Psychotherapy with somatosensory stimulation for endometriosis-associated pain: The role of the anterior hippocampus

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Abstract

Background: Endometriosis is a gynecological disorder affecting 6-10% of all women in their reproductive age. There is an emerging view in the literature that psychological trauma plays a central role in the pathogenesis of pelvic pain, one of the core symptoms of endometriosis. Here, we report central nervous mechanisms of a novel combination of psychotherapy and somatosensory stimulation that has recently shown remarkable effects in reducing pain, anxiety and depressive symptoms in these patients.

Methods: We conducted a randomized controlled trial. 67 patients with severe endometriosis-associated pain (maximum pain: 7.6 ± 2.0, average pain: 4.5 ± 2.0 on a 10-point numeric rating scale) were included in the study and randomly allocated to intervention (35 pat.) or wait-list control (32 pat.). Resting-state functional magnetic resonance imaging was used to assess brain connectivity of these patients at baseline, after three months of therapy and after six months. The analysis focused on the hippocampus.

Results: We identified a cortical network comprising the right anterolateral hippocampus – a region modulating the hypothalamic-pituitary-adrenal (HPA) axis – and somatosensory, viscerosensory and interoceptive brain regions. Regression analysis showed that reduction in connectivity predicted therapy-induced improvement in patients' anxiety.

Conclusions: We have identified a putative neurobiological mechanism underlying the potent combination of psychotherapy and somatic stimulation in treating symptoms of endometriosis.

(Trial name: "Cortical Plasticity in a Complex Intervention for Endometriosis", https://clinicaltrials.gov/ct2/show/NCT01321840, registration number: NCT01321840)

Introduction

Endometriosis is a common gynecological disorder characterized by endometrial tissue outside the uterus that affects 6-10% of all women in their reproductive age (1-2). The most frequent symptoms are dysmenorrhea, chronic pelvic pain, dyspareunia, and infertility (3). Despite decades of research the disease mechanism is poorly understood. In particular the notoriously weak correlation between the severity of organic pathology and reported pain intensity still puzzles clinicians and scientists alike (4-6). One possible explanation is the growing evidence for psychological factors and central nervous alterations contributing to the course of endometriosis-associated pain. In particular, several studies have shown that anxiety, depression, catastrophizing and previous traumatic experiences have a profound influence on pain severity and pain-related disability in endometriosis and related symptoms (7-10). Furthermore, central sensitization processes have been demonstrated for endometriosis-associated pain (11) and dysmenorrhea (12) as have functional and structural brain changes (13-15). These observations imply that psychotherapy aimed at reducing trauma, catastrophizing, anxiety and stress might be a valuable tool in the treatment of endometriosis-associated pain.

Here we report the results of a randomized waitlist-controlled trial investigating the central nervous mechanisms of a novel combination of psychotherapy and somatosensory stimulation that exploits the interrelation of bodily sensations and painful memories and has recently shown remarkable effects in reducing pain, anxiety and depressive symptoms in patients with endometriosis-associated pain (16-17).

We measured treatment-associated changes in brain connectivity by means of resting-state functional magnetic resonance imaging (rsfMRI). Contemplating probable targets for the trauma-centered therapy studied here, we chose the hippocampus as starting point for our analysis. It is one of the central regions in memory and trauma (18-19). Furthermore, hippocampal alterations are a frequent finding in traumatized patients and have been shown to be reversible by psychotherapy (20-21). We were particularly interested in the anterior part of the hippocampus, which besides memory is involved in imagination (22), emotions (18-19), anxiety (23-24), and the transition from acute to chronic pain (25). To study functional connectivity of the hippocampus, we applied a recently validated method to segment brain regions into functionally independent subregions and derive whole-brain connectivity of such regions (26-27).

We hypothesized that patients receiving the intervention would show altered functional connectivity between the anterior hippocampus and the rest of the brain and that these changes would reflect improvement of their symptoms.

Methods and Materials

Participants

We included female patients aged 18-40 years with a clinically proven history of endometriosis, who were currently suffering from pelvic pain, and had sufficient knowledge of German language. Sample size calculation revealed that 30 patients per group would be sufficient to answer the primary study questions (see Supplementary Methods for details).

Exclusion criteria were MRI contraindications, hormonal treatment during the month before enrollment, as well as drug or alcohol addiction. All patients were free to take analgesics, as needed. The study was conducted according to the Declaration of Helsinki. All participants gave written informed consent in accordance with the guidelines of the ethics committee of the Klinikum Rechts der Isar, Munich, Germany, who had approved the study protocol. The trial is registered at ClinicalTrials.gov (NCT01321840).

Study design and procedures

This study was a randomized controlled clinical trial with patients being randomly assigned to either an intervention group or a waiting-list control group. The randomized study period was three months. After this time, the intervention group was free to continue treatment, and the control group could also start receiving treatment. All patients were seen by a gynecologist prior to randomization and were cared for, as required, during the waiting-list period.

The trial involved three study visits, which were scheduled at baseline, three months, and six months (see **Figure 1**). At each study visit, patients were seen by a gynecologist. A 5-minute electrocardiogram measurement to derive heart rate variability measures (reported elsewhere) was acquired in the gynecological department. Questionnaires to assess pain and other symptoms of endometriosis, as well as anxiety, stress, depressive symptoms, and health-related quality of life were completed immediately before the MRI exam, which comprised structural and functional MRI scans.

To minimize the influence of sex hormone fluctuations on brain function (28-29), great care was taken to schedule MRI exams between days two and six of the patients' menstrual cycle (self-report). This was successful for 95% of the 165 measurements (4/2/1 measurements were performed on days 1/7/8, resp.).

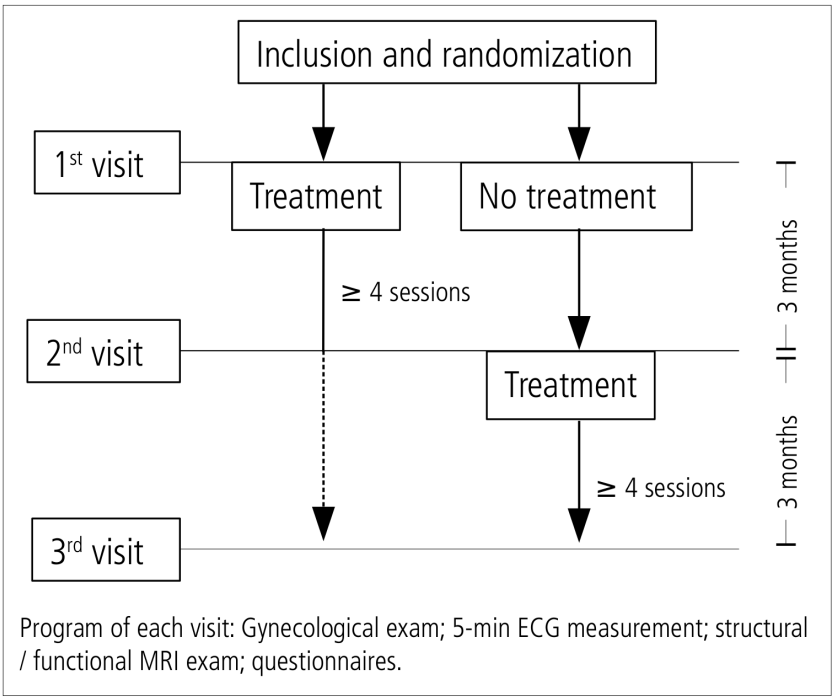

Figure 1: Study design. The randomized phase was three months from the first visit. During this time subjects in the treatment group received at least four therapeutic sessions. After the second study visit the control group also received treatment.

Clinical outcomes

The following secondary outcomes were used for the analyses in this paper: (1) maximum pain, (2) average pain, both assessed retrospectively over the last four weeks by means of a numeric rating scale (NRS) ranging from 0 (“no pain”) to 10 (“worst pain imaginable”), (3) anxiety and depression assessed by the Hospital Anxiety and Depression Scale (30), (4) trait anxiety and stress assessed by the State-Trait Anxiety Inventory (31), (5) health-related quality of life assessed by the 12-Item Short-Form Health Survey (32). To control for acute effects, we further included pain and state anxiety ratings from the day of the measurement as assessed by NRS and STAI, resp. The use of analgesics was recorded throughout the trial and did not differ between groups.

Psychotherapy with somatosensory stimulation

The intervention used in this study (16) was an integrative psychotherapy combining elements from hypnotherapy (33), mindfulness-based psychotherapy (34), cognitive behavioral therapy (35), and problem-solving therapy (36). A strong emphasis is placed on the joint involvement of mind and body in the therapeutic process. To achieve this, the approach employs diagnostic concepts and stimulation methods from Traditional Chinese Medicine (TCM) (37). All patients

were treated in an outpatient setting by the same therapist (ASA), a medical specialist for psychosomatic medicine and TCM. The pre-defined minimum number of treatments in the randomized period was 4 times. However, most patients received more treatments. A typical one-on-one treatment session takes 60 minutes and topics arise from the current wishes and needs of the patient. The central theme of the therapy are somatic markers, i.e feelings of pressure, tension, or pain. Thus, each therapeutic session starts by the therapist asking the patient to report present worries and accompanying bodily sensations (e.g. pain, tension, pressure etc). These somatic markers are used as a path to painful memories of adverse life experiences (e.g. death of a close relative or friend, sexual abuse, domestic violence). A list of such life experiences identified by the therapist can be found in **Tables S1** and **S2**. Memories are then uncovered using hypnotic techniques. Once a memory surfaces, it often triggers strong emotional reactions that patients are encouraged to express. Surfacing memories are treated as if they were present experiences and the patient is encouraged to develop appropriate solutions from the present perspective. This may either resolve the problem or uncover a deeper emotional conflict, which is then treated again by the same approach. The therapist uses acupuncture and related techniques (moxibustion, cupping) in combination with psychotherapeutic techniques to resolve the current symptoms. For example, if a patient reports pain in the lower abdomen while remembering humiliation by a close relative, the patient is asked for her inner needs while visualizing this situation. At the same time acupuncture point CV3 (~1.5 cm above the symphysis) is stimulated by moxibustion. This typically induces immediate feelings of warmth in the lower abdomen and often leads to spontaneous symptom relief. The goal of each session is to render the patient into a stable and relaxed state, free of pain and negative emotions by resolving intrusive memories of adverse life experiences.

Imaging

Data were acquired on a 3T Magnetom Verio MRI scanner (Siemens Medical Solutions, Erlangen, Germany) using the body coil for RF transmission and an 8 channel phased-array head coil for signal reception. For resting-state functional MRI we acquired a time series of 300 T2*-weighted gradient echo (GE) echo-planar images (EPI) with TR/TE/FA = 2sec/30ms/90° and an isotropic voxel size of 3 mm (acquisition matrix of 64x64, 35 slices, 0.6mm gap). For anatomical reference and to screen for brain lesions, we used 3D T1-weighted magnetization prepared (MPRAGE) data (FA/TE/TR/TI = 9°/2.98ms/2300ms/900ms) with 1 mm isotropic spatial resolution (acquisition matrix 256x256x160) and T2-weighted FLAIR images (FA/TE/TR/TI = 180°/136ms/8560ms/2500ms) with a voxel size of 0.8x0.7x4.0mm^3 (acquisition matrix of 320x288, 29 slices, 0.4mm gap).

Data analysis

Functional connectivity analysis was centered around the hippocampus. An overview of the analysis steps is given in **Figure 2**.

Preprocessing

Data were preprocessed using SPM8 (Wellcome Department of Imaging Neuroscience, UCL, London, UK, http://www.fil.ion.ucl.ac.uk/spm), FSL5.0 (Oxford Centre for Functional MRI of the Brain, Oxford, UK, http://www.fmrib.ox.ac.uk/fsl/), and scripts. Anatomical images were segmented using SPM SEGMENT. Gray and white matter maps were used for brain extraction and anatomical images were normalized to MNI space using a 12-parameter affine transformation (FSL FLIRT) followed by non-linear warping (FSL FNIRT) with a warp resolution of ten millimeters. Functional images were corrected for head motion by realigning each volume to the middle volume of the run. After temporal high-pass filtering with a 0.01Hz cut-off and brain extraction (FSL BET) the data were denoised (FSL FIX) (38). After manual training of the FIX classifier on a subset of 12 of our datasets, noise components were automatically detected and their unique variance was regressed out. Filtered functional images were co-registered to the anatomical scan, using FLIRT with boundary-based registration (39), and non-linearly transformed to MNI space using transformation parameters of the anatomical scans. Images were up-sampled to 2mm isotropic resolution and spatially smoothed by a Gaussian kernel of 5 mm FWHM.

Functionally independent hippocampal subregions

Functionally independent subregions of the hippocampus were identified using masked independent component analysis (26-27) of the temporally concatenated data of all subjects at baseline and three months. Analysis was restricted within the bilateral hippocampus by a mask derived from the Harvard-Oxford atlas, using a tissue probability threshold of 50%. Preprocessed functional data were projected into a 10-dimensional subspace using probabilistic principal component analysis after voxel-wise de-meaning and normalization by the voxel-wise variance (40). The number of dimensions was pre-defined and based on our previous research (27). The goal was to partition the hippocampus into five subregions per hemisphere. Whitened observations were decomposed into spatial maps and time-courses using a fixed-point iteration technique optimizing for non-Gaussian spatial distributions (41). Resulting group-level component maps were divided by the standard deviation of the residual noise and thresholded by fitting a mixture model to the histogram (40).

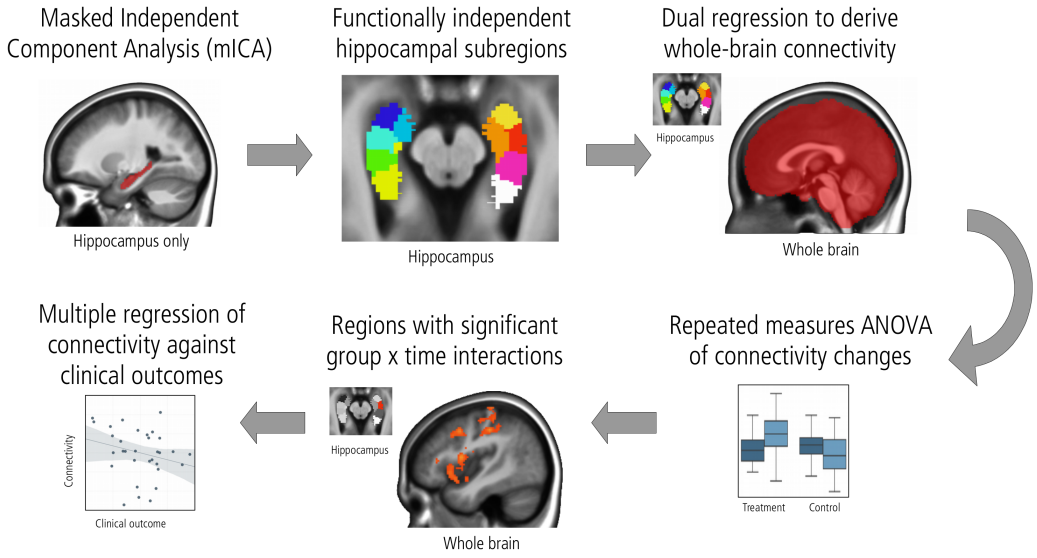

Figure 2: Flow diagram of relevant analysis steps. Functional segmentation of the hippocampus using masked independent component analysis with a bilateral hippocampal mask was followed by a modified dual regression approach, where subject-level time-courses were extracted for each hippocampal subregion to derive cortical and subcortical regions with significant functional connectivity. In a repeated-measures analysis of variance we identified those regions that showed differential connectivity changes between intervention and control group (i.e. a significant group x time interaction). Averaged connectivity values from these regions were then tested for their association with clinical outcomes at baseline. Those outcomes that predicted connectivity at baseline were followed up by regressing changes in connectivity after three and six months against changes in these clinical outcomes.

Whole-brain connectivity of hippocampal subregions

Multivariate functional connectivity between hippocampal subregions and the whole brain was assessed using a modified dual regression approach (42, 26). This analysis uses group-level ICA results to derive single-subject versions of each independent component (IC). These can then be used for within- and between-subject analyses (40). The first regression step used a general linear model (GLM) with the spatial maps of all 10 hippocampal ICs as design matrix. It was used to derive subject-specific time-courses for each hippocampal IC in a process that effectively averages the time-courses of all voxels, while weighting each voxel with the respective z-value of the IC at that voxel. The second regression step used a GLM with these subject-specific time-courses as design matrix to identify subject-specific whole-brain spatial maps of voxels associated with them. Group-level maps showing whole-brain functional connectivity of the 10 hippocampal subregions were obtained by passing the unthresholded single-subject parameter estimates and variance maps up to mixed-effects analysis using FSL FLAME1. A supplementary one-sample t-test was used as group-level design matrix to display group-averaged connectivity and the resulting z-maps were thresholded at a value of $z > 5$ after conventional thresholds had proven too liberal.

Repeated measures analysis of variance

Since we were interested in therapy-related changes of functional connectivity, we repeated the above-mentioned mixed-effects analysis using a repeated measures analysis of variance (ANOVA) as group-level design matrix. We included the within-subjects factor “time” and the between-subjects factor “group” and tested for a significant interaction that would indicate a differential connectivity change in the intervention and control group. Brain maps were thresholded using cluster correction for multiple comparisons. The cluster forming threshold was $z > 2.3$ and cluster-size threshold was $p < 0.05$, Bonferroni-corrected for the number of ICs (i.e. $p < 0.005$). Mean connectivity values and their standard deviations were extracted for each IC and each subject using a mask of all significant voxels on subjects’ z-maps. Because a recent paper had shown problems with cluster-based thresholding procedures (43), we also repeated our analysis using a much more conservative threshold of $z > 3.1$ and $p < 0.005$.

Since significant group differences were found at baseline, we calculated additional analyses of covariance (ANCOVAs) on the mean connectivity values. By including baseline connectivity values as covariates, we excluded the possibility that interactions were driven by baseline differences.

Regression of connectivity and clinical outcomes

To explore possible connections between clinical outcomes and functional connectivity, we used multivariable linear regression analysis. A first regression was conducted for the baseline values, with acute, maximum and average pain, state and trait anxiety, age, anxiety and depression, as well as physical and mental health scores of the SF-12 entered as independent variables, and functional connectivity of the rAL hippocampus entered as dependent variable. Ratings of acute pain and state anxiety from the day of the measurement were added to the list of pre-defined clinical outcomes to rule out the possibility that our results reflect short-term changes in brain function, whereas age had been shown to influence hippocampal connectivity (44). Stepwise multivariable regression was then performed using bidirectional elimination and Akaike’s information criterion for model selection.

To limit the number of parallel tests in later statistical analyses, only those clinical outcomes that predicted connectivity at baseline were followed up further. Thus, the two significant predictors as derived from this model (i.e., maximum pain and trait anxiety) were entered as independent variables in multivariable regression analyses of the changes in functional connectivity of the rAL hippocampus after three and six months, respectively (separated by group).

Results

Participants

67 patients with endometriosis-associated pain were recruited for the study. All patients had histologically confirmed endometriosis and the average time since last confirmation was 2.7 years. Patients were randomly allocated to the intervention group or the control group (flow chart in **Figure S1**). Both groups were comparable with regard to all baseline characteristics except for HADS anxiety (see **Table 1**). Data from 60 patients (30 per group) were available after three months. At that time, participants in the intervention group had received 8.7 ± 2.1 (mean ± SD) treatments. After six months, data from 40 patients (20 per group) were available and participants in the intervention and control group had received 16.1 ± 4.2 and 10.5 ± 5.0 treatments, respectively. No patient took hormones or had surgery during the study period of six months.

Clinical outcomes

Therapy-induced changes in clinical outcomes have been reported in detail elsewhere (16). In summary, after three months we found significantly larger improvements for all of the above-mentioned outcomes in the treatment group as compared to controls (all $p<0.05$). Effect sizes were medium to large. After six months, following delayed intervention, improvements seen in control patients almost equaled those observed in patients, who had receive immediate intervention (all $p>0.3$).

Table 1: Demographic, diagnostic, and pain characteristics of the study participants

Variable	Treatment Group (n=30)	Control Group (n=30)	p-Value[†]
Age, mean years (SD)	35.2 (4.7)	36.4 (4.8)	0.341
BMI, mean (SD)	23.2 (3.6)	22.2 (2.4)	0.200
Endometriosis Stage, ASRM Score (%)			0.231
I	2 (7%)	3 (10%)	
II	10 (33%)	9 (30%)	
III	7 (23%)	13 (43%)	
IV	11 (37%)	5 (17%)	
Time since diagnosis, mean years (IQR)	4.7 (1.5-6.4)	5.1 (1.0-7.3)	0.451
Time since last histologic confirmation of endometriosis, mean years (IQR)	2.2 (0.0-3.3)	3.1 (1.0-6.0)	0.155
Pain outcomes (NRS, 0-10)			
Maximal pain (last 4 weeks), mean (SD)	7.5 (2.1)	7.7 (2.0)	0.684
Average pain (last 4 weeks), mean (SD)	4.9 (2.1)	4.1 (1.9)	0.133
Disease-related Quality of Life			
SF-12 - Physical health sum score, mean (SD)	45.6 (7.4)	42.5 (7.4)	0.110
SF-12 – Mental health sum score, mean (SD)	42.1 (11.4)	40.8 (9.8)	0.639
Depression, Anxiety, Stress			
Trait Anxiety, Stress (STAI), mean (SD)	42.8 (9.1)	48.0 (11.2)	0.061
Anxiety (HADS), mean (SD)	7.6 (3.4)	9.8 (3.4)	0.017
Depression (HADS), mean (SD)	5.4 (2.8)	5.5 (3.0)	0.981

[†] Two-sided *t*-test, Mann-Whitney *U* test, or χ^2-test.

Abbreviations: SD, Standard deviation; IQR, Interquartile range; BMI, Body mass index; ASRM, American Society for Reproductive Medicine; DSF, Pain Questionnaire of the German Society for the Study of Pain; SF-12, 12-Item Short-Form Health Survey; STAI, State-Trait Anxiety Inventory, HADS, Hospital Anxiety and Depression Scale.

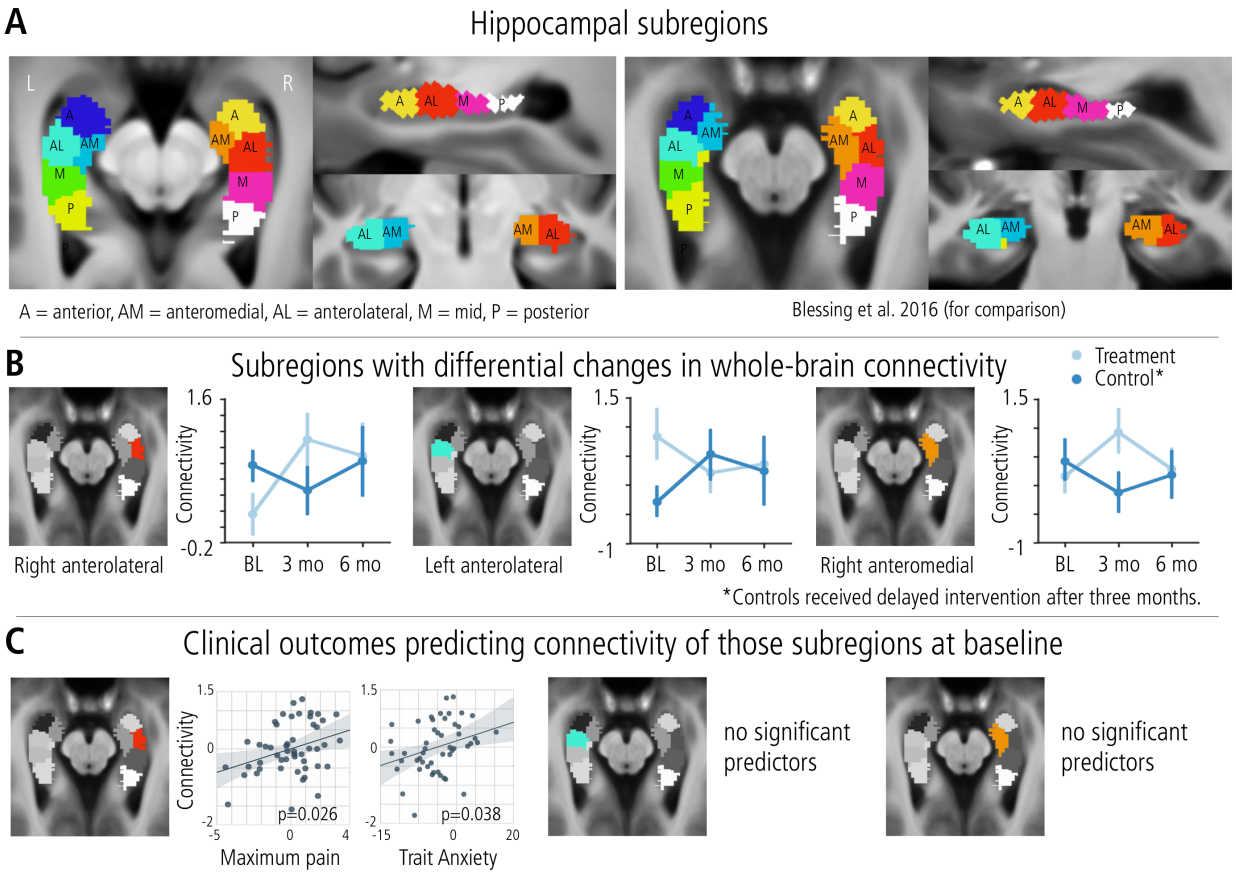

Figure 3: Functional connectivity of the hippocampus and its relation to treatment effects of psychotherapy with somatosensory stimulation. (A) Segmentation of the bilateral hippocampus into functionally-independent subregions by masked ICA. The analysis identified three regions in the anterior, one in the mid, and one in the posterior hippocampus closely replicating earlier results obtained in healthy subjects (25). (B) Hippocampal subregions whose whole-brain connectivity changed differentially in the intervention and control group between baseline and three months. Connectivity values shown as mean ± 95% CI. Note that all three regions lie in the anterior part of the hippocampus and that group differences vanish after controls have received delayed treatment. (C) Multivariable regression with clinical outcomes showed that the right anterolateral hippocampus was the only subregion whose whole-brain connectivity at baseline was predicted by long-term clinical outcomes, namely maximum pain and trait anxiety. Abbreviations: BL, baseline; mo, months.

Functional connectivity of the hippocampus

Functional connectivity analysis showed a segregation of the hippocampus into three anterior, one mid and one posterior region, closely reproducing recent results obtained in healthy subjects (27) (**Figure 3A, Table S3**). All hippocampal subregions showed significant whole-brain functional connectivity (**Figure S2**). For three subregions, namely the right and left anterolateral (rAL, lAL), and right anteromedial (rAM) hippocampus, ANOVA identified cortical networks whose hippocampal connectivity showed a significant interaction between the factors of group and time (**Figures 3B, 4A and 4C, Table S4**). When using more conservative thresholds, only the left

anterolateral subregion showed this interaction (**Figure S3**). All interactions were still significant, when we controlled for group differences at baseline. As hypothesized, all three regions were located in the anterior part of the hippocampus. Multivariable regression identified a model with the predictors acute pain, maximum pain, trait anxiety, anxiety, and depression for the dependent variable of whole-brain connectivity of the rAL hippocampus. This model yielded a significant regression equation ($F(5,44) = 2.792$, $p = 0.028$), with an R^2 of 0.241. The only significant single predictors were maximum pain ($t = 2.312$, $p = 0.026$) and trait anxiety ($t = 2.135$, $p = 0.038$), which together explained 18.2 percent of the variance (**Figure 3C**). None of the other two hippocampal subregions (lAL, rAM) had significant predictors.

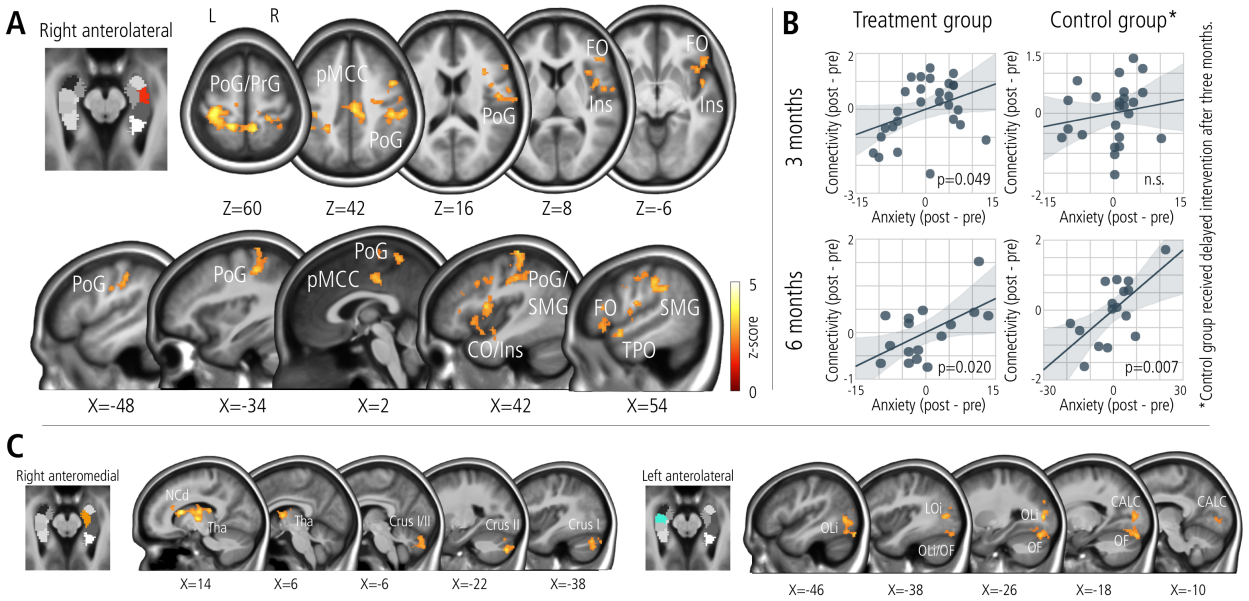

Figure 4: (A) The cortical network associated with the right anterolateral hippocampus showing differential connectivity between treatment and control group. Involved regions include primary and secondary somatosensory cortex, supramarginal gyrus, posterior midcingulate cortex, right fronto-insular cortex and temporal pole. (B) Reduced functional connectivity between this network and the right anterolateral hippocampus was associated with therapy-induced reductions in trait anxiety. Three months after therapy onset, treatment but not control group showed a significant association of connectivity reduction and clinical improvement of anxiety. After six months, when the control group had received delayed intervention, the association was significant in both groups. (C) The cortical networks associated with the right anteromedial an left anterolateral hippocampus showing differential connectivity between treatment and control group. Abbreviations: PoG, postcentral gyrus; pMCC, posterior midcingulate cortex; CO, central opercular cortex; Ins, insula; SMG, supramarginal gyrus; TPO, temporal pole, NCd, caudate nucleus; Tha, thalamus; OLi, lateral occipital cortex, inferior division; OF, occipital fusiform gyrus; CALC, intracalcarine cortex.

Changes in connectivity and clinical outcomes

The network associated with the rAL hippocampus comprised bilateral primary somatosensory cortex, right secondary somatosensory cortex extending to supramarginal gyrus, right fronto-insular cortex and temporal pole, as well as posterior midcingulate cortex (**Figure 4A**). Changes in averaged connectivity values of these regions were predicted by clinical changes in trait anxiety but not maximum pain as shown by multivariable regression. After three months, the prediction of a decrease in rAL connectivity by clinical reduction of anxiety was significant in the treatment group ($t = 2.058$, $p = 0.049$), but not in the control group ($t = 0.793$, $p = 0.436$). After six months, when controls had received delayed intervention, both, treatment and control group showed this association ($t = 2.613$, $p = 0.020$, and $t = 3.173$, $p = 0.007$, resp.) (**Figure 4B**).

Discussion

We have studied a combination of psychotherapy and somatic stimulation for endometriosis-associated pain using functional brain imaging. This novel combination produces remarkable reductions in pain, anxiety and depressive symptoms up to complete symptom relief in patients with endometriosis (16-17). As a long-term follow-up has shown, the effects are stable and have large effect sizes (16). Our analysis aimed at elucidating underlying mechanisms and was centered around the hippocampus as a probable target for this kind of therapy. We used a novel method to identify functionally independent hippocampal subregions as well as their whole-brain connectivity, and found connectivity changes that differed between treatment and control group at the end of the randomization phase. Three distinct hippocampal subregions showed therapy-related changes, namely the left and right anterolateral and the right anteromedial regions. As hypothesized, all were located in the anterior part of the hippocampus. This was expected because of the central role that emotions and painful memories play in our therapeutic approach (16). In particular, patients are encouraged to express their emotional reactions triggered by memories that surface during the therapeutic sessions. The important contribution of the anterior hippocampus to the processing of emotions and affective memories is well established (18-19). Furthermore, a recent review has emphasized revisiting of autobiographical memories as one of its core functions (22). Finally, the anterior hippocampus plays a central role in anxiety (23-24, 46), a personality trait with great significance for pelvic pain (7-10) and chronic pain in general (47-51).

We found evidence that a distinct anterior hippocampal region, the right anterolateral hippocampus, mediates therapy-associated reductions in anxiety. This is in good agreement with a recent study by Shackman and colleagues that identified the right anterolateral hippocampus as the neural substrate of anxiety mediating activity of the hypothalamic-pituitary-adrenal (HPA) axis

(24). A central role of the hippocampus in mediating HPA axis activity is well established in animals (52) and we have recently demonstrated functional connectivity between the anterior hippocampus and hypothalamic regions, including the periventricular nucleus (PVN) in humans (27). Hypocortisolism, a biomarker of HPA axis dysfunction, is an important factor in the pathophysiology of stress-related bodily disorders (53) and has been reported in patients with endometriosis and pelvic pain by several studies (54-56). Furthermore, the authors of these studies have linked the dysfunctional HPA axis with pain and anxiety (55) as well as with previous traumatic experiences (56). There is an emerging view in the recent literature that trauma is common among patients with endometriosis-associated pain and may play a central role in the pathogenesis of chronic pelvic pain (7-10).

In our study, the anterolateral hippocampus showed strong connectivity with septal nuclei at baseline (**Fig. S2**), which underscores the involvement of the anterolateral hippocampus in the so-called septo-hippocampal system, as proposed by Gray and McNaughton (57). This neurobiological system is believed to respond to situations of conflict or uncertainty by evoking responses aimed at conflict resolution, which may involve upregulation of arousal, inhibition of behavioral programs, and modulation of salience and attention to stimuli. Anxiety in this context is a response to potential danger that has evolved in order to prevent the organism from going into potentially dangerous situations (57-58). We found a cortical network whose connectivity with the anterolateral hippocampus was reduced, when patients experienced reduction of their anxiety symptoms. It comprised large parts of the primary and secondary somatosensory cortex, as well as the right anterior and mid insula and adjacent opercular areas. The involvement of somatosensory regions gives a first hint, why a combination of psychotherapy and somatosensory stimulation may be useful (16-17), although this was not directly assessed by our study. As Bannerman points out, sensory stimuli may act as occasion-setting cues in the hippocampus to enable selection of the correct body reaction when there is competition between concurrently available response choices (58). We believe that the therapeutic approach studied here exploits the interrelation of bodily sensations and traumatic or painful memories, and applies somatosensory stimulation to modify these somatic markers and resolve the associated emotional conflicts (16-17). The stimulation may help access deeper physiological programs in the septo-hippocampal system with its connection to the hypothalamus and brainstem. Functional imaging studies have shown that acupuncture can alter activity in the hippocampus (59-60), amygdala (59-61), hypothalamus (60-61) and brainstem (60-62). Thus, it is conceivable that concomitant somatic stimulation may reduce arousal during traumatic memory retrieval and help disconnect the memory from the bodily reaction.

We did not find direct evidence to explain the most striking therapeutic effect, i.e. pain reduction (16). Connectivity of the somatosensory network with the anterolateral hippocampus was associated with maximum pain at baseline, but clinical improvements did not predict connectivity changes as in the case for anxiety. This may be related to our choice of region of interest. Focusing the analysis on other areas, like the insula, or the thalamus may help to gain a better understanding of this therapeutic effect. However, there are several ways how improvements in anxiety and pain may be related. Tang and colleagues found that trait anxiety similar to state anxiety augments pain sensitivity (47), potentially by directing one's attention towards pain (63). Ploghaus and colleagues have shown that this exacerbation of pain by anxiety is mediated by a hippocampal network involving somatosensory areas (49). As Davis points out, patients with chronic pain may be in a "stuck" state of self-referential thought or focus on their pain (64). Finally, there is recent evidence of a critical role of hippocampal connectivity in the transition from acute to chronic pain (25).

Some limitations of the current study should be noted. Most women with endometriosis-associated pain have multifactorial causes of pelvic pain (musculoskeletal, bladder, central nervous system), and we did not explore those in detail. Furthermore, we compared the intervention to a waiting-list control group. The major limitation of such a design is that the observed improvements cannot be clearly attributed to specific treatment components. Waiting list control groups, however, control for important confounders such as regression to the mean, spontaneous improvement, and unidentified co-interventions (65). Furthermore, the lack of a matched control group with comparable chronic pain symptoms limits the inferences that can be made about the relevance to endometriosis. Finally, we used a step-wise multivariable regression to identify clinical parameters whose changes predicted changes in functional connectivity. Such analysis must be considered exploratory, as it does not fully control for multiple comparisons.

In conclusion, this study has identified a putative mechanism underlying the clinically potent combination of psychotherapy and acupuncture point stimulation in treating endometriosis-associated pain. The mechanism involves alterations in a cortical network of the right anterolateral hippocampus and somatosensory / interoceptive brain regions. Connectivity of this network reflects patients' anxiety and may be linked to activity of the HPA axis.

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References

1. Giudice LC. Clinical practice. Endometriosis. The New England journal of medicine 2010;362(25):2389-2398.
2. Rogers PA, D'Hooghe TM, Fazleabas A, Giudice LC, Montgomery GW, Petraglia F, Taylor RN. Defining future directions for endometriosis research: workshop report from the 2011 World Congress of Endometriosis In Montpellier, France. Reproductive sciences 2013;20(5):483-499.
3. Soliman AM, Yang H, Du EX, Kelley C, Winkel C. The direct and indirect costs associated with endometriosis: a systematic literature review. Human Reproduction (2016);31(4):712-722.
4. Vercellini P, Fedele L, Aimi G, Pietropaolo G, Consonni D, Crosignani PG. Association between endometriosis stage, lesion type, patient characteristics and severity of pelvic pain symptoms: a multivariate analysis of over 1000 patients. Hum Reprod 2007;22:266-271.
5. Allen C, Hopewell S, Prentice A, Gregory D. Nonsteroidal anti-inflammatory drugs for pain in women with endometriosis. The Cochrane database of systematic reviews 2009(2):CD004753.
6. Triolo O, Lagana AS, Sturlese E. Chronic pelvic pain in endometriosis: an overview. Journal of clinical medicine research 2013;5(3):153-163.
7. Latthe P, Mignini L, Gray R, Hills E, Khan K. Factors predisposing women to chronic pelvic pain: systematic review. BMJ 2006;332:749.
8. As-Sanie S, Clevenger LA, Geisser ME, Williams DA, Roth RS. History of abuse and its relationship to pain experience and depression in women with CPP. Am J Obstet Gynecol 2014;210:317.e1-8.
9. Seth A, Teichman JM. Differences in the clinical presentation of interstitial cystitis/painful bladder syndrome in patients with or without sexual abuse history. J Urol. 2008;180(5):2029-2033.
10. Pope CJ, Sharma V, Sharma S, Mazmanian D. A Systematic Review of the Association Between Psychiatric Disturbances and Endometriosis. Journal of obstetrics and gynaecology Canada : JOGC 2015;37:1006-15.
11. Bajaj P, Bajaj P, Madsen H, Arendt-Nielsen L. Endometriosis is associated with central sensitization: a psychophysical controlled study. The journal of pain : official journal of the American Pain Society 2003;4(7):372-380.

12. Iacovides S, Baker FC, Avidon I, Bentley A. Women with dysmenorrhea are hypersensitive to experimental deep muscle pain across the menstrual cycle. The journal of pain : official journal of the American Pain Society 2013;14(10):1066-1076.
13. As-Sanie S, Kim J, Schmidt-Wilcke T, Sundgren PC, Clauw DJ, Napadow V, Harris RE. Functional Connectivity Is Associated With Altered Brain Chemistry in Women With Endometriosis-Associated Chronic Pelvic Pain. J Pain 2016;17(1):1-13.
14. Brawn J, Morotti M, Zondervan KT, Becker CM, Vincent K. Central changes associated with chronic pelvic pain and endometriosis. Human Reproduction Update 2014;20(5):737–747.
15. As-Sanie S, Harris RE, Napadow V, Kim J, Neshewat G, Kairys A, Williams D, Clauw DJ, Schmidt-Wilcke T. Changes in regional gray matter volume in women with chronic pelvic pain: A voxel-based morphometry study. Pain 2012;153(5):1006-14.
16. Meissner K, Schweizer-Arau A, Limmer A, Preibisch C, Popovici RM, Lange I, de Oriol B, Beissner F. Psychotherapy With Somatosensory Stimulation for Endometriosis-Associated Pain - A Randomized Controlled Trial. Obstetrics & Gynecology (2016); 128(5): 1134-1142.
17. Meissner K, Bohling B, Schweizer-Arau A. Long-term effects of traditional Chinese medicine and hypnotherapy in patients with severe endometriosis - a retrospective evaluation. Forschende Komplementärmedizin 2010;17(6):314-320.
18. LaBar KS, Cabeza R. Cognitive neuroscience of emotional memory. Nat Rev Neurosci 2006;7:54-64.
19. LeDoux JE. Emotional memory systems in the brain. Behavioural Brain Res 1993;58:69-79.
20. Levy-Gigi E, Szabó C, Kelemen O, Kéri S. Association among clinical response, hippocampal volume, and FKBP5 gene expression in individuals with posttraumatic stress disorder receiving cognitive behavioral therapy. Biol Psychiatry. 2013;74(11):793-800.
21. Lindauer RJ, Vlieger EJ, Jalink M, Olff M, Carlier IV, Majoie CB, Den Heeten GJ, Gersons BP. Effects of psychotherapy on hippocampal volume in out-patients with post-traumatic stress disorder: a MRI investigation. Psychol Med. 2005 Oct;35(10):1421-31.
22. Zeidman P, Maguire EA. Anterior hippocampus: the anatomy of perception, imagination and episodic memory. Nat Rev Neurosci. 2016 Mar;17(3):173-82.
23. Bannerman DM, Rawlins JN, McHugh SB, Deacon RM, Yee BK, Bast T, Zhang WN, Pothuizen HH, Feldon J. Regional dissociations within the hippocampus - memory and anxiety. Neurosci Biobehav Rev. 2004;28(3):273-83.
24. Shackman AJ, Fox AS, Oler JA, Shelton SE, Davidson RJ, Kalin NH. Neural mechanisms underlying heterogeneity in the presentation of anxious temperament. Proc Natl Acad Sci U S A 2013;110(15):6145-50.

25. Mutso AA, Petre B, Huang L, Baliki MN, Torbey S, Herrmann KM, Schnitzer TJ, Apkarian AV. Reorganization of hippocampal functional connectivity with transition to chronic back pain. J Neurophysiol 2014;111(5):1065-76.
26. Moher Alsady T, Blessing E, Beissner F. MICA – A toolbox for masked independent component analysis of fMRI data. Hum Brain Mapp 2016; 37(10):3544–3556.
27. Blessing EM, Beissner F, Schumann A, Brünner F, Bär KJ. A data-driven approach to mapping cortical and subcortical intrinsic functional connectivity along the longitudinal hippocampal axis. Hum Brain Mapp 2016;37(2):462-76.
28. Protopopescu X, Butler T, Pan H, Root J, Altemus M, Polanecsky M, McEwen B, Silbersweig D, Stern E. Hippocampal structural changes across the menstrual cycle. Hippocampus 2008;18(10):985-8.
29. van Wingen GA, van Broekhoven F, Verkes RJ, Petersson KM, Bäckström T, Buitelaar JK, Fernández G. Progesterone selectively increases amygdala reactivity in women. Molecular psychiatry 2008;13(3):325-33.1.
30. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta psychiatrica scandinavica 1983;67:361-70.
31. Spielberger C, Gorsuch R, Lushene R, Vagg P, Jacobs G. Manual for the state-trait anxiety inventory (form Y). Palo Alto (CA): Consulting Psychologists Press; 1983.
32. Ware JE, Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: Construction of Scales and Preliminary Tests of Reliability and Validity. Medical Care 1996;34(3):220-233
33. Erickson MH, Rossi EL. Hypnotherapy, an exploratory casebook. North Stratford, New Hampshire: Irvington Publishers; 1979.
34. Kabat-Zinn J. An outpatient program in behavioral medicine for chronic pain patients based on the practice of mindfulness meditation: theoretical considerations and preliminary results. General hospital psychiatry 1982;4:33-47.
35. Butler AC, Chapman JE, Forman EM, Beck AT. The empirical status of cognitive-behavioral therapy: a review of meta-analyses. Clinical psychology review 2006;26:17-31.
36. Haley J. Problem-solving therapy. San Francisco: Jossey-Bass; 1987.
37. Kaptchuk TJ. OMD. The Web That Has No Weaver. New York: Congdon and Weed, Inc; 1983.
38. Salimi-Khorshidi G, Douaud G, Beckmann CF, Glasser MF, Griffanti L, Smith SM. Automatic denoising of functional MRI data: Combining independent component analysis and hierarchical fusion of classifiers. Neuroimage 2014;90:449-68.

39. Greve DN, Fischl B. Accurate and robust brain image alignment using boundary-based registration. Neuroimage 2009;48(1):63-72.
40. Beckmann CF, Smith SM. Probabilistic independent component analysis for functional magnetic resonance imaging. IEEE Trans Med Imaging 2004;23:137–152.
41. Hyvarinen A. Fast and robust fixed-point algorithms for independent component analysis. IEEE Trans Neural Netw 1999;10:626–634.
42. Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, Matthews PM, BeckmannCF, Mackay CE. Distinct patterns of brain activity in young carriers of the APOE-ε4 allele. PNAS 2009, 106(17):7209-14.
43. Eklund A, Nichols TE, Knutsson H. Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. PNAS 2016, 113(28):7900-5.
44. Grady CL, McIntosh AR, Craik FIM. Age-Related Differences in the Functional Connectivity of the Hippocampus During Memory Encoding. Hippocampus 2003;3:572–586.
45. Leech R, Braga R, Sharp DJ. Echoes of the brain within the posterior cingulate cortex. J Neurosci 2012;32:215–222.
46. Bannerman DM, Grubb M, Deacon RMJ, Yee BK, Feldon J, Rawlins JNP. Ventral hippocampal lesions affect anxiety but not spatial learning. Behavioural Brain Research 2003;139:197-213.
47. Tang J, Gibson SJ. A Psychophysical Evaluation of the Relationship Between Trait Anxiety, Pain Perception, and Induced State Anxiety. J Pain 2005;6(9):612-619.
48. Moix J, Kovacs FM, Martín A, Plana MN, Royuela A, The Spanish Back Pain Research Network. Catastrophizing, State Anxiety, Anger, and Depressive Symptoms Do Not Correlate with Disability when Variations of Trait Anxiety Are Taken into Account. A Study of Chronic Low Back Pain Patients Treated in Spanish Pain Units [NCT00360802]. Pain Medicine 2011;12:1008–1017.
49. Ploghaus A, Narain C, Beckmann CF, Clare S, Bantick S, Wise R, Matthews PM, Rawlins JN, Tracey I. Exacerbation of pain by anxiety is associated with activity in a hippocampal network. J Neurosci 2001;21(24):9896-903.
50. McCracken LM, Gross RT. The Role of Pain-Related Anxiety Reduction in the Outcome of Multidisciplinary Treatment for Chronic Low Back Pain: Preliminary Results. Journal of Occupational Rehabilitation 1998;8(3):179-189.
51. McWilliams LA, Cox BJ, Enns MW. Mood and anxiety disorders associated with chronic pain: an examination in a nationally representative sample. Pain 2003;106(1–2):127–133.

52. Jacobson L, Sapolsky R. The Role of the Hippocampus in Feedback Regulation of the Hypothalamic-Pituitary-Adrenocortical Axis. Endocrine Reviews 1991;12(2):118-134.
53. Heim C, Ehlert U, Hellhammer DH. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Psychoneuroendocrinology 2000;25(1):1-35.
54. Petrelluzzi KF, Garcia MC, Petta CA, Grassi-Kassisse DM and Spadari-Bratfisch RC. Salivary cortisol concentrations, stress and quality of life in women with endometriosis and chronic pelvic pain. Stress 2008;11:390-7.
55. Quiñones M, Urrutia R, Torres-Reverón A, Vincent K, Flores I. Anxiety, coping skills and hypothalamus-pituitary-adrenal (HPA) axis in patients with endometriosis. J Reprod Biol Health 2015;3:2.
56. Heim C, Ehlert U, Hanker JP, Hellhammer DH. Abuse-Related Posttraumatic Stress Disorder and Alterations of the Hypothalamic-Pituitary-Adrenal Axis in Women With Chronic Pelvic Pain. Psychosomatic Medicine 1998;60:309-318.
57. Gray JA, McNaughton N. The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septo-Hippocampal System (2 ed). Oxford: Oxford University Press; 2000.
58. Bannerman DM, Sprengel R, Sanderson DJ, McHugh SB, Rawlins JNP, Monyer H, Seeburg PH. Hippocampal synaptic plasticity, spatial memory and anxiety. Nat Rev Neurosci 2014;15:181-192.
59. Hui KK, Liu J, Makris N, Gollub RL, Chen AJ, Moore CI, Kennedy DN, Rosen BR, Kwong KK. Acupuncture modulates the limbic system and subcortical gray structures of the human brain: evidence from fMRI studies in normal subjects. Hum Brain Mapp 2000;9(1):13-25.
60. Napadow V, Dhond R, Park K, Kim J, Makris N, Kwong KK, Harris RE, Purdon PL, Kettner N, Hui KK. Time-variant fMRI activity in the brainstem and higher structures in response to acupuncture. Neuroimage 2009 Aug 1;47(1):289-301.
61. Napadow V, Kettner N, Liu J, Li M, Kwong KK, Vangel M, Makris N, Audette J, Hui KKS. Hypothalamus and Amygdala Response to Acupuncture Stimuli in Carpal Tunnel Syndrome. Pain 2007;130(3):254-266.
62. Beissner F, Deichmann R, Henke C, Bär KJ. Acupuncture - Deep pain with an autonomic dimension? NeuroImage. 2012; 60(1):653-60.
63. Payne KA, Binik YM, Amsel R, Khalifé S. When sex hurts, anxiety and fear orient attention towards pain. European Journal of Pain 2005;9:427–436.
64. Davis KD, Moayedi M. Central mechanisms of pain revealed through functional and structural MRI. Journal of Neuroimmune Pharmacology 2013;8:518–534.

65. Hrobjartsson A, Kaptchuk TJ, Miller FG. Placebo effect studies are susceptible to response bias and to other types of biases. Journal of clinical epidemiology 2011;64:1223-9.