



Portraying emotions at their unfolding: A multilayered approach for probing dynamics of neural networks

Gal Raz^{a,d,e}, Yonatan Winetraub^{a,b}, Yael Jacob^{a,d}, Sivan Kinreich^{a,c}, Adi Maron-Katz^{a,d}, Galit Shaham^f, Ilana Podlipsky^a, Gadi Gilam^{a,c}, Eyal Soreq^{a,c,g}, Talma Hendler^{a,c,d,*}

^a Functional Brain Center, Tel Aviv Sourasky Medical Center, 6 Weizmann Street, Tel Aviv 64239, Israel

^b Adi Lautman Interdisciplinary Program for Outstanding Students, Tel Aviv University, Tel Aviv 69978, Israel

^c School of Psychological Sciences, Tel Aviv University, Tel Aviv 69978, Israel

^d Physiology and Pharmacology Department, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

^e Film and Television Department, Tel Aviv University, Tel Aviv 69978, Israel

^f Psychology Department, The Hebrew University of Jerusalem, Jerusalem, Israel

^g Screen Based Art Department, Bezalel Academy of Arts and Design, Jerusalem, Israel

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ABSTRACT

Dynamic functional integration of distinct neural systems plays a pivotal role in emotional experience. We introduce a novel approach for studying emotion-related changes in the interactions within and between networks using fMRI. It is based on continuous computation of a network cohesion index (NCI), which is sensitive to both strength and variability of signal correlations between pre-defined regions. The regions encompass three clusters (namely limbic, medial prefrontal cortex (mPFC) and cognitive), each previously shown to be involved in emotional processing. Two sadness-inducing film excerpts were viewed passively, and comparisons between viewer's rated sadness, parasympathetic, and inter-NCI and intra-NCI were obtained. Limbic intra-NCI was associated with reported sadness in both movies. However, the correlation between the parasympathetic-index, the rated sadness and the limbic-NCI occurred in only one movie, possibly related to a "deactivated" pattern of sadness. In this film, rated sadness intensity also correlated with the mPFC intra-NCI, possibly reflecting temporal correspondence between sadness and sympathy. Further, only for this movie, we found an association between sadness rating and the mPFC–limbic inter-NCI time courses. To the contrary, in the other film in which sadness was reported to commingle with horror and anger, dramatic events coincided with disintegration of these networks. Together, this may point to a difference between the cinematic experiences with regard to inter-network dynamics related to emotional regulation. These findings demonstrate the advantage of a multi-layered dynamic analysis for elucidating the uniqueness of emotional experiences with regard to an unguided processing of continuous and complex stimulation.

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Introduction

Dynamism has been a key issue in the field of empirical research of emotion since its early days. The notion that exploration of the temporal characteristics of emotions is crucial to their psychological conceptualization is a red thread running through classical and contemporary theories (e.g. those of James (1884), Schachter and Singer (2000), Lazarus and Folkman (1984), Frijda (1986), Scherer (2001), Russell (2003)). These theories have paid much attention to temporal aspects of emotional processes, such as order, duration, latency, sequentiality and simultaneity. Biologically oriented researchers of emotion (e.g. Cannon, 1929; Damasio et al., 1996; LeDoux, 1996; MacLean, 1955; Papez, 1937) have reformulated this

issue into a neuroscientific problem, proposing various models of temporal relations between different emotional processes, which are instantiated in sub-cortical and cortical regions.

In contemporary theory (e.g. Barrett, 2006; Frijda, 1986; Mesquita et al., 2010; Scherer, 2009) the interactive and contextualized nature of emotional dynamism is increasing in prominence. Emotions are regarded less as a reflex-like deterministic execution of automatic or innate "affect programs", and more as processes which are dynamically emerging and interactively shaping vis-à-vis changing internal and external factors. Neuroscientifically, this focus on interacting processes is related to a system level perspective, which highlights the unfolding *integration* and *disintegration* of neural networks. Researchers such as Scherer (2009), Lewis (2005), and Barrett (2006) maintain that emotions wax and wane in correspondence with neural synchronization and de-synchronization respectively, assuming that synchronization allows a coherent response from the interacting networks. Specifically, Barrett (2006, 2009) highlights two interacting

* Corresponding author at: Functional Brain Imaging Unit, Tel Aviv Sourasky Medical Center, 6 Weizmann Street, Tel Aviv 64239, Israel. Fax: +972 3 6973080.

E-mail address: talma@tasmc.health.gov.il (T. Hendler).

processes, which are hypothesized to be underpinned by distinct brain systems: (a) core affect—a continuous stream of neurophysiological data, experienced as a feeling of pleasantness/unpleasantness with a varying degree of arousal; (b) conceptualization—attribution of the core affect to specific mental contents, which facilitates the categorization and conscious elaboration of the affective state. Barrett further maintains that with a growing extent of re-entrance between the systems that render these processes during emotional experience, neural activities are constrained and tunneled into a coherent “solution” in terms of interpretation and action plans.

While the theoretical interest in dynamism of emotions and the related neural network dynamics is growing, the empirical inquiry into these issues appears disproportionately limited, especially in human neuroscience (cf. Frijda, 2009). Neuroimaging studies have tended to focus on peaks of emotional intensity, rather than on its unfolding, thus experientially “chunking” the changing feelings into supposedly static emotion states (Scherer, 2009). A considerable limitation of the empirical scope on dynamism particularly appears in studies, which introduce instantaneous emotional cues, such as images of facial expressions and abrupt aversive stimuli.

As theoretical emphasis has been placed on the dynamics of coordination within and between neural networks rather than on the change of local activation, an analysis tool for probing inter-regional crosstalk in the brain is required. Functional connectivity, coherence and synchronization are such measures of neural coupling. This has been established by numerous studies of various brain functions and dysfunctions, using mainly functional Magnetic Resonance Imaging (fMRI), ElectroEncephaloGraphy (EEG) and MagnetoEncephaloGraphy (MEG). In these cases, the neural correlate of a specific function is not assumed to be a result of activation in a group of brain regions, but rather of one or more connection paths between regions (Sporns, 2010).

Experience of emotions has also been correlated with certain patterns of synchronization and coherence in EEG (Garcia-Garcia et al., 2010; Keil et al., 2007), and functional connectivity measures in fMRI, mostly between the amygdala and cortical regions (e.g. Lerner et al., 2009; Morris et al., 1999; Williams, 2006). Further, the strength of such relations has been reported to covary with factors such as personality traits (Cremers et al., 2010; Matsumoto et al., 2006), and psychopathological states (Admon et al., 2009; Bleich-Cohen et al., 2009; Chen et al., 2008). However, while these studies examined coupling of brain activities during emotional experience, the dynamics of such coupling remains largely unexplored. Similar to other research in the field, most of the studies that examine the impact of emotion on fMRI correlation and EEG synchronization of brain signals used instantaneous stimuli (mainly brief presentation of affective images), thus limiting the scope of research to transient affects. While some studies (Aftanas et al., 1998; Eryilmaz et al., 2010) have introduced prolonged experimental stimuli (e.g., video clips, personalized recall), their authors report on functional connectivity or synchronization indices computed over the entire recording period rather than tracing the dynamic unfolding of these measures. Moreover, while the anatomical delineation of the networks of interest is a central issue for theories of emotion, the spatial resolution of EEG does not allow such specification. On the other hand, fMRI studies have often focused on the correlativity of one or a few regions of interest, but have not accounted for temporal aspects of networking.

Alternatively, here we used a dynamic measure of correlativity within and between anatomically defined groups of regions, which can be functional referred to as networks. This study, in line with contemporary theories of emotion, aims to characterize the dynamic interactions within and between brain networks, which instantiate processing of distinct aspects of the emotional experience. The anatomical definition of the networks was based on the findings of a comprehensive meta-analysis of 162 emotion studies (Kober et al., 2008), which clustered six distributed groups of regions according

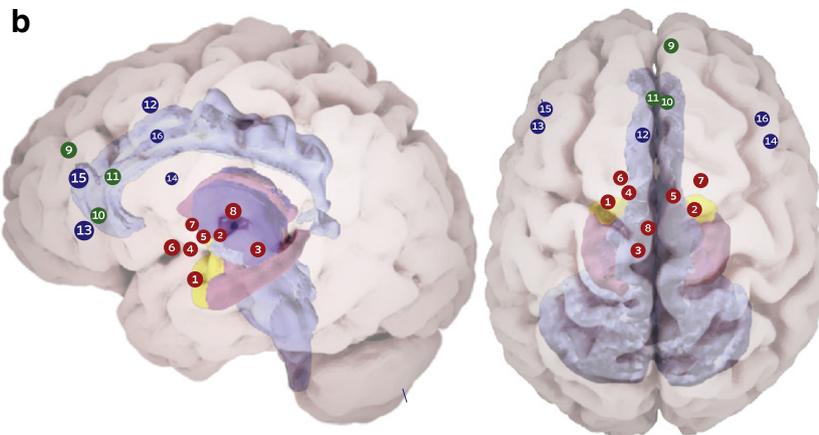
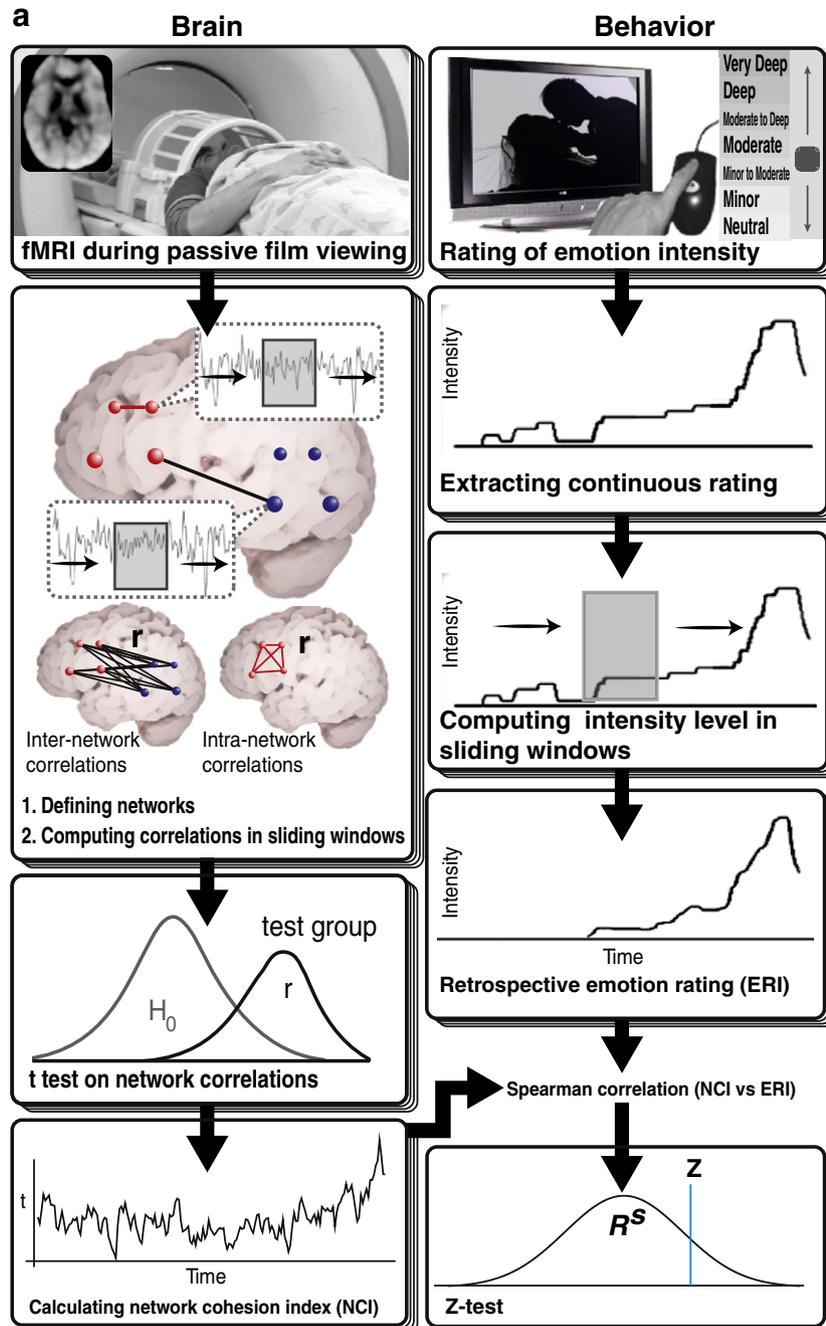
to the extent to which they were significantly co-activated across studies. Importantly, while the data-driven clustering algorithm used in this meta-analysis was blind to the assigned functions of the regions, the resulting clusters appear to correspond with distinct aspects of emotional processing beyond stimulus type. Rather than accounting for the co-activity of these functional networks across studies, in this study we were interested in their unfolding co-activity during an experience of the same emotional stimulus.

Since the “conceptual act model” (Barrett, 2006) provides specific predictions of the dynamic coordination between basic affective and higher cognitive processes, we selected three of the region groups, which have been shown to play a role in these functions in the context of emotional processing (for coordinates see Table S2): (a) *Core limbic group* (from here on referred to as the *limbic network*). In our study, this network is comprised of the periaqueductal gray, ventral striatum nuclei, amygdala, hypothalamus, and central medial thalamus. These regions have been related to quick, automatic, and often preattentive, “low-level” appraisals of the affective value of emotional stimuli, as well as to modulation of autonomic emotional reactions (cf. Kober et al., 2008; LeDoux, 1996). (b) *Cognitive/motor group* (*cognitive network*)—includes lateral prefrontal cortical areas and the pre-supplementary motor area. These regions have been associated with cognitive appraisal of emotional information (e.g. conceptualization) and with cognitive control over emotional reactions, including allocation of attention, inhibition, and the selection of appropriate action (e.g. Kober et al., 2008; Pessoa, 2008). (c) The *medial prefrontal group* (*mPFC network*)—encompassing the pre-genual and rostromedial anterior cingulate cortex (ACC) and the dorsomedial prefrontal cortex (dmPFC). The mPFC has been implicated in the monitoring of one's own or other's affective states in a social context (e.g. Northoff et al., 2006; Ochsner and Gross, 2008), as well as empathy (Shamay-Tsoory, 2008). Anatomical evidence (e.g. Öngür and Price, 2000) has suggested that the regions in this network mediate cortical visceromotor output to the hypothalamus and brainstem, and a plethora of functional evidence (cf. Nakao et al., 2009) associates mPFC structures with regulation of emotions. While both lateral and medial prefrontal regions have been shown to play a key role in emotion regulation, mPFC seems to be more dominant in forms of regulation, which do not involve cognitive reappraisal of the emotional stimuli (Ochsner and Gross, 2008).

In order to empirically tackle the issue of dynamism of these networks, we applied the following steps: (a) dynamically manipulating emotions by utilizing prolonged and complex stimuli by movie clips; (b) developing a method for probing changes in the coordinated activities within and between functional networks; (c) comparing the resultant temporal patterns of connectivity with corresponding behavioral and physiological indices of the emotional reaction.

In keeping with the conceptual act model, we were interested in emotions, which apparently engage both “low” and “high” levels of processing, and are also readily elicited by motion pictures. Sadness, which involves well-known autonomic reactions, as well as cognitive processing of social information (cf. Averill, 1968), seems to meet these criteria, and therefore it was selected as a case study. To note, a previous meta-analysis reported that ACC and dorsomedial areas, which are included in the mPFC network described above, are specifically implicated in sadness (Murphy et al., 2003). Following this rationale, two excerpts from the films *Stepmom* (Columbus, 1998) and *Sophie's Choice* (Pakula, 1982, here termed *Sophie*) were used in the study. Both excerpts, which were previously shown to effectively induce sadness (Goldin et al., 2005; Oatley, 1996), present a fatal separation of a mother from her children. In *Sophie*, the act of separation is forced by a Nazi officer; in the farewell scene from *Stepmom*, the separation of a terminally-ill mother from her children is discussed as a future event.

To test the unfolding of co-activity of the three brain networks of interest, we propose a new index, probing the dynamics of coordination both within a defined network (intra-network cohesion index;



intra-NCI) and between networks (inter-network cohesion index; inter-NCI). Cohesion is measured here in a way that reflects both the strength of the average correlations between signals in a group of regions and the variation about this average, with higher values for correlations that are narrowly distributed about a high average. The temporal patterns of the NCIs are then compared with corresponding time courses of behavioral and physiological indices of emotionality (see Fig. 1 for an outline of the procedure).

It was expected that these indices of emotionality will covary when testing brain networks, which are associated with emotional processing in general, and sadness in specific. Assuming this coupling to be consistent across the two instances of cinematic sadness induction (i.e. *Sophie* and *Stepmom*), we specifically tested the following hypotheses: (a) the intra-NCI of the limbic and mPFC networks will covary with behavioral and physiological indices of emotional reaction to the films as an indication of their key involvement in the basic processing of affective information; and (b) the inter-NCI of limbic-cognitive networks will correlate with the continuous behavioral indices, indicating enhanced conceptual processing of affect during an intense emotional experience and reduced processing as it wanes.

Methods

Material and procedure

Induction of emotional experience

Two video excerpts, taken from the commercial films *Sophie's Choice* (Pakula, 1982) and *Stepmom* (Columbus, 1998), were used in this study. In the scene from *Sophie*, a mother is forced by a Nazi officer to choose which of her two children lives and which dies. The clip from *Stepmom* included two farewell scenes in which a mother talks with her children about her future death from a terminal disease. The durations of the clips were 10:00 and 8:27 min, respectively, and their display was preceded and followed by a 3-minute epoch during which the participants passively gazed at an all-black slide.

Retrospective self-reporting of emotional experience

Emotion label rating. An inventory containing 76 emotion labels was created on the basis of a comprehensive list of emotion words suggested by Shaver et al. (2001). The labels were translated to Hebrew and presented along with their corresponding annotations, adapted from the Rav-Milim Hebrew dictionary (Choueka et al., 1997). After scanning, the participants rated the intensity to which they experienced each labeled emotion on a seven point Likert-like scale, consulting the annotations in case of unclarity.

Continuous emotion rating. The participants continuously reported on shifts in intensity of sadness they have experienced while watching the clip during scanning, i.e. retrospectively. The data were acquired via designated homemade software. By using the computer-mouse, the subjects indicated changes in their felt intensity of sadness in respect with a vertical scale continuously presented on the screen. The scale included 7 levels of sadness—from neutral to very deep—each containing 3° of change (21° in total). The feedback was sampled at the rate of 10 Hz.

Electrocardiography recording

Electrocardiography (ECG) was recorded continuously during scanning via an MRI-compatible system. The sampling rate was

5000 Hz. The measurements were obtained using a BrainAmp ExG MR psychophysiological monitoring system (BrainProducts, Munich, Germany). For each participant, Ag/AgCl electrodes were attached to the right and left side of the chest.

fMRI acquisition

Structural and functional scans were performed using a GE 3 T Signa Excite echo speed scanner with an 8-channel head coil, and a resonant gradient echoplanar imaging system. The scanner is located at the Wohl Institute for Advanced Imaging at the Tel-Aviv Sourasky Medical Center. A T1-weighted 3D axial spoiled gradient echo (SPGR) pulse sequence (TR/TE = 7.92/2.98 ms, flip angle = 15°, pixel size = 1 mm, FOV = 256 × 256 mm, slice thickness = 1 mm) was applied to provide high-resolution structural images. Functional whole-brain scans were performed in an interleaved top-to-bottom order, using a T2*-weighted gradient echo planar imaging pulse sequence (TR/TE = 3000/35 ms, flip angle = 90°, pixel size = 1.56 mm, FOV = 200 × 200 mm, slice thickness = 3 mm, 39 slices per volume).

General procedure

All the participants in this study were healthy volunteers without known history of neurological or psychiatric disorder with at least 12 years of education living in Israel most of their life with Hebrew as their spoken language. All participants signed an informed consent form approved by the ethical committees of the Tel Aviv Sourasky Medical Center. The participants in Experiment 1 additionally signed an informed consent form approved by the department of psychology at the Tel Aviv University. For a summary of details on the different experiments included in this study, see Table S1.

Experiment 1: validation of the behavioral measure

To examine the reliability and validity of our tool for retrospective moment-to-moment emotion rating we used a test-retest design with the same rating software. 20 healthy volunteers (13 females; 23.18 ± 2.56 years) were randomly and equally assigned to participate in either the reliability or validity protocols and viewed one of the clips mentioned above. In both test subgroups, participants were randomly and equally assigned to view either *Sophie's Choice* or *Stepmom*.

The reliability of the behavioral measure was tested on a group of 10 participants. These participants went through three sessions during which the same clip was presented to them on a computer screen. Following the first and second sessions, the participants were asked to fill two personality questionnaires: the Sensitivity to Punishment and Sensitivity to Reward Questionnaire (Torrubia et al., 2001) and the NEO Five-Factor Inventory (Costa and McCrae, 1992), also serving as a buffer in this procedure. In the second and third sessions, but not in the first, the participants were asked to retrospectively report on their emotional experience during the first session, as described in the main text. Test-retest reliability was determined by calculating the moment-by-moment correlation between the ratings performed during the second and third sessions over both film clips. Together, these series showed high moment-by-moment correlation (average $r = 0.93$), indicating a considerable reliability.

To examine the construct validity of the tool, we tested whether the retrospective emotion rating indeed fits with the participants' report of emotional effect during the initial viewing. 10 additional participants were instructed to rate their felt sadness intensity

Fig. 1. Analysis approach. (a) Schematic description of the computation of the network cohesion index, the behavioral index, and the comparison between them (see Methods). (b) The locations of the ROIs encompassing the limbic (red), mPFC (green), and cognitive (blue) network: 1 and 2—L and R amygdala; 3—periaqueductal gray; 4 and 5 L and R hypothalamus; 6 and 7—L and R ventral striatum; 8—central medial thalamus; 9—dorsomedial PFC; 10—pregenual ACC; 11—rostradorsal ACC; 12—pre-SMA; 13 and 14—R and L inferior frontal gyrus; 15 and 16—L and R dorsolateral prefrontal cortex (coordinates in Table S2). The regions are projected on a 3 D model of brain anatomy adopted from the SPL-PNL Brain Atlas (Talos et al., 2008) registered to the *Talairach coordinate system* and visualized using 3D slicer (Pieper et al., 2004) and Maya (Autodesk Inc., San Rafael, CA).

throughout two sessions during which the same clip was presented. During the first session, the participants were requested to rate the intensity of sadness they were *currently* feeling, while in the second session they were asked to retrospectively report on sadness experienced during the first session. Other instructions were identical to those given to the other group, as described in the previous paragraph. Between the sessions the participants filled the SPSRQ questionnaire. Moment-by-moment correlation between the on-line and the retrospective ratings was calculated over both film clips.

Analyzed together, the average moment-by-moment correlation between the data series was 0.89, indicating that retrospective rating considerably reflects of reported experience of the initial viewing. These findings are in line with the reports of [Hutcherson et al. \(2005\)](#), who reported an average r of 0.86 between online and retrospective ratings, which were obtained using a similar method.

Experiment 2: fMRI and retrospective emotional rating

The fMRI data were acquired from 31 right-handed healthy volunteers (16 females; 26.47 ± 4.79 years) during passive viewing of the two film excerpts described above, displayed in a counterbalanced order across participants and intersected by 10 min of anatomical scans. ECG was recorded during the scan for 13 of these subjects (7 females; 26.6 ± 5.59 years) as described above ([Material and procedure section](#)). To avoid distraction during film viewing, continuous emotional rating was obtained only retrospectively immediately following scanning in a quiet room using a computer screen and a mouse in a similar manner as described above (see [Methods](#)).

Experiment 3: physiological characterization of the emotional experience

To further characterize the physiological reaction to the clips with regard to retrospective emotional rating, ECG was recorded outside the scanner from additional group of 26 healthy volunteers (15 females; 25.23 ± 2.7 years). In addition, emotional rating and labeling were performed retrospectively in a similar manner to Experiment 2.

Data preprocessing and analysis

Behavioral indices

Emotion label rating. Overall, 51 self-report questionnaires (25 females; 25.75 ± 3.68 years and 23 females; 25.87 ± 3.81 years for *Sophie* and *Stepmom*, respectively) were included in the analysis after 5 of the forms were excluded for each film due to incorrect completion. The median values and the frequency of their rating higher than minimal were computed for each label.

Rated sadness intensity. The rating was collected from 64 (35 females; 25.55 ± 3.68 years) and 59 (31 females; 25.87 ± 3.81 years) subjects across the three experiments described above. Technical problems prevented the inclusion of 3 and 8 ratings for *Sophie* and *Stepmom*, respectively.

Parasympathetic index

The high-frequency (0.15 to 0.4 Hz) component of the power spectrum of heart rate variability (HF–HR) is considered to represent an autonomic parasympathetic vagal influence on the sino-atrial node of the heart ([Camm et al., 1996](#)). Significant changes in HF–HR power were demonstrated during the experience of various emotions, including sadness ([Kreibig, 2010](#)). We therefore considered the fluctuations in this frequency band as indicating an emotional response. Preprocessing of ECG was done offline using Matlab software (MathWorks Inc.) to yield a continuous heart rate (HR) index, based on RR interval analysis. The ECG signal was analyzed offline using Matlab software (MathWorks Inc.) to yield a continuous heart rate (HR) index, based on R–R interval analysis. Artifacts related to the MR gradients were removed from all the ECG datasets using the

FASTR algorithm ([Niazy et al., 2005](#)) implemented in FMRIB plug-in for EEGLAB ([Delorme and Makeig, 2004](#)) and provided by the University of Oxford Centre for Functional MRI of the Brain (FMRIB). The resulting clean data was downsampled to 250 Hz.

R peak detection using the FMRIB plug-in for EEGLAB. A trained rater monitored the performance of the algorithm, hand-correcting mis-marking of R peaks. The average correction rate over subjects was 0.45% (sd: 1.01%, maximum: 5.78%). The inter-beat intervals or RR intervals were obtained as differences between successive R-wave occurrence times. A sliding window approach was used to correct the time series for irregular RR intervals. Irregular ectopic RR intervals were defined as intervals shorter than 2 standard deviations within a window of 21 s. These intervals were considered abnormal beats and their RR value was set to be the average RR interval within that window. A cubic spline interpolation was used to obtain an equidistantly sampled time series of RR intervals.

HF–HR calculation included Hilbert transform ([Le Van Quyen et al., 2001](#); [Peng et al., 2004](#)) to explore the dynamics in the high frequency component. A time series of the instantaneous power is the parasympathetic index we use in our investigation of HF component of the RR time series: first the equidistantly sampled RR time series was band pass filtered to the HF frequency band (0.15–0.4 Hz), then the Hilbert transform was applied to it and the result was squared to obtain instantaneous power estimate. The obtained HF estimates were Z-transformed to allow inter-subject comparisons.

Relationships between behavioral and parasympathetic indices

When using Spearman's rank test to compare continuous time series, such as the behavioral and physiological indices, dependencies between sequential samples should be minimized to avoid violation of the assumption of independence among samples. Temporal autocorrelations resulting from the cyclic nature of the signal is a source of such dependencies. Since the HF–HR signal was high pass filtered at 0.15 Hz, the longest duration of such a cycle in this signal is $\frac{1}{0.15} = 6.67$ s. In other words, any information with dependency time constant higher than 6.67 s was removed from the signal following the filtering. However, the information in windows shorter than 6.67 s may still be dependent due to the cyclic nature of the signal. In that case, resampling the signal in time windows longer than 6.67 guarantees independence. Therefore, the HF–HR signal was averaged in non-overlapping windows of 7 s. To allow the comparison of the time series of sadness rating with this physiological index, the median values of the former were also computed in non-overlapping windows of 7 s. The correlations between physiological and behavioral data were calculated for each subject, yielding for each pair of series one value. A two sided Z-test was used to test whether the correlation between the pair is 0, as Spearman correlation values are approximately normally distributed.

fMRI network indices

Preprocessing. Seventeen (9 females in both cases; 26.12 ± 4.66 and 26.56 ± 4.66 years in *Sophie* and *Stepmom*, respectively) participants were included for final analysis of the brain data, and 7 participants were included in the analysis of the simultaneous ECG (4 females; 24.14 ± 3.06 and 3 females; 25.93 ± 3.94 years for *Sophie* and *Stepmom*, respectively). Since the viewing of the onset of the clip may engage emotion-related neural processing associated with appraisal of the novelty, and not with its content, the rest epochs and additional seven first TRs recorded during the film viewing (approximately the time span of hemodynamic response to the onset of the film) were excluded from the statistical analysis.

Preprocessing and statistical analysis were performed using Brain-Voyager (BV) QX version 2.1.2.1545. Head motions were corrected by rigid body transformations, using 3 translation and 3 rotation

parameters. The middle volume served as a reference in this procedure. Trilinear interpolation was applied to detect head motions, which were corrected using sinc interpolation. Slice scan time correction was performed using sinc interpolation. The temporal smoothing process included linear trend removal and usage of high pass filter of 0.008 Hz. 6 mm FWHM Gaussian spatial smoothing was used to reduce noise. The voxel size of the SPGR images was standardized to $1 \times 1 \times 1$ mm using trilinear interpolation. Structural maps were then transformed into Talairach space and manually coregistered with the corresponding functional maps.

Brain data of 13 of the participants were excluded from analysis due to various technical problems during scanning. Head motion exclusion criteria were > 1.5 mm translational or $> 1.5^\circ$ rotation in any of the axes. For each of the cinematic conditions, one data set was discarded due to these criteria. 6 of the ECG data sets for each film were discarded due to technical problems and poor quality of ECG signal.

Selection of regions of interest (ROIs) was anatomically based on the work of Kober et al. (2008) with few modifications (see Supplements). The final location of regions included in our study and their coordinates are presented in Fig. 1b and Table S2. The transformation of the MNI coordinates to Talairach space was performed using the Brett transform (Brett et al., 2002). The ROIs were delineated on the basis of these coordinates, using 6 mm Gaussian smoothing kernel.

Computation of intra-network cohesion index (intra-NCI)

To analyze the dynamics of the functional connectivity within each of the networks, we first extracted the signal of each ROI (sig_{nr}) from the raw data, using a Gaussian mask with a radius of 3 mm around the seed coordinates of the ROI. Next, a set of all pair-wise Pearson correlation values were calculated for each subject, network, and time window. Cohesion indices were computed over time windows of 30 s (10 TRs) in keeping with the findings of an *in silico* study of functional connectivity at multiple time scales (Honey et al., 2007). In this study, the authors simulated dynamic brain activity and estimated the related BOLD signal on the basis of anatomical connectivity data. Dynamic time-dependent patterns of functional connectivity were observed in the simulated data when sampled in sliding windows of 30 s (but not of 240 s). Accordingly, we expected to find function-related connectivity fluctuations in our data using a similar time frame.

The correlation values computed over these windows were then transformed using Fisher's transformation, resulting in values that are approximately normally distributed, with mean at the indicated correlation. For each time window Δt : $[t * \Delta, (t + 1) * \Delta]$ and each subject (sub), $sub = 1, 2, \dots, N$, and each one of the networks $k = 1, 2, 3$ we define the set of Fisher-transformed pair-wise correlations between regions i and j belonging to network k

$$R_k^{sub}(\Delta t) = \{R_{ij}^{sub}(\Delta t) | i, j \in k\} \quad (1)$$

$$R_{ij}^{sub}(\Delta t) = \text{arctanh} \left[\text{corr} \left(sig_i(\Delta t), sig_j(\Delta t) \right) \right]. \quad (2)$$

For each subject, network region ($nr_1 \dots nr_n \in K$) and time window (Δt) we calculated a *network cohesion index* (NCI_k^{sub}), being the t-statistic on the set of R values (Eq. (3)). A right-tailed t-statistic with the null hypothesis of $\mu_k = 0$ was computed. This measurement takes into consideration the standard deviation of the correlation values. This was required in order to make sure that a high mean correlation value is not the result of a single strong connection.

$$NCI_k^{sub}(\Delta t) = \frac{R_k^{sub}(\Delta t)}{se(R_k^{sub}(\Delta t))}. \quad (3)$$

Here we made use of the t-statistic like measure, which is also proportional to the inverse of the square root of the coefficient of

variation, as it yields high values when the mean correlation between the regional signals in the network is high and the variability in the correlations is low. Note that this measure cannot be used for testing the hypothesis of 0 correlation, because a BOLD signal across the brain is known to be highly correlated, as are the pairs themselves. It serves merely as an indicator for both the strength and distribution of these correlations. A different approach was adopted in order to validate the significance of our downstream results (see below).

Computation of inter-network cohesion index (inter-NCI)

Cohesion indices were calculated also for each pair of networks in the same manner, while considering only correlation values between regions that are not in exclusive networks. Once again, the score was calculated using a right-tailed t-statistic assuming null hypothesis of mean 0 and unknown variance. For each pair of regions ($K_k, K_{k'}$) we defined:

$$R_{k'k''}(\Delta t) = \left\{ R_{pq}^{sub}(\Delta t) \mid p \in K_k, q \in K_{k'}, k' \neq k'' \right\}. \quad (4)$$

Having defined intra-network and inter-network functional connectivity indices, we next examined the relationships between the temporal pattern of these indices and the corresponding behavioral and physiological indices.

Examining the relationship between NCI and behavioral index

To fit the sampling method applied on the brain data we resampled the behavioral data. Median values of the rated sadness were computed in time windows of 7 time repetitions (TRs) with an overlap of 6 TRs (21 and 18 s respectively). Spearman ranked correlation coefficient (R^s) between the resampled behavioral and the cohesion time series (NCI_k^{sub}) were computed for each subject separately. Spearman coefficient was used here due to the fact that the relationship between the values of the emotion rating and BOLD signals need not be linear.

When it came to testing, in order to reduce the dependence between values in different time windows, only samples of non-overlapping time windows were utilized. Calculating Spearman's correlation coefficient yielded a correlation value for each subject (sub), $sub = 1, 2, \dots, N$ and network (k), $k = 1, 2, 3$. Two-tailed Z-test was used to test whether the average correlation is different from 0, yielding one p-value for each network.

Examining the relationship between NCI and the parasympathetic index

Instantaneous HF estimate was downsampled to allow its comparison with the network cohesion. The signal was averaged across time windows of 7 time repetitions (TRs) with an overlap of 6 TRs (21 and 18 s respectively), using a similar window averaging approach as applied for the NCI. Since only 7 valid ECG recordings of the scanned subjects were available, and the statistic power that can be achieved when performing individual comparisons in such a small sample group is low, the NCIs and the parasympathetic index were compared at the group level (rather than individually as in the case of the behavioral index). To moderate the effect of outlier data in this small group, the medians rather than the mean values were compared. Spearman's ranked correlation coefficient between the two time series was calculated for each network and between networks. Only samples of non-overlapping time windows were compared.

Correction for multiple comparisons

Adjusting for multiplicity, over all the 24 NCI-behavioral and NCI-parasympathetic comparisons, was done by controlling the false discovery rate (FDR) at level 0.05. The procedure used for that purpose is the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995), which is valid even under dependency for normally distributed test statistics as is the case in the current study (Benjamini and

Yekutieli, 2001; Reiner-Benaim, 2007). As for the two research questions (on NCI relations with the behavioral index on one side and the parasympathetic index on the other), each comprising of a family of 12 hypotheses, the combined evidence per family was derived using the Benjamini–Hochberg procedure. Both families were significant so no further selection was needed. In such a case each family can be tested separately at 0.05 level, and this was done using again the Benjamini–Hochberg procedure. The procedure assures FDR control on the average over the selected families.

Estimating the spatial specificity of the results

In order to examine the extent to which the observed relationships are part of a “whole brain effect” (i.e., their specificity in the brain space), we compared the resulting correlation values with corresponding values generated by an identical analysis of a set of random groups of regions in the gray matter. The original coordinates were randomly rotated and translocated in a sampling space, which included gray matter voxels. This gray matter mask was generated by applying a segmentation algorithm (BrainVoyager QX version 2.1.2.1545) on the anatomical data collected for the study. The randomization of the stereotactic coordinates was carried out in a way, which preserved the Euclidean distances between the nodes of the original network with a precision of 1 mm.

The cohesion indices for the random groups of regions and the behavioral and physiological indices were compared using a similar protocol as described above. This procedure was repeated for one thousand times. The specificity of the tested result was defined as the proportion of the random cases with lower Z value (behavioral) or R^s in the sampling space.

Percent signal change computation during limbic–mPFC NCI minima

A complementary post-hoc analysis of the percent of BOLD signal change was performed to further examine the neural activations coinciding with dips in limbic–mPFC NCI in the case of *Sophie*. We examined data recorded during 8 cinematic events, which were reported to elicit at least “minor” intensity of sadness during the film viewing. The first time window during which the median rating of sadness intensity was null, was used as an emotional baseline, thus, the baseline condition included the first ten TRs.

The signals were Z-transformed across all time points, and a mean baseline signal level was then calculated for each region in the core limbic and the mPFC networks. The percent signal change was calculated for each network (k), subject (sub) and local minimum (min) as follows:

$$PSC_{k,min,sub} = \frac{\frac{1}{n} \sum_{i=1}^n (\bar{X}_{k,i} - \bar{X}_{k,baseline})}{\bar{X}_{k,baseline}} \times 100 \quad (5)$$

where \bar{X}_k is the mean level of the BOLD signal within the network k , i is the index of the TR within a certain window, n is the number of TRs in the window (in this case, 7), and $\bar{X}_{k,baseline}$ is the signal in network k averaged over the baseline window. A two-tailed t -test was performed on the PSC over subjects to assess the significance of the results. FDR correction was applied to the resulting p values of (16 comparisons) comparisons (Benjamini and Hochberg, 1995; Benjamini and Yekutieli, 2001)

Results

Behavioral and physiological characterization of the emotional impact of the film excerpts

To identify the most commonly used labels for the emotional experience we used a specially designed inventory administered to all participants ($n = 51$, see Methods). Fig. 2a and Table S3 present the

labels of the emotions that were reported by the participants to be most intensively experienced during the viewing of each of the excerpts. The highest rate was obtained for “sadness”, “compassion” and “mercy” in both films (5 out of 7 in median, corresponding to the category “moderate to high intensity”). On the other hand, “sympathy” was additionally top-rated only in *Stepmom*, while “horror”, “hate”, “fear”, and “anger” were reported high (4 out of 7 in median) only in *Sophie*.

To characterize the dynamic aspects of the subjective emotional experience evoked by the films, we recorded retrospective continuous reports on sadness intensity during a second viewing of the film outside the scanner. Fig. 2b shows the median time course obtained for all participants for this rating (across experiments; $n = 64$ and $n = 59$ for *Sophie* and *Stepmom*, respectively). The peak intensity levels indicate an effective induction of sadness, which was significantly higher for *Sophie* than for *Stepmom* ($p < 0.005$, Mann–Whitney, two-tailed test) rating 85.7% (i.e. deep sadness) and 59.5% (i.e. “moderate to deep sadness”) of the maximal value, respectively. The emotional response to the excerpts differed not only in terms of the maximal values, but also in the patterns of its unfolding in time—a monotonic increase in *Sophie* vis à vis double-humped shape in *Stepmom*.

As evident in Figs. 2d and e, for each of the films, similar patterns were obtained for participants whose first time viewing had taken place inside (i.e. Experiment 2; $n = 16$, $n = 17$ in *Sophie* and *Stepmom*, respectively) or outside (i.e. Experiments 1 and 3; $n = 22$, $n = 19$ in *Sophie* and *Stepmom*, respectively) the scanner. The high correlation between the median time-courses obtained from these different study groups ($R^s = 0.95$, $p < 5 \times 10^{-37}$, $R^s = 0.9$, $p < 5 \times 10^{-23}$ for *Sophie* and *Stepmom*, respectively) also indicates a similarity in their emotional experience.

The behavioral data was compared with the parasympathetic index for a group who viewed the excerpts outside the scanner (i.e. Experiment 3; $n = 24$, $n = 19$ for *Sophie* and *Stepmom*, respectively). The temporal pattern of the *parasympathetic index*, which was obtained during the first uninterrupted film viewing is presented in Fig. 2c (see Methods for details on calculations), and is overlaid with the retrospective sadness rating in Fig. 2d. To note, the within-film similarity of parasympathetic patterns, which is evident for the two independent groups tested inside or outside the scanner ($R^p = 0.384$, $p < 0.001$ and $R^p = 0.46$, $p < 0.0002$ for *Sophie* and *Stepmom*, respectively) indicates the reliability of this measurement.

The individual parasympathetic index and sadness ratings were positively correlated for *Stepmom* ($Z = 3.84$, $p < 0.0002$) but not for *Sophie* ($Z = -1.90$, $p < 0.06$). Importantly, similar relationships ($Z = 3.46$, $p < 0.0006$) were observed for a separate group who viewed *Stepmom* inside the scanner (i.e. Experiment 2).

Correction for multiple comparisons

Adjusting for the multiplicity of the tests of the association between the cohesion index with the other two indices was done by controlling the false discovery rate at level 0.05 ($q_{FDR}^{BH} \leq 0.05$), using the Benjamini–Hochberg procedure (see Methods, Data preprocessing and analysis section). The comparisons yielded four p -values smaller than 0.0042, which are statistically significant (for the entire list of p -values, see Table S4). When viewing the research questions as our relevant frame of inference, one family of hypotheses about the associations between the cohesion and behavioral indices, and the second family about associations between the cohesion and the parasympathetic indices, we find that both are statistically significant after adjusting for multiplicity. We can therefore add an analysis within each family, adjusting separately for multiplicity using the Benjamini–Hochberg procedure. We find that a fifth hypothesis with p -value of 0.0163 (on an association between limbic intra-NCI and the behavioral index in the case of *Sophie*) can also be rejected (see Data preprocessing and analysis section).

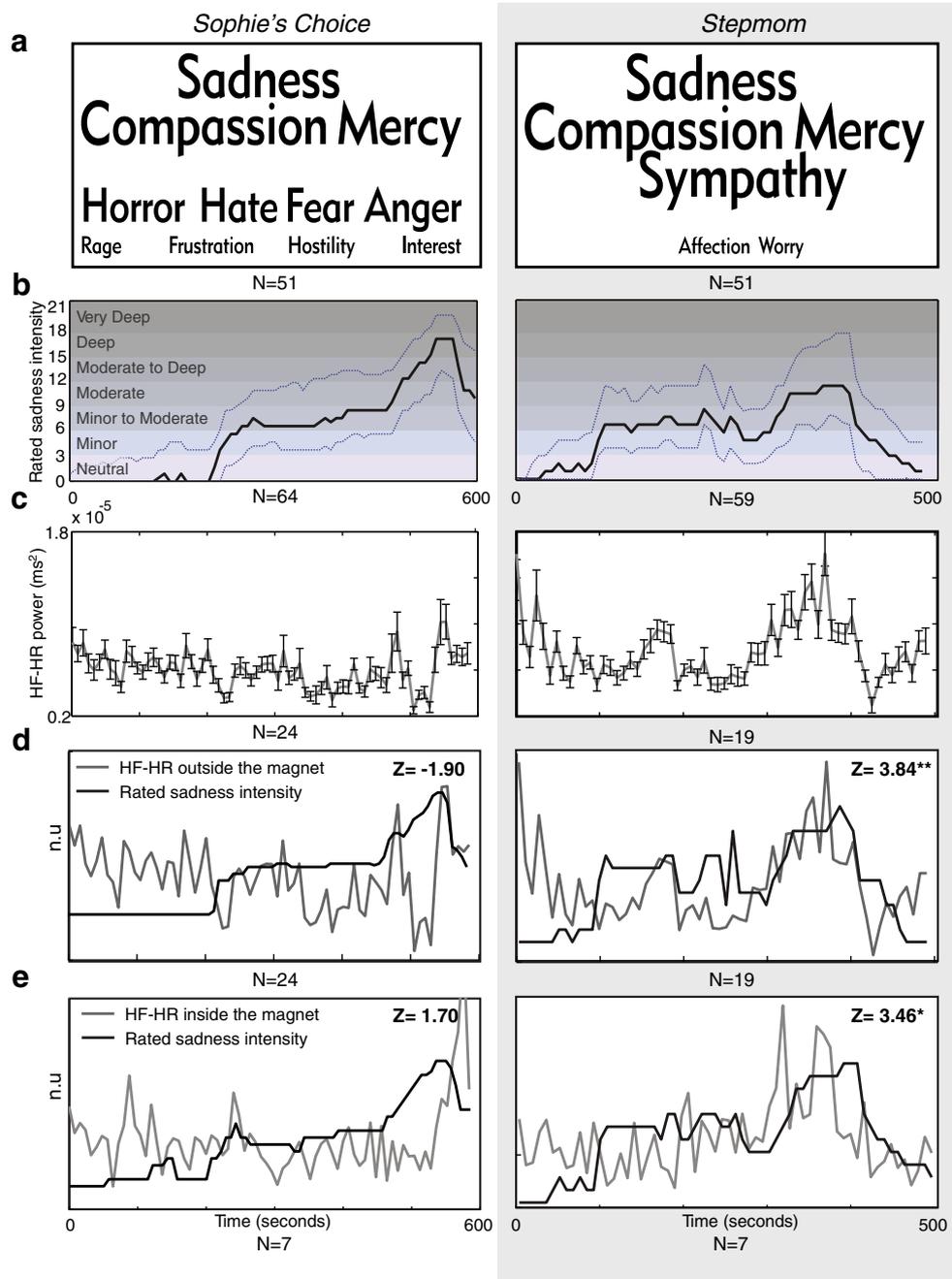


Fig. 2. Behavioral and physiological reactions to the film excerpts. (a) Emotion label rating—font size is proportional to the median value of the rating (the maximal size corresponds to the level of 5 out of 7). Only labels rated higher than 1 in median are presented (see Table S3 for more details). (b) Rated sadness intensity—median values (black) and inter-quartile range (blue) of rated sadness intensity. (c) Parasympathetic index (mean ± standard error) in a group of subjects whose ECG data were recorded while viewing the film excerpts outside the scanner. (d and e) Parasympathetic (mean) and behavioral (median) indices for the groups of subjects whose ECG was recorded outside (d) and inside (e) the scanner. Z values for tests on R^2 coefficients between the behavioral and parasympathetic indices are presented in (d). * $p < 0.006$; ** $p < 0.0002$.

Relations between dynamics of NCI and rated sadness intensity and a complementary percent signal change analysis

A comparison between the temporal patterns of intra-NCI and the behavioral index revealed both similarity and difference between the emotional states elicited by the two film excerpts.

In line with our first hypotheses, for both films the pattern of the rated sadness intensity significantly correlated with the time course of the limbic intra-NCI ($Z = 2.40$, $p < 0.02$, $q_{FDR}^{BH} \leq 0.05$; $Z = 2.86$, $p < 0.005$, $q_{FDR}^{BH} \leq 0.05$ for *Sophie* and *Stepmom*, respectively, Fig. 3a i and ii and Table 1). To test the specificity of this association, a bootstrapping analysis was performed. Corresponding temporal patterns

of network cohesion were computed for 1000 clusters of voxels whose coordinates were randomly chosen. For each of the random clusters, the NCI time course was compared with the ratings of sadness intensity (see Data preprocessing and analysis section for more details). In the case of *Sophie*, the association between the limbic NCI and the rated sadness intensity was stronger than 99.4% of the associations measured for the randomized clusters, indicating its high specificity (Fig. S1). A lower specificity of 84.4% for the limbic-behavioral association was found in *Stepmom*.

Similar analyses were performed for the mPFC and cognitive networks. The pattern of the rated sadness intensity significantly correlated with mPFC intra-NCI in *Stepmom* but not in *Sophie* ($Z = 3.24$,

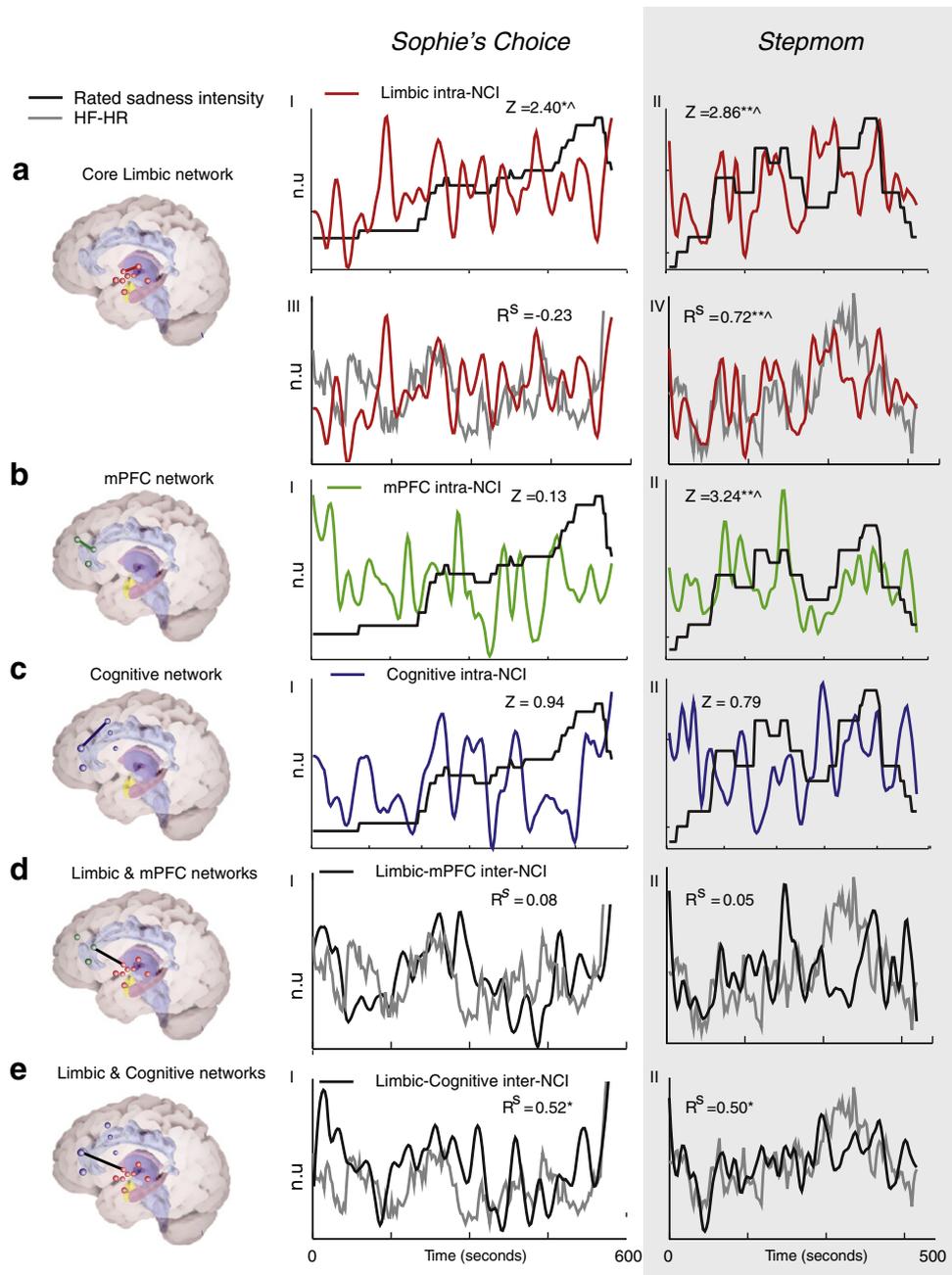


Fig. 3. Relations between indices. (a–c) Comparison between network cohesion (colored), behavioral (black) and parasympathetic (gray) indices. (d and e) Comparison between inter-network cohesion (black) and parasympathetic (gray) indices. The median values of the indices are presented (NCI is smoothed with Loess of 0.08; for presentation of the unsmoothed signal, see Fig. S2). Z and R^S values for tests on individual behavior–NCI and group parasympathetic–NCI comparisons, respectively, are shown. * $p < 0.05$; ** $p < 0.005$, ^ $q_{FDR}^{BH} \leq 0.05$.

Table 1
Z values for tests of individual Spearman's coefficients between rated sadness intensity and network cohesion.

| | Limbic | Cognitive | MPF | Limbic–cognitive | Limbic–MPF | Cognitive–MPF |
|----------------|----------------------|-----------|----------------------|------------------|-----------------------|---------------|
| <i>Sophie</i> | 2.40 ^{*,^} | 0.94 | 0.13 | 1.40 | 1.20 | 0.42 |
| <i>Stepmom</i> | 2.86 ^{**,^} | 0.79 | 3.24 ^{**,^} | 1.94 | 3.68 ^{***,^} | 1.84 |

q_{FDR}^{BH} stands for FDR adjusted q-value using the BH procedure (see Methods, fMRI network indices section)

* $p < 0.05$.
 ** $p < 0.005$.
 *** $p < 0.0005$.
 ^ $q_{FDR}^{BH} \leq 0.05$.

$p < 0.002$, $q_{FDR}^{BH} \leq 0.05$; and $Z = 0.13$, $p < 0.89$, respectively, Fig. 3b i and ii). The bootstrapping analysis indicated a 97.2% specificity of this association (Fig. S1). For both films there were no significant associations between cognitive intra-NCI and the patterns of reported sadness (see Fig. 3c and Table 1).

Significant association between the fluctuations of inter-NCI and the pattern of continuous rated sadness intensity was found to be significant only in one case: mPFC–limbic inter-NCI in *Stepmom* ($Z = 3.68$, $p < 0.0003$, $q_{FDR}^{BH} \leq 0.05$; Fig. 4). The specificity of this association in the brain space was 97.1% (Fig. S1). In contrast, not only that limbic–mPFC inter-NCI were not found to be significantly associated in the case of *Sophie*, but in fact some dips in the median value of

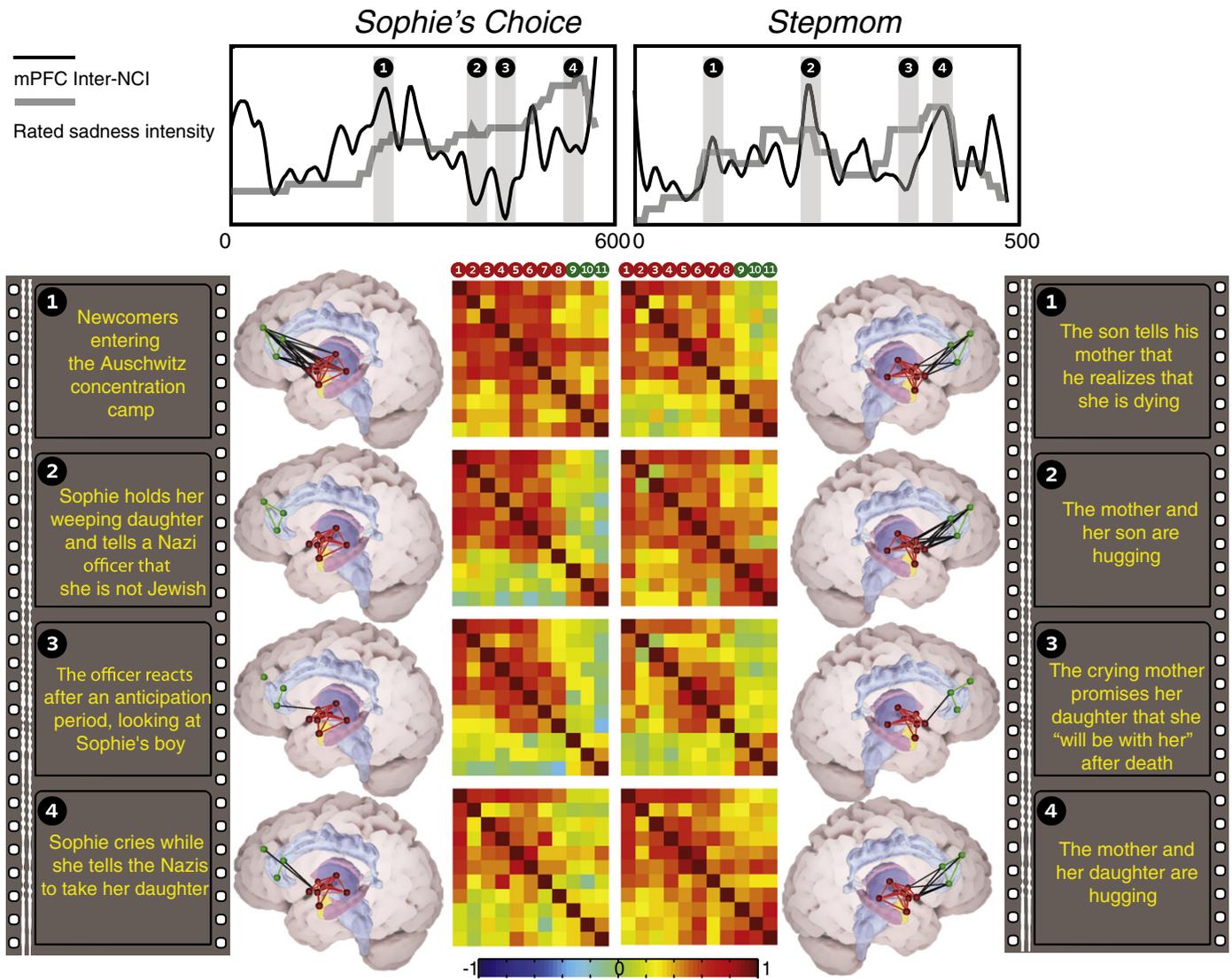


Fig. 4. Descriptive examination of local extrema in the course of limbic–mPFC inter-NCI. Peaks and minima of interest are numbered at the top panel, and the corresponding brain data and cinematic events are represented below (outer panels). Textual description rather than images are presented due to copyright reasons. More details may be obtained directly from the authors upon request. The heat maps (at the center) represent matrices of correlations between ROIs (see Fig. 1 for nomenclature) in the corresponding time windows. For each pair of ROIs, the median correlation values are computed over the group of subjects. The inter-NCI computation was based on these matrices. For each pair-wise connection, a t -test was performed on the set of Fisher-transformed R^p coefficients, computed across participants with the null hypothesis of $\mu_R = 0$. The lines indicate connections, which were found to be significantly correlated ($p < 0.01$, FDR corrected) in this test. For video illustration of the changing correlation matrices in relation to cinematic content, see Video S1.

this neural index were evident especially throughout a period, which was reported to induce increased sadness intensity.

To further examine these events of uncoupling, we tested which of the networks activates/deactivates during these dips in *Sophie*. A complementary post-hoc analysis of the percent of BOLD signal change within the limbic and the mPFC networks during local minima of limbic–mPFC inter-NCI was performed (see Supplements for more details on analysis, and Fig. S3 for the selected time points). Of 8 minima analyzed for *Sophie*, 3 significant ($q_{FDR}^{BH} < 0.05$) elevations of core limbic activity were found. One significant activation ($q_{FDR}^{BH} < 0.05$) of the mPFC network was also observed in the case of another limbic–mPFC inter-NCI minimum.

Relations between dynamics of NCI and the parasympathetic index

The limbic intra-NCI, which was significantly associated with the behavioral index in the case of *Stepmom*, also significantly correlated with the parasympathetic index for that film excerpt, but not for

Sophie ($R^s = 0.72$, $p < 0.003$, $q_{FDR}^{BH} \leq 0.05$; $R^s = -0.23$, $p < 0.35$, respectively, Fig. 3a iii and iv and Table 2). This association in *Stepmom* was found to be highly specific in the brain space, as lower NCI-parasympathetic associations were found in 99.8% of the randomized clusters (Fig. S1).

Lastly, for both films a correlation between cognitive–limbic inter-NCI and the parasympathetic index was observed at $p < 0.05$ ($R^s = 0.50$ and $R^s = 0.52$ in *Stepmom* and *Sophie*, respectively),

Table 2
Spearman r coefficients for correlation between the parasympathetic index and network cohesion.

| | Limbic | Cognitive | MPF | Limbic–cognitive | Limbic–MPF | Cognitive–MPF |
|----------------|---------------------|-----------|-------|------------------|------------|---------------|
| <i>Sophie</i> | –0.23 | 0.34 | 0.36 | 0.52* | 0.08 | 0.45 |
| <i>Stepmom</i> | 0.72** [^] | –0.04 | –0.37 | 0.50* | 0.05 | 0.30 |

* $p < 0.05$.
** $p < 0.005$.
[^] $q_{FDR}^{BH} \leq 0.05$.

showing considerable specificity (95.7% and 97.9% in *Stepmom* and *Sophie*, respectively). However, both correlations did not survive the correction for multiple comparisons.

Discussion

In line with the growing emphasis of theories of emotion on the dynamism of synchronization within and between distinct neural networks, we propose a novel approach for a multi-layered characterization of emotional experiences as they unfold. The findings presented above point to the potential of this approach to reveal commonalities, as well as key specificities, of emotional experiences induced by prolonged stimuli. Correspondences in results across the behavioral, autonomic and brain levels suggest a rich and complex, but also reconcilable, picture of the processes taking place during the emotional experiences elicited by the two film excerpts used in this study.

Multi-layered dynamics of emotional response related to the limbic network

The theme of separation of a mother from her children, which is presented in both of the film scenes, was commonly reported by viewers to induce primarily “sadness” in both cases (Fig. 2a). Accordingly, for both films the development of the rated sadness intensity over time significantly correlated with the temporal pattern of cohesion within the limbic network. To the extent to which signal similarity between regions indicates their coordinated functioning, these findings support our first hypothesis, based on the conceptual act model (Barrett, 2006). In both experimental conditions, dynamics of cohesion within the limbic network, which is assumed to mediate core aspects of emotion including the evaluation of the affective value of the stimulus and the triggering of bodily responses to it, was associated with the rated sadness intensity level. Our findings suggest that the involvement of this limbic network in affective processing crosses experimental cinematic conditions, which are reported to differ in the mixture of emotions they elicit. It is still open to further investigation whether the relation found between the limbic cohesion and the aware emotional rating contradicts prior work showing that the amygdala activity and connectivity is most prominent during implicit processing (e.g. Williams, 2006).

When parasympathetic index is considered, a difference between the cinematic experiences arises. While in *Stepmom*, this autonomic index significantly correlated with the time courses of both the rated sadness and the limbic cohesion, no such associations were found for *Sophie*. These findings may be interpreted in light of an observation made in a recent meta-analytical study (Kreibig, 2010), according to which the widely-shared lay concept of sadness conflates at least two distinct major patterns of autonomic modulations: an “activating sadness response”, which implicates increased cardiovascular sympathetic control, versus a “deactivating sadness response” (observed in most of the studies that induce sadness using films) involving a decrease in cardiac activity, mostly driven by a parasympathetic control. The limbic–parasympathetic–behavioral association in *Stepmom* indicates that the emotional experience elicited by this film excerpt better fits with the later “parasympathetic mode” of sadness. This interpretation is congruent with the emotion labeling profiles of these excerpts. In the case of *Stepmom*, sadness was reported by the participants to commingle with the related feelings of sympathy and affection. Notably, the tendency to sympathize with fictional characters during film viewing was previously related to parasympathetic activity (Fabes et al., 1993). Alternatively, emotions of horror, fear, and anger, which are associated with an immediate urge to act, were reported to accompany feelings of sadness while viewing *Sophie*. Further studies could examine the involvement of the sympathetic system in such a case.

Multi-layered dynamics of emotional responses related to the mPFC network cohesion

Another key difference between the two experimental conditions regards behavioral and brain aspects of social cognition. “Sympathy”, which was rated high in *Stepmom* but not *Sophie*, denotes in the inventory we used “fondness; positive attitude; sharing the emotions of the other”. We therefore suspect that its elicitation involved processes of social cognition during the film viewing. This may correspond with the finding of a significant positive correlation between the mPFC intra-NCI and the rated sadness intensity only in *Stepmom*. The emergence of this coupling when witnessing the sorrow of others fits with a body of evidence which links medial prefrontal structures with empathic processing. A number of neuropsychiatric, neuroimaging and lesion studies have indicated that more ventral aspects of the mPFC have a key role in appreciation of emotional states of the other (affective theory of mind), while a more dorsal locus is also involved in mentalizing the state of another person, possibly a more cognitive aspect of empathic processing (Shamay-Tsoory, 2008). Lastly, the dorsal ACC has been implicated in appraisal and expression of negative affects (Etkin et al., 2010a). Thus, the correspondence between the mPFC–NCI and the rated sadness indicates that the affective response in *Stepmom* may be guided by the encoding of the character’s emotional states and the evaluation of relevance to the viewer.

Since the empathy-related emotions of “Compassion” and “Mercy” were also rated high in *Sophie*, it is not clear why mPFC–limbic coupling with sadness rating was not evident in this case. While a direct examination of this question is yet to be carried out, the difference between the ways in which the loss is presented in the films may provide a clue. In *Stepmom*, the separation of the mother from her children is a future imagined event, while in *Sophie* it is overtly presented as an event which is taking place at present. In the first case, sadness intensification possibly coincides with mentalizing processes, while in the second it may couple with the process of “sensorimotor resonance”, which relies on brain circuits related to processing of empathy through simulation, including the pars opercularis and the anterior insula (Lamm et al., 2011). Specifically, this apparent neuro-behavioral difference between *Stepmom* and *Sophie* may align with the empirically supported distinction between empathy systems driven by cognitive and somatic related processes (Shamay-Tsoory, 2008) with a prominent role of the mPFC only in the former.

Ebb and flow of the cross talk between prefrontal cortical networks

At odds with our second hypothesis, the behavioral index was not found to be significantly associated with cognitive–limbic inter-NCI in either of the cinematic conditions. Thus, the findings do not support the notion that the reported intensification of emotion is necessarily associated with an increasing correlativity between the limbic and the cognitive networks. It is possible that the relations between the inter-network cohesion and the awareness of emotional intensity are non-linear or non-monotonic (see the descriptive “reverse engineering” analysis presented below), and therefore the regression method adopted in this study fails to capture such an effect. Alternatively, cognitive and limbic processing may cohere linearly for short time intervals following emotional stimuli, and then quickly de-coordinate. Such a process is largely unobservable in fMRI-based functional connectivity analysis due to the limited temporal resolution of this technique, and call for further investigation via EEG/MEG-based methods.

While the temporal pattern of limbic–cognitive inter-NCI was not found to be related to the time course of reported sadness intensity, we did find indications for its association with parasympathetic activity. For both movies, this association showed high specificity in the brain space, though its significance at the 0.05 level did not survive

correction for multiple comparisons. While further examination of this association in a larger group of subjects is needed, our results indicate a possible coupling between the parasympathetic reaction and the dynamics of cognitive–limbic crosstalk. This coupling may reflect a dependency of a cognitive processing of limbic information on parasympathetic modulation of arousal. Alternatively, parasympathetic reaction to the cinematic content may result from top-down cortical processes mediated by lateral prefrontal structures, included in the cognitive network. In line with this interpretation is a study, which demonstrated that cognitive re-appraisal of emotional stimuli involves the coupling of apparent regulatory effect of the ventrolateral PFC on the amygdala with parasympathetically-mediated constriction of the pupil (Johnstone et al., 2007).

An examination of the case of the coordination between the medial PFC and the limbic networks indicates additional dissimilarity between the cinematic experiences elicited by the two films. This association between the limbic–mPFC inter-NCI and the behavioral index was statistically significant in *Stepmom*, but not in *Sophie* (Fig. 4). When examining the data from a descriptive perspective, the peaks of the rated sadness intensity temporally coincide with peaks of limbic–mPFC inter-NCI in *Stepmom*. The corresponding cinematic events include notable moments of mother–child physical bonding (see Fig. 4 and Supplements for details).

While a peak of limbic–mPFC inter-NCI also corresponds to an early phase of rated sadness intensification in *Sophie* (as Sophie and her children arrive to the concentration camp), other major reported emotional events are not accompanied with such a global peak. In fact, prominent limbic–mPFC inter-NCI local minima in *Sophie* coincide with significant dramatic events (see Fig. 4 and supplements).

What may be the functional meaning of such local minima and peaks of inter-NCI? Anatomically, the medial PFC and structures of the limbic network are directly connected: highly ordered innervations of specific hypothalamic and PAG substructures have been mapped in detail in the rat's brain (Floyd et al., 2000, 2001); other animal studies have also shown that the ventromedial prefrontal cortex (VMPFC) and dorsal aspects of the ACC project to the nucleus accumbens and adjacent areas in the ventral striatum (Ernst and Fudge, 2009) and dense projections reciprocally connect the mPFC and the ACC with the amygdala (Kim et al., 2011).

In terms of the functionality of such connections, accumulating evidence from human neuroimaging studies indicates that the DMPFC, the dorsal extension of the VMPFC, and the ACC are activated when one appraises an affective state, probably through interaction with core limbic structures (Barrett et al., 2007; Ochsner and Gross, 2008). This interaction is thought to mediate introspection and monitoring of one's own stream of feelings (Barrett et al., 2007), and accordingly hypo-activity of the DMPFC and ACC was shown to be associated with alexithymia, a deficiency in the ability to attend emotional states (Aleman, 2005; Heinzel et al., 2010).

Another explanation may be derived from a line of research, which has related this limbic cortical connection to the process of emotional regulation. This assumption has been based on animal studies demonstrating an inhibitory effect of medial PFC projections on output from the central nucleus of the amygdala (Milad and Quirk, 2002; Quirk et al., 2003). Furthermore, a line of neuroimaging studies in humans has reported that successful regulation of emotion involves simultaneous increase in mPFC and ACC activity along with decreased amygdala (Kim et al., 2011), and ventral striatum activity (Phan et al., 2005; Staudinger et al., 2009).

In contrast to the congruency of the reports on medial PFC and limbic activity relative to baseline conditions, evidence of their functional connectivity during emotional regulation is less clear in terms of the direction of the correlativity. Increased coupling between the amygdala and the DMPFC/pregenual cortex in this condition was demonstrated (Banks et al., 2007; Erk et al., 2010). Moreover, this inter regional connectivity was stronger for subjects who reported

successful reduction of negative affect (Banks et al., 2007), and weaker for patients with a major depressive disorder (Erk et al., 2010). On the other hand, several fMRI studies of emotion regulation reported on negative correlativity between the amygdala and the rostral ACC/pregenual cortex—an effect which is not evident among anxiety disorder patients (Etkin et al., 2006, 2010b).

Considering the contradicting evidence reviewed above, limbic–mPFC inter-NCI minima could reflect either down-regulation of limbic activity via mPFC projections or decoupling of these networks in a state of under-regulation. In order to examine these options, a complementary post-hoc calculation of the percent of signal change in the mPFC and core limbic networks was obtained for *Sophie* (Fig. S3). The analysis revealed significantly increased core limbic signal (but not of mPFC activation) for three out of five limbic–mPFC NCI minima, observed during the interaction between Sophie and the Nazi officer. To note, two of these significant effects were measured at the climax of the scene, as indicated by the rated sadness intensity (Fig. 4, Fig. S3). One case of mPFC (but not limbic) increased activation was found in an earlier time point, corresponding to the presentation of a line of newcomers to the Auschwitz concentration camp (see Fig. S3).

How may these findings contribute to the interpretation of mPFC–limbic NCI dips, coinciding with highly emotional events in *Sophie*? A recent review on post-traumatic stress disorder (PTSD)-related pathologies of emotion modulation (Lanius et al., 2010) may provide a clue. Based on functional imaging studies, Lanius and colleagues distinguish between two forms of emotion dysregulation in PTSD: hyperarousal of limbic regions due to their under-regulation by mPFC structures, and disengagement with emotional content, which is entailed with excessive inhibition of limbic activity following activation of the mPFC. Furthermore, another recent review, which focuses on studies of emotion regulation (Ochsner and Gross, 2008), suggests that medial (rather than lateral) PFC regions are more active when subjects utilize methods of detachment and distancing. Thus, the combination of mPFC–limbic discoordination with the mPFC activation during the presentation of the entrance of the Jews to the Auschwitz concentration camp (see the upper image from *Sophie* in Fig. 4) may reflect emotion regulation via disengagement while coping with this cinematic content. On the other hand, the dips coinciding with limbic activation, especially during the event of forced separation of Sophie from her daughter (see the lower image from *Sophie* in Fig. 4), may indicate unregulated limbic bursts, while facing a traumatic content.

In summary, the significant correlation between the limbic–mPFC inter-NCI and the emotional rating in *Stepmom* indicates that in this case, sadness involves regulated processes of mentalization and introspection. Together with the evidence of the associations between brain, behavior and parasympathetic indices, the findings suggest that the emotional experience elicited by this film excerpt corresponds with a parasympathetic profile of “deactivated sadness”, governed by cognitive processes. Conversely, it is possible that abrupt effects of dysregulated limbic arousal and dissociation drive emotional experience in key moments of *Sophie*, resulting in non-monotonic relations between the time course of the cohesion and the reported emotional intensity.

Caveats and future directions

The strength of the reported associations, although significant, is modest in some cases. This shortcoming may result for various reasons, which should be taken into account in a future work. First, as previously mentioned, the behavioral, physiological and neural indices may also be related in non-linear and non-monotonic fashions, producing effects which were undetected by our methods. Further, time-shifts between the indices may appear due to causal relationships and other factors, such as hemodynamic delay.

Accounting for the hemodynamic delay between a certain stimulus and the peak of the signal, which reflect a response to it, is a common practice in studies that test the *levels* of BOLD signal. However, BOLD *correlativity* may behave differently in time. The signals of two hypothetical ROIs, which are reactive to a certain stimulus, may become correlated immediately in response to it and maintained while the hemodynamic reaction is reaching its peak and afterwards. In the absence of an established assumption about the nature of the temporal alignment of the cohesion and behavioral indices, no lag between the indices was introduced in the current study. However, this issue is to be examined in future work. As for the temporal alignment of the physiological and behavioral indices, shifting forward the HF–HR time series seems to be theoretically plausible. The parasympathetic response may at least partially reflect pre-attentive processing of an emotional input, whether in a form of a reflexive and sub-cognitive reaction in Jamesian notions or as a prompt result of a primary non-conscious appraisal. The conscious assessment of the emotional intensification may accordingly lag after this physiological reaction. Thus, the temporal relations between the rated emotional intensity and the HF–HR index have yet to be explored.

Furthermore, while the correlations between the indices were computed over the entire experimental epoch, significant relations may last for shorter time periods. Thus, additional analysis methods (e.g. cross-correlation and spectral methods), which are sensitive for such effects, may offer complementary information on the relationships between these processes. Moreover, the anatomical delineation of the regions of interest in this work relied on meta-analytical data, which was averaged over many brains. This method of functional localization is insensitive to individual differences. A semi-supervised approach for delineating seed regions on the basis of signal similarity at the individual level may improve such sensitivity.

Regardless, the behavioral, physiological, and brain indices should not be expected to fully overlap, since they provide information on different and nonsynonymous aspects of the emotional experience. Therefore, rather than expecting full correspondence between the indices, it seems more productive to treat them as related components of a complex image of emotional experience. The examination of the variability in these sets of relations accordingly provides important clues regarding the uniqueness of certain emotional states.

The duration of the time-frame in which functional connectivity is examined should also be taken into consideration. While in this work, connectivity fluctuations were monitored at a time scale of seconds, inter-network synchronization events, triggered by processing of emotion-related information occur within milliseconds. Methods for intracranial measurement of neural activity provide the opportunity to trace such events. Slower fluctuations in network synchronization measured intracranially may be compared with cohesion indices, computed for a similar cinematic stimulus on the basis of fMRI data.

As for the lower specificity of the measured effects in the brain space in the case of the limbic NCI in *Stepmom*, it may indicate a wide-scale association between inter-regional correlativity and the specific temporal patterns of the reported sadness. It is possible that this finding results from a global pattern of enhanced multi-systemic synchrony in moments of high emotional intensity. Since emotional arousal involves the activation of multiple processes, it may also increase the functional correlativity within other relevant circuits. In that case, a global effect is expected to overshadow the specific effect in the tested network.

Finally, it is worth noting the possibility of meaningful emotional events, which are neurally represented not as a unidirectional increase or decrease in the cohesion index of a specific network, but rather as a “split” of the network into sub-components with opposing patterns of functional connectivity. An intra-network cohesion index, which relates to the entire network, will probably fail to capture such effects (although it should be evident when the correlation matrices of the networks are examined, as in Fig. 4). Indeed, similarly to

other research methods, the approach presented above is limited by the definition of the elementary unit of analysis—the single network in this case. Thus, theoretically or empirically justifiable hypotheses regarding patterns of dynamics of functional connectivity between sub-components of a certain network (e.g. striatal structures vs. amygdala in the core limbic network) may be tested using the same analysis protocol with re-defined sets of ROIs.

Conclusions

In keeping with contemporary theoretical concerns in the field of emotion research, this work presents a new approach for monitoring the dynamics of connectivity within and between functional networks. Tested on two sadness inducing films, the subjective feeling was significantly coupled with the cohesion of a core limbic network for both excerpts. However, the limbic cohesion and the rated sadness correlated with the parasympathetic index only in *Stepmom*, suggesting a specific autonomic profile for the emotional state elicited by this movie. Unlike the case of the limbic cohesion, the relations between the mPFC cohesion and the rated sadness varied across cinematic conditions, providing insights about the uniqueness of the neural dynamics as related to affective-social processing. Lastly, the cohesion between the limbic and mPFC networks demonstrated either integration or disintegration at points of prominent dramatic events during the movies. Only in *Stepmom* did this correlate with the behavior, pointing to different dynamics of emotional regulation during the viewing of these films. Together, the findings suggest that unraveling the relations between various indices of emotional response may contribute to the understanding of the individual ways in which we experience emotions.

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References

- Admon, R., Lubin, G., Stern, O., Rosenberg, K., Sela, L., Ben-Ami, H., Hendler, T., 2009. Human vulnerability to stress depends on amygdala's predisposition and hippocampal plasticity. *Proc. Natl. Acad. Sci.* 106, 14120.
- Aftanas, L.I., Lotova, N.V., Koshkarov, V.I., Popov, S.A., 1998. Non-linear dynamical coupling between different brain areas during evoked emotions: an EEG investigation. *Biol. Psychol.* 48, 121–138.
- Aleman, A., 2005. Feelings you can't imagine: towards a cognitive neuroscience of alexithymia. *Trends Cogn. Sci.* 9, 553–555.
- Averill, J.R., 1968. Grief: its nature and significance. *Psychol. Bull.* 70, 721–748.
- Banks, S.J., Eddy, K.T., Angstadt, M., Nathan, P.J., Phan, K.L., 2007. Amygdala frontal connectivity during emotion regulation. *Social cognitive and affective neuroscience* 2, 303–312.
- Barrett, L.F., 2006. Are emotions natural kinds? *Perspect. Psychol. Sci.* 1, 28.
- Barrett, L.F., 2009. Variety is the spice of life: a psychological construction approach to understanding variability in emotion. *Cognit. Emot.* 23, 1284–1306.
- Barrett, L.F., Mesquita, B., Ochsner, K.N., Gross, J.J., 2007. The experience of emotion. *Annu. Rev. Psychol.* 58, 373.
- Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B Methodol.* 289–300.
- Benjamini, Y., Yekutieli, D., 2001. The control of the false discovery rate in multiple testing under dependency. *Ann. Stat.* 1165–1188.
- Bleich-Cohen, M., Strous, R.D., Even, R., Rotshtein, P., Yovel, G., Iancu, I., Olmer, A., Hendler, T., 2009. Diminished neural sensitivity to irregular facial expression in first-episode schizophrenia. *Hum. Brain Mapp.* 30, 2606–2616.

- Brett, M., Johnsrude, I.S., Owen, A.M., 2002. The problem of functional localization in the human brain. *Nat. Rev. Neurosci.* 3, 243–249.
- Camm, A.J., Malik, M., Bigger, J.T., Breithardt, G., Cerutti, S., Cohen, R.J., Coumel, P., Fallen, E.L., Kennedy, H.L., Kleiger, R.E., et al., 1996. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation* 93, 1043–1065.
- Cannon, W.B., 1929. Bodily changes in pain, hunger, fear and rage. *South. Med. J.* 22, 870.
- Chen, S.S., Tu, P.C., Su, T.P., Hsieh, J.C., Lin, Y.C., Chen, L.F., 2008. Impaired frontal synchronization of spontaneous magnetoencephalographic activity in patients with bipolar disorder. *Neurosci. Lett.* 445, 174–178.
- Choueka, Y., Freidkin, U., Hakohen, H.A., Zachi-Yannay, Y., 1997. *Rav Milim: A Comprehensive Dictionary of Modern Hebrew*. Center for Educational Technology, Israel: Bar-Ilan University, Miskal.
- Columbus, C., 1998. *Stepmom*.
- Costa Jr., P.T., McCrae, R.R., 1992. Revised neo personality inventory (neo pi-r) and neo five-factor inventory (neo-ffi).
- Cremers, H.R., Demenescu, L.R., Aleman, A., Renken, R., van Tol, M.J., van der Wee, N.J., Veltman, D.J., Roelofs, K., 2010. Neuroticism modulates amygdala–prefrontal connectivity in response to negative emotional facial expressions. *Neuroimage* 49, 963–970.
- Damasio, A.R., Everitt, B.J., Bishop, D., 1996. The somatic marker hypothesis and the possible functions of the prefrontal cortex. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 351, 1413–1420.
- Delorme, A., Makeig, S., 2004. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J. Neurosci. Methods* 134, 9–21.
- Erk, S., Mikschl, A., Stier, S., Ciaramidaro, A., Gapp, V., Weber, B., Walter, H., 2010. Acute and sustained effects of cognitive emotion regulation in major depression. *J. Neurosci.* 30, 15726.
- Ernst, M., Fudge, J.L., 2009. A developmental neurobiological model of motivated behavior: anatomy, connectivity and ontogeny of the triadic nodes. *Neurosci. Biobehav. Rev.* 33, 367–382.
- Eryilmaz, H., Van De Ville, D., Schwartz, S., Vuilleumier, P., 2010. Impact of transient emotions on functional connectivity during subsequent resting state: a wavelet correlation approach. *NeuroImage* 3, 2481–2491.
- Etkin, A., Egner, T., Peraza, D.M., Kandel, E.R., Hirsch, J., 2006. Resolving emotional conflict: a role for the rostral anterior cingulate cortex in modulating activity in the amygdala. *Neuron* 51, 871–882.
- Etkin, A., Prater, K.E., Hoef, F., Menon, V., Schatzberg, A.F., 2010a. Failure of anterior cingulate activation and connectivity with the amygdala during implicit regulation of emotional processing in generalized anxiety disorder. *American Journal of Psychiatry* 167, 545–554.
- Etkin, A., Prater, K.E., Hoef, F., Menon, V., Schatzberg, A.F., 2010b. Failure of anterior cingulate activation and connectivity with the amygdala during implicit regulation of emotional processing in generalized anxiety disorder. *Am. J. Psychiatry* 167, 545.
- Fabes, R.A., Eisenberg, N., Eisenbud, L., 1993. Behavioral and physiological correlates of children's reactions to others in distress. *Dev. Psychol.* 29, 655.
- Floyd, N.S., Price, J.L., Ferry, A.T., Keay, K.A., Bandler, R., 2000. Orbitomedial prefrontal cortical projections to distinct longitudinal columns of the periaqueductal gray in the rat. *J. Comp. Neurol.* 422, 556–578.
- Floyd, N.S., Price, J.L., Ferry, A.T., Keay, K.A., Bandler, R., 2001. Orbitomedial prefrontal cortical projections to hypothalamus in the rat. *J. Comp. Neurol.* 432, 307–328.
- Frijda, N.H., 1986. *The Emotions*. Cambridge Univ Pr, Cambridge.
- Frijda, N.H., 2009. Emotions, individual differences and time course: reflections. *Cognit. Emot.* 23, 1444–1461.
- Garcia-Garcia, M., Yordanova, J., Kolev, V., Domínguez-Borrás, J., Escera, C., 2010. Tuning the brain for novelty detection under emotional threat: the role of increasing gamma phase-synchronization. *Neuroimage* 49, 1038–1044.
- Goldin, P.R., Hutcherson, C.A., Ochsner, K.N., Glover, G.H., Gabrieli, J.D., Gross, J.J., 2005. The neural bases of amusement and sadness: a comparison of block contrast and subject-specific emotion intensity regression approaches. *Neuroimage* 27, 26–36.
- Heinzel, A., Schäfer, R., Müller, H.W., Schieffer, A., Ingenhag, A., Eickhoff, S.B., Northoff, G., Franz, M., Hatzel, H., 2010. Increased activation of the supragenual anterior cingulate cortex during visual emotional processing in male subjects with high degrees of alexithymia: an event-related fMRI study. *Psychother. Psychosom.* 79, 363–370.
- Honey, C.J., Kötter, R., Breakpear, M., Sporns, O., 2007. Network structure of cerebral cortex shapes functional connectivity on multiple time scales. *Proc. Natl. Acad. Sci.* 104, 10240–10245.
- Hutcherson, C.A., Goldin, P.R., Ochsner, K.N., Gabrieli, J.D., Barrett, L.F., Gross, J.J., 2005. Attention and emotion: does rating emotion alter neural responses to amusing and sad films? *Neuroimage* 27, 656–668.
- James, W., 1884. What is an emotion? *Mind* 9, 188–205.
- Johnstone, T., van Reekum, C.M., Urry, H.L., Kalin, N.H., Davidson, R.J., 2007. Failure to regulate: counterproductive recruitment of top-down prefrontal-subcortical circuitry in major depression. *J. Neurosci.* 27, 8877.
- Keil, A., Stolarova, M., Moratti, S., Ray, W.J., 2007. Adaptation in human visual cortex as a mechanism for rapid discrimination of aversive stimuli. *Neuroimage* 36, 472–479.
- Kim, M.J., Loucks, R.A., Palmer, A.L., Brown, A.C., Solomon, K.M., Marchante, A.N., Whalen, P.J., 2011. The structural and functional connectivity of the amygdala: from normal emotion to pathological anxiety. *Behavioural Brain Research* 223, 403–410.
- Kober, H., Barrett, L.F., Joseph, J., Bliss-Moreau, E., Lindquist, K., Wager, T.D., 2008. Functional grouping and cortical-subcortical interactions in emotion: a meta-analysis of neuroimaging studies. *Neuroimage* 42, 998–1031.
- Kreibig, S.D., 2010. Autonomic nervous system activity in emotion: a review. *Biol. Psychol.* 84, 394–421.
- Lamm, C., Decety, J., Singer, T., 2011. Meta-analytic evidence for common and distinct neural networks associated with directly experienced pain and empathy for pain. *Neuroimage* 54, 2492–2502.
- Lanius, R.A., Vermetten, E., Loewenstein, R.J., Brand, B., Schmahl, C., Bremner, J.D., Spiegel, D., 2010. Emotion modulation in PTSD: clinical and neurobiological evidence for a dissociative subtype. *Am. J. Psychiatry* 167, 640.
- Lazarus, R.S., Folkman, S., 1984. *Stress, Appraisal, and Coping*. Springer Publishing Company.
- Le Van Quyen, M., Foucher, J., Lachaux, J.P., Rodriguez, E., Lutz, A., Martinerie, J., Varela, F.J., 2001. Comparison of Hilbert transform and wavelet methods for the analysis of neuronal synchrony. *J. Neurosci. Methods* 111, 83–98.
- LeDoux, J., 1996. *The Emotional Brain*. New York.
- Lerner, Y., Papo, D., Zhdanov, A., Belozersky, L., Hendler, T., 2009. Eyes wide shut: amygdala mediates eyes-closed effect on emotional experience with music. *PLoS One* 4, e6230.
- Lewis, M.D., 2005. Bridging emotion theory and neurobiology through dynamic systems modeling. *Behav. Brain Sci.* 28, 169–194.
- MacLean, P., 1955. The limbic system ('visceral brain') and emotional behavior. *Arch. Neurol. Psychiatry* 73, 130.
- Matsumoto, A., Ichikawa, Y., Kanayama, N., Ohira, H., Iidaka, T., 2006. Gamma band activity and its synchronization reflect the dysfunctional emotional processing in alexithymic persons. *Psychophysiology* 43, 533–540.
- Mesquita, B., Barrett, L.F., Smith, E.R., 2010. Emoting: a contextualized process. *The mind in context*, pp. 83–104.
- Milad, M.R., Quirk, G.J., 2002. Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature* 420, 70–74.
- Morris, J.S., Öhman, A., Dolan, R.J., 1999. A subcortical pathway to the right amygdala mediating "unseen" fear. *Proc. Natl. Acad. Sci.* 96, 1680–1685.
- Murphy, F.C., Nimmo-Smith, I., Lawrence, A.D., 2003. Functional neuroanatomy of emotions: a meta-analysis. *Cogn. Affect. Behav. Neurosci.* 3, 207.
- Nakao, T., Takezawa, T., Miyatani, M., Ohira, H., 2009. *Psychologia* 52, 93–109.
- Niazy, R.K., Beckmann, C.F., Lannetti, G.D., Brady, J.M., Smith, S.M., 2005. Removal of fMRI environment artifacts from EEG data using optimal basis sets. *Neuroimage* 28, 720–737.
- Northoff, G., Heinzel, A., de Greck, M., Bermpohl, F., Dobrowolny, H., Panksepp, J., 2006. Self-referential processing in our brain—a meta-analysis of imaging studies on the self. *Neuroimage* 31, 440–457.
- Oatley, K.G., 1996. Emotions, rationality and informal reasoning. In: Oakhill, J., Garnham, A. (Eds.), *Mental Models in Cognitive Science: Essays in Honour of Phil Johnson-Laird*. Psychology Press, East Sussex, pp. 175–196.
- Ochsner, K.N., Gross, J.J., 2008. Cognitive emotion regulation. *Curr. Dir. Psychol. Sci.* 17, 153–158.
- Öngür, D., Price, J.L., 2000. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cereb. Cortex* 10, 206–219.
- Pakula, A.J., 1982. *Sophie's Choice*.
- Papez, J.W., 1937. A proposed mechanism of emotion. *Arch. Neurol. Psychiatry* 38, 725.
- Peng, C.K., Henry, I.C., Mietus, J.E., Hausdorff, J.M., Khalsa, G., Benson, H., Goldberger, A.L., 2004. Heart rate dynamics during three forms of meditation. *Int. J. Cardiol.* 95, 19–27.
- Pessoa, L., 2008. On the relationship between emotion and cognition. *Nat. Rev. Neurosci.* 9, 148–158.
- Phan, K.L., Fitzgerald, D.A., Nathan, P.J., Moore, G.J., Uhde, T.W., Tancer, M.E., 2005. Neural substrates for voluntary suppression of negative affect: a functional magnetic resonance imaging study. *Biol. Psychiatry* 57, 210–219.
- Pieper, S., Halle, M., Kikinis, R., 2004. 3D slicer. *Biomedical Imaging: Nano to Macro, 2004: IEEE International Symposium On*, pp. 632–635.
- Quirk, G.J., Likhtik, E., Pelletier, J.G., Paré, D., 2003. Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. *J. Neurosci.* 23, 8800.
- Reiner-Benaim, A., 2007. FDR control by the BH procedure for two-sided correlated tests with implications to gene expression data analysis. *Biom. J.* 49, 107–126.
- Russell, J.A., 2003. Core affect and the psychological construction of emotion. *Psychol. Rev.* 110, 145–172.
- Schachter, S., Singer, J.E., 2000. Cognitive, social and physiological determinants of emotional state. *Emotions in social psychology: essential readings*, p. 76.
- Scherer, K.R., 2001. Appraisal considered as a process of multilevel sequential checking. *Appraisal processes in emotion: theory, methods, research*, pp. 92–120.
- Scherer, K.R., 2009. The dynamic architecture of emotion: evidence for the component process model. *Cognit. Emot.* 23, 1307–1351.
- Shamay-Tsoory, S.G., 2008. Empathic processing: its cognitive and affective dimensions and neuroanatomical basis. In: Decety, J., Ickes, W. (Eds.), *The Social Neuroscience of Empathy*. MIT Press, Cambridge, Mass., pp. 215–232.
- Shaver, P., Schwartz, J., Kirson, D., O'Connor, C., 2001. Emotion knowledge: further exploration of a prototype approach. *Emotions in Social Psychology: Essential readings* 26–56.
- SpOrnS, O., 2010. Brain networks and embodiment. *The mind in context*, p. 42.
- Staudinger, M.R., Erk, S., Abler, B., Walter, H., 2009. Cognitive reappraisal modulates expected value and prediction error encoding in the ventral striatum. *Neuroimage* 47, 713–721.
- Talos, I.-F., Jakab, M., Kikinis, R., Shenton, M., 2008. SPL-Brain Atlas [WWW Document]. URL <http://www.slicer.org/publications/item/view/12652008>.
- Torrubia, R., Avila, C., Moltó, J., Caseras, X., 2001. The sensitivity to punishment and sensitivity to reward questionnaire (SPSRQ) as a measure of Gray's anxiety and impulsivity dimensions. *Pers. Individ. Differ.* 31, 837–862.
- Williams, L.M., 2006. Mode of functional connectivity in amygdala pathways dissociates level of awareness for signals of fear. *J. Neurosci.* 26, 9264–9271.