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The cerebello-hypothalamic tract:
The relationship between estradiol, neuroticism, and cerebellar white matter.

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Abstract. Many reproductive aged women experience symptoms of Premenstrual Syndrome and Premenstrual Dysphoric Disorder. Nonetheless, research on the mechanisms behind these symptoms is limited. In the current paper, it is proposed that the steroid hormone estradiol is associated with these emotional symptoms, using neuroticism scores. In addition, it is expected that estradiol is related to cerebellar white matter volume, and the cerebello-hypothalamic tract (CHT). The CHT is a white matter projection, directly connecting the cerebellum with the hypothalamus. Data from a longitudinal cohort study at Leiden University Medical Centre, the "Braintime" project was used to examine the associations. MRI examinations were performed in twenty subjects, using a 3-tesla 3T unit, and the CHT was exposed using Diffusion Tensor Imaging. The CHT was found unilaterally in 11 cases (n_{left} = 9 and n_{right} = 2), and bilaterally in 7 cases. However, no significant relations were found between estradiol and neuroticism, estradiol and cerebellar white matter volumes, and cerebellar white matter volumes and neuroticism. Nevertheless, a significant negative correlation was found between the CHT fiber count and white matter volume in cerebellar lobule IV. Furthermore, results showed that participants in which the CHT was found bilaterally scored significantly higher on the NEO-PI-R Depression scale. Hence, further research could examine whether there are significant associations between estradiol, white matter volume, and the CHT, taken age and individual ovulatory phases into account in data collection.

Key words: Cerebello-hypothalamic tract, cerebellum, white matter, estradiol, neuroticism, Diffusion Tensor Imaging, PMDD.

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Layman's summary

Veel vrouwen ervaren negatieve emoties rond de laatste week voor de menstruatie. Men gaat er vanuit dat dit te maken heeft met twee hormonen: estradiol en progesteron. Deze hormonen nemen in de laatste twee weken voor de menstruatie sterk toe, waarna ze plotseling dalen rond de menstruatie. Er wordt gedacht dat deze plotselinge daling te maken heeft met de ervaren emoties, maar deze mechanismen worden weinig onderzocht. Het huidige onderzoek heeft daarom gekeken naar de relatie tussen het hormoon estradiol en gevoeligheid voor het ervaren van negatieve emoties (ook wel "neuroticisme" genoemd). Specifiek is er gekeken naar de relatie tussen estradiol en het cerebellum, welke ook wel bekend staat als "de kleine hersenen". Het cerebellum lijkt namelijk gevoelig te zijn voor het hormoon estradiol. Ook wordt het cerebellum de afgelopen twee decennia steeds vaker in verband gebracht met emoties. We hadden dan ook verwacht dat de hoeveelheid verbindingen (ofwel "witte stof") in het cerebellum mogelijk gerelateerd zou zijn aan de mate waarin estradiol aanwezig is. Hier hebben wij echter geen significant effect voor gevonden. Daarnaast vonden wij ook geen significante relatie tussen estradiol en neuroticisme. Dit kan echter te maken hebben met de opzet van het huidige onderzoek, dus vervolg onderzoek is noodzakelijk.

Het andere doel dat we met het huidige onderzoek hadden was om een specifieke verbinding, de “cerebello-hypothalamic tract”, in kaart te brengen in het menselijke brein. Deze verbinding koppelt het cerebellum aan een van de emotiekernen in het brein, de hypothalamus. De cerebello-hypothalamic tract, of afgekort de CHT, is voorheen al vaker gevonden in verschillende dierenbreinen (zoals apen, katten en boomspitsmuizen). Maar, pas in 2019 werd er voor het eerst een artikel gepubliceerd waarin de CHT was gevonden in het menselijke brein. Ons onderzoek is daarmee een van de eerste waarin de CHT bij mensen in kaart is gebracht, en dat is gelukt in 18 van de 20 scans van menselijke breinen. Omdat de CHT het cerebellum dus verbind aan de emotiekern “de hypothalamus”, waren wij benieuwd of deze verbinding in verband kon worden gebracht met het ervaren van negatieve emoties. We hebben in deze kleine groep geen significante relaties kunnen vinden tussen de hoeveelheid projectiebanen van de CHT en neuroticisme en estradiol. Echter zijn er wel aanwijzingen van een dergelijk verband, dus vervolg onderzoek in een grotere groep vrouwen is van toegevoegde waarde. Wat we overigens wel hebben gevonden, maar niet van te voren hadden voorspeld, is dat er een verschil lijkt te zijn tussen vrouwen met één CHT verbinding en twee CHT verbindingen. Vrouwen met twee CHT verbindingen (één aan beide kanten van het brein), scoorden namelijk significant hoger op de depressie-vragen uit de neuroticisme vragenlijst. Dit is een interessante bevinding, maar vervolg onderzoek is nodig om dit te bevestigen. Het huidige onderzoek draagt dus bij aan het begrip van de achterliggende mechanismen van de ervaren emoties rond de menstruatie, en biedt potentie voor vervolg onderzoek om de gevonden resultaten te kunnen ontkrachten of bevestigen.

Introduction

20 to 80 percent of reproductive aged women across the globe suffer from Premenstrual Syndrome (PMS) symptoms (Hamilton et al., 1984; Rapkin & Mikacich, 2013). Besides, up to 7% (Rapkin et al., 2019) of women are affected by the more severe disorder Premenstrual Dysphoric Disorder (PMDD), which is included in the DSM-5 under Depressive Disorders (American Psychiatric Association, 2013). Women affected by PMDD repeatedly experience "mood lability, irritability, dysphoria, and anxiety symptoms" (Orff & Parry, 2017, p.197). In addition, symptoms of depressed mood, such as eating and sleeping problems, lack of interest in daily activities, concentration problems, and feelings of hopelessness are experienced (Orff & Parry, 2017). In PMDD, symptoms arise during the premenstrual phase of the ovulatory cycle and women experience a rapid decrease of symptoms at, or shortly after, the onset of menses (Orff & Parry, 2017). Since mood disturbances are also experienced during pregnancy (e.g. Buckwalter et al., 1999), postpartum (e.g. Buckwalter et al., 1999; Nonacs & Cohen, 1998) or during menopause (e.g. Soares et al., 2004; Toffol et al., 2015) it led to a commonly assumed theory, namely that strong fluctuations of ovarian steroid hormones, predominantly estradiol and progesterone, are related to disturbances in mood (e.g., Barth et al., 2015;Rapkin et al., 2019). However, given the limited research on the mechanisms behind the occurrence of these symptoms, alternative explanations are proposed.

Estradiol and PMDD

Several of the alternative explanations are related to the levels of the ovarian steroid hormone estradiol. This, due to its influence on broad range of regions in the brain, such as the amygdala, hypothalamus, and hippocampus, which have high densities of estrogen receptors (Barth et al., 2015). Estradiol, amongst others, is produced and regulated by the hypothalamic-pituitary-gonadal (HPG) axis (Brann et al., 1995). The hypothalamus secretes gonadotrophin-releasing hormone

(GnRH), which stimulates the anterior pituitary to produce luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These gonadotropins are released into the bloodstream, and initiate ovarian follicle growth after being transported to the ovaries. Mature ovarian follicles secrete the steroid hormones estradiol and progesterone, which initiate a surge of GnRH, followed by a peak of LH and FSH leading to ovulation (Brann et al., 1995). After ovulation, levels of estradiol and progesterone steadily increase during the luteal phase, after which they suddenly drop at the onset of menses around day 28 of the ovulatory cycle (Brann et al., 1995; See Figure 1).

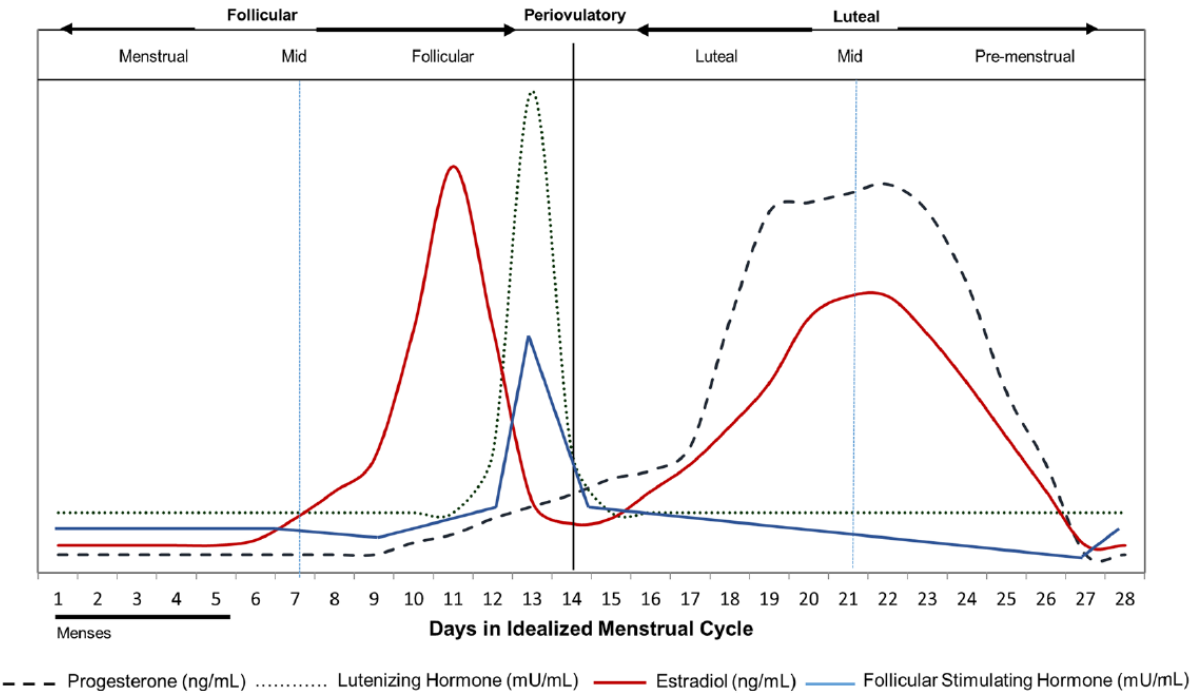

Figure 1. Female steroid hormone levels during the phases of the ovulatory cycle. Reprinted from "Menstrual cycle rhythmicity: metabolic patterns in healthy women" by Draper et al., 2018, "*Scientific reports",* 8(1), p. 2.

In 1989, Hammerbäck et al. examined the severity of PMS symptoms and the cooccurring hormone levels. Using daily blood samples during the luteal and follicular phase, they found that high levels of plasma estradiol and progesterone during the luteal phase seemed to be related to more severe symptoms of PMS. Similarly, Seippel and Bäckström (1998) also analyzed the relationship

between luteal phase estradiol and symptoms of PMS. Again, through the examination of daily blood samples of women with symptoms of PMS during the luteal and follicular phase, they found that women with high levels of luteal estradiol experienced more negative symptoms.

However, a recent meta-analysis showed contradictory findings (Amiel Castro et al., 2021). The meta-analysis by Amiel Castro et al. (2021) examined the role of the HPG axis in female mood disorders. Included studies found indications that women with symptoms of PMDD might have lower early luteal phase estradiol levels than women without PMDD symptoms. Hence, Amiel Castro et al. (2021) concluded that low levels of estradiol during the luteal phase are associated with symptoms of PMDD. However, note that the meta-analysis by Amiel Castro et al. (2021) did not find a significant relation between symptoms of PMDD and levels estradiol in the luteal phase (p = .37, 95 % CI [-0.29;0.11]). This could be caused by the results of the included studies, of which all but one failed to find significantly lower levels of estradiol in the luteal phase. However, because heterogeneity was in fact significant (p = .004), and the predictive value of the analyses seemed to be moderate to high (I^2 = 51 %), Amiel Castro et al. (2021) conclude that low levels of estradiol are related to the experience of PMDD symptoms.

Nonetheless, note that the studies above were merely of correlational nature. However, Segebladh et al. (2015) examined the effect of PMDD treatments in women diagnosed with PMDD through an experimental study. To study the effects, patients were administered the GnRH agonist Leuprolide during the luteal phase of the ovulatory cycle. As a result, the natural cycle was blocked through the inhibition of the pituitary gland. Subsequently, different combinations of estradiol and progesterone were administered. Results showed that treatment in which only gel estradiol was administered was most effective in alleviating symptoms of PMDD (Segebladh et al., 2015). Thus, since increasing the estradiol levels in women with PMDD seemed to decrease symptoms of

PMDD, it might provide indirect evidence for lower levels of estradiol in women with PMDD. Furthermore, when a combination of gel estradiol with progesterone was administered, symptoms recurred (Segebladh et al., 2015). This could imply that progesterone might be heightened in women with PMDD. The association between lower levels of estradiol and increased levels of progesterone has also been suggested by Yen et al. (2019). They propose that, given the finding of lower levels of estradiol during the early luteal phase, early luteal estradiol might moderate the effect of early luteal progesterone, which they found was elevated in women with symptoms of PMDD.

However, Segebladh et al. (2015) also found, slightly contradictory to the previous results, that anxiety and depressive symptoms showed a dose-dependent increase when estradiol was administered. Therefore, Segebladh et al. (2015) suggest that in addition to the fluctuating levels of estradiol and progesterone, the serum concentration and the estradiol/progesterone ratio may impact symptoms in women with PMDD. Nevertheless, the relation between estradiol and mood disorders such as major depressive disorder (MDD) has also been studied (Henningson et al., 2015). Similar to Segebladh et al. (2015), Henningsson et al. (2015) used a GnRH agonist; yet results were contradictory. Henningsson et al. (2015) found that with the reduction of estradiol to menopausal levels by the GnRH agonist, subclinical depressive symptoms increased significantly. In conclusion, levels of estradiol, and possibly progesterone, might be altered in women with symptoms of PMDD; yet, evidence is weak, and directions of effects are ambiguous.

Box 1. The cerebellum

The cerebellum is a brain structure that consists of two hemispheres. In Figure 2, unfolded maps of the cerebellar hemispheres are portrayed. As Figure 2A. shows, the cerebellum is divided into three lobes. At the top, in red, the anterior lobe is shown. Underneath, it shows the posterior lobe in yellow, and the flocculonodular lobe in blue. In addition to the division in lobes, the cerebellum has further been divided into lobules. Specifically, ten lobules (I - X) are distinguished (Stoodley & Schmahmann, 2016). These lobes and lobules are clustered into three systems (Figure 2B.): the Vestibulocerebellum which receives input from the brainstem and has been related to balance and posture; the Spinocerebellum, which obtains input from the spinal cord and has been associated with muscle tone, limb position, and movements; and lastly the Cerebrocerebellum, which seems to be connected to the cerebral cortex (Klein et al., 2016).

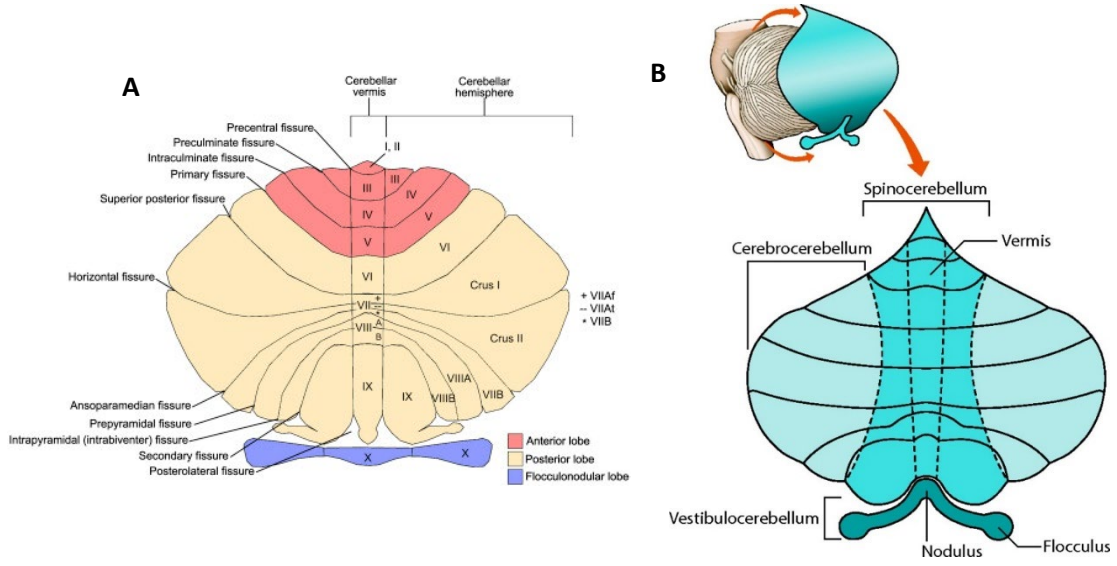

Figure 2. A). Reprinted from "Functional linguistic topography of the cerebellum", by Stoodley, C. J., & Schmahmann, J. D., 2016, "*The Linguistic Cerebellum*", p. 316. B) Reprinted from "Nonmotor functions of the cerebellum: an introduction", by Klein, A. P., Ulmer, J. L., Quinet, S. A., Mathews, V., & Mark, L. P., 2016, *"American Journal of Neuroradiology",* 37, p. 1006.

The cerebellum and estradiol

In 2020, Fitzgerald et al. published a case study in which they suggest that levels of estradiol during the ovulatory cycle might be associated with the functional connectivity in the cerebellum. The cerebellum is a brain structure divided into two hemispheres consisting of multiple lobules (see Box 1. The cerebellum). One of the main cell types in the cerebellum are Purkinje cells (Dieni et al., 2020; Hedges et al., 2018), which are sensitive to steroid hormones such as estradiol (Dieni et al., 2020; Hedges et al, 2012; Tsutsui & Haraguchi, 2020). Hence, an association between estradiol and the cerebellum seems possible.

Furthermore, estradiol is believed to not only be associated with activation in the brain as depicted above, but it might also have organizing effects in the brain. Multiple studies show a protective effect of estradiol on white matter (e.g. Curry & Heim, 1966; Dominguez et al., 2018) through stimulation of myelination (Curry & Heim, 1966; Luo et al., 2016). Curry and Heim (1966) found that administered estradiol in Neonatal rats enhanced myelination, especially in the hypothalamus. According to Gerstner et al. (2007) and Acs et al. (2009) "Estradiol promotes neuroprotection of oligodendrocytes and Schwann cells in cell culture and demyelination by cuprizone" [1] (Meeker et al., 2020 p.11). However, Kranz et al. (2017) examined the effects of hormone replacement therapy in transgender persons on white matter microstructures. They found correlations between hormone fluctuations and changes in white matter structure, in both male-to-female and female-to-male persons. Interestingly, when structurally receiving high doses of estradiol, the Fractional Anisotropy[2] (FA) values significantly decreased and the mean diffusivity (MD) values significantly increased. This means that there is more free space for water molecules to travel,

[1] Cuprizone is a copper chelator that results in oligodendrocyte death and, therefore, to demyelination (Torkildsen et al., 2008).
[2] FA is a measurement in Diffusion Tensor Imaging (DTI) which quantifies white matter integrity (Kochunov et al., 2007; Kochunov et al., 2012).

leading to less optimal transmission in the white matter structures (Assaf & Pasternak, 2008; Kochunov et al., 2007). Thus, since high FA values and low MD values seem to represent increased myelination and fiber organization in white matter (Alexander et al., 2007), increasing levels of estradiol might have a negative effect on white matter organization based on the results by Kranz et al. (2017). In sum, it remains unclear whether estradiol could be positively or negatively associated with white matter structure.

The cerebellum and emotion

For decades, it has been believed that the cerebellum is primarily involved in motor processes. However, the cerebellum has increasingly been associated with non-motor processes, such as emotional and cognitive processes (e.g. Klein, et al., 2016; Schutter & Van Honk, 2005). Hence, the cerebellum has been divided into three segments (Figure 3; Timmann et al., 2010). First, the lateral section (the Cerebrocerebellum) is believed to be involved in cognitive processing.

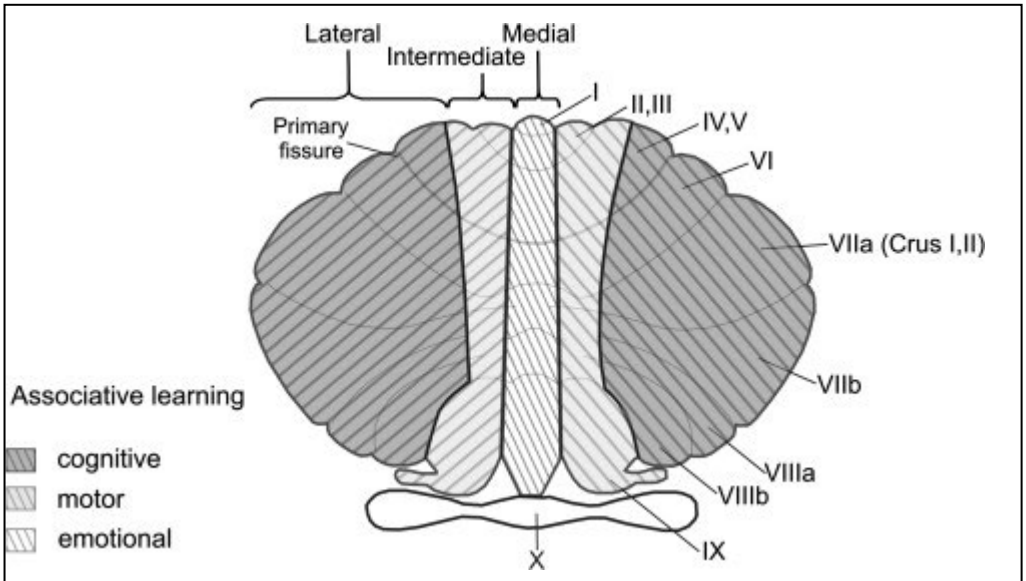

Figure 3. Reprinted from "The human cerebellum contributes to motor, emotional and cognitive associative learning. A review", by Timmann, D., Drepper, J., Frings, M., Maschke, M., Richter, S., Gerwig, M., & Kolb, F. P. , 2010, "*Cortex", 46(7), p.846*

Secondly, the intermediate segment, part of the Spinocerebellum, has been associated with motor processes. And lastly, the medial section, consisting of the vermis, is believed to be involved in emotional processing (Timmann et al., 2010).

Besides the suggested involvement in emotional processes, the cerebellum has also been associated with multiple mood disorders, such as MDD (Depping et al., 2018; Lupo et al., 2019; Minichino et al., 2014; Yucel et al., 2012) and PMDD (Rapkin et al., 2011; Rapkin et al., 2014). Using fMRI, an association between MDD and cerebellar activity was found (Adamaszek et al., 2017; Fitzgerald et al., 2008). In the meta-analysis by Fitzgerald et al. (2008), they concluded that an increase in resting state activity was found in the anterior cerebellum in patients with MDD, compared to healthy controls. The increase in cerebellar activity was also found by Guo et al. (2013), and they interpreted the increase as a compensatory effort of the cerebellum for the areas in the cerebrum to which that part of the cerebellum is connected. In addition to MDD, cerebellar activity was also examined in women with and without symptoms of PMDD (Rapkin et al., 2011). Using positron emission tomography, Rapkin et al. (2011) found an increase in cerebellar activity in women with PMDD symptoms, compared to women without PMDD symptoms. Furthermore, this increase in cerebellar activity is correlated with self-reported deterioration of mood (Rapkin et al., 2011). Thus, it seems that cerebellar activity might be altered in patients with mood disorders such as MDD and PMDD.

Moreover, the association between mood disorders and the cerebellum was not only found through functional connectivity, but there also seem to be structural differences in the cerebellum (Depping et al., 2018; Frodl et al., 2008; Peng et al., 2011). In healthy volunteers, it was found that smaller cerebellar volumes were negatively related to depression and anxiety scores on a neuroticism questionnaire (Schutter et al., 2012). Furthermore, a significantly smaller cerebellar volume has

also been found in patients diagnosed with MDD (Escalona et al., 1993), in addition to decreased volume in the anterior cerebellar vermis (Shah et al., 1992), and decreased cerebellar grey matter density (Frodl et al., 2008; Peng et al., 2011). Nevertheless, an increase in cerebellar volume was also found (Depping et al., 2018). Specifically, in patients diagnosed with MDD, the volume of lobule IX of the cerebellum seemed to be increased (Depping et al., 2016). The cerebellar lobule IX is known for partaking a role in non-motor functions (Guell & Schmahmann, 2019), such as emotional processing (Depping et al., 2016; Berman et al., 2013). Based on the increased grey matter volume, Depping et al. (2016) speculate that it could indicate an ineffective functional state in lobule IX. In addition, structural cerebellar differences in patients diagnosed with MDD have also been found by Zeng et al. (2012). Specifically, Zeng et al. (2012) found significantly decreased white matter volumes in the left posterior cerebellum in patients with MDD, compared to healthy non-depressed subjects. The posterior cerebellum has been associated with primary emotion processing (Adamaszek et al., 2017; Baumann & Mattingley, 2012) and the cognitive aspects of emotion processing (Adamaszek et al., 2017). Based on their findings, Zeng et al. (2012) speculate that reduced white matter volume in the left posterior cerebellum might contribute to the functional abnormalities observed in depression. Furthermore, the decreased white matter volume in the posterior cerebellum was not significant when comparing volumes of recovered patients (after antidepressant therapy) and healthy non-depressed subjects (Zeng et al., 2012). This could suggest that the reduction in left posterior cerebellar white matter volume might occur concurrently with symptoms of MDD.

Besides structural cerebellar differences in MDD, structural differences in the cerebellum are also proposed in women experiencing symptoms of PMDD; yet, not many studies have been conducted. A study by Berman et al. (2013) examined grey matter volume in the posterior cerebellum in women with symptoms of PMDD. Their results showed that the grey matter volume in the posterior

cerebellum was significantly larger in women with symptoms of PMDD, than in women without symptoms. Again, the posterior cerebellum is related to primary emotion processing (Adamaszek et al., 2017; Baumann & Mattingley, 2012). Thus, it could carefully be speculated that this increase might indicate ineffective functionality in (parts of) the posterior cerebellum. However, note that the sample in the study by Berman et al. (2013) was fairly small (12 PMDD, 13 controls). In addition, Berman et al. (2013) found a significant interaction effect with age: Increased grey matter volume was only found in women with PMDD symptoms over the age of thirty. These results therefore need to be interpreted with caution. Nonetheless, it might carefully be speculated that structural cerebellar differences in women with symptoms of PMDD could be possible. Unfortunately, cerebellar white matter volumes in women experiencing PMDD symptoms currently seem to remain, to the best of our knowledge, uninvestigated.

The Cerebello-Hypothalamic Tract

The cerebellum seems to be directly connected to the hypothalamus, which is a part of the HPG axis (Brann et al., 1995) and one of the emotion centers in the brain (Rolls, 2015), through a white matter fiber bundle called the cerebello-hypothalamic tract (CHT). The CHT has previously been found in animals (Haines et al., 1984), such as squirrel monkeys (Haines & Dietrichs, 1984), cats (Dietrichs, 1983), tree shrew (Haines et al., 1985) and brown greater galago's (Dietrichs & Haines, 1984); however, little is known about the CHT in humans. Çavdar et al. (2019) found evidence for the CHT using diffusion tensor tractography in adults. Diffusion Tensor Imaging (DTI) tractography showed that the fibers from the superior cerebellar peduncle (SCP) reached the red nucleus of the midbrain through the cerebral aqueduct. Additionally, several uncrossed SCP fibers reached the ipsilateral posterior hypothalamic area. Thus, it seems that the human cerebellum might possibly be directly connected to affective brain regions through the CHT.

The current study

In sum, not much is known about the underlying mechanisms of emotional disorders related to the ovulatory cycle, such as PMDD. However, the ovarian steroid hormone estradiol, produced by the HPG axis, has been associated with the functional connectivity in the cerebellum and is also associated with organizing effects in the brain. Furthermore, the cerebellum has increasingly been associated with emotions and multiple mood disorders, both through functional connectivity and microstructure. In addition, the cerebellum might also in humans, be directly connected through the CHT to the hypothalamus, which is a part of the HPG axis and one of the primary emotion centers in the brain. Therefore, the current exploratory study will examine the association between estradiol, cerebellar white matter volume, negative emotions, and the CHT.

To examine the relationship between negative emotions in a healthy population, self-reports on trait Neuroticism can be used. The personality trait Neuroticism is related to the frequent experience of negative emotions and negative beliefs about one's personal ability to cope with challenges (Barlow et al., 2014). Furthermore, trait Neuroticism is a solid risk factor for mood disorders such as depression and anxiety (Kendler & Myers, 2010; Kotov et al., 2010; Schutter et al., 2012).

Hence, the purpose of this study is, first of all, to examine whether we can find the CHT in Diffusion Weighted Imaging scans similar to Çavdar et al. (2019). Secondly, it is explored whether levels of estradiol and neuroticism scores can be associated with cerebellar white matter volume and the CHT, as could be expected from the theory above. We expect to find the CHT in all DWI scans, replicating findings by Çavdar et al. (2019). Furthermore, based on the meta-analysis by Amiel Castro et al. (2021), we expect to find a negative association between levels of estradiol and neuroticism scores. Besides, even though literature is inconsistent, it is expected that there is a

positive association between estradiol and white matter volume, based on studies by, for example, Curry and Heim, (1966), Dominguez et al. (2018), and Luo et al. (2016). Therefore a positive relation between estradiol and the CHT is expected as well. Furthermore, given the expected negative association between estradiol and neuroticism, and the finding of reduced cerebellar white matter in patients with MDD by Zeng et al. (2012), it is expected that there is a negative relation between white matter and neuroticism, in addition to a negative relation between the CHT and neuroticism.

Methods

Participants

The volunteers in this study were 258 healthy children and young adults who participated in a longitudinal cohort study at Leiden University Medical Centre, the "Braintime" project (Braams et al., 2015; Peper et al., 2013; Peters et al., 2014; Wolfs et al., 2022). Participants were between 8 to 26 years of age, with a mean age of 14.2 (*SD* = 3.8). In the current study, only female participants were included (*n* = 122) with a mean age of 14.3 years (*SD* = 3.4). Participants were recruited from Dutch Elementary Schools, High Schools, and Leiden University. All participants or their caretakers signed the consent form, and the project was approved by the ethical review board from Leiden University.

Materials

Hormone levels

Estradiol levels were determined from morning saliva samples collected at home on the day of MR-scanning (for details, see Peper et al., 2014). Postmenarcheal participants collected saliva samples during the early follicular phase (day 7) when estradiol and progesterone levels are low.

This, to control for hormone fluctuations during the ovulatory cycle. In addition, when oral contraceptives were used (n = 16), participants collected saliva samples on the last day of their stopping period (day 7). Participants using hormonal contraception without stopping period were excluded from the study by Peper et al. (2014). The Department of Clinical Chemistry of the Free University Medical Centre assayed saliva samples for estradiol levels, with a lower limit of detection of 0.1 pg/mL, "using an enzyme linked immunosorbent assay (DRG Instruments, Marburg, Germany). Interessay CV was 8% and 15% at 10 and 40 pg/L, respectively" (Peper et al., 2014, p.1046).

Neuroticism

Various dimensions of the subscale "neuroticism" of the Dutch version of the Revised NEO Personality Inventory questionnaire (NEO-PI-R; Costa & McCrae, 2008), were used to rate neuroticism scores of participants. Specifically the dimensions "anxiety", "depression", and "impulsiveness" were used, all dimensions containing 8 items. Items were rated on a 5-point scale by participants, ranging from 0 (*Strongly Disagree*) to 5 (*Strongly Agree*). Previous work using the Dutch NEO-PI-R found Cronbach's alphas between .86 and .92 (Hoekstra et al., 1996), which can be seen as highly reliable. Out of the 122 female participants, 41 did not fill in the NEO-PI-R Neuroticism questionnaire. Hence, they were excluded from the analyses in which neuroticism scores were used.

Structural MRI acquisition

Structural magnetic resonance images were acquired using a 3-Tesla Philips Achieva MRI system (Philips Healthcare, Best, The Netherlands) at Leiden University Medical Center. The high resolution T1-weighted structural image scans were created with a T1 Turbo Field Echo (T1-TFE) and with the following parameters: repetition time = 9.76 ms, echo time = 4.59 ms, flip angle = 8°,

slices = 140, voxel size = 0.875 × 0.875 × 1.2 mm^3, field of view = 224 × 168 × 177 mm^3, scanning time = 296 s. Furthermore, all scans were reviewed and cleared by a radiologist (Peper et al., 2014).

Diffusion Weighted Imaging

Diffusion Weighted Imaging (DWI) scans were created with the following parameters (Paper et al., 2014, p.1045):

> 30 diffusion-weighted volumes with different noncollinear diffusion directions (Jones et al., 1999) with b-factor 1,000 s/mm2 and 5 diffusion-unweighted volumes (b-factor 0 s/mm2); parallel imaging SENSE factor = 3; flip angle = 90 degrees; 75 slices of 2 mm; no slice gap; reconstruction matrix 128 × 128; Field of view (FOV) = 240 × 240 mm; TE = 69 ms; TR = 7,315 ms; total scan duration = 271 s per DWI set. The second DWI set had identical parameter settings as used for the first set except that it was acquired with a reversed *k*-space readout direction enabling the removal of susceptibility artifacts during postprocessing (Andersson et al., 2003). During scanning, the FOV was angulated according to the anterior commissure-posterior commissure line, and diffusion gradients were adjusted accordingly during data processing. Subsequently, diffusion scans were realigned to the averaged b0 scan and corrected for motion, eddy current, and susceptibility distortions (Andersson and Skare, 2002; Andersson et al., 2003).

White matter volume extraction

T1 weighed images were used for anatomical reference and for the selection of regions of interest in cerebral white matter. T1 weighted images were acquired with the following parameters (Peper et al., 2014, p.1045):

> T1 scanning parameters were as follows: 3D FFE using parallel imaging; TR/TE 10 ms/4.6 ms; FOV 240 3 240 mm, 200 slices, 0.75 mm isotropic voxel size. Tissue classification,

including automatic segmentation of gray/white/csf tissue was performed using the Freesurfer suite (V5, http://surfer.nmr.mgh.harvard.edu/) [Fischl et al., 2004]. Freesurfer was also used for automatic segmentation of the cortex into 68 distinct regions (34 per hemisphere) and the subcortical gray matter into 14 (7 per hemisphere) distinct regions. Intrahemispheric white matter tracts between frontal and temporal lobes and subcortical brain areas were selected (Fig. 1), and the following pathways (with mean [SD] streamlines) were reconstructed: (1) subcorticosubcortical (2,125 6 295 streamlines), (2) subcortico-frontal (2,276 6 470 streamlines), (3) subcortico-temporal (865 6 243 streamlines), (4) fronto-frontal (1,646 6 335 streamlines), (5) fronto-temporal (619 6 203 streamlines), and (6) temporo-temporal (1,182 6 291 streamlines) connections. Connections within the occipital lobe (794 6 407 streamlines) were analysed as "control" tracts. To access the level of white matter complexity of reconstructed white matter connections, FA, MD, RD, and LD values along aggregated white matter pathways were averaged and used for further analyses.

Procedure

To examine the CHT, twenty sMRI scans were selected based on level of estradiol. Specifically, the DWI scans from 10 participants with the highest levels of estradiol (m_{age} = 17.53 , *SD* = 4.47) and 10 participants with the lowest levels of estradiol (m_{age} = 11.51, *SD* = 4.01) were selected. This way, a broad range of levels of estradiol could be used given the current dataset, creating a maximal difference in the analyses. After selection of the DWI scans, the scans were transformed as described below.

Data reduction and analyses

Diffusion scans were transformed using ExploreDTI (Leemans et al., 2009) in the following manner: (1) the first dimension was flipped, (2) a .mat file was created, with permute gradient

components y x z, and sign of gradient components were flipped to x -y z, (3) scans were corrected for motion, eddy current, and susceptibility distortions. Additionally, whole brain tractography was performed using DTI, with a seedpoint resolution of 2 2 2 mm, a FA threshold of .2, and an angle threshold of 60 degrees. Furthermore, tractfiles were converted to .trk files, in order to visualize the CHT using the program TrackVis (Wang et al., 2007) and estimated B0 images were created. Using TrackVis (Wang et al., 2007), the cerebello-hypothalamic fibers were visualized.

Moreover, IBM SPSS Statistics 26 (IBM Corp., 2017) is used to perform statistical analyses. All analyses were performed with an alpha level of .05 to determine significance and were two-tailed. First, using a Mann-Whitney U Test, we examined differences between the 20 selected female participants with highest and lowest levels of estradiol on age, ranges of levels of estradiol, CHT fiber count, cerebellar white matter volume, and total cerebellar volume. Furthermore, Spearman's Rho correlations, controlling for age and total cerebellar white matter volume, were used to examine the relation between estradiol, neuroticism and cerebellar white matter volumes in lobules I, III, IV, V, VI, Crus I, Crus II, VIIB, VIIIA, VIIIB, IX, and X. Additionally, using Spearman's Rho correlations, the relation between estradiol and the CHT fiber count, and neuroticism and the CHT fiber count are examined. Spearman's Rho correlations are used when examining CHT fiber count, due to its suitability for not normally distributed values. Moreover, based on the study by Çavdar et al. (2019), a Mann-Whitney U Test was used to examine whether there is a significant difference between the participants in which the CHT fibers were found unilateral and bilateral in levels of estradiol and neuroticism scores.

Results

For levels of estradiol, the assumption of normality was not met; the Shapiro-Wilk test is significant with $p = .030$. Also, the right CHT fiber count was significant with $p = .004$. For other values, the Shapiro-Wilk test was non-significant. However, due to the significancy in estradiol and the right CHT fiber count, a non-parametric Mann-Whitney U Tests was performed. The Mann-Whitney U Tests showed that there is a significant difference in levels of estradiol between the 20 selected participants with highest (Mean Rank = 15.50, $n = 10$) and lowest (Mean Rank = 5.50, $n = 10$) levels of estradiol, $U = .00$, $z = -3.780$ (corrected for ties), $p < .001$, two tailed, $r = .85$, which can be interpreted as a large effect. In addition, a significant difference in age between the 20 selected participants with highest (Mean Rank = 13.70, $n = 10$) and lowest (Mean Rank = 7.30, $n = 10$) levels of estradiol was found, $U = 18.00$, $z = -2.419$ (corrected for ties), $p = .016$, two tailed, $r = .54$, which can be interpreted as a large effect. Furthermore, no significant difference were found on cerebral white matter volume, total cerebral volume, and CHT fiber count between the 20 selected participants (Table 1).

Estradiol and Neuroticism

When examining the assumption of normality, a significant Shapiro-Wilk test of normality was found for estradiol, $p < .001$. In addition, significant Shapiro-Wilk tests of normality were found for the NEO Fear scale ($p = .003$), NEO Depression scale ($p < .001$), and NEO Neuroticism Total ($p = .010$). Hence, these values were not normally distributed. The NEO Impulsivity scale was non-significant with $p = .457$. Due to the not normally distributed values, a nonparametric Spearman's Rho correlation is used. Spearman's Rho indicated that there were no significant relations between levels of estradiol and neuroticism scores on all subscales and total neuroticism value, after controlling for age and total cerebellar white matter volume (Table 2).

Estradiol and cerebellar white matter volume

Assessing the assumption of normality, a significant Shapiro-Wilk test of normality was found for estradiol, $p < .001$. In addition, significant Shapiro-Wilk tests of normality were found for all values of cerebellar white matter volume per lobule. Hence, these values were not normally distributed and a non-parametric Spearman's Rho correlation was used. No significant Spearman's Rho correlations were found between levels of estradiol and cerebellar white matter volumes in all examined lobules after controlling for age and total cerebellar white natter volume (see Table 3).

Cerebellar white matter volume and neuroticism

Examining the assumption of normality, the Shapiro-Wilk tests of normality for the NEO Impulsivity scale was non-significant with $p = .457$. All other values were significant; therefore, a nonparametric Spearman's Rho correlation is used. Spearman's Rho indicated that there were no significant relations between cerebellar white matter volumes in all examined lobules and neuroticism scores after controlling for age and total cerebellar white natter volume (see Table 4).

The Cerebello-Hypothalamic Tract

Results show that the cerebello-hypothalamic tract (CHT; Figure 4) was found in 18 out of 20 DWI scans. Specifically, the CHT was found unilaterally $n = 11$ times (61.11 %), of which $n_{left} = 9$ and $n_{right} = 2$. Furthermore, 38.89 % of participants ($n = 7$) seemed to have a bilateral CHT ending in the hypothalamus (See Appendix 1, Cerebello-Hypothalamic Tracts).

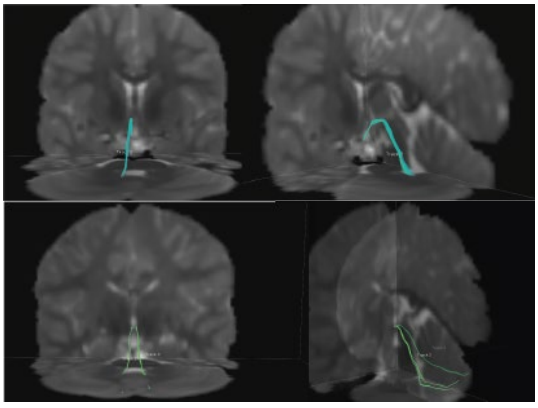

Figure 4. The image above shows one of the unilateral cerebello-hypothalamic tracts. The image below depicts a bilateral CHT.

When assessing the assumption of normality, a non-significant Shapiro-Wilk test of normality was found for the left CHT fiber count, $p = .550$. However, for the right CHT fiber count, values were not normally distributed; a significant Shapiro-Wilk test of normality was found, $p = .004$. Hence, a nonparametric Spearman's Rho correlation was used. When controlled for age and total cerebellar white matter volume, Spearman's Rho indicated the presence of a negative correlation between ranked right CHT fiber count and white matter volume in cerebellar lobule IV, $r_s = .-92$, $p = .028$, two-tailed. No other significant correlations were found (see Table 5).

In addition, after controlling for age and total cerebellar white matter volume, Spearman's Rho indicated that there were no significant relations between both left and right CHT fiber counts and levels of estradiol and the neuroticism scales (see Table 6).

Differences between Bilateral and Unilateral CHT on Estradiol and Neuroticism

Furthermore, differences between participants in which the CHT was found bilaterally and unilaterally has been examined. For levels of estradiol, the assumption of normality is met; the Shapiro-Wilk test is not significant with $p = .095$ for low estradiol and $p = .135$ for high estradiol. However, for the CHT fiber count, the assumption of normality was not met; the Shapiro-Wilk test was significant with $p = .049$ for unilateral CHT and $p = .030$ for bilateral CHT. Therefore, non-parametric Mann-Whitney U Tests were performed.

The Mann-Whitney U Test showed that there is no significant difference in levels of estradiol between participants in which the CHT was found bilaterally (Mean Rank = 8.14, $n = 7$) or unilaterally (Mean Rank = 10.36, $n = 11$), $U = 29.00$, $z = -.860$ (corrected for ties), $p = .390$, two tailed, $r = .20$. Nevertheless, participants in which the CHT was found bilaterally (Mean Rank = 8.70, $n = 5$) scored significantly higher on the NEO Depression scale than participants in which the CHT was found unilaterally (Mean Rank = 3.75, $n = 6$), $U = 1.50$, $z = -2.48$ (corrected for ties), p

= .013, two tailed, r = .75. This effect can be described as large (Cohen, 1988). Other scores on the NEO-P-R scales did not differ significantly between groups (see Table 7).

Discussion

The current study attempted to explore and provide insight in the cerebello-hypothalamic tract (CHT) using Diffusion Tensor Imaging (DTI) techniques. In addition, it was examined whether levels of estradiol and neuroticism scores could be associated with cerebellar white matter volume and the CHT fiber count. Moreover, differences in levels of estradiol and neuroticism scores between participants with an unilateral or a bilateral CHT were examined.

Estradiol and neuroticism

Based on the meta-analysis by Amiel Castro et al. (2021), a negative association between levels of estradiol and neuroticism scores were expected to be found. However, after controlling for age and total cerebellar white matter volume, no significant relation between estradiol and NEO neuroticism scores were found.

A possible explanation worth investigating is the difference between phases of the ovulatory cycle in which levels of estradiol were collected. In the "Braintime" project (Braams et al., 2015; Peper et al., 2013; Peters et al., 2014; Wolfs et al., 2022), levels of estradiol were measured during the early follicular phase in postmenarcheal participants, when levels of estradiol are on their lowest. This way, it was attempted to control for hormone fluctuations during the ovulatory cycle. However, it could be possible that the current levels of estradiol might not correspond to the levels of early luteal phase estradiol that were examined by Amiel Castro et al. (2021). Hence, it might be possible that the relation between estradiol and the experience of negative emotions that are captured by the NEO neuroticism scales, can only be found with at least early luteal phase levels of estradiol. However, in a study by Miller et al. (2010) no differences in neuroticism scores were

found between the luteal phase and follicular phase in participants without symptoms of PMDD. Therefore, the difference in ovulatory cycle phase in which estradiol was collected might not be of influence; however, what might be more important is that in the current study, healthy participants were used. Miller et al. (2010), in addition to Berlin (2001), also found that scores on trait neuroticism did not differ between phases in the ovulatory cycle in asymptomatic controls, but that they did differ in women with symptoms of PMDD. Therefore, it could possibly be assumed that not finding a significant correlation between levels of estradiol and neuroticism scores, might be based on the specific sample that was used in the current study.

However, another explanation for not finding significant relations between estradiol and neuroticism scores, might be that trait neuroticism is not suitable to predict symptoms of PMDD. Amiel Castro et al. (2021) found a negative relation between levels of estradiol and symptoms of PMDD. In the current study, the negative emotions experienced in PMDD were tried to be captured using trait neuroticism questionnaire scores in healthy subject who are not selected based on symptoms of PMDD. A recent study by Hamidovic et al. (2022) showed that, besides low interest in daily activities and difficulty concentrating, no other symptoms of PMDD were significantly associated with trait neuroticism. Therefore, in an asymptomatic sample, another measure than trait neuroticism might be more useful in detecting associations between levels of estradiol and negative emotions.

Estradiol and cerebellar white matter volume

Furthermore, it was expected that there would be a positive association between estradiol and white matter volume (Acs et al., 2009; Curry & Heim, 1966; Dominguez et al., 2018; Gerstner et al., 2007; Luo et al., 2016). Yet, no significant correlations were found between levels of estradiol and cerebellar white matter volumes, after controlling for age and total white matter volume in the

cerebellum. This is not in accordance with our expectation. Nonetheless, the non-significant results do indicate that there might be both positive and negative correlations between estradiol and white matter volume in different lobules. Hence, further research could examine whether this pattern holds if significant effects can be found.

Furthermore, finding merely non-significant results could have occurred due to the current sample. The subjects in the current sample were not all postmenarcheal, which might have influenced the results. Levels of estradiol increase during adolescence, and neural changes occur during adolescence through the influence of steroid hormones (Herting et al., 2014). Thus, there might be an age specific effect of white matter volume development across adolescence (Herting et al., 2014; Ladouceur et al., 2012). Therefore, further research could examine the association between estradiol and cerebellar white matter volumes, taken age of participants and individual ovulatory phases into account in data collecting. For example, levels of estradiol and DWI scans from postmenarcheal women could be examined during all phases in the ovulatory cycle. Thereby, a clearer pattern of the association could be found.

Cerebellar white matter volume and neuroticism

A negative association between white matter volume and neuroticism scores was expected, based on the expected negative association between estradiol and neuroticism, and the finding of reduced cerebellar white matter in patients with MDD by Zeng et al. (2012). However, no significant correlations were found between white matter volumes in the examined cerebellar lobules and the NEO-PI-R Neuroticism questionnaire scores in the current subjects. Nonetheless, even though results were all non-significant, most correlations do in fact show a negative direction of effects. Given the limitations of the current asymptomatic sample with a broad range of age, and the development of white matter volume across adolescence (Herting et al., 2014; Ladouceur et al.,

2012), it could be possible that the association between white matter volume and neuroticism could be found in an older, symptomatic sample.

The Cerebello-Hypothalamic Tract

We expected to find the CHT in all DWI scans, replicating findings by Çavdar et al. (2019). In the current study, the CHT was found in eighteen out of twenty DWI scans. Furthermore, similar to the study by Çavdar et al. (2019), the current study found the CHT more often unilaterally. Specifically, the CHT was found unilaterally $n = 11$ times (61.11 %), of which $n_{left} = 9$ and $n_{right} = 2$. Furthermore, 38.89 % of participants ($n = 7$) seemed to have a bilateral CHT ending in the hypothalamus. In addition, the CHT seemed to project ipsilaterally in all cases; again, similar to findings by Çavdar et al. (2019). However, in animals, both ipsilateral (Haines et al., 1985) and contralateral (Haines et al. 1984) projections of the hypothalamus were found ex-vivo. Hence, it could be possible that there are artifacts, such as problems with crossing fibers, in the current scanning techniques. Currently used DTI technique often fail to reconstruct crossing or kissing fibers (Frigo et al., 2019). Therefore, it could be possible that the crossing fibers of the CHT are reconstructed inadequately, resulting in the CHT being reconstructed only ipsilaterally in humans. New techniques as proposed by Frigo et al. (2019) might reduce these artifacts of fiber tracking in the future. However, examining the CHT ex-vivo in humans might provide more clarity as well.

As indicated above, the CHT could not be determined convincingly in two DWI scans. The fiber bundles seemed to end in the thalamus, instead of bending towards or ending in the hypothalamus. Therefore, these scans were excluded from further analyses of the CHT. However, it could be possible that the reconstructed fibers did not extend into the hypothalamus due to artifacts in DTI. After the thalamus, the fiber bundle needs to make a strong curve downwards toward the hypothalamus. In fiber tractography, there is a maximum limit of degrees for angles, in order for

fibers to be reconstructed reliably (Knösche et al., 2015). In the current study the maximum curvature of fibers was determined at an angle threshold of 60 degrees. Other degrees, 30, 45, 50, and 70 degrees, were examined as well; Yet, for the CHT, 60 degrees seemed optimal, and is commonly used in DTI (Knösche et al., 2015). However, it could be possible that in the two excluded cases, the CHT could have been found with a slightly higher angle threshold. When fibers have a stronger curvature than the predetermined angle threshold, the fibers are not reconstructed correctly (Mukherjee et al., 2008); Yet, with a higher angle threshold, the number of false tracks increase dramatically (Mukherjee et al., 2008). In sum, in order to find the CHT in most subjects, the angle threshold was determined at 60 degrees; however, it could be possible that individual differences in curvature might lead to the inability to find the CHT in some subjects.

The CHT and white matter volume

After controlling for age and total cerebellar white matter volume, a negative correlation between the right CHT fiber count and white matter volume in cerebellar lobule IV was found. The cerebellar lobule IV has been associated with emotional processing (E et al., 2011). Specifically, activity in the right lobule IV is significantly correlated to negative emotion processing, when compared with positive emotions (E et al., 2011). However, besides a potential functional relation with negative emotion, it might also be possible that the correlation occurred due to crossing or kissing fibers (Frigo et al., 2019; Watts et al., 2003). The CHT projects closely to lobule IV in order to project to the hypothalamus. Therefore, when the volume in lobule IV is high, the probability of crossing or kissing fibers might potentially increase, and the currently used DTI technique could have failed to reconstruct crossing or kissing fibers (Frigo et al., 2019).

The CHT, estradiol, and neuroticism

Based on the expected positive relation between estradiol and white matter volume, a positive relation between estradiol and the CHT fiber count was expected as well. However, no significant relation was found between estradiol and white matter volume. This could have occurred due to limitations in age and ovulatory phase in which levels of estradiol were collected in participants in the current study. In addition, no significant relation between both left and right CHT fiber counts and levels of estradiol were found. Again, due to the limitations in collecting levels of estradiol, it might be that estradiol levels are not defined enough in order to find an effect in the white matter fiber count from the CHT.

Furthermore, a negative relation between the CHT and neuroticism was expected based on the expected association between reduced white matter volume and neuroticism scores. However, no significant results were found, similar to the non-significant association between white matter volume and neuroticism. Yet again, this might have occurred due to the age of the current sample (Herting et al., 2014; Ladouceur et al., 2012),

Differences between unilateral and bilateral CHT projections

Given the finding that the CHT seemed to project both unilaterally as bilaterally, differences in estradiol levels and neuroticism scores between participants in which the CHT was found bilaterally and unilaterally have been explored. Levels of estradiol did not seem to differ between groups; Yet, participants in which the CHT was found bilaterally scored significantly higher on the NEO depression scale, than participants in which the CHT was found unilaterally. A possible explanation worth exploring might be the influence of age on the finding. Models by Romero et al. (2021) showed that the cerebellum develops strongly during the first 17 years, in an inverted U-shape way (Tiemeier et al., 2010; Wierenga et al., 2014). Specifically, cerebellar development is

mainly characterizes by an increase in white matter volume (Østby et al., 2009). With the increase in white matter, it might be possible that the CHT fibers become more prominent and easier to detect during scanning. Besides, during adolescence, the risk for the development of depressive symptoms increases with age, specifically for female adolescents (Salk et al., 2018). Hence it would be interesting to explore whether there is a difference in age between participants in which the CHT was found unilateral (expected to be younger) and bilateral (expected to be older). Since this is one of very few studies examining the CHT in humans, a subsequential exploration of data has been conducted to explore this theory. Results showed no significant difference in age between participants in which the CHT was found bilaterally (Mean Rank = 11.29, n = 7) or unilaterally (Mean Rank = 8.36, n = 11), U = 26.00, z = -1.132 (corrected for ties), p = .258, two tailed, r = .60. Which could be interpreted as a large effect (Cohen, 1988). Thus, given the underpowered sample, further research could examine whether this pattern holds in a larger sample. If the pattern holds, it might provide new insight in the development of depressive symptoms in adolescent girls.

Conclusion

In conclusion, the cerebello-hypothalamic tract (CHT) was found both unilateral and bilateral, with an ipsilateral preponderance, similar to findings by Çavdar et al. (2019). Furthermore, despite our expectations, estradiol levels and neuroticism scores could not be significantly associated with cerebellar white matter volume and the CHT. Moreover, NEO-PI-R Depression scores were significantly higher in participants with a bilateral CHT; Yet, no differences in levels of estradiol and age were found between participants with an unilateral or a bilateral CHT. Hence, further research could examine whether there are significant associations between estradiol, white matter volume, and the CHT, taken age and individual ovulatory phases into account in data collection.

Besides, examining the CHT ex-vivo in the human brain could provide more clarity in the direction of the projection without limitations of DTI techniques.

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Tables

Table 1

Mann-Whitney U Test for Differences in Age, level of Estradiol, Total Cerebellar White Matter Volume, Total Cerebellar Volume, and CHT fiber count between the 20 selected Female Participants with Highest and Lowest levels of Estradiol

Expression	*n*	*Mean Rank*	*U*	*p*	*r*
Age			18.00	.016*	.54
Low Estradiol	10	7.30			
High Estradiol	10	13.70			
Level of Estradiol			.000	< .001**	.85
Low Estradiol	10	5.50			
High Estradiol	10	15.50			
Total Cerebellar White Matter Volume			47.00	.821	.05
Low Estradiol	10	10.20			
High Estradiol	10	10.80			
Total Cerebellar Volume			50.00	1.000	-
Low Estradiol	10	10.50			
High Estradiol	10	10.50			
CHT fiber count left hemisphere			25.50	.624	.12
Low Estradiol	6	7.75			
High Estradiol	10	8.95			
CHT fiber count right hemisphere			8.50	.897	.04
Low Estradiol	6	4.92			
High Estradiol	3	5.17			

Note. * $p < .05$, p = two tailed.

Table 2

Spearman's Rho Correlation Coefficients between Estradiol and Neuroticism scales.

	NEO Fear scale	NEO Depression scale	NEO Impulsivity scale	NEO Neuroticism Total
Estradiol				
r_s	.049	-.041	.094	.036
p	.555	.620	.255	.667

Note. * $p < .05$. *p* = two-tailed. Controlled for age and total cerebellar volume.

Table 3

Spearman's Rho Correlation Coefficients between Cerebellar White Matter Volumes (cm3) and Estradiol.

	r_s	*p*
Lobule I	.066	.348
Lobule III	-.025	.718
Lobule IV	.013	.857
Lobule V	-.064	.362
Lobule VI	.054	.439
CRUS I	-.129	.065
CRUS II	-.072	.300
Lobule VIIA	-.056	.420
Lobule VIIB	-.023	.738
Lobule IX	.121	.084
Lobule X	.004	.955

Note. * $p < .05$, *p* = two-tailed. Controlled for age and total cerebellar volume.

Table 4

Spearman's Rho Correlation Coefficients between Cerebellar White Matter Volumes (cm3) and Neuroticism scales.

	NEO Fear scale	NEO Depression scale	NEO Impulsivity scale	NEO Neuroticism Total
Lobule I				
r_s	.066	-.019	.012	.028
P	.415	.811	.879	.730
Lobule II				
r_s	-.096	.010	-.035	-.061
p	0233	.900	.663	.451
Lobule IV				
r_s	-.017	-.065	-.019	-.036
p	.835	.421	.814	.656
Lobule V				
r_s	-.099	-.001	.102	.003
p	.217	.992	.203	.973
Lobule VI				
r_s	.084	-.041	-.074	-.018
p	.300	.615	.359	.827
Lobule Crus I				
r_s	.032	.149	.104	.129
p	.695	.064	.195	.109
Lobule Crus II				
r_s	-.105	.045	.065	.008
p	.193	.578	.421	.919
Lobule VIIB				
r_s	-.036	-.026	-.084	-.065
p	.658	.746	.297	.418
Lobule VIIIA				
r_s	.063	.049	-.072	.033
p	.438	.542	.371	.686
Lobule VIIIB				
r_s	-.058	-.064	-.036	-.075
p	.475	.430	.659	.350
Lobule IX				
r_s	.067	-.043	-.121	-.039
p	.404	.597	.133	.632
Lobule X				
r_s	-.042	-.027	.028	.004
p	.600	.733	.731	.963

Note. * $p < .05$. *p* = two-tailed. Controlled for age and total cerebellar volume.

Table 5

Spearman's Rho Correlation Coefficients between Cerebello-Hypothalamic Tract Fiber Count and Cerebellar White Matter Volumes (cm3)

	Left CHT	Right CHT
Lobule I		
r_s	-.266	-.849
p	.666	.069
Lobule II		
r_s	.130	-.306
p	.834	.616
Lobule IV		
r_s	-.754	-.918
p	.141	.028*
Lobule V		
r_s	.171	-.658
p	.783	.227
Lobule VI		
r_s	.357	-.203
p	.555	.744
Lobule Crus I		
r_s	.052	-.700
p	.933	.188
Lobule Crus II		
r_s	-.656	-.077
p	.229	.903
Lobule VIIB		
r_s	-.437	.406
p	.462	.498
Lobule VIIIA		
r_s	-.377	-.191
p	.532	.758
Lobule VIIIB		
r_s	-.288	.390
p	.638	.517
Lobule IX		
r_s	-.459	-.078
p	.436	.900
Lobule X		
r_s	.481	.696
p	.412	.192

Note. * $p < .05$. *p* = two-tailed. Controlled for age and total cerebellar volume.

Table 6

Spearman's Rho Correlation Coefficients between Cerebello-Hypothalamic Tract Fiber Count and Estradiol and the NEO-PI-R Neuroticism scales.

	Left CHT	Right CHT
Estradiol		
r_s	-.470	-.579
p	.689	.607
NEO Fear scale		
r_s	.974	-.235
p	.145	.849
NEO Depression scale		
r_s	.712	.309
p	.495	.800
NEO Impulsivity scale		
r_s	-.897	.006
p	.292	.996
NEO Neuroticism Total		
r_s	-	-
p	-	-

Note. * $p < .05$. p = two-tailed. Controlled for age and total cerebellar volume.

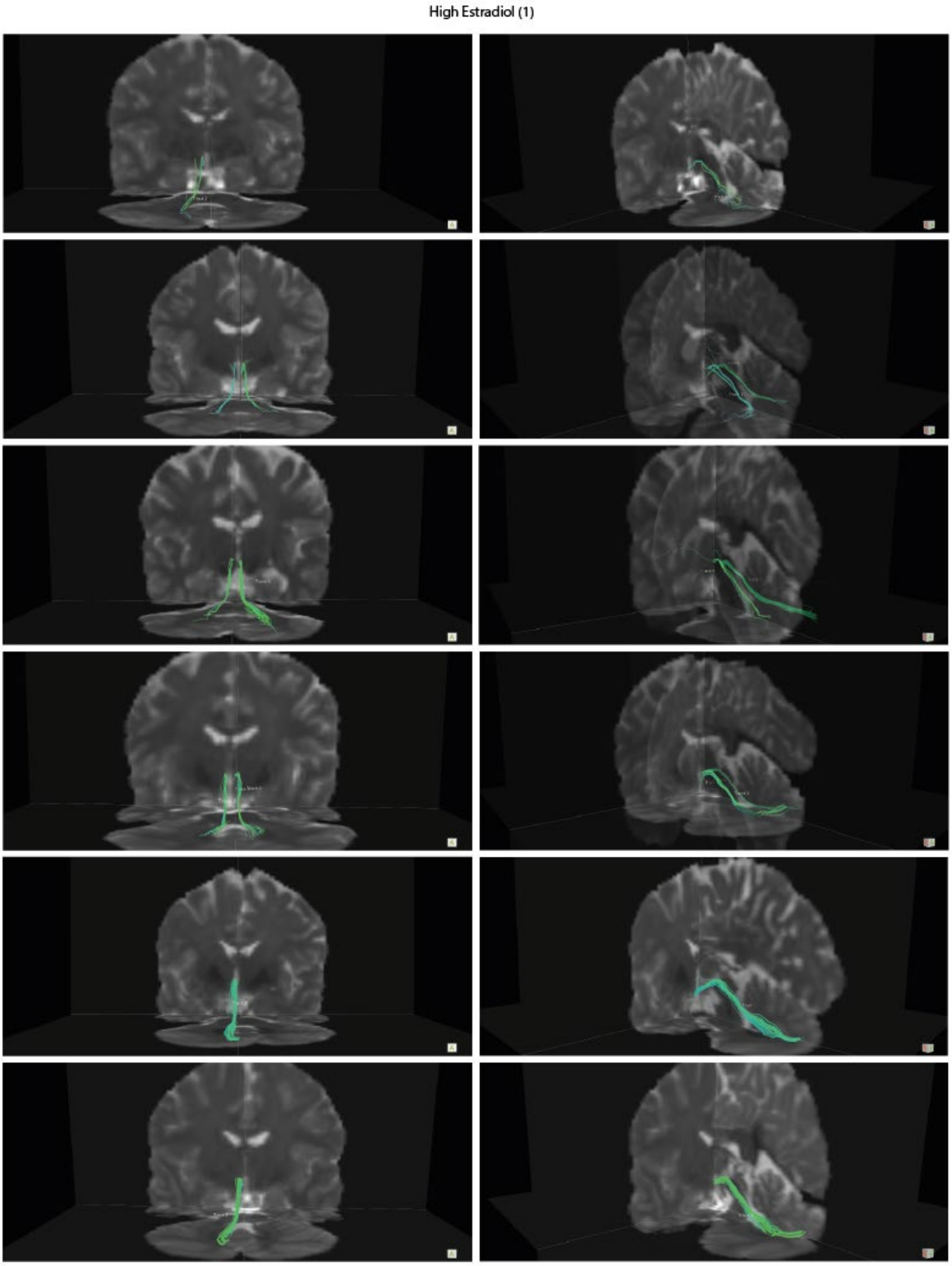
Table 7

Mann-Whitney U Test for Cerebello-Hypothalamic Tract Unilateral versus Bilateral groups

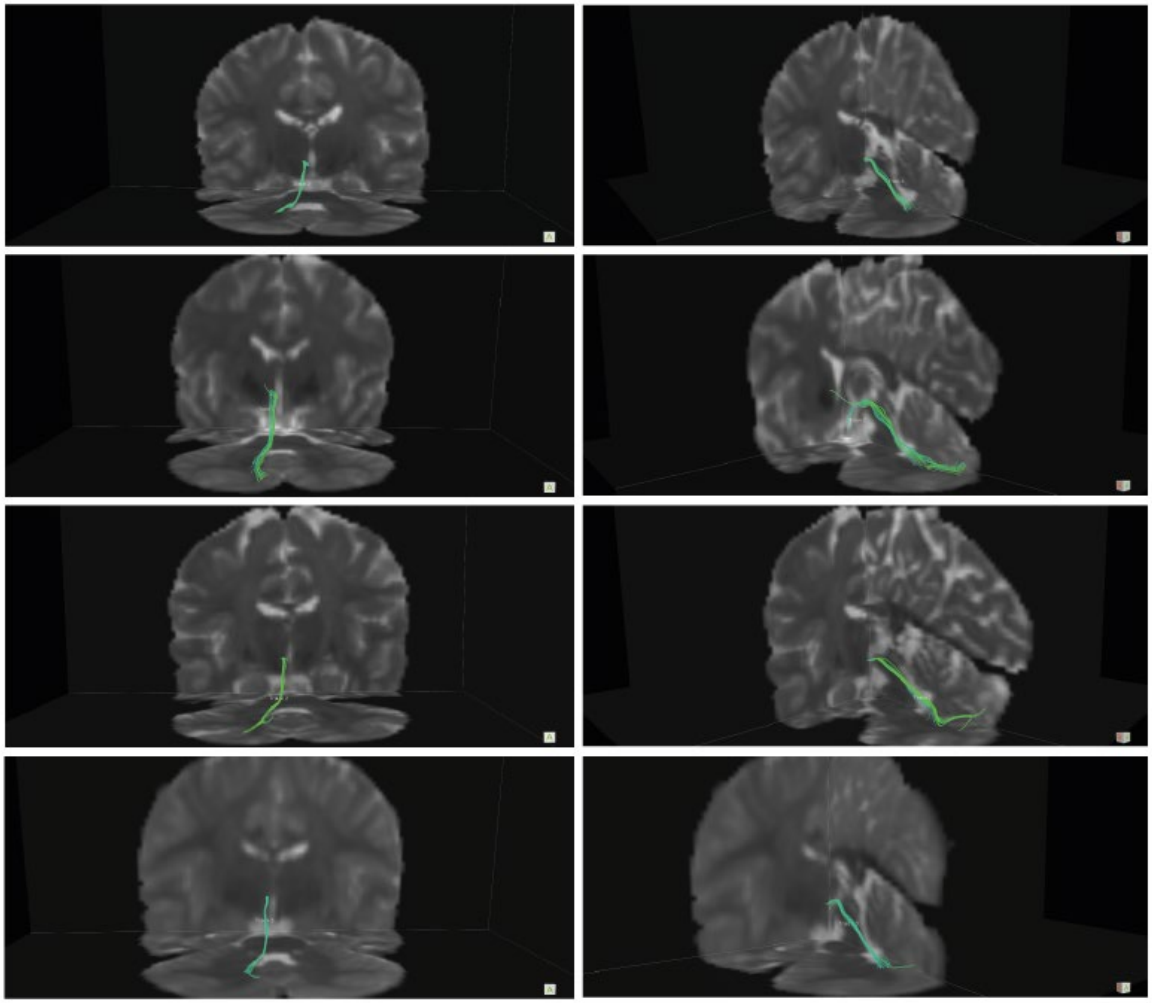
	n	*Mean Rank*	*U*	*p*	*r*
NEO Fear scale			10.00	.359	.28
Unilateral CHT	6	5.17			
Bilateral CHT	5	7.00			
NEO Depression scale			1.50	.013*	.75
Unilateral CHT	6	3.75			
Bilateral CHT	5	8.70			
NEO Impulsivity scale			5.50	.080	.53
Unilateral CHT	6	4.42			
Bilateral CHT	5	7.90			
NEO Neuroticism Total			6.00	.100	.50
Unilateral CHT	6	4.50			
Bilateral CHT	5	7.80			

Note. * $p < .05$. p = two tailed.

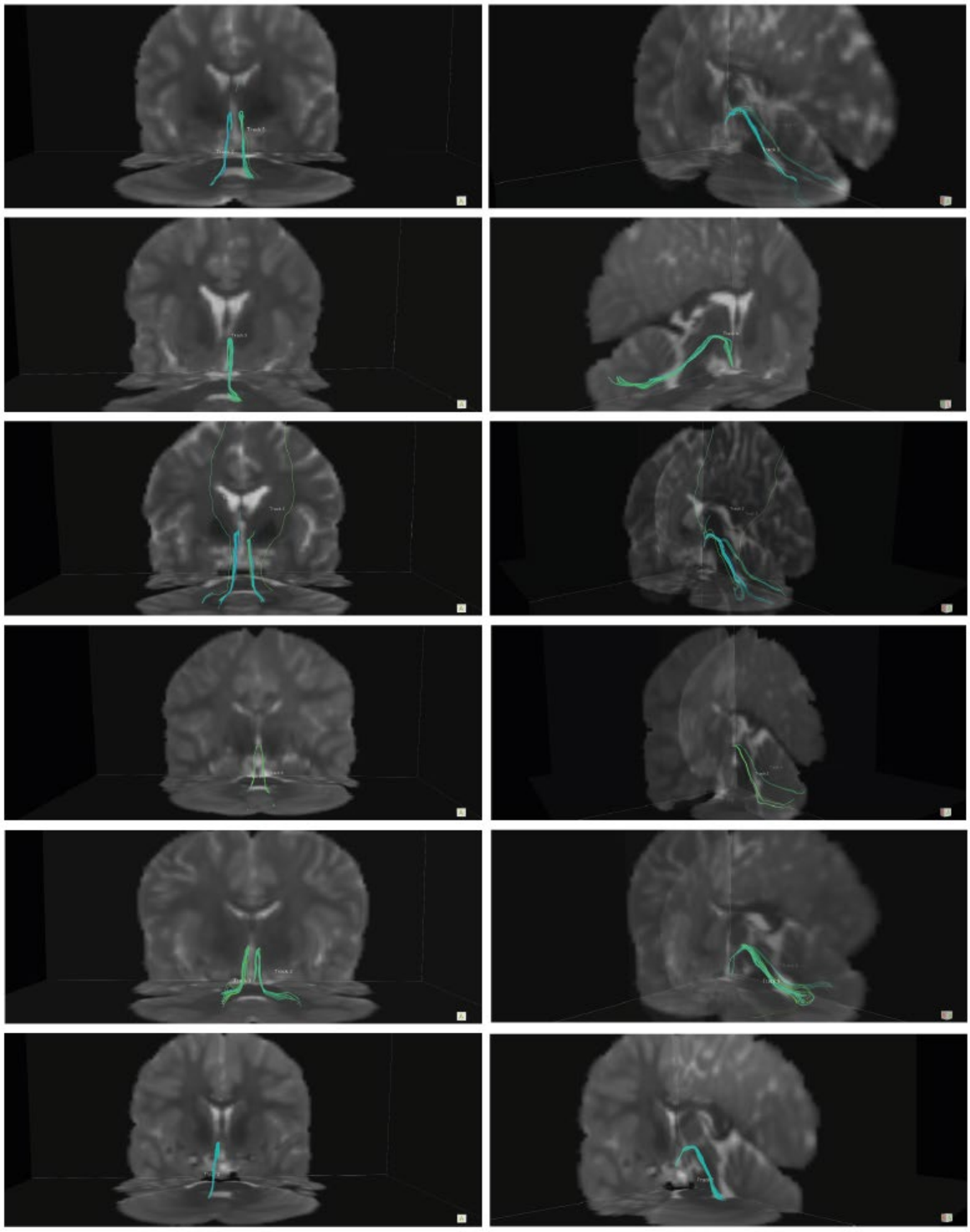
Appendix 1 Cerebello-Hypothalamic Tracts



High Estradiol (2)



Low Estradiol (1)



Low Estradiol (1)