PLACEBO ANALGESIA: NEUROMODULATION OF PAIN-RELATED EFFECTIVE CONNECTIVITY

By

LANDREW SAMUEL SEVEL

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To my wonderful parents for their boundless love and support

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DACC	Dorsal anterior cingulate cortex
DCM	Dynamic causal modeling
DLPFC	Dorsolateral prefrontal cortex
PA	Placebo analgesia
PAG	Periaqueductal gray
P-INS	Posterior insula
RACC	Rostral anterior cingulate cortex
THAL	Thalamus

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By
Landrew Samuel Sevel

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The use of placebos to decrease subjective ratings of painful stimuli is well documented; however, the neural mechanisms underlying endogenous pain modulation (i.e., placebo analgesia) are not yet fully understood. This study used functional magnetic resonance imaging (fMRI) data from 30 healthy subjects, and dynamic causal modeling (DCM) to investigate changes in inter-regional connectivity associated with the placebo analgesic (PA) response in pain-related brain regions. Before scanning, subjects were conditioned to expect less heat pain at 2-of-4 sites on their feet (i.e., the PA). VAS pain ratings from the scanning session revealed a significant difference between the painful and placebo sites. However, no significant differences in brain activation between conditions were observed with traditional GLM analyses. These results are indicative of the insensitivity of the GLM to appropriately model BOLD responses to rapidly changing stimuli used in our protocol. DCM was then used to estimate and compare models of neural networks involved with endogenous pain

modulation. The results indicate that PA was associated with significant, bilateral changes in the influence of brain regions involved in attentional, expectation, and evaluative processes including the dorsolateral prefrontal cortex (dlPFC) and dorsal anterior cingulate cortex (dACC). In the right hemisphere PA was associated with a substantial increase in the influence the periaqueductal grey (PAG) received from the dlPFC (i.e., dlPFC→PAG). In the left hemisphere, PA was associated with significant changes among the dlPFC→dACC and dACC→thalamus connections. These findings highlight the subtle, but crucial, differences between the processing and modulation of pain, and signal to future studies the importance of nuanced analytical approaches that are sensitive to temporal shifts in pain-related processes.

CHAPTER 1
INTRODUCTION

Chronic pain is a significant health concern, affecting over 100 million Americans and resulting in over $600 billion in lost income and healthcare costs,[1, 17] however, powerful treatments for chronic pain remain elusive. One way to mitigate this problem is through the enhancement of currently available treatments. Placebo analgesia (PA) is an endogenous process that can effectively reduce an individual's pain.[34] Furthermore, PA is seen as an acceptable treatment by patients who have learned that they have received a placebo.[8] PA, however, is a complex and multifaceted phenomenon that is influenced by multiple psychological constructs and mediated by multidimensional neuronal systems. Given this complexity, the neural mechanisms that underlie PA and the factors that predict an individual's placebo response remain poorly understood. Early investigations of PA that used functional MRI (fMRI) associated PA with the modulation of neural activity among pain-related brain regions. Nuanced analytical methods that investigate the temporal development of PA's are necessary to better understand the dynamic changes among brain regions involved in endogenous pain modulation.

Psychological Mechanisms of Placebo Analgesia

Placebo analgesia is a complex phenomenon, which has been linked to the pain-modulatory processes of classical conditioning, expectation, and anxiety. Through classical conditioning, the repeated pairing of an inert, placebo substance with a non-painful stimulus forms a conditioned association between the placebo and analgesia. Pairing the conditioned, placebo substance with a painful stimulus then results in decreased pain.[51] Although expectations may be produced directly through conditioning,

processes of expectation may also produce analgesia before stimulus conditioning.[52] Anxiety is also known to influence pain. For example, subjects reporting high levels of anxiety may experience stress-induced analgesia. Additionally, subjects reporting low levels of anxiety tend to expect less distress and report lower levels of pain.[30, 33] Studies have also suggested that dispositional factors such as optimism[18] or cognitive-evaluative processes such as expectation-effectiveness comparisons[25] mediate placebo effectiveness.

Cortical Pathways of Pain and Placebo Analgesia

The experience of pain is multidimensional, encompassing sensory, cognitive and affective components,[25] this complexity is reflected in neural responses to painful stimuli. Experimental and clinical studies of brain activation related to pain processing identify a diffuse network of brain regions encompassing ascending pain pathways and descending pain modulatory systems.[31] Various ascending pathways are thought to differentiate unique aspects of pain such as sensation, unpleasantness, and affect.[31, 32] For example, the spinothalamic tract, vital for sensory and affective processing, sends projections from the spinal cord through the thalamus to the primary and secondary somatosensory cortices, and then to regions such as the insular cortex, amygdala, and posterior parietal cortices.[32] Higher-order brain regions, such as the anterior cingulate cortex (ACC) and prefrontal cortex (PFC), are involved in cognitive and affective dimensions of pain. The ACC and PFC also contribute to the modulation of pain via descending projections to the periaqueductal grey and rostral ventromedial medulla (RVM).[5, 7] fMRI studies of pain are consistent with these ascending and descending pathways and also offer insights into the modulation of pain via placebo analgesia.[33]

The neural modulation of pain processing effected by PA is multifaceted. Multiple studies have associated PA with reductions in BOLD activity in pain-related brain such as the thalamus, somatosensory cortices, insula, and anterior cingulate cortex.[13, 34, 53] Increased activity in regions responsible for cognitive control and evaluative processes, such as the dorsolateral prefrontal cortex (dlPFC), orbitofrontal cortex (OFC), and rostral anterior cingulate cortex (rACC), has also been observed in anticipation of and during placebo.[34, 53] The DLPFC, rACC, amygdala, periaqueductal grey (PAG), and RVM comprise a widely studied descending pain modulatory pathway that ultimately projects to the dorsal horn of the spinal cord. Activation of this pathway involves the release of neurotransmitters associated with pain modulation including endogenous opioids, noradrenaline and serotonin.[5, 7, 32, 38] Although previously seen as indirectly involved in pain modulation,[38] μ-opioid release in the dorsal anterior cingulate cortex (dACC) has been shown to mediate placebo effectiveness via expectation-outcome comparisons.[25] The neural complexity of pain processing and modulation suggests that studying these processes with network connectivity analysis approaches may provide vital insight into the mechanisms of the pain modulation.

Effective Connectivity: Dynamic Causal Modeling

Effective connectivity, is an estimation of the influence one brain region or network exerts on another.[14] Using fMRI data, models of effective connectivity help clarify the inter-regional relationships associated with changes identified in the blood oxygen-level dependent (BOLD) signal.

There are multiple methods for estimating effective connectivity. One such approach, structural equation modeling (SEM), models effective connectivity via changes in the observed hemodynamic covariance structure between brain regions.[27]

SEM has been applied to the study of pain in both healthy and clinical populations.[10, 11] In an SEM analysis of placebo analgesia in chronic pain patients, Craggs and colleagues[10] demonstrated that, compared to a baseline painful condition, the inter-regional relationships among pain-related brain regions were drastically altered during the experience of placebo analgesia. However, the data in this study for the baseline painful and PA conditions were collected on separate visits. Thus, whether these same changes occur among healthy individuals, and whether the BOLD response to rapidly presented thermal stimuli could distinguish pain and PA processes from a single scanning session remains unclear.

Dynamic causal modeling (DCM),[16] another method for estimating effective connectivity, differs from SEM in a number of advantageous ways.[27] In DCM, the influence of experimental manipulations on a network of brain regions is modeled at the neuronal level. The Balloon-Windkessel model is then used to translate modeled neuronal activity into hemodynamic responses,[16] that can be compared to the observed regional BOLD responses acquired in fMRI.[15, 16] This process allows for the comparison of competing models of neural dynamics and produces mechanistically interpretable effective connectivity parameter estimates. DCM offers interpretational ease in the sense that it readily allows the estimate of the effects of multiple experimental stimuli or cognitive, contextual variables on inter-regional dynamics. These advantages make DCM ideal for studying the unique impacts of painful and placebo analgesic stimuli on pain-related effective connectivity during the same fMRI scanning session.

The application of DCM to the study of pain has thus far been very limited. To the best of our knowledge, there have been no DCM studies of placebo analgesia.

Furthermore, many studies of PA have utilized experimental paradigms in which the stimulation of painful and PA sites were temporally distant, preventing a more robust understanding of PA neural processes. Therefore, the present study examined the effects of a placebo analgesic stimulation on brain activation (random effects general linear model) and effective connectivity (DCM) compared to baseline, painful stimulation. Rapid succession of experimental conditions (painful vs. PA stimulation) was used to allow for a dynamic understanding of PA-related modulation. Based upon our previous work investigating the placebo analgesic response, we hypothesized that: 1) during the experience of PA compared to pain processing, decreased BOLD activation would be found in regions commonly associated with pain experience (thalamus, insula, primary and secondary somatosensory cortices, ACC) and increased BOLD activation would be found in regions associated with descending pain modulation (dlPFC and ACC) and 2) PA would modulate descending pain-related, inter-regional connectivity parameters from regions such as the dlPFC, dACC, and rACC.

CHAPTER 2
METHODS

The data used in the present study were collected as part of a larger, NIH-funded project (grant number: 5R01AT001424) designed to investigate the mechanisms of placebo analgesia. During a screening visit, “pain” and “placebo” temperatures were identified for each subject. Subjects then completed three fMRI scanning visits designed to assess establish baseline neural response to thermal quantitative sensory testing (QST), identify the neural correlates of placebo analgesia (placebo imaging visit) and assess the durability of the placebo response over time. Subjects completed an initial demographics questionnaire and during each visit, completed two self-report questionnaires, the State-Trait Anxiety Inventory (STAI) and the Pennebaker Inventory of Limbic Languidness (PILL) and provided subjective, VAS ratings of their pain during QST. Only fMRI data and subjective ratings from subjects’ placebo imaging visit were analyzed in the present study.

The present study utilized a within subjects design to assess differences in brain activation and network effective connectivity during painful and placebo analgesic stimulation. The parent study was approved by the University of Florida Institutional Review Board and all participants provided written informed consent.

Participants

MRI data from 30, healthy individuals were used in this study (mean age = 22.27 years, SD = 2.90 years). Eleven participants identified as Caucasian, eight as Asian, five as Hispanic, six as African American, and one as Native Hawaiian or other Pacific Islander (one identified as both African American and Hispanic). Exclusion criteria included: 1) current participation in another research protocol that could interfere with or

influence the present study (i.e. other studies of pain) 2) use of prescription or non-prescription drugs that might impact pain-processing that could not be stopped seven days prior to testing (e.g. NSAIDs, antihistamines, antidepressants, anti-convulsants, migraine medications, and cough suppressants) 3) history of psychiatric, psychological, neurologic, or other disorders (e.g. diabetes, thyroid disease, gastrointestinal/liver disease (other than IBS), collagen vascular disease, focal or systemic neurological disease, malignancy, seropositive for HIV, or documented psychiatric disorders) 4) current chronic pain condition 5) positive pregnancy test result 6) possession of metal in the head, neck or abdominal cavity 7) current medical condition that would contraindicate participation in this study 8) inability to provide informed consent.

Experimental Materials

Thermal stimuli were delivered to two locations on the surface of each foot with an MR compatible, computer-controlled Medoc Thermal Sensory Analyzer (TSA-2001, Ramat Yishai, Israel). This is a peltier-element-based stimulator, capable of producing stimuli across a range of temperatures (33-51°C). A Visual Analog Scale (VAS) was used in the acquisition of subjective pain ratings. The VAS was anchored on the left with “No pain” and on the right with “The most imaginable pain.”

Experimental Procedures

To account for individual differences in pain perception, each subject underwent a series of QST calibration trials during the screening visit to determine pain and placebo temperatures. In these trails, subjects received a series of thermal pulses on the dorsal aspect of the foot starting at 43°C and increasing by 1°C until a subject’s tolerance or 51°C was reached. Subjects rated their pain intensity after each pulse. The

highest temperature with a VAS score ≤20 was used as the placebo temperature, and the lowest temperature with a score ≥40 and ≤60 was used as the painful temperature.

During the first part of the placebo visit, subjects were conditioned to expect less pain from thermal stimuli applied to two sites of their feet where an inert cream had been applied. Specifically, an inert cream was applied on two of four sites (placebo sites) of the dorsal aspects of the feet and subjects were then told: “The agent you have just been given is known to significantly reduce pain in some patients.” During the subsequent eight conditioning trials, thermal stimuli were applied in a random order using the placebo temperature (VAS ≤ 20) on the placebo sites and painful temperature on the others (40 ≤ VAS ≥ 60). Directly following the conditioning, subjects completed an MRI scanning session, which included one anatomical, and three fMRI scans. During each scan, subjects received 16 thermal pulses (4s, ~12s inter-stimulus interval, Figure 2-1), half on placebo sites, in a random order. All thermal pulses were presented at the painful temperature in each scan. Following each stimulus, subjects rated their pain using an electronic VAS.

Data Acquisition and Image Processing

The parameters for the T1-weighted structural MRI included: saggital orientation (XYZ dimension= 256*256*180; FOV [ap,fh,rl - mm] =240, 240, 180; slice thickness [mm] =1; gap thickness = 0; voxel dimension [mm]= 1.0*1.0*1.0; repetition time [ms] =8.1, FA=8). Parameters for the subsequent fMRI scans were: trans-axial orientation, echo planar acquisition (XYZ dimension = 80*80*39; FOV [ap,fh,rl - mm]=240, 114, 240; slice thickness [mm] =3; gap thickness = 0; voxel dimension [mm]= 3*3*3; repetition time [ms] =2000, FA=80). To prevent issues related to field inhomogeneity, four dummy

volumes were acquired and discarded at the beginning of each scan, which lasted 5:40, and resulted in 486 volumes per subject.

Data were analyzed using SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK) with MATLAB 2011b (MathWorks, Sherbon, MA, USA). Functional images were slice-time corrected and realigned to the middle volume of each sequence to compensate for head movements. Anatomical and functional images were then coregistered using the functional images as reference. The functional data were then spatially normalized to MNI space and smoothed with an isotropic 6-mm Gaussian kernel (FWHM).

A random-effects general linear model (RFX-GLM) was used to identify cortical regions wherein pain and placebo stimuli onset were significantly convolved with the hemodynamic response function (HRF). The first-level analyses included the canonical HRF, and also temporal and dispersion derivatives, which model small differences in peak response latency and peak response duration, respectively. Inclusion of these informed basis functions allows for inter-subject and inter–voxel response variation. The planned contrasts for the first-level analysis included the main effect for each condition, pain and placebo, and the difference between them. Using RFX-GLMs, the first-level contrast images were analyzed using one-sample t-tests ($p \leq 0.05$), and adjusted for multiple comparisons with the family-wise error correction (FWE). Group-level areas of activation identified by RFX-GLM and previous functional studies of pain[7, 10, 34, 36, 52, 53] were used to guide region of interest (ROI) selection for the DCM analyses. To account for individual variability in the BOLD response, for each subject, data were extracted for each ROI on a per scan basis.

DCM (DCM12, Wellcome Trust Centre for Neuroimaging, London, UK) was used to estimate the effective connectivity among brain regions involved in processing pain-related information, and the changes in effective connectivity that corresponded with the PA response. Neural pathways identified in functional and anatomical studies were used to inform the creation of a theoretically informed model of how painful stimuli are processed (Figure 2-2).[3, 4, 6, 7, 21, 31, 32, 38, 41, 50] To be included in DCM analyses, individual subjects were required meet the following criteria: 1) supra-threshold ($p \leq 0.05$, uncorrected) activation within 9 mm of the group peak in all ROIs 2) activation in all ROIs in two of three fMRI scans. ROIs were created as 6mm spheres using SPM12 on a contrast of combined activation in response to painful and PA stimuli.

DCM model comparison and parameter inference proceeded in two steps. 1) To identify the winning model in each hemisphere, Bayesian model selection (BMS) [39, 45] was used to compare hypothesized models. The winning model demonstrated the highest posterior probability (expected probability of that model being observed in a randomly selected subject) and highest exceedance probability (certainty that a model is more likely than any other of those tested, given the data). 2) Bayesian model averaging (BMA) [29] was used to identify the group- and subject- level parameter coefficients of the winning model. Post-hoc, one-sample t-tests on each parameter class, experimental inputs, endogenous connections, and modulatory parameters, were conducted to determine parameter consistency at the subject-level, and Bonferroni corrected for multiple comparisons separately for each class.[43]

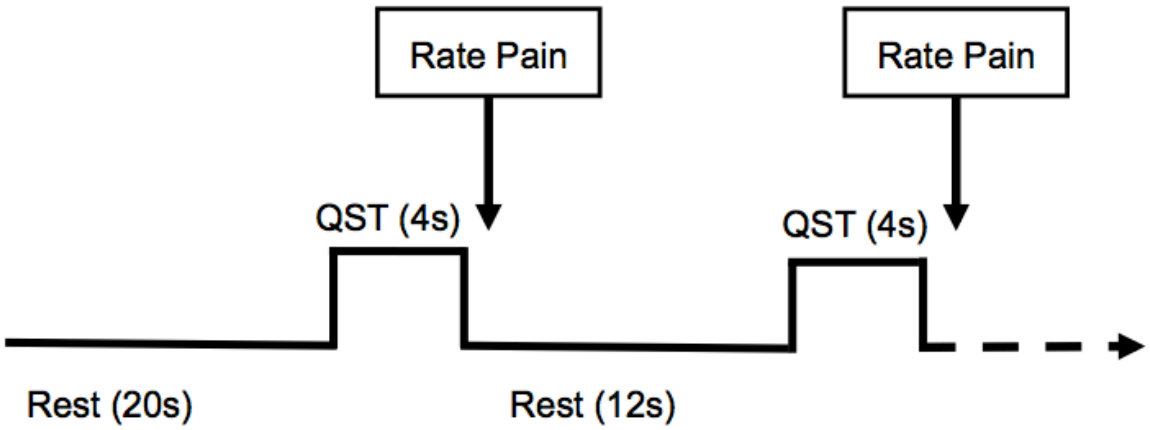

Figure 2-1. fMRI experimental protocol. Subjects completed three fMRI scans. Each scan consisted of 16 four second QST trials on randomly ordered painful and placebo sites of the foot with a ~12 second inter-stimulus interval. Directly after each QST trial, subjects provided a VAS rating of their pain.

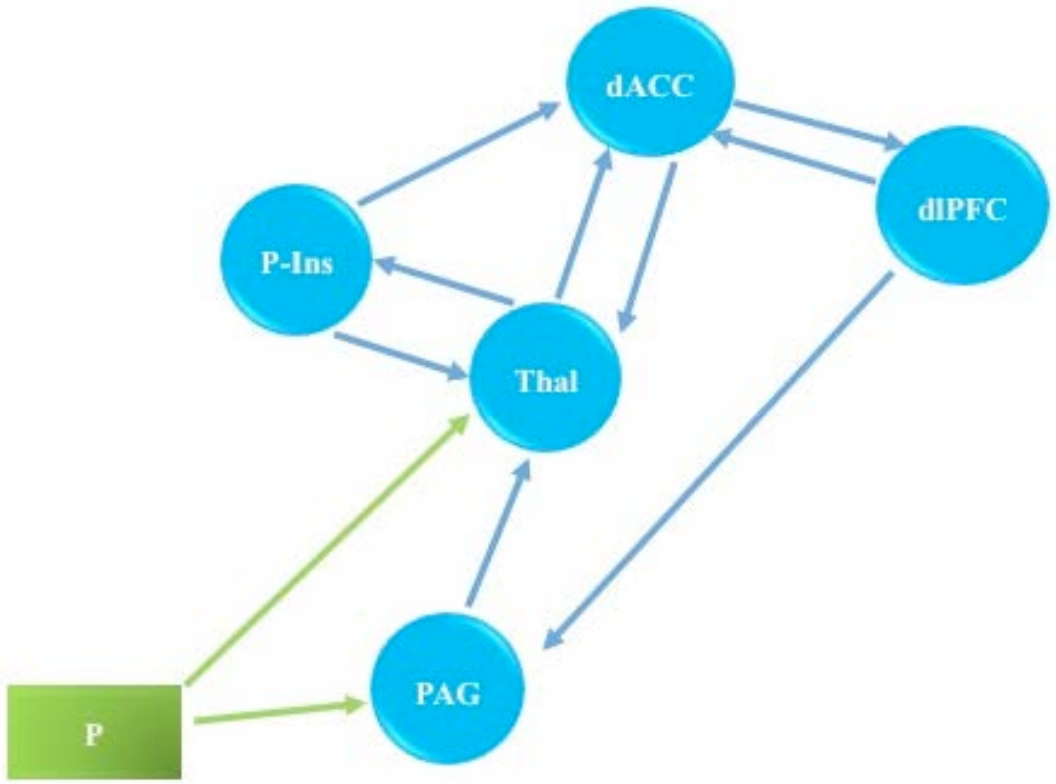

Figure 2-2. Theory-driven model of endogenous pain processing. Green rectangles and arrows represent the influence of painful stimuli while blue circles and arrows represent ROIs and their endogenous connections. Abbreviations: P, painful stimulation; PA, placebo analgesic response; PAG, periaqueductal gray; Thal, thalamus; P-Ins, posterior insula; dACC, dorsal anterior cingulate cortex, dlPFC, dorsolateral prefrontal cortex.

CHAPTER 3
RESULTS

Random Effects General Linear Model Brain Activations

Mean VAS pain ratings at painful and PA sites of the foot were 48.49 (SD=18.49) and 41.87 (SD=16.90), respectively. A significant main effect was observed when comparing mean VAS ratings for each condition [mean difference = 6.63, t (29) = 3.91, p≤0.001, *d* =0.97], RFX-GLM did not identify significant differences in brain activation between the baseline and PA conditions. Significant activations due to thermal stimulation were observed when viewing the combination response due to both conditions ($p \leq 0.05$, FWE). Activation was observed in regions including the bilateral thalamus, posterior insula, secondary somatosensory cortex, dorsal anterior cingulate cortex, and supplementary motor area (see Figure 3-1). Activation was also seen in the brainstem, including the PAG, and right anterior insula.

Dynamic Causal Modeling

The pain-related regions chosen for DCM included the PAG, thalamus, posterior insula, dACC, and dlPFC. Coordinates based upon group maxima identified by RFX-GLM are listed in Table 3-1. Four bilinear, deterministic DCMs were specified for comparison in BMS (see Figure 3-2). All models contained the same underlying structure of endogenous connections. Pain was assumed to act as an experimental input to the thalamus and PAG. Specified endogenous connections functioned to explain how painful stimuli are processed by this set of regions via ascending projections from the thalamus and PAG to subcortical structures (posterior insula) and finally to cingulate and prefrontal structures, and descending pathways from the dlPFC and dACC functioned to explain pain modulation.[3,4,6, 7, 21, 31 32, 38, 41, 50]

The models compared differed in their estimation of the modulation of pain-related effective connectivity during PA. Model one (M1) was a baseline model of pain processing model, and proposed no modulatory effects of PA. The same endogenous structure was used in all subsequent models. Model two (M2) additionally estimated the potential changes in descending projections of the dACC to the thalamus during PA. Model three (M3) estimated the potential changes in descending projections from the dlPFC (dlPFC→PAG and dlPFC→dACC) during PA. Model four (M4) estimated PA-related changes in both descending projections from the dlPFC and dACC, as such it is a combination of the modulatory effects in M2 and M3. Models were estimated separately for each hemisphere. Thirteen of 30 subjects met DCM inclusion criteria of supra-threshold activation at each VOI in at least two of three fMRI scans.

BMS identified M4 as the best fitting model in both the right and left hemispheres. Posterior and exceedance probabilities for each model can be seen in Table 3-2. Parameter estimates for M4 calculated in BMA for models of each hemisphere can be seen in Tables 3-3, 3-4, and 3-5. Significant differences in parameter estimates between hemispheres were not observed in any parameter class.

Consistency of parameter estimates across subjects was assessed with *post-hoc* one-sample t-tests independently for each parameter class (Tables 3-3, 3-4, and 3-5). Experimental pain inputs were highly, significantly consistent across subjects. Significant endogenous connections were seen bilaterally in the Thal→P-Ins, Thal→dACC, dACC→dlPFC connections. In the right hemisphere, the P-Ins→dACC connection was additionally significantly consistent; this connection trended toward significance in the left hemisphere (uncorrected p=0.057, corrected threshold p=0.05).

Strong, average modulatory effects of PA were observed in each hemisphere. Bilaterally, negative influence was observed in the descending modulation of the dlPFC→PAG and dACC→Thal connections while positive modulation was observed in the dlPFC→dACC. Assessment of modulatory parameters in the left hemisphere revealed significant modulations of the dACC→Thal and dlPFC→dACC connections. In the right hemisphere only the dlPFC→PAG modulatory parameter was significantly different from zero.

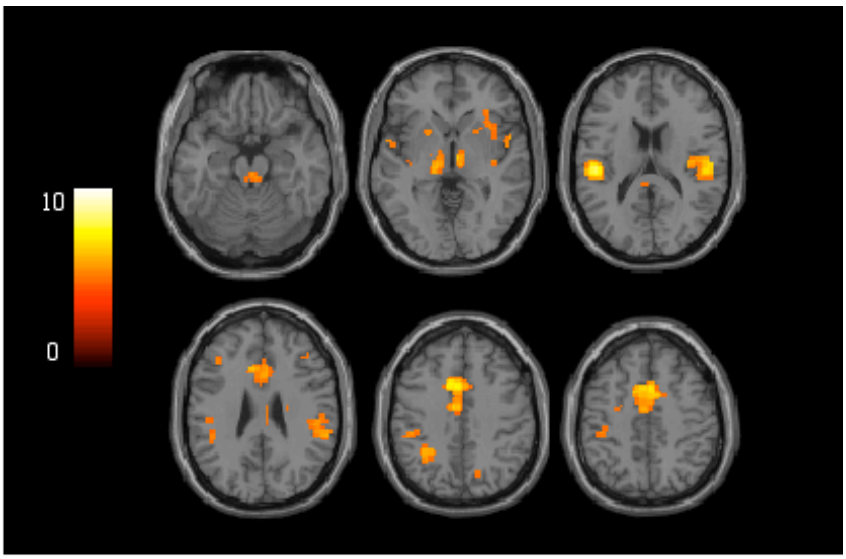

Figure 3-1. Significant activation (p≤0.05, FWE) in response to combined painful and PA stimuli.

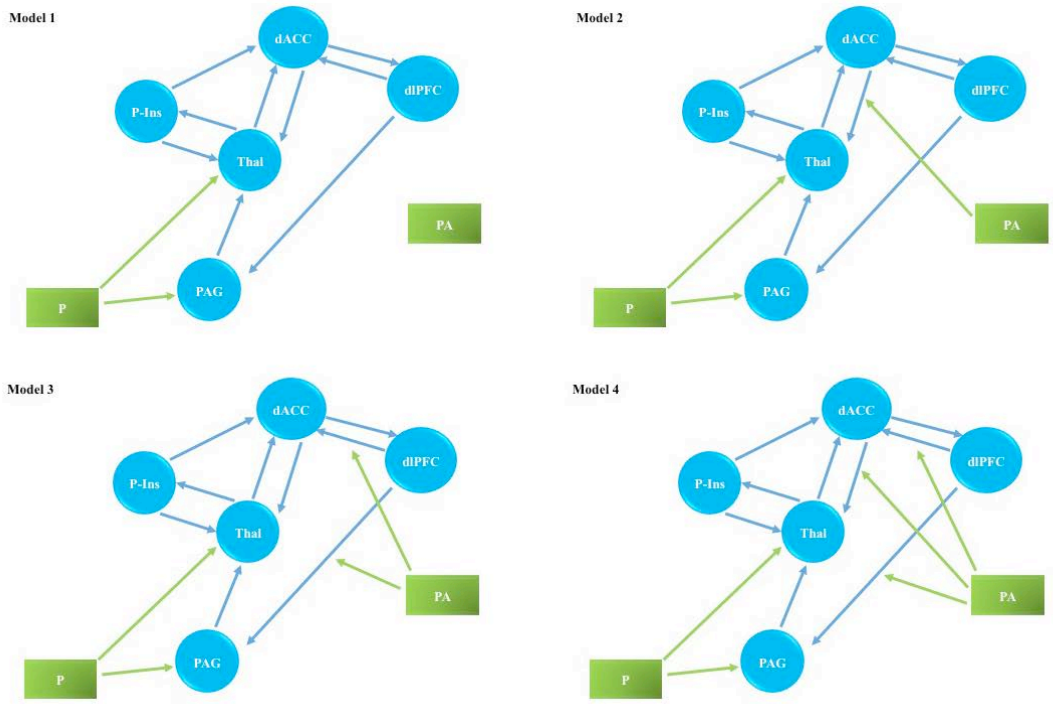

Figure 3-2. Models of pain processing and placebo-related pain modulation compared in BMS. Green rectangles and arrows represent the influence of experimental conditions, while blue circles and arrows represent ROIs and their endogenous connections. Abbreviations: P, painful stimulation; PA, placebo analgesic stimulation; PAG, periaqueductal gray; Thal, thalamus; P-Ins, posterior insula; dACC, dorsal anterior cingulate cortex, dlPFC, dorsolateral prefrontal cortex.

Table 3-1. Group-level ROI Peak MNI Coordinates

	Right Hemisphere				Left Hemisphere			
	X	*y*	*z*	Peak t	*x*	*y*	*Z*	Peak t
PAG	6	-28	-19	6.13	6	-28	-19	6.13
Thal	15	-13	5	6.91	-15	-19	-2	6.99
P-Ins	45	-19	17	6.21	-45	-25	14	10.08
dACC	9	11	41	5.86	-9	8	44	8.54
dlPFC	42	35	26	5.96	-39	29	26	6.08

Note: All regional activations are significant at $p \leq 0.05$ FWE. Abbreviations: PAG, periaqueductal gray; Thal, thalamus; P-Ins, posterior insula; dACC, dorsal anterior cingulate cortex, dlPFC, dorsolateral prefrontal cortex.

Table 3-2. Bayesian Model Selection Results

	Right Hemisphere				Left Hemisphere			
	M1	M2	M3	M4	M1	M2	M3	M4
Posterior Probability	0.06	0.06	0.28	0.59	0.06	0.07	0.23	0.63
Exceedance Probability	0.00	0.00	0.08	0.92	0.00	0.00	0.04	0.96

Table 3-3. Experimental Input Parameter Estimate Means and Standard Deviations

Input Region	Right Hemisphere Mean (SD)	t	Left Hemisphere Mean (SD)	t
PAG	0.18(0.10)	6.10*	0.17(0.11)	5.24*
Thal	0.19(0.07)	9.68*	0.14(0.10)	5.24*

*p≤0.05, Bonferroni corrected. Abbreviations: PAG, periaqueductal gray; Thal, thalamus.

Table 3-4. Endogenous Connection Parameter Estimate Means and Standard Deviations

	Right Hemisphere		Left Hemisphere	
Parameter	Mean (SD)	t	Mean (SD)	t
PAG→Thal	0.29(0.59)	1.76	0.12(0.61)	0.70
Thal→P-Ins	0.42(0.28)	5.28*	0.36(0.22)	5.93*
Thal→dACC	0.59(0.23)	9.38*	0.46(0.22)	7.97*
P-Ins→Thal	0.14(0.23)	2.20	0.08(0.13)	2.24
P-Ins→dACC	0.45(0.37)	4.36*	0.26(0.28)	3.36
dACC→Thal	-0.19(0.24)	-2.85	-0.15(0.19)	-2.85
dACC→dlPFC	0.37(0.13)	10.10*	0.32(0.30)	3.81*
dlPFC→PAG	-0.18(0.25)	-2.51	0.03(0.30)	0.30
dlPFC→dACC	0.10(0.20)	1.74	-0.03(0.10)	-0.93

*p≤0.05, Bonferroni corrected. Abbreviations: PAG, periaqueductal gray; Thal, thalamus; P-Ins, posterior insula; dACC, dorsal anterior cingulate cortex, dlPFC, dorsolateral prefrontal cortex.

Table 3-5. Modulatory Parameter Estimate Means and Standard Deviations

	Right Hemisphere		Left Hemisphere	
Modulated Parameter	Mean (SD)	t	Mean (SD)	t
dACC→Thal	-0.60(0.99)	-2.21	-0.96(1.17)	-2.97*
dlPFC→PAG	-1.59(1.98)	-2.90*	-0.65(1.65)	-1.41
dlPFC→dACC	1.09(1.70)	2.31	1.10(1.13)	3.52*

*p≤0.05, Bonferroni corrected. Abbreviations: PAG, periaqueductal gray; Thal, thalamus; dACC, dorsal anterior cingulate cortex, dlPFC, dorsolateral prefrontal cortex.

CHAPTER 4
DISCUSSION

Placebo analgesia has been shown to alter neural activity of brain regions involved in the processing and modulation of pain as well as the effective connectivity among these regions.[10, 32] The present study examined the effects of rapid, random succession of painful stimuli applied to unconditioned and placebo conditioned sites of the foot on: 1) overall brain activation via RFX-GLM and 2) inter-regional connectivity via DCM. The results showed that although no significant differences in neural activation between conditions were identified, PA was associated with significant modulatory effects on the strength of connectivity parameters among regions associated with the descending modulation of pain.

Placebo Effect in the Absence of RFX-GLM Differences

Previous studies of placebo analgesia found decreased activation in pain-related brain regions and increased activity in regions associated with the modulation of pain.[23, 34, 52] The present study, however, did not identify RFX-GLM differences in BOLD activation between conditions. Our results suggest that sole reliance on the RFX-GLM may prevent the observation of subtle changes in BOLD activation during PA in certain experimental contexts. Nonetheless, a clear explanation for the disparity between our findings and previous PA research is needed.

One possible explanation is the random and rapid changes of experimental condition utilized in the present study. Earlier studies of placebo analgesia utilized both greater stimuli durations and longer inter-stimulus intervals. For example, comparisons in BOLD activity between pain-processing and PA made in Wager and colleagues[52] were based upon 15 single-condition blocks lasting 30 seconds each while Price, et al.[34]

compared data collected in different fMRI scanning sessions. Another explanation is that RFX-GLM may not be sufficiently sensitive to detect changes in our design given the relatively slowness of the HRF compared to precipitating neural events. Also, the short stimulation intervals and ISI used in the present study may have allowed for blurring of condition-specific responses during GLM response convolution.

It is additionally possible that within group differences in placebo response prevented RFX-GLM from identifying condition-related differences in BOLD activation. A study by Eisenbruch and colleagues[13] found significant differences between placebo responders and nonresponders during stimulus anticipation, pain experience, and in neural correlations with BOLD activity. To investigate whether similar differences exist in our sample, an independent samples t-test was performed to determine whether the size placebo response impacted neural activity. Placebo responders and nonresponders were identified by a median split in VAS ratings. No significant differences in BOLD activation were found between placebo responders and nonresponders. This suggests that differences in placebo response did not impact RFX-GLM results.

Modulation of Effective Connectivity Due to PA

The results of the present study support a model of neural activity that elucidates the neural underpinnings of placebo analgesia. BMS clearly identified the same model of pain-related neural activity in five regions (PAG, thalamus, posterior insula, dACC, dlPFC) and the modulation of specific descending connections associated with PA. Consistent with current models of ascending pain pathways, the endogenous inputs of the baseline condition to the PAG and thalamus were highly significant.[7,30,31] Although the winning model contained both ascending and descending endogenous connections,

the significance of only ascending endogenous parameter estimates may reflect the relative inactivity of the descending pathways during the baseline, painful condition. This is contrasted by the significant modulatory effect observed in both hemispheres during PA. It can be postulated that only during the experience of PA do these connections become active in the modulation of the individual's pain experience.

Our results additionally suggest PA is associated with unique neural modulation in each hemisphere. Both the dlPFC and dACC have shown to affect the release of endogenous opioids in the modulation of pain[25,53] and the results of the present study provide additional support for the influence of prefrontal processes in lower-level pain modulation, however, differently in each hemisphere. The modulation of the dlPFC→PAG connection in the right hemisphere is suggestive of the involvement of attentional or expectation-related processes. This pathway has also been implicated to involved modulation of pain through the RVM to the dorsal horn.[30] The importance of the dACC, reflected in significant dlPFC→dACC and dACC→Thal modulatory parameter estimates, is suggestive of error detection and evaluative processes such as expectation-effectiveness comparisons.[25] Specifically, Pecina and colleagues[25] demonstrated endogenous opioid release in the dACC mediated placebo effectiveness while dlPFC involvement was only linked to expectation. Additionally, these hemispheric differences suggest a need for future studies to further investigate the laterality of pain modulation.

Sensitivity of DCM to Subtle, Neural Changes

The powerful modulatory effects of PA identified by our DCM findings have a number of implications for the understanding of endogenous pain processes. The elucidation of PA-related modulation by DCM but not the conventional RFX-GLM

suggests that pain-modulation requires network-level investigation. Other processes in addition to placebo analgesia, such as language deficits in the context of aphasia, [41] were previously identified as effecting changes network interactions in the absence of GLM activation differences. As DCM offers the unique advantage of modeling effective connectivity on the scale of neuronal rather than hemodynamic interactions,[16] it has the power to identify processes at the neuronal level that are not discernable purely from hemodynamic comparisons.[19] Based upon our findings, methods such as DCM offer a more robust understanding of pain modulation than conventional techniques.

Strengths and Limitations

As far as we are aware, this is the first study to examine changes in effective connectivity due to PA with DCM. Although other effective connectivity approaches were used to study PA and pain modulation, [10] the rapid succession of pain and placebo site stimulation used in study allowed insight into the subtle nature of descending modulatory network activation. To our knowledge, this is the first study of PA to suggest that changes in pain-related effective connectivity rather than or in the absence of regional hypoactivation may explain placebo effects. The results of the present study provide valuable insight into PA-related neural processes in healthy individuals. As prior studies have implicated different pain modulatory functioning in individuals with chronic pain,[7, 11] future studies are encouraged to examine the impact of chronic pain conditions on the processes illuminated by this study.

Limitations present in this study are also important to note. Although DCM was able to successfully identify neural modulatory parameters active in the experience of PA, the stimulus and inter-stimulus intervals may have been too brief to disambiguate condition-related changes in BOLD activity via RFX-GLM. To address the sensitivity of

pain- and placebo-related processing differences, future studies could investigate the impact of paradigm timing-related manipulations on condition-related BOLD activation differences and underlying effective connectivity.

A second limitation of the study is the relatively small number of subjects who met DCM inclusion criteria. These criteria required supra-threshold activation in all ROIs in at least two sessions. The failure of the majority of subjects (56%) to meet these criteria could have multiple explanations. One possibility is that data collected in one fMRI scan did not have enough power to identify significant pain-related activations as many of our previous studies have utilized data concatenated across multiple sessions.[9, 33] This procedure adds statistical power, however, is contraindicated in SPM analyses due to between-session signal bleed-over. Another possibility is that the included subjects exhibited more robust paradigm-related activations. However, a follow-up, independent samples t-test revealed no significant differences in BOLD activation in response to experimental stimuli between those included and excluded from DCM analysis. Alternatively, The 17 excluded subjects may have exhibited high within session variability such that consistency of activation as required for DCM inclusion was not obtained, thus questioning the reliability of within subject activations. There are numerous factors that can strongly influence single subject fMRI reliability;[20] we recommended that future studies investigate the impact of these factors on the study of pain and its modulation. Nonetheless, the results of the 13 subjects entered into DCM analyses proved to be significant and theoretically informative.

Conclusion

In conclusion, our results support evidence of the involvement of afferent inhibition, possibly due to endogenous opioid release, in pain-modulatory neural

systems due to PA. Additionally, these modulations were only visible when observed on the level of inter-regional, neural interactions, suggesting that changes in effective connectivity between regions rather than differences in magnitude of activation are fundamental to the neural underpinnings of the placebo response.

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BIOGRAPHICAL SKETCH

Landrew Sevel was born and raised in Columbus, Ohio. Before beginning graduate studies in the University of Florida's clinical and health psychology doctoral program, he graduated Summa cum Laude from Bowling Green State University (BGSU), where he majored in music performance and psychology. At BGSU, he conducted research in the Mindful Behavior Therapies and Psychophysiology Lab. Landrew's research interests predominantly include investigating dynamic neural correlates of cognitive, pain-modulatory processes. His future, career aspirations include employment in an academic hospital setting, allowing for dedication to clinical practice and neuroimaging research activities.