Name, institution, and contact details of the applicant (one person only) and a list of the scientists or institutions involved in the research project (max. 2 A4 pages) CVs and lists of publications need not be submitted.

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INM-7: The proposed work is embedded in a multidisciplinary working team combining knowledge in the field of neuropsychology, structural and functional MRI analysis, computational neuroscience and machine learning.

Dr. Kaustubh Patil, head of the working group “Applied Machine Learning”

Rick Betzel is an associate professor at the Psychological and Brain Sciences Department of Psychological and Brain Sciences at Indiana University Bloomington, USA. He is an expert in principles of time-varying functional network reconfiguration and its relationship to ongoing cognitive processes and one of the developers of the edge time series approach which we will employ within the project suggested here.

Dynamic Cognition: Movies as a Window into Sex Differences in the Brain

Project Description

Background and Research Question

Functional brain imaging, especially fMRI, has been widely used to investigate sex differences in the brain. Such differences in structural and functional organization are crucial for understanding healthy development, aging, and the manifestation of psychiatric and neurological disorders [2, 12]. Thus, a deeper understanding of sex differences in the brain and their underlying mechanisms is essential for understanding both healthy behavior and psychopathology.

In this proposal, we employ novel brain imaging methodology utilizing naturalistic viewing (NV), i.e. watching movie clips in the scanner, to broaden knowledge of sex differences in brain function. While it is common knowledge that women and men often react differently to films, our study moves beyond stereotypes ("women prefer emotions, men prefer action") to examine in detail how brain activity and functional connectivity (FC) differ when viewing diverse scenes. For example, women may process subtle social cues in dialogue differently, whereas men may respond more strongly to visual foreshadowing of danger. By systematically analyzing such responses, we aim to advance understanding of cognitive sex differences beyond the current state of research.

Despite decades of work, our knowledge of sex differences in the brain remains incomplete. Some consensus exists for cognitive domains such as language or spatial processing, yet others argue that male and female brains are more alike than different [17].

Classical studies used task-based (TB) fMRI, yielding domain-specific but low-ecological insights [27, 30, 31]. However, due to the highly controlled and artificial nature of the tasks, ecological validity of task-related fMRI is usually very low and does not reflect cognitive sex differences as observed in daily life More recently, resting-state (RS) fMRI has been applied, in which fMRI data is acquired while subjects relax in the scanner without any specific task demand or visual or auditory stimulation. Earlier RS studies examined group differences in FC patterns between women and men. More recently, machine learning (ML) methods have been applied to move beyond group averages: sex classification approaches use RS data to predict the sex of individual subjects and then infer which brain networks contribute most to distinguishing females from males.

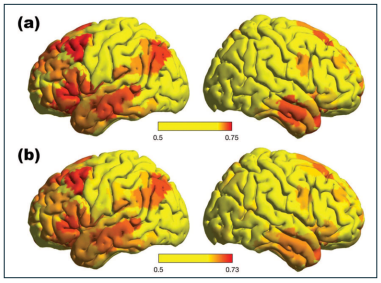

Fig. 1: ROI-based sex classification shows high accuracies for (a) within sample CV and (b) across sample classification.

Our own ML work identified regionally specific networks with predictive power strongest in higher-level regions for language, social cognition, and emotion processing [32, 35, 34]. However, RS primarily reflects intrinsic, trait-like brain organization.

What remains largely unexplored are sex differences in the "brain in action" when engaging with complex, multimodal input resembling real life. The present proposal aims to contribute to closing this gap in knowledge by applying the newly emerging NV approach to examine sex-specific brain responses in ecologically valid contexts. NV focuses on cognitive processes in dynamic, temporally extended, naturalistic contexts, which are much more akin to situations which the brain must deal with in real life.

Importantly, as opposed to RS, all participants are exposed to the same stimulus, for which content and timing is known and can be used in the analyses.

NV approaches offer complex, dynamic and ongoing stimulation similar to experiences in everyday situations, where low-level (audiovisual) and high-level (cognitive and emotional) content vary fluidly, creating a multimodal and immersive experience [26], offering the opportunity to capture dynamic neural processing in ecologically valid contexts [28] and has been shown to enhance reliability and identifiability compared to RS [19].

Our lab has pioneered NV analyses. We developed the Topography-based Predictive Framework (TOPF) [20], which extracts individual-specific evoked topographies and links them to behavior using ML. Applied to NV data, TOPF achieved up to 80% accuracy in sex classification, with predictive regions tied to emotion, language, and higher cognition. These promising results highlight the potential of NV to uncover novel sex differences.

Surprisingly, to our knowledge, no study has yet used NV to systematically examine sex differences. In a previous publication [5], we have compared the potential of movies for the study of individual brain differences to a cardiac stress-test, i.e. to potentially provide a standardized way to study the whole organ while it works

to compare function across different levels of intensity and demands. We therefore expect NV to expose sex differences across a wide range of real-life-like situations—social interactions, face and emotion perception [26], or complex narratives—that TB or RS approaches cannot capture. While isolated cognitive processes as examined in TB fMRI might not reveal significant sex differences, the complex interplay of these processes - as engaged during movie watching - could highlight more pronounced differences between women and men. Careful stimulus annotation will further allow us to identify the specific features and networks driving these differences.

Using advanced neuroimaging and analysis methods, we aim to detect subtle but meaningful sex-related differences in brain activity and FC, that can inform our understanding of broader cognitive and behavioral differences between females and males. First, we will study FC patterns that emerge over several minutes of movie watching. Then, we will zoom in to identify specific events that trigger differences between women and men, and examine how these differences unfold in brain networks over time.

This multi-layered approach — combining aggregated and time-resolved FC, network dynamics, and brain activity — offers a richer view of sex differences in the brain function. Results from the proposed project can shed new light on why cognitive and behavioral patterns differ between women and men, and why certain neurological and psychiatric disorders present differently across the sexes. Such knowledge may improve diagnostic precision and personalized treatments, and support sex-specific strategies in healthcare and education.

We are uniquely positioned to realize this project, as our lab has already acquired the Ju-MOVIES dataset, a rich NV fMRI resource with over 130 participants. It combines extended movie stimuli, hormone measures, and detailed scene annotations, providing an unparalleled foundation for uncovering sex differences in the brain.

For clarity, throughout this proposal "sex" refers to self-reported biological sex. We acknowledge that "gender identity", i.e. the subjective identification of an individual as female, male, or one of the other gender identities which might be also fluid, also plays a significant role, but this lies beyond the scope of the present project.

Data: The Ju-MOVIES dataset

The proposed work builds on the Ju-MOVIES dataset, which has already been acquired and is uniquely suited for investigating sex differences with a NV approach. Its richness and design make it an ideal foundation for the present project. Over the course of the project, further data will be collected. The paradigm comprises seven Hollywood movie excerpts (8 - 10 minutes each) selected to capture diverse social interactions, complex situations, and evolving emotions ("Dirty Dancing", "Scream", "Dead Poets Society", "Forrest Gump", "Dead Man Walking", "Life is Beautiful", "The Good, the Bad, the Ugly"), as well as 12 shorter clips and two RS scans of about 9 minutes.

Stimuli were chosen to be long enough for participants to grasp the context and empathize with characters, ensuring ecological validity.

So far, data from 135 healthy participants (68 males, 18 - 35 years) have been collected on a 3T Siemens Prisma scanner with a 64-channel head coil, using a T2w multiband echo planar imaging sequence with the following parameters: repetition time (TR) = 980ms, echo time (TE) = 30ms, flip angle = 70°, field of view (FOV) = 207 x 207mm, voxel size=2.2 x 2.2 x 2.0mm3, number of slices: 64, multiband acceleration factor=4, phase encoding direction=AP, FoV=207mm). A mirror fixed on the head coil allows participants to see a screen used to display the movies. In-ear headphones are used for ear protection and to deliver the movie sound. Additionally, a structural T1w image is acquired using an MP-RAGE sequence (TR=2000ms, TE=2.45ms, TI=900ms, flip angle=8°, FoV: 256mm) yielding 1mm3 voxels. Alongside fMRI and structural imaging, saliva samples were collected and analyzed for levels of cortisol, estradiol, progesterone, and testosterone to account for hormone-related variability. Oral contraceptive use was documented in women. The movies are richly annotated: emotion ratings from 44 additional participants (23 males, age 20-30 years) for the six basic emotions (happiness, fear, surprise, sadness, disgust and anger [6]), sampled at 10 Hz confirmed that the stimuli evoke a wide spectrum of affective states. Further annotations by two independent raters include scene content: faces, bodies, male / female presenting characters, ethnicity of characters, presence of children, adults, crowds, hands, buildings, vehicles, food, landscapes, animals, plants, movement, social interactions, place (inside or outside / urban vs. non-urban), time of day (day or night), weather, presence of music and camera movements, enabling fine-grained mapping of movie features to neural

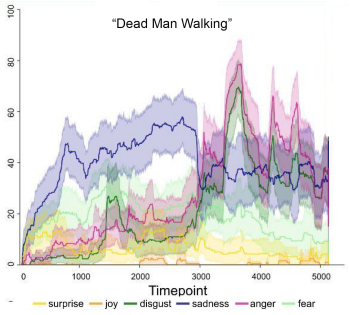

Fig. 2: Exemplary emotion annotation for one of the movie stimuli..

responses. Altogether, Ju-MOVIES offers a rare combination of naturalistic stimulation, hormone measures, and detailed scene-level annotations, providing an exceptionally strong basis for the proposed project.

Work Program and Research Methods

The overarching goal of the proposed project is to complement existing research on sex differences in the brain by employing a NVapproach. This method promises new insights into sex differences in brain responses to complex, dynamically evolving situations—much closer to the demands the brain faces in real life. Our results can go beyond findings from both TB and RS studies and provide perspectives that previous approaches could not achieve. Specifically, we will identify which types of complex situations depicted in the movie stimuli give rise to the most pronounced sex differences. In subsequent steps, we will disentangle which narrative events within the movies drive these differences and examine how female and male brains respond differentially to the evolving storyline. We expect that this innovative approach, combining analyses of temporally aggregated FC, event-related responses, network dynamics and brain activity will yield important new insights into the multi-faceted spectrum of sex differences in the brain in action.

WP1will extend existing RS findings by examining sustained FC during NV across time periods of several minutes. Using a temporally aggregated FC approach, as commonly applied to RS data, this work will identify sex differences in "action brain states" and their underlying networks.

WP2 will build on this by employing a time-resolved FC approach to detect temporally specific events that drive sex differences in the experience of complex situations. Leveraging detailed annotations of the movie content, we will characterize which types of situations give rise to pronounced sex differences and reveal sex-specific cognitive strategies in processing them.

WP3 will focus on brain activation patterns rather than FC. Here, we will extract typical female and male brain responses within each brain region to the dynamically evolving movie content. This analysis will complement WP1 and WP2 by providing a direct account of how women's and men's brains differentially respond to unfolding narrative events.

**** maybe change titles WP1: Beyond Resting State – Sustained Action States in Naturalistic Viewing (focus: stable, aggregated brain states under movie watching vs RS)

WP2: Going Dynamic – Sex Differences in Time-Resolved Functional Connectivity (focus: event-level, moment-to-moment sex differences during movies)

WP3: Shared but Unique – Sex-Specific Neural Responses to Dynamic Emotions (focus: typical male/female response patterns to emotional movie content)

WP 1: The Brain in Action - The Naturalistic Viewing Action State (NV-AS)

Aim: Identify sex differences in sustained, content-driven brain "action states" during NV and compare their predictive power against RS connectivity.

Open Question: Do sex-specific movie feature networks differ systematically, and if so, what do these differences reveal about distinct cognitive strategies in women and men?

RS studies have shown that sex can be classified with high accuracy from temporally aggregated FC patterns [3, 24, 32, 35, 34]. Because RS reflects brain activity in the absence of external stimulation, these patterns are often interpreted as proxies for intrinsic brain organization—capturing traits rather than actual states.

In this WP, we extend beyond RS by examining sustained FC patterns evoked by complex movie narratives. Unlike RS, these naturalistic viewing action states (NV-ASs) are content-driven: the brain's functional organization adapts to ongoing events in the film. Since the content is precisely known, we can link it to FC patterns and identify sex-specific differences in how these states unfold. Thus, this WP will provide insights into which kinds of complex situations elicit the strongest sex differences in brain networks. By focusing on the brain "in action," we aim to capture sex differences in the overall experience of complex situations—an approach that is fundamentally different from both task-based studies (specific, isolated processes) and RS studies (intrinsic organization during free thought). We hypothesize that putting the brain into action will highlight sex differences more strongly than the unconstrained state of mind wandering [29].

Methodologically, we will adopt and extend our previous sex classification pipeline [32]. fMRI data will be parcellated [25], parcel-wise time courses extracted, and FC patterns computed. For each parcel, FC with the rest of the brain will serve as features for sex classification using cross-validation (CV), yielding a spatial map

of classification accuracies for RS and each NV-AS (i.e., each movie). Importantly, and in contrast to the majority of previous studies, we will incorporate hormone levels and oral contraceptive (OC) status in women as confounds in all prediction analyses to ensure that observed differences reflect sex per se rather than hormone-related variability. We will also implement rigorous strategies to prevent confound leakage [15], ensuring robust and unbiased results.

WP 1.1: Comparing sex classification accuracies between RS and NV-ASs

As a first step, we will compare sex classification performance between RS and NV-AS (averaged across all movies) within each parcel using corrected resampled *t*-tests [22]. Higher accuracies during NV relative to RS would indicate that sex differences emerge more strongly when the brain is "in action" rather than in its intrinsic resting organization.

In addition, the spatial distribution of highly discriminative parcels in NV will highlight the brain networks underlying these sex differences. Their associated cognitive domains will be characterized using functional decoding [10].

While sex differences in RS have often been reported in the default mode network (DMN) [32, 36], we expect NV-AS to reveal effects in higher cognitive or task-general networks [16]. Moreover, we hypothesize that sex classification based on NV-AS will outperform RS, consistent with findings for other phenotypes [8, 29] and with evidence that NV enhances individual identifiability over RS [19].

WP 1.2: Which movie features drive sex classification accuracies?

To examine which kinds of complex situations maximize sex differences in the brain, we will characterize each movie clip by a set of visual and auditory features across its duration. These will include low-level properties such as mean motion energy, visual brightness, and auditory loudness, as well as higher-level properties such as number of faces, social interactions, and spoken words, which can be automatically extracted [21, 23], alongside additional features from our manual annotations.

We will then use a multiple regression approach to compare sex classification accuracies across the whole brain between the different movie clips. This analysis will delineate which movie features drive classification performance for each brain parcel. Because higher classification accuracy reflects more pronounced sex differences, this procedure will yield a "movie feature profile" that explains which features evoke the strongest differences. By clustering parcels according to their feature profiles, we will identify brain networks in which sex differences are jointly driven by specific features. We hypothesize that some of these networks will align with well-established cognitive domains (e.g., language, spatial cognition [14, 18]), whereas others will involve more generalized, task-independent resources [16]. We expect domain-specific networks to achieve higher classification accuracies than domain-general ones.

Finally, in an exploratory step, we will compare the expression of these networks in females and males. By computing the first principal component of each network's FC pattern separately for women and men, we will derive "typical" female and male network signatures for each movie feature cluster. This analysis will reveal sex-specific cognitive strategies in response to complex, life-like situations.

WP 2: Going dynamic: Sex differences in time-resolved FC

Aim: Determine which specific narrative events drive sex differences in FC and reveal sex-specific cognitive strategies in processing complex situations.

Open Questions: How do naturalistic sex differences observed here relate to findings from traditional TB paradigms (e.g., isolated face viewing)? Do the same brain regions emerge, or do new networks appear under ecologically valid conditions?

While WP1 examines sex differences in sustained "action states" evoked by movies, WP2 takes advantage of one of the main strengths of naturalistic viewing: its continuous, dynamic variation in content. Unlike RS or aggregated NV analyses, here the goal is to dissect the evolving brain responses to specific narrative events within the movies. Because all participants experience the same time-locked stimulus, the impact of movie content on the brain can be examined directly and with temporal precision. This enables us to move beyond asking whether men and women differ "on average" in their processing of complex situations, and instead pinpoint *which moments* in the evolving narrative trigger sex-specific brain network configurations. In other words, this WP shifts the focus from "what kind of situations" (WP1) to "exactly when and how" sex differences manifest during real-life-like experiences.

Methodologically, fMRI data will again be parcellated, and parcel-wise time courses used to compute moment-by-moment co-fluctuations between regions using the edge time series (eTS) approach [1, 7]. This effectively "unwraps" FC into its temporal evolution, yielding a time series of co-fluctuation magnitudes for each edge.
To identify dynamic FC differences between females and males during movie watching, we will identify time points within the movies, at which females' and males' FC patterns are most distinct. To do so, we will employ a ML approach at each time point to classify sex based on the individual time resolved FC patterns. Given that the dimensionality of these features, i.e. the number of edges, is extremely high, we will first employ a principal component analysis (PCA) for dimensionality reduction. Then, for each time point, the first n (e.g. 50) principal components will be used as features to train a sex classifier for which classification accuracy will be determined by use of a CV approach. Again, hormone levels and OC status will be included in the models as confounds to control for hormone related variability and possible confound leakage will be assessed and controlled for [15].
Significant time points—those at which classification exceeds chance level ($p < 0.05$ by permutation test)—will be taken as markers of narrative events that drive the strongest sex differences. To characterize these events, movie annotations will be used to code the presence of discrete features (e.g., faces, bodies, animals, scene cuts) and continuous features (e.g., luminance, audio intensity). Each significant time point will thus be linked to a detailed movie feature vector. We will then cluster significant time points across movies according to their feature vectors, thereby identifying categories of scenes that consistently evoke sex-specific differences. For each cluster, typical female and male FC patterns will be computed as the first principal component across participants, and thresholded to highlight the most discriminative connections and key network nodes. This will allow us to identify brain networks that respond differently in women and men to particular types of naturalistic events.
Altogether, WP2 will reveal which narrative elements most strongly drive sex differences in time-resolved FC and how these differences are expressed at the network level. While some of the observed patterns are expected to overlap with domains already known from TB studies (e.g., sex differences in face perception), this dynamic, ecologically valid approach will provide a much richer picture—capturing sex-specific strategies for processing complex, multimodal real-life situations. Sex specific brain network patterns will potentially shed light on different cognitive strategies used by females in males in dealing with complex real life-like events.

WP 3: Shared but Unique: Examining Sex-Specific Responses to Dynamic Emotions

Aim: To characterize how female and male brains differ in their regional activation patterns to evolving movie content, complementing connectivity-based analyses from WP1 and WP2.

Open Questions: Which brain regions exhibit fundamentally different time-resolved responses to emotional content between females and males, and are these differences consistent across movie narratives? Are specific emotional categories (e.g., fear, anger, happiness) more likely to induce divergent neural responses between females and males?

A major advantage of NV paradigms over RS is that all participants are exposed to the same stimulus, producing synchronized neural responses across individuals, while still preserving meaningful inter-individual differences [9, 29]. This WP capitalizes on that property to focus directly on the evolution of neural activation patterns during movie watching, asking whether and how females' and males' brains differ in their responses to dynamic, emotionally rich content. Since movies are especially effective in eliciting strong emotions [13, 33], and sex differences in emotion perception and regulation are well documented [4, 11], we focus here on differences in emotional brain responses unfolding over naturalistic narratives. This extends prior work that typically examined only isolated emotions in artificial tasks.

Methodologically, we will employ the Topography-based Predictive Framework (TOPF) recently developed in our lab [20]. For each brain region, TOPF extracts the shared time course of brain responses across participants (via PCA) and quantifies how strongly each individual expresses this response. In the present context, we will compute separate shared response time courses for women and men, while regressing out hormone levels to ensure that results reflect sex differences rather than hormone-driven variability.

Analyses will proceed in two steps. First, we will directly compare the typical female and male time courses

for each brain region to identify regions where the evoked responses fundamentally diverge between sexes. To probe whether such differences are driven by emotions depicted in the movies, we will correlate the sex-specific shared response with existing emotion annotations (six basic emotions). High correlations with particular emotions (e.g., fear, happiness) will reveal which categories most strongly drive sex differences.
Second, for regions where overall time courses do not differ, we will test whether the *intensity* of response expression differs between sexes using two-sample t-tests. This will identify regions in which females and males process the narrative similarly in form but differently in strength. These regions are expected mainly in higher-order cognitive areas, and functional decoding will be used to interpret their domain-specific relevance [10].

Finally, we will assess whether whole-brain patterns reveal sex-typical processing of dynamic narratives. For each subject, we will compute the similarity of their brain response to the sex-specific shared response across all regions, and classify subjects accordingly. High classification accuracy would indicate the existence of typical female and male whole-brain response patterns; failure to classify would suggest that individual variability outweighs sex as the organizing principle. Should this not be the case it would speak to the brain patterns being driven by individual factors over sex.

Altogether, WP3 will identify sex-specific regional and whole-brain patterns in the perception of dynamic emotions, providing novel insights into how females and males process emotionally charged, real-life-like situations.

*** noch sortieren

Altogether, the proposed project will provide an unprecedented, ecologically valid view of sex differences in the brain by combining sustained and dynamic connectivity analyses with regional activation patterns and hormone-sensitive modulation. With our uniquely suited Ju-MOVIES dataset as a foundation, this work will not only advance fundamental understanding of how female and male brains process complex, real-life-like situations, but also deliver insights with direct relevance for mental health, personalized medicine, and sex-specific healthcare strategies.

Altogether, the proposed project will open a new chapter in the study of sex differences in the brain by moving beyond artificial laboratory settings to naturalistic, dynamic experiences. Leveraging our unique Ju-MOVIES dataset, we will uncover how female and male brains process the complexity of real life as it unfolds on screen. In doing so, we aim not only to advance basic neuroscience, but also to lay the groundwork for a future in which insights into sex-specific brain function help shape more precise diagnostics, personalized treatments, and ultimately more equitable strategies in medicine, education, and mental health.

By combining novel naturalistic viewing paradigms with state-of-the-art neuroimaging and analysis methods, this project moves beyond traditional approaches and opens a new path for understanding sex differences in the human brain. Leveraging our unique Ju-MOVIES dataset, we will reveal how female and male brains engage with the complexity of real-life-like experiences as they unfold on screen.

In doing so, the project not only advances fundamental neuroscience but also establishes a framework with far-reaching implications: from improving diagnostic accuracy to inspiring personalized medicine and education, and ultimately contributing to more equitable strategies in healthcare and society. We envision this work as a pioneering step toward a future where insights into sex-specific brain function directly inform how we understand, treat, and support human diversity.

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