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Hypothalamus volume in men: Investigating associations with paternal status, self-reported caregiving beliefs and adult attachment style

*Long M.[1,2], Puhlmann L.[2,3], Vrtička P.[2,4]

[1] *Alberta Children's Hospital Research Institute, University of Calgary, Canada*

[2] *Research Group "Social Stress and Family Health", Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany*

[3] *Leibniz Institute for Resilience Research, Mainz, Germany*

[4] *Centre for Brain Science, Department of Psychology, University of Essex, Colchester, UK*

*Corresponding Author

Madison Long, MSc

Alberta Children's Hospital Research Institute, University of Calgary, Canada

Alberta Children's Hospital; 28 Oki Drive NW; Calgary; T3B6A8; AB Canada

madisonmaylong@gmail.com

Tel: +1 (541) 321-0045

Abstract

Most studies on mammalian caregiving and attachment have focused on the mother-child relationship, particularly in humans. Yet, changing societal roles of male caregivers have highlighted the necessity for research with fathers.

In this study, we examined the volume of the hypothalamus, an important subcortical brain area for caregiving and attachment, in a sample of N=50 fathering (child age 5-6 years) and N=45 non-fathering men using a novel technique to identify the human hypothalamus in 3T MRI. Furthermore, we employed three self-report measures to assess interindividual differences in adult attachment style across all men and caregiving beliefs in fathers.

While we did not observe any significant difference in hypothalamus volume between fathers and non-fathers or associations between hypothalamus volume and self-reported adult attachment style across all men, self-reported caregiving beliefs were positively related to total hypothalamus volume in fathers. A follow-up analysis showed that fathers' self-reported belief that a father's role is important to child development was specifically related to tuberal hypothalamus volume, while self-reported enjoyment of spending time with the child was not associated with volume in hypothalamus sub-regions.

Together, these findings suggest that interindividual variability in self-reported caregiving beliefs in fathers is related to brain structure, warranting further research.

Key Words: Hypothalamus, structural MRI, fatherhood, caregiving, attachment

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Introduction

Until the early 1990's, fathers were typically cast as either breadwinners or playmates rather than nurturing figures (Collins & Russell, 1991; Forehand & Nousiainen, 1993; Rohner & Veneziano, 2001). The past decade, however, has brought a surge of research acknowledging the neurobiological underpinnings as well as effects of paternal caregiving on child development (Bretherton, 2010; Feldman et al., 2019; Glasper et al., 2018; Kim et al., 2014; Liu et al., 2016; Swain et al., 2014). Even so, a dearth of scientific investigation on fathers as caregivers and attachment figures persists, especially from a social neuroscience perspective. The inclusion of fathers in caregiving and attachment research is also important from an ethical perspective, acknowledging the competency of fathers with regard to raising their children.

The establishment and maintenance of caregiving and attachment relationships lie at the core of social interactions and learning. Infant attachment styles were first described by John Bowlby, Mary Ainsworth, and Silvia Bell, and are considered an evolutionarily adaptive set of behaviors which keep offspring in close physical proximity to a caregiver such as a parent (Ainsworth & Bell, 1970; Ainsworth, 1964; Bowlby, 1973; Fraley et al., 2005). Attachment behavior is also understood to persist into adulthood as individuals age and form bonds with significant others such as romantic partners (Brennan et al., 1998; Fraley et al., 2005, 2005; Fraley, 2019; Mikulincer & Shaver, 2007; Vrtička & Vuilleumier, 2012). Self-reported adult attachment style, which is a main focus of this manuscript, may therefore reflect both adults' present cognitions about attachment as well as attachment style established in and persisting from childhood (Fraley, 2019). Related to attachment is the complementary construct of caregiving (Canterberry & Gillath, 2012; Mikulincer & Shaver, 2007). While the aim of attachment is to seek support from significant others, a caregiving response provides that support. Accordingly, the attachment neurobehavioral system in one individual is activated as a distress response (to an internally or externally derived threat), and the caregiving neurobehavioral

system in the significant other is in turn activated to help alleviate the first individual's distress through emotion and allostatic co-regulation (Atzil et al., 2018; Long et al., 2020).

Attachment and caregiving behavior are thought to arise from a chorus of activation in multiple neural networks. We recently proposed a functional neuro-anatomical model of (organized) human attachment (*NAMA*) based on associations between interindividual differences in attachment as well as structural and functional neuroimaging data (Long et al., 2020; see also Vrtička, 2017; Vrtička & Vuilleumier, 2012). *NAMA* proposes that attachment behavior is maintained by four brain networks: approach, aversion, emotion regulation, and mental state representation. Here, we focus on the approach and aversion networks, which are thought to include (amongst others) the ventral tegmental area, substantia nigra, ventral striatum, ventromedial orbitofrontal cortex, pituitary, and the hypothalamus as parts of the approach network, and the anterior cingulate cortex, insula, amygdala, anterior temporal pole, hippocampus and hypothalamic-pituitary adrenal (HPA) axis as parts of the aversion network (see also Feldman, 2017; Fisher et al., 2006; Insel & Young, 2001; Swain et al., 2014). The prototypical role of the approach network in the context of caregiving and attachment is to encode social interactions with significant others (i.e., parents, children, and romantic partners) as inherently rewarding and soothing. On the other hand, the aversion network likely encodes stressful social (and non-social) stimuli as unpleasant. The approach and aversion networks are likely in dynamic balance, modulated by endocrine pathways mediated through the action of cortisol, oxytocin, and vasopressin, hormones whose function is dependent on the hypothalamus (Dudás, 2013, du Vigneaud et al., 1954; Scharrer, 1990.) Given the central role of the hypothalamus in modulating the NAMA approach and aversion networks, it is a structure of primary interest when investigating attachment and caregiving - also because interindividual variation in approach and aversion network function may be partially attributable to interindividual differences in caregiving and attachment behavior (Long et al., 2020; Vrtička, 2017; Vrtička & Vuilleumier, 2012).

Interindividual differences in adult attachment are often described in terms of the three organized or resolved attachment styles: secure, anxious (insecure-ambivalent/resistant), and avoidant (insecure-dismissive) (Fraley et al., 2000; Shaver & Hazan, 1987), which are defined by corresponding approach and aversion strategies. Similarly, caregivers tend to align their support-giving behavior with a particular approach strategy (Collins et al., 2010; Mikulincer & Shaver, 2007). *NAMA* predicts a decrease in approach network activation, and upregulated aversion network activation, for avoidant individuals in caregiving and attachment contexts (for a review, see Long et al., 2020). While data on anxious attachment and approach network activity is not yet conclusive, complementary behavioral data suggests that an anxious caregiver might respond to a significant other's distress with heightened social approach and helping behavior, sometimes even when not immediately necessary (Canterberry & Gillath, 2012). Although our understanding of relationships between approach/aversion network function and caregiving and attachment is growing, there remains a lack of evidence regarding its structure, especially in men and fathers.

Here we focus specifically on the hypothalamus, a sub-cortical brain region which likely plays a key modulatory role in approach and aversion and has been implicated as a core neural structure underlying both parental and romantic love (Bartels & Zeki, 2004). The hypothalamus contains numerous sub-structures including the medial preoptic area (MPOA) as well as the supraoptic (SON) and paraventricular (PVN) nuclei. Anatomically, the SON and PVN span the anterior and tuberal regions of the hypothalamus (Dudás, 2013) where they produce and release oxytocin (du Vigneaud et al., 1954; Scharrer, 1990), an affiliative hormone implicated in the development of attachment bonds (Carter, 2014; Fisher et al., 2006; Insel & Young, 2001). Additionally, these nuclei are involved in the production and release of corticotropin releasing hormone (CRH; Dudás, 2013), a central messaging hormone in the HPA stress response. The oxytocin and HPA systems of the hypothalamus are theorized to have a modulatory effect on the approach network, which may ultimately relate to interindividual differences in caregiving and attachment behaviors in humans. Recent developments in structural

magnetic resonance imaging (MRI) methods have now made it possible to identify the human hypothalamus, its subregions (i.e., anterior, tuberal, and posterior), and estimate its volume in-vivo (Makris et al., 2013) (**Figure 1 A and B**).

Research from non-human mammals indicates that the hypothalamus is a plastic structure at the onset of paternity and plays a key role in pair-bonding and parenting behavior for both males and females. In particular, the anterior hypothalamus and its nuclei are often implicated in rodent parenting behavior. For example, the function of the anterior hypothalamus area is implicated in the inhibition and onset of maternal behavior in rats (Bridges et al., 1999). Moreover, structural changes in the supraoptic nucleus were observed in association with motherhood (Theodosis & Poulain, 1984). While the evidence is sparser for males, hormonal and cellular changes have been observed in the paternal hypothalamus in response to parenthood across several species of bi-parental rodents (see Horrell et al., 2020 and Saltzman & Ziegler, 2014 for an overview). Endocrine changes associated with fatherhood include an increase in oxytocin binding (Parker et al., 2001) and vasopressin expression (Wang et al., 2000) in the hypothalamus - and are not found in non-fathering males. Changes in cell survival rate in the ventromedial hypothalamus were observed in male prairie voles, indicating changes in plasticity and hypothalamus morphology are associated with fatherhood (Lieberwirth et al., 2013). Of note, two studies that compared paternal and non-paternal male California mice found no difference in morphometry of the medial preoptic region of the hypothalamus (Gubernick et al., 1993; Horrell et al., 2019). However, this does not preclude morphometric differences between father and non-fathers in other areas of the hypothalamus, especially given that parenthood induced changes in hypothalamic cells include an upregulation of gene expression related to plasticity (Seelke et al., 2018). Overall, the hypothalamus appears to be an important structure underlying both maternal and paternal caregiving.

MRI research in humans has also revealed similarities in the substrates of maternal and paternal caregiving (Abraham et al., 2014; Kim et al., 2010, 2014), as well as an important role of the hypothalamus in social approach/aversion, caregiving, and attachment contexts. Both mothers (Kim et

al., 2010) and fathers (Kim et al., 2014) showed increased volume in the hypothalamus during the first few months after their first child was born, in addition to changes in other cortical and subcortical regions. Functional brain networks related to parenting were observed to be similar across mothers and fathers but was especially similar across parents in a primary caregiving role regardless of parent sex (Abraham et al., 2014). Differences in brain structure between men with and without children have also been observed; a comparison of cortical thickness in fathers and non-fathering men revealed regional differences (Orchard et al., 2020). This is somewhat in contrast to other findings which indicated no changes in paternal cortical volume before and after becoming a parent (Hoekzema et al., 2017). Concerning approach, aversion, and the hypothalamus specifically, one study observed that lower hypothalamus volume in men was predictive of low pro-sociality (Tost et al., 2010). While multiple within-subjects or between-subjects studies have examined some combination of relationships between the brain, hypothalamus, fatherhood, and social approach/aversion, there are presently no studies comparing hypothalamus morphometry between fathers and non-fathers in humans, despite resounding agreement on the importance of the hypothalamus in attachment and caregiving.

Morphological differences in regional hypothalamus volume observed via MRI may reflect cellular level changes in neural structure, such as neuronal number or dendritic spine arborization and pruning. Recently, a combined analysis of MRI and two-photon microscopy indicated that nucleus size and cell density partly explained variation in volume observed with MRI (Asan et al., 2021). Cellular level morphometry may correspond with function and ultimately explain variations in behavior. Given the functional differences observed in the hypothalamus in both human and non-human caregiving mammals (de Jong et al., 2009; Parker et al., 2001; Strathearn et al., 2009; Wang et al., 2000), and the previous finding of increased hypothalamus volume after becoming a father (Kim et al., 2014), we expected that greater hypothalamus volume in fathers would ultimately correspond with altered function and increased positive feelings toward attachment and caregiving interactions.

Our study is the first to examine structural correlates between the human male hypothalamus and parenting in the later post-partum period and one of few to address the relationship between hypothalamus structure, caregiving beliefs, and adult attachment style in men. Using a novel approach to segment the hypothalamus in 3T MRI (Makris et al. 2013), we characterized hypothalamus volume in a sample of N=95 men. Because fully automatic segmentation of the hypothalamus in 3T MRI is not yet possible (Baroncini, 2012), this method of hypothalamus measurement represents a competitive technique for studying the hypothalamus in humans. Moreover, with our sample's composition of N=50 fathers and N=45 men with no children, we related hypothalamus volume to self-reported adult attachment style across all men. In fathers alone, we furthermore characterized the relationship between hypothalamus volume and self-reported caregiving beliefs. Hypotheses and the analysis plan for the present investigation were registered and made publicly available online at https://aspredicted.org/5uj5y.pdf. However, given the lack of extant evidence on the paternal hypothalamus, especially in the late postpartum period, other analyses presented here were exploratory.

As per our online registration, we first hypothesized that fathers of five-year old children would have greater anterior hypothalamus volume than men without children. We also planned to test between-group differences in total as well as tuberal and posterior hypothalamus volume but without specific expectations for the outcome. Our second hypothesis about the relationship between hypothalamus volume and adult attachment style can be broken into two parts: 1) We thought that self-reported adult attachment security would be positively related to anterior hypothalamus volume in all men, and 2) we believed that this relationship would be stronger in fathers - given the interconnected nature of the attachment and caregiving systems and an assumption that fathers' attachment and caregiving systems are activated on a more regular basis than non-fathering men. Our third hypothesis was also comprised of two parts. We believed that 1) self-reported enjoyment of the child and 2) beliefs about the importance of a father's role would be positively associated with anterior hypothalamus volume in fathers.

Our study's focus on the anterior hypothalamus as a region of interest stems from previous findings in both human and rodent literature. Histological examinations of the human hypothalamus indicate that the anterior hypothalamus contains nuclei responsible for oxytocin and CRH function (Carter, 2014; Dudás, 2013; Fisher et al., 2006; Insel & Young, 2001; du Vigneaud et al., 1954; Scharrer, 1990). These anterior hypothalamic nuclei likely perform modulatory functions for the approach and aversion networks that support attachment and caregiving behavior (Long et al., 2020). Moreover, in both male and female rodents, functional and structural changes in the anterior hypothalamus and its nuclei have been observed in response to parenthood (Bridges et al., 1999; Parker et al., 2001; Saltzman & Ziegler, 2014; Theodosis & Poulain, 1984). While previous studies of paternal brain structure in humans indicated that the hypothalamus is likely a plastic region (Kim et al., 2014), ours is the first to employ a method of segmentation specific enough to delineate the hypothalamus itself and its subregions in-vivo. Thus, the present study is the first to probe whether, as in rodents, human paternal anterior hypothalamus structure differs between fathers and non-fathers, and whether interindividual differences in adult attachment or caregiving relate to structural differences in the anterior hypothalamus.

Methods

Participants

Fathers' data was acquired as part of the D-CARE study, an investigation of the behavioral, biological, and brain substrates of fathers' adult attachment style and caregiving beliefs performed at the Max Planck Institute of Human Cognitive and Brain Sciences (MPI-CBS) in Leipzig, Germany. A total of N=68 fathers of 5-to-6-year old children were recruited for the D-CARE study from the general population. Inclusion criteria included being aged 23-55 years, right-handed, physically healthy with no history of psychiatric illness (including current drug or alcohol abuse), and having no difficulties reading or writing in German. Of those N=68 fathers recruited for the D-CARE study, MRI data was

available from N=50 fathers. N=12 fathers could either not be admitted to the MRI scanner due to counter indications (N=11) or had to be excluded from further analysis due to incidental MRI findings (N=1). An additional N=6 fathers dropped out from the study prior to MRI scanning due to various reasons. The D-CARE study was approved by the Ethics Committee of the University of Leipzig and fathers gave informed consent for both themselves and their children before participation. Fathers were remunerated financially for each visit while children received two small presents plus a participation certificate. The control sample was pragmatically sampled from a previous independent longitudinal study conducted at the MPI-CBS (the ReSource Project; Singer et al., 2016). Of a total N=198 participants available in the ReSource sample, we selected all participants meeting the following criteria for our control sample: male, aged 23-55 years, no children, complete ECR-RD data. This yielded a total control sample of N=45 non-fathering men.

Demographic characteristics for the sample are provided in **Table 1.** Briefly, the two groups of men did not differ significantly in education or total brain volume. However, they did differ significantly in age, monthly household income, and marital status. We therefore controlled for all the above demographic characteristics in our main analyses. Additionally, we repeated our analyses with a matched subgroup of fathers and non-fathers (N=28 each) to ensure the integrity of our results.

Questionnaires

Descriptive statistics for all questionnaires can be viewed in **Supplemental Table S1**.

Adult Attachment Style

To measure self-reported adult attachment style, we used a German translation of the Experiences in Close Relationships questionnaire revised (ECR-RD; Ehrenthal et al., 2009; original English version: Fraley et al., 2011). The ECR-RD measures adult attachment style on two subscales: avoidance and anxiety. Participants rate items on a 7-point Likert scale (1 = not at all true for me; 7 = very true for me). For both subscales, higher scores indicate greater levels of attachment anxiety or

avoidance, respectively. A low score on both subscales is thought to indicate attachment security. Scale reliability was high for both ECR-RD anxiety (α = 0.881) and avoidance (α = .994).

Caregiving Beliefs

We measured fathers' attitudes toward caregiving with a German version of the Caregiving Experiences Questionnaire (CEQ-D: Nguyen et al., in preparation; CEQ: Brennan et al., 2013; Røhder et al., 2019). The CEQ-D yields independent scores for 4 subscales (Delight, 18 items; Helplessness, 12 items; Role Reversal, 4 items; Heightened Caregiving, 5 items). All items are rated on a 5-point Likert scale (1=not at all characteristic; 5=very characteristic). The present analyses used scores for the Delight subscale of the CEQ-D, which was best suited to evaluate caregiving beliefs related to the father's self-reported enjoyment of the child, with higher scores indicating a greater enjoyment. In our study, scale reliability for the CEQ-D Delight was α = 0.665, which has been considered adequate, moderate, or sufficient in previous studies (Taber, 2018). Of note, this fell below our pre-determined threshold of α = 0.7 as described in the online registration. Results involving the CEQ-D Delight should thus be interpreted somewhat cautiously.

We furthermore utilized the Role of the Father Questionnaire (ROFQ-D; translated into German and adapted to fathers of preschoolers after consultation with Rob Palkovitz: see Nguyen et al., under review / preprint; original English version: Palkovitz, 1984). The ROFQ-D captures participants' beliefs and values around being a father. Fathers rated 15 items on a five-point Likert scale (5 = agree strongly, 1= disagree strongly). Higher scores indicate a greater belief that fathers are capable, should be sensitive to their children, and should be involved in their development. The ROFQ has successfully been used in father research and was found to be related to key domains of paternal involvement in fathers of infants (Bronte-Tinkew et al., 2006) and preschoolers (McBride & Rane, 1996). Scale reliability for the ROFQ-D in our sample was high at α = 0.85.

For information on outliers, variable distribution and frequencies, simple correlations between questionnaire variables, as well as normality and homoscedasticity of residuals and variance inflation factors in multiple regressions, please see **Supplemental Tables S1 and S2**, and **Supplemental Figures S1, S2, and S3**.

MR image acquisition, pre-processing, and hypothalamus segmentation

This cross-sectional study included a single anatomical MRI scan per participant. For both fathers and non-fathering men, a T1-weighted MPRAGE anatomical image was acquired at the MPI-CBS in Leipzig, Germany. For the D-CARE sample of fathers, images were acquired on a 3T Siemens Skyra with a 32-channel head coil using the following parameters: 176 slices, voxel size = $1mm^3$, TR=2300 msec, TE=2.98 msec, Flip angle=9°, FOV=256 mm. Control subjects from the ReSource sample were scanned on a 3T Siemens Verio with identical parameters except for a 2° divergence in flip angle.

We processed T1-weighted anatomical images in NIFTI format for volumetric analysis of the hypothalamus (Makris et al., 2013) using the FreeSurfer software package, version 5.1.0. First, T1-weighted images were fed to the recon-all processing pipeline, which performed automated intensity normalization, skull stripping, tissue segmentation and parcellation, and cortical reconstruction following previously described steps (Dale et al., 1999; Fischl et al., 1999). In VP of the human hypothalamus, trained raters manually identify the volume of the hypothalamus in a T1-weighted image using anatomical landmarks (**Appendix A**). The protocol allows for sub-division of the hypothalamus into the rostral to caudal subregions defined by hypothalamic anatomy: anterior, tuberal, and posterior (Dudás, 2013; Makris et al., 2013; see **Figure 1 A and B**). All subregions are further divisible by hemisphere and their superior and inferior portions. In the present study, three independent raters (including ML) completed the VP protocol for each of the MRI scans. Using FreeSurfer's statstotable function we extracted Parcellation Units (Pus; voxel counts) for each segmented region. In

the present study, each subject's hypothalamus was identified independently by each rater and PUs were averaged across two or three raters to obtain a reliable measure of hypothalamus volume. Inter-rater reliability (IRR) was assessed using a two-way mixed, average measures, absolute agreement intraclass correlation coefficient (ICC). ICCs above 0.75 for the total hypothalamus and the three main subregions (anterior, tuberal, and posterior) were considered excellent (Cicchetti, 1994) and of sufficient quality for use in the study. Training, reliability, and data collection phases for this method are described in Appendix B of the Supplemental material for this paper. The ICC's for the final sample of N=95 hypothalami were all excellent (ICC >.78; **Supplemental Table S3**).

Hypothesis Testing

All statistical analyses were performed using the statistical programming software R (version 3.6.2, R Core Team, 2019). For each group of tests, we applied a false discovery rate (FDR; Benjamini & Hochberg, 1995) correction for multiple comparisons at the .05 level. Participants' age, education, average household income, marital status, and total brain volume were used as control variables in all analyses. We further controlled for child's sex in analyses of the father sample.

To test our first hypothesis, we used an ANCOVA to compare total and regional hypothalamus volumes in fathers and men with no children. This was a slight deviation from the t-test described in our registered analysis plan but allowed us to control for sociodemographic variables in the model. Our second hypothesis was tested in two parts: We 1) used regression with ECR-RD anxiety and avoidance as main effects, and 2) tested the effect of parenting status on the relationship between ECR-RD scores and hypothalamus volume by adding ECR-RD anxiety by parenting status and ECR-RD avoidance by parenting status interaction terms to the model. The above models were tested once with total hypothalamus volume as the dependent variable and again with anterior hypothalamus volume as the dependent variable. For our third hypothesis, we tested two regression models for each of the self-reported caregiving measures (CEQ-D Delight and ROFQ-D), one with total hypothalamus volume as

the dependent variable and the second with anterior hypothalamus volume as the dependent variable in the father subsample.

Results

Regarding the between-group comparison (non-fathering men, fathers) of hypothalamus volume, we found no difference in total ($F(1,84)=0.34$, $p=0.56$, $\eta^2=0.003$) or regional (Anterior: $F(1,84)=0.061$, $p=0.81$, $\eta^2=0.001$; Tuberal: $F(1,84)=0.08$, $p=.78$, $\eta^2=0.001$; Posterior: $F(1,84)=.08$, $p=.78$, $\eta^2=0.001$) hypothalamus volume between fathers and non-fathers when controlling for education, age, average household income, marital status, and total brain volume (**Figure 2 and Supplemental Table S5**).

As noted previously, the sample of fathers and non-fathers significantly differed on age, income, and marital status. As such, we repeated the above analysis for between-group differences in hypothalamus volume with a subsample of N=28 fathers and a matched sample of non-fathering men. These matched subgroups did not differ in age, income, education, or total brain volume. However, they still differed significantly on marital status (**Table 2**). Our findings did not change in light of the matched subgroup analyses; we found no difference between fathers and non-fathers on total or regional hypothalamus volume (**Supplemental Tables S6, S7**).

In two steps, we then probed whether 1) adult attachment style was related to hypothalamus volume, and 2) whether there was an effect of parenting status. For our whole sample of men, we observed a negative relationship between attachment anxiety and total hypothalamus volume, but this relationship was not significant after correction for multiple comparisons ($\beta=-41.13$, $p=.033$, $q=.013$, $\eta^2=.038$; **Supplemental Table S4 and Supplemental Figure S4**). We also did not find any significant relationship between total hypothalamus volume and attachment avoidance, or between anterior hypothalamus volume and either attachment anxiety or avoidance (**Supplemental Table S4**). Furthermore, in testing for the effect of parenting status, we again did not find any significant effects

after correction for multiple comparisons; there was no significant association between parenting status and total hypothalamus volume, and no interaction between self-reported adult attachment style and parenting status predicting total hypothalamus volume (**Supplemental Table S4**).

However, we found that fathers' self-reported caregiving beliefs (ROFQ-D) and enjoyment of the child (CEQ-D Delight) were significantly related to total hypothalamus volume and remained so after correction for multiple comparisons. Both CEQ-D Delight (β=41.291, p=0.024, q=0.047, η^2=0.095) and ROFQ-D (β=43.384, p=0.023, q=0.047, η^2=0.108; **Supplemental Table S4 and Figure 3A, B**) scores were positively related with total hypothalamus volume when controlling for father age, average household income, marital status, education, biological child sex, and total brain volume. These results indicate that for every 8.19 point increase in ROFQ-D score, there was a 3.2% (43.384 mm^3) increase in father's total hypothalamus volume, and for every 3.34 point increase in CEQ-D Delight, there was a 3% (41.291 mm^3) increase in total hypothalamus volume. ROFQ-D and CEQ-D Delight, respectively, explained about 11% and 9.5% of the variance in total hypothalamus volume for our sample of fathers, increasing our confidence in these findings. However, contrary to our hypothesis, we found no notable relationships between our predictors and anterior hypothalamus volume in fathers. To further probe what might be driving the significant relationship between our caregiving measures and total hypothalamus volume, we tested two exploratory models for tuberal and posterior hypothalamus volume. ROFQ-D significantly positively related to tuberal hypothalamus volume (β=68.202, p<0.005, q<0.02, η^2=0.189; **Figure 3C**), indicating that for every 8.19 point increase in ROFQ-D score, tuberal hypothalamus volume increased by 13.5% (68.2 mm^3). ROFQ-D scores explained roughly 19% of the variance in tuberal hypothalamus volume. On the other hand, CEQ-D Delight scores were not significantly related to either tuberal or posterior hypothalamus volume (**Supplemental Table S4**).

Discussion

Caregiving and attachment are grounded in complementary behavioral and neural systems that are crucial for social interaction and learning throughout life. Recent re-considerations of attachment theory and changing societal roles of male caregivers have highlighted the necessity for research with fathers. We found no significant differences in hypothalamus volume between men with and without children. Additionally, we examined relationships between hypothalamus volume and self-reported adult attachment style in all men and found a non-significant negative relationship between total hypothalamus volume and attachment anxiety. Lastly, we found that total hypothalamus volume was significantly positively related with both self-reported enjoyment of interacting with their child and self-reported beliefs about the importance of a father's role. In an exploratory follow-up analysis, we showed that self-reported beliefs about the importance of a father's role was specifically related to greater tuberal hypothalamus volume. To our knowledge, this is the first study to relate hypothalamus volume to fathers' caregiving beliefs, adult attachment style, and to compare hypothalamus volume between men with and without children.

Hypothalamus Volume in Fathering versus Non-Fathering Men

We found no difference between fathers and non-fathering men when it came to hypothalamus volume. This was contrary to our hypothesis that a) there would be a difference as such, and more specifically, b) that fathers would have greater anterior hypothalamus volume than non-fathers. To clarify this finding and reduce between-group variance in age and income, we conducted a supplementary matched subgroup analysis. Our finding of no difference between men with and without children was unchanged. This suggests that there is no difference in hypothalamus structure between non-fathers and fathers in the late post-partum period.

An alternative explanation for our null finding regarding hypothalamus volume in fathers versus non-fathering men is that a cross-sectional comparison may not capture changes occurring within an individual before and after becoming a father. In other words, volume at a particular moment in time

may be not as meaningful as relative changes in volume across time. Previous results from studies of both humans and rodent models present a regionally heterogeneous picture of neural plasticity in the peripartum and early postpartum periods. Neuroimaging studies of human fathers during the peripartum period have shown mixed results for paternal plasticity and have found that paternal hypothalamus volume increased over the first 4 months of being a father (Kim et al., 2014), while parenting-related regions of the cortex did not change from before to after becoming a father (Hoekzema et al., 2017) – although the latter study did not examine the hypothalamus specifically. One rodent study indicated changes in both behavior and neurogenesis in the ventromedial hypothalamus of paternal prairie voles, suggesting fathering-specific neural and behavioral plasticity (Lieberwirth et al., 2013). Studies of bi-parental rodents have also revealed hypothalamus-specific endocrine changes, including increases in vasopressin gene expression (Wang et al., 2000) and increases in oxytocin binding (Parker et al., 2001). Taken together, these studies suggest widespread neural plasticity occurring through the early postpartum period. To our knowledge, our study has provided the first data surrounding the paternal hypothalamus in the late postpartum period. While our results suggest that there are no meaningful differences in hypothalamus volume between fathers of five-to-six-year old children and non-fathers, longitudinal research is needed to discern whether important dynamic changes in hypothalamus volume occur in the father later on in a child's life.

Hypothalamus Volume and Adult Attachment Style in Men

Our findings did not reveal any significant relationship between self-reported adult attachment style and hypothalamus volume, both across all men and when probing a possible interaction with parenting status. Although we observed a negative correlation between attachment anxiety and total hypothalamus volume for all men, this correlation was not statistically significant after correction for multiple comparisons ($q = 0.13$). These findings only partially confirmed our hypotheses as we predicted a) a positive relation between secure adult attachment and hypothalamus volume in men in

general, and b) an association between adult attachment, parenting status, and hypothalamus volume more specifically.

We are only aware of one study to date that specifically examined hypothalamus structure in association with interindividual differences relating to approach behavior in humans, and described that lower hypothalamus volume was predictive of low pro-sociality (Tost et al., 2010). Other available data suggested more specific relations between hypothalamus structure and function related to parenthood, as increases in hypothalamus volume were found in both mothers (Kim et al., 2010) and fathers (Kim et al., 2014) in the first few months after their first child was born. Furthermore, hypothalamus activation was higher for securely versus avoidantly attached mothers when viewing pictures of their own versus an unknown infant, and such hypothalamus activation was indirectly related to peripheral oxytocin during free interaction with the own child (Strathearn et al., 2009). In the present study, we therefore assumed that more secure adult attachment style in men would be positively related to hypothalamus volume, and that such association may be strengthened in fathering men, whose caregiving system is likely to be more strongly engaged. However, we only found preliminary evidence supporting a negative relationship between adult attachment anxiety (but not avoidance) and total hypothalamus volume in men, regardless of parenting status. As this study is the first that reports an association between adult attachment (anxiety) and hypothalamus volume in general, and in middle aged men more specifically, more research is needed to extend and clarify the observed patterns.

Regarding the absence of an interaction between self-reported adult attachment style, parenting status, and hypothalamus volume in the present study, recent research suggests that attachment can be context dependent. For example, an individual's behavior may be characterized by secure attachment tendencies when interacting with one person but reflect more anxious attachment tendencies when interacting with another. This notion reflects the presence of considerable heterogeneity of attachment across relationships – for example, relationships with one's parents versus romantic partner versus child (Collins, 2001; Collins et al., 2004; Fraley, 2019; Fraley et al., 2011, 2015; Klohnen et al., 2005;

Sibley & Overall, 2008). Future studies may therefore benefit from including several measures assessing interindividual variation in specific caregiving and attachment contexts.

Hypothalamus Volume and Caregiving Beliefs in Fathers

Regarding our models of self-reported caregiving beliefs and hypothalamus volume in fathers, we found that both greater paternal beliefs in the importance of a father's role (as measured by the ROFQ-D) and greater enjoyment of interacting with the child (as measured by the CEQ-D Delight subscale) were positively related to total hypothalamus volume. However, when further specifying these relations, caregiving measures were not associated with anterior hypothalamus volume as we had predicted. Rather, self-reported belief in the importance of a father's role (ROFQ-D) was positively related to tuberal hypothalamus volume. Self-reported enjoyment of the child (CEQ-D Delight) was not related to regional hypothalamus volumes. This suggests that more widespread, rather than focal, differences in hypothalamus structure could relate to differences in enjoyment of the child, while structures located in the tuberal hypothalamus in particular may associate with beliefs about the importance of a father's role.

There is limited evidence on the role of the hypothalamus in human parental behavior and the neural substrates of paternal care. Our hypothesis that anterior hypothalamus volume would relate to self-reported caregiving beliefs was therefore based on studies of maternal behavior (Bridges et al., 1999; Rogers & Bales, 2019) and general indications that the anterior hypothalamus partly contains the SON and PVN. However, the SON and PVN additionally span into the tuberal hypothalamus (Handa et al., 1994; Makris et al., 2013; Nieuwenhuys et al., 2008; Tobet et al., 2009). Our new results indicate that the tuberal hypothalamus may play a role related to caregiving beliefs in fathers. Because the SON and PVN produce and release the neuromodulators oxytocin and CRH, the tuberal hypothalamus may serve as a seat for regulation of the approach network which in turn may affect caregiving behavior and

beliefs. Further neuroendocrine studies of oxytocin and CRH in paternal caregiving behavior are needed to assess this possible link.

To our knowledge, ours is the first study to report interindividual variation of hypothalamus volume in fathers, providing a valuable contribution to the knowledge base for hypothalamic structure in human paternal care. These novel findings in fathers are congruent with similar observations from studies with mothers that indicated greater maternal positive perception of her baby to be related to larger hypothalamus volume (Kim et al., 2010). While our findings in fathers are congruent with this research in mothers, we note both a difference in developmental timing (perinatal period versus 5 to 6 years post-partum) and that the hypothalamus is a sexually dimorphic subcortical region with regard to both structure and function (for an overview, see Dumais & Veenema, 2016; Kudielka & Kirschbaum, 2005; Swaab et al., 2003). Our findings therefore provide an important basis for future research on the paternal hypothalamus, as well as direct comparisons of hypothalamic structure and function in mothers versus fathers.

The present findings additionally dovetail with another recent observation from a study of the same sample of fathers. Fathers participating in D-CARE additionally underwent a functional near-infrared spectroscopy (fNIRS) hyperscanning protocol together with their 5-to-6 year old children to assess father-child interpersonal neural synchrony (Nguyen et al., 2020). Nguyen et al. report that fathers with higher scores on the ROFQ-D showed a greater degree of interpersonal neural synchrony in bilateral dorsolateral prefrontal cortex and left temporo-parietal junction when engaging in cooperative problem-solving with their children. Interpreted together, fathers with stronger positive attitudes towards their role as a parent as well as reporting more positive characteristics of interactions with their children may also display distinct functional and structural neural traits both with regard to interpersonal neural synchrony and hypothalamus volume.

More generally, our finding that hypothalamus volume was related to a psychobehavioral construct (i.e., caregiving beliefs) has some foundation in previous research, which has linked

structural differences in the hypothalamus to brain function and behavior. Multiple studies showed differences in hypothalamus volume for individuals with a mood disorder as opposed to healthy controls (Schindler et al., 2018; also see Schindler et al., 2012 for an overview). Furthermore, cellular-level structural changes in the hypothalamus have been linked to differences in hypothalamus function (Hatton, 1997), lending credibility to the idea that structural differences detectable via MRI (such as volume) may underlie functional changes, which, in turn, could promote differential behavioral phenotypes in constructs such as caregiving qualities.

Limitations

Control and father samples were recruited as part of two independent studies and were not perfectly matched (age was especially different between the two). Being different in age (with the sample of non-fathers being older) might also indicate some underlying difference in men who become fathers and those who live into adulthood without becoming fathers. However, all analyses were controlled for age, education, income, marital status, and total brain volume to amend any influence of these demographic differences, and two additional analyses were carried out in a better matched sample of participants (N=28 each).

Another potential limitation of the study is its reliance on self-report measures to characterize participants' adult attachment style and caregiving beliefs. Future studies would benefit from the additional use of interview-based measures of adult attachment representation such as the Adult Attachment Interview (AAI) and observational measures of parenting behavior. Moreover, this study is cross-sectional and correlational. Therefore we cannot not account for changes that may occur in attachment, caregiving, and/or hypothalamus volume throughout the lifespan, or provide any indication of directionality or causality.

The aim of this study was very specific, to investigate relationships between self-reported attachment, caregiving beliefs, and hypothalamus volume. We did not collect information about the

primary caregiving status of the father or about non-parenting factors such as personality characteristics. While the circumscribed scope of the study can be counted among limitations in that we cannot completely rule out alternative explanations for our findings, it also lends credibility to our results. For example, our main hypotheses and corresponding analyses were pre-determined as part of an online registration. Deviations from this plan were slight and are well-described in this manuscript. Of our three main hypotheses, only one was supported by our findings and we have reported all results accordingly. The specificity of our results is also suggested by the fact that while the ROFQ-D and CEQ-D Delight scales were not correlated with one another (Supplementary Table S2) both were significantly related to hypothalamus volume. While measuring and analyzing additional variables was outside the scope of the present study, future studies may want to consider potential underlying factors such as personality traits and primary caregiving status in investigations of caregiving, attachment, and brain structure.

Finally, although structural MRI scans in fathers and non-fathering men were obtained on different scanners, scanning parameters were identical, except for a 2° divergence in flip angle. Since hypothalamus volume was manually identified, our approach was also robust against scanner-induced differences for example in image intensity, which may systematically affect automated segmentation algorithms. We are therefore confident that our approach is robust against any scanner-induced differences.

Conclusions

We first characterized and compared hypothalamus volume between men with and without children and assessed relations between hypothalamus volume and self-reported adult attachment style across all men. In doing so, we did not find any differences in hypothalamus volume between fathers and non-fathers, and no significant relations between hypothalamus volume and adult attachment style across all men. However, in a subsequent step, our study uncovered a positive relationship between

fathers' hypothalamus volume and self-reported caregiving beliefs, namely enjoyment of interacting with their child and beliefs about the importance of a father's role in child development. After further investigation, we found that self-reported beliefs about the fathers role were positively related to tuberal hypothalamus volume in particular. The present work therefore supports the notion that hypothalamus structure may be associated with interindividual differences in caregiving beliefs and behavior.

Table 1

Sample demographic characteristics and between-group comparisons

		Whole sample	Father sample	Control sample	Significance of between-group tests
Adult Age (years)		42.35	39.08	45.98	F(1, 93)=30.53, $p<0.001$
Child Age (years)			5.36		NA
Biological Child Sex	Male		29		NA
	Female		21		
Total Brain Volume (mm^3)		1217769	1219028	1216370	F(1, 93)=0.002, p=0.88
Marital status	Married	49	34	7	X^2(2, 95)=33.651, $p<0.001$
	Not married	50	12	38	
Education	Less than high school	2	1	1	X^2(4, 95) = 6.18, p=0.1859
	High school diploma (not eligible for university)	13	7	6	
	Abitur (high school diploma eligible for university)	18	13	5	
	University or university of applied sciences	53	27	26	
	Higher degree	9	2	7	
Monthly Household Income (euros)	less than 1500	6	2	4	X^2(6, 95) =15.916, p=0.014
	1500 – 1999	16	3	13	
	2000 – 2999	18	9	9	
	3000 – 3999	16	12	4	
	4000 – 4999	11	4	7	
	5000 and up	21	13	8	
	Preferred not to answer	3	3	0	

Note: Table reports means (adult age, child age, total brain volume) and frequencies (biological child sex, marital status, education, income.) NA= not applicable.

Table 2

Demographic characteristics and between-group comparisons for matched subgroups

		Subsample (N=56)	Father sample (n=28)	Control sample (n=28)	Significance of between-group tests
Adult Age (years)		41.04	40.11	41.96	F(1, 54)=1.54, *p*=0.22
Child Age (years)			5.36		NA
Biological Child Sex	Male		15		NA
	Female		13		
Total Brain Volume (mm^3)		1211964	1209046	1214882	F(1, 54)=0.058, *p*=0.811
Marital status	Married	27	22	5	X^2(1, 56)=18.309, *p<0.001*
	Not married	29	7	23	
Education	High school diploma (not eligible for university)	8	4	4	X^2(3, 56) =3.43, *p*=0.3299
	Abitur (high school diploma eligible for university)	10	7	3	
	University or university of applied sciences	33	16	17	
	Higher degree	5	1	4	
Monthly Household Income (euros)	less than 1500	2	1	1	X^2(6, 56) =8.67, *p*=0.193
	1500 – 1999	11	2	9	
	2000 – 2999	10	5	5	
	3000 – 3999	12	9	3	
	4000 – 4999	7	3	4	
	5000 and up	14	8	6	

Note: Table reports means (adult age, child age, total brain volume) and frequencies (biological child sex, marital status, education, income.) NA= not applicable

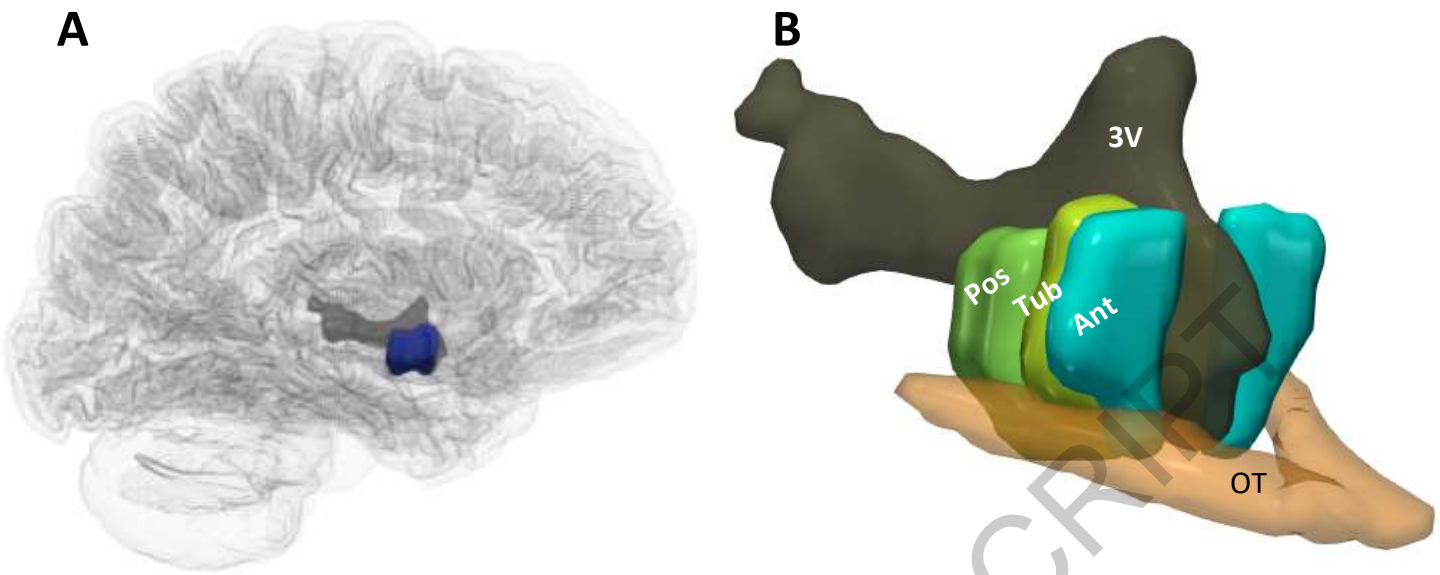

Figure 1. The human hypothalamus (panel A, blue) is located near the basal forebrain. It is situated medially of the optic tracts (panel B; OT) and lateral of the third ventricle (3V) with three rostral to caudal sub-regions: anterior (Ant), tuberal (Tub), and posterior (Pos). Images were created using a combination of FreeSurfer (Dale et al., 1999; Fischl et al., 1999), ITK-SNAP (Yushkevich et al., 2006), and ParaView (Ayachit, 2015) as described by Madan, 2015.

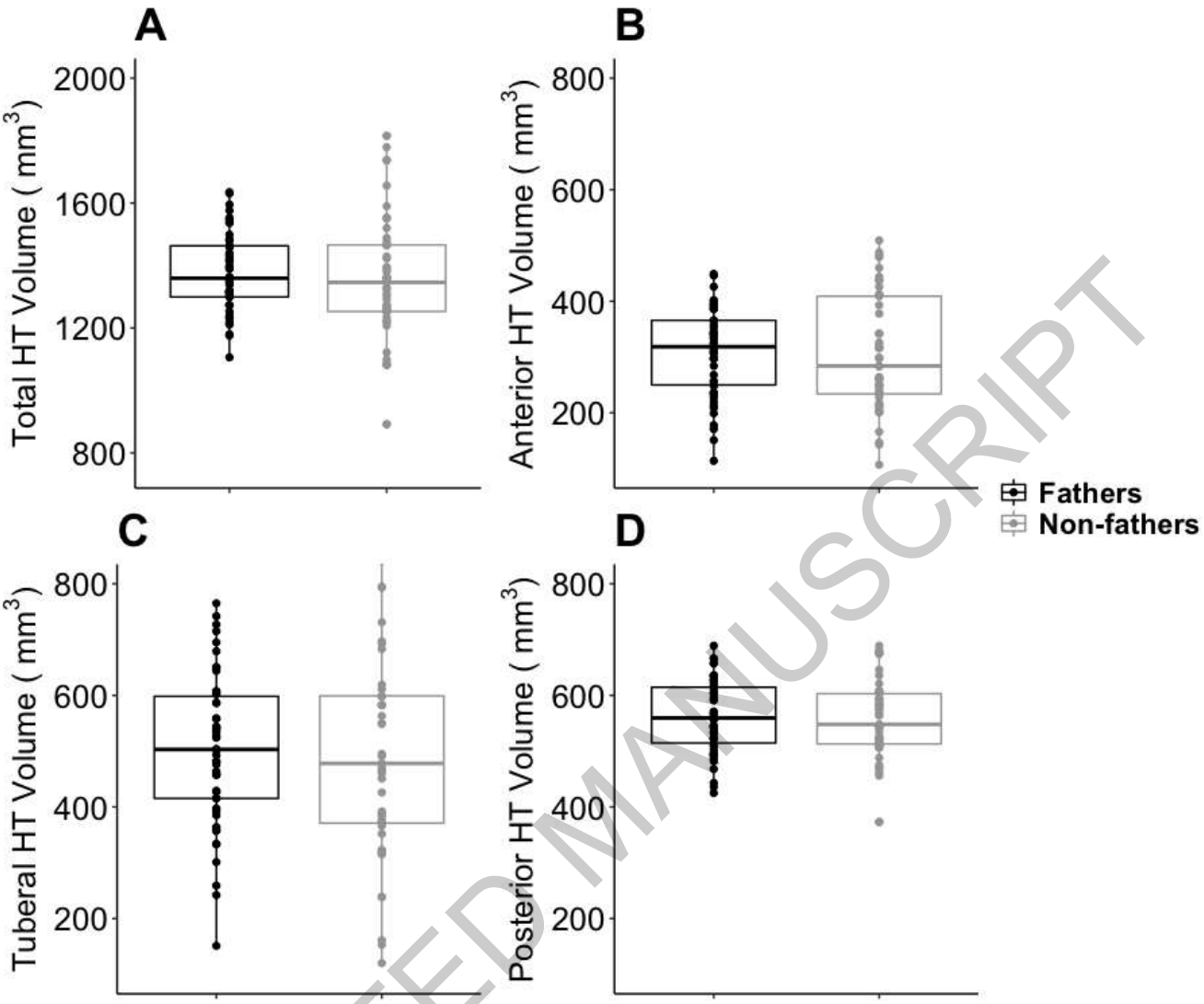

Figure 2. Boxplots of raw measurements of total (A), anterior (B), Tuberal (C), and Posterior (D) hypothalamus volumes in fathers and non-fathers. The pattern of data indicates similar average total and regional hypothalamus volumes across both groups. HT = Hypothalamus

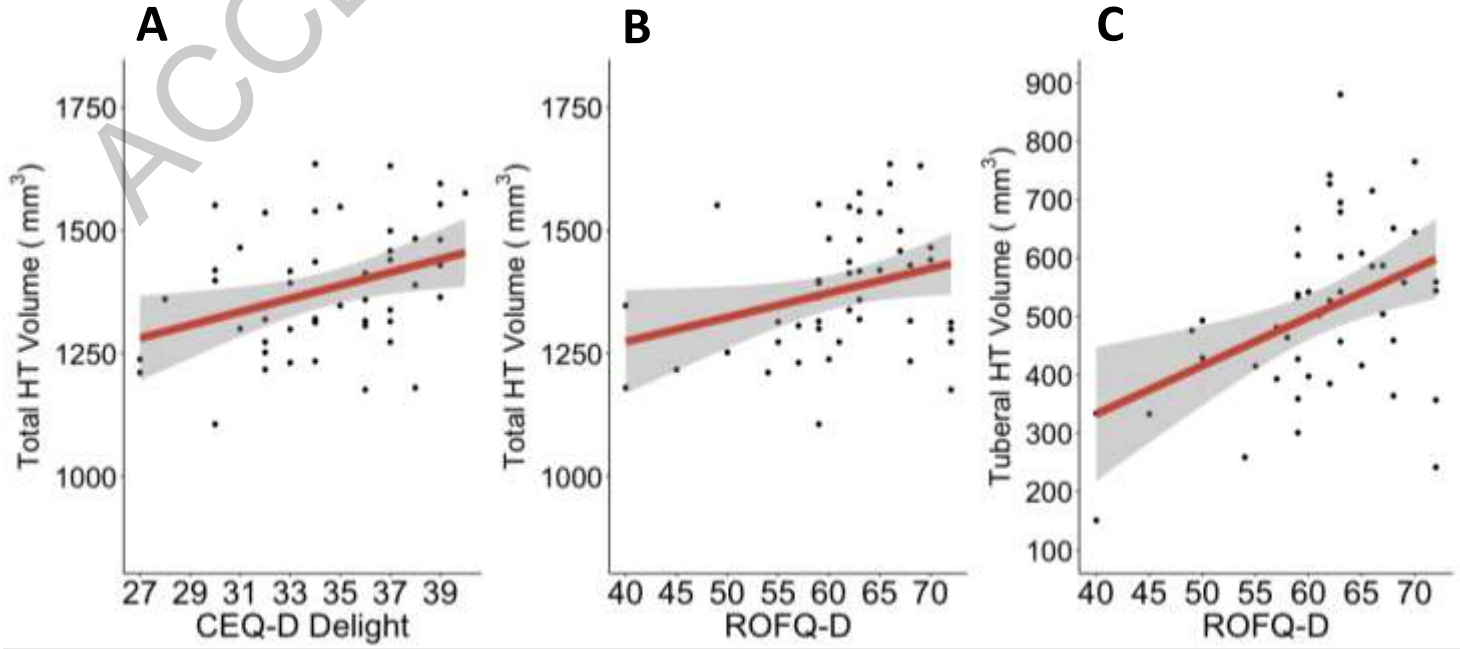

Figure 3. Association between parenting attitudes and total hypothalamus volume. We found significant positive relationships between total hypothalamus volume and father's enjoyment of the child (CEQ-D Delight) (A) and belief of the importance of a father's role (ROFQ-D) (B). We further found a strong positive relationship between the tuberal hypothalamus and ROFQ-D scores (C). The red line represents the estimated association based on linear regression analysis; shaded areas are 95% CIs; dots show raw data. HT = Hypothalamus.

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Supplemental Material

Supplemental Table S1

Descriptive statistics for questionnaire data

	Sample	Min	Max	Mean	SD
ECR-RD Anxiety	All	18	83	43.26	13.65
	Fathers	21	73	42.85	13.65
	Controls	18	83	43.69	13.8
ECR-RD Avoidance	All			41.33	15.97
	Fathers	18	87	38.83	15.6
	Controls	18	83	43.69	16.11
CEQ-D Delight	Fathers	27	40	34.42	3.34
ROFQ-D	Fathers	31	72	60.84	8.19

Note: We observed one outlier in the ROFQ-D (value of 31). To ensure that our results were not driven by this individual we ran the regression analysis both with and without winsorization of the individual value. The results were unchanged between analyses. Figure 3A depicts the relationship between total hypothalamus volume and ROFQ-D scores with the winsorized value.

Supplemental Table S2

Correlation coefficients of questionnaire variables

	ECR-RD Anxiety	ECR-RD Avoidance	ROFQ-D	CEQ-D Delight
ECR-RD Anxiety	1			
ECR-RD Avoidance	.586**	1		
ROFQ-D	-0.198	-0.141	1	
CEQ-D Delight	.319*	0.209	0.235	1

*Note: *p<0.01, **p<0.001*

Supplemental Table S3

Intraclass correlation coefficients for paired raters

	Rater pairing	
	ML and DB	**ML and MT**
Anterior	0.781	0.803

Tuberal	0.844	0.909
Posterior	0.881	0.884

Supplemental Table S4

Results for regression analyses of attachment, caregiving, and hypothalamic volume.

Model	Variable	β	p-value	q-value	η^2	VIF
Total Hypothalamic Volume (attachment; all subjects)	ECR-RD Anxiety	-41.13	0.033	0.13	0.038	1.29
	ECR-RD Avoidance	15.57	0.428	0.57	0.012	1.38
	Education	3.62			< 0.001	1.05
	Adult Age	-22.94			0.028	1.22
	Income	7.1			0.003	1.72
	Marital Status	0.26			0.003	1.32
	Brain Volume	55.14			0.104	1.08
Anterior Hypothalamic Volume (attachment; all subjects)	ECR-RD Anxiety	-9.79	0.431	0.57	0.007	1.29
	ECR-RD Avoidance	7.26	0.571	0.57	0.004	1.38
	Education	12.77			0.018	1.05
	Adult Age	-7.46			0.006	1.22
	Income	3.3			0.001	1.72
	Marital Status	-5.05			0.002	1.32
	Brain Volume	10.51			0.012	1.08
Total Hypothalamic Volume (attachment; parenting status added)	ECR-RD Anxiety	-61.732	0.014	0.14	0.038	2.129
	ECR-RD Avoidance	11.501	0.647	0.954	0.015	2.218
	Parenting Status	-2.763	0.954	0.954	0.003	2.092
	ECR-RD Anxiety X Parenting Status	52.016	0.228	0.954	0.018	3.198
	ECR-RD Avoidance X Parenting Status	-7.615	0.861	0.954	<0.001	3.106
	Education	6.458			< 0.001	1.133
	Adult Age	-20.288			0.023	1.455
	Income	9.827			0.004	1.242
	Marital Status	-2.845			0.002	1.814
	Brain Volume	55.609			0.104	1.091
Anterior Hypothalamic Volume (attachment; parenting Status added)	ECR-RD Anxiety	-4.158	0.781	0.954	0.007	2.129
	ECR-RD Avoidance	3.741	0.807	0.954	0.005	2.218
	Parenting Status	3.612	0.902	0.954	<0.001	2.092
	ECR-RD Anxiety X Parenting Status	-18.531	0.48	0.954	0.003	3.198

	ECR-RD Avoidance X Parenting Status	15.076	0.568	0.954	0.004	3.106
	Education	12.4			0.019	1.133
	Adult Age	-8.194			0.004	1.455
	Income	1.609			0.001	1.242
	Marital Status	-5.955			0.002	1.814
	Brain Volume	9.932			0.012	1.091
Total Hypothalamus Volume (caregiving)	ROFQ-D	43.384	0.023	0.048	0.108	1.287
	CEQ-D Delight	41.291	0.024	0.048	0.095	1.127
	Education	0.349			0.012	1.239
	Father Age	-52.884			0.072	1.373
	Income	36.789			0.04	1.603
	Marital Status	-23.848			0.008	1.396
	Brain Volume	44.052			0.116	1.029
	Child's Sex	-12.265			0.002	1.316
Anterior Hypothalamus Volume (caregiving)	ROFQ-D	-12.403	0.375	0.5	0.026	1.287
	CEQ-D Delight	0.836	0.95	0.95	< 0.001	1.127
	Education	10.892			0.036	1.239
	Father Age	-8.589			< 0.001	1.372
	Income	17.106			0.004	1.603
	Marital Status	-45.192			0.056	1.396
	Brain Volume	16.708			0.044	1.03
	Child's Sex	-2.472			< 0.001	1.316
Tuberal Hypothalamus Volume (caregiving)	ROFQ-D	68.202	< 0.005	<0.02	0.189	1.287
	CEQ-D Delight	28.962	0.194	0.315	0.048	1.127
	Education	-19.508			0.006	1.239
	Father Age	-44.003			0.069	1.372
	Income	5.019			0.007	1.603
	Marital Status	40.312			0.013	1.396
	Brain Volume	16.858			0.013	1.03
	Child's Sex	-17.395			0.003	1.316
Posterior Hypothalamus Volume (caregiving)	ROFQ-D	-12.481	0.294	0.315	0.019	1.287
	CEQ-D Delight	11.461	0.315	0.315	0.014	1.127
	Education	9.106			0.029	1.239
	Father Age	-0.181			0.005	1.372
	Income	14.43			0.016	1.603

Marital Status	-18.792	0.019	1.396
Brain Volume	10.454	0.025	1.03
Child's Sex	7.545	0.002	1.316

Note: q-value = significance after correction for multiple comparisons, η2 = effect size, VIF = variance inflation factor.

Supplemental Table S5

Results of ANOVA to test for between group differences in hypothalamus volume.

		F-value	p-value	η^2
Total hypothalamus	Group	0.338	0.5625	0.003
	Education	0.058		0.001
	Father Age	2.294		0.023
	Income	0.374		0.004
	Marital Status	0.376		0.004
	Brain Volume	10.442		0.107
Anterior hypothalamus	Group	0.061	0.806	0.001
	Education	1.624		0.019
	Father Age	0.379		0.004
	Income	0.017		< 0.001
	Marital Status	0.315		0.004
	Brain Volume	1.179		0.013
Tuberal hypothalamus	Group	0.081	0.777	0.001
	Education	0.322		0.004
	Father Age	0.45		0.005
	Income	0.098		0.001
	Marital Status	0.124		0.001
	Brain Volume	2.391		0.027
Posterior hypothalamus	Group	0.081	0.7761	0.001
	Education	0.06		0.001
	Father Age	0.989		0.011
	Income	0.206		0.002
	Marital Status	0.054		0.001
	Brain Volume	4.671		0.052

Note: η2 = effect size

Supplemental Table S6
Results of ANOVA of matched subgroups to test for between group differences in hypothalamus volume.

		F-value	p-value	η^2
Total hypothalamus	Group	0.748	0.3914	
	Education	2.854		
	Adult Age	4.553		
	Income	0.068		
	Marital Status	2.642		
	Brain Volume	3.766		
Anterior hypothalamus	Group	0.022	0.883	
	Education	0.507		
	Father Age	0.199		
	Income	0.350		
	Marital Status	0.469		
	Brain Volume	0.109		
Tuberal hypothalamus	Group	0.522	0.474	
	Education	0.913		
	Father Age	0.810		
	Income	0.260		
	Marital Status	2.032		
	Brain Volume	0.169		
Posterior hypothalamus	Group	0.045	0.832	
	Education	0.003		
	Father Age	1.941		
	Income	0.674		
	Marital Status	1.644		
	Brain Volume	5.369		

Note: η2 = effect size

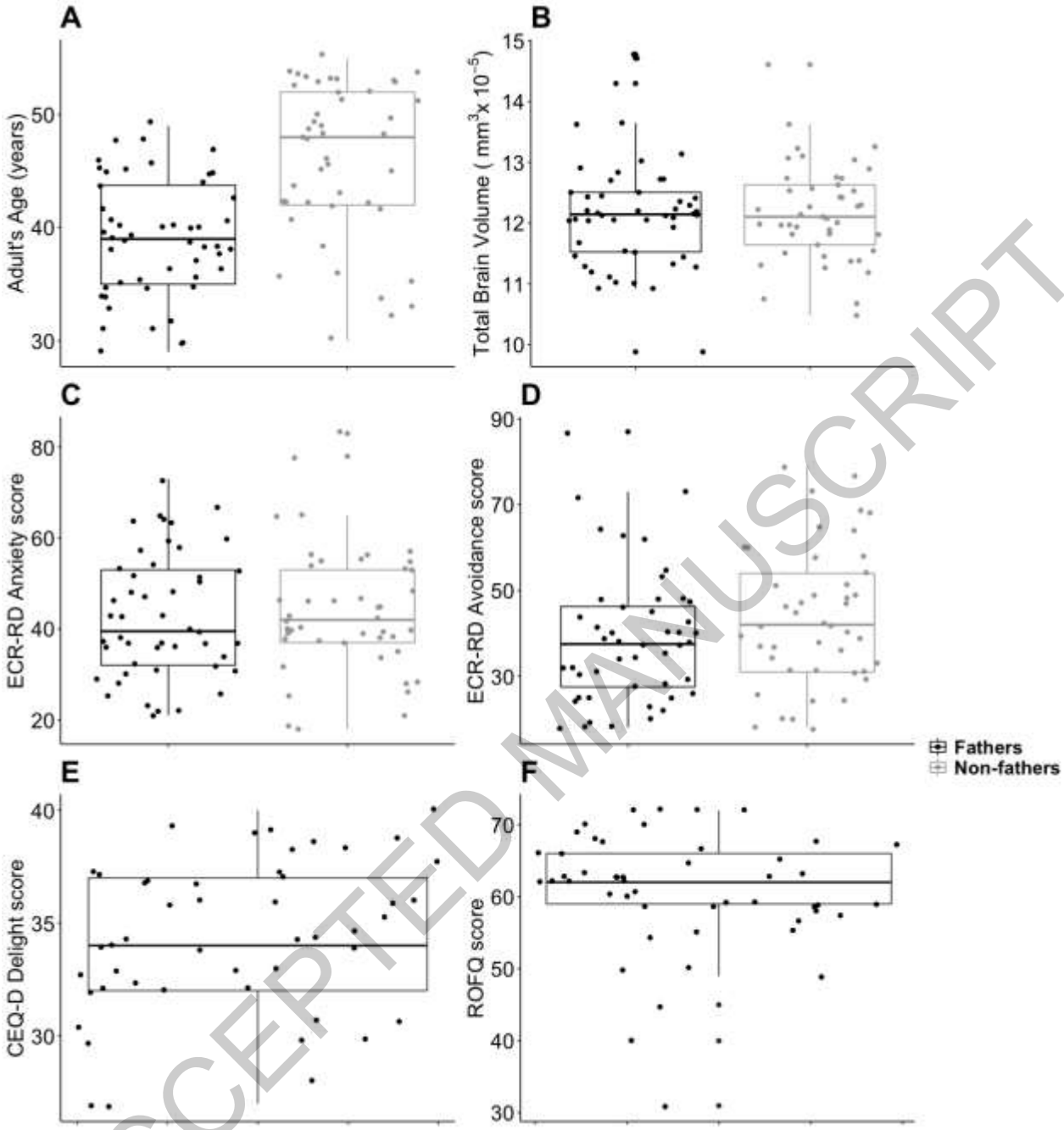
Supplemental Table S7

Results for matched subgroup regression analyses of attachment, caregiving, and hypothalamic volume.

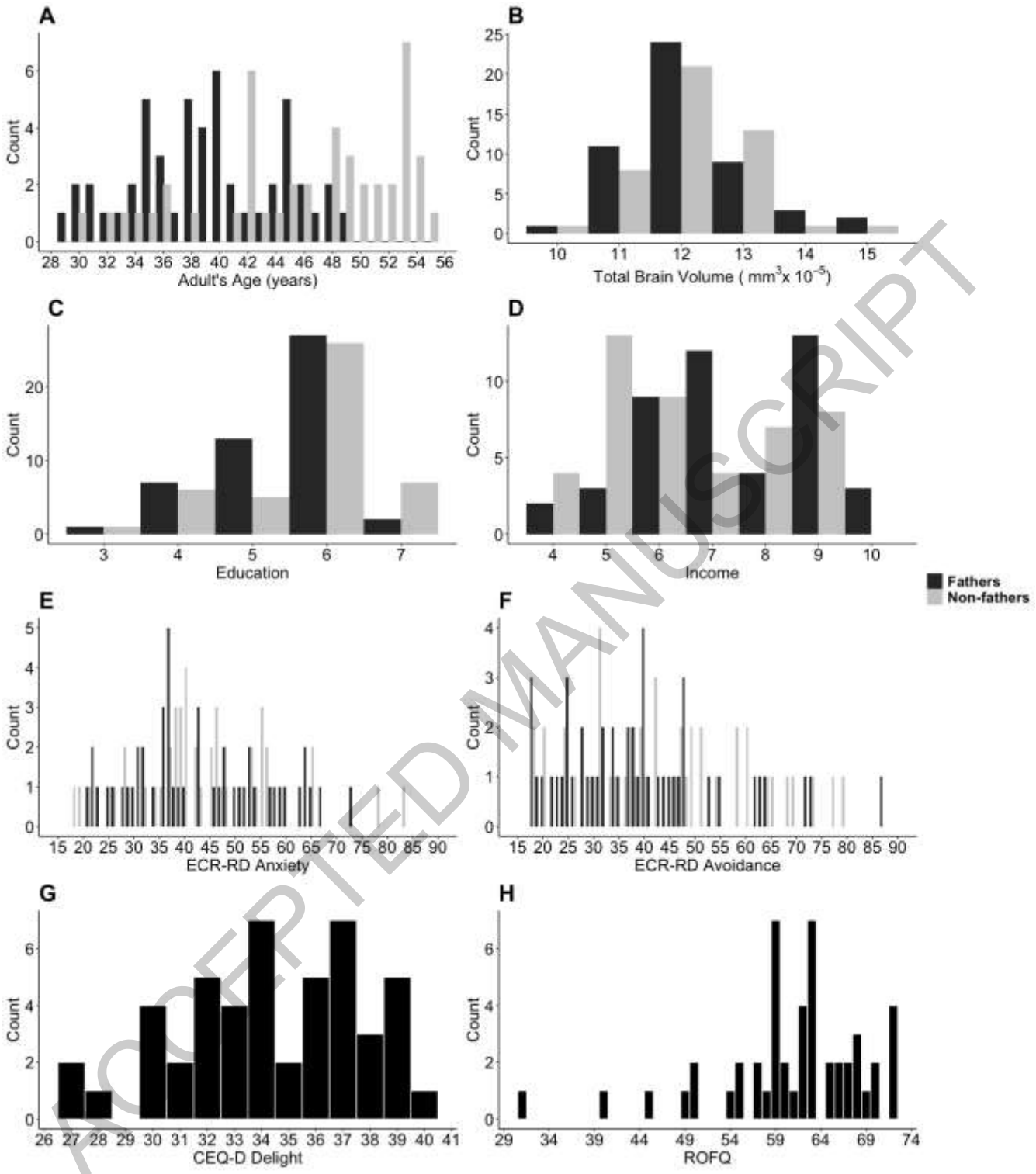
Model	Variable	β	p-value	q-value	η^2	VIF
Total Hypothalamic Volume (both groups)	ECR-RD Anxiety	-11.417	0.5774		0.002	1.304
	ECR-RD Avoidance	21.781	0.3018		0.016	1.371
	Education	21.001			0.044	1.101
	Adult Age	-44.249			0.088	1.239
	Income	7.944			0.008	1.144
	Marital Status	-18.673			0.012	1.178
	Brain Volume	33.032			0.049	1.142
Anterior Hypothalamic Volume (both groups)	ECR-RD Anxiety	10.952	0.49		0.019	1.305
	ECR-RD Avoidance	8.295	0.61		0.003	1.371
	Education	8.874			0.013	1.101
	Adult Age	-9.637			0.002	1.239
	Income	11.063			0.01	1.144
	Marital Status	-21.009			0.011	1.178
	Brain Volume	0.961			< 0.001	1.142
Total Hypothalamic Volume (Parenting Status added)	ECR-RD Anxiety	36.171	0.38		0.002	5.31
	ECR-RD Avoidance	-15.943	0.68		0.023	4.75
	Parenting Status	-68.117	0.15		0.012	1.81
	ECR-RD Anxiety X Parenting Status	-56.357	0.24		0.007	4.09
	ECR-RD Avoidance X Parenting Status	55.187	0.24		0.022	3.71
	Education	27.513			0.056	1.18
	Adult Age	-40.867			0.071	1.33
	Income	7.245			0.004	1.23
	Marital Status	-64.009			0.042	1.79
	Brain Volume	30.04			0.044	1.15
Anterior Hypothalamic Volume (Parenting Status added)	ECR-RD Anxiety	2.54	0.94		0.019	5.31
	ECR-RD Avoidance	15.46	0.62		0.003	4.75
	Parenting Status	-14.45	0.71		>0.001	1.81
	ECR-RD Anxiety X Parenting Status	12.01	0.75		0.001	4.09

ECR-RD Avoidance X Parenting Status	-7.41	0.85	0.001	3.73
Education	9.58		0.013	1.18
Adult Age	-9.71		0.002	1.33
Income	9.71		0.01	1.23
Marital Status	-28.14		0.014	1.79
Brain Volume	0.83		>0.001	1.15

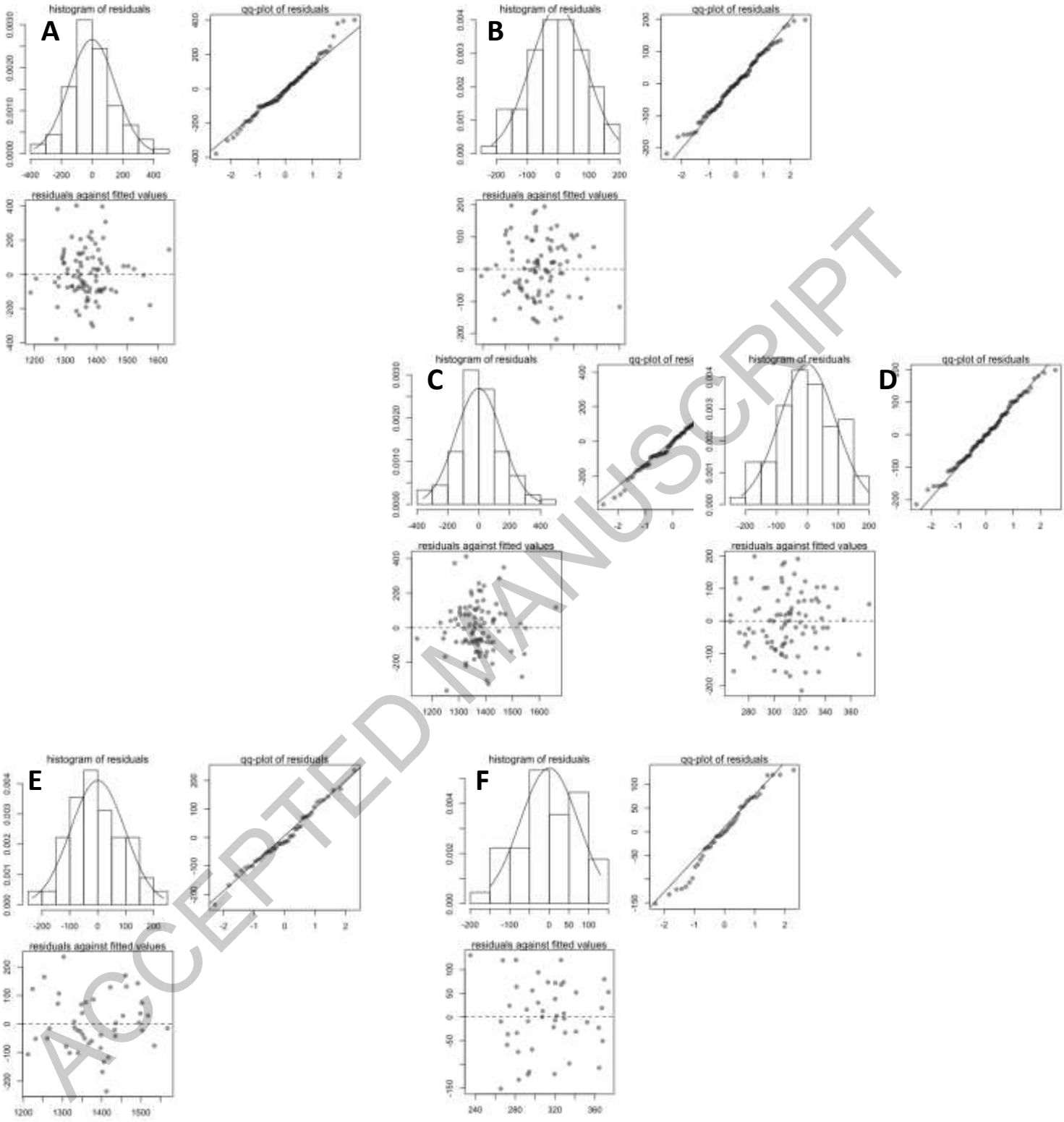
Note: q-value = significance after correction for multiple comparisons, η2 = effect size, VIF = variance inflation factor.



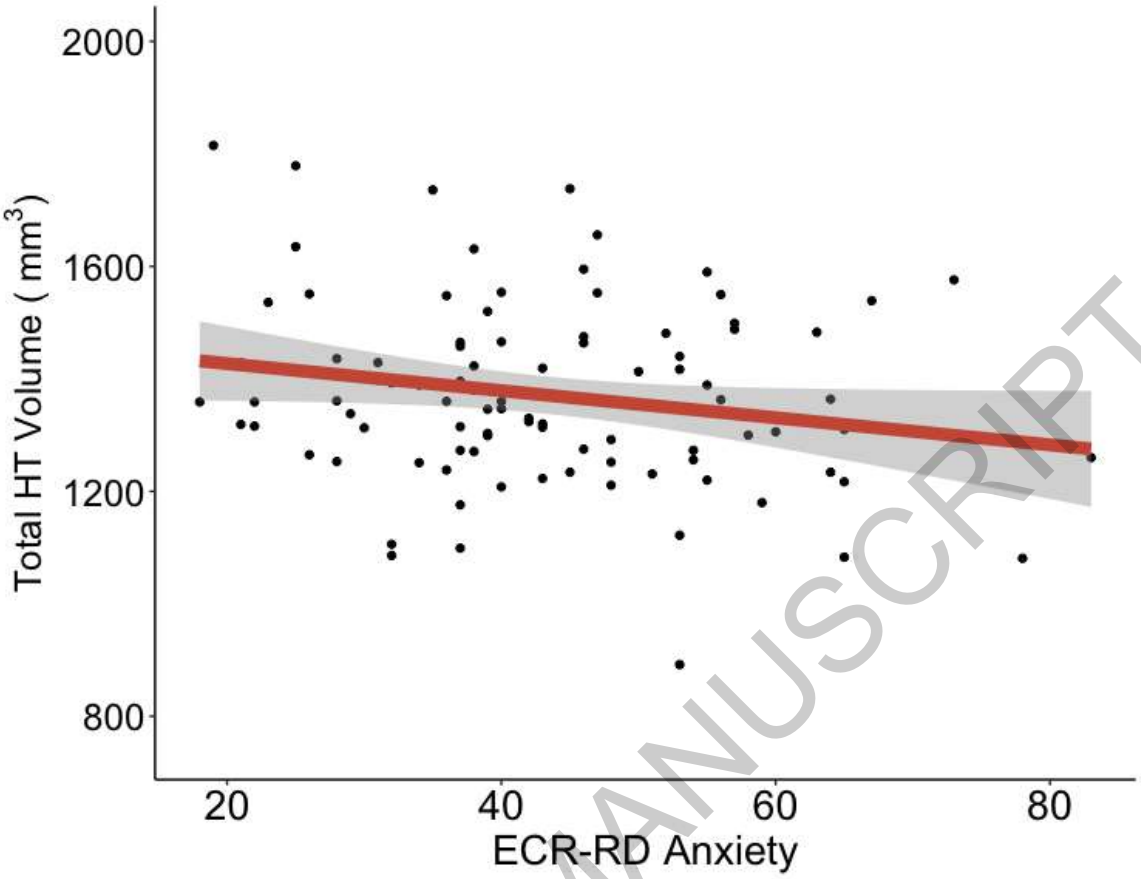
Supplemental Figure S1. Boxplots showing raw data for continuous covariates, including adult age (A), total brain volume (B), ECR-RD Anxiety (C) and Avoidance (D), CEQ-D Delight (E) and ROFQ-D (F). Data points are jittered to avoid over-plotting.



Supplemental Figure S2. Histograms showing variable distributions for covariates, adult age (A), total brain volume (B), Education (C), Income (D), ECR-RD Anxiety (E) and Avoidance (F), CEQ-D Delight (G) and ROFQ-D (H).



Supplemental Figure S3. Diagnostic plots assessing assumptions for regression models testing hypothesis 2.1 (total hypothalamus (A), anterior hypothalamus (B)), hypothesis 2.2 (total hypothalamus (C), anterior hypothalamus (D)), hypothesis 3 (total hypothalamus (E), anterior hypothalamus (F)).



Supplemental Figure S4. Non-significant negative association between adult attachment anxiety and total hypothalamus volume (q = 0.13). For our entire sample of men, we found a negative relationship between total hypothalamus volume and adult attachment anxiety scores. The red line represents the estimated association based on linear regression analysis; shaded areas are 95% CIs; dots show raw data.

Appendix A: **Protocol for Manual Segmentation of the Hypothalamus**

-DCARE-

Set-up (You should only have to take these steps once!)

1. Open the ~/.bashrc script in your home directory. If you do this in the GUI, hi CTRL+H to show hidden files.
2. Edit the ~/.bashrc script to include the following lines:

```
export SUBJECTS_DIR=/data/pt_01958/DCARE_Hypothalamus/
export PILOT_DIR=/data/pt_01958/PILOT_Hypothalamus/
export subjid=NULL
export colorfile1=/data/pt_01958/PILOT_Hypothalamus/Fiss.txt
export colorfile2=/data/pt_01958/PILOT_Hypothalamus/DCARE_Hypo.txt
alias FS='FSL FREESURFER --subjectsdir /data/pt_01958/DCARE_Hypothalamus/'
```

   These lines of code set up shortcuts to folders and files which you will use later.

3. Open a terminal window and type:

```
source ~/.bashrc
```

   This ensures that your computer knows you made changes to the script.

Open the subject in FreeView

1. Open a new terminal window
2. To open **FSL/Free**
3. **Surfer** type:

```
FS
```

4. Navigate to the desired directory

```
cd name/of/path   (or cd $PILOT_DIR)
```

5. Open the subject

```
bash load.sh <subjectID>
```

6. FreeView will open in coronal view (Figure 1.) For now, de-select the volume, “aparc+aseg.”
7. Basic navigation in FreeView:

To move through the selected volume, use the page (bild) up/down keys.

To zoom in on the image, use the scroll on the mouse.

To move the image within your field of view, press down on the scroll and move the mouse.

To change to a different view, use the buttons in the toolbar at the top (coronal, saggital, and axial views...different screen configurations.)

To draw, select the voxel edit tool, the desired volume, and the correct color from the color lookup table. Click/ hold the left mouse button to draw.

Erasing is similar to drawing. Just hold Shift + left mouse and move the cursor over the area to be erased.

Figure 1. The initial view when opening a subject in FreeView

Segment the Hypothalamus Part 1: Define the ROI

1. Using the coronal view, define the most anterior and posterior slices of the ROI (Figure 2.) This is the range of slices on which you will draw the hypothalamus ROI.
   a) Note the slice numbers in your own spreadsheet for every subject.
   b) Anterior boundary: Includes the slice where the **anterior commisure (AC)** is clearly and continuously visible
   c) Posterior boundary: Includes the most posterior parts of the **mammillary bodies (MB)** (check this by switching between coronal and saggital view)
2. Select the voxel edit tool and erase FreeSurfer's automated output for the basal forebrain on the range of slices you have identified in step 1 (Figure 3.)

Use volume "hypo_rois.nii."

To erase more quickly, increase the brush size.

3. Identify boundaries between the 3 sections of the hypothalamus (**anterior, tuberal, posterior; Figure 4**.) You can mark the sections slice by slice with a single dot of color if it's helpful. Use volume "hypo_rois.nii."
   a) **Anterior** hypothalamus: includes all slices where **AC** is still visible. Sometimes this is only 1 or 2 slices.
   b) **Tuberal** hypothalamus:
      1. Anterior boundary: Includes the first slice where **AC** is no longer the most prominent WM structure (as opposed to the Fornix). In other words, use the "two-out-of-three rule." If two of the three sections of AC (left, right, and center) are still visible, the slice is anterior. If it's fewer than two sections, the slice is tuberal.
      2. Posterior boundary: includes all slices before the **MB** appear
   c) **Posterior** hypothalamus: Includes full extent of the **MB**. If the MB have begun one one side of the brain, the whole slice is considered posterior. Flip between coronal and saggital view to determine the start of the MB.
4. Draw the **Hypothalamic Fissure in saggital view.** This will define the **superior border** of the tuberal and posterior hypothalamus (Figure 5.) Use the volume "hypo_fiss.nii"
   a) Draw the left and right fissure separately, on the most **lateral** slices where the fissure itself is still visible. Look for the "shadow" under the **thalamus**.
   b) The fissure cups the **thalamus**
   c) Inferior boundary is the end of the **Cerebral Spinal Fluid (CSF)**
   d) When viewed coronally, the colors for the fissure should appear on the edges of the third ventricle.
5. Segment the **Third Ventricle** using FreeSurfer's automated output volume "aparc+aseg" as a guide. Draw the ventricle manually on the "hypo_rois.nii" volume. Draw the ventricle on all slices in the range you identified in step 1. As you complete the ROI you may edit the boundaries of the ventricle slightly.

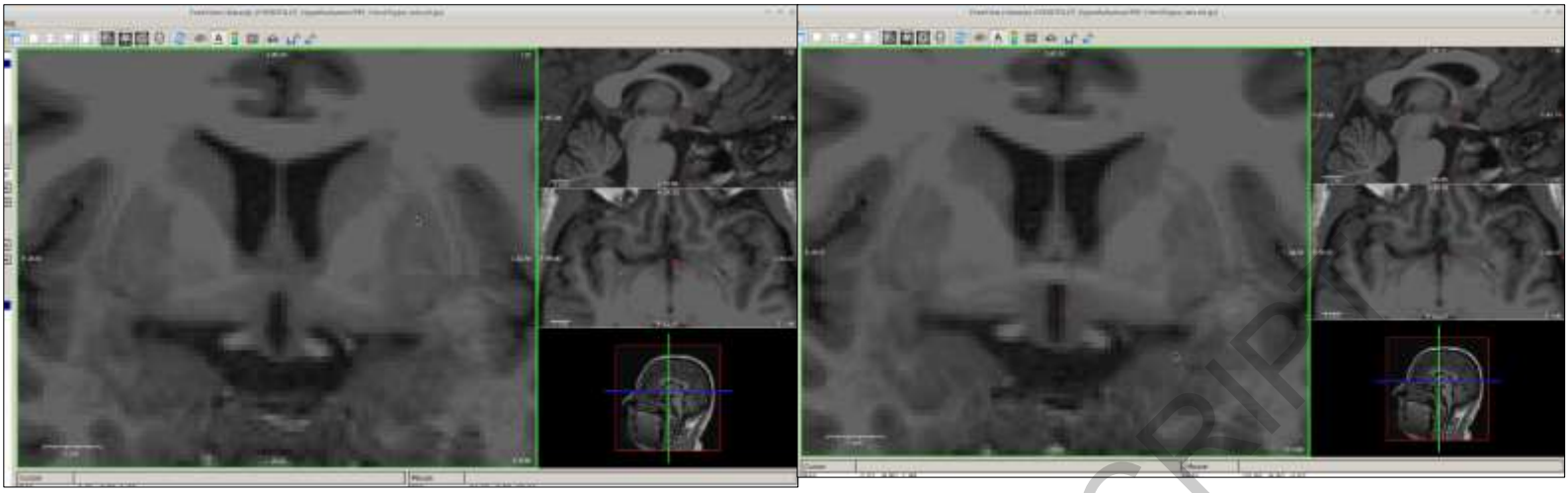


Figure 2a. From Left: 1) The AC is emerging but not yet continuously visible. 2) Moving one slice posterior, the AC is now continuously visible. This would be the first Anterior slice.

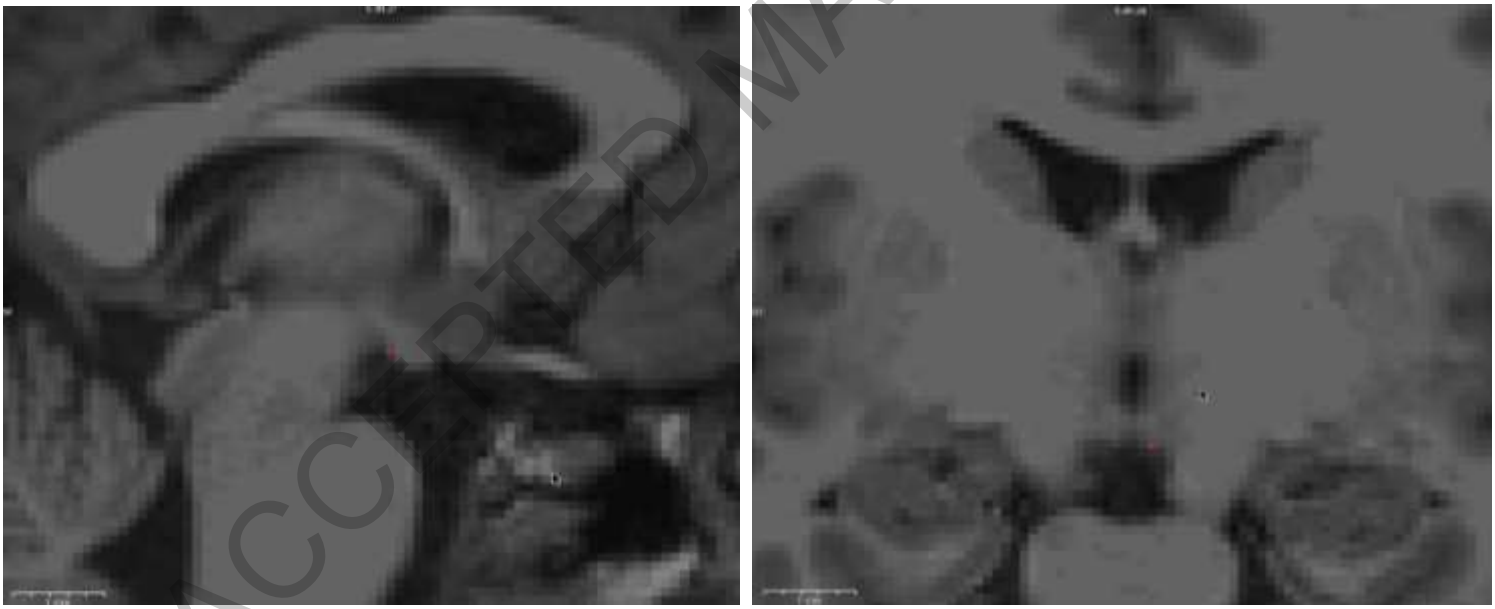
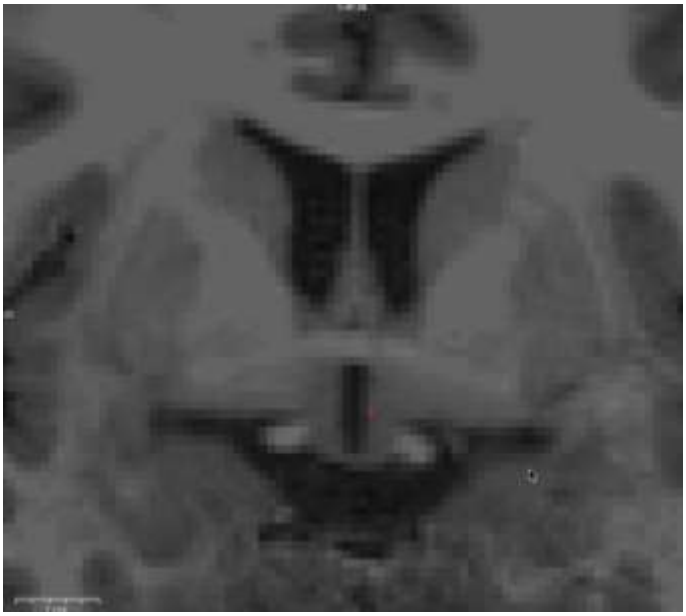


Figure 2b. Locating the most Posterior slice. From Left: 1) In saggital view, put the crosshairs on the last voxel of the MB. 2) Without moving the crosshairs, return to coronal view. This is the last Posterior slice.

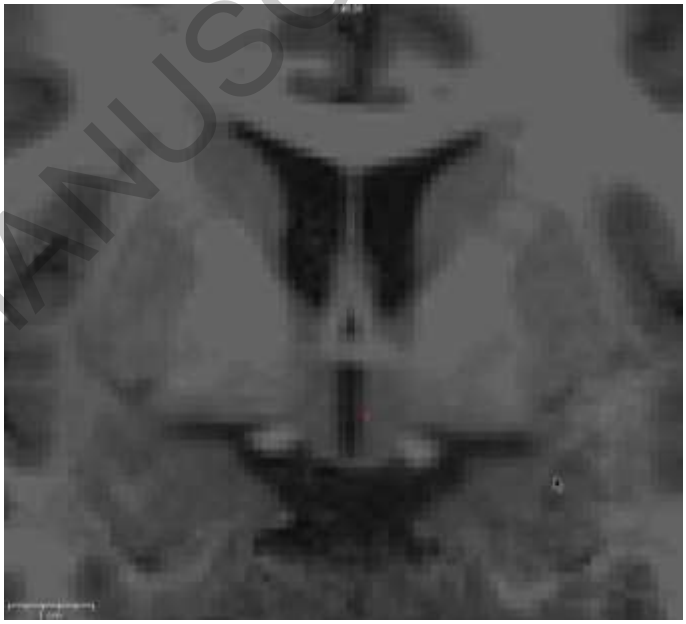


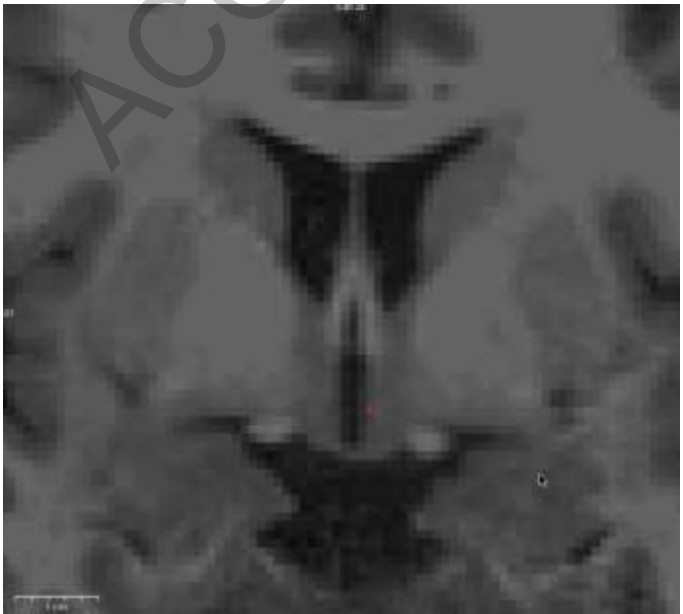
1) First Anterior slice, AC is continuously visible.

2) A second Anterior slice where the AC is beginning to fade into the Fornix but is still visible.

3) First tuberal slice, AC is no longer visible and columns of the Fornix are clearly present.

Figure 4a: Transition from Anterior to Tuberal Hypothalamus. From top:





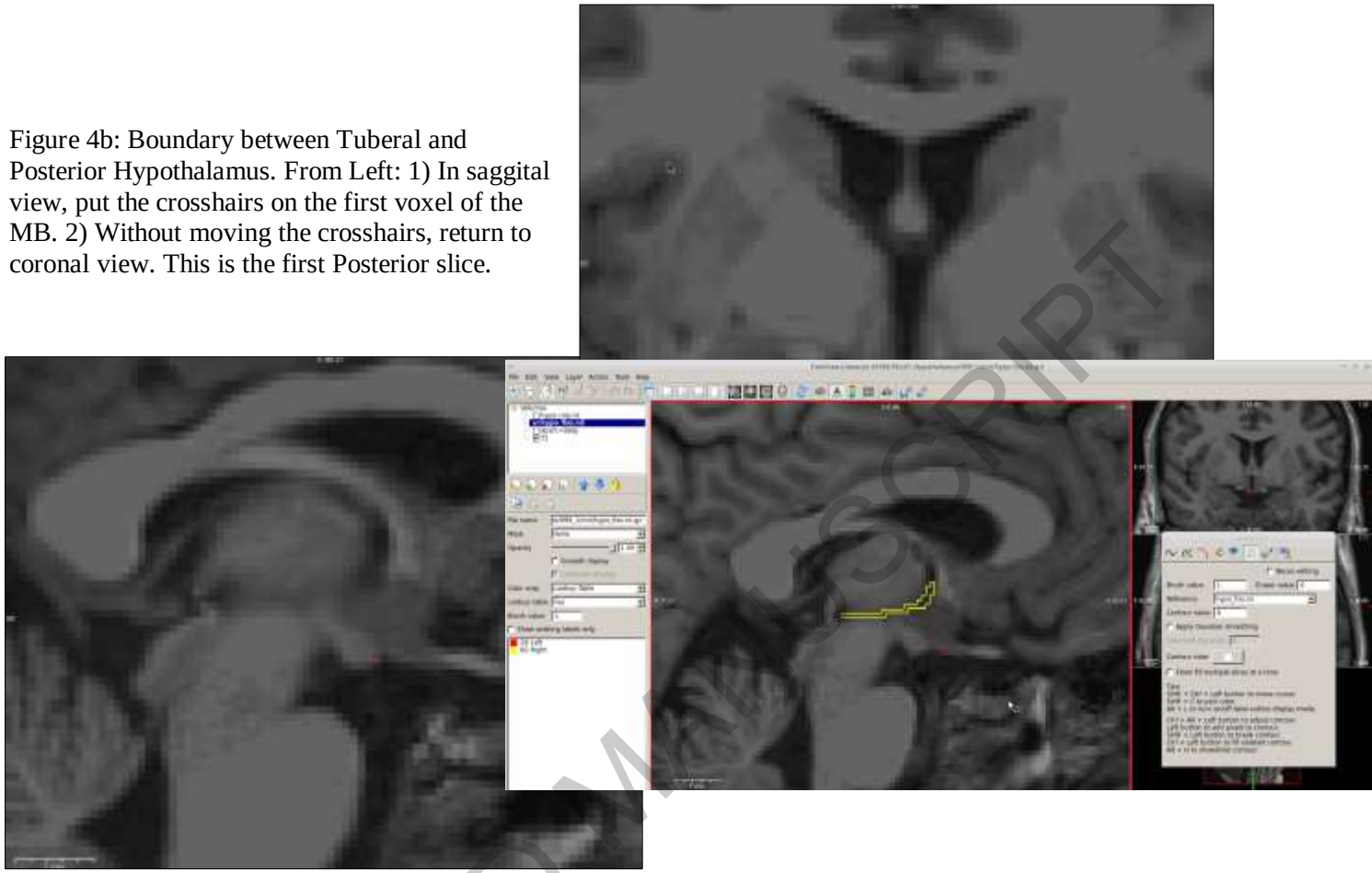


Figure 4b: Boundary between Tuberal and Posterior Hypothalamus. From Left: 1) In saggital view, put the crosshairs on the first voxel of the MB. 2) Without moving the crosshairs, return to coronal view. This is the first Posterior slice.

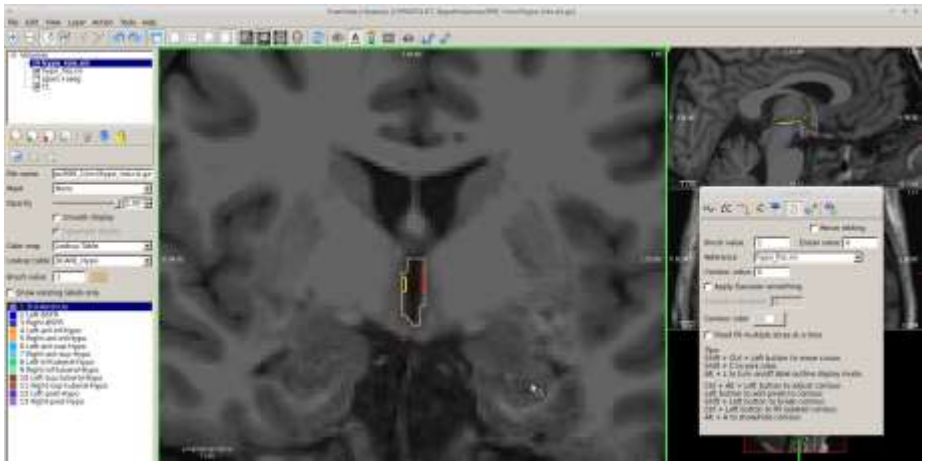


Figure 5. Segmenting the Hypothalamic Fissure.

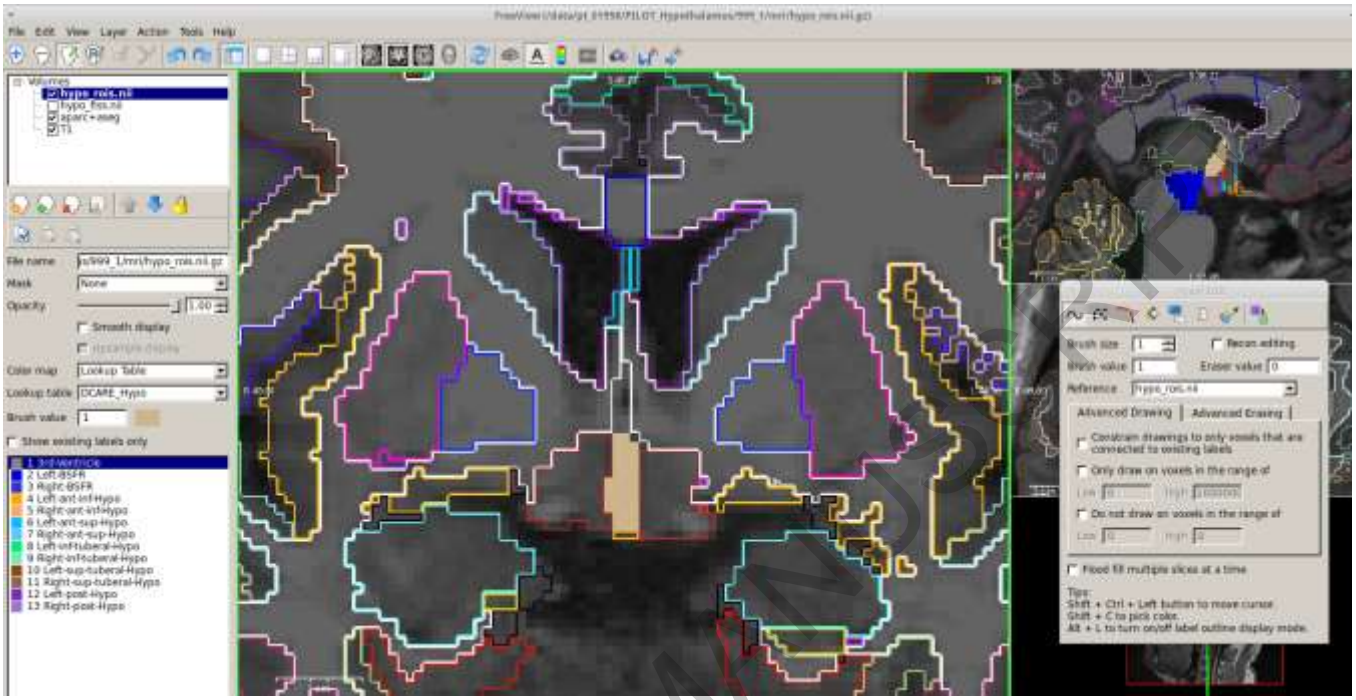


Figure 6. Segmenting the Third ventricle using aparc+aseg overlay as a guide.

Segment the Hypothalamus Part 2: Complete the ROI

1. Fill the **anterior hypothalamus** using specified colors for left, right, superior, and inferior anterior hypothalamus. The defining boundaries are:
    a. ***Superior***: **Anterior Commisure**
    b. ***Lower Bound of Superior Segment***: Floor of the **Basal Forebrain.** In other words, bring the superior section down to the row above the darkest voxels.
    c. ***Upper Bound of Inferior Segment***: Floor of the **Basal Forebrain.** In other words, bring the inferior section up to the height of the darkest voxels.
    d. ***Inferior***: Superior horizontal line of the **Optic Chiasm** or (after separation of the chiasm into the optic tracts) inferior horizontal line of the **optic tracts**
    e. ***Medial***: Third ventricle
    f. ***Lateral***: Vertical line of the Optic tracts or Optic Chiasm
2. Fill the **tuberal hypothalamus** using specified colors for left, right, superior, and inferior tuberal hypothalamus. The defining boundaries are:
    a. ***Superior***: Horizontal line of the **Fornix** or **Hypothalamic Fissure**
    b. ***Lower bound of Superior Segment***: Floor of the **Basal Forebrain.** In other words, bring the superior section down to the row above the darkest voxels.
    c. ***Upper bound of Inferior Segment***: Floor of the **Basal Forebrain.** In other words, bring the inferior section up to the height of the darkest voxels.
    d. ***Inferior***: Inferior horizontal line of the optic tracts or (after separation of the **infundibular stalk**) the CSF.
    e. ***Medial***: **Third ventricle**
    f. ***Lateral***: Grey/ white matter boundary from manual inspection with FreeView contour tool (see next section.) Be sure to include just enough around the optic tracts to include the **supra-optic and infundibular nuclei**.
3. Fill the **posterior hypothalamus** using specified colors for left and right posterior hypothalamus.
    a. ***Superior***: Horizontal line of the **Hypothalamic Fissure**
    b. ***Lower bound of Superior Segment***: N/A
    c. ***Upper bound of Inferior Segment***: N/A
    d. ***Inferior***: Lower extent of the **Mammilary Bodies**
    e. ***Medial***: **Third ventricle**
    f. ***Lateral***: **Grey/ white matter boundary** from manual inspection with FreeView contour tool (see next section.)

FreeView Contour Tool

The Freeview contour tool defines a line between gray and white matter based on intensity value differences per voxel. You may choose a certain intensity value (e.g. 100) as a threshold for how conservative the contour defines the gray/white matter borders. Check the border yourself. In case the shape Freesurfer provides is not accurate, edit the output manually. If there is considerable noise in the T1, you may choose to smooth the border by checking the option “Apply Gaussian smoothing”(SD=1).

To use:

1. Select the contour tool
2. Choose T1 as reference volume
3. Ctrl+Alt+left mouse button, then move mouse to adjust contour value

Appendix B: **Description of training and reliability phases for hypothalamus segmentation protocol**

Inter-rater reliability (IRR) was achieved in three phases: training, reliability, and data collection. In the training phase, raters learned about hypothalamic anatomy and familiarized themselves with the FreeSurfer and Freeview software. To complete this phase, raters were required to complete a practice set of five brains drawn from the D-CARE sample. Communication between the raters during the training phase was highly encouraged as to facilitate the raters coming to consensus on segmentation decisions for the set of practice brains. The training phase lasted approximately 20 hours: 10 hours spent in didactic training and receiving hands-on assistance, 10 hours spent working semi-independently to segment the five practice brains.

In the reliability phase, each rater independently segmented a set of 10 brains drawn from the D-CARE sample. None of these 10 brains was used in the training phase. Each set of 10 was identical between raters. To complete the reliability phase, the raters needed to achieve excellent IRR for the three sub-regions and total hypothalamus. Raters achieved a high degree of IRR for anterior (ICC=.855), tuberal (ICC=.888), posterior (ICC=.781), and total (ICC=.809) hypothalamus. The reliability phase was completed over approximately 40 hours.

In the data collection phase, all three raters segmented the first 28 available brains for the D-CARE sample (Long, 2019). To increase efficiency, the remaining 67 hypothalami were segmented by two of the three original raters.