Hypothalamus volume and fatherhood:

similarity across men and interindividual differences among dads

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Abstract

Most studies on mammalian caregiving and attachment have focused on the mother-child relationship, particularly in humans. Yet, recent re-considerations of attachment theory and changing societal roles of male caregivers have highlighted the necessity for research with fathers. In this pre-registered study (https://aspredicted.org/5uj5y.pdf), we examined the structure of the hypothalamus – an important subcortical brain area for caregiving and attachment behavior – in a sample of N=95 fathering (child age 5-6 years) and non-fathering men. To do so, we used a recently developed technique to accurately and efficiently identify the human hypothalamus in 3T MRI and calculate hypothalamus volume. Furthermore, we employed several self-report measures to assess interindividual differences in attachment style across all men, and caregiving specifically in fathers. While we found no difference in hypothalamus volume between fathers and non-fathers, fathers' interindividual variation in caregiving style was related to hypothalamus volume. Specifically, we observed that fathers who held greater belief in the importance of their role as a father and reported more enjoyment of interacting with their child had greater total hypothalamus volume. This finding suggests that there is interindividual variability in the association between brain structure and caregiving style in fathers, warranting further research.

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

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1 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 importance and neurobiological effects of paternal caregiving (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 research 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. Overall, research on fathers brings the fields of caregiving, attachment, human development, and social neuroscience closer to an understanding of the underlying neurobehavioral systems' function across caregiver types.

The establishment and maintenance of attachment relationships through activation of the attachment behavioral system lies at the core of social interactions and learning. Attachment behavior was first described by John Bowlby, Mary Ainsworth, and Silvia Bell and is 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). Related to the attachment system is a complementary caregiving system (Canterberry & Gillath, 2012; Mikulincer & Shaver, 2007). While the attachment system's purpose is to seek support from an attachment figure, the purpose of the caregiving system is to provide that support. Accordingly, the attachment system in one individual is activated as a distress response to an internally or externally derived event that is appraised as a threat, and the caregiving system in a second individual is activated to help alleviate the first individual's distress through emotion and allostasis 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 systems. We recently proposed a functional neuro-anatomical model of human attachment (*NAMA*) based on associations between interindividual differences in attachment and structural as well

as 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 or modules: approach, aversion, emotion regulation, and mental state representation. Here, we focus on the module for approach and reward, which is thought to include (amongst others) the ventral tegmental area, substantia nigra, ventral striatum, ventromedial orbitofrontal cortex, pituitary, and the hypothalamus (see also Feldman, 2017; Fisher et al., 2006; Insel & Young, 2001; Swain et al., 2014). The prototypical role of the approach module 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. In doing so, activation of this module increases the likelihood that two individuals will seek physical proximity to one another, particularly if one of them is in need. It is theorized that interindividual variation in approach module function may at least partially be attributable to interindividual differences in caregiving and attachment behavior (Long et al., 2020; Vrtička, 2017; Vrtička & Vuilleumier, 2012).

Interindividual differences in attachment are often described in terms of the three organized or resolved attachment orientations: secure, anxious, and avoidant (Fraley et al., 2000; Shaver & Hazan, 1987). Similarly, attachment-informed theories of caregiving suggest that caregiving behavior tends to align with one of the three attachment orientations. In other words, caregivers tend to align their support-giving behavior with a particular social approach strategy (Collins et al., 2010; Mikulincer & Shaver, 2007). Secure caregiving is characterized by empathic concern with the caregiver employing proximity seeking to reduce another person's suffering (Collins et al., 2010). In contrast, secondary caregiving strategies are thought to be deactivating (avoidant) or hyper-activating (anxious) in nature and motivated by the caregiver's desire to alleviate their own personal distress - caused by the negative emotional state of a significant other (Collins et al., 2010) . An avoidant caregiver might decrease their social approach behavior to evade a stressful stimulus and correspondingly, *NAMA* predicts a decrease in approach system activation for avoidant individuals in caregiving contexts. On the other hand, 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). Anxious caregiving may also be accompanied by commensurate heightened activation in the approach module.

Here we focus specifically on the hypothalamus, a sub-cortical brain structure that plays a key role in social approach and has been implicated as a core neural structure underlying both parental and romantic love (Bartels & Zeki, 2004). The human hypothalamus is known to be sexually dimorphic (Swaab et al., 2003) and to vary with both gender and sexual orientation (Swaab et al., 1992).Through its interactions with the pituitary gland the hypothalamus serves as a link between central nervous and endocrine systems (Ramón y Cajal, 1909). It contains the supraoptic and paraventricular nuclei, which produce and release oxytocin (du Vigneaud et al., 1954; Scharrer, 1990), an affiliative hormone implicated in the development of attachment bonds (Carter, 2014; Carter et al., 2006; Fisher et al., 2006; Insel & Young, 2001). Additionally, these nuclei are involved in the production and release of corticotropin releasing hormone (CRH; Carter et al., 2006; Dudás, 2013), a central messaging hormone in the physiological stress response. Interpreted in the context of *NAMA*, the oxytocin and HPA stress systems of the hypothalamus are theorized to have a modulatory effect on the approach module, which may ultimately relate to interindividual differences in caregiving and attachment behavior in humans. Recent developments in structural magnetic resonance imaging (MRI) methods have now made it possible to identify the hypothalamus, its subregions (anterior, tuberal, and posterior), and estimate its volume (Makris et al., 2013) (**Figure 1 A and B**). A robust body of literature from non-human mammals indicates that the hypothalamus plays a key role in pair-bonding and parenting behavior. As with humans, research on the hypothalamus in the context of caregiving and attachment in rodents has largely focused on mothers and their offspring and cohesively suggests that structural, functional, and hormonal changes all occur in relation to parenting. 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 alongside other physiological changes associated with motherhood such as lactation (Theodosis & Poulain, 1984).

While the literature on males is sparser, the importance of the hypothalamus and its associated hormones in caregiving and attachment related contexts remains apparent. One study found that introducing moderate (but not high) amounts of CRH in the hypothalamus facilitated pair-bonding in male prairie voles (Carter et al., 2006). Moreover, across several species of bi-parental rodents (i.e. species in which both mother and father contribute to offspring care), hormonal and cellular changes were observed in the paternal hypothalamus in response to parenthood (see Saltzman & Ziegler, 2014 for a summary). In paternal meadow voles, this included an increase in oxytocin binding in subregions of the anterior hypothalamus as compared to non-fathering males (Parker et al., 2001).

Regarding hypothalamus structure and function in humans, MRI research has revealed an important role of the hypothalamus in social approach and attachment- and caregiving-related contexts. For example, both mothers (Kim et al., 2010) and fathers (Kim et al., 2014) showed increases in hypothalamus volume in the first few months after their first child was born. Additionally, securely attached mothers were found to have greater midbrain volume, including the hypothalamus, than insecurely attached mothers (Kim et al., 2010). In an fMRI study of mothers and their infants, securely as opposed to avoidantly attached mothers showed greater activation in the hypothalamus when viewing images of their own infant and such pattern was indirectly related to peripheral oxytocin concentration during free play with the own infant (Strathearn et al., 2009). To our knowledge, only one study to-date has specifically examined hypothalamus structure as it relates to interindividual differences within the approach module in healthy human men. This study observed that lower hypothalamus volume was predictive of low pro-sociality (Tost et al., 2010). Such findings can be interpreted as an indication that avoidant attachment- and caregiving-like qualities might be negatively related to hypothalamus volume in men. However, it is still unknown whether there is an association between interindividual differences in adult caregiving and/or attachment and hypothalamus volume in fathers.

1.5 Present study

Using a novel approach 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 differences in hypothalamic volume to interindividual variation in romantic attachment across all men and in fathers alone, we characterized the relationship between hypothalamus volume and interindividual variation in caregiving style. All hypotheses were preregistered at https://aspredicted.org/5uj5y.pdf.

First, we predicted that fathers would have greater anterior hypothalamus volume than non-fathering men. Functions of the tuberal and posterior hypothalamus identified to date are not directly related to the experience of becoming a father. Nonetheless, since the function of the human hypothalamus in caregiving and attachment is generally still poorly understood, we additionally tested for volumetric differences between fathers and non-fathers in these remaining sub-regions and the total hypothalamus in a set of exploratory analyses. Next, we hypothesized that there would be a positive relationship between self-reported romantic attachment security and anterior hypothalamus volume for both fathering and non-fathering men. We further predicted that the relationship between romantic attachment and anterior hypothalamus volume would be moderated by parenting status (i.e. being a father). Lastly we hypothesized that there would be a positive relationship between fathers' self-reported perception of their role as a parent and anterior hypothalamus volume, and that there would be a positive relationship between self-reported enjoyment of interacting with the own child and anterior hypothalamus volume. For all questions, we also performed pre-registered analyses with total hypothalamus volume without directional hypotheses.

2 Methods

2.1 Participants

The data reported here was acquired as part of the D-CARE study, an investigation of the behavioral, biological, and brain substrates of paternal attachment and caregiving 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 data analyzed here also include a control sample of male participants aged 23-55 with no children whose MRI and questionnaire data was previously collected during the pre-treatment visit of an independent longitudinal study conducted at the MPI-CBS (the ReSource Project; Singer et al., 2016). 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 analyses.

2.2 Questionnaires

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

2.2.1 Attachment

To measure self-reported adult romantic attachment, 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 romantic attachment 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.

2.2.2 Caregiving styles

We measured fathers' attitudes toward caregiving with a German version of the Caregiving Experiences Questionnaire (CEQ-D; Nguyen et al., in preparation; original English version: Brennan et al., 2013). The CEQ-D comprises 40 items rated on a 5-point Likert scale (1=not at all characteristic; 5=very characteristic). The present analyses used the Delight subscale of the CEQ-D. This subscale was constructed to measure the caregiver's enjoyment of the child with higher scores indicating a greater enjoyment of time spent with their own child.

We furthermore utilized the Role of the Father Questionnaire (ROFQ; 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).

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**.

2.3 MR Image Acquisition, Pre-processing, and Hypothalamus Delineation

This cross-sectional study included a single anatomical MRI scan per participant. For both fathers and non-fathering men (controls), 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**).

2.4 Significance 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.

For our first hypothesis, we deemed it necessary to diverge from the preregistered simple t-test analysis of group-level differences in hypothalamus volume. To account for the significant between-group differences in age, average household income, and marital status, we instead performed an analysis of covariance (ANCOVA) that allowed us to control for these covariates. All other hypotheses were addressed by means of multiple regression analyses in line with the preregistration.

For each hypothesis, two separate analyses were conducted for the outcome variables anterior and total hypothalamus volume. Participants' age, education, average household income, marital status, and total brain volume were used as control variables in all analyses. For our second set of hypotheses, ECR-RD scales anxiety and avoidance were used as predictors, either as main effect or with additional interaction terms for parenting status and anxiety and avoidance scores, respectively. For our third

hypothesis, ROFQ-D and CEQ-D Delight scores were used as additional predictors and analyses were furthermore controlled for biological child sex.

3 Results

We found no difference in total ($F(1,84) = 0.34$, $p = 0.56$, $\eta^2 = 0.003$) or regional hypothalamus volume (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: $F(1,84) = .08$, $p = .78$, $\eta^2 = 0.001$) between fathers and non-fathers when controlling for education, age, average household income, marital status, and total brain volume (**Figure 2 and Supplemental Table S4**).

In two steps we then probed whether 1) attachment orientation 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 S5 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 S5**). Finally, 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 romantic attachment and parenting status predicting total hypothalamus volume (**Supplemental Table S5**).

Regarding our caregiving measures in fathers, we found several relationships with total hypothalamus volume that remained significant after correction for multiple comparisons. Both ROFQ-D ($\beta = 43.384$, $p = 0.023$, $q = 0.047$, $\eta^2 = 0.108$) and CEQ-D Delight ($\beta = 41.291$, $p = 0.024$, $q = 0.047$, $\eta^2 = 0.095$; **Supplemental Table S5 and Figure 3**) 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 one standard deviation increase in ROFQ-D score, there was a 3.2% (43.384 mm^3) increase in father's total hypothalamus volume, and for every one standard deviation 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 the findings. Again, we found no notable relationships between our predictors and anterior hypothalamus volume in fathers.

4 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. This study characterized hypothalamus volume in a sample of healthy adult men and found no significant differences between those with and without children. Additionally, we tested relationships between hypothalamus volume and interindividual variation in romantic attachment (anxiety and avoidance) in all men and found a non-significant negative relationship between total hypothalamus volume and attachment anxiety. Lastly, and most interestingly, we tested the relationship between hypothalamus volume and interindividual variation in caregiving style for fathers only. Here, we found that total hypothalamus volume was significantly positively related with both enjoyment of interacting with the own child and beliefs about the importance of a father's role. To our knowledge, this is the first study to characterize and compare hypothalamus volume between men with and without children and to relate hypothalamus volume to fathers' caregiving style. Each finding and relevant caveats are discussed separately below.

4.1 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. Our null finding does not preclude the presence of functional differences in the hypothalamus between fathers and non-fathers. However, it does suggest that if anatomical changes in the paternal hypothalamus occur, they do so on a finer scale than what we were able to observe via volumetric MRI analysis of the hypothalamus in fathers of 5-to-6 year old children in our sample.

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. Previous results from studies of both humans and rodent models present a heterogeneous picture; neuroimaging studies of human fathers during the peripartum period have shown mixed results for paternal plasticity. Kim and colleagues found that paternal midbrain volume increased over the first 4 months of being a father (2014). On the other hand, Hoekzema and colleagues observed that the paternal cortex did not show structural plasticity before and after becoming a father (2017), although this 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) altered corticosterone function and HPA-axis reactivity (Harris & Saltzman, 2013), and increases in oxytocin binding (Parker et al., 2001). Taken together, these studies suggest regionally heterogeneous neural plasticity in the peripartum period along with hormone-specific endocrine changes for mammalian fathers. Future research of father-specific neural signatures would benefit from longitudinal designs and combined measurement of both neural and endocrine measures.

4.2 Hypothalamus Volume and Romantic Attachment in Men

Our findings did not reveal any significant relationship between romantic attachment 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 comparison ($q = .13$). These findings only partially confirmed our hypotheses as we predicted a) a positive relation between secure romantic attachment and hypothalamus volume in men in general, and b) an association between romantic 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 social 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 midbrain 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 romantic attachment security in men may be generally 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 for our first partial hypothesis, and specifically so for romantic attachment anxiety (but not avoidance): in men, regardless of parenting status, total hypothalamus volume tended to be decreased as a function of the degree of romantic attachment anxiety. As this study is the first that reports an association between romantic 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.

What is regarding the absence of an interaction between romantic attachment, parenting status, and hypothalamus volume in the present study, a point of nuance is provided by recent research on attachment suggesting that attachment can be context dependent. For example, an individual's behavior may be characterized by secure attachment tendencies when interacting with a specific other person but reflect more anxious attachment tendencies when interacting with another person. 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.

4.3 Hypothalamus Volume and Caregiving Style in Fathers

Regarding our models of caregiving style and hypothalamus volume in fathers, we found that a greater belief held by the father that his role is important to his child's development (as measured by the ROFQ-D) was associated with larger total hypothalamus volume. Additionally, greater enjoyment of interacting with the child (as measured by the CEQ-D Delight subscale) was associated with larger total hypothalamus volume. These novel findings in fathers are congruent with similar observations from studies with mothers that indicated greater maternal sensitivity to be related to larger midbrain volume, including the hypothalamus (Kim et al., 2010). While our findings in fathers are congruent with research in mothers, we note both a difference in developmental timing (perinatal period versus 5 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 an 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 style) 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 were linked to differences in hypothalamus function, (Hatton, 1997) lending credibility to the idea that structural differences detectable via MRI (such as volume) may underly functional changes, which, in turn, could promote differential behavioral phenotypes in constructs such as caregiving style.

4.4 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). However, all analyses were controlled for age, education, income, marital status, and total brain volume to amend any influence of these demographic differences. 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. Moreover, the study is cross-sectional and does not account for changes that may occur in attachment, caregiving, and hypothalamus volume throughout the lifespan.

4.5 Conclusions

We characterized and compared hypothalamus volume between men with and without children. While hypothalamus volume did not differ between groups, our study uncovered a positive relationship between hypothalamus volume, caregiving enjoyment, and belief about the importance of the paternal role. The present work supports the notion that the hypothalamus is an important structure underlying caregiving behavior in human men and that hypothalamus structure may associate with interindividual differences in caregiving beliefs and behavior.

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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.

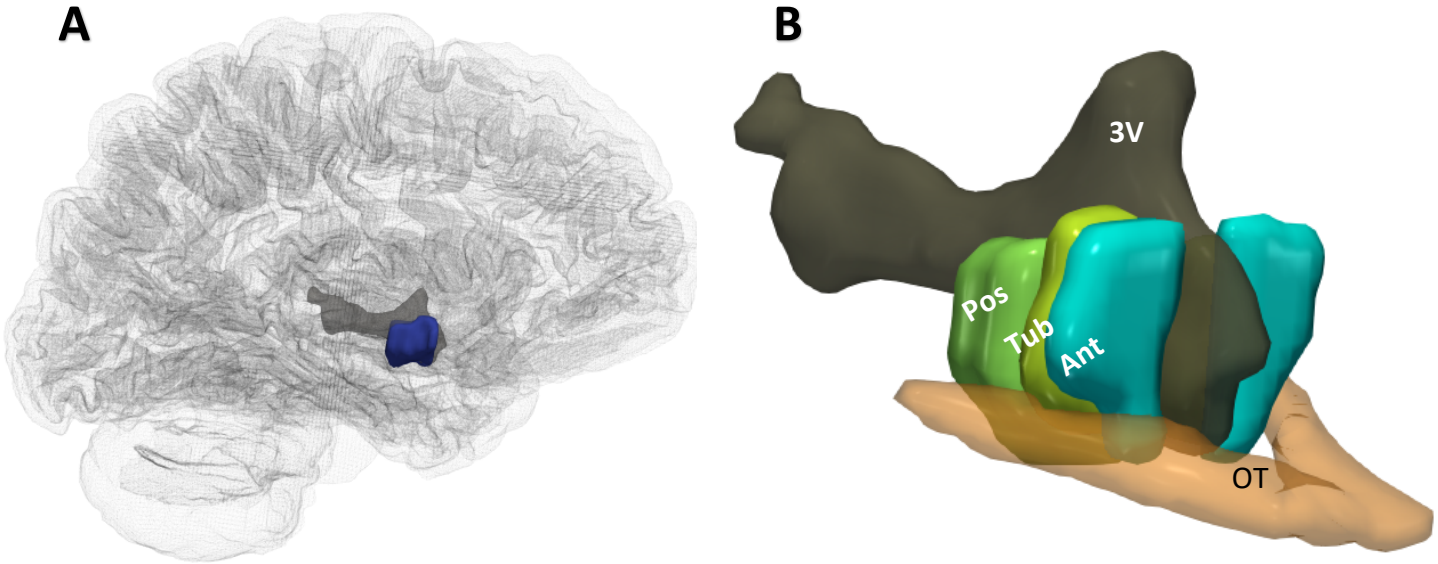


Figure 1. The human hypothalamus (panel A, blue) is located within the basal forebrain. It is situated medially of the optic tracts (panel B; OT) and lateral of the third ventricle (3V) with three rostral to caudal sub-regions: anterior (Ant), tuberal (Tub), and posterior (Pos). Images were created using 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.

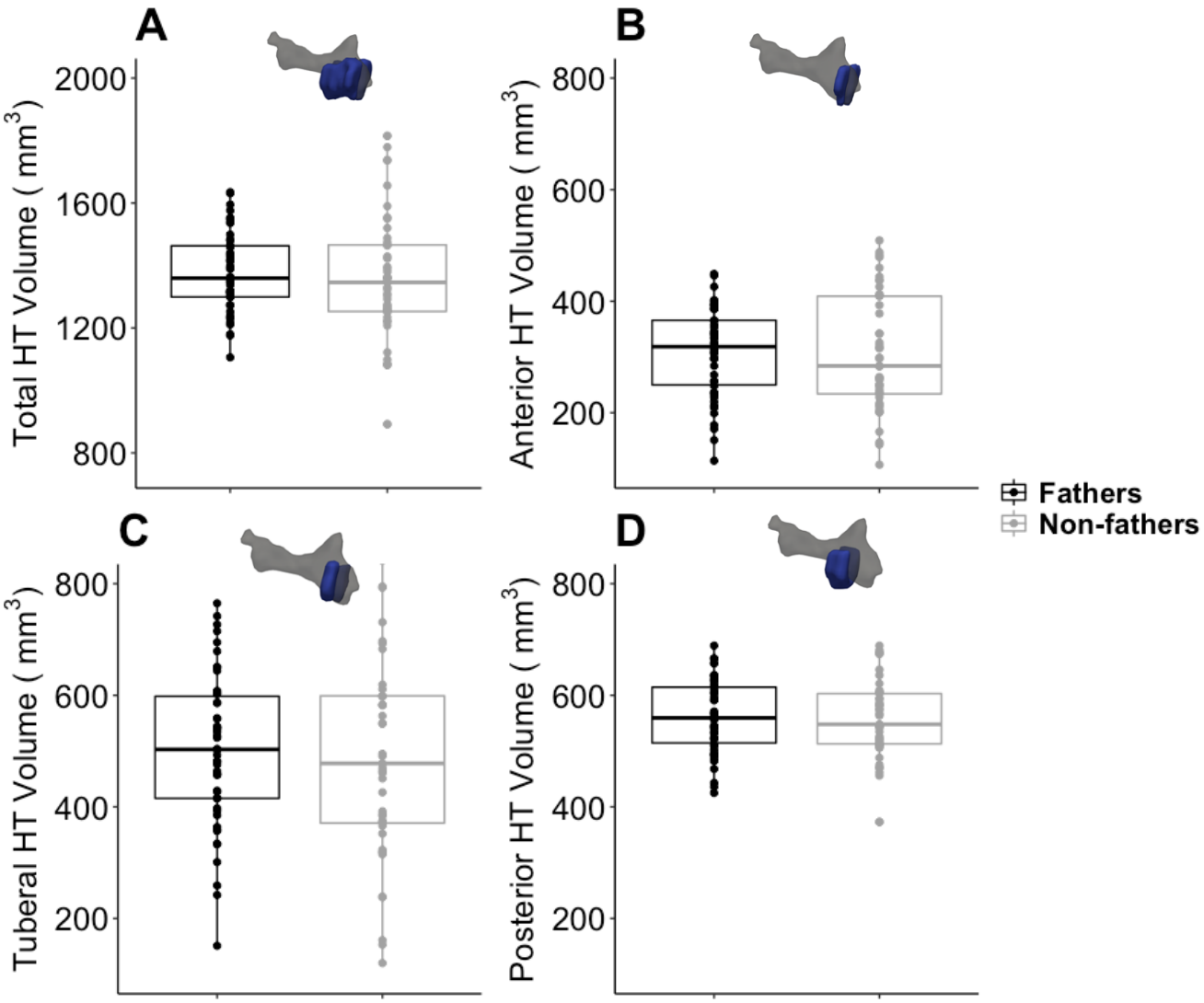


Figure 2. Boxplots of raw measurements of total (A), anterior (B), Tuberal (C), and Posterior (D) hypothalamus volumes in fathers and non-fathers. The pattern of data indicates similar average total and regional hypothalamus volumes across both groups. HT = Hypothalamus

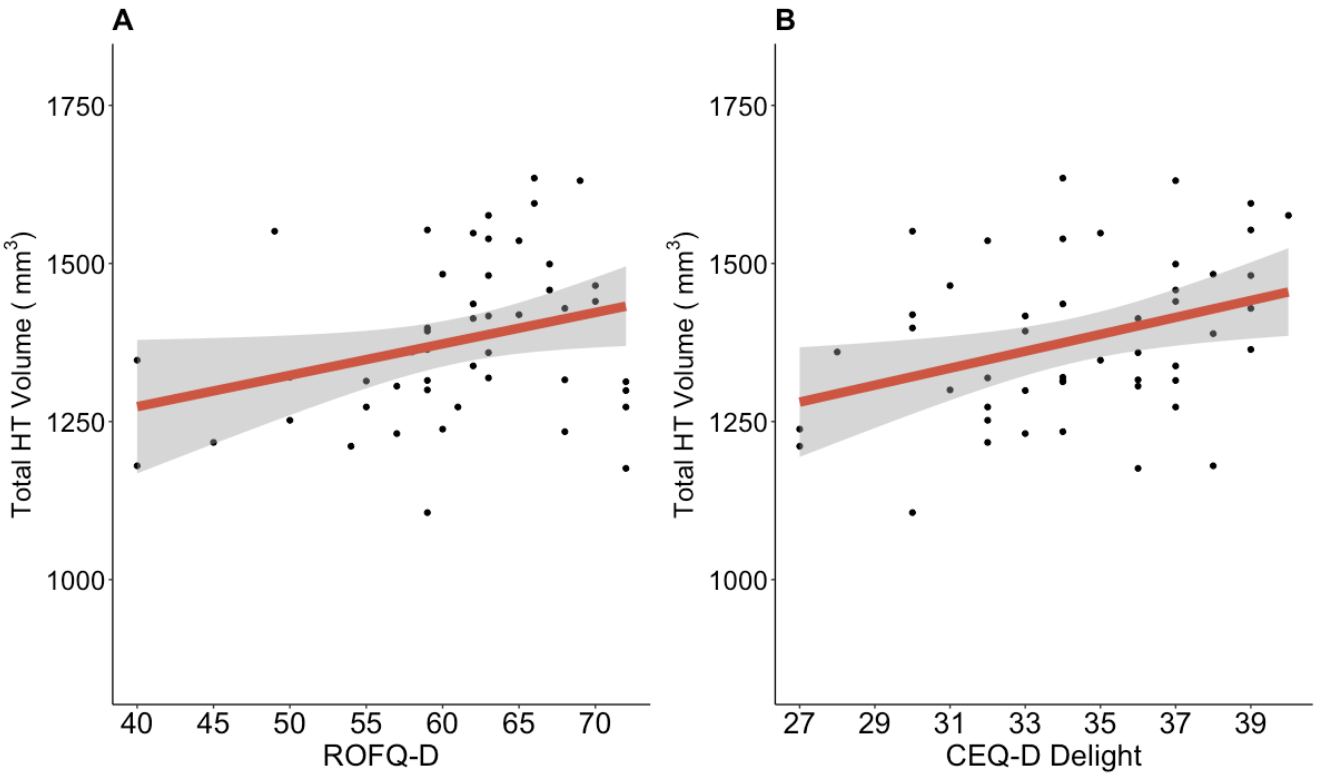


Figure 3. Association between parenting attitudes and total hypothalamus volume. We found significant positive relationships between total hypothalamus volume and participants' belief of the importance of a father's role (ROFQ-D) (A) as well as father's enjoyment of the child (CEQ Delight) (B). The red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

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 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

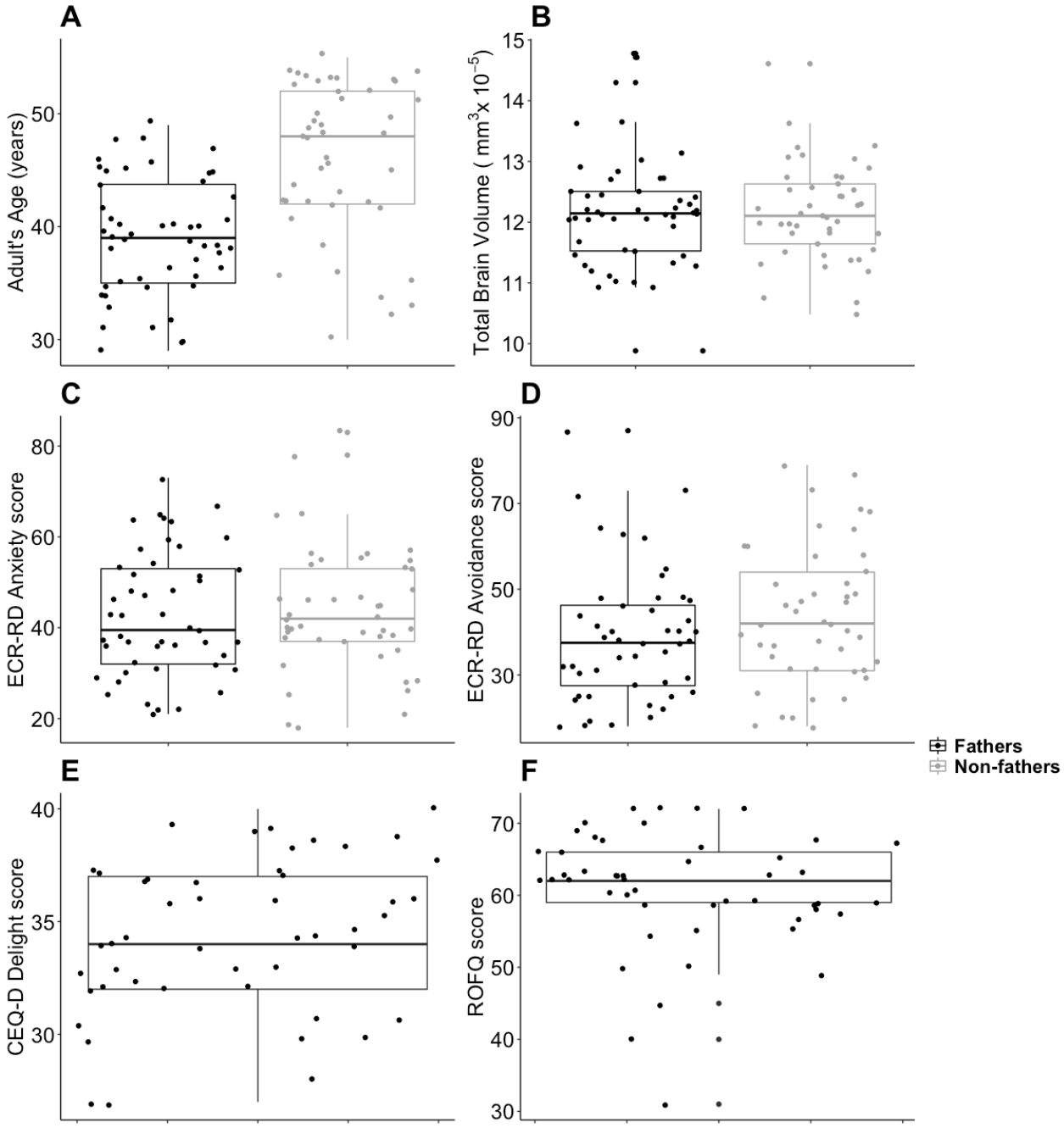
Supplemental Table S5

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

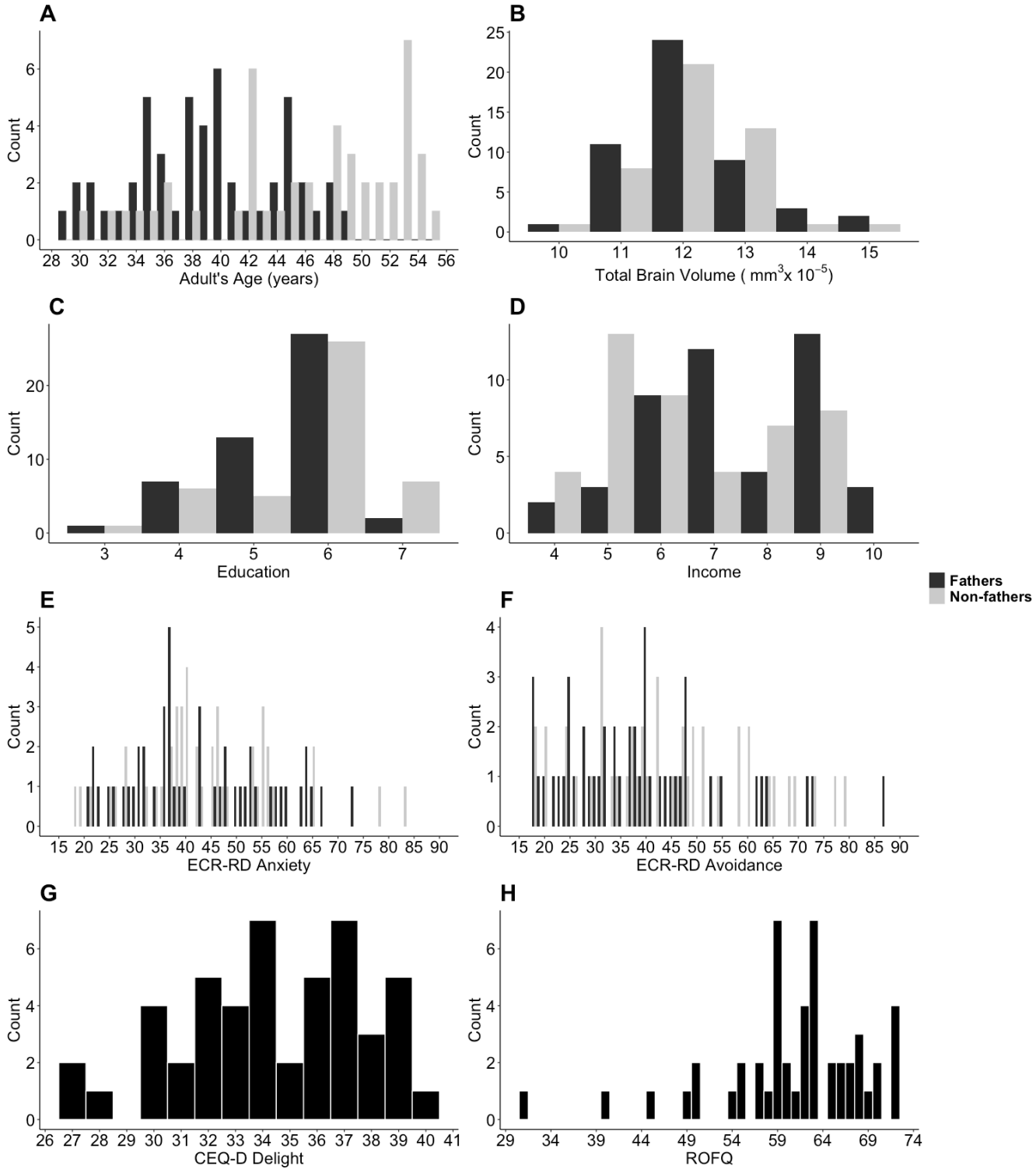
Model	Variable	β	p-value	q-value	η^2	VIF
Total Hypothalamic Volume (all subjects)	ECR-RD Anxiety	-41.13	0.033	0.13	0.038	1.29
	ECR-RD Avoidance	15.57	0.428	0.57	0.012	1.38
	Education	3.62			< 0.001	1.05
	Adult Age	-22.94			0.028	1.22
	Income	7.1			0.003	1.72
	Marital Status	0.26			0.003	1.32
	Brain Volume	55.14			0.104	1.08
Anterior Hypothalamic Volume (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 (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 (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

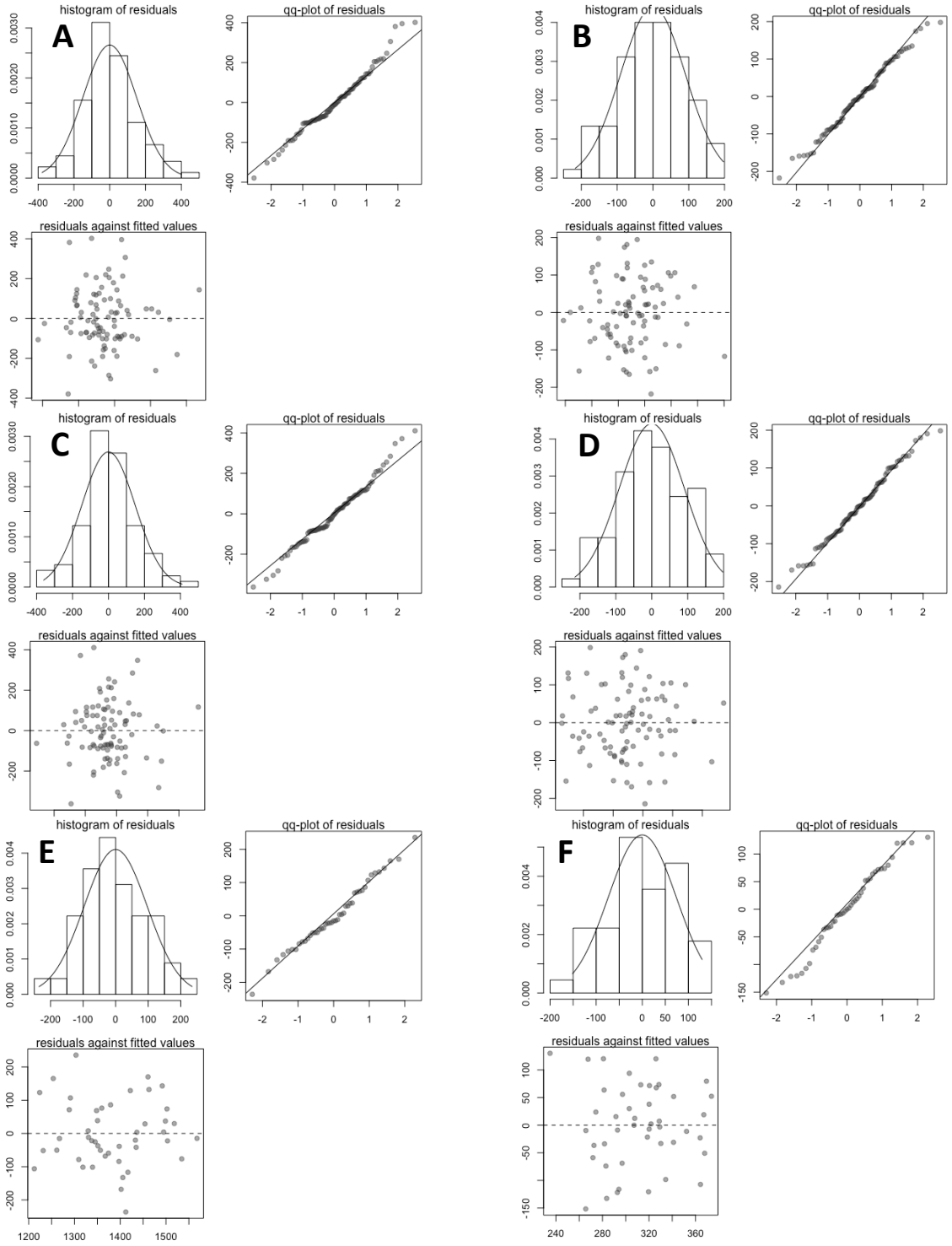
Note: q-value = significance after correction for multiple comparisons, η2 = effect size, VIF = variance inflation factor.



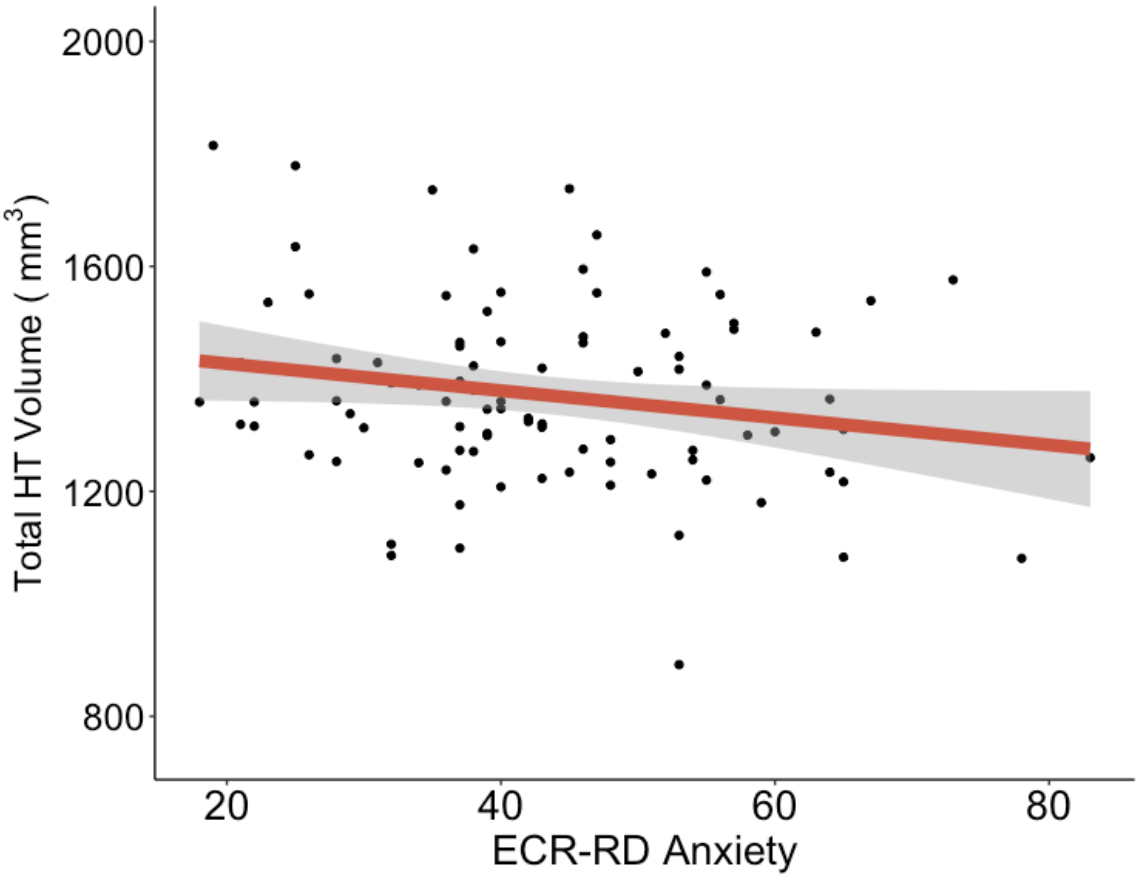
Supplemental Figure S1. Boxplots showing raw data for continuous covariates, including adult age (A), total brain volume (B), ECR-RD Anxiety (C) and Avoidance (D), CEQ-D Delight (E) and ROFQ-D (F). Data points are jittered to avoid over-plotting.



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 romantic 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 romantic 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.

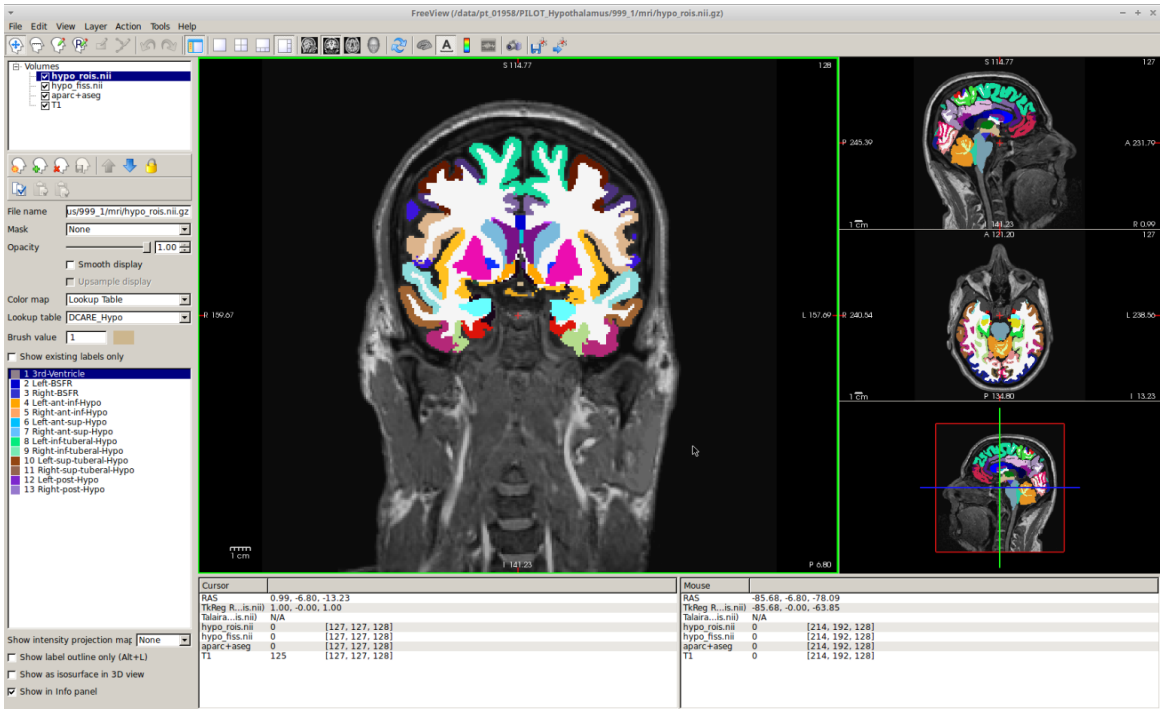


Figure 1. The initial view when opening a subject in FreeView

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.

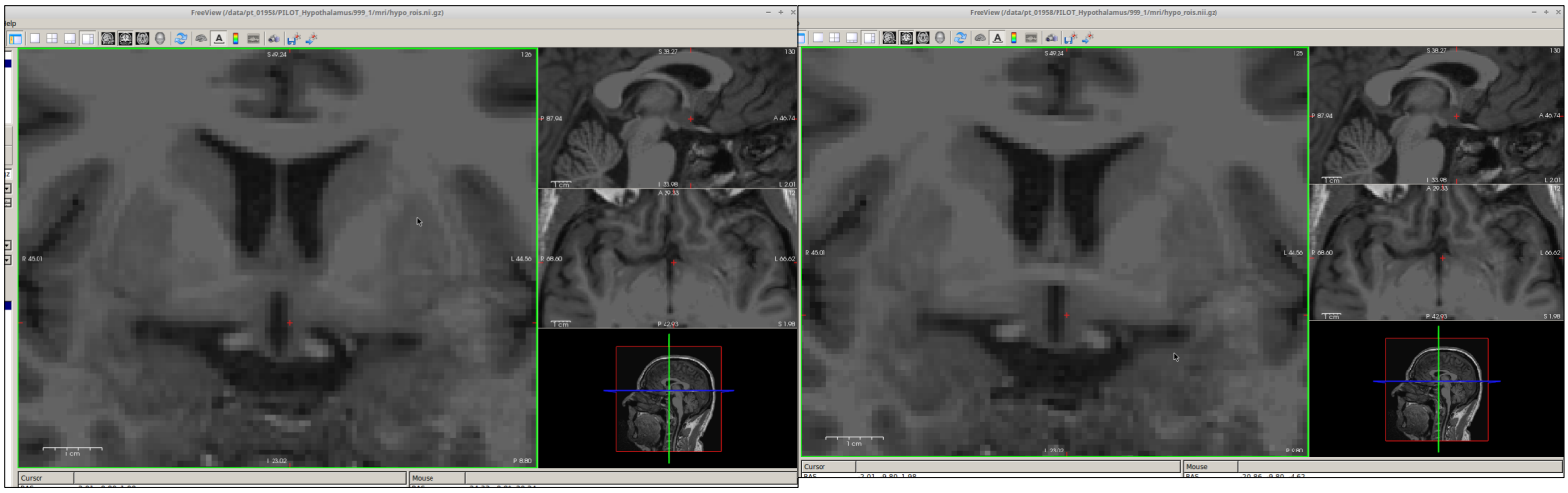


Figure 2a. From Left: 1) The AC is emerging but not yet continuously visible. 2) Moving one slice posterior, the AC is now continuously visible. This would be the first Anterior slice.

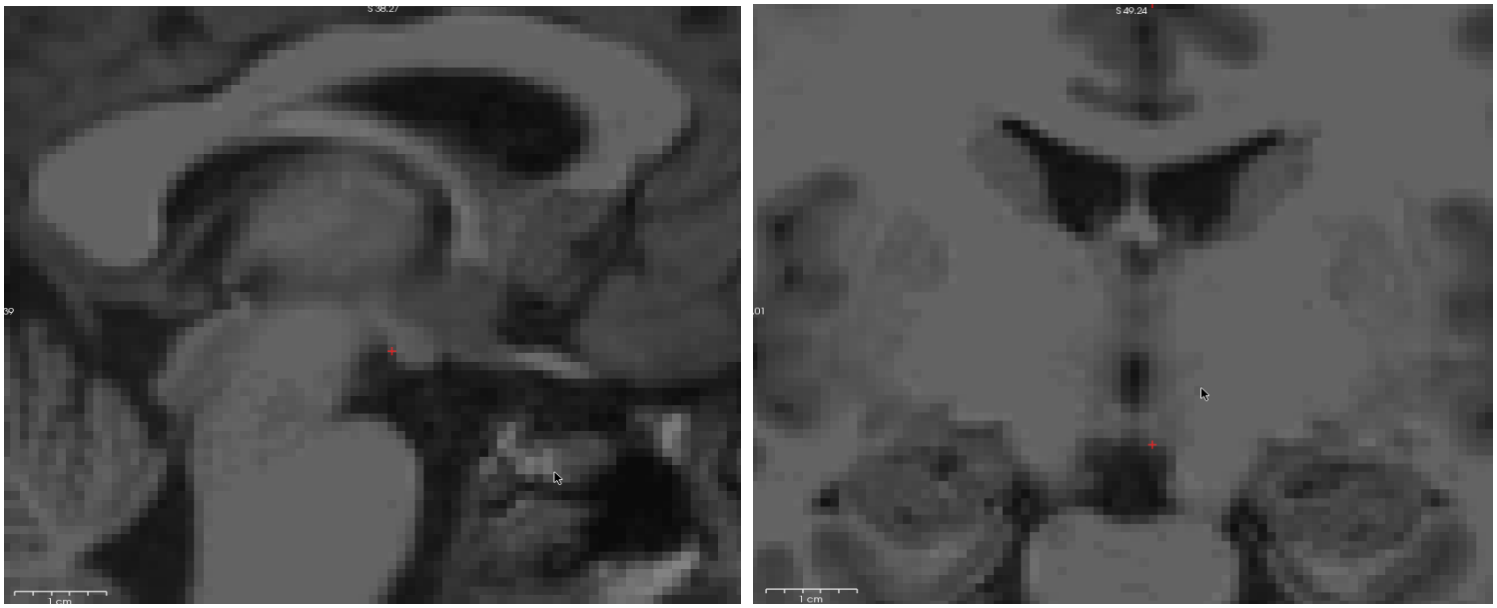


Figure 2b. Locating the most Posterior slice. From Left: 1) In saggital view, put the crosshairs on the last voxel of the MB. 2) Without moving the crosshairs, return to coronal view. This is the last Posterior slice.

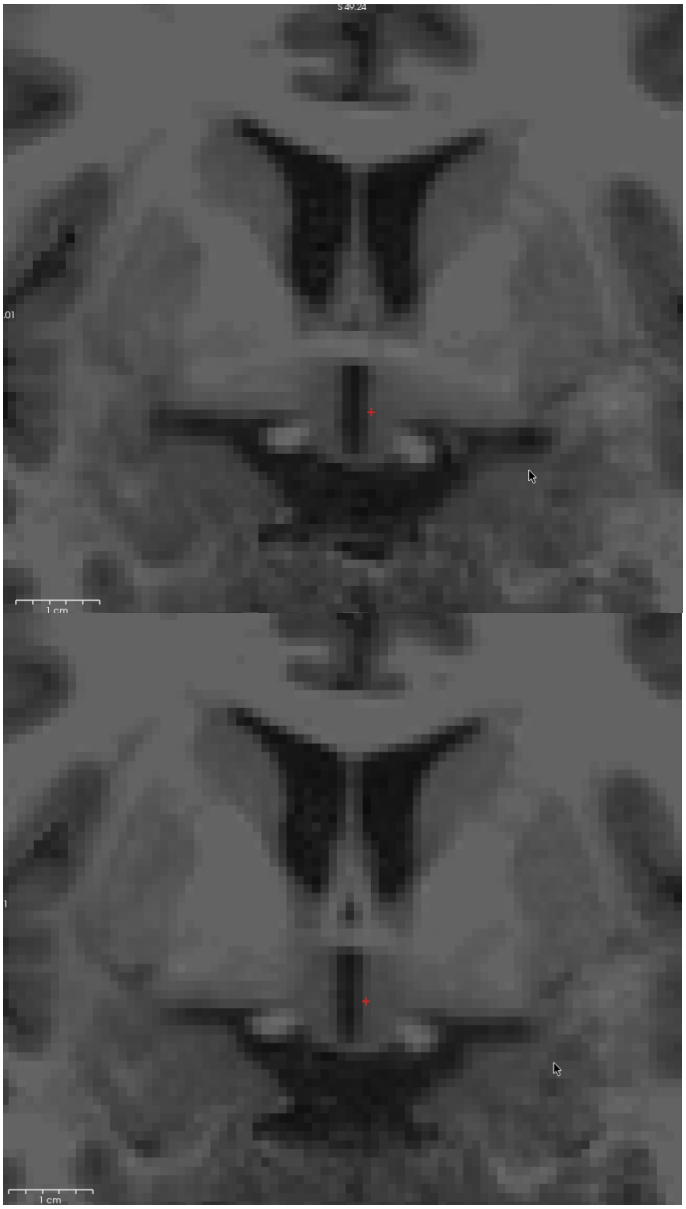


Figure 4a: Transition from Anterior to Tuberal Hypothalamus. From top:

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.

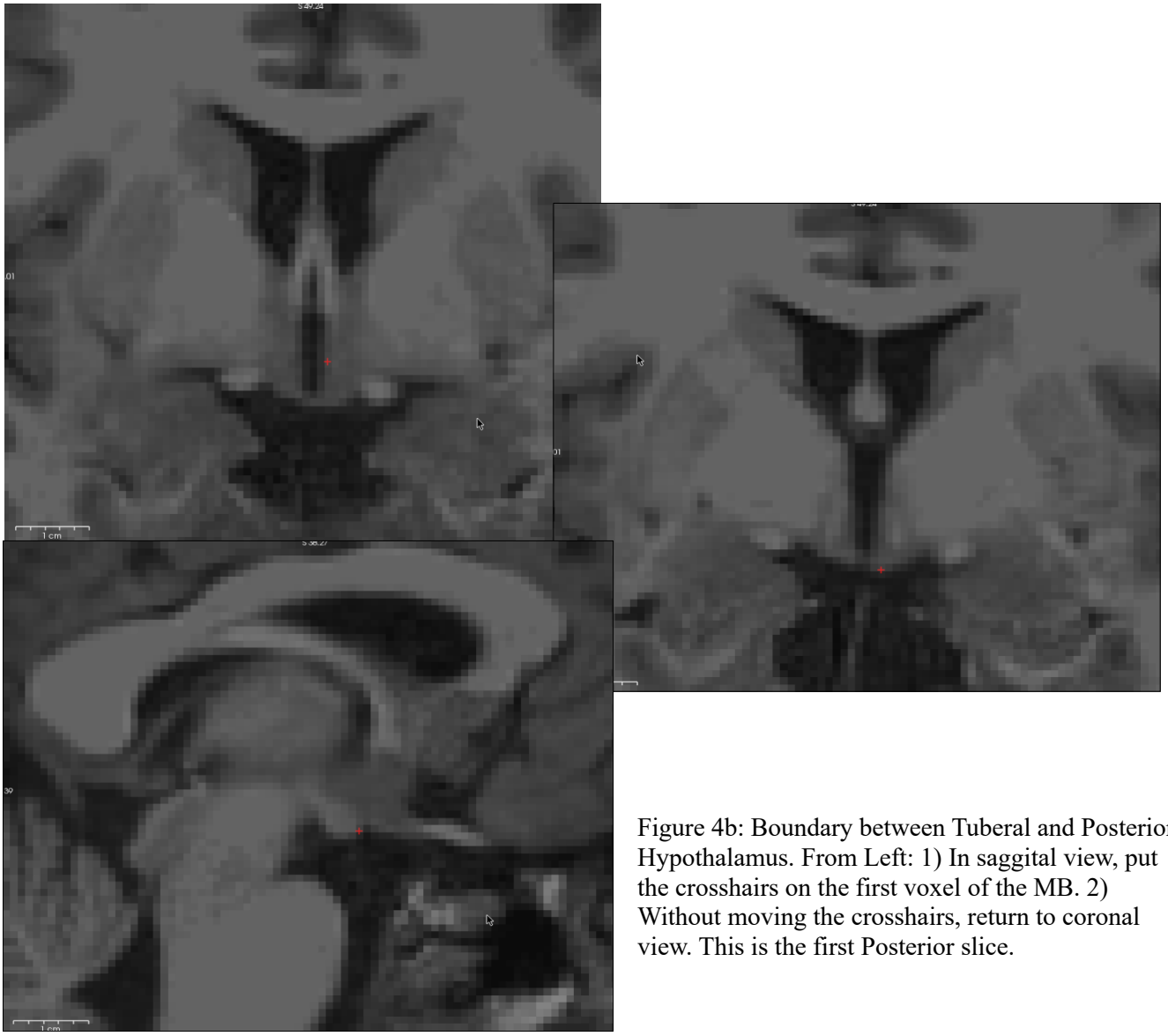
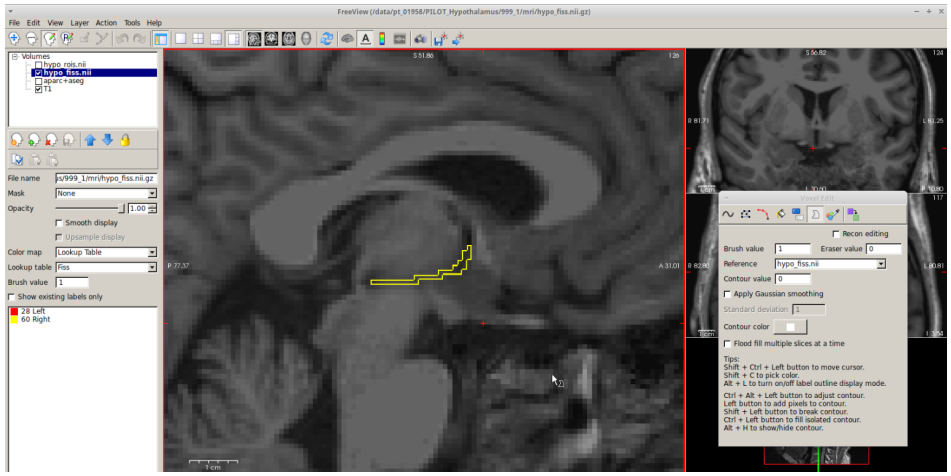


Figure 4b: Boundary between Tuberal and Posterior Hypothalamus. From Left: 1) In saggital view, put the crosshairs on the first voxel of the MB. 2) Without moving the crosshairs, return to coronal view. This is the first Posterior slice.



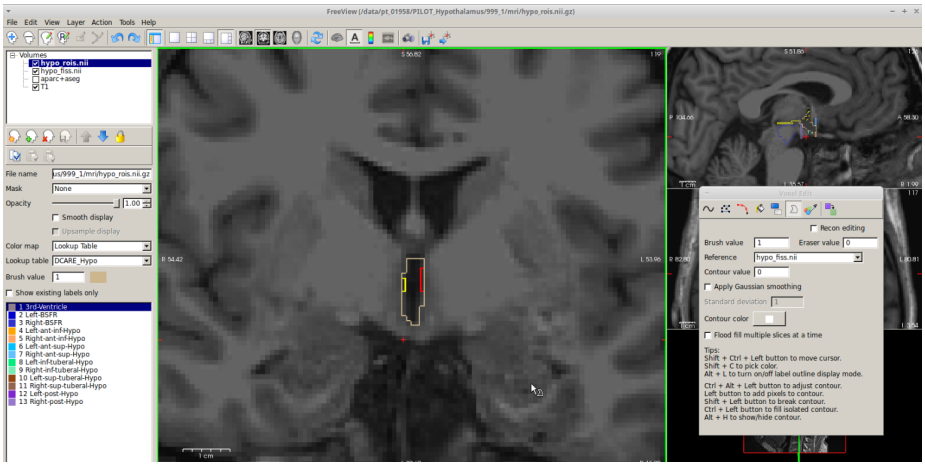


Figure 5. Segmenting the Hypothalamic Fissure.

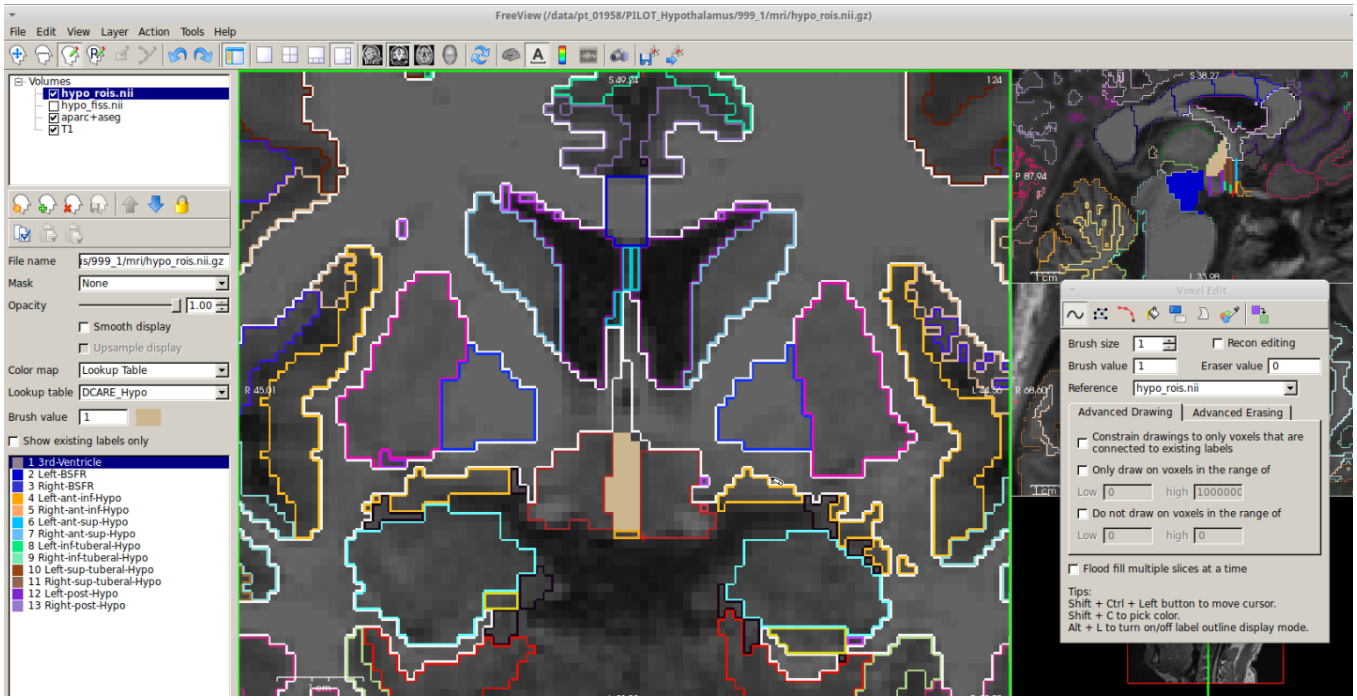


Figure 6. Segmenting the Third ventricle using aparc+aseg overlay as a guide.

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.