested to affect specific brain regions that might be implicated in mood disorders. For example, the hippocampus and hypothalamus have been shown to be affected by seasonal changes in photoperiod. In particular, a smaller volume of the hippocampus was associated with shorter photoperiods in the winter months compared to summer12–14, and higher gene expression and hormonal activity of the hypothalamus were associated with longer photoperiod days in summer compared to winter15. In addition, the brainstem has been shown to be associated with seasonal changes. In particular, in Rana temporaria L., the size of the nuclei of the medulla oblongata cells controlling lipofuscin in pigment was significantly associated with changes in photoperiod during the annual cycle, and that higher volume of the nuclei of the medulla oblongata was detected in July and lower volume was detected in March16. Moreover, photoperiodic changes have been shown to influence the midbrain dorsal raphe serotonin neurons. For example, mice exhibit increased firing

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rates, levels of mood neurotransmitters (serotonin and norepinephrine), and responsiveness to noradrenergic stimulation when exposed to longer photoperiod days compared to those exposed to shorter photoperiod days. Furthermore, in humans, longer photoperiod days were significantly associated with a higher density of dopamine neurons, tyrosine hydroxylase TH (the rate-limiting enzyme in dopamine synthesis) neurons, dopamine transporter (DAT) neurons, and DAT and TH neurons immunoreactivities in the midbrain compared to short photoperiod days. It has been suggested that the different densities of dopamine and TH neurons could be due to neurogenesis, a new generation of neuron cells, in the brain, including midbrain. Together, these studies suggest that changes in photoperiod directly affect the volume of the medulla oblongata, or affect the density of serotonergic and dopaminergic midbrain neurons, which in turn may lead to morphology changes of the brainstem. Therefore, we hypothesize that longer photoperiod days are associated with larger brainstem substructure volumes and shorter photoperiod days are associated with smaller brainstem substructure volumes.

Changes in the brainstem substructure volumes have been found to be linked to the pathophysiology of several mood disorders. Photoperiodic changes in the brainstem substructures may contribute to seasonally occurring phenotypes such as seasonal affective disorder (SAD) where symptoms such as depression and fatigue are present in the winter season with full remission taking place in the summer season. Phenotypical and morphological brainstem, particularly midbrain, changes in adult mice exposed both prenatally and postnatally to longer photoperiods are associated with decreased depressive-like and anxiety-like behaviours (by decreasing immobility during the forced swim test and time spent in the close arms of the elevated zero maze) compared to those exposed to short photoperiods. Overall, this evidence suggests that seasonal changes in brainstem substructures are linked to depressive-like behaviours.

In light of this, a cross-sectional study within a large population cohort, was conducted to analyse the links between seasonal variation in photoperiod with seasonal variation in mood and seasonal variation in brainstem volume. The aim was to explore whether seasonal variation in depressive symptoms including low mood, anhedonia, tenseness and tiredness was mediated by brainstem or substructure volumes.

Results

Participant characteristics. Nine thousand and two hundred and eighty-nine participants (51.8% females, 48.1% males ages ranging from 44 and 79 years (mean = 62.4, SD = 7.4)) taken from the UK biobank cohort were included in this study. High resolution three-dimensional T1 weighted images were collected from all participants. The MRI scans were acquired between May 2014 and December 2016 with the date of scan recorded for each participant. All 9,289 participants completed a touchscreen questionnaire on their mood in the two weeks prior to the MRI assessment. Participants lived in approximately equal proportions north and south of the scanning centre with a mean distance of 31.1 km North or South. Photoperiod for each participant’s date of scan was measured based on the location of residence of each participant. The average range of observed photoperiod is from 7.25 hours in winter to 17.22 hours in summer. The range for each mood measure was from 0 to 3 (where 0 = not at all and 3 = nearly every day). Demographic characteristics are presented in Table 1.

Tests of seasonality of brainstem substructure volumes using cosinor models. To test for seasonality, we used a cosinor analysis to examine brainstem substructure volumes. We found significant cosinor terms for all brainstem volumes including midbrain, pons, medulla and whole brainstem in all participants, and when separated into females and males, p < 0.001 (See Table 2). The acrophase peak (greatest volume) for the midbrain, pons, medulla and whole brainstem volumes occurred in July for all participants and males while in June for females.

Association of photoperiod with depressive symptoms. Negative binomial regression was conducted to investigate the association between photoperiod and depressive symptoms including low mood, anhedonia, tenseness, tiredness and total depressive score. For all participants there was a significant negative correlation between photoperiod and low mood (p < 0.05). When corrected for age, ethnicity, living area (urban or rural) and Townsend deprivation index the correlation in low mood did not remain significant (See Table 3). No significant correlations between photoperiod and anhedonia, tenseness, tiredness and total depressive score for all participants were seen. In females, photoperiod was negatively correlated only with low mood and anhedonia (p = 0.03) when corrected for age, ethnicity, living area (urban or rural) and Townsend deprivation index. When the p-value was Bonferroni corrected (0.05/15 = 0.003), there were no significant correlations between photoperiod and low mood and anhedonia in females. In males, no significant correlations between photoperiod and any of these depressive symptoms (low mood, anhedonia, tenseness, tiredness and total depressive score) were seen either before or after correction for the above confounders.

Association of brainstem substructure volumes with photoperiod. There were significant correlations between brainstem and substructure volumes and photoperiod in all participants and both females and males separately when the p-value was corrected for multiple comparisons (p < 0.003). To further explore the observed association between brainstem volume and photoperiod, a multiple linear regression model was applied to better understand the variance in the brainstem volume when accounting for known confounds. Age and total brain volume (TBV) covariates were found to associate with brainstem and substructure volumes, and that age was negatively correlated with all brainstem volumes (p < 0.001), while TBV was positively correlated with all brainstem volumes in all participants and both females and males. Thus, these covariates were entered in separate blocks in a hierarchical regression model to account for their confounding effects. When photoperiod was corrected for these two covariates, there were significant correlations between brainstem and substructure volumes and photoperiod in all participants and both females and males separately with the p-value corrected for multiple comparisons (p < 0.003). Photoperiod was positively correlated with whole brainstem r²(9289) = 0.021, medulla...
There were significant correlations between brainstem and substructure volumes and depressive symptoms including low mood, anhedonia and total depressive symptoms in all participants but only in females when the group was categorised by sex (corrected for age, TBV, ethnicity, living area and Townsend deprivation index). For all participants, low mood, anhedonia and total depressive score were negatively correlated with all brainstem volumes except the midbrain and SCP, \( p < 0.05 \) for all (See Table 5). When the p-value was Bonferroni corrected (0.05/75 =

### Variable Mean (SD)

| Number of all participants | 9289 | Age (years) 62.4 (7.4) |
|----------------------------|------|------------------------|
| Low mood                   | 0.21 (0.50) |
| Anhedonia                  | 0.19 (0.45) |
| Tenseness                  | 0.24 (0.52) |
| Tiredness                  | 0.57 (0.74) |
| Total depressive score     | 1.21 (1.76) |
| Townsend deprivation score | −2.01 (2.56) |
| Number of females          | 4817  |
| Age (years)                | 61.7 (7.2) |
| Low mood                   | 0.24 (0.54) |
| Anhedonia                  | 0.20 (0.51) |
| Tenseness                  | 0.26 (0.54) |
| Tiredness                  | 0.65 (0.78) |
| Total depressive score     | 1.35 (1.87) |
| Townsend deprivation score | −1.96 (2.57) |
| Number of males            | 4472  |
| Age (years)                | 63.1 (7.5) |
| Low mood                   | 0.17 (0.45) |
| Anhedonia                  | 0.18 (0.47) |
| Tenseness                  | 0.22 (0.50) |
| Tiredness                  | 0.49 (0.68) |
| Total depressive score     | 1.07 (1.63) |
| Townsend deprivation score | −2.05 (2.55) |

| Living area, N (%)          |      |
|----------------------------|------|
| Rural                      | 671  (7.22) |
| Urban                      | 8553 (92.07) |

| Ethnic background, N (%)    |      |
|----------------------------|------|
| White                      | 8728 (93.96) |
| Black                      | 29 (0.31) |
| Mixed                      | 271 (2.91) |
| Asian                      | 191 (2.05) |
| Chinese                    | 30 (0.32) |
| Other                      | 40 (0.43) |
| Photoperiod in hours       | 13.003 (3.05) |

| Brain volumes              |      |
|----------------------------|------|
| Whole brainstem (mm³)      | 25341.6 (3008) |
| Midbrain (mm³)             | 6026.7 (645) |
| Pons (mm³)                 | 14750.3 (1916) |
| Medulla (mm³)              | 4321.4 (565) |
| WMV (cm³)                  | 498.57 (59.3) |
| GMV (cm³)                  | 628.04 (55.03) |
| TBV (cm³)                  | 1125.92 (109.43) |

**Table 1.** Characteristics of the UK Biobank participants. Abbreviations: SD; Standard Deviation; N: number; WMV; White Matter Volume, GMV; Grey Matter Volume, TBV; Total Brain Volume.
0.0006), only the correlation between medulla and total depressive symptom remained significant (p < 0.0006). In females, whole brainstem and pons were associated with low mood, anhedonia and total depressive score, and the medulla volume was associated with low mood, anhedonia, tenseness and total depressive score, whereas midbrain volume was significantly associated with only anhedonia, p < 0.05 for all (See Table S5). When the p-value was Bonferroni corrected (0.05/75 = 0.0006), the correlations between anhedonia and whole brainstem and pons remained significant (p < 0.0006). No significant correlations between SCP volume and all depressive symptoms including low mood, anhedonia, tenseness, tiredness and total depressive score were seen in females. No significant correlations between all brainstem substructure volumes and low mood, anhedonia, tenseness, tiredness and total depressive score were seen in males.

**Mediation analysis.** A mediation analysis was performed to examine whether brainstem volumes mediate the relationship between photoperiod and depressive symptoms including low mood and anhedonia in females (Fig. 2). Negative binomial regression analysis (corrected for age, ethnicity, living area, Townsend deprivation index and TBV) was used to test path-correlations. Photoperiod and the hypothesised mediator(s) (whole brainstem, midbrain, pons and medulla volumes) were significantly associated (Table 4). In addition, whole brainstem, pons and medulla volumes were significantly related to both low mood and anhedonia, while midbrain volume was significantly related to anhedonia (Table 5). To test whether volume reduced the associations of photoperiod and anhedonia or photoperiod and low mood, whole brainstem, midbrain, pons and medulla volumes were added separately as predictors to negative binomial regression models. We found that: (1) the association between photoperiod and anhedonia was reduced and no longer significant when whole brainstem, midbrain, pons and medulla were included; (2) larger whole brainstem, midbrain, pons and medulla volumes which in turn were related to reporting reduced anhedonia, p = 0.114 for whole brainstem, p = 0.027, CI [-0.057 to 0.002] and p = 0.066 for midbrain, p = 0.024, CI [-0.054 to 0.005] and p = 0.100 for pons, and (3) β = -0.022, CI [-0.053 to 0.007] and p = 0.127 for medulla. (2) the association between photoperiod and low mood was reduced and no longer significant when whole brainstem, pons and medulla were included, β = -0.026, CI [-0.059 to 0.002] and p = 0.072 for whole brainstem, β = -0.027, CI [-0.060 to 0.001] and p = 0.062 for pons and β = -0.024, CI [-0.057 to 0.005] and p = 0.097 for medulla, while the association between photoperiod and low mood remained significant and did not reduce when midbrain was included, β = -0.030, CI [-0.063 to -0.002] and p = 0.040. Because these results satisfy the requirements of the mediation analysis, we examined whether whole brainstem, midbrain, pons and medulla significantly mediate the relationship between photoperiod and anhedonia, and whether whole brainstem, pons and medulla significantly mediate the relationship between photoperiod and low mood.

To formally test the mediation, we used a bias corrected and accelerated bootstrap method (PROCESS macro in SPSS). The indirect effects were significant (See Table 6) meaning that longer photoperiod days were associated with (1) larger whole brainstem, midbrain, pons and medulla volumes which in turn were related to reporting reduced anhedonia, β = -0.046, CI [-0.114 to -0.027] and p = 0.001 for whole brainstem, β = -0.030, CI [-0.104 to -0.003] and p = 0.037 for midbrain, β = -0.047, CI [0.010 to -0.026] and p = 0.001 for pons, and β = -0.043, CI [-0.093 to -0.019] and p = 0.003 for medulla, and (2) larger whole brainstem, pons and medulla

| Outcome | Multivariate generalized regression estimates | Acrophase (Month) | Amplitude |
|---------|-----------------------------------------------|--------------------|-----------|
|         | Multivariate generalized regression estimates |                    |           |
|         | Cosine coefficient b | (SE) | P | Sine coefficient b | (SE) | P | AIC | AAIC |           |
| a) All participants | | | | | | | | | |
| Midbrain (n = 9289) | -67.61 | 6.19 | <0.001 | 26.92 | 6.19 | <0.001 | -127.26 | July | 72.7 |
| Pons (n = 9289) | -255.05 | 12.81 | <0.001 | 94.11 | 21.37 | <0.001 | -143.89 | July | 271.8 |
| Medulla (n = 9289) | -115.98 | 7.16 | <0.001 | 43.30 | 7.02 | <0.001 | -277.9 | July | 123.8 |
| Brainstem (n = 9289) | -441.75 | 32.76 | <0.001 | 166.20 | 32.10 | <0.001 | -193.01 | July | 471.9 |
| b) Females | | | | | | | | | |
| Midbrain (n = 4817) | -57.93 | 7.82 | <0.001 | 31.27 | 7.63 | <0.001 | -62.74 | June | 65.8 |
| Pons (n = 4817) | -251.40 | 28.58 | <0.001 | 120.82 | 27.92 | <0.001 | -85.71 | June | 278.9 |
| Medulla (n = 4817) | -110.33 | 9.40 | <0.001 | 50.40 | 9.18 | <0.001 | -151.80 | June | 121.3 |
| Brainstem (n = 4817) | -422.66 | 42.60 | <0.001 | 204.09 | 41.61 | <0.001 | -110.05 | June | 469.3 |
| c) Males | | | | | | | | | |
| Midbrain (n = 4472) | -85.15 | 9.43 | <0.001 | 24.14 | 9.26 | 0.009 | -80.48 | July | 88.5 |
| Pons (n = 4472) | -265.05 | 33.17 | <0.001 | 68.30 | 32.56 | 0.036 | -61.70 | July | 273.7 |
| Medulla (n = 4472) | -125.91 | 10.80 | <0.001 | 36.83 | 10.61 | 0.001 | -136.48 | July | 131.2 |
| Brainstem (n = 4472) | -479.60 | 49.89 | <0.001 | 131.53 | 48.97 | 0.007 | -91.40 | July | 497.3 |

Table 2. Cosinor parameters for brainstem substructure volumes in all participants, females and males. Generalized linear regression coefficients (b), robust standard error (SE), probability of significance (p) for cosine and sine transformations of the month of scan for: (a) all participants, (b) females and (c) males. Models were adjusted for age and TBV. AIC values refer to the differences between this model and a model excluding sine and cosine terms but including all the covariates (age and TBV). Seasonality of outcome variables (whole brainstem and substructure volumes except SCP) is inferred from (1) the significance (p < 0.05) of the cosine and sine generalized linear regression coefficients and (2) improved model fit included cosinor terms by reduced AIC value (AAIC).
Table 3. Associations between photoperiod and all depressive symptoms in all participants, females and males. Negative binomial regression coefficients (b), robust standard error (SE) and incidence rate ratios (IRR) for association between photoperiod (corrected for age, ethnicity, living area and Townsend deprivation index) and low mood, anhedonia, tenseness, tiredness and total depressive score. Significant associations (p < 0.05) are shown in bold.

| Model | b (SE) | IRR | p |
|-------|--------|-----|---|
| **All participants** | | | |
| Low mood (n = 9289) | -0.015 (0.008) | 0.984 | 0.053 |
| Anhedonia (n = 9289) | -0.013 (0.008) | 0.987 | 0.122 |
| Tensioness (n = 9289) | -0.007 (0.007) | 0.993 | 0.339 |
| Tiredness (n = 9289) | 0.002 (0.005) | 1.002 | 0.700 |
| Total depressive score (n = 9289) | -0.005 (0.004) | 0.995 | 0.247 |
| **Females** | | | |
| Low mood (n = 4817) | -0.022 (0.010) | 0.978 | 0.038 |
| Anhedonia (n = 4817) | -0.025 (0.011) | 0.975 | 0.033 |
| Tensioness (n = 4817) | -0.014 (0.010) | 0.986 | 0.175 |
| Tiredness (n = 4817) | 0.000 (0.007) | 1.000 | 0.980 |
| Total depressive score (n = 4817) | -0.010 (0.006) | 0.990 | 0.111 |
| **Males** | | | |
| Low mood (n = 4472) | -0.009 (0.012) | 0.991 | 0.487 |
| Anhedonia (n = 4472) | 0.001 (0.012) | 1.001 | 0.920 |
| Tensioness (n = 4472) | 0.000 (0.011) | 1.000 | 0.985 |
| Tiredness (n = 4472) | 0.003 (0.008) | 1.003 | 0.721 |
| Total depressive score (n = 4472) | -0.001 (0.006) | 0.999 | 0.914 |

Discussion

We have shown that brainstem volumes are associated with photoperiod in humans. Interestingly, we found that in females whole brainstem, pons, and medulla volumes mediated the relationship between photoperiod and both anhedonia and low mood, while midbrain volume mediated the relationship between photoperiod and anhedonia only. No mediation effects for the other depressive symptoms were found in females. No mediation effects were found in males. These findings are the first to demonstrate the mediating effects of brainstem volumes on the seasonal variability of mood and anhedonia.

We also found that photoperiod was associated with depressive symptoms including low mood and anhedonia in females but not in males, where longer photoperiod days were associated with reporting reduced low mood and anhedonia. This however did not remain after Bonferroni correction. This association has been previously reported^1^, Lyall et al., study had significantly greater statistical power (n = up to 80,000) which may explain why the association no longer remains significant after correction.

To our knowledge no previous animal or human studies have reported seasonal variations in brainstem volumes with only a small number focusing on the association between photoperiod and the density of serotonergic and dopaminergic neurons and binding transporters in the brainstem, especially midbrain or raphe nuclei as described above20,21,17,18,30. However, circadian rhythms are generated and maintained by a neural clock that is regulated by midbrain raphe nuclei in the suprachiasmatic nucleus (SCN)^31^, Therefore, any circadian clock disruptions caused by changes in photoperiod may alter midbrain raphe nuclei which in turn may lead to morphology changes of the brainstem. Our findings support the notion that changes in photoperiod change the brainstem substructure volumes.

The biological mechanisms behind changes in brainstem volumes in mood disorders are still unclear. Previous studies22-24 that have shown that individuals with major depressive disorder (MDD) showed increased whole brainstem and midbrain volumes compared to healthy controls. In addition, previous studies25 have shown that the echogenicity of the brainstem raphe nuclei is altered in patients with unipolar depressive disorders (UDD)
Table 4. Linear correlations between brainstem substructure volumes (corrected for age and total brain volume) and photoperiod in all participants, females and males. Abbreviations; SCP: Superior Cerebellar Peduncle; r: Pearson correlation (standardized regression coefficient); B: regression coefficient (mm$^3$/hour); SE: standard error; M: mean; SD: standard deviation; p: probability of significance.

| Volume M (SD) | r     | B (SE) | p     |
|---------------|-------|--------|-------|
| **All participants (n = 9224)** | | | |
| Whole brainstem | 25341.6 (3008) | 0.147 | 105.6 (7.3) | <0.001 |
| Midbrain | 6026.7 (645) | 0.123 | 16.5 (1.3) | <0.001 |
| SCP | 243.13 (44) | 0.064 | 0.807 (0.13) | <0.001 |
| Pons | 147.25 (1916) | 0.127 | 60.7 (4.9) | <0.001 |
| Medulla | 4321.4 (565) | 0.175 | 27.5 (1.6) | <0.001 |
| **Females (n = 4817)** | | | |
| Whole brainstem | 24086.9 (2547) | 0.155 | 104.5 (9.5) | <0.001 |
| Midbrain | 5701.1 (507) | 0.120 | 14.7 (1.7) | <0.001 |
| SCP | 228.27 (38) | 0.064 | 0.742 (0.16) | <0.001 |
| Pons | 14023.9 (1667) | 0.138 | 62.0 (6.4) | <0.001 |
| Medulla | 4133.5 (500) | 0.181 | 27.0 (2.1) | <0.001 |
| **Males (n = 4472)** | | | |
| Whole brainstem | 26693.1 (2879) | 0.146 | 110.6 (11.1) | <0.001 |
| Midbrain | 6377.5 (592) | 0.140 | 20.0 (2.1) | <0.001 |
| SCP | 259.13 (45) | 0.069 | 0.933 (0.20) | <0.001 |
| Pons | 15532.7 (1859) | 0.121 | 69.7 (7.4) | <0.001 |
| Medulla | 4523.7 (561) | 0.176 | 28.9 (2.4) | <0.001 |
Table 5. Associations between brainstem substructure volumes and depressive symptoms in all participants, females and males. Negative binomial regression coefficients (b), robust standard error (SE) for association between brainstem substructure volumes (corrected for age, ethnicity, TBV, living area and Townsend deprivation index) and low mood, anhedonia, tenseness, tiredness and total depressive score in (a) all participants, (b) females and (c) males. Significant associations (p < 0.05) are shown in bold.

compared to healthy controls. Our finding of a negative association between brainstem volumes and depressive symptoms (low mood, anhedonia and total depressive score) in a large population cohort adds to this evidence.

Further, previous studies30,33,34 have suggested that seasonal changes in serotonin (5-hydroxytryptamine; 5-HT) expression, which is mainly synthesized by several nuclei of the midbrain and pons such as dorsal raphe nucleus and locus coeruleus31 could be the molecular mechanism that drives this correlation. They found that individuals with seasonal affective disorder showed higher cerebral serotonin transporter binding in winter, but not in summer, compared to healthy controls, and this change in serotonin transporter binding was positively associated with severity of depressive symptoms. Together, these results support the notion of the role of the brainstem in regulating related-mood processing.

In addition, the result of the association of mood or depressive symptoms including low mood and anhedonia with season in only females before correction for multiple comparisons is consistent and supported by several previous studies32,36,45 in which females were found to have more hospital admissions due to winter depression and also to report higher prevalence of depression or depressive symptoms during shorter photoperiod days in the winter months compared to males. Little is known about the mechanisms underlying sex-related differences in
Figure 2. The mediation model demonstrates that the relationship between photoperiod and anhedonia in females was mediated by whole brainstem (a), midbrain (b), pons (c) and medulla (d) and the relationship between photoperiod and low mood was mediated by whole brainstem (e), pons (g) and medulla (h), while the relationship between photoperiod and low mood was not meditated by midbrain (f). Standardized beta values are shown on the model paths. a and b included in the model show the direct effects of the relationship between photoperiod and depressive symptoms. c and c’ show the total and the indirect effects of the relationship between photoperiod and anhedonia as well as low mood without and with the mediators respectively. *p < 0.05, **p < 0.001.
seasonality of mood though this phenomenon has been widely investigated. One possibility is that the sex-related differences in seasonal variation in mood could be due to the differences in cortico-limbic mood regulation network, which includes the hippocampus, amygdala, prefrontal and anterior cingulate cortices and anterior thalamic nuclei, between males and females. Interestingly, the features of subgenual anterior cingulate cortex (sgACC), which have been shown to elevate the metabolic activity in the presence of depression, dysfunction in mood disorders are different between males and females, and females exhibit higher levels of reactivity compared to males. Therefore, it is possible that the corticolimbic network of mood regulation in females is more affected by photoperiod than males, leading to impact the mood status during the year between sexes. Another possibility could be explained by the difference in cortisol hormone and inflammatory stress responses, which have been linked to the prevalence of depression. It has been shown that females have greater cortisol and inflammatory stress responses are more sensitive to depressed mood when inflammation is present. Together, these studies suggest neuroanatomical and/or hormonal sex-related differences that could be the mechanisms underlining seasonal variation in mood between sexes.

The current study design has three limitations. First, our study was cross-sectional in which participants were measured only once rather than at different times over the year, therefore the brainstem volumes measured represent inter-individual variance not change. Making a causal statement about seasonal change of an individual brainstem volume would require a longitudinal study. Second, depressive symptom scores were taken from questions about feelings over the previous two weeks and may be subject to recall bias and also gender biases in reporting mood. Third, we included all data available in the January 2017 brain imaging data release. This means that we included participants who may have medical or psychiatric issues related to their brain such as stroke, Alzheimer’s disease, congenital or acquired structural brain defects.

To conclude, our study is the first to demonstrate that brainstem volumes fully mediate the seasonal variability of depressive symptoms. We further showed that this mediating effect is only present in females. This finding advances our understanding of brainstem morphology and suggests it may be an important neural substrate in the pathophysiology of seasonal mood disorders. This finding adds to the evidence supporting the role of photoperiod on brain structural plasticity which will have implications for future investigations of changes in mood associated with human exposure to variations in natural and artificial light.

### Methods

**Participants.** From 2006–2010, 502,655 participants aged 37–73 years were recruited into the UK Biobank cohort. Participants attended one of 22 assessment centres across the UK and completed a range of lifestyle, demographic, health and mood questionnaires, cognitive assessments and physical measures, and subsequently cohort. Participants attended one of 22 assessment centres across the UK and completed a range of lifestyle, health and mood questionnaires, cognitive assessments and physical measures, and subsequently completed cognitive assessments and physical measures. Negative binomial regression coefficients (b), robust standard error (SE), bootstrapping confidence interval (CI), lower level and upper level confidence interval (LL and UL) for the mediation analysis of brainstem substructure volumes on the relationship between photoperiod and depressive symptoms. Significant associations (p < 0.05) are shown in bold.

| Low mood | Anhedonia |
|----------|-----------|
|          |           |
| b (SE)   | Bootstrap CI [LL to UL] | p   | b (SE)   | Bootstrap CI [LL to UL] | P   |
| Brainstem | −0.0063 (0.002) | −0.0112 to −0.0018 | 0.004 | −0.0072 (0.002) | −0.0120 to −0.0027 | 0.001 |
| Midbrain | −0.0027 (0.001) | −0.0062 to 0.0007 | 0.112 | −0.0036 (0.001) | −0.0074 to −0.0001 | 0.037 |
| Pons     | −0.0054 (0.002) | −0.0096 to −0.0014 | 0.005 | −0.0063 (0.002) | −0.0106 to −0.0025 | 0.001 |
| Medulla  | −0.0083 (0.002) | −0.0139 to −0.0028 | 0.001 | −0.0079 (0.002) | −0.0137 to −0.0024 | 0.002 |

Table 6. Mediation analyses examining the relationship between photoperiod and both low mood and anhedonia in females via whole brainstem, midbrain, pons and medulla volumes.
Volumetric analysis and segmentation. Volumetric processing and segmentation were performed using a development version of the FreeSurfer v6.0 software package (http://surfer.nmr.mgh.harvard.edu), with brainstem segmentation. We chose FreeSurfer for segmentation due to its good reproducibility in brainstem segmentations compared to other methods. FreeSurfer was used to process the data including averaging volumetric T1 weighted images, motion correction, transformation to Talairach image space, nonuniform intensity normalization for intensity inhomogeneity correction, removal of non-brain tissues using hybrid watershed, and segmentation of subcortical volumetric structures; white matter and deep grey matter. FreeSurfer was used to segment the brainstem subfield volumes (medulla oblongata, pons, superior cerebellar peduncle and midbrain). Briefly, 39 MRI scans were manually delineated to highlight the whole brainstem, together with manual labelling and MNI atlas. All segmentations for the brainstem were visually checked for errors. No manual interventions were performed on the data.

Statistical analysis. Statistical analyses were conducted using SPSS version 24, with an alpha for all analyses of \( p = 0.05 \). We tested the seasonal pattern of photoperiod (as a continuous measure of day length) and we found that it follows a sinusoidal pattern. No further transforms were applied. To investigate the association of photoperiod with depressive symptoms including low mood, anhedonia, tenseness, tiredness and total depressive symptoms score, a negative binomial regression model was used. Likelihood ratio tests for these depressive scores showed that over-dispersion was greater than 1, i.e. their variance was greater than their mean. We investigated the seasonality of brainstem volumes using a cosinor generalized regression analysis with both sine and cosine month as the time variable. We assessed whether the seasonal pattern of the brainstem is sinusoidal, by comparing a model including sine and cosine month transformations and the covariates of age and TBV with models excluding sine and cosine month transformations. We determined two specific criteria for indicating the significance of seasonality or improved model fit and these were (1) significance of sine and/or cosine (cosinor) terms (\( p < 0.025 \)), with amplitude significantly greater than zero and (2) lower Akaike Information Criterion (AIC) for the model including the cosinor terms. The amplitude of the cosinor model (or curve) was calculated as:

\[
A = \sqrt{\beta^2 + \gamma^2}
\]

where \( \beta \) and \( \gamma \) are cosine and sine generalized regression coefficients respectively. Finally, the Acrophase (\( \phi \); peak of cosinor model) in month of scan was calculated from:

\[
\phi = 12 \times \tan^{-1}(\gamma/\beta) + 1
\]

Pearson (bivariate) correlations between photoperiod and brainstem substructure volumes as well as age, and total brain volume (TBV) were performed, with significance levels Bonferroni-corrected for multiple comparisons and set at \( p = 0.003 \). To investigate the predictability for each of these independent variables for the brainstem subfield volumes, single linear regression models for each were created. There were significant correlations between brainstem subfield volumes and age, total brain volume, ethnicity, living area (urban or rural).
and Townsend deprivation index, therefore, these predictors were included as covariates in a multiple regression model. For the mediation analysis, negative regression analysis was used here to test whether brainstem or substructure volumes mediate the relationship between photoperiod and low mood as well as anhedonia. Eight separate mediation analyses were conducted to examine whole brainstem, midbrain, pons and medulla volumes as mediators in the relationship between photoperiod and both anhedonia and low mood, after controlling for the relevant covariates mentioned above. The mediation analysis applied here used the standard three-variable path model\textsuperscript{33}, and a bootstrapping test (with 5000 samples to compute the 95% confidence interval) for the statistical significance of the model using the "PROCESS" method\textsuperscript{34} in SPSS version 24.

Data availability

The datasets processed and analysed during the current study are available from the online open access UK Biobank repository (https://www.ukbiobank.ac.uk/). This research was conducted under the UK Biobank Resource under Application Number 24089 (PI Waiter).

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References

1. Harmatz, M. G. et al. Seasonal Variation of Depression and Other Moods: A Longitudinal Approach. J. Biol. Rhythm. 15, 344–350 (2000).
2. Bartko, J. J. & Kasper, S. Seasonal changes in mood and behavior: a cluster analytic approach. Psychiatry Res. 28, 227–239 (1989).
3. Hegde, A. L. & Woodson, H. Prevalence of seasonal changes in mood and behavior during the winter months in central Texas. Psychiatry Res. 62, 265–271 (1996).
4. Lyall, L. M. et al. Seasonality of depressive symptoms in women but not in men: A cross-sectional study in the UK Biobank cohort. J. Affect. Disord. 229, 296–305 (2018).
5. Rosenthal, N. E. et al. Seasonal affective disorder: A description of the syndrome and preliminary findings with light therapy. Arch. Gen. Psychiatry 41, 72–80 (1984).
6. Pirk, E. et al. Epidemiology and socioeconomic impact of seasonal affective disorder in Austria. Eur. Psychiatry 32, 28–33 (2015).
7. Levitt, A. J., Boyle, M. H., Joffe, R. T. & Baumal, Z. Estimated Prevalence of the Seasonal Subtype of Major Depression in a Canadian Community Sample. Can. J. Psychiatry 45, 650–654 (2000).
8. Kuehner, C. Gender differences in unipolar depression: an update of epidemiological findings and possible explanations. Acta Psychiatr. Scand. 106, 163–174 (2003).
9. Lucht, M. J. & Kasper, S. Gender differences in seasonal affective disorder (SAD). Arch. Womens Ment. Health 2, 83–89 (1999).
10. Walton, J. C. et al. Photoperiod-mediated impairment of long-term potentiation and learning and memory in male white-footed mice. Neuronsci. 175, 127–132 (2011).
11. Friberg, O., Bjorgevoll, B., Amponsah, B. & Pallesen, S. Associations between seasonal variations in day length (photoperiod), sleep timing, sleep quality and mood: a comparison between Ghana (5°) and Norway (69°). J. Sleep Res. 21, 176–184 (2012).
12. Miller, M. A. et al. Photoperiod is associated with hippocampal volume in a large community sample. Hippocampus 25, 534–543 (2015).
13. Clayton, N. S., Reboreda, J. C. & Kaelin, L. A. Seasonal changes of hippocampus volume in parasitic cowbirds. Behavioural Process. 41, 237–243 (1997).
14. Workman, J. L., Manny, N., Walton, J. C. & Nelson, B. R. Short day lengths alter stress and depressive-like responses, and hippocampal morphology in Siberian hamsters. Hormones Behav. 60, 520–528 (2011).
15. Adam, C. L. et al. Photoperiod Regulates Growth, Puberty and Hypothalamic Neuropeptide and Receptor Gene Expression in Female Siberian Hamsters1. Endocrinol. 141, 4349–4356 (2000).
16. Dziubek, K., Krawczyk, S., Lach, H. & Staroma, W. Changes in nuclear volume of neurotensin containing lipofuscin pigment in the medulla oblongata of the brain of Rana temporaria L. in the annual cycle. Chromobiology 8, 325–332 (1981).
17. Green, N. L., Jackson, C., Iwamoto, H., Tackenberg, M. & McMahon, D. Photoperiod Programs Dorsal Raphe Serotonergic Neurons and Affective Behaviors. Curr. Biol. 25, 1389–1394 (2015).
18. Aumann, T. D. et al. Differences in Number of Midbrain Dopamine Neurons Associated with Summer and Winter Photoperiods in Humans. PLoS One 11, e0158847 (2016).
19. Zhao, M. et al. Evidence for neurogenesis in the adult mammalian substantia nigra. Proc. Natl Acad. Sci. U S Am. 100, 7925 (2003).
20. Tomas, D. et al. Environmental Modulations of the Number of Midbrain Dopamine Neurons in Adult Mice. Journal of Visualized Experiments, 52329 (2015).
21. Aumann, T. D., Tomas, D. & Horne, M. K. Environmental and behavioral modulation of the number of substantia nigra dopamine neurons in adult mice. Brain Behav. 3, 617–625 (2013).
22. Han, K. et al. Alterations in the brainstem volume of patients with major depressive disorder and their relationship with antidepressant treatment. J. Affect. Disord. 208, 68–75 (2016).
23. Soriano-Mas, C. et al. Cross-Sectional and Longitudinal Assessment of Structural Brain Alterations in Melancholic Depression. Biol. Psychiatry 69, 318–325 (2011).
24. Qi, H. et al. Gray Matter Volume Abnormalities in Depressive Patients With and Without Anxiety Disorders. Med. 93, e345 (2014).
25. Wirz-Justice, A. Seasonality in affective disorders. Gen. Comp. Endocrinol. 258, 244–249 (2018).
26. Partonen, T. In Seasonal affective disorder (Oxford Univ. Press, Oxford [u.a.], 2001).
27. Arendt, J. & Middleton, B. Human seasonal and circadian studies in Antarctica (Halley, 75 degrees S). Gen. Comp. Endocrinol. 258, 250–258 (2018).
28. Bronson, F. H. Are humans seasonally photoperiodic? J. Biol. Rhythm. 19, 180–192 (2004).
29. Stiemann, J. K., Green, N. H., Reddy, N. & McMahon, D. G. Sequential Photoperiodic Programming of Serotonin Neurons, Signaling and Behaviors During Prenatal and Postnatal Development. Front. Neurosci. 13, 459 (2019).
30. McMahon, B. et al. Seasonal difference in brain serotonin transporter binding predicts symptom severity in patients with seasonal affective disorder. Brain: a J. Neurol. 139, 1605–1614 (2016).
31. Glass, D. J., Grossman, G. H., Farnbauch, L. & DiNardo, L. Midbrain Raphe Modulation of Nonphotic Circadian Clock Resetting and 5-HT Release in the Mammalian Suprachiasmatic Nucleus. J. Neurosci. 23, 7451–7460 (2003).
32. Becker, T. et al. Reduced echogenicity of brainstem raphe specific to unipolar depression: A transcranial color-coded real-time sonography study. Biol. Psychiatry 38, 180–194 (1995).
33. Molendijk, M. L. et al. Serum BDNF Concentrations Show Strong Seasonal Variation and Correlations with the Amount of Ambient Sunlight, PLoS One 7 (2012).
34. Nexon, L., Potrel, V. J., Cléssie, D., Pevet, P. & Raison, S. Complex regional influence of photoperiod on the myenteric functioning of the dorsal and median raphe serotoninergic system in the Syrian hamster. Eur J. Neurosci. 30, 1790–1801 (2009).
35. Suhail, K. & Cochrane, R. Seasonal changes in affective state in samples of Asian and white women. Soc. Psychiatry Psychiatr. Epidemiol. 32, 149–157 (1997).
36. Seney, M. L. & Siblee, E. Sex differences in mood disorders: perspectives from humans and rodent models. Biol. sex. differences 5, 17 (2014).
37. Drevets, W. C., Savitz, J. & Trimble, M. The subgenual anterior cingulate cortex in mood disorders. CNS Spectr. 13, 663–681 (2008).
38. Bale, T. L. & Epperson, C. N. Sex differences and stress across the lifespan. Nat. Neurosci. 18, 1413–1420 (2015).
39. Moieni, M. et al. Trait sensitivity to social disconnection enhances pro-inflammatory responses to a randomized controlled trial of endotoxin. Psychoneuroendocrinology 62, 336–342 (2015).
40. Niles, A. N., Smirnova, M., Lin, J. & O’Donovan, A. Gender differences in longitudinal relationships between depression and anxiety symptoms and inflammation in the health and retirement study. Psychoneuroendocrinology 95, 149–157 (2018).
41. Sudlow, C. et al. UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. PLoS Med. 12, e1001779 (2015).
42. Spitzer, R. L., Kroenke, K. & Williams, J. B. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire. JAMA 282, 1737 (1999).
43. Miller, K. L. et al. Multimodal population brain imaging in the UK Biobank prospective epidemiological study. Nat. Neurosci. 19, 1523–1536 (2016).
44. Iglesias, J. E. et al. Bayesian segmentation of brainstem structures in MRI. NeuroImage 113, 184–195 (2015).
45. Velasco-Annis, C., Akhondi-Asl, A., Stamm, A. & Warfield, S. K. Reproducibility of Brain MRI Segmentation Algorithms: Empirical Comparison of Local MAP PSTAPLE, FreeSurfer, and FSL-FIRST. J. Neuroimaging 28, 162–172 (2018).
46. Fischl, B. FreeSurfer. NeuroImage 62, 774 (2012).
47. Ségonne, F. et al. A hybrid approach to the skull stripping problem in MRI. NeuroImage 22, 1060–1075 (2004).
48. Fischl, B. et al. Whole Brain Segmentation. Neuron 33, 341–355 (2002).
49. Dale, A. M., Fischl, B. & Sereno, M. I. Cortical Surface-Based Analysis: I. Segmentation and Surface Reconstruction. NeuroImage 9, 179–194 (1999).
50. Buckner, R. L. et al. A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. NeuroImage 23, 724–738 (2004).
51. Cameron, A. C. & Trivedi, P. K. Econometric Models Based on Count Data: Comparisons and Applications of Some Estimators and Tests. J. Appl. Econom. 1, 29–53 (1986).
52. Dobson, A. J. & Barnett, A. G. Analysing Seasonal Health Data. (2010).
53. Shrout, P. E. & Bolger, N. Mediation in experimental and nonexperimental studies: New procedures and recommendations. Psychological methods 7, 422–445 (2002).
54. Preacher, K. & Hayes, A. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav. Res. Methods 40, 879–891 (2008).