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.

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# 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.

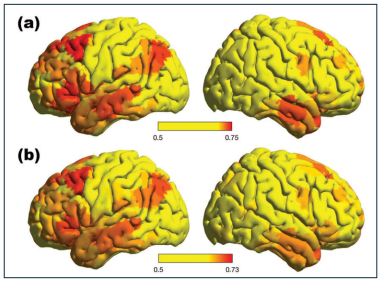

*Fig. 1: ROI-based sex classification shows high accuracies for (a) within sample CV and (b) across sample classification.*

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

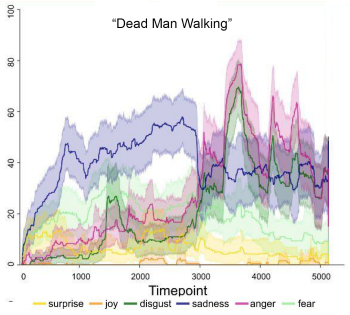


*Fig. 2: Exemplary emotion annotation for one of the movie stimuli..*

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.

**WP1**will 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.

## 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 to reveal sex-specific cognitive strategies in processing complex situations.

WP 1 will provide insights into sex differences in the overall NV-AS induced by different movies, thus identifying sex differences in brain networks evoked over the temporally aggregated state of perceiving a complex situation. However, one of the main advantages of using movies in the study of individual differences is that their content varies dynamically and that due to the shared stimulus experienced by all participants the impact of the dynamic movie content on the brain can be directly analyzed. Making use of this, the present WP will further disentangle the temporally aggregated FC pattern of the NV-AS into time resolved FC patterns at each time point of the movie. Going beyond characterizing "state type" movie content as a whole, here the main aim is to directly identify specific events within the movies that result in distinct time-point specific FC patterns that differ between females and males. These, in turn, can be further analyzed through the use of temporally resolved rather than movie wide annotations.

To achieve this goal, we will again parcellate the fMRI data and compute a mean activation time course for each parcel. Instead of considering the NV-AS, we will now decompose static FC patterns into moment-by- moment, single repetition time (TR) co-fluctuation patterns [1, 7]. Basically, the Pearson correlation which is above used as a measure of temporally aggregated FC between pairs of brain regions is "temporally unwrapped" by calculating the element-wise product of each pair of nodes' z-scored time series. For each edge, i.e. connection between two brain parcels, this results in a time series - typically referred to as "edge time series" (eTS) - representing the instantaneous co-fluctuation magnitude between the pairs, or simply speaking, the development of FC over time [1]. To identify dynamic FC differences between females and males during movie watching, we

will identify time points within the movies, at which females' and malesÄ 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].

Using permutation tests we will identify those time points, for which classification accuracy differs significantly ($p < 0.05$) from chance, thus identifying events within each movie for which female and male time-resolved FC differs most. This approach offers the unique opportunity to link temporally specific sex differences in FC to movie content, thus identifying specific situations to which the sexes react differently. To characterize these situations, annotations of the movies will be used to identify movie events driving sex differences in time specific FC together with sex specific FC patterns driven by these features. To this aim, for each of the significantly different time points, the presence or absence of specific movie features (e.g. human bodies, faces, animals, scene cuts) will be coded binary, while continuous features (like luminance and audio level) will be coded as continuous numbers, thus creating a movie feature vector for each significant time point. We assume that typical sex differences in time resolved FC patterns will repeat across similar types of scenes in the different movies. Therefore, we will cluster the time points inducing significant differences between females and males according to their feature vectors, thus revealing parts of the movies that evoke sex difference during scenes with similar content. Then, for each of the time point clusters, we will compute a typical female and male FC pattern as the first component of a PCA across the FC patterns assigned to this cluster. This will identify typical female and male FC patterns in response to movies content associated with this cluster of movie scenes.

Finally, we will identify the pattern of brain regions that are most strongly involved in the typical female and male FC patterns for each cluster. Firstly, we will threshold the typical female and male FC patterns to identify most important edges in the sex-specific networks. Then, for each brain region, we will compute the weighted sum of all edges connected to that region and identify most important network nodes by selecting those for which summed FC strength exceeds the mean + 2 standard deviations across all nodes. This procedure will identify the sex specific spatial distribution of brain networks evoked by specific scenes in the movies.

Altogether, this procedure will shed light on which features within the movies induce most pronounced differences between females and males. Together with a set of features describing the movie scenes, we will identify female and male typical brain networks responding to these situations. While we expect to identify cognitive domains that match those identified in classical TB studies, the present approach will not only identify differences in specific isolated cognitive domains but reveal sex differences in cognitive strategies of dealing with certain complex situations. For example, while TB studies have identified sex differences in specific brain regions (like the fusiform face area) when participants viewed pictures of faces presented in isolation, our approach will depict sex differences in the brain when faces are encountered in a multimodal situation like in real life. Consideration of the brain networks evoked differently in females and males will shed light on sex specific neuronal patterns in perceiving faces.

**Main Goal: Identify temporally specific movie events driving sex differences in the experience of real life- like stimuli and related differences in time-resolved FC patterns.**

**Open Question - Relation to Classical Findings: How do naturalistic sex differences observed here relate to findings from traditional TB paradigms (e.g., isolated face viewing)? Are the same brain regions implicated, or do new networks emerge under ecologically valid conditions?**

### WP 3: Shared but unique: Examination of the sex specific shared response to dynamic emotions

**WP3 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.**

One of the main advantages of NV paradigms over the RS approach is that all participants are exposed to the same stimulus, resulting in a synchronization of the neural response across participants. However, at the same time, substantial individual differences within this response are preserved [9, 29]. In this WP, instead of looking at time resolved FC patterns, we will focus directly on the evolution of the neural response over time to examine in more detail, whether and how females' and males' brains differ in response to the dynamic movie content. Considering that sex differences in emotion perception and regulation have often been suggested in

the literature (e.g. [4, 11]) and that movies are particularly effective in inducing strong negative and positive emotions [13, 33], we will focus on the sex differences in the brain's reaction to the evolving emotional content over the course of the movies' narratives. Results from this WP will extend existing findings on sex differences in emotion perception which have mostly only focused on single emotions in rather artificial situations.
To do so, we will employ the new analysis approach (TOPF, [20]) which has recently been developed in our lab. Simply speaking, for each brain region, this methodological approach extracts the shared brain response time course across a group of participants (through use of a PCA), which is evoked by watching the movie. Furthermore, the individual expression of this shared response is computed, which indicates to what extent the individual brain reaction matches the typical brain response across the group. In the present context, we will employ an adjusted "two group" version of TOPF. Instead of identifying the shared brain response across all participants, we will identify typical brain responses for females and males separately. Importantly, to control for hormone related variance within the data, we will regress each subject's time series on their hormone levels and use the residuals, i.e. the part of the signal not explained by hormones, for computation of the shared response. For each brain region we will compare the typical female and male response in two steps: Firstly, we will check whether the typical female response is significantly different from the typical male response. This will identify brain regions, in which the evoked brain response to movie differs fundamentally between females and males. To further explore whether depicted emotions are the driving factor in differential brain activation patterns in females and males we will employ the existing emotion annotation of our movies with respect to the six basic emotions. For each of the brain regions which display significantly different time courses for female and males, we will correlate the female and male time course with the emotion annotation to find out which emotions drive the differences. High correlation between the female respectively male shared response and annotation of specific emotions will identify emotions within the movies that drive sex differences in certain parts of the brain.
For brain regions, where the overall sex specific brain responses do not differ significantly, we will compare the individual expressions of this response by a two-sample t-test. This will identify brain regions, which react to the narrative of the movie in a similar way, but in which the intensity of the experience differs between females and males. We expect to find both types of regions mainly in higher cognitive regions, which we will further explore through the use of functional decoding [10]. Finally, we aim to find out whether, across the whole brain, females and males differ fundamentally in their perception of the evolving narrative of the movies. To this aim, for each subject, we will compute the similarity of individual brain response to the sex-specific typical response for each brain region. Then we will calculate a summary score (over all regions) to determine the sex of the subject by assigning them to the class with the higher overall similarity score. If this classification works with high accuracy, it can be taken to indicate, that there are typical female and male whole brain patterns in the perception of complex narratives. Should this not be the case it would speak to the brain patterns being driven by individual factors over sex.
**Main Goal: Identify sex-specific patterns in neural responses to dynamic emotional content during NV, revealing how females and males process emotionally charged situations differentially.**
**Open Question 1 - Which Brain Regions Show Fundamental Sex Differences? Which brain regions exhibit fundamentally different time-resolved responses to emotional content between females and males, and are these differences consistent across movie narratives?**
**Open Question 2 - Which Emotions Drive Neural Divergence? Are specific emotional categories (e.g., fear, anger, happiness) more likely to induce divergent neural responses between females and males?**

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