Connecting Inter-Individual Differences in Attachment to Hypothalamic Volume in Fathers

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Abstract

Neural correlates of adult attachment (usually differentiated into secure, anxious, and avoidant) and caregiving have been increasingly studied in mothers and, to a lesser extent, in fathers. Studies of parental attachment in both humans and rodents have identified the hypothalamus as a potential source of individual difference in attachment and caregiving behavior. While longitudinal brain imaging studies of human fathers have suggested that the hypothalamus is a plastic region, it is unknown whether hypothalamic volume is associated with inter-individual differences in paternal attachment. The present study used self-report measures of attachment and caregiving in combination with volumetric parcellation, a structural magnetic resonance imaging method, of the human hypothalamus to examine the relationship between individual differences in attachment, caregiving, and hypothalamic volume (total and anterior). In a sample of 28 healthy fathers, we found that inter-individual differences in fathers' romantic attachment, but not caregiving, was related to differences in anterior hypothalamic volume. Specifically we found that secure attachment was positively related to anterior hypothalamic volume while anxious attachment was negatively related to anterior hypothalamic volume. We also found a trend-level negative relationship between avoidant attachment and anterior hypothalamic volume. These results bolster previous findings in both men and women that implicate the hypothalamus as an important neural structure in attachment behavior. Moreover, these results indicate that the hypothalamus may be a source if inter-individual differences in attachment orientation in fathers.

Keywords: Attachment orientation, fathers, hypothalamus, structural MRI, volumetric parcellation

1. Introduction

Sociality is a core component of human nature as evidenced by the variety of social bonds that humans form throughout life. Whether it's dependence on a caregiver during infancy, forming friendships in childhood and adolescence, pair bonding in adulthood, or becoming bonded to one's own offspring, humans are social in every stage of life. At the core of social learning is the development of attachment relationships and the attachment behavioral system. 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 caregiving attachment figure such as a parent (Ainsworth & Bell, 1970; Ainsworth, 1964; Bowlby, 1973; Fraley, Brumbaugh, & Marks, 2005). Due to the involvement of the attachment system in both parent-child and romantic relationships (Feldman, 2017), parents are of particular interest when investigating the attachment system. Parents have a lifelong accumulation of attachment experiences from childhood, when they formed an attachment bond to their own caregiver(s), to adulthood, when they became attached to a romantic partner and then had children themselves.

Initial descriptions of attachment relationships focused on the mother-child bond. However, recent developments in attachment research, including advances from the field of social neuroscience, have expanded our understanding of attachment relationships (Feldman, 2017; Fisher, Aron, & Brown, 2006; Insel & Young, 2001; Vrtička, 2017). It is now considered that the attachment system is active not only in the parent-child relationship, but also in other affiliative relationships such as between romantic partners (Feldman, 2017). Importantly, cultural changes in western societies have come to incorporate fathers as attachment figures for their children. Until the early 1990's, fathers were typically cast as either a breadwinner or a playmate

rather than a nurturing figure (Collins & Russell, 1991; Forehand & Nousiainen, 1993; Rohner & Veneziano, 2001). The past decade has brought a surge of research acknowledging fathers in attachment and caregiving roles with their children (Bretherton, 2010; Feldman, Braun, & Champagne, 2019; Kim et al., 2014; Liu et al., 2016; Swain et al., 2014). Even so, a dearth of research on fathers as attachment figures persists, making fathers a population of great interest in present and future studies of attachment. Not only is the inclusion of fathers in caregiving and attachment related research important from an ethical perspective, validating the competency of fathers as attachment figures, but also brings the field of attachment research closer to an understanding of what the attachment and caregiving systems looks like across caregivers.

1.1 Attachment and Caregiving Behavioral Systems

As described above, attachment behavior is thought to be an evolutionarily adaptive set of behaviors which are prototypically thought to keep offspring in close physical proximity to a care-providing attachment figure such as a parent in times of distress (Bowlby, 1973; Fraley et al., 2005). Proximity seeking to an attachment figure is understood to be a mechanism for allostatic regulation as the attachment figure can assist the attached individual in returning to homeostasis after experiencing stressful event (Atzil & Barrett, 2017). Related to the attachment system is a reciprocal 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. For example, when a hungry infant's attachment system becomes active and they begin to cry, a parent's caregiving system activates, prompting them to feed and soothe the baby. A similar pattern exists in the attachment bonds formed between adults. An adult individual's attachment system might become active after

experiencing a stressor, such as conflict at work, and they might seek the support of their romantic partner. In return, the partner might provide comfort and care to that individual by hugging them or offering to prepare the individual's favorite dinner. In either case, the attachment system in one individual becomes active as a distress response to an internal or externally derived event and the caregiving system in a second individual helps to alleviate the first individual's distress. Attachment and caregiving share an underlying behavioral component that compels two individuals to be close to one another, social approach.

1.2 The Social Neuroscience of Individual Differences in Attachment and Caregiving

A core component of attachment theory is the existence of three resolved attachment orientations: secure, anxious, and avoidant (Fraley, Waller, & Brennan, 2000; Hazan & Shaver, 1987). It is thought that these orientations reflect individual differences in the attachment system arising from early experiences in attachment relationships (Vrtička, 2017) and persisting into adulthood. Similarly, attachment-informed theories of caregiving suggest that caregiving behavior tends to align with one of the three orientations and may, to an extent, reflect the types of attachment bonds an individual has experienced throughout life (Canterberry & Gillath, 2012; Mikulincer & Shaver, 2007). Individual differences in attachment have long been studied from a behavioral and self-report perspective. More recently, insights from the field of social neuroscience have allowed attachment researchers to investigate not only the neural mechanisms underlying the attachment and caregiving systems, but also aspects within these systems that may give rise to individual differences (Vrtička, 2017; Vrtička & Vuilleumier, 2012).

Attachment and caregiving behavior arise from a chorus of activation in multiple neural systems, namely those for social approach and reward, social aversion, emotion regulation, and

theory of mind (see Vrtička, 2017 and Vrtička & Vuilleumier, 2012 for a complete review). The present study focuses on a particular sub-cortical brain structure, the hypothalamus, which 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). In the following sections, I briefly summarize the role of the social approach neural system (SApNS) as it relates to individual differences in attachment followed by a discussion of the role of the hypothalamus in social approach specifically. Of note, the majority of neuroscientific evidence for individual differences in human attachment comes from studies with adult participants. Given the type of available evidence and our study's focus on fathers' individual differences in attachment and caregiving, the remainder of this manuscript will focus on attachment in adulthood.

1.2.1 The Social Approach Neural System

Underlying both the attachment and caregiving behavioral systems is the neural system for social approach and reward (Feldman, 2017; Fisher et al., 2006; Insel & Young, 2001; Swain et al., 2014) which includes the ventral tegmental area, substantia nigra, ventral striatum, ventromedial orbitofrontal cortex, pituitary, and the hypothalamus (Vrtička, 2017). The prototypical role of the SApNS in the context of attachment and caregiving is to encode social interactions with significant others (ie. parents, children, and romantic partners) as inherently rewarding. In doing so, activation of the SApNS increases the likelihood that two individuals will seek physical proximity to one another such as what was described in the examples above. However, inter-individual differences exist in SApNS function and it is theorized that these differences partially underlie individual differences in attachment and caregiving behavior (Vrtička, 2017; Vrtička & Vuilleumier, 2012).

When experiencing a stressful event, a securely attached adult will typically seek proximity to a significant other such as a romantic partner (Mikulincer & Shaver, 2007). As a form of social approach, proximity seeking is understood to be driven in part by the SApNS (Noriuchi, Kikuchi, & Senoo, 2008; Swain, Lorberbaum, Kose, & Strathearn, 2007; Xu et al., 2011; Zeki, 2007). In contrast, insecurely attached adults tend to employ secondary attachment strategies: hyper-proximity seeking, characteristic of anxious attachment, or distancing and inhibition of attachment responses in the case of avoidant attachment (Mikulincer & Shaver, 2007). Additionally, it is theorized that because secure, anxious, and avoidant attachment orientations are largely characterized by differences in social approach behavior, differences in SApNS function is fundamental to the emergence and maintenance of attachment orientation (Vrtička, 2017; Vrtička & Vuilleumier, 2012). It is theorized that, as compared to secure individuals, anxiously attached individuals show increased activation of the SApNS in attachment interactions while avoidant individuals show a relative decrease in activation (Vrtička, 2017). While this pattern of SApNS deactivation is relatively well documented for avoidant attachment, the above mentioned pattern for SApNS activation and anxious attachment has only been observed in one study (Poore et al., 2012) and as should be interpreted conservatively pending further investigation.

Similar to the strategies associated with the three core attachment orientations, caregivers tend to align with a particular social approach strategy (Collins, Ford, Guichard, Kane, & Feeny, 2010; Mikulincer & Shaver, 2007). Secure caregiving is aligned with a strategy of empathic concern during which the caregiver present in an attachment-based interaction employs proximity seeking with the intention of reducing the other person's suffering (Collins et al., 2010). In contrast to secure caregiving, it is theorized that secondary caregiving strategies are

motivated by a desire to alleviate personal distress caused by the distress of a significant other (Collins et al., 2010) for example, seeing your romantic partner cry. Secondary caregiving strategies are either deactivating (avoidant) or hyper-activating (anxious) in nature. Accordingly, an avoidant caregiver might decrease their social approach behavior to avoid the stressful stimulus. Correspondingly, a decrease in SApNS activation for avoidant individuals in caregiving contexts would be expected (Vrtička, 2017). On the other hand, an anxious caregiver might respond to a significant other's distress with heightened social approach and sometimes helping behavior (Canterberry & Gillath, 2012). Anxious caregiving may also be accompanied by commensurate heightened activation in the SApNS however, this is largely speculative. It is theorized that the SApNS is partially modulated by means of the hormone oxytocin (Vrtička, 2017; Vrtička & Vuilleumier, 2012). The relationship between the SApNS and oxytocin are discussed in further detail below as they relate to the hypothalamus.

1.2.2 The Hypothalamus

At work in the SApNS is a small but influential subcortical structure called the hypothalamus. Anatomists divide the hypothalamus into three rostral to caudal sub-regions: anterior, tuberal, and posterior (Figure 1; Dudás, 2013; Makris et al., 2013). Recent developments in structural MRI methods have made it possible to identify the hypothalamus, its sub-regions, and estimate its volume (Makris et al., 2013). Of particular interest to this study is the anterior hypothalamus which contains the supraoptic and paraventricular nuclei. As alluded to above, these nuclei produce and release oxytocin (Makris et al., 2013), an affiliative hormone implicated in the development of attachment bonds (Carter, 2014; Carter et al., 2006; Fisher et al., 2006; Insel & Young, 2001). These nuclei are also implicated in production of corticotropin

releasing hormone (CRH; Carter et al., 2006; Dudás, 2013). It is understood that oxytocin produced in the hypothalamus can down-regulate HPA axis function (Carter et al., 2006) further indicating that the CRH and oxytocin systems within the hypothalamus may be intertwined. Taken together, these two mechanisms are theorized to have modulatory effect on the SApNS (Vrtička, 2017; Vrtička & Vuilleumier, 2012) which may ultimately relate to differences in caregiving and attachment behavior in humans.

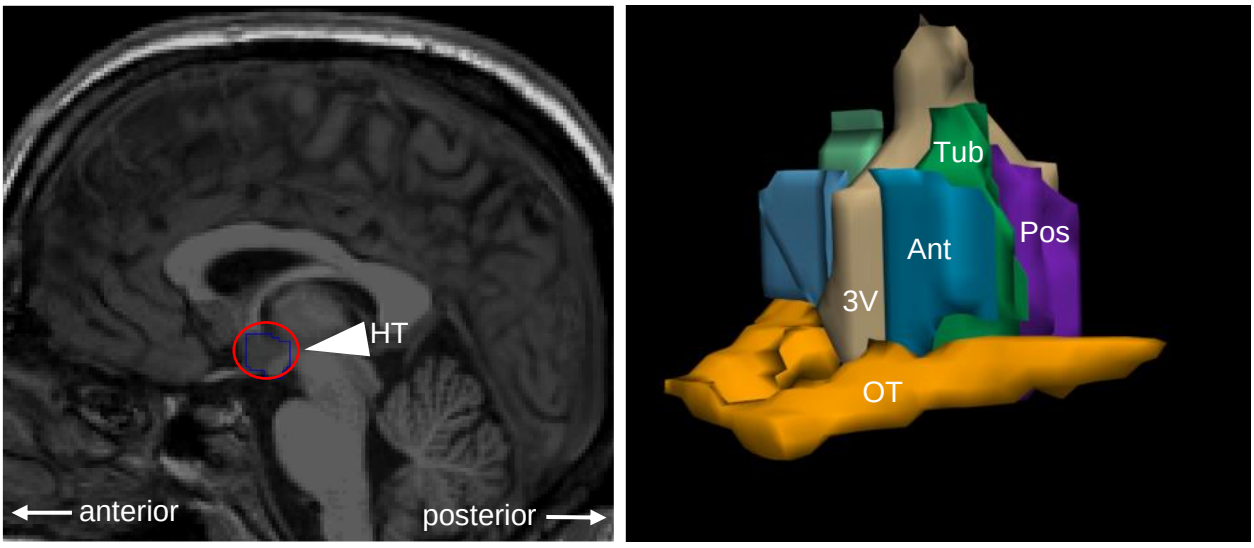

Figure 1. The human hypothalamus (HT; circled in red) viewed sagitally in a structural MRI scan (left) and a 3D rendering of the hypothalamus (right) with optic tracts (OT) and third ventricle (3V) for reference. The hypothalamus is segmented by the three main sub-regions, anterior (Ant), tuberal (Tub), and posterior (Pos).

A robust body of literature from non-human animal models indicates that the hypothalamus plays a key role in pair-bonding and parenting behavior in both males and females. As with humans, research on the hypothalamus and attachment contexts in rodents has largely focused on mothers and their offspring. This research cohesively suggests that structural,

functional, and hormonal changes all occur in the hypothalamus in relation to parenting. For example, the function of the anterior hypothalamic area is implicated in the inhibition and onset of maternal behavior in rats (Bridges, Mann, & Coppeta, 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 is more sparse for males, the importance of the hypothalamus and it's associated hormones in 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 rodent (ie. species in which both mother and father contribute to offspring care), hormonal and cellular changes are observed in the paternal hypothalamus in response to parenthood (Saltzman & Ziegler, 2014). In paternal meadow voles, this includes an increase in oxytocin binding in sub-regions of the anterior hypothalamus as compared to non-fathering males (Parker, Kinney, Phillips, & Lee, 2001). Indeed, oxytocin and the hypothalamus appear to play a key role in the adaptation to fatherhood in male prairie voles (Kenkel, Suboc, & Sue Carter, 2014).

While certain measures of oxytocin in humans are mere proxy to hypothalamic function, we include that evidence here for its potential relationship to the hypothalamus and modulation of the SApNS. Evidence has shown that plasma oxytocin positively correlates with affectionate contact and positive engagement with offspring in both mothers and fathers (Apter-Levi, Zagoory-Sharon, & Feldman, 2014; Feldman, 2012). In a sample of mothers already mentioned above (Strathearn, Fonagy, Amico, & Montague, 2009), secure mothers had greater oxytocin responsiveness as compared to avoidant mothers after interacting with their babies, and this was

further correlated with hypothalamic activation in response to images of their own infant's face. In certain cases, oxytocin may facilitate father-infant bonding (Rilling & Young, 2014). One study in particular found that the application of intranasal oxytocin (which simulates the effects of naturally circulating plasma oxytocin (Gossen et al., 2012)) increased fathers' responsiveness to their toddler during an interaction task as compared to a placebo group (Naber, van IJzendoorn, Deschamps, van Engeland, & Bakermans-Kranenburg, 2010). A recent epigenetic study of the oxytocin receptor gene (OXTR) indicated that hypermethylation of the OXTR promotor region is associated with avoidant attachment in young adults (Ein-Dor, Verbeke, Mokry, & Vrtička, 2018). Taken together, the above findings suggest that oxytocin plays a role in the modification of the SApNS and in turn, inter-individual differences in attachment. While we do not measure oxytocin in the present study, the available literature on oxytocin, attachment, and caregiving illustrates a possible underlying mechanism for differences in attachment orientation that is biologically related to the hypothalamus.

Focusing specifically on hypothalamic structure and function in humans, MRI research has revealed an important role of the hypothalamus in social approach and attachment-related contexts. For example, both mothers (Kim et al., 2010) and fathers (Kim et al., 2014) showed increases in hypothalamic volume in the first few months after their first child was born. Additionally, secure mothers showed greater midbrain volume, including the hypothalamus, than insecure 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 (Strathearn et al., 2009). To our knowledge, only one study to-date has specifically examined hypothalamic structure as it relates to individual differences in the SApNS in healthy men. This study observed that lower hypothalamic volume was predictive

of low pro-sociality (Tost et al., 2010). This finding can be interpreted as an indication that avoidant attachment-like qualities might be negatively related to hypothalamic volume in men. However, it is still unknown whether there is an association between adult attachment orientation and hypothalamic volume in fathers. The results above emphasize the importance of the hypothalamus in the SApNS as it relates to inter-individual differences in attachment.

1.3 Present Study

1.3.1 Research Questions

Given our interest in the role of the hypothalamus as it relates to individual differences in both attachment and caregiving, our research questions were twofold: first, what is the relationship between fathers' attachment orientation and hypothalamic volume and second, what is the relationship between fathers' caregiving orientation and hypothalamic volume?

1.3.2 Hypotheses

Regarding attachment, we proposed the following: In line with previous research in mothers (Kim et al., 2010), we hypothesized that secure attachment would be positively related to total hypothalamic volume (A1). Additionally, given the specific role of the anterior hypothalamus in oxytocin production and the well-established connection between oxytocin and the SApNS, we hypothesized that secure attachment would be positively related to anterior hypothalamic volume (A2). Building on the finding from Tost et al. 2010 which showed lower social approach tendencies relating to lower hypothalamic volume in men, we further hypothesized that avoidant attachment would be negatively related to total hypothalamic volume (A3) and similarly that avoidant attachment would be negatively related to anterior hypothalamic

volume (A4).

Regarding caregiving, we had several hypotheses which correspond with the attachment hypotheses above. Namely, we believed that secure caregiving would be positively related to total hypothalamic volume (C1), as well as to anterior hypothalamic volume (C2), and that avoidant caregiving would be negatively related to both total (C3) and anterior (C4) hypothalamic volume.

1.3.3 Broader Relevance

While clear physiological differences exist between biological mothers and fathers, such as the experience of pregnancy and lactation, it is theorized that maternal and paternal caregiving involve homologous neural underpinnings (Feldman et al., 2019; Wynne-Edwards, 2001). However, given the relative lack of father-specific research on attachment and caregiving, this statement remains speculative. The present study focuses on fathers as a means of bringing attachment research into the 21st century by acknowledging the increasingly familiar role of men as caregivers for their children. Additionally, the present study aims to understand the role of the hypothalamus in characterizing inter-individual differences in attachment and caregiving in fathers.

2. Methods

2.1 Participants

This investigation of attachment and the paternal hypothalamus was conducted as part of the D-CARE study, an investigation of the behavioral, biological, and brain substrates of paternal attachment. The study is led by Dr. Pascal Vrticka of the Social Stress and Family Health (SSFH) Lab at the Max Planck Institute of Human Cognition and Brain Sciences (MPI-CBS) in Leipzig, Germany. Fathers of 5-year old children were recruited for the D-CARE study (N=66) from three sources: an internal database at MPI-CBS, flyers posted at public childcare facilities in Leipzig, and flyers posted at the University of Leipzig campus. To be eligible for participation, fathers needed to be between 23-55 years of age, right-handed, physically healthy, have no history of psychiatric illness (including current drug or alcohol abuse), and have no difficulties reading or writing in German. Additionally, fathers participating in the MRI portion of the study were required to have no contraindications for MRI. Data collection for D-CARE is ongoing and thus, for the purpose of this investigation, only participants with complete MRI and questionnaire data by February 28th, 2019 (N=28) were included in the present study of the hypothalamus.

2.2 MRI Acquisition

This cross-sectional study included a single MRI visit. Participants completed online self-report questionnaires prior to arriving at the lab for the MRI session. For the study of the hypothalamus, a T1-weighted MPRAGE anatomical image was acquired on a Siemens Skyra 3T MRI scanner at the MPI-CBS in Leipzig, Germany using the following parameters: 176 slices, voxel size = 1mm^3, TR=2300 msec, TE=2.98 msec, Flip angle=9°, FOV=256 mm. Complete

MRI parameters for the MPRAGE can be viewed in Appendix A. As part of the larger D-CARE study, subjects also participated in two functional MRI paradigms: a cyberball task and an emotional face recognition task utilizing photos of the subject's own child and a gender-matched other child from the D-CARE sample. At the time of the MRI visit, D-CARE participants had also completed an fNIRS visit for a separate part of the study. Analysis for these additional aspects of the D-CARE project are ongoing and are not further discussed in this manuscript.

2.3 Image Processing and Volumetric Parcellation

As part of the standard MRI preprocessing pipeline used in the SSFH Lab, MPRAGE images were converted from DICOM to NIFTI format using the SPM 12 software package. We further prepared the images for volumetric analysis of the hypothalamus (Makris et al., 2013) using the FreeSurfer software package. First, images were fed to the recon-all processing pipeline which outputs a reconstruction of the brain's cortical surface, total volume, and volume estimates of certain sub-cortical structures (excluding the hypothalamus). Images were additionally processed in FreeSurfer using a custom script developed by Dr. Nikos Makris at Massachusetts General Hospital to specifically identify the subcortical region known as the basal forebrain and encompasses, among other structures, the hypothalamus. The resulting image can then be used in Volumetric Parcellation (VP) of the hypothalamus.

In VP of the human hypothalamus, trained raters use anatomical landmarks to manually identify the volume of the hypothalamus in a 3-D structural MRI scan, such as the T1-weighted MPRAGE utilized in this study (Figure 2). The VP protocol used in this study was adapted by Madison Long, Dominique Troost, and Lal Koyuncu with supervision from Dr. Elseline Hoekzema and Dr. Sarah Burke at Leiden University. The protocol allows for segmentation of

the hypothalamus into several sub-regions: Superior and inferior anterior hypothalamus, superior and inferior tuberal hypothalamus, and posterior hypothalamus. All sub-regions can also be divided by the left and right hemisphere for a total of ten sub-regions. The sub-regions can also be binned to create larger functional sub-regions (ie. superior and inferior anterior hypothalamus can be collapsed into the total anterior hypothalamus). Please see Appendix B for the complete VP protocol used in this study, including a detailed description of the anatomical landmarks.

In our study, three raters (including Madison Long) completed the VP protocol. All raters were masters-level students in the SSFH lab at the MPI-CBS. Using Freeview, a built-in data visualizer included in the FreeSurfer software, raters navigate through each 3-D brain image to locate the hypothalamus. For each subject, raters first identified the anterior and posterior boundaries of the hypothalamus by locating the anterior commissure and mammillary bodies, respectively. Next, raters determined the superior boundary of the hypothalamus by marking the hypothalamic fissure. Lastly, raters segmented the boundaries of the anterior, tuberal and posterior hypothalamus. For an experienced rater, the entire VP protocol requires one hour or less per brain to complete.

To extract a measure of hypothalamic volume to be used in further analysis, we used FreeSurfer's statstotable function which extracts Parcellation Units (PUs). PUs are 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 the three raters to obtain a reliable measure of hypothalamic volume.

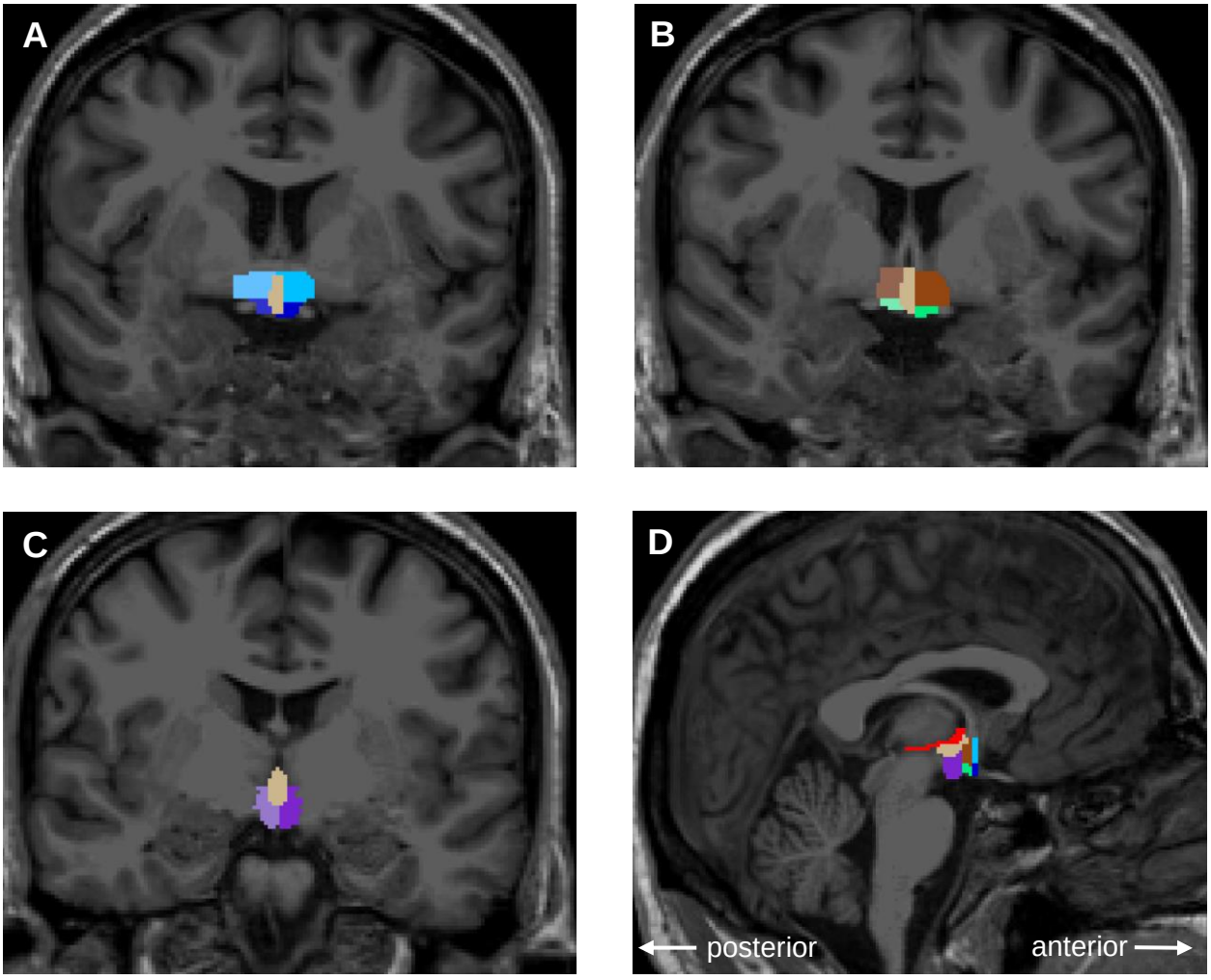

Figure 2. Example of a hypothalamus segmented for this study including the anterior (A; light and dark blue), tuberal (B; brown and green) and posterior (C; purple) sub-regions, and the total hypothalamus (D; including previously mentioned colored sub-regions, third ventricle (tan) and hypothalamic fissure (red)). Images A-C are in coronal view. Image D is in saggital view.

2.3.1 Inter-rater reliability and preparation of hypothalamic volume variable

Inter-rater reliability (IRR) was assessed using a two-way mixed, average measures, absolute agreement intraclass correlation coefficient (ICC). In keeping with commonly used ICC

interpretations (Cicchetti, 1994), ICCs above 0.75 for the total hypothalamus and the three main sub-regions (anterior, tuberal, and posterior) were considered excellent and of sufficient quality for use in the study.

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 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. Once all 28 brains were segmented, IRR was assessed again using the same average measures, mixed, two-way intraclass correlation coefficient. Initial ICC's for the sample were good (posterior ICC = .711) or excellent (anterior ICC = .781, tuberal ICC = .876, and total ICC = .845). As the goal was to achieve and maintain excellent (ICC>.75) IRR for this study, raters were asked to improve posterior reliability. To do this, raters referred back to their lab

notes, recorded during the initial segmentation of the 28 brains, to identify possible points of disagreement. After discussion, raters independently made edits to their sets of brains and reliability was re-calculated. After edits, IRR increased to excellent in all regions. The ICCs for the final sample of 28 hypothalami were: Anterior = .781 , tuberal= .883 , posterior= .825, and total= .876.

2.4 Questionnaires

2.4.1 Attachment

Father attachment orientation was measured by self-report via a German version of the Experiences in Close Relationships scale revised (ECR-RD; German version: (Ehrenthal, Dinger, Lamla, Funken, & Schauenburg, 2009; original English version: Fraley, Heffernan, Vicary, & Brumbaugh, 2011). This scale measures attachment orientation in an adult's current romantic relationship on two sub-scales: attachment avoidance and attachment anxiety. The ECR includes 18 items (9 for anxiety and 9 for avoidance). Subjects rate all 18 items on a 7-point likert scale (1 = not at all true for me; 7 = very true for me). The ECR is scored by averaging the responses for each sub-scale. For the dimensions, higher scores indicate higher levels of avoidance or anxiety respectively. A low score in both dimensions is thought to indicate attachment security. Scale reliability was high for both attachment anxiety (alpha = .881) and attachment avoidance (alpha = .944).

2.4.2 Caregiving

We used a German translation of the Caregiving Experiences Questionnaire (CEQ-D; German version: Nguyen et al., in preparation; original English version: Brennan, George, &

Solomon, 2013) to measure caregiving qualities in this study. Subjects rate scale items on a 5-point likert scale (1=not at all characteristic; 5=very characteristic). The CEQ has been scored in two different ways, using either a five-factor sub-scale structure (Brennan et al., 2013) or a four-factor sub-scale structure (Røhder et al., 2019)

Initially we planned to use the five-factor sub-scale structure described in the flagship study (2013) wherein Brennan, George, & Solomon identified sub-scales for delight, discouraging closeness, separation anxiety, helplessness, and role reversal. We felt that, conceptually, sub-scales for discouraging closeness and separation anxiety could serve as caregiving-related counterparts to the avoidant and anxious sub-scales from the ECR. However, scale analysis for the five-factor CEQ in our sample showed poor reliability. Instead, we adopted a four-factor structure for the CEQ which was first described in a recent study with Danish mothers (Røhder et al., 2019). The four-factor structure had improved reliability in our sample for the sub-scales of interest: enjoyment (alpha=.665), heightened caregiving (alpha=.648), and helplessnes (alpha=.84). As such, we decided to use these sub-scales in further analyses. However, with the loss of the sub-scale for discouraging closeness, we determined that we would not be able to test hypotheses C3 and C4 relating to avoidant caregiving as initially planned.

2.5 Data exploration and treatment of variables

All variables of interest (ECR-avoidance, ECR-anxiety, CEQ-heightened caregiving, CEQ-helplessness, CEQ-enjoyment, father age, total brain volume, anterior hypothalamic volume, and total hypothalamic volume) were examined for outliers and distribution normality. Descriptive statistics for all variables are presented in Table 1. Regarding outliers, we set a threshold of three standard deviations above or below the mean and thus determined that there were no outliers in our sample for any of the variables. Regarding normality, we found that ECR-

avoidance was positively skewed (Kolmogorov-Smirnov=.175, sig=.028; Shapiro-Wilk=.914, sig=.024). Consequently, we took the natural log of the ECR-avoidance variable to use in further analysis. Because ECR-avoidance and ECR-anxiety are corresponding constructs that are intended for simultaneous use in analyses, we also took the natural log of ECR-anxiety.

Next, we standardized all predictor (ECR-avoidance, ECR-anxiety, CEQ-heightened caregiving, CEQ-helplessness, CEQ-enjoyment) and control variables (father age, and total brain volume). We used the resulting z-scores in further analysis.

Finally, we computed simple correlations between the predictor variables and covariates to test for collinearity (Table 2). We observed a moderate, significant, negative correlation between father age and total brain volume (R=-.418, $p<.05$) and a slight positive correlation between CEQ-Enjoyment and CEQ-Heightened Caregiving (R=.459, $p<.05$). No significant relationship was found between the ECR dimensions and the CEQ sub-scales. Importantly, we found a strong and significant correlation between ECR-anxiety and ECR-avoidance (R=.839, $p<.001$), indicating high collinearity and thus, that these two variables should not be used simultaneously in our regression analyses as planned. Instead, we computed an ECR-composite variable wherein low scores indicated attachment insecurity and higher scores indicated greater attachment security. The ECR-composite score was computed by inverting the mean of the raw ECR-anxiety and ECR-avoidance so that a higher value would indicate more security. The need for this composite variable was prohibitive for evaluating hypotheses A3 and A4 as planned. Instead, we determined that any analyses conducted using the separate variables for ECR-avoidance and ECR-anxiety should be considered exploratory.

Table 1
Descriptive statistics

Variable	Mean	Std. Dev.	Minimum	Maximum
ECR-Avoidance	2.28	0.96	1	4.83
ECR-Anxiety	2.44	0.84	1.16	4.33
ECR-Composite	2.36	0.86	1.08	4.17
CEQ-Heightened	1.85	0.61	1	3
CEQ-Helplessness	2.07	0.52	1.33	3.08
CEQ-Enjoyment	4.91	0.38	4.33	5.56
Father Age (Years)	38.39	5.63	29	49
Total Brain Volume (cm^3)	1229.02	94.68	1090	1470
Total Hypothalamic Volume (mm^3)	1409.13	125.63	1152.67	1635.67
Anterior Hypothalamic Volume (mm^3)	314.1	79.61	170.67	449.33

Table 2
Correlation matrix to test for collinearity between variables

	ECR-Avoidance	ECR-Anxiety	ECR-Composite	CEQ-Enjoyment	CEQ-Heightened	CEQ-Helplessness	Father Age	Total Brain Vol.	Total Hypothalamic Vol.
ECR-Avoidance	1								
ECR-Anxiety	.839**	1							
ECR-Composite	.951**	.924**	1						
CEQ-Enjoyment	0,009	-0,077	0,027	1					
CEQ-Heightened	-0,019	0,081	0,097	.453*	1				
CEQ-Helplessness	0,281	0,258	0,278	-0,349	-0,175	1			
Father Age	-0,307	-0,116	-0,209	-0,265	-0,023	0,209	1		
Total Brain Vol.	0,292	0,224	0,241	0,324	0,113	-0,225	-.418*	1	
Total Hypothalamic Vol.	-0,004	-0,01	0,018	.403*	0,255	-0,097	-0,026	0,237	1
Anterior Hypothalamic Vol.	-0,173	-0,318	-0,269	0,089	-0,093	-0,294	-0,099	.399*	0,156

Note. * =Correlation is significant at the 0.05 level (2-tailed); ** =Correlation is significant at the 0.01 level (2-tailed).

3. Results

To test our hypotheses, we computed a series of regression analyses. We controlled for total brain volume and father age in all models. For a summary of the results, including Beta values both for variables of interest and control variables, please see Table 3.

3.1 Caregiving Models

To test hypothesis C1, that secure caregiving and total hypothalamic volume would be positively related, we computed a regression model with CEQ-enjoyment, CEQ-heightened caregiving, and CEQ-helplessness as predictors and total hypothalamic volume as the dependent variable. This model explained relatively little variance (R^2=.201) and the test of the overall model was non-significant (F(2,22)=1.104, *p*=.386).

Next, to test hypothesis C2, that secure caregiving and anterior hypothalamic volume would be positively related, we computed a model with the same CEQ predictors as above and anterior hypothalamic volume as the outcome. As before, this model explained relatively little variance (R^2=.246) and the test of the overall model was non-significant (F(5,25)=1.433, *p*=.252).

3.2 Attachment Models

3.2.1 ECR-Composite Models

To test hypothesis A1, that secure attachment and total hypothalamic volume would be positively related, we computed a regression model with the ECR-composite score as a predictor

Table 3.

Summary of results from regression analyses

Model	Variable	F-value	R^2	Beta	P value	Corr. P value
Caregiving and Total Hypothalamic Vol.		1.104	0.201		0.386	N/A
	CEQ-Enjoyment			0.362	0.138	N/A
	CEQ-Heightened			0.083	0.703	N/A
	CEQ-Helplessness			0.056	0.79	N/A
	Total Brain Vol.			0.179	0.418	N/A
	Father Age			0.136	0.533	N/A
Caregiving and Anterior Hypothalamic Vol.		1.433	0.24		0.252	N/A
	CEQ-Enjoyment			-0.031	0.895	N/A
	CEQ-Heightened			-0.17	0.426	N/A
	CEQ-Helplessness			-0.264	0.2	N/A
	Total Brain Vol.			0.418	0.06	N/A
	Father Age			0.118	0.576	N/A
ECR-Composite and Total Hypothalamic Vol.		0.543	0.064		0.658	0.877
	ECR-Composite			0.032	0.878	0.878
	Total Brain Vol.			0.28	0.217	N/A
	Father Age			0.085	0.703	N/A
ECR-Composite and Anterior Hypothalamic Vol.		3.455	0.302		0.032	0.08
	ECR-Composite			0.384	0.04	0.08
	Total Brain Vol.			0.505	0.014	N/A
	Father Age			0.031	0.871	N/A
ECR-Anxiety and Anterior Hypothalamic Vol.		4.09	0.338		0.018	0.038
	ECR-AX			-0.428	0.019	0.038
	Total Brain Vol.			0.524	0.01	N/A
	Father Age			0.07	0.706	N/A
ECR-Avoidance and Anterior Hypothalamic Vol.		2.687	0.251		0.069	0.092
	ECR-AV			-0.315	0.109	0.109
	Total Brain Vol.			0.496	0.019	N/A
	Father Age			0.011	0.956	N/A

and total hypothalamic volume as the dependent variable. This model explained very little of the variance in the data (R^2=.064) and the test of the overall model was non-significant (F(3,24)=.543, *p*=.658).

To test hypothesis A2, that secure attachment and anterior hypothalamic volume would be positively related, we computed another regression model with the ECR-composite variable as predictor and anterior hypothalamic volume as the outcome. This model explained a fair amount of the variance in the data (R^2=.302) and the initial test of the overall model was significant (F(3,24)=3.455, *p*=.032). Specifically, ECR-composite score was positively related to anterior hypothalamic volume (Beta=.384, *p*=.04; Figure 3). This indicates that, for the range of our model, a one standard deviation increase (.86 points) in ECR-composite score is matched by a 30.57 voxel increase in anterior hypothalamic volume.

Lastly, we applied a False Discovery Rate (FDR) correction for multiple comparisons with the Benjamini-Hochberg procedure with an FDR threshold of .05 to the set of tests using the ECR-composite variable. Specifically we corrected for four tests: the significance test of each full regression model (2 tests) and for the significance test for the ECR-composite variable within each model (2 tests). The resulting corrected p-values were all non-significant (overall model: *p*=.08; ECR-composite specifically: *p*=.08). However, given the relatively high R^2 value for the model including ECR-composite and anterior hypothalamic volume, we proceeded with the exploratory analyses described in the next section.

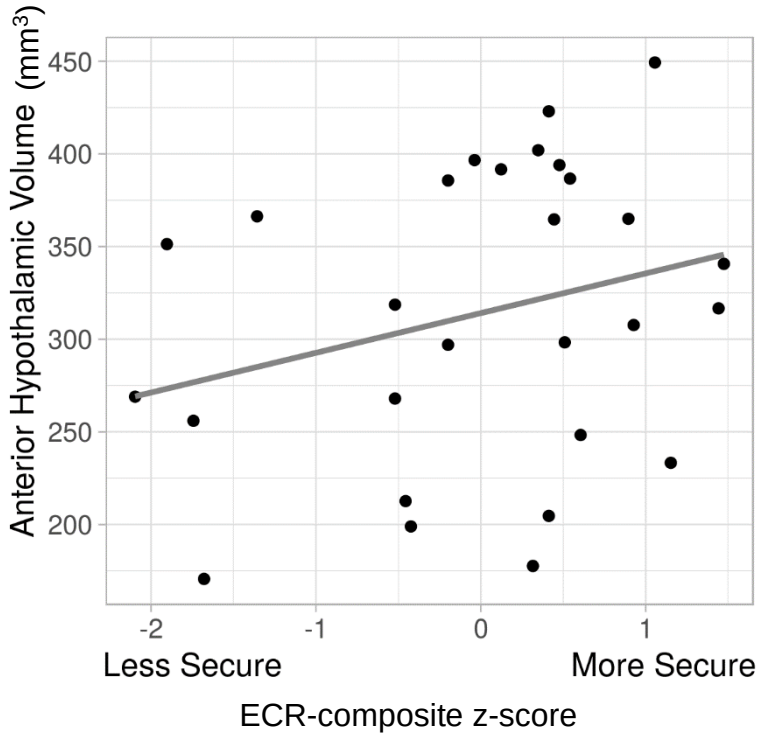

Figure 3. We found a positive relationship between ECR-composite score and anterior hypothalamic volume (Beta=.384, *p*=.04, corrected *p*=.08) while controlling for father age and total brain volume indicating a positive relationship between anterior hypothalamic volume and attachment security.

3.2.2 Exploratory ECR Models

Given the above finding that our regression model with the ECR-composite score explained roughly 30% of the sample's variance in anterior hypothalamic volume, we explored the distinct relationships that ECR-anxiety and ECR-avoidance may have with anterior hypothalamic volume. To this end, we computed two final regression models.

First we computed a model with ECR-anxiety as predictor and anterior hypothalamic volume as outcome. This model explained a fair amount of variance (R^2=.338) and the test of the

overall model was significant (F(3,24)=4.090, *p*=.018). Specifically, we found a significant negative relationship between ECR-anxiety and anterior hypothalamic volume (Beta=-.428, *p*=.019; Figure 4). This indicates that, for the range of our model, a one standard deviation increase (.96 points) in ECR-anxiety score is matched by a 34.07 voxel decrease in anterior hypothalamic volume.

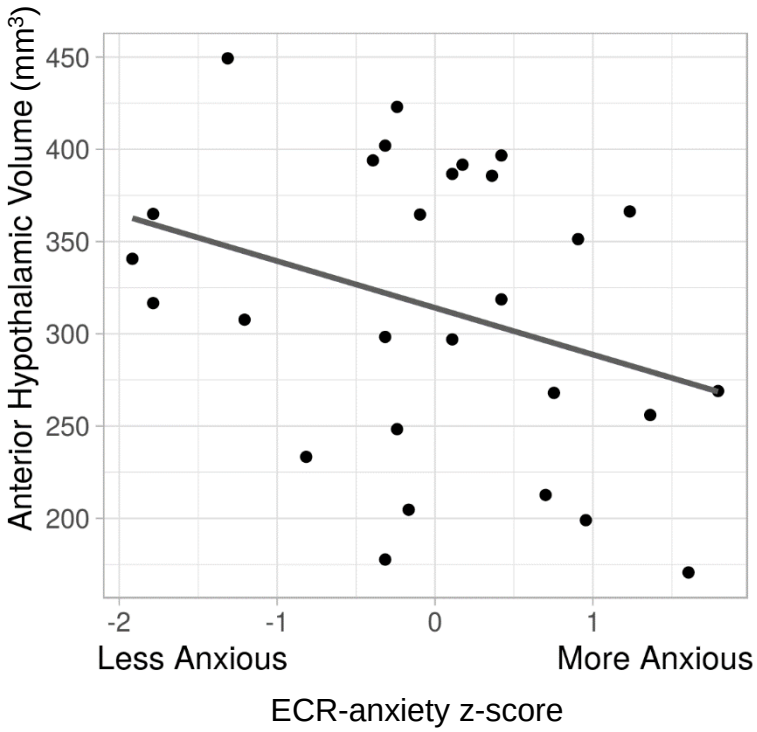

Figure 4. We found a negative relationship between ECR-anxiety score and anterior hypothalamic volume (Beta=-.428, *p*=.019, corrected *p*=.038) while controlling for father age and total brain volume.

Next, we computed a model with ECR-avoidance as predictor and anterior hypothalamic volume as outcome. This model explained a small amount of variance (R^2 = .251) and the test for the overall model indicated a non-significant trend (F(3,24)=2.687 , *p*=.069; Figure 5).

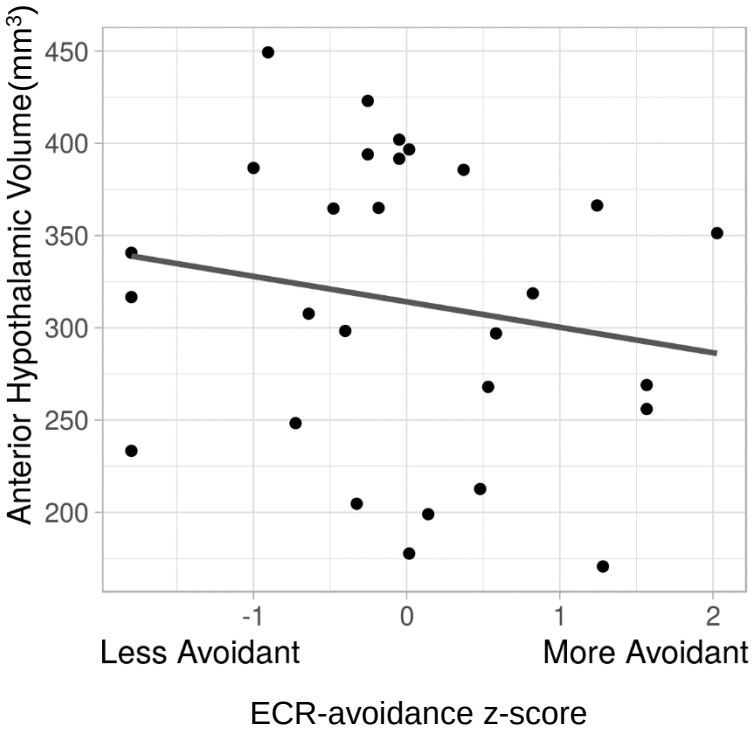

Figure 5. We found a negative trend between ECR-avoidance score and anterior hypothalamic volume (Beta=-.315) while controlling for father age and total brain volume.

As above, we applied an FDR correction for multiple comparisons (threshold=.05) to our set of exploratory tests. Specifically we corrected for four tests: the significance test of each full regression model (2 tests) and for the significance test of the anxiety or avoidance variable within each model respectively (2 tests). The results which were previously significant remained so under this correction (overall model for ECR-anxiety and anterior hypothalamic volume: *p*=.038; ECR-anxiety specifically: *p*=.038)

4. Discussion

In this study, we found evidence to evaluate the following attachment hypotheses about fathers: secure attachment would be positively related to total hypothalamic volume (A1), secure attachment would be positively related to anterior hypothalamic volume (A2) and avoidant attachment would be negatively related to anterior hypothalamic volume (A4). Furthermore, we evaluated two caregiving hypotheses. First, that secure caregiving would be positively related to total hypothalamic volume (C1) and second, that secure caregiving would be positively related to anterior hypothalamic volume (C2).

We found evidence to support hypothesis A2, that fathers' attachment security would be positively related to anterior hypothalamic volume. In a set of exploratory analyses, we observed that anterior hypothalamic volume may be negatively related to attachment anxiety. Additionally we found a trend-level negative relationship between attachment avoidance and anterior hypothalamic volume. While this evidence is in partial support of hypothesis A4, this result should be interpreted cautiously as the treatment of the variables was determined after examination of the data. We found no support for our caregiving hypotheses and no support for our hypotheses regarding total hypothalamic volume. Each finding is discussed in detail below.

4.1 Evaluation of Attachment Findings

4.1.1 Hypotheses about Attachment Security

We found that in a sample of fathers of five-year old children, attachment security was positively related to anterior hypothalamic volume while controlling for total brain volume and father age. This regression model explained approximately 30% of the variance in anterior hypothalamic volume in our sample and initially reached statistical significance. However, it did

not survive an FDR correction for multiple comparisons. Despite this, the high R^2 value effectively supports our hypothesis. Our finding in human fathers is consistent with previous findings in human mothers which support that greater attachment security typically coincides with greater hypothalamic volume (Kim et al., 2010). One difference however, is that our findings were specific to the anterior hypothalamus and not for the hypothalamus as a whole. We acknowledge that, at present, there are a multitude of ways to measure the volume of sub-cortical structures including voxel-based morphometry (Kim et al., 2010, 2014), automated and semi-automated algorithms (Wolff et al., 2018), and manual parcellation (Makris et al., 2013). To our knowledge, no published studies have used volumetric parcellation to examine the relationship between hypothalamic volume and adult attachment. We believe that the varied methodological approaches may be partially responsible for the variance in research outcomes available in the literature. In our case, manual volumetric parcellation of the hypothalamus allowed us to separate the structure into three functional sub-regions and thus, we were able to have a more fine-grained look at the potential role of the anterior hypothalamus in attachment security than other available methods. While we believe that our finding of a positive relationship between attachment security and anterior hypothalamic volume to be theoretically well-supported, we acknowledge that our study's lack of support for a positive relationship between attachment security and total hypothalamic volume is discrepant with previous literature. As such, we believe that a necessary future direction is a direct replication of our findings, as well as near replication with samples from more varied populations.

4.1.2 Attachment Anxiety and Avoidance

This study also found that attachment anxiety is negatively related to anterior

hypothalamic volume. This is a very novel finding given that, to our knowledge, relationships between attachment anxiety and the hypothalamus have not been previously examined. Specifically, our regression model for attachment anxiety, which controlled for father age and total brain volume, explained nearly 34% of the variance in anterior hypothalamic volume in our sample and reached statistical significance. Moreover the finding survived an FDR correction for multiple comparisons, indicating a lower probability that this result arises as a type I error. Given the exploratory nature of this part of our investigation and the relative novelty of the finding, direct methodological replication and re-testing of this model in a hypothesis-driven study is necessary to corroborate this discovery.

Regarding attachment avoidance, we observed a trend-level, negative relationship to anterior hypothalamic volume. In our sample of fathers, the model for attachment avoidance while controlling for total brain volume and father age explained approximately 25% of the variance in anterior hypothalamic volume. While our model did not reach statistical significance, the trend we observed is in line with findings from a previous study in non-fathering men which showed a negative relationship between hypothalamic volume and avoidant-like traits. As discussed in section 4.1.1 above, our findings diverge from previous studies in that we observed a relationship between attachment avoidance and the anterior sub-region of the hypothalamus rather than the hypothalamus as a whole.

At the start of our study, we hypothesized that avoidant attachment would be negatively related to both total hypothalamic volume (A3) and anterior hypothalamic volume (A4). However, during data exploration, we found that our variables for attachment avoidance and attachment anxiety were highly collinear. This precluded us from performing a regression analysis containing both anxiety and avoidance as simultaneous predictors and thus we do not

consider our findings with avoidant attachment to be hypothesis driven. As such, these hypotheses should be investigated as part of a future replication study.

Another facet of the collinearity of anxiety and avoidance in our sample is that most subjects scored relatively low in both dimensions of the ECR. In other words, we had a sample of fathers who are securely attached to their romantic partners. Given this, it may be that repeating this investigation in a more attachment diverse sample (ie. a sample of healthy fathers with a broader range of anxious and avoidant attachment traits) would yield a stronger effect for the relationship between both avoidance and anxiety, and anterior hypothalamic volume. However, this interpretation should be considered cautiously as it presses on one of the main limitations of regression analysis: limited scope. It may be that a multiple linear regression describes our data from secure fathers relatively well but would not fit a broader range of values for the ECR variables we examined. Given the lack of current literature on attachment anxiety and the hypothalamus, we are both excited and skeptical of the present finding and look forward to re-testing this new hypothesis in the future with a more attachment diverse sample. On the other hand, while our model for attachment avoidance did not reach significance, we believe that there is a strong theoretical argument for its extension in an attachment diverse sample. To our knowledge, two studies have reported differences in either hypothalamic structure (Tost et al., 2010) and or function (Strathearn et al., 2009) on the basis of avoidant attachment traits. Again, we look forward to the replication of our models to further test this hypothesis.

4.2 Evaluation of Caregiving Hypotheses

We began this investigation with four hypotheses related to caregiving, namely that C1) Secure caregiving will be positively related to total hypothalamic volume, C2) Secure caregiving

will be positively related to anterior hypothalamic volume, C3) Avoidant caregiving will be negatively related to total hypothalamic volume, and C4) Avoidant caregiving will be negatively related to anterior hypothalamic volume. However, as described above, our caregiving measure did not yield a clear scale for avoidant caregiving as initially expected and as such, we were not able to test hypotheses C3 and C4 in this study.

Regarding hypotheses C1 and C2, we found no evidence to support a relationship between secure caregiving and either total hypothalamic volume or anterior hypothalamic volume in fathers. Additionally during data exploration, we found no correlation between the ECR and CEQ variables. While it may be that this is a true null result, this would be in contradiction to consensus across research in both humans and non-human animals indicating a relationship between caregiving and the hypothalamus (Bridges et al., 1999; Kim et al., 2010, 2014; Strathearn et al., 2009). Moreover, given that the attachment and caregiving systems are inter-related (N. L. Collins et al., 2010; Mikulincer & Shaver, 2007), it seems unlikely that we would observe a relationship with hypothalamic volume for one variable but not the other. This outcome could be attributed to measurement error in the CEQ or the low sample size (N=28) for this preliminary study. Additional limitations are discussed in the following section.

4.4 Strengths, Limitations and Future Directions

4.4.1 Measurement of Attachment and Caregiving

One of the primary limitations of this study comes from the restrictions imposed by the self-reported caregiving and attachment measures. The ECR is a reliable measure of adult attachment (Fairchild & Finney, 2006; Sibley, Fischer, & Liu, 2005). In our case, the limitation of this measure lies in the fact that our sample of interest was fathers and the ECR is not

designed to elicit information about adults' experiences in childhood attachment relationships or to obtain details about an adult's caregiving relationship to a child. While the ECR is still informative about, in our case, a father's current attachment relationship to a romantic partner, it is not informative about the other types of attachment relationships that a father has had throughout life such as with parents or previous romantic partners. This is important because, in recent years, evidence has emerged to suggest that attachment orientation varies between attachment relationships for a given individual (Liu, 2008). As such, an individual does not have one single attachment style. Rather, an individual's attachment orientation as we have discussed it in this manuscript may be a culmination of the styles that the individual employs within and between attachment relationships. To truly understand the relationship between attachment orientation and the hypothalamus, we must examine patterns of attachment behavior across attachment relationships. To this end, further attachment studies should employ multiple modes of measurement for attachment orientation. The larger DCARE study includes such a measure, the Adult Attachment Interview (AAI; George, Kaplan, & Main, 1996). However, results from this measure were not available at the time when data analysis for the study of the hypothalamus occurred. One future direction to expand the findings of the present study and to contribute to collective knowledge about the stability versus specificity of attachment orientation across relationships is to compare the results of the ECR-R and the AAI for the subjects in our study.

Additionally, this study sheds light on the need for an attachment-informed measure of caregiving orientation that is comparable to an existing measure of attachment such as the ECR. This imagined caregiving measure would therefore yield a score for avoidant caregiving and a score for anxious caregiving with a low score on both dimensions being regarded as caregiving security. In this way, comparable measures of adult attachment and caregiving could be obtained

in future studies of parents. From a statistical perspective, having similarly structured measures of caregiving and attachment would allow for parsimonious integration of these measures in future models of caregiving and attachment.

4.4.2 Design of the present study

This study is cross-sectional and thus provides no evidence for a direction of causality, ie. that differences in anterior hypothalamic volume cause differences in attachment orientation or on the contrary, that differences in attachment orientation cause hypothalamic volume. However, we speculate that the co-occurrence of individual difference in anterior hypothalamic volume and attachment orientation may be reflective of relevant differences in the underlying functional processes of the hypothalamus. In the future, the SSFH Lab plans to integrate findings from the structural study of the hypothalamus in fathers with findings from the other functional fMRI tasks used in the larger D-CARE study. This may help to close the gap in our understanding of the connection between hypothalamic structure and function in humans and specifically, the role of the hypothalamus in paternal attachment and caregiving.

4.4.3 Manual Volumetric Parcellation

In the present study, we successfully implemented manual volumetric parcellation of the human hypothalamus with a high degree of inter-rater reliability. This makes us hopeful for the use of this method in future studies. However, this method is not without its drawbacks. At present, manual volumetric parcellation is time and labor intensive. Using our study as a rough guide, each brain requires a cumulative 3 hours to segment the hypothalamus split across three raters. Additionally, to achieve high inter-rater reliability, it was necessary that multiple raters

rate each brain and take the average of measures. The use of average measures and a corresponding average measures ICC ensured that inter-rater reliability was of sufficient quality. Unfortunately, the flagship study for this method (Makris et al.) did not publish exact details for the ICC used (ie. average measures or single measures) which makes comparisons of inter-rater reliability across studies difficult.

Future studies employing this method are advised to consider the time and labor investment needed to collect high-quality data and to report exact details of the ICC used to assess inter-rater reliability. Alternative methods are also available (ie. semi-automated method Wolff et al., 2018 and whole-brain based voxel-based morphometry Kim et al., 2010, 2014) however, our study illustrates that manual volumetric parcellation of the hypothalamus is a viable method to assess hypothalamic volume in future studies.

4.4.4 Stronger and more specific hypotheses

Though we had a largely hypothesis driven study, we did not make any specific predictions as to the magnitude of difference we would observe in hypothalamic volume by attachment orientation. Using the information gleaned in this study, a subsequent replication/ extension study could re-test the models we used here and specifically look for meaningful differences in the magnitude of difference in hypothalamic volume observed across the sample.

4.5 Conclusions

We speculate that the differences we observed in hypothalamic volume may reflect differences in hypothalamic function which ultimately underlie inter-individual differences in paternal attachment. This claim is corroborated by several existing studies which indicated

attachment-related differences in hypothalamic volume (Kim et al., 2010; Tost et al., 2010) and function (Strathearn et al., 2009). These suggested differences in hypothalamic function might be related to the role of the hypothalamus in oxytocin production which, in turn, relates to control of the SApNS and social approach behavior. To be even more specific, differences in human hypothalamic structure and function detected via MRI may be indicative of underlying differences in the size or function of one or more hypothalamic nuclei. Considering findings from studies on the hypothalamus in rodents, we speculate that in the case of the anterior hypothalamus, volumetric differences could be indicative of differences in the supraoptic or paraventricular nuclei and perhaps differences in functional oxytocin production in these structures. In summary, the present study contributes to a growing pool of knowledge about the neural substrates of attachment orientation in an under-studied group: fathers. Our study has provided evidence that the anterior hypothalamus may be source of inter-individual differences in attachment in fathers.

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Appendix A

Parameters for MPRAGE Acquisition

Eigenschaften

Prio Rekonstr.	Aus
Bilder in den Viewer laden	Aus
Automatischer Kinostart	Aus
Automatische Bildspeicherung	Ein
Bilder in die Mini-Segmente laden	Aus
Bilder in großes Bildsegment laden	Aus
Inline-Anzeige automatisch öffnen	Aus
Inline-Anzeige automatisch schließen	Aus
Start measurement without further preparation	Aus
Auf Start duch Benutzer warten	Ein
Start measurements	Einmal messen

Routine

3D-Block-Gruppe	1
3D-Blöcke	1
Distanzfaktor	50 %
Position	Isozentrum
Orientierung	Sagittal
Phasenkod.-Richt.	A >> P
AutoAlign	---
Phasen-Oversampling	0 %
Schicht-Oversampling	0,0 %
Schichten im 3D-Block	176
FoV Auslese	256 mm
FoV Phase	93,8 %
Schichtdicke	1,00 mm
TR	2300,0 ms
TE	2,98 ms
Mittelungen	1
Verknüpfungen	1
Filter	Verzeichn. Korr.(3D), Prescan Normalisierung
Spulenelemente	HEA;HEP

Kontrast - Allgemein

TR	2300,0 ms
TE	2,98 ms
Magn. Präparation	Nichtsel. IR
TI	900 ms
Flipwinkel	9 Grad
Fettunterdr.	Keine
Wasserunterdr.	Keine

Kontrast - Dynamisch

Mittelungen	1
Mittelungsmodus	Langzeit
Rekonstruktion	Betrag
Messungen	1
Mehrere Serien	Aus

Auflösung - Allgemein

FoV Auslese	256 mm
FoV Phase	93,8 %
Schichtdicke	1,00 mm
Basis-Auflösung	256
Phasen-Auflösung	100 %
Schicht-Auflösung	100 %
Phasen Partial Fourier	Aus
Schicht Partial Fourier	Aus

Auflösung - Allgemein

Interpolation	Aus

Auflösung - iPAT

PAT Modus	Keiner

Auflösung - Filter Bild

Image Filter	Aus
Verzeichn. Korr.	Ein
Modus	3D
Ungefilterte Bilder	Ein
Prescan Normalisierung	Ein
Ungefilterte Bilder	Aus
Normalisierung	Aus
B1-Filter	Aus

Auflösung - Filter Rohdaten

Rohdaten	Aus
Elliptischer Filter	Aus

Geometrie - Allgemein

3D-Block-Gruppe	1
3D-Blöcke	1
Distanzfaktor	50 %
Position	Isozentrum
Orientierung	Sagittal
Phasenkod.-Richt.	A >> P
Schicht-Oversampling	0,0 %
Schichten im 3D-Block	176
FoV Auslese	256 mm
FoV Phase	93,8 %
Schichtdicke	1,00 mm
TR	2300,0 ms
Mehrschichtmodus	Einzelmess.
Serie	Verschachtelt
Verknüpfungen	1

Geometrie - AutoAlign

3D-Block-Gruppe	1
Position	Isozentrum
Orientierung	Sagittal
Phasenkod.-Richt.	A >> P
AutoAlign	---
Initiale Position	Isozentrum
L	0,0 mm
P	0,0 mm
H	0,0 mm
Initiale Rotation	0,00 Grad
Initiale Orientierung	Sagittal

Geometrie - Navigator

System - Verschiedenes

Positionierungsmodus	REF
Tischposition	H
Tischposition	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Kanalkombination	Adaptive Combine

Appendix B

Protocol for Manual Volumetric Parcellation of the Human Hypothalamus

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/FreeSurfer** type:

   ```
   FS
   ```

3. Navigate to the desired directory

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

4. Open the subject

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

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

6. 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.

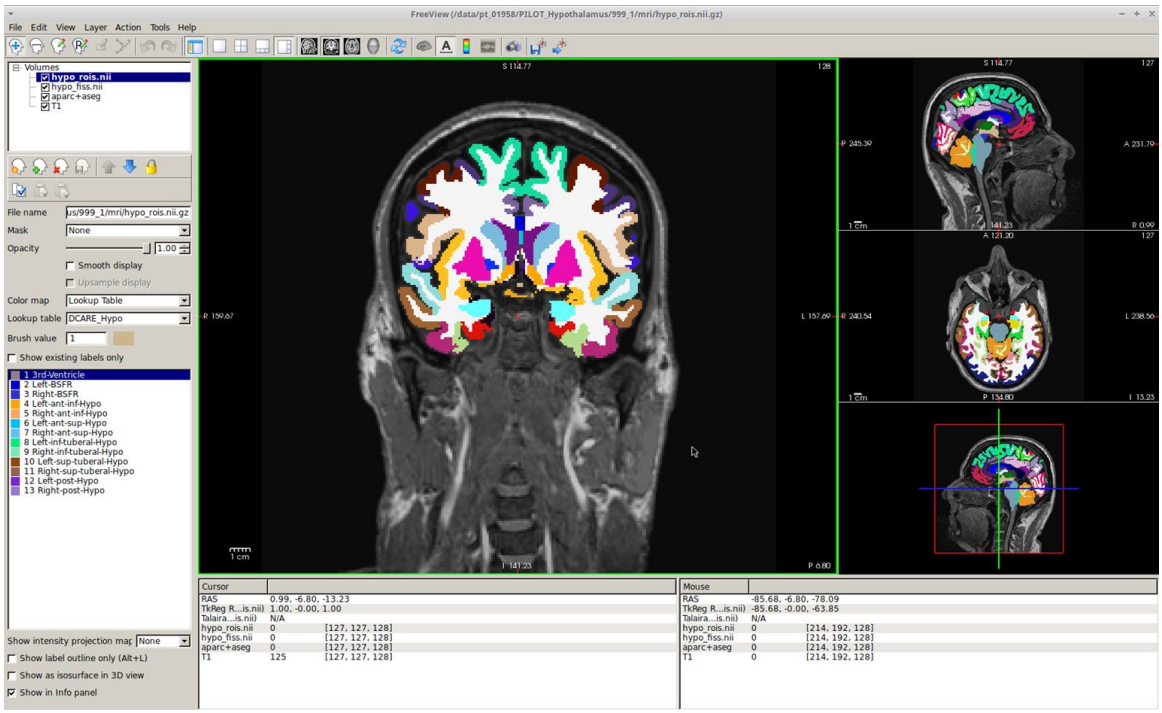

Figure 1. The initial view when opening a subject in FreeView

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.

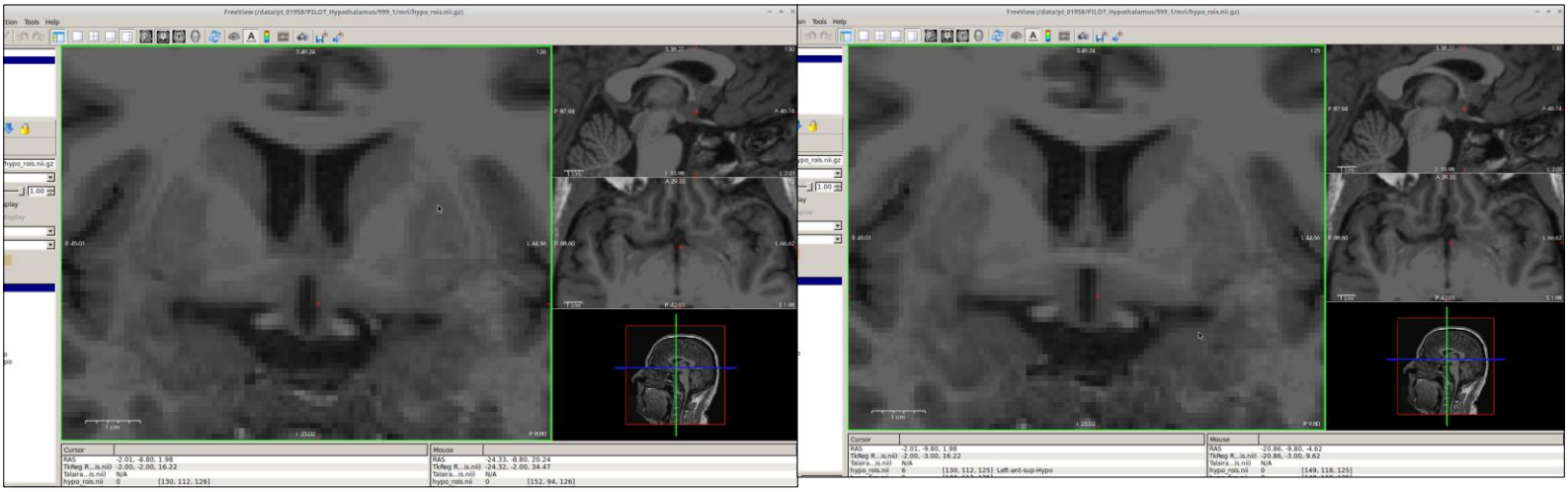

Figure 2a. From Left: 1) The AC is emerging but not yet continuously visible. 2) Moving one slice posterior, the AC is now continuously visible. This would be the first Anterior slice.

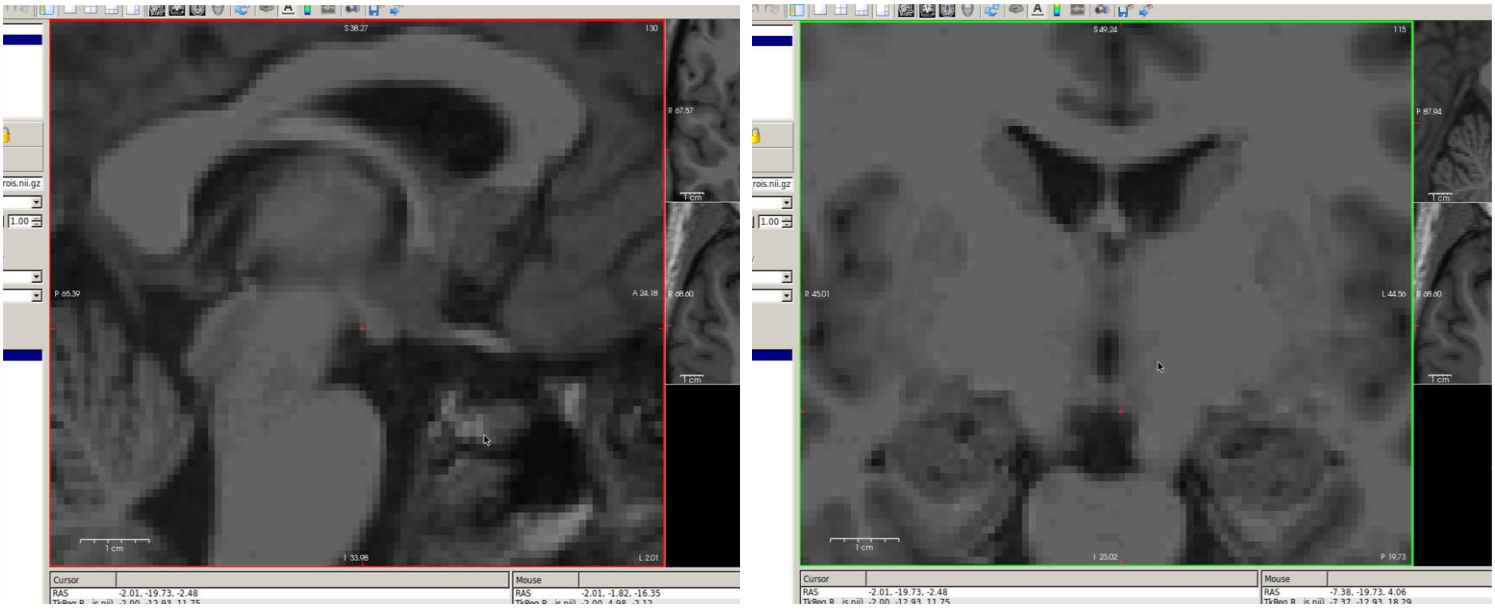

Figure 2b. Locating the most Posterior slice. From Left: 1) In saggital view, put the crosshairs on the last voxel of the MB. 2) Without moving the crosshairs, return to coronal view. This is the last Posterior slice.

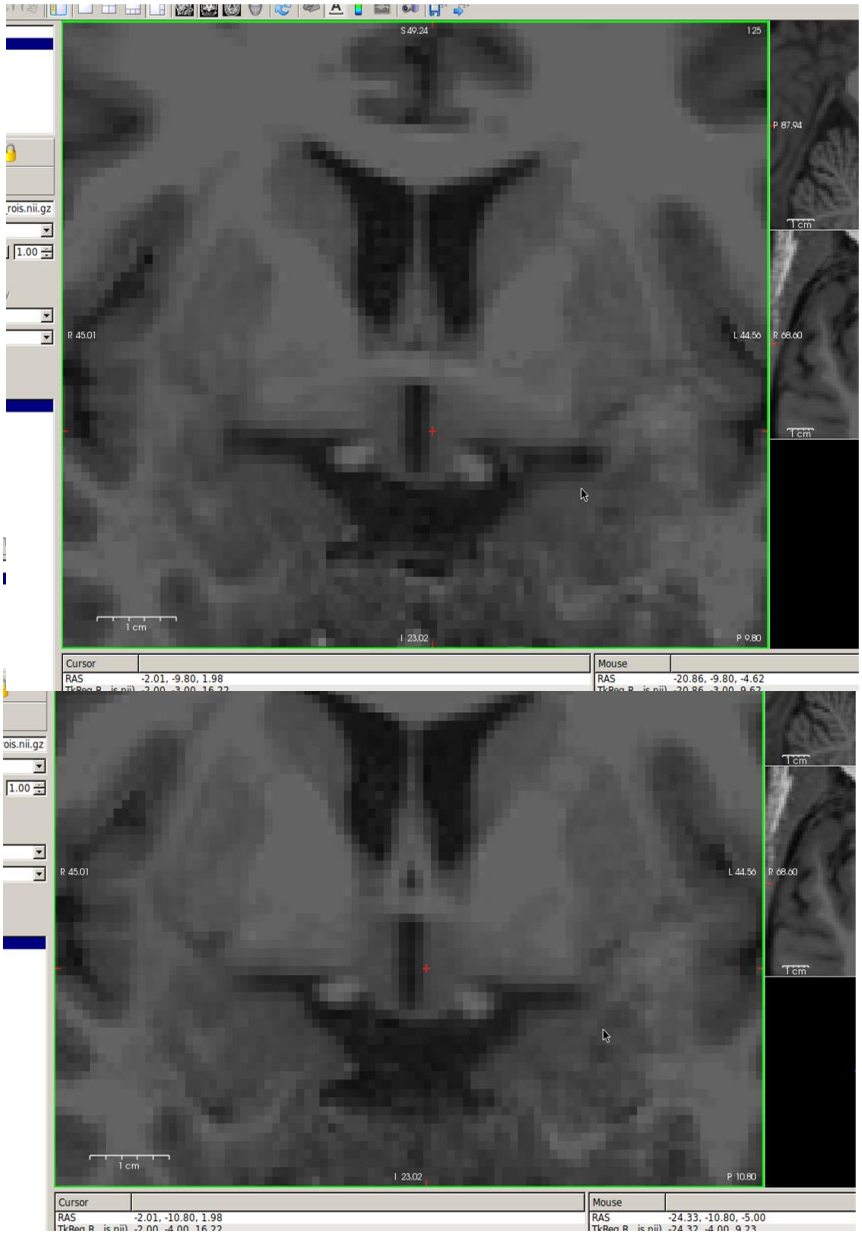

Figure 4a: Transition from Anterior to Tuberal Hypothalamus. From top:

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.

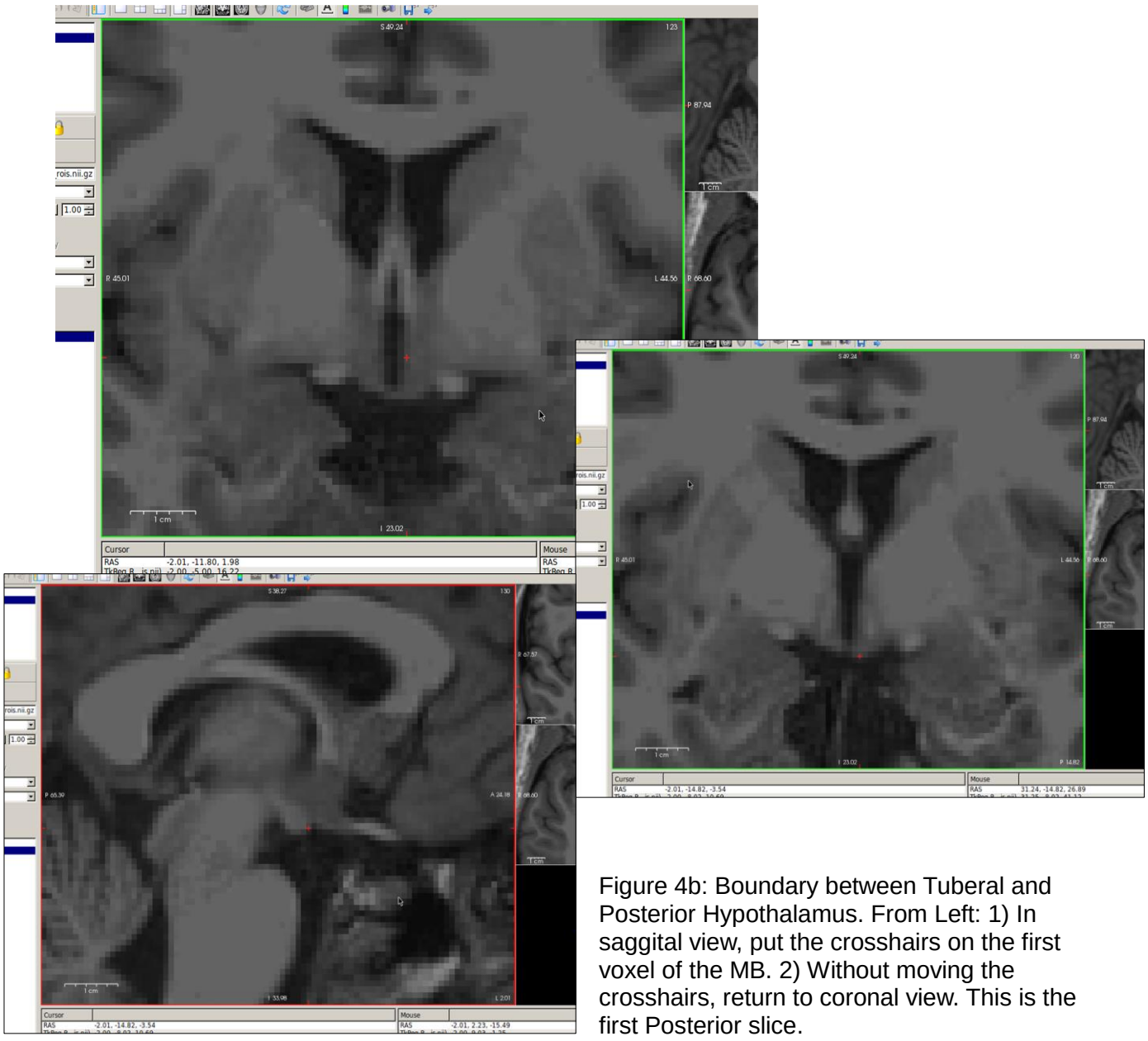

Figure 4b: Boundary between Tuberal and Posterior Hypothalamus. From Left: 1) In saggital view, put the crosshairs on the first voxel of the MB. 2) Without moving the crosshairs, return to coronal view. This is the first Posterior slice.

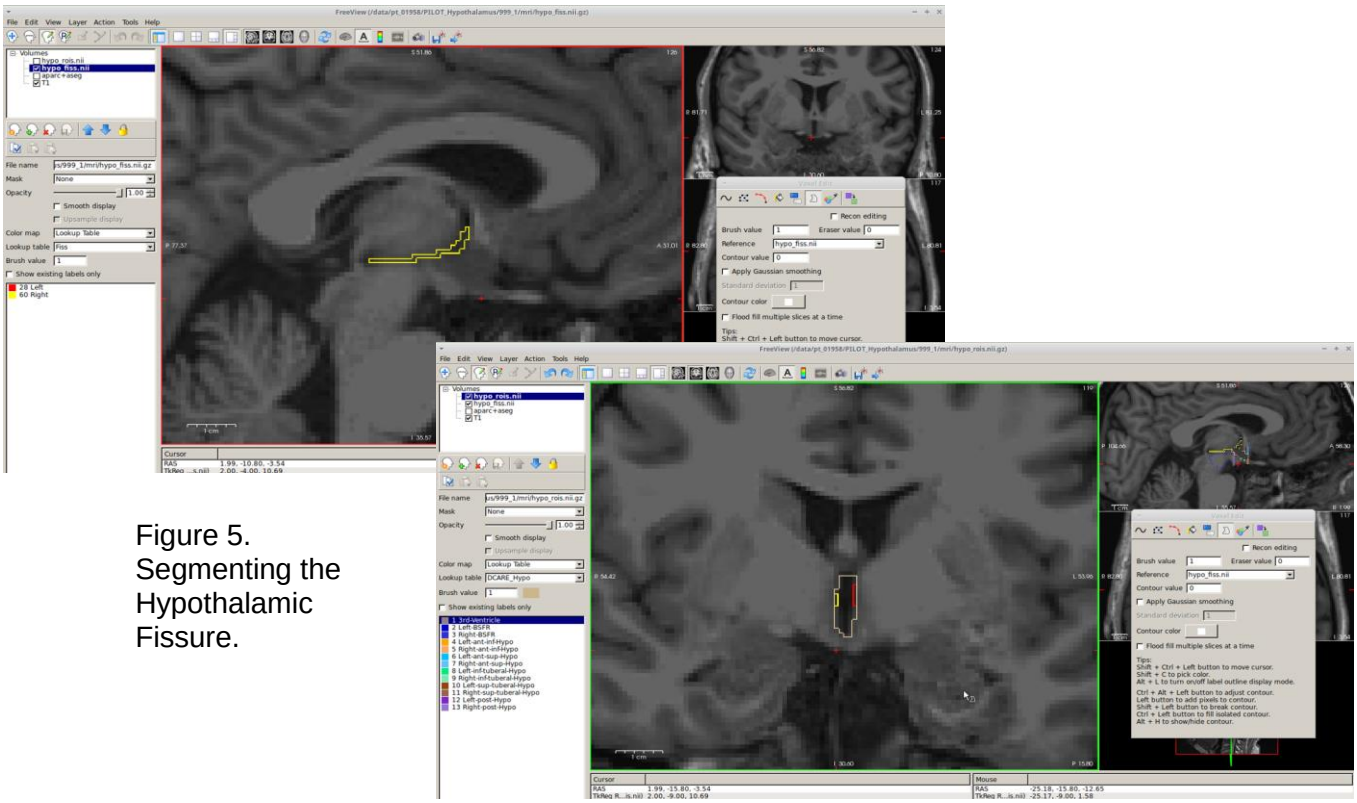

Figure 5. Segmenting the Hypothalamic Fissure.

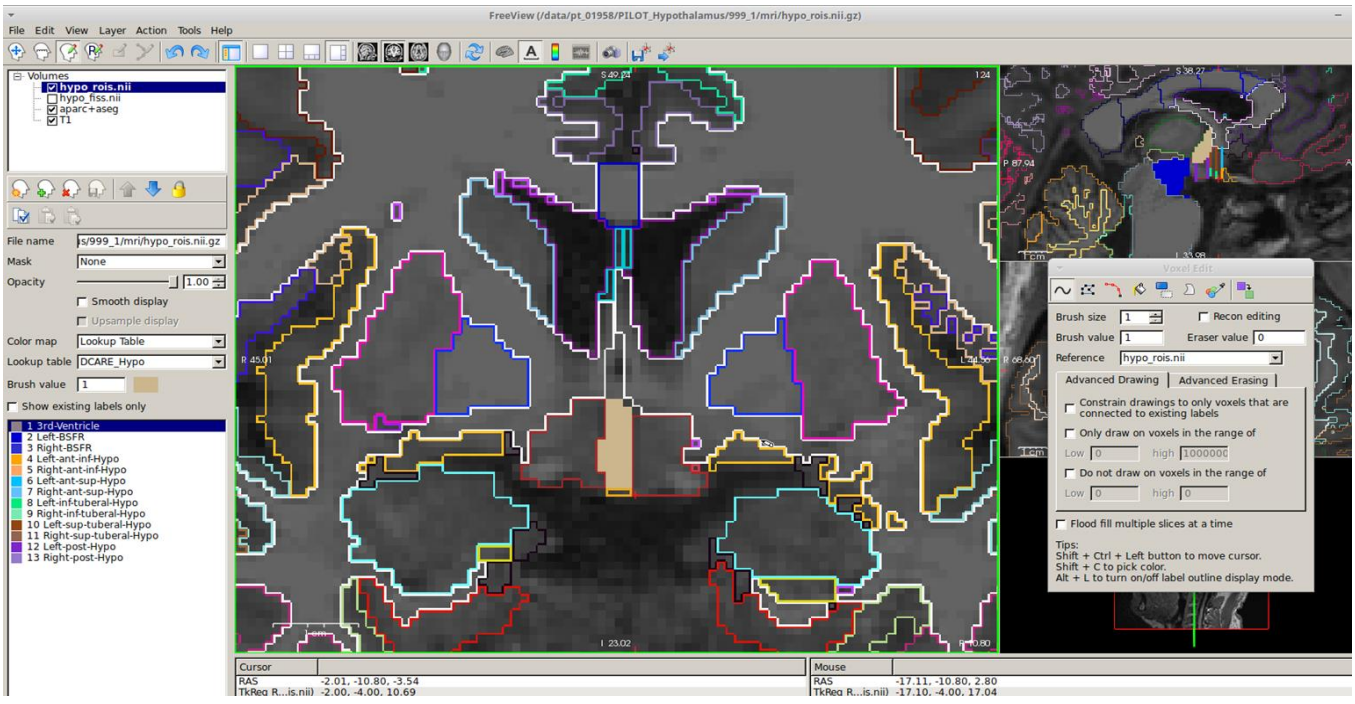

Figure 6. Segmenting the Third ventricle using aparc+aseg overlay as a guide.

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

Ctrl+Alt+left mouse button, then move mouse to adjust contour value