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Exploring neuroendocrine influences on the sensorimotor-association axis in a female and a male individual

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ABSTRACT

Human neuroimaging studies consistently show multimodal patterns of variability along a key principle of macroscale cortical organization—the sensorimotor-association (S-A) axis. However, little is known about day-to-day fluctuations in functional activity along this axis within an individual, including sex-specific neuroendocrine factors contributing to such transient changes. We leveraged data from two densely sampled healthy young adults, one female and one male, to investigate intra-individual daily variability along the S-A axis, which we computed as our measure of functional cortical organization by reducing the dimensionality of functional connectivity matrices. Daily variability was greatest in temporal limbic and ventral prefrontal regions in both participants, and was more strongly pronounced in the male subject. Next, we probed local- and system-level effects of steroid hormones and self-reported perceived stress on functional organization. Beyond shared patterns of effects, our findings revealed subtle and unique associations between neuroendocrine fluctuations and intra-individual variability along the S-A axis in the female and male participants. In sum, our study points to neuroendocrine factors as possible modulators of intra-individual variability in functional brain organization, highlighting the need for further research in larger samples to assess the sex specificity of these effects.

Keywords: brain organization, functional connectivity, steroid hormones, dense sampling, individual variability

1. INTRODUCTION

Patterns of functional connectivity are considered to be broadly stable, trait-like features of the human brain, both within and between individuals (Damoiseaux et al., 2006; Gratton et al., 2018; Power et al., 2011). In particular, ubiquitous patterns of functional connectivity across cortical structure and function seem to reflect a major principle of brain organization, also known as the sensorimotor-association (S-A) axis (Margulies et al., 2016; Sydnor et al., 2021). More specifically, this axis of functional organization differentiates unimodal primary regions, such as the visual and the sensorimotor cortices, from heteromodal association regions involved in higher order cognitive functions, such as regions in frontal, parietal, and temporal cortices, including the medial prefrontal cortex, superior temporal sulcus, and precuneus. However, beyond the

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consistency and robustness of functional networks arranged along this axis lies a subtle—yet notable—degree of intra-individual variability (Park et al., 2012), suggesting that the S-A axis also has dynamic properties, even at rest. Given that the brain is an endocrine organ, susceptible to transient endogenous fluctuations in the levels of different steroid hormones across sexes (Banks, 2012), such fluctuations may influence the dynamic reconfiguration of functional networks underpinning intra-individual variability, ultimately supporting flexible cognition and behavior (Shine & Poldrack, 2018). Neuroendocrine processes are thus likely involved in variability of functional brain organization within an individual in a sex-specific manner (Shansky & Murphy, 2021)—yet how remains unclear.

In the adult mammalian endocrine system, the production of gonadal steroid hormones differs between the sexes. In females of reproductive age, a major source of daily variability in gonadal steroids is dictated by the ovarian cycle, which is responsible for the cyclical production of estradiol and progesterone over the 4–5-day rodent estrous cycle and the monthly human menstrual cycle (Eliot et al., 2023). In humans, both sexes are also subject to cyclical changes in endogenous steroid hormone levels following the 24-h circadian rhythm, whereby testosterone and cortisol production peaks in the morning and steadily declines throughout the day (Dabbs Jr, 1990; Fries et al., 2009). Although steroid hormones are not exclusive to either sex, females generally present higher concentrations of estrogens and progesterone, and males generally present higher concentrations of testosterone (Bale & Epperson, 2017), which explains why research primarily focuses on the predominant hormones of each sex accordingly. Despite these substantial differences in steroid hormone concentrations between males and females, we lack a formal understanding of how sex-specific neuroendocrine mechanisms may interact with human brain organization.

Cross-species evidence points to steroid hormones as potent neuromodulators. Receptors for steroid hormones are expressed throughout the brain, particularly in the hippocampus and medial temporal lobe (González et al., 2007; Loy et al., 1988; Meffre et al., 2013). A foundational study in female rats detected a 30% increase in dendritic spine density of hippocampal CA1 pyramidal neurons on the day of ovulation (peak estradiol) relative to 24 h later (peak progesterone) (Woolley et al., 1990), suggesting estradiol's role in enhancing synaptic plasticity in CA1 neurons (Brinton, 2009; Galea et al., 2017; Hao et al., 2003; Hara et al., 2015), while progesterone appears to inhibit this effect (Woolley & McEwen, 1993). Androgens, such as testosterone, also appear to influence medial temporal lobe morphology, for example, by inhibiting apoptosis in hippocampal neurons (Nguyen et al., 2010). Similarly, findings in humans have linked gonadal steroid levels to changes in brain structure, for example, through effects of estradiol and progesterone levels on hippocampal morphology over the menstrual cycle (Taylor et al., 2020; Zsido et al., 2023). Studies have also reported associations between diurnal steroid hormone fluctuations (including testosterone, estradiol, and cortisol) and total brain volume, gray matter volume, and cortical thickness (Murata et al., 2024), as well as associations between testosterone levels and cortical thickness during puberty in regions with high androgen receptor density (Bramen et al., 2012). Moreover, functional magnetic resonance imaging (fMRI) studies have revealed associations between human steroid hormone levels and functional brain activity at rest. Studies with samples varying in size and sampling frequency suggest that changes in functional connectivity in women are associated with fluctuating levels of endogenous steroid hormones, such as estradiol and progesterone, over the menstrual cycle (Arélin et al., 2015; Avila-Varela et al., 2024; Hidalgo-Lopez et al., 2021), as well as contraceptive-dependent levels of exogenous steroid hormones (Engman et al., 2018; Petersen et al., 2014). In men, group analyses have revealed changes in resting-state network connectivity related to exogenous increases in testosterone levels (Votinov et al., 2020; Westlye et al., 2017). Although considerable evidence from animal and human research supports the role of gonadal steroid hormones in modulating brain structure and function, whether and how sex-specific endogenous fluctuations in steroid hormones contribute to daily variability in functional brain organization remains poorly understood.

Gonadal hormones further have the ability to modulate the stress response through tight interactions between the hypothalamic–pituitary–gonadal (HPG) and hypothalamic–pituitary–adrenal (HPA) axes, which are the neuroendocrine axes, respectively, producing gonadal and adrenal (i.e., cortisol) hormones (Viau, 2002). As such, gonadal steroids are thought to contribute to sex differences in the stress response through their activational and organizational effects on the brain throughout the lifespan (Bale & Epperson, 2015). For example, circulating estradiol levels in female rodents appear to elevate cortisol levels during both threatening and non-threatening situations, leading to a more robust HPA axis response relative to male rodents (Oyola & Handa, 2017). In humans, estradiol levels have also been shown to modulate healthy female functional activity across key regions of the stress circuitry, including the hippocampus, bilateral amygdala, and hypothalamus—an effect that was not observed in women with major depressive disorder, suggesting an association between affective dysfunction and the dysregulation of hormonal effects on stress-related activity (Jacobs et al.,

2015). In fact, given that stress contributes to mechanisms of plasticity and vulnerability by physiologically remodeling neural architecture (McEwen et al., 2015), sex differences in the stress response are thought to contribute to differences in the prevalence of affective psychiatric disorders (Oyola & Handa, 2017). Moreover, cortisol responsivity seems to both vary (Maki et al., 2015) and differentially interact with perceived stress (Duchesne & Pruessner, 2013) at different stages of the menstrual cycle, highlighting the importance of also considering effects related to subjective self-reported cognitive experience. As such, psychosocial and physiological stress levels should be considered as potential neurocognitive and neuroendocrine factors affecting dynamic changes in functional brain organization via sex-specific mechanisms.

Over the last decade, dense sampling has emerged as a method to investigate the stability and variability of functional brain organization by repeatedly scanning smaller sets of individuals across longer periods of time. Based on the premise that not enough neuroimaging data are collected per individual—yielding estimates with high measurement error (Poldrack, 2021)—recent initiatives such as the MyConnectome Project (Laumann et al., 2015; Poldrack et al., 2015) and the Midnight Scan Club (Gordon et al., 2017) have demonstrated the utility of dense sampling, further inspiring the collection of several related precision fMRI datasets, reviewed in Gratton et al. (2020). These studies revealed fine-grained features unique to the individual, adding a layer of detail and specificity that is otherwise overlooked in group-averaged data (Poldrack et al., 2015). Aiming to demonstrate the reliability of resting-state functional connectivity patterns, these pioneering studies focused on assessing the within-subject stability—rather than variability—of the functional connectome (Gratton et al., 2018; Seitzman et al., 2019). As such, they did not investigate co-varying factors and mechanisms that may contribute to day-to-day intra-individual variability in functional brain activity, nor did they investigate the effects of sex as a biological variable in their analyses (Shansky & Murphy, 2021). In fact, many dense sampling studies so far have focused their analyses on fMRI data without probing underlying mechanisms or behavioral associations—with some exceptions (e.g., reporting associations between mood fluctuations and functional connectivity patterns; Mirchi et al., 2019).

Recently, a dense sampling and deep phenotyping approach has been applied on a 23-year-old female (28&Me study; Pritschet et al., 2020) and a 26-year-old male (28&He study; Grotzinger et al., 2024), who were tested over 30 consecutive days in time-locked study sessions including brain imaging, venipuncture, salivary sampling, and self-report mood questionnaires. These studies—as well as subsequent studies using the female dataset (e.g., De Filippi et al., 2021; Fitzgerald et al., 2020; Greenwell et al., 2023; J. M. Mueller et al., 2021)—measured day-to-day changes in functional brain activity, reporting associations between hormonal fluctuations and the reorganization of functional networks. However, neither of these studies have been used to directly compare intra-individual variability across sexes, nor have sex-specific research designs been applied to probe and compare neuroendocrine effects in the female and male subjects in relation to major principles of brain organization, such as the S-A axis. In fact, increasing evidence supports the premise of using low-dimensional measures of functional connectivity to study variations in sensory-to-association hierarchical patterns of intrinsic cortical organization (Bernhardt et al., 2022; Huntenburg et al., 2018; Margulies et al., 2016; Royer et al., 2024). Conceptually, the S-A axis has been shown to reflect both developmental (Sydnor et al., 2021) and evolutionary (Valk et al., 2022; Xu et al., 2020) mechanisms, aligning with microstructural variation (Burt et al., 2018; Paquola & Hong, 2023; Saberi et al., 2023; Valk et al., 2022), as well as capturing organizational differences between the sexes (Serio et al., 2024). Methodologically, the S-A axis has demonstrated suitable levels of reproducibility, predictive validity, and test–retest reliability (Hong et al., 2020; Knodt et al., 2023). As such, studying daily intra-individual variability along the S-A axis as well as associated unique neuroendocrine factors in a female and a male would allow to contextualize subtle intra-individual changes in the functional connectome at a meaningful organizational level.

In the current work, we capitalize on a dense sampling approach to investigate intra-individual variability along the S-A axis in two healthy young adults, one male and one female, from the aforementioned openly available datasets (Grotzinger et al., 2024; Pritschet et al., 2020), probing and comparing both distinct and shared female and male neuroendocrine factors (i.e., steroid hormone levels), as well as perceived stress, associated with daily variability in functional brain organization. We first applied a dimensionality reduction algorithm to daily functional connectivity matrices in order to compute the S-A axis. After quantifying intra-individual variability along the S-A axis, we directly compared patterns of variability between the participants, and further decoded these patterns with publicly available multimodal brain maps. Next, we probed local- and system-level effects of day-to-day changes in hormone levels and perceived stress on the S-A axis in both participants. Here, we conducted two sets of analyses probing different forms of potential sex specificity by design. First, we specifically assessed effects of steroid hormones that are most predominant

within each sex (i.e., estradiol and progesterone in the female participant, testosterone in the male participant), as well as cortisol in the male participant given its availability and given that its production follows circadian fluctuation patterns similar to testosterone. Second, we tested for effects of common steroid hormones (i.e., estradiol and testosterone), allowing a direct comparison of effects across the female and male participants. As such, rather than systematically testing for statistical differences between the sexes, our study design capitalizes on sex as a biological variable to investigate particularly relevant as well as common neuroendocrine factors that may underpin intra-individual variability along a major principle of functional cortical organization in female and male single individuals.

2. METHODS

The current study relies on the use of open data, whose methods have already been reported elsewhere in detail (see Grotzinger et al. (2024) and Pritschet et al. (2020) for the original publications).

2.1. Participants and study design

Our sample (N = 2) consisted of one female (23 years; data available at https://openneuro.org/datasets/ds002674/versions/1.0.5; Pritschet et al., 2020) and one male (26 years; data available at https://openneuro.org/datasets/ds005115/versions/1.0.0; Grotzinger et al., 2024), both right-handed and Caucasian, with no history of endocrine disorders, neuropsychiatric diagnoses, or head injuries. The female participant reported a history of regular menstrual cycles (occurring every 26–28 days on average, with no missed periods). As such, through 30 consecutive days of data collection, the study design and duration aimed to capture the full breadth of a menstrual cycle, in order to capture the full range of possible variation in endogenous estradiol and progesterone levels. Effectively, since the first day of data collection was not aligned with a specific day or phase of the menstrual cycle, the experimental sessions spanned two cycles. The female participant also refrained from taking hormone-based medication in the 12 months preceding data collection. Participants gave written informed consent for studies that were originally validated by the University of California, Santa Barbara Human Subjects Committee.

The original study designs for the collection of the female and male data slightly differed and are fully reported in Pritschet et al. (2020) and Grotzinger et al. (2024), respectively. Here, we report the original and complete study designs although we use only part of the collected data for our analyses in order to maximize consistency and comparability between the participants (see our data inclusion criteria below). For 30 consecutive days, both participants underwent behavioral assessments, assessments for hormone analysis (including serological and salivary assessments), and brain structural and fMRI in time-locked sessions. Experimental sessions for the female participant occurred exclusively in mid-to-late morning, whereas sessions for the male participant took place in the early morning for the first 10 days, in both the morning and evening for the following 10 days, then exclusively in the late evening for the last 10 days, for a total of 40 sessions, as shown in Figure 1. Due to blood sampling restrictions, the male participant's serological assessments were conducted in the morning session for the first 15 days and in the evening session for the last 15 days, while salivary samples were collected at every session. Each session started with a behavioral assessment consisting of self-report questionnaires including the Perceived Stress Scale (PSS; adapted to reflect past 24 h), consisting of 10 questions measuring the level of appraised stress from life situations on a 5-point Likert scale from 0 ("never") to 4 ("very often"), for a total PSS score ranging from 0 (low stress) to 40 (high stress) (Cohen et al., 1983).

The time-locked collection of steroid hormones was conducted in a study-specific manner. For the female participant, steroid hormone samples were collected via venipuncture at 10:00 a.m. ± 30 min. For the male participant, salivary sampling and venipuncture were collected at 7 am for morning sessions and at 8 pm for evening sessions. Following safety guidelines, blood was drawn only once on days with two sessions (i.e., in the morning for experimental days 11–15 and in the evening for days 16–20). Endocrine samples were collected after abstaining from food or drink consumption (including caffeine and excluding water) for at least 2 h (female participant), at least 8 h (male participant, morning sessions), and at least 1.5 h (male participant, evening sessions).

2.2. Steroid hormone measurements

For the female participant, serum levels of gonadal steroid hormones (17β-estradiol, progesterone, and testosterone), as well as pituitary gonadotropins (luteinizing hormone (LH) and follicle stimulating hormone (FSH)), were sampled. For the male participant, both serum and salivary levels of total testosterone and cortisol were sampled, as well as serum levels of 17β-estradiol. The saliva sample (~2 mL) was collected over 5–10 min of passive drooling at every session, before storing the sample in a plastic cryovial at -20°C until assayed. Saliva concentrations of testosterone and cortisol were

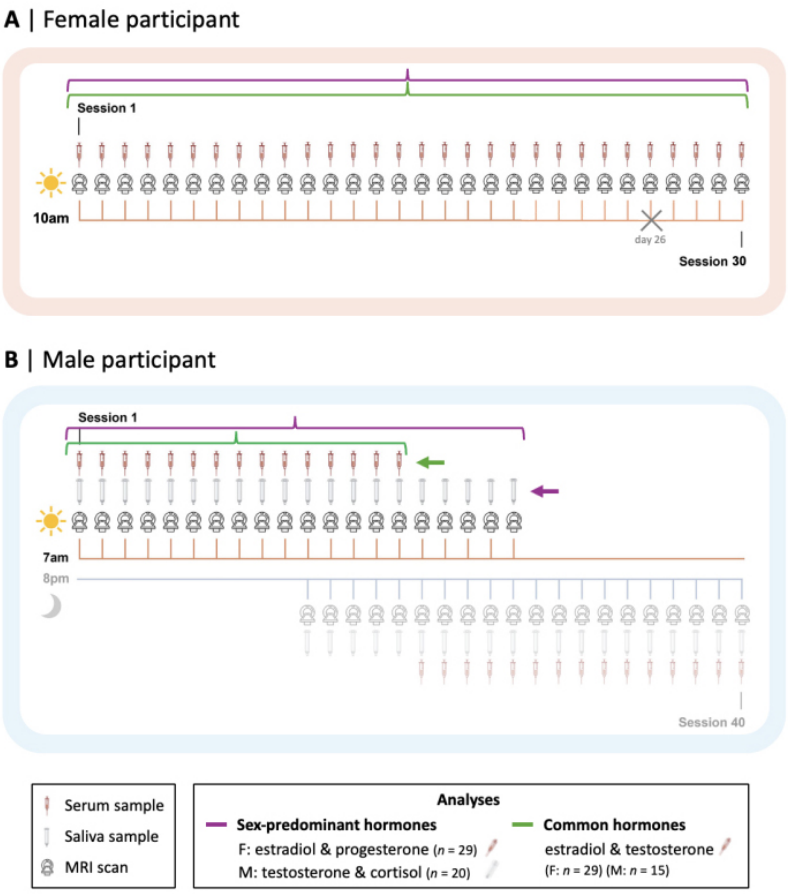

Fig. 1. Schematic representation of the experimental designs and analyses inclusion criteria. (A) Female (F) participant; (B) Male (M) participant. For the female participant, experimental day 26 was excluded from all analyses due to compromised functional magnetic resonance imaging (MRI) data; for the male participant, only morning session data were analyzed. Figure adapted from Grotzinger et al. (2024).

determined using enzyme immunoassay at the Brigham and Women's Hospital Research Assay Core.

For both participants, a 10 cc blood sample was collected per session by a licensed phlebotomist via the insertion of a saline-lock intravenous line into the dominant or non-dominant forearm and the use of a vacutainer SST (BD Diagnostic Systems). The serum samples were first allowed to clot at room temperature for 45–60 min, then centrifuged (2,100 x g for 10 min) and aliquoted into three 2 mL microtubes. The samples were then stored at -20 °C until assayed. At the Brigham and Women's Hospital Research Assay Core, liquid chromatography-mass spectrometer (LCMS) was used to determine serum concentration for all steroid hormones, and an immunoassay was used to determine serum concentration for all gonadotropins in the female participant (i.e., FSH and LH).

Assay sensitivities, dynamic range, and intra-assay coefficients of variation, respectively, were as follows: estradiol, 1 pg/mL, 1–500 pg/mL, <5% relative standard deviation (RSD); progesterone, 0.05 ng/mL, 0.05–10 ng/mL, 9.33% RSD; testosterone, 1.0 ng/dL, <4% RSD; testosterone, 1.0 ng/dL, 1–200 ng/dL, <2% RSD; cortisol, 0.5 ng/mL, 0.5–250 pg/mL, <8% RSD. Gonadotropin levels were determined using chemiluminescent assay (Beckman Coulter), with assay sensitivity, dynamic range, and the intra-assay coefficient of variation as follows: FSH, 0.2 mIU/mL, 0.2–200 mIU/mL, 3.1–4.3%; LH, 0.2 mIU/mL, 0.2–250 mIU/mL, 4.3–6.4%.

2.3. MRI acquisition

Both participants underwent a 1 h-long MRI scan at every session, conducted on a Siemens 3T Prisma scanner with a 64-channel phased-array head coil. Structural anatomical images were acquired using a T1-weighted magnetization prepared rapid gradient echo (MPRAGE) sequence (TR = 2,500 ms, TE = 2.31 ms, TI = 934 ms, flip angle = 7°, 0.8 mm thickness) and a gradient echo field-map (TR = 758 ms, TE1 = 4.92 ms, TE2 = 7.38 ms, flip angle = 60°). Resting-state fMRI images were acquired with a T2*-weighted multiband echo-planar imaging (EPI) sequence measuring the blood-oxygen-level-dependent

(BOLD) contrast (TR = 720 ms, TE = 37 ms, flip angle = 52° (female participant) and 56° (male participant), multiband factor = 8; 72 oblique slices, voxel size = 2 mm^3). The resting-state scans lasted 10 and 15 min for the female and male subjects, respectively. To reduce head motion, both participants' heads were secured in a 3D-printed custom-fitted foam head case. Overall head motion was minimal for both participants, with a daily mean framewise displacement (FWD) below 130 μm in the female and below 80 μm in the male.

2.4. fMRI preprocessing

The preprocessing of fMRI data was performed in MATLAB using the Statistical Parametric Mapping 12 (SPM12, Wellcome Trust Centre for Neuroimaging, London) software and is fully reported by Pritschet et al. (2020) and Grotzinger et al. (2024). The preprocessing pipeline was identical for both participants. In short, to correct for head motion and geometric deformations, functional images were realigned and unwarped, followed by a coregistration of the mean motion-corrected images to the anatomical images. The Advanced Normalization Tool's (ANTs) multivariate template construction was used to normalize all scans to a subject-specific template (Avants et al., 2011). The functional data were subsequently smoothed using a 4 mm full-width half maximum (FWHM) isotropic Gaussian kernel. To account for fluctuations in signal intensity across time and space, global signal scaling (median = 1,000) was applied and voxel-wise time series were detrended linearly. After removing the effects of five sources of physiological noise (cerebrospinal fluid and white matter signal) as well as head motion, the residual BOLD signal was extracted from each voxel. A Volterra expansion of translational/rotational motion parameters was used to model head motion based on the Friston-24 approach, which accounts for the nonlinear and autoregressive effects of head motion on the BOLD signal (Friston et al., 1996). In the current study, we did not apply further global signal regression.

2.5. Functional connectivity and the S-A axis of functional organization

Throughout this work, we used the Schaefer 400-region cortical parcellation (Schaefer et al., 2018) as well as its associated Yeo-Krienen seven functional network solution including the visual, somatomotor, dorsal attention, ventral attention, limbic, frontoparietal, and default-mode networks (Yeo et al., 2011). As reported by Pritschet et al. (2020) and Grotzinger et al. (2024), the first eigenvariate across functional volumes was used to extract a regional summary time series in order to compute functional connectivity for each scanning session (Friston et al., 2006). Then, using a maximal overlap discrete wavelet transform, these regional time series were decomposed into different frequency bands. We used low-frequency fluctuations in wavelets 3–6 (~0.01–0.17 Hz) for our subsequent connectivity analyses (Patel & Bullmore, 2016). The spectral association between time series data from each region was estimated with magnitude-squared coherence, yielding a 400 x 400 functional connectivity matrix for each experimental session, indicating the strength of functional connectivity between all pairs of regions (false discovery rate (FDR)-corrected at $q < 0.05$). Coherence was chosen to measure interregional functional connectivity because it avoids contamination by physiological noise given that it is not sensitive to the shape of the regional hemodynamic response function, which can vary as a function of vascular differences (Sun et al., 2004).

We then applied diffusion map embedding, a nonlinear dimensionality reduction algorithm, on the functional connectivity matrices in order to generate low-dimensional representations of macroscale functional organization (Margulies et al., 2016). Diffusion map embedding compresses high-dimensional data into low-dimensional "gradients" or axes describing the global structure of the data, along which data points that are highly associated are clustered closer together (i.e., they have similar loadings on the axes), and data points that have low association are further apart (Coifman & Lafon, 2006). To this end, we used the BrainSpace Python toolbox (Vos de Wael et al., 2020) to generate 10 gradients with the following parameters: 90% threshold (i.e., only considering the top 10% row-wise z-values of functional connectivity matrices, representing each seed region's top 10% of maximally functionally connected regions), $\alpha = 0.5$ (α controls whether the geometry of the set is reflected in the low-dimensional embedding—i.e., the influence of the sampling points density on the manifold, where $\alpha = 0$ (maximal influence) and $\alpha = 1$ (no influence)), and $t = 0$ (t controls the scale of eigenvalues). First, for both participants separately, mean gradients were computed by reducing the dimensionality of their mean functional connectivity matrices (i.e., averaged across study sessions). Then, using the same parameters, we computed "daily" gradients, that is, for each scanning session. In order to maintain comparability for intra-individual analyses, the daily gradients were aligned to their respective mean gradients (i.e., per participant) using Procrustes alignment. Finally, for data from each experimental session, we took the well-replicated principal gradient explaining the most variance in the data and spanning from sensorimotor to association regions (Margulies et al., 2016), which we labeled the S-A axis and used to represent functional cortical organization. In our analyses,

we refer to S-A axis loadings, which represent each cortical region's position on the S-A axis.

2.6. Data inclusion

The female subject's fMRI data collected on experiment day 26 appeared to be compromised, with the original publication of the dataset reporting that it was markedly dissimilar to the other study sessions (Pritschet et al., 2020). We could confirm this dissimilarity when computing and plotting the S-A axis and comparing it with the mean S-A axis (averaged across study sessions, excluding day 26), $r = 0.41$, $p_{spin} < 0.001$ (see Supplementary Fig. 1 for a visual representation of the female fMRI data on day 26 compared with fMRI data averaged across study sessions). There was also a notable difference in the variance explained in the functional connectivity data by the S-A axis when the S-A axis was computed from the functional connectivity matrix on day 26 (23.7% of variance explained) as opposed to being computed from the mean functional connectivity matrix (excluding day 26; 33.95% variance of explained). For these reasons, we excluded day 26 of the female dataset from our analyses. Furthermore, considering that study designs slightly differed for the two participants, we conducted our analyses on only a part of the data that were originally collected—see Figure 1 for a schematic representation of the experimental designs and analyses inclusion criteria. In our first set of analyses considering sex-predominant steroid hormones, for the female participant, we chose to include serum levels of estradiol and progesterone, as these steroid hormones are the most potent and studied endocrine neuromodulators in females ($n = 29$). For the male participant, we chose to include morning salivary levels of testosterone, as this steroid hormone is a more potent endocrine neuromodulator in males, as well as cortisol given its availability and given that its production follows circadian fluctuation patterns similar to testosterone ($n = 20$). To note, there were some differences in the hormones originally analyzed and available in the participants' datasets: Cortisol levels were not provided for the female participant and progesterone levels were not provided for the male participant. Furthermore, we chose morning salivary samples for the male participant (rather than serum/evening samples) in this first set of analyses in order to maximize our sample size ($n = 20$) while maintaining intra-individual consistency and keeping the time of data collection comparable between participants. Although serum hormone measurements are known to be more accurate, we confirmed the validity of the salivary hormone measurements (and thus their comparability with serum levels) in the male participant by correlating serum and salivary levels for testosterone ($r = 0.90$, $p = 0.001$) and cortisol ($r = 0.92$, $p = 0.001$). In our second set of analyses, aimed at comparing the local- and system-level effects of common steroid hormones (i.e., estradiol and testosterone) between participants, we used morning serum hormone levels for the male participant in order to increase comparability with the female serum hormone levels (still $n = 29$) at the cost of decreasing male sample size ($n = 15$). We further conducted supplementary analyses with reduced female samples ($n = 20$ for analyses on sex-predominant steroid hormones; $n = 15$ for analyses on common steroid hormones) to increase comparability with the male sample sizes of $n = 20$ and $n = 15$ for the respective analyses. We subsampled the female data points in a manner that evenly covered the entire 30-day experimental period (excluding day 26). Specifically, we included the following experimental days: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 17, 19, 20, 22, 23, 25, 27, 28, 30 (for $n = 20$) and 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 (for $n = 15$).

2.7. Statistical analyses

For each participant, intra-individual daily variability in functional organization was computed by taking the standard deviation of each parcel's S-A axis loading across study sessions. Spearman-rank correlation was used to test the similarity of the two participants' intra-individual variability maps, followed by a spin-permutation test with 1,000 spherical rotations to control for spatial autocorrelation (Alexander-Bloch et al., 2018). To test for inter-individual differences in intra-individual variability, we first quantified differences in variance by subtracting the standard deviation of male S-A axis loadings from the standard deviation of female S-A axis loadings within each cortical region. This subtraction was solely conducted to obtain the directionality of effects, whereby a negative subtraction result in a given cortical region indicated greater male variance in S-A axis loadings across experimental sessions and a positive value conversely indicated greater female variance. Then, to assess the statistical significance of these regional differences in variance between the subjects in each cortical region, we used the Levene's test for equality of variances, which tests the null hypothesis that the variance of two sets of data is equal. Here, we further applied FDR correction ($q < 0.05$) to control for multiple comparisons across the 400 cortical regions.

To probe other factors that might be associated with intra-individual variability in functional organization, we tested, for each participant, the Spearman-rank correlation between intra-individual daily variability in S-A axis loadings and 19 brain maps from the openly available Neuromaps database (https://github.com/netneurolab/neuromaps; Markello et al., 2022). We conducted a

spin-permutation test with 1,000 spherical rotations for each correlation analysis to control for spatial autocorrelation (Alexander-Bloch et al., 2018), and then applied FDR correction ($q < 0.05$) to control for multiple comparisons across the 19 tests conducted per subject. The following 19 brain maps were selected for their hypothesized relevance to intra-individual variability in S-A axis loadings: The first principal component of the 123 Neurosynth terms in the Cognitive Atlas, which represents meta-analytically derived brain functions associated with cortical areas (Yarkoni et al., 2011); the first principal component computed for the top 1,000 genes displaying the greatest variation in expression between cortical gyri of two brains recorded in the Allen Human Brain Atlas (Hawrylycz et al., 2012); metabolic measures such as glucose, oxygen, and cerebral blood flow (Vaishnavi et al., 2010); receptor densities of dopamine (Alakurtti et al., 2015), acetylcholine (Bedard et al., 2019), serotonin (Beliveau et al., 2017), norepinephrine (Ding et al., 2010), and glutamate (DuBois et al., 2016); structural measures obtained from the Human Connectome Project S1200 release (Van Essen et al., 2013), including group average cortical myelin that was quantified using MRI T1-weighted/T2-weighted ratio (Glasser et al., 2016) and cortical thickness; electrophysiological MEG power distributions from six frequency bands, also obtained from the Human Connectome Project S1200 release (Van Essen et al., 2013), including alpha (8–12 Hz), beta (15–29 Hz), delta (2–4 Hz), low gamma (30–59 Hz), high gamma (60–90 Hz), and theta (5–7 Hz); and a representation of evolutionary expansion, based on the cortical surface area expansion from macaque to human (Hill et al., 2010).

To probe associations between daily changes in brain organization and fluctuating levels of steroid hormones and perceived stress, we used linear mixed effects models in complementary local- and system-level approaches. Our local-level approach involved testing for local effects (i.e., in each cortical region) of steroid hormones and perceived stress on S-A axis loadings, using FDR correction to control for multiple comparisons of the tested effects across the 400 cortical regions ($q < 0.05$). Local-level effects are informative from a statistical and mathematical perspective, illustrating local shifts in the position of cortical regions on the S-A axis in relation to changes in steroid hormone and perceived stress levels. Local-level effects also allow the statistical comparison of brain-wide patterns of regional effects across participants via the Spearman rank correlation of *t*-maps (i.e., *t*-values across all cortical regions), using spin-permutation testing with 1,000 spherical rotations to correct for spatial autocorrelation.

Our system-level approach involved investigating effects of steroid hormone levels and perceived stress on measures of network topology, which describe the physical organization of nodes in networks and of networks along the S-A axis. For this, we computed measures of within- and between-network dispersion, as described in previous work (Bethlehem et al., 2020; Serio et al., 2024). Within-network dispersion is defined as the sum of the Euclidean distances squared between network nodes (represented by the parcel S-A axis loadings) to the network centroid (quantified by the median of S-A axis loadings for parcels belonging to the same network), for which a higher value indicates a wider distribution of a given network's nodes along the S-A axis, indicating greater segregation of the network. Between-network dispersion is defined as the Euclidean distance between network centroids, for which a higher value indicates that networks are more segregated from one another along the S-A axis. Within-network dispersion was computed for each of the seven Yeo–Krienen functional networks (Yeo et al., 2011), and between-network dispersion was computed for each of the 21 possible network pairs. Then, to test for effects of hormone levels and perceived stress on measures of within- and between-network dispersion, we used the same linear mixed effects models that we used to test for local effects. In order to assess statistical significance, we corrected for multiple comparisons, at Bonferroni-corrected thresholds of $p < 0.004$ (0.025/7) for the within-network effects and $p < 0.001$ (0.025/21) for the between-network effects. For effects that survived Bonferroni correction, we further tested for their spatial specificity. Specifically, for each model, we generated a null distribution of *t*-values for the given effect using spin permutation testing (1,000 spherical permutations) of the Schaefer 400 parcellation scheme, thus shuffling the network labels (Alexander-Bloch et al., 2018). We thus controlled for spatial autocorrelation by assessing our empirical *t*-values against our generated null distributions, with a significance threshold of $p_{spin} < 0.05$. Although system-level effects can only be qualitatively compared between participants, they are biologically informative and interpretable, capturing associations between hormone levels and changes in network topology, namely changes in integration and segregation within and between functional networks.

In order to account for the longitudinal structure of our data (i.e., single subject data collected over consecutive days of testing), we used the above-mentioned linear mixed effects models including "experimental sessions" as a random effect to capture associations within repeated measures without assuming independence between observations. We also considered different sets of hormones as covariates in our linear mixed effects models, for both the local- and system-level approaches, in two sets of analyses. Our first set of analyses aimed to test effects of sex-predominant steroid hormones. As

such, estradiol and progesterone levels were included as covariates in the model testing hormonal effects in the female participant (see Supplementary Materials, Formula 1), and testosterone and cortisol levels were included as covariates in the model testing hormonal effects in the male participant (see Supplementary Materials, Formula 2). Separate models were used to account for the local effects of perceived stress (PSS score; see Supplementary Materials, Formula 3). This decision was made *a priori* on the assumption that perceived stress may covary with the different steroid hormone levels tested in the female and male subjects to different degrees depending on the hormone (see Supplementary Fig. 2 for correlations between PSS scores and steroid hormone levels in both subjects). This may lead to varying levels of shared variance between perceived stress and steroid hormones and, consequently, including perceived stress in the main models may differentially impact both the resulting hormonal and perceived stress effects. We, therefore, opted for running independent models separating effects by modality (steroid hormones levels vs. self-reported perceived stress) in our main analyses, in order to minimize bias and increase the comparability of effects between the participants. We nevertheless conducted supplementary analyses to show effects yielded by models including both steroid hormones and perceived stress compared with effects yielded by our main, separate models. Our second set of analyses aimed to test effects of common steroid hormones (i.e., estradiol and testosterone) in both participants (see Supplementary Materials, Formula 4). As such, estradiol and testosterone were included as covariates in the models tested for both participants.

3. RESULTS

3.1. Daily variability in steroid hormone levels and perceived stress

In the female participant (n = 29; we excluded experimental day 26 from all analyses given that the fMRI data collected on that day appeared to be compromised—as further reported in our Methods data inclusion criteria), daily serum steroid hormone fluctuations followed expected patterns throughout the menstrual cycle (Fig. 2A). Estradiol levels (mean = 84.26 ± 53.74 pg/mL) showed typical increases and decreases, peaking on day 13 of the menstrual cycle (corresponding to the ovulatory window), while progesterone levels (mean = 5.25 ± 5.84 ng/mL) were low during the follicular phase (before ovulation) and high

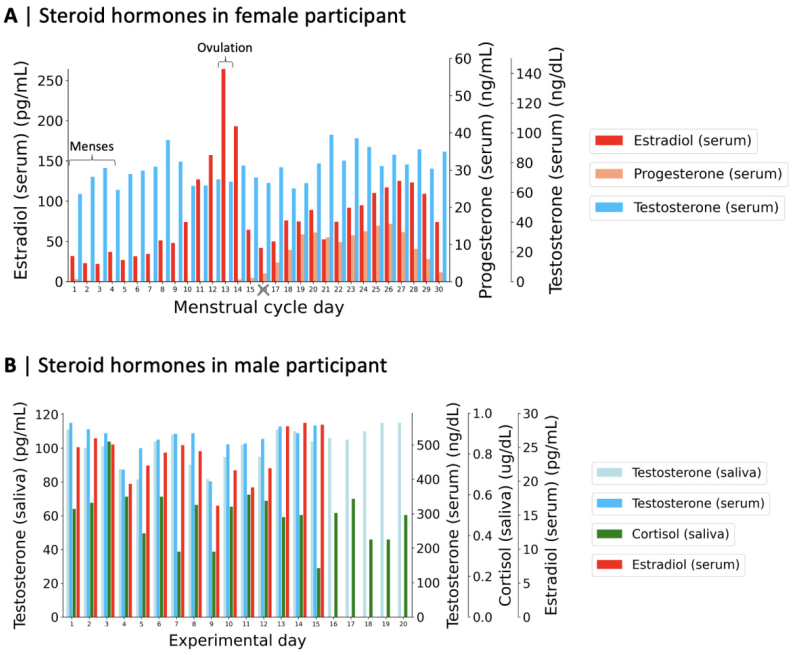

Fig. 2. Daily variability in steroid hormone levels. (A) Estradiol, progesterone, and testosterone serum levels in the female participant, capturing the full spectrum of hormonal variation across the menstrual cycle (n = 29), as originally reported by Pritschet et al. (2020). Note that the menstrual cycle days shown here do not correspond to the experimental sessions, which were rearranged to begin at menstruation for this visualization. Experimental day 26 (corresponding to menstrual cycle day 16) was excluded from all analyses, see Methods for more detail on our data inclusion criteria; (B) Total testosterone and cortisol salivary levels across experimental days (n = 20), as well as estradiol and testosterone serum levels across experimental days (n = 15) in the male participant as originally reported by Grotzinger et al. (2024).

during the luteal phase (after ovulation). Fluctuations in serum testosterone levels (mean = 76.72 ± 10.51 ng/dL) did not follow any particular or expected pattern. The steroid hormone levels of the female subject have been previously reported elsewhere (Pritschet et al., 2020). In the male participant, fluctuating levels of waking salivary steroid hormones (n = 20), that is, testosterone (mean = 101.61 ± 9.98 pg/mL) and cortisol (mean = 0.50 ± 0.13 ug/dL), as well as daily serum steroid hormones (n = 15), that is, testosterone (mean = 513.4 ± 45.4 ng/dL) and estradiol (mean = 23.77 ± 3.46 pg/mL), did not follow any particular or expected pattern between morning sessions (Fig. 2B). Evening experimental sessions allowed to confirm normative circadian patterns of higher testosterone and cortisol levels in the morning relative to the evening in the male participant, although we excluded data acquired during evening sessions to control for time of day in our analyses (see Methods for more detail on our data inclusion criteria). The steroid hormone levels of the male participant have been previously reported elsewhere (Grotzinger et al., 2024).

Self-reported perceived stress was measured with the perceived stress scale (PSS), where PSS scores can range from 0 (low stress) to 40 (high stress). We found no statistically significant difference in PSS scores between the female (mean score = 8.28 ± 6.59) and the male (mean score = 10.10 ± 2.10) participants, as measured by the Mann–Whitney U Test, U = 211.5, p = 0.11.

3.2. Intra- and inter-individual daily variability in functional cortical organization

We computed the S-A axis as our measure of functional cortical organization at each study session in both subjects. For this, we used diffusion map embedding, a non-linear data reduction algorithm, to reduce the dimensionality of the 400 x 400 functional connectivity matrices, representing the pairwise strength of functional connectivity between Schaefer 400 cortical regions (Schaefer et al., 2018). We thus computed, for each study session within both subjects, the well-replicated principal gradient explaining the most variance in the data (33.95% in the female participant, 35.47% in the male participant)—spanning from unimodal sensorimotor regions to transmodal association regions (Margulies et al., 2016)—which we defined as the S-A axis. Figure 3A and B show the mean S-A axes of the female and male participants, respectively, computed by applying diffusion map embedding to the mean daily functional connectivity matrices (averaged across study sessions) within each participant. We used the S-A axis to represent functional cortical organization throughout our analyses, where S-A axis loadings represent each of 400 cortical regions' positions along this low-dimensional axis of functional cortical organization.

We then probed subtle daily changes in functional cortical organization. For both participants, intra-individual daily variability in S-A axis loadings was quantified using standard deviation (Fig. 3C, D). We found a statistically significant spatial association between the cortex-wide patterns of female and male intra-individual variability (Spearman's rank: r = 0.29, p_{spin} < 0.001). Both participants displayed greatest variability in temporal limbic and ventral prefrontal regions, extending further across the cortex in the male participant. To quantify inter-individual differences in intra-individual variability, we subtracted male from female standard deviations by cortical parcel (Fig. 3E). We then assessed the statistical significance of the inter-individual differences across cortical regions with Levene's test for equality of variances and found statistically significant greater local intra-individual variability exclusively in the male participant, namely in about 5% of cortical regions (19 out of 400) distributed across functional networks (Fig. 3F). Given the discrepancies in sample sizes for the female (n = 29) and male (n = 20) participants, we conducted supplementary analyses with a reduced female sample of n = 20 daily measurements. In Supplementary Figure 3C, we show that patterns of daily variability in the S-A axis of the female reduced sample are similar to those yielded by the full female sample (Fig. 3C), r = 0.80, p_{spin} < 0.001. With comparable female and male sample sizes, a greater number of cortical regions passed the significance threshold for inter-individual differences in intra-individual variability, namely 25% of cortical regions (100 out of 400), as illustrated in Supplementary Figure 3F.

To further interpret intra-individual functional variability, we explored its association with brain features such as gene expression, meta-analytic functional activations subserving behavior and cognition, metabolism, neurotransmitter receptor distribution, brain structure and function (electromagnetic waves), as well as patterns of evolutionary expansion. We thus decoded patterns of intra-individual variability in S-A axis loadings by testing their associations with 19 independent maps of brain features from the publicly available Neuromaps database (Markello et al., 2022)—see Methods for more information about each map. For this, we computed the Spearman-rank correlation between each brain feature map and both the female and male intra-individual variability maps separately (Fig. 3G). Here, we only found statistically significant associations that survived spin permutation testing as well as false discovery rate correction (FDR; q < 0.05) for the male participant. Specifically, patterns of male intra-individual variability were negatively associated with patterns of overall gene expression, glucose and oxygen metabolism, myelin, and magnetoencephalography (MEG) alpha activity, illustrating that regions with higher

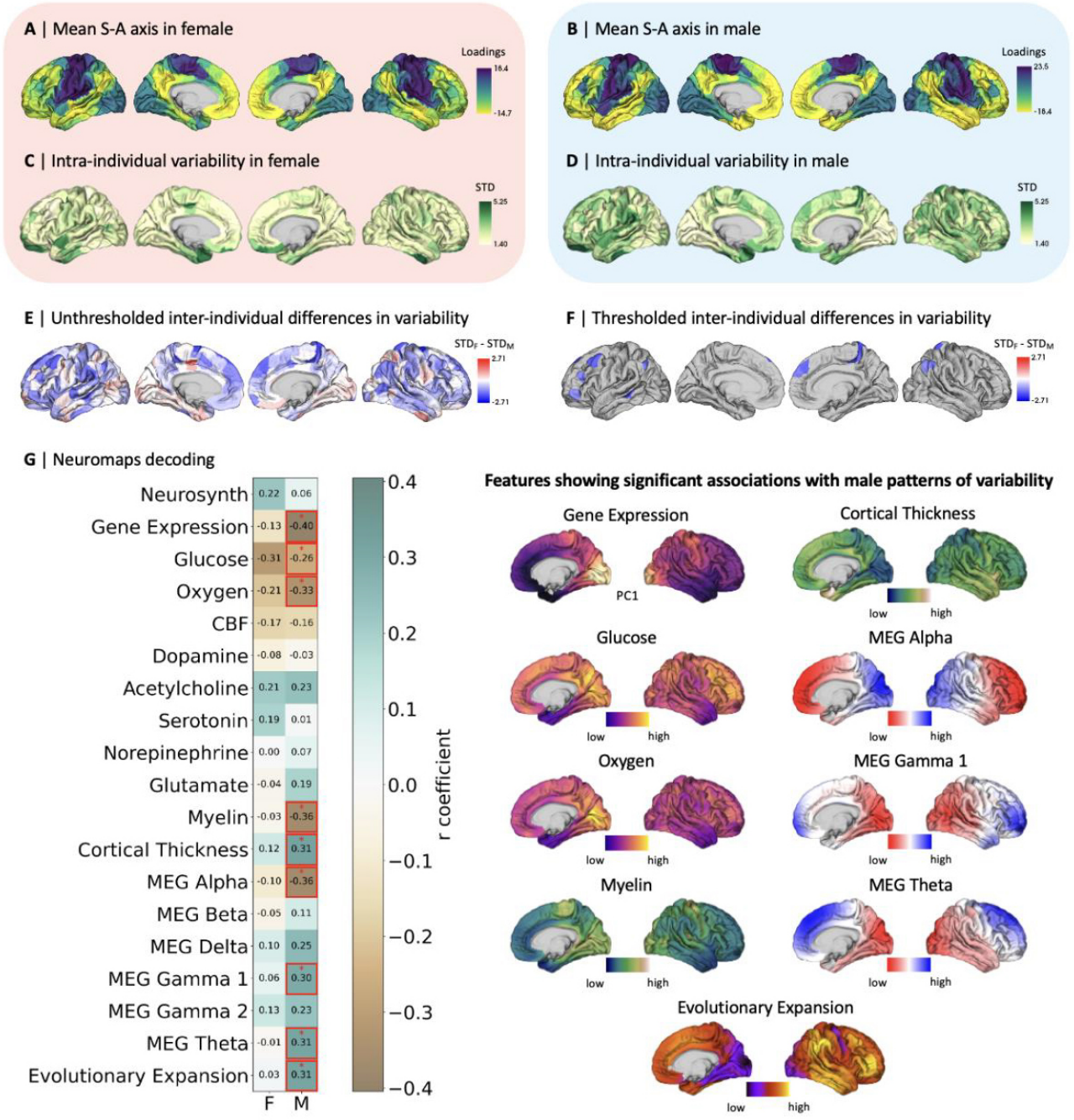

Fig. 3. Intra- and inter-individual daily variability in functional cortical organization. (A) Mean sensorimotor-association (S-A) axis loadings across 29 days in the female participant; (B) Mean S-A axis loadings across 20 days in the male participant; (C) Intra-individual variability in S-A axis loadings quantified by standard deviation (STD) in the female participant; (D) Intra-individual variability in S-A axis loadings quantified by STD in the male participant; (E) Inter-individual differences in intra-individual variability quantified by the subtraction of male from female intra-individual variability; (F) Thresholded inter-individual differences in intra-individual variability, displaying inter-individual difference in intra-individual variability in false discovery rate (FDR)-corrected parcels ($q < 0.05$) showing statistically significant differences as resulted by the Levene's test for equality of variances, namely in about 5% of cortical regions (19 out of 400); (G) Spearman-rank correlations between patterns of intra-individual variability in the female (F) and male (M) participants and 19 brain feature maps sourced from the Neuromaps database, where red * and boxes indicate statistically significant correlations after spin permutation testing and FDR correction ($q < 0.05$). Brain feature maps showing statistically significant associations with the male participant's intra-individual variability are displayed. MEG, magnetoencephalography.

variability are regions typically displaying lower metabolism, myelin intensity, and MEG alpha activity. To note, the directionality of the association with gene expression patterns is irrelevant given that the gene expression map represents the first principal component computed over the expression of 1,000 genes from the Allen Human Brain Atlas (Hawrylycz et al., 2012), which should be understood as an axis of variability in the similarity of gene expression profiles rather than a measure with meaningful directionality. Furthermore, patterns of male intra-individual variability were positively associated with patterns of cortical thickness, MEG gamma 1 activity, MEG theta activity, and evolutionary expansion, illustrating that regions with higher variability are regions typically displaying greater cortical thickness, MEG gamma 1 and theta activity, and greater relative cortical surface area expansion from macaque to human. Again, we conducted supplementary analyses to decode patterns of intra-individual variability in S-A axis loadings for a reduced female sample (n = 20) and still found no statistically significant associations between female daily variability and the tested brain features (Supplementary Fig. 3G).

3.3. Effects of sex-predominant steroid hormones and perceived stress on functional cortical organization

In order to investigate factors potentially underlying dynamic intra-individual daily changes in functional organization, we tested for local effects of steroid hormone levels and perceived stress on the S-A axis loadings by independently fitting different linear mixed effects models in both participants, including the random effect of experimental sessions to model the longitudinal structure of our data. Our first set of analyses included steroid hormones that are most predominant within each sex (i.e., estradiol and progesterone in the female participant, testosterone in the male participant), as well as cortisol in the male participant given its availability and given that its production follows circadian fluctuation patterns similar to testosterone. We thus included serum estradiol and progesterone levels as covariates in the model for the female participant (n = 29), and included salivary testosterone and cortisol levels as covariates in the model for the male participant (n = 20). Separate additional models were used to account for the local effects of perceived stress (PSS score) in both participants independently. We applied statistical corrections for multiple comparisons across the 400 cortical regions for each tested effect, using FDR correction (q < 0.05). t-maps of tested local effects are displayed in Figure 4, where a positive t-value denotes a positive association between hormone levels and S-A axis loadings, and a negative t-value conversely denotes a negative association. In the female participant, while estradiol did not show statistically significant local-level effects on S-A axis loadings (Fig. 4A), progesterone showed statistically significant effects in 1.3% of cortical regions (5 out of 400; Fig. 4B), and perceived stress

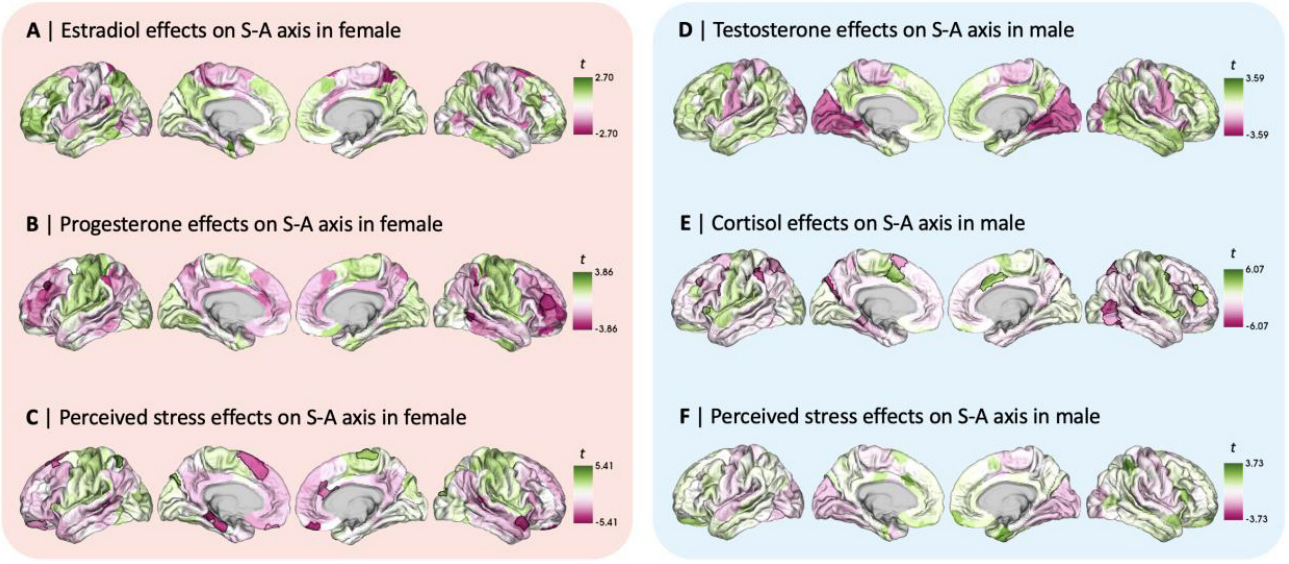

Fig. 4. Local-level effects on sensorimotor-association (S-A) axis loadings in the female and male participants. Unthresholded t-maps of linear mixed effects model results showing patterns of local effects of (A) Estradiol, (B) Progesterone, and (C) Perceived stress on S-A axis loadings in the female participant; unthresholded t-maps of linear mixed effects model results showing patterns of local effects of (D) Testosterone, (E) Cortisol, and (F) Perceived stress on S-A axis loadings in the male participant. Delineated cortical regions show statistically significant effects following false discovery rate (FDR) correction (q < 0.05), which was used to control for multiple comparisons across the 400 cortical regions.

showed statistically significant effects in 5% of cortical regions (20 out of 400; Fig. 4C). In the male participant, while testosterone (Fig. 4D) and perceived stressed (Fig. 4F) showed no statistically significant local-level effects on S-A axis loadings, cortisol showed statistically significant effects in 9.3% of cortical regions (37 out of 400; Fig. 4E). Furthermore, the female and male participants showed overall different patterns of perceived stress effects on functional organization, as indicated by the negative association between female and male spatial patterns of PSS score effects on S-A axis loadings, r = -0.50, p_{spin} = 0.001. See Supplementary Figure 4 for comparisons of the local-level results reported in Figure 4 (independently testing effects of steroid hormone levels and perceived stress in separate models) and local-level results yielded by models including both steroid hormone levels and perceived stress as covariates in the same model. In Supplementary Figure 5, we also show that patterns of local-level effects of sex-predominant steroid hormones and perceived stress in a reduced female sample (n = 20) are similar to those yielded by the full female sample (illustrated in Fig. 4A–C).

For a more interpretable characterization of daily changes in S-A axis loadings (i.e., local shifts in the position of cortical regions along the S-A axis), we tested for system-level effects of hormone levels and perceived stress on changes in network topology, that is, changes in the topographical organization of functional networks along the S-A axis (Fig. 5A). For this, we independently computed measures of both within- and between-network dispersion across study sessions for both participants as done in previous work (Bethlehem et al., 2020; Serio et al., 2024), based on the Yeo-Krienen seven functional network solution (Yeo et al., 2011). Within-network dispersion quantifies the spread of cortical regions within each of the seven networks along the S-A axis, with higher values of within-network dispersion indicating higher segregation of regions within a given network. Between-network dispersion quantifies the pairwise distance between a given pair of functional networks along the S-A axis, with higher values of between-network dispersion indicating a higher segregation of the two given networks. We thus computed measures of within-network dispersion for all 7 functional networks, and measures of between-network dispersion for all possible pairwise combinations of the 7 networks (i.e., 21 pairs of networks in total). Then, we fitted the same linear mixed effects models used to test for local effects on the S-A axis, separately testing for steroid hormone and perceived stress effects on all measures of within- and between-network dispersion. In Figure 5B–E, we summarized patterns of all tested system-level effects on functional network dispersion along the S-A axis, using heatmaps to highlight the directionality of effects, where positive t-values are illustrated in purple and negative t-values in brown, respectively, indicating segregation and integration effects. Overall, we found a few statistically significant effects. In the female participant, we found an association between progesterone and increased segregation between the frontoparietal and limbic networks, as well as associations between perceived stress and increased segregation of the frontoparietal network relative to both the limbic and dorsal attention networks (Fig. 5D). In the male participant, we found an association between testosterone and increased integration within the somatomotor network (Fig. 5C), as well as associations between testosterone and increased integration between the visual and dorsal attention networks, and associations between cortisol and increased integration of the ventral attention network relative to both the dorsal attention and the visual networks (Fig. 5E). More broadly, notable patterns of within-network dispersion effects in the female participant were that estradiol generally displayed patterns of greater integration (particularly within association networks), whereas progesterone exclusively displayed patterns of greater segregation within all networks (Fig. 5B). In the male participant, testosterone predominantly displayed patterns of greater integration within networks, while cortisol was exclusively associated with patterns of greater within-network segregation (Fig. 5C). Strikingly, effects of perceived stress showed opposite patterns across the subjects: In the female participant, perceived stress was exclusively associated with increased within-network segregation, while being exclusively associated with increased within-network integration in the male participant. The detailed statistical results for all analyses of system-level effects on functional organization are summarized in Supplementary Tables 1–3. For further system-level results yielded by models including both steroid hormone levels and perceived stress as covariates, see Supplementary Figure 6. In Supplementary Figure 7A and C, we also show that patterns of system-level effects of sex-predominant steroid hormones and perceived stress in a reduced female sample (n = 20) are similar to those yielded by the full female sample, illustrated in Figure 5B and D.

3.4. Effects of common steroid hormones on functional cortical organization

After testing for the hypothesized effects of steroid hormones that are most predominant within each sex, our second set of analyses tested for effects of common steroid hormones (i.e., estradiol and testosterone) on functional organization in both participants, to allow a direct comparison of effects across participants. We thus

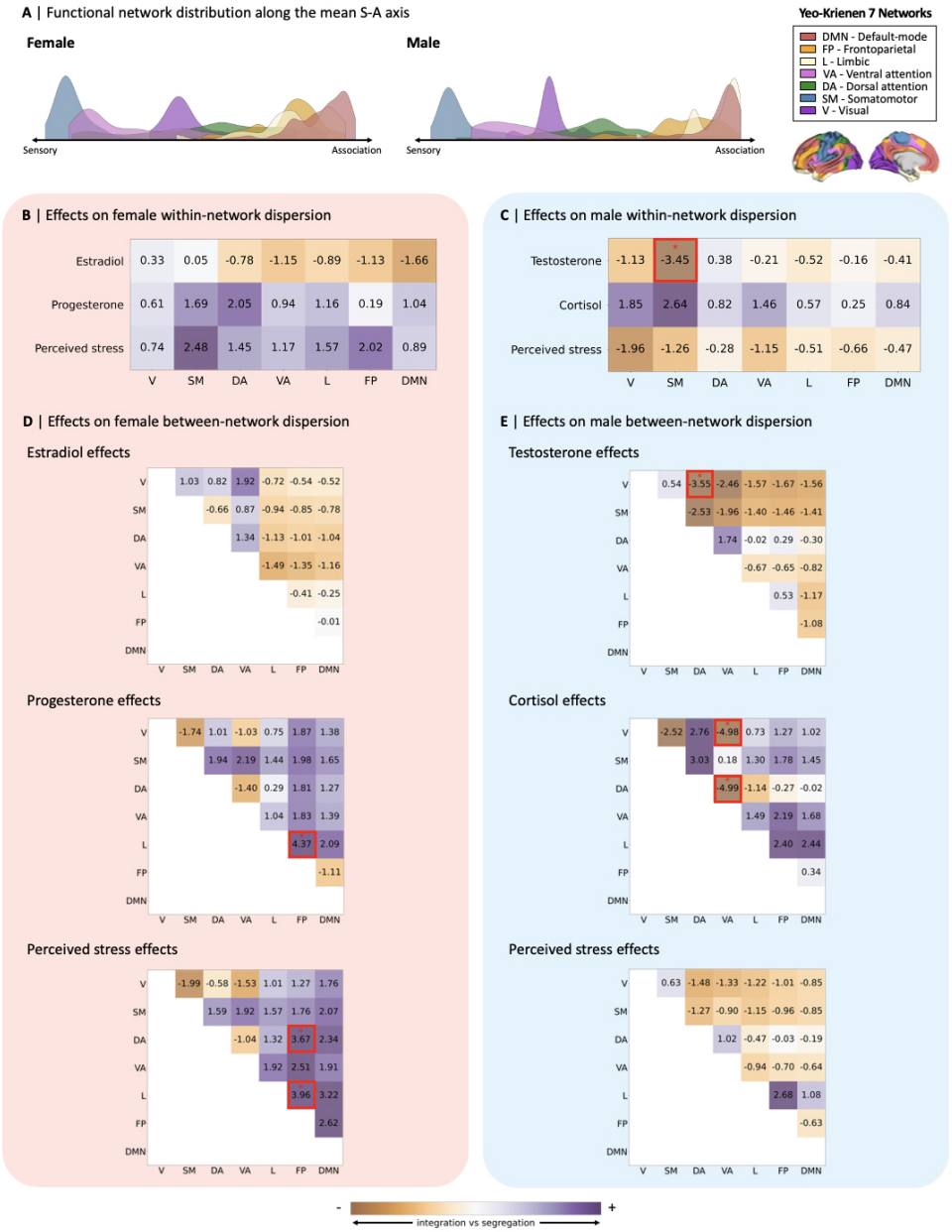

Fig. 5. System-level effects of sex-predominant steroid hormones and perceived stress on functional cortical organization. (A) Visualization of the distribution of the seven Yeo–Krienen functional networks along the mean sensorimotor-association (S-A) axis in the female and male participants. Heatmaps summarizing the *t*-values for system-level effects across functional networks of estradiol, progesterone, and perceived stress on the female participant's within- (B) and between- (D) network dispersion, and effects of testosterone, cortisol, and perceived stress on male participant's within- (C) and between- (E) network dispersion. *t*-values were obtained from linear mixed effects models including different sets of covariates, namely estradiol and progesterone (for female hormone effects), testosterone and cortisol (for male hormone effects), and perceived stress only (for both female and male, tested separately). Red * and boxes indicate statistical significance of effects corrected for multiple comparisons, at Bonferroni-corrected thresholds of $p < 0.004$ (0.025/7) for the within-network dispersion effects and $p < 0.001$ (0.025/21) for the between-network dispersion effects, as well as corrected for spatial autocorrelation via spin-permutation testing (1,000 permutations). Positive *t*-values represent higher segregation and negative *t*-values represent higher integration effects. V, visual; SM, somatomotor; DA, dorsal attention; VA, ventral attention; L, limbic; FP, frontoparietal; DMN, default-mode network.

included estradiol and testosterone as covariates in a new linear mixed effects model that we independently tested for each participant in order to directly compare the local-level effects of these common steroid hormones on S-A axis loadings. To increase comparability between participants, we used serum hormone levels for the male participant in this set of analyses, at the cost of a smaller sample size (n = 15; see Methods section for more detail on our data inclusion criteria and the availability of steroid hormones per subject). As done for the analyses testing for sex-predominant steroid hormone effects, we applied statistical corrections for multiple comparisons across the 400 cortical regions for each tested effect, using FDR correction ($q < 0.05$). t-maps of tested local effects are displayed in Figure 6, where a positive t-value denotes a positive association between hormone levels and S-A axis loadings, and a negative t-value conversely denotes a negative association. Estradiol showed statistically significant effects on S-A axis loadings in 0.25% of cortical regions (1 out of 400) in the female participant (Fig. 6A) and no statistically significant effects in the male participant (Fig. 6B). The comparison of brain-wide patterns of local effects (i.e., unthresholded t-values) of estradiol in the female and male participants revealed a small statistically significant association (r = 0.28, p_{spin} = 0.003; Fig. 6C). Testosterone showed statistically significant effects on the S-A axis loadings in 35% of cortical regions (140 out of 400) in the female participant (Fig. 6D) and effects in 0.25% of cortical regions (1 out of 400) in the male participant (Fig. 6E). The comparison of brain-wide patterns of local effects of testosterone in the female and male participants revealed a medium statistically significant association (r = 0.57, p_{spin} = 0.001; Fig. 6F). In Supplementary Figure 8, we also show patterns of local-level effects of common steroid hormones in a reduced female sample (n = 15), which are somewhat weaker for estradiol and somewhat stronger for testosterone effects relative to results yielded by the full sample.

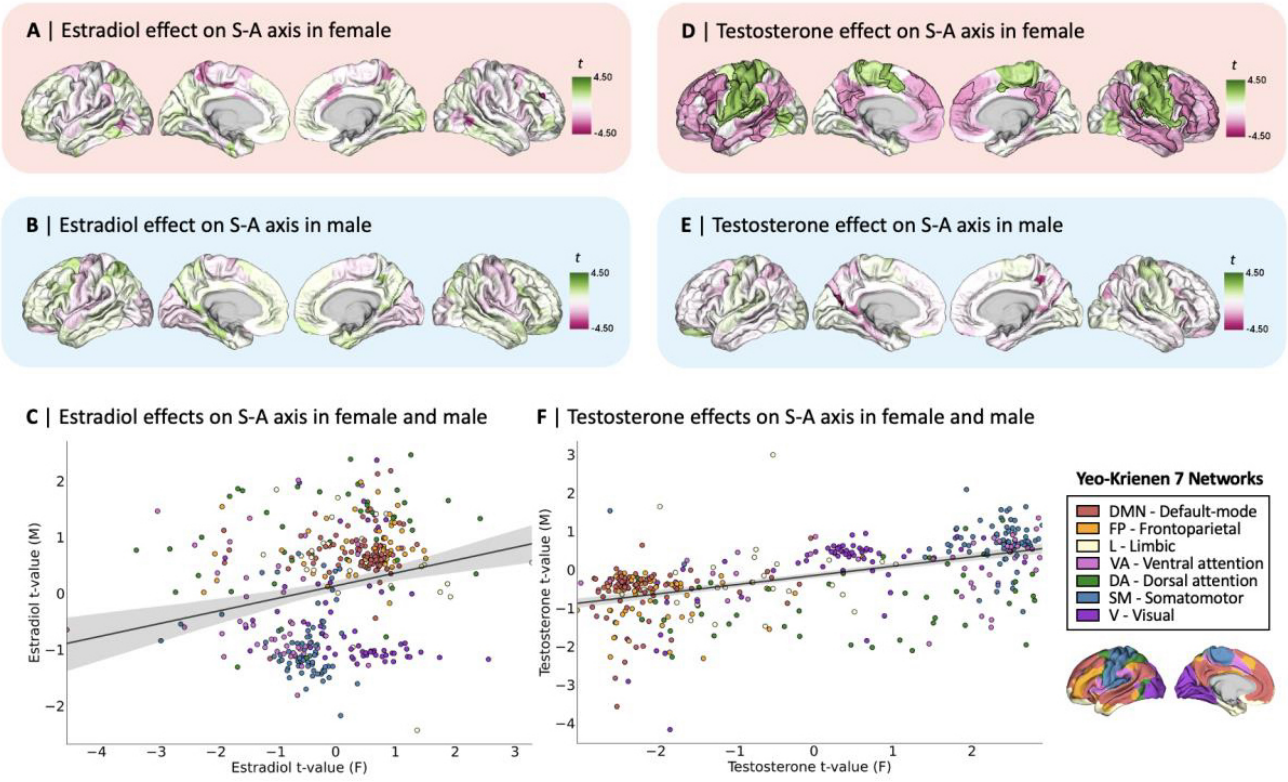

Fig. 6. Local-level effects of estradiol and testosterone on functional organization in female and male participants. Unthresholded t-maps of linear mixed effects model results showing patterns of local effects of estradiol effects on S-A axis loadings in the (A) Female and (B) Male participants. (C) Scatterplot displaying the spatial correlation between patterns of local estradiol effects on S-A axis loadings in the female participant (F; x-axis) and in the male participant (M; y-axis), r = 0.28, p_{spin} = 0.003; colors denote the seven Yeo–Krienen functional networks. Unthresholded t-maps of linear mixed effects model results showing patterns of local effects of testosterone on S-A axis loadings in the (D) Female and (E) Male participants. (F) Scatterplot displaying the spatial correlation between patterns of local testosterone effects on S-A axis loadings in the female participant (x-axis) and in the male participant (y-axis), r = 0.57, p_{spin} = 0.001. Delineated cortical regions show statistically significant effects following false discovery rate (FDR) correction ($q < 0.05$), which was used to control for multiple comparisons across the 400 cortical regions.

For a more interpretable characterization of effects of common steroid hormones on S-A axis loadings, we tested for system-level effects of estradiol and testosterone on changes in the topographical organization of functional networks along the S-A axis (Fig. 7A). In Figure 7B–E, we summarized patterns of all system-level effects on functional network dispersion along the S-A axis, using heatmaps to highlight the directionality of effects. Here, we did not find any statistically significant effects of steroid hormones on within- or between-network dispersion, although patterns of estradiol effects in the female and testosterone effects in the male remained stable relative to effects yielded by models including sex predominant hormones. Notably, we observed diverging patterns effects between the two subjects. For example, in the male participant, estradiol was associated with greater integration within sensory networks, opposite to female patterns of estradiol associations with greater segregation within sensory networks. The detailed statistical results for system-level effects of estradiol and testosterone on functional organization in both participants are summarized in Supplementary Tables 4 and 5. In Supplementary Figure 9A and C, we also show patterns of system-level effects of common steroid hormones in a reduced female sample (n = 15), which are overall somewhat weaker relative to results yielded by the full sample.

4. DISCUSSION

In the current work, we used a dense sampling approach to investigate neuroendocrine factors that may be associated with intra-individual daily variability in functional brain organization in two deeply phenotyped young adults, one female and one male. Different from previous work using dense sampling, we computed a low-dimensional representation of patterns of resting-state functional connectivity—the S-A axis, spanning from unimodal sensorimotor regions to transmodal association regions—to quantify subtle daily intra-individual variability along this key hierarchical principle of functional cortical organization and directly compared variability across subjects. Overall, participants showed unique cortical patterns of intra-individual variability in S-A axis loadings, with similar cortical areas (i.e., temporal limbic and ventral prefrontal regions) displaying the largest amount of variability across participants and male variability extending further across the cortex. We also found statistically significant greater intra-individual variability exclusively in the male participant relative to the female participant, as well as associations between male whole-brain patterns of intra-individual variability and a range of brain features pertaining to brain metabolism, structure, electrophysiology, genetics, and phylogeny. Our analyses also revealed some statistically significant local- and system-level effects of steroid hormones and perceived stress on functional organization, which—while demonstrating some shared patterns—also exhibited unique divergences between the female and male participants under study. Collectively, our findings suggest subtle inter-individual differences in intra-individual daily variability along a major principle of functional cortical organization and hint at unique neuroendocrine processes for which sex specificity should be further investigated in larger, more diverse samples.

By establishing daily intra-individual variability in the functional cortical organization, steroid hormone levels, and perceived stress of two densely sampled individuals (Grotzinger et al., 2024; Pritschet et al., 2020), our findings highlight the dynamic nature of brain function, embedded in equally dynamic endocrine (i.e., steroid hormones) and cognitive systems (i.e., perceived stress) under study. We found that patterns of intra-individual variability did not follow a particular sensory-to-association differentiation, unlike previous reports of greater within-subject variability in lower order unimodal regions and greater between-subject variability in higher order transmodal regions (Laumann et al., 2015; S. Mueller et al., 2013). Nevertheless, intra-individual variability was greatest in temporal limbic and ventral prefrontal regions in both participants, which to some extent replicates previous findings of greater variability in the limbic network of 30 densely sampled individuals (Chen et al., 2015). Although greater variability in the limbic network may in part reflect the lower signal-to-noise ratio typically observed in temporal regions during fMRI scans (Arnold Anteraper et al., 2018), limbic regions are also known for their remarkable plasticity, which has been linked to laminar patterns of structural variability (García-Cabezas et al., 2019). The temporal lobe is a cortical area that is particularly dense with steroid receptors (González et al., 2007; Loy et al., 1988; Meffre et al., 2013), whose volume has been shown to vary as a function of steroid hormone levels (Hoekzema et al., 2017; Taylor et al., 2020; Zeydan et al., 2019; Zsido et al., 2023). Altogether, our findings suggest unique whole-brain patterns of subtle daily intra-individual changes along a major principle of functional cortical organization, with some similarities across participants in the regions displaying the greatest amount of variability.

Higher variability in S-A axis loadings was exclusively observed in the male participant when statistically testing for inter-individual differences in intra-individual variability along this low-dimensional measure of functional cortical organization. Given that we only sampled one individual of each sex, we cannot generalize this finding to the group level. Nevertheless, an evolutionary

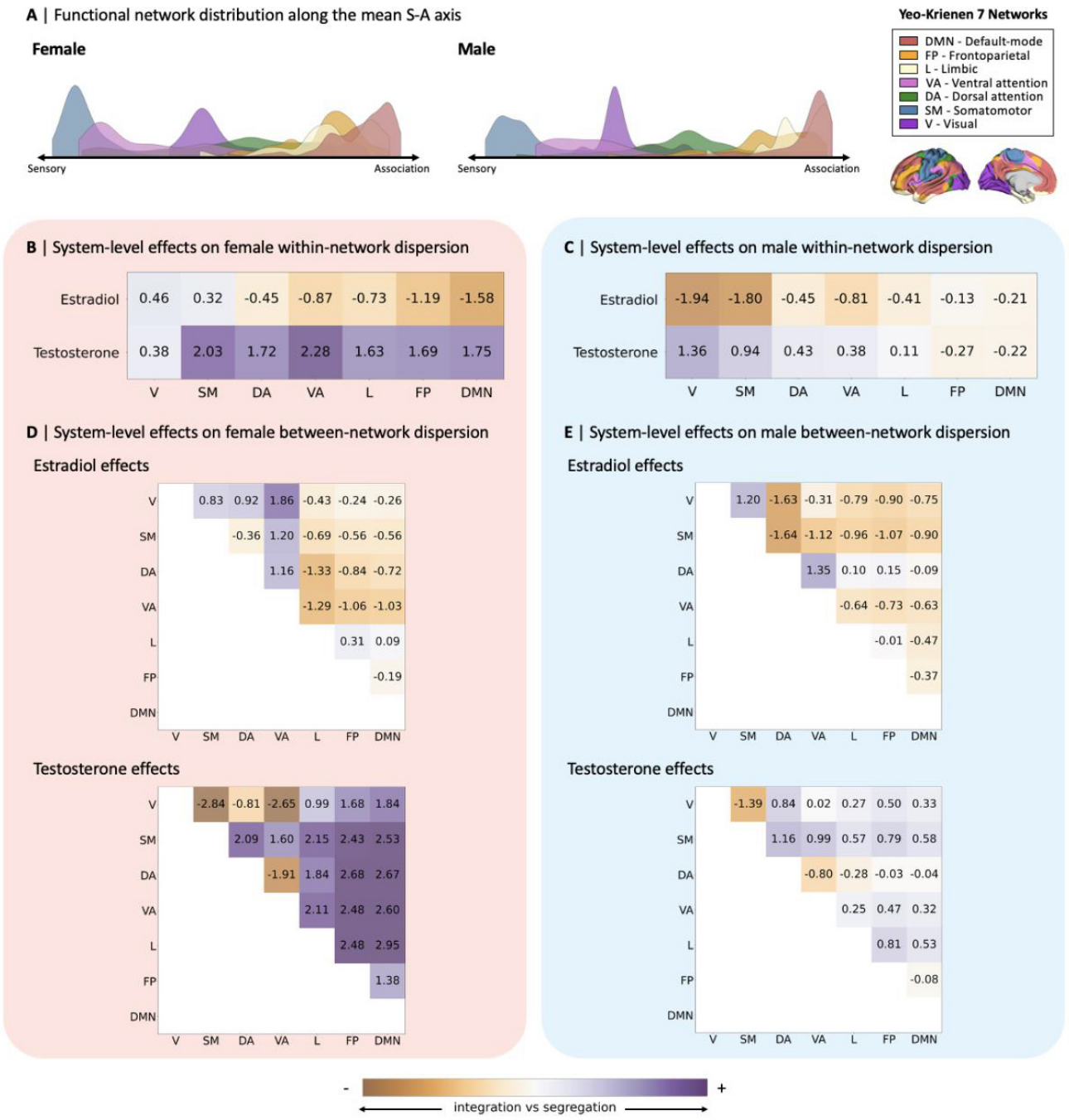

Fig. 7. System-level effects of estradiol and testosterone on within- and between-network dispersion in female and male participants. (A) Visualization of the distribution of the seven Yeo–Krienen functional networks along the mean sensorimotor-association (S-A) axis in the female and male participants. Heatmaps summarizing the *t*-values for system-level effects across functional networks of estradiol and testosterone for the female participant's within- (B) and between- (D) network dispersion, and the male participant's within- (C) and between- (E) network dispersion. *t*-values were obtained from linear mixed effects models including estradiol and testosterone as covariates in both the female and male models, tested separately per participant. None of the tested effects were statistically significant after correction for multiple comparisons, that is, at Bonferroni-corrected thresholds of $p < 0.004$ (0.025/7) for the within-network effects and $p < 0.001$ (0.025/21) for the between-network effects. Positive *t*-values represent higher segregation and negative *t*-values represent higher integration effects. V, visual; SM, somatomotor; DA, dorsal attention; VA, ventral attention; L, limbic; FP, frontoparietal; DMN, default-mode network.

hypothesis supporting greater male variability in both biological and cognitive phenotypes has long been formulated (Darwin, 1888) and has more recently been empirically supported by different measures of brain structure across the lifespan (Bethlehem et al., 2022; Forde et al., 2020; Wierenga et al., 2022). Greater male variability is thought to potentially result from constraints imposed by genetic architecture, namely the heterogametic nature of male sex chromosomes (XY) as opposed to identical sex chromosomes in females (XX) (Reinhold & Engqvist, 2013). In fact, our exploratory analyses showed that intra-individual variability in the male participant was further associated with patterns of overall gene expression, as well as patterns of glucose and oxygen metabolism, myelin, cortical thickness, MEG alpha, MEG gamma 1, MEG theta activity, and evolutionary expansion. It is important to note that these different brain features were obtained from openly available datasets representing group averages rather than being specific to the individuals under study. Yet, these multilevel features are theoretically pertinent to functional organization and may thus plausibly contribute to intra-individual functional variability, as suggested by our findings. For example, metabolic substrates such as glucose and oxygen are directly related to the brain's energy expenditure and local changes in hemodynamics, thus relevant to the measurement of the blood-oxygen-level-dependent (BOLD) signal (Buxton, 2010). Furthermore, the comparison of MEG and fMRI signals—that is, local field potentials and the BOLD response, respectively—is conceptually plausible given that both predominantly pertain to postsynaptic (dendritic, rather than axonal) signaling (E. L. Hall et al., 2014). Finally, patterns of evolutionary cortical expansion have previously been shown to reflect spatial patterns of variability along the S-A axis (Buckner & Margulies, 2019; Valk et al., 2022; Xu et al., 2020) as well as inter-individual variability in functional connectivity (S. Mueller et al., 2013). Interestingly, the fact that associations between intra-individual variability and the tested brain features were only statistically significant in the male participant suggests that sources of variability may differ between the two participants. We indeed only observed a low association in patterns of intra-individual variability between our participants, consistent with previous research suggesting that—beyond some shared patterns of variability—a larger proportion of intra-individual daily changes in functional organization is unique to the individual (Laumann et al., 2015; S. Mueller et al., 2013). Our findings thus point to unique multilevel factors associated with intra-individual variability, for which sex specificity should be further investigated in larger samples.

To probe the possible multilevel underpinnings of daily variability along our low-dimensional measure of functional cortical organization, we tested both local- and system-level effects of steroid hormone levels and self-reported perceived stress in the female and male participants separately. Overall, we found some effects that survived our statistical corrections. At the local level, we observed a few minor effects of steroid hormone levels on S-A axis loadings in both participants, which were spread across functional networks. When comparing effects of common steroid hormones between the two participants, we observed some similarities—but also divergence—in patterns of effects, suggesting some level of inter-individual differences. Similarly, patterns of local-level perceived stress effects on the S-A axis loadings were negatively correlated between the participants, and system-level analyses further show that perceived stress showed patterns of exclusively increased within-network segregation in the female participant and increased within-network integration in the male participant (although effects did not pass our statistical significance thresholds). This is possibly in line with previous findings of diverging associations between stress and functional connectivity across the sexes, with one study, for example, reporting no relationship between perceived stress and functional connectivity at rest in men as opposed to women (Archer et al., 2018). The literature further points to the possibility of different stress mechanisms across the sexes, as seen through varying and interacting effects of cortisol levels and perceived stress in females throughout the menstrual cycle (Duchesne & Pruessner, 2013; Maki et al., 2015), as well as associations between resting functional connectivity and both testosterone and cortisol concentrations in males (Ilkevič et al., 2024; Kiem et al., 2013).

System-level analyses further revealed some interestingly diverging patterns of effects on network topology across the participants, with statistically significant effects in the female reflecting greater segregation of more "association" networks, while statistically significant effects in males exclusively reflected greater integration of more "sensory" networks. Although these findings denote variability within—rather than between—individuals, and are derived from only one subject of each sex, they are somewhat reminiscent of previously reported sex differences in functional connectivity. For instance, the literature has most consistently reported greater functional connectivity in females in association networks such as within regions belonging to the DMN (Allen et al., 2011; Biswal et al., 2010; Bluhm et al., 2008) and greater functional connectivity in males within somatomotor regions (Biswal et al., 2010; Scheinost et al., 2015). Our previous work also suggests, from the perspective of connectivity profiles, that

females make stronger connections within the DMN and males make stronger connections involving the somatomotor network (Serio et al., 2024). Furthermore, our findings of diverging patterns of effects on network topology between the female and male participants, yielded by measures of network dispersion, can be meaningfully interpreted as topological changes in functional communities becoming more similar or different along the hierarchical domains of the S-A axis (Bethlehem et al., 2020; Serio et al., 2024). Functional network integration and segregation are more generally considered to be important indicators of network topological structure and reconfiguration underpinning cognition, as they have been associated with a range of cognitive functions as well as changes in brain states, arousal, and energy expenditure (Shine & Poldrack, 2018). Altogether, our findings underscore heterogeneous and widespread patterns of effects that are not specific to a set of regions or networks. The divergence of findings between the participants further suggests individual differences in neuroendocrine and stress effects on network topology, hinting at processes that may vary across sexes but require systematic and statistical testing in larger samples.

Despite the insights gained through our study, some limitations should be acknowledged. First, our small sample of two young healthy adults—one female (n = 29) and one male (n = 20/15)—not only entails low statistical power, likely underpinning the scarcity of statistically significant effects in some analyses of our study, but also precludes the generalization of results. On the one hand, although the study design effectively captured a snapshot of the full range of possible endogenous female steroid hormone variation across consecutive days dictated by the menstrual cycle, more data would be required for both the female and male participants to generalize within-subject findings across longer periods of time. On the other hand, larger heterogeneous samples of different and diverse individuals are required to generalize findings at the population level. We used data that were collected with a high sampling frequency, conscious of the trade-off of data depth (i.e., repeated deep phenotyping in the same individuals) over breadth (i.e., across multiple individuals). As previously eloquently formulated: "Just as no single brain is representative of a population, no group-averaged brain represents a given individual" (Laumann et al., 2015). Our focus was thus not to yield generalizable findings per se, but to probe fine-grained intra-individual effects that provide a multilevel account of factors potentially influencing intra-individual variability in functional organization. In fact, recent work from our group has highlighted sex differences in functional organization in a large sample (N = 1,000), which could not be explained by differences in cortical morphometry (Serio et al., 2024), requiring a deeper investigation of other potential explanations, such as neuroendocrine and neurocognitive factors. Although our group has also observed sex differences in isocortex and hippocampus microstructure related to self-reported female menstrual cycle stage and hormonal contraceptive use (Küchenhoff et al., 2024), those measures were only proxies of female hormone levels, and establishing neuroendocrine mechanisms strictly requires endocrine samples. Our current study thus allowed us to bridge both methodological and conceptual gaps with a deeply phenotyped sample, providing insights into intra-individual variability that was not otherwise possible in large samples and thus highlighting the complementary nature of both approaches (Poldrack, 2021).

Second, with respect to interpreting sex effects in our current study, we cannot determine the extent to which the observed inter-individual differences in intra-individual variability may be explained by sex-specific mechanisms as opposed to broader individual differences. By only including one individual of either sex, we cannot assume the degree to which our participants may be representative of the greater female and male populations, respectively, with further increasing evidence suggesting that sex should actually be treated as a continuous variable in biological research (Neuhoff, 2022; Wiersch & Weis, 2021). Furthermore, there were differences in the available data collected for the female and the male participants, which both constrained the scope of our analyses (i.e., progesterone effects only tested in the female and cortisol effects only tested in the male) and may have further resulted in systematic differences in the observed effects (i.e., due to methodological differences in data collection). However, our data inclusion criteria specifically aimed to minimize differences and thus maximize the comparability of effects across participants. The outstanding differences in sample size (i.e., more female data points) and hormone sampling method (i.e., saliva vs. serum) should have had minimal impact on the results, as indicated by our supplementary analyses on reduced female samples, as well as by the high correlations of testosterone and cortisol levels measured in the male participant's saliva and serum. In terms of the differences in hormones focused on for our female and male subjects, future work exploring the dynamics of all major steroid hormones across the sexes will yield a stronger understanding of the interplay between the endocrine and nervous systems. Another limitation related to the categorical conceptualization of biological sex is that we did not consider possible effects of steroid hormones on functional organization in gender-diverse individuals who challenge the notion of binary female–male categories. In fact, steroid hormone levels are not fixed and may be

dynamically affected by gendered social experiences (Hyde et al., 2019), as well as a range of more general environmental factors that go beyond sex and gender identity, such as sleep, nutrition, caffeine consumption, physical exercise, and stress (Chichinadze & Chichinadze, 2008; Glover et al., 2022; D. C. Hall, 2001; Ives et al., 2011; Kraemer & Ratamess, 2005; Lord et al., 2014; Roney & Simmons, 2015; Sisti et al., 2015; Wrzosek et al., 2020). As such, a larger sample capturing greater variability across females, males, and individuals in general is necessary to establish the degree of sex specificity of the effects tested in our study, as well as to further assess possible gender-specific effects (Dhamala et al., 2024). Our study is, however, novel in probing and comparing both distinct and shared female and male neuroendocrine effects on the S-A axis in densely sampled individuals.

Third, it could be contended that our findings of intra-individual daily variability in functional cortical organization may be capturing random noise in our data. However, we explicitly made methodological decisions aimed at reducing biases and noise particularly pertaining to daily variability and endocrine effects. First, we used the S-A axis as our measure of functional cortical organization, which—through its low dimensionality and thresholding—has been shown to have greater test–retest reliability than more commonly used measures of unthresholded edge-wise functional connectivity (Hong et al., 2020; Knodt et al., 2023). In terms of data design, study sessions were time locked, and food and caffeine intake prior to study sessions was strictly controlled through abstinence in order to limit confounding physiological effects (Grotzinger et al., 2024; Pritschet et al., 2020). Steroid hormones are also thought to potentially induce physiological artifacts, such as local changes in cerebral blood perfusion (Ghisleni et al., 2015), which could be mistaken as cognitively pertinent changes in brain function (Laumann & Snyder, 2021). However, various steps were taken in the preprocessing of our fMRI data—such as global signal scaling and linear detrending of voxel-wise time series—to account for temporal and spatial fluctuations in signal intensity and to remove the effects of head motion and physiological noise features such as cerebral spinal fluid from the BOLD signal. We also used coherence as our measure of functional connectivity, which is known for its robustness to temporal variability in regional hemodynamics as well as its measurement of time series covariances in frequencies outside the spectrum prone to contamination by physiological noise (Sun et al., 2004). As such, the intra-individual daily changes in variability observed in our study are likely to reflect meaningful fluctuations in signal beyond noise. Nevertheless, more research is required to assess the directionality of hormonal effects on functional brain organization, specifically testing causality in statistical relationships and further probing mechanistic biological explanations of lagged hormonal effects, which are reported elsewhere (Pritschet et al., 2020) but go beyond the scope of our study.

All in all, by observing subtle daily changes along a low-dimensional measure of functional cortical organization in two densely sampled healthy young adults, co-occurring with fluctuations in steroid hormone levels and perceived stress, our findings underscore the importance of holistically considering the brain as an organ embedded in an extensive network of interacting endocrine and psychophysiological systems. By observing diverging patterns of effects in a female and a male individual, we highlight the need for research to systematically test for sex effects, particularly considering the sex specificity of neuroendocrine mechanisms (Shansky & Murphy, 2021). Importantly, by showing that a male individual is as subject to hormone-related fluctuations in functional brain organization as a female, we debunk the deeply rooted belief that endocrine variability is an exclusively female concern, which has led to the historical underrepresentation of women from research studies (Jacobs, 2023). Going forward, giving equal consideration to both sexes—as well as combining dense sampling approaches with large population-based studies—is necessary to gain a thorough understanding of neuroendocrine and neurocognitive processes underlying variability along principles of functional brain organization in health and disease.

DATA AND CODE AVAILABILITY

All data needed to evaluate the conclusions in the paper are present in the paper and the Supplementary Materials. We obtained data from the open-access 28&Me sample (Pritschet et al., 2020), available at https://openneuro.org/datasets/ds002674/versions/1.0.5, and the 28&He sample (Grotzinger et al., 2024), available at https://openneuro.org/datasets/ds005115/versions/1.0.0.

The code used to conduct the analyses presented in this manuscript is available at https://github.com/biancaserio/MC_gradients. The code used to preprocess the data used in this manuscript is available at https://github.com/tsantander/PritschetSantander2020_NI_Hormones. The general code and tutorials for functional gradient decomposition can further be found at https://brainspace.readthedocs.io/en/latest/index.html.

AUTHOR CONTRIBUTIONS

Conceptualization of original study design and data collection: L.P., H.G., and E.G.J. Conceptualization of the current study and analyses: B.S. and S.L.V. Main analyses and visualizations: B.S. and D.Y. Writing—original

draft: B.S. and D.Y. Writing—review and editing: B.S., D.Y., L.P., H.G., E.G.J., S.B.E., S.L.V. Supervision: S.L.V.

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DECLARATION OF COMPETING INTEREST

Authors declare that they have no competing interests.

SUPPLEMENTARY MATERIALS

Supplementary results can be found in the Supplementary Materials. Supplementary material for this article is available here: https://doi.org/10.1162/imag_a_00474.

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