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# Neural processes underpinning pain perception: genetic, temporal, and behavioral factors

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Cover illustration: My resting-state functional connectivity. Colors as in Study I

# Neural processes underpinning pain perception: genetic, temporal, and behavioral factors

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

**Gránit Kastrati**

The thesis will be defended in public at Karolinska Institutet, 13th of May

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To my beloved mother

POPULÄRVETENSKAPLIG SAMMANFATTNING

Smärta är en subjektiv upplevelse som i grunden är adaptiv då den varnar oss om faror i vår omgivning. Hur hjärnan bearbetar signaler som sedan upplevs som smärta är förnärvarande inte helt kända. Vi har kunskap om vilka områden i hjärnan som är involverade, men inte hur deras interaktion ger upphov till smärta. För att öka vår förståelse för de mekanismer som ligger till grund för smärta är det relevant att förstå vad som bidrar till att den varierar – exempelvis kan människor skilja sig åt i hur de upplever smärta. En sådan källa till variation är vårt genetiska bidrag till hjärnfunktion. Det här kan studeras genom en klassisk tvillingdesign som låter oss undersöka hur mycket av variationen i hjärnaktivitet som kan förklaras av genetik. En annan källa till variation är de fluktuationer som sker under en smärtupplevelse – alltså hur hjärnaktivitet varierar över tid. Målet med det här projektet var att undersöka ärftlighet i neurala processer vid förväntningar och bearbetning av smärtsamma stimuli, samt att förstå hur nätverk i hjärnan interagerar över tid under en smärtupplevelse.

Resultaten visade på ärftlighet i centrala områden i smärtbearbetning och även i konnektivitetsmönster, som tyder på ärftlighet i integrering över multipla nätverk. Heritabilitet påvisades även under rädsloinlärning – det vill säga på hjärnaktivitet som föregår smärta - i områden som anses vara centrala både för rädsla och smärta, inklusive områden i hjärnans smärtreglerande system. Vidare visar resultaten hur nätverk integrerar sinsemellan över tid vid experimentell smärta. Resultaten visar även att det är viktigt att följa hjärnaktivitet med hög temporal upplösning. De här resultaten kan komma att användas för att utveckla biomarkörer samt för att förstå varför vissa har större risk att utveckla långvarig smärta.

ABSTRACT

Pain is an alarm system – warning us of dangers in the environment – yet becomes problematic when it transitions into chronic pain. It is defined, according to the International Association of Pain as “An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage”. In advancing our knowledge of the underlying mechanisms of acute pain, it is relevant to understand sources of variability in pain perception. One such source is the genetic influence on brain function. This can be studied using a classic twin design to infer the proportion of variance in brain activation attributed to genetics. Another source of variation pertains to the temporal fluctuations in brain activity that could track pain processing. This was studied here using time-varying functional connectivity. Furthermore, since pain arises through large-scale interactions in the brain – the purpose here is to study pain and related processes through network neuroscience. Specifically, how functionally specialized – or segregated – neural structures of the brain integrate to shape pain.

List of scientific papers

**I.** **Kastrati, G**., Rosén, J., Thompson, W.H., Chen, X., Larsson, H., Nichols, T.E., Tracey, I., Fransson, P., Åhs, F., & Jensen, K.B. (2022). Genetic Influence on Nociceptive Processing in the Human Brain-A Twin Study. *Cerebral Cortex* **32**(2): 266-274.

**II.** **Kastrati, G**., Thompson, W.H., Schiffler, B., Fransson, P., & Jensen K.B. (2022). Brain Network Segregation and Integration during Painful Thermal Stimulation. *Cerebral Cortex.* bhab464.

**III.** **Kastrati G**, Lagerbäck T, Thompson, et al. Brain networks, back morphology and gait 14 years after surgery for disc herniation in adolescence. *In Manuscript*.

**IV.** **Kastrati, G**., Rosén, J., Fredrikson, M., Chen, X., Kuja-Halkola, R., Larsson, H., Jensen, K.B., & Åhs, F. (2022). Genetic influences on central and peripheral nervous system activity during fear conditioning. *Translational Psychiatry.* **12**(1): 95.

**CONTENTS**

LIST OF ABBREVIATIONS

APACE – Accelerated Permutation Inference for the ACE model
BOLD – Blood-Oxygen-Level-Dependent
DMN – Default-Mode Network
FC – Functional Connectivity
fMRI – functional Magnetic Resonance Imaging
GLM – General Linear Model
IASP – International Association for the Study of Pain
I/S – Integration and Segregation
MZ & DZ – Monozygotic and dizygotic
NPS – Neurologic Pain Signature
PAG - Periaqueductal Grey
PCC/MCC/ACC – Posterior/Mid/Anterior Cingulate Cortex
RPN – Resting-state Pain susceptibility Network
SCR – Skin Conductance Responses
SID – Segregation and Integration Difference
SPM – Statistical Parametric Mapping
TVFC – Time-Varying Functional Connectivity

# 1. INTRODUCTION

Pain is a phenomenal experience that occurs against a backdrop of a complex cognitive, social, sensory, and emotional landscape. The neuroimaging community is well underway in understanding the principles by which the brain processes information about the world – including the body – and what might have gone astray when pain simply won't go away. This thesis attempts to increase our understanding of how distinct specialized brain regions integrate to give rise to pain. Therefore, the terms integration and segregation (abbreviated as I/S) will be repeated throughout the thesis to stress the fundamental organizational nature of the brain in generating complex experiences such as pain. This thesis begins with the definition of pain and related notions. It then introduces the neuroimaging technique that underlies the thesis and how to measure integration in the brain through functional connectivity (FC). After introducing fundamental concepts, the thesis describes advances in pain neuroimaging in terms of brain networks before considering fluctuations in FC. Then follows a description of the derivation of genetic influences on brain activation and FC.

# 2. LITERATURE REVIEW

## *2.1 What is pain?*

Pain is defined by the International Association for the Study of Pain (IASP) as: "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage." The definition emphasizes the inherent subjectivity of pain by using the term experience. There is also a multidimensional aspect to pain as it is part sensory and part emotional. This multidimensionality means that pain informs the brain on a large scale.

Acute pain is adaptive (for instance, it allows us to protect the integrity of our bodies) yet has adverse effects when it becomes persistent. Someone can be said to have chronic pain if it is present over 50% of the time over six months or when it persists for three months. There is evidence that neural pain processing differs between acute and chronic pain (*1*). Pain can be studied by experimentally inducing pain or tracking spontaneous pain in the case of chronic pain. Though the neuroimaging field may one day be able to say objectively whether one is in pain or not – currently, the assessment of the intensity of the experienced pain is given by subjective ratings. Here, **Studies I-II** and **IV** included experimental pain in healthy participants. The participants in **Study III** had been surgically operated on for disc herniation on average 14 years ago, and some reported lingering pain.

Finally, pain is distinct from nociception, which according to IASP, is defined as the neural encoding of noxious stimuli. This may involve autonomic processes or behavioral expressions but does not imply a subjective perception of pain. **Study I** investigated nociception since no subjective reports of pain were collected, while **Study II** examined subjective pain. **Study III** used a task-free design (described below), while **Study IV** investigated anticipatory effects in a fear conditioning paradigm.

## *2.2. An overview of neuroimaging*

Investigations of the neural processes underlying pain involve many methods that provide different perspectives on the brain. This thesis used functional Magnetic Resonance Imaging (fMRI) to derive brain signals related to neuronal activity. This section begins with a description of the signals derived with fMRI and the methods for studying the brain's functional organization. Then follows a description of some of the discoveries that have been made in cognitive neuroscience.

### *2.2.1. fMRI*

Brain function can be studied by way of blood-oxygen-level-dependent (BOLD) signals using fMRI (*2, 3*) (**Figure 1**). The term brain activation refers to locations representing increased/decreased neural activation. In network neuroscience, one can relate BOLD signals from distinct brain regions to build a network of pairwise associations reflecting the degree of integration in the brain. This latter approach

aims to understand the brain's functional organization that generate cognition, emotion, perception, and action.

The BOLD signal has advantages and disadvantages. It is an indirect measure of neuronal activity based on the relative difference in oxygenated and deoxygenated hemoglobin between active and inactive neurons and their difference in magnetic susceptibility. While neurons signal within milliseconds, the cerebral blood flow, or the hemodynamic response, is sluggish, with a peak after a few seconds following the activation of neurons. The relation between neuronal activity and hemodynamic activity is well-established (*4*). Thus, the difference between two conditions can be interpreted as a difference in neuronal activity (*5*). fMRI is slow compared to other techniques for measuring brain function. Other techniques that rely on electromagnetic properties of the brain, such as electroencephalography and magnetoencephalography, are faster and more direct measures of neural activity, sensitive to fluctuations on the order of milliseconds. On the other hand, fMRI is a whole-brain method that can capture signals at a higher spatial resolution. However, the sluggishness of the BOLD signal imposes a limit on the temporal resolution of fMRI. Despite these limits, fMRI has significantly advanced our understanding of the brain (*5*).

*2.2.2. FC: measuring functional integration*

The BOLD signal tells us about relative increases and decreases in activation across the brain during an fMRI session. FC refers to the pairwise interrelations between these time series, allowing for investigating the degree of integration between spatially remote brain regions (*6*). Brain regions can be defined in several ways; as individual voxels, by grey matter boundaries, or through resting-state FC profiles (*7-9*) (**Figure 1**). The definitions used in this thesis are described under Methodological Considerations.

BOLD signals extracted from brain regions can be used to form relational structures or networks that can be investigated with methods from network science (Figure 1) (*10*). Two signals are related by some function that captures their similarity or distance, with the most common similarity measure being the Pearson correlation, although there are several other measures (*11*). FC does not imply that the brain regions have direct structural connectivity. Instead, it can be seen as a way to assess the degree of correlation between two brain regions, ultimately interpreted as neural communication (*12*) or as functional integration (*6*).

The network of interconnectivity among regions across the brain is often called the functional connectome – the functional network architecture underpinning cognition, perception, emotion, and action (*13*). Studies in FC (and structural connectivity) reveal that the brain is organized into a hierarchy of smaller networks or modules - tightly connected groups of brain regions (*14-17*). The smaller networks integrate information within the network, for instance within the visual network, forming functionally specialized or segregated networks (*18*). Integration between networks in turn support complex functions (*17-19*).

In neuroimaging, a distinction is made between resting-state and task-evoked FC (*14*). With brain activity measured when volunteers lay idle in the scanner, called resting-state fMRI, it is possible to measure the brains' baseline architecture (spontaneous fluctuations). However, this baseline is poorly understood from a psychological perspective (*20*). Nevertheless, FC measured from task-based fMRI reveals task-evoked changes from the baseline intrinsic networks. This could mean that task-evoked reconfigurations of brain networks use the brains' baseline architecture as a point of departure. However, there could also be interactions between evoked and baseline activity (*20*). **Studies I, II,** and **IV** use task-based fMRI, while **Study III** used resting-state fMRI. Furthermore, the functional brain architecture revealed through FC can be seen as dynamics around the structural connectome (*18*). In other words, the structural connectome constrains FC. Compared to resting-state FC, task-related reconfigurations are relatively small (*14*). Our brains already have a great capacity to make small but significant changes to meet task demands.

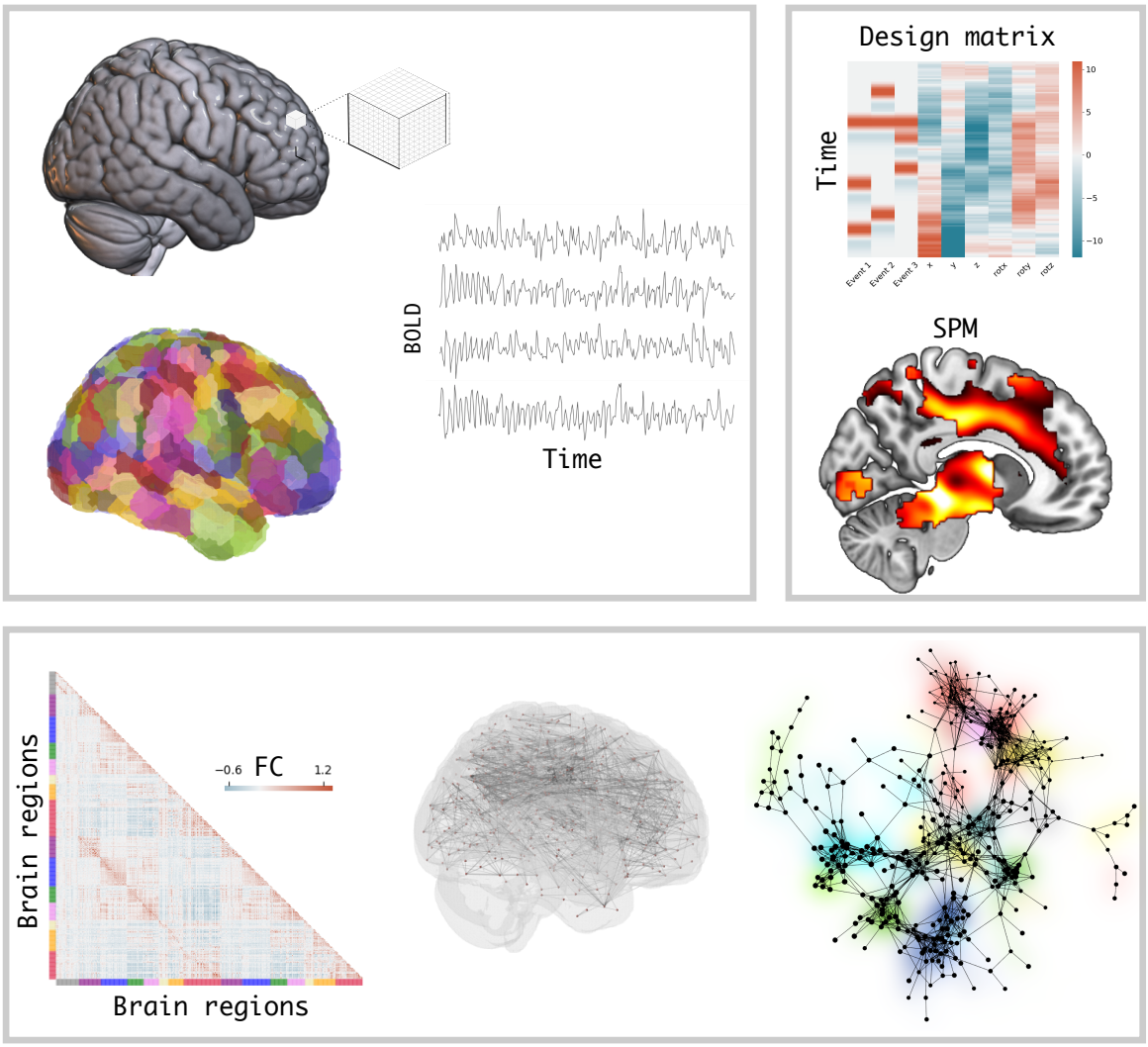


**Figure 1. From brain activation to brain networks.** *Top left frame*: In raw form, blood-oxygenation-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) signals are represented as voxels (3D grid) (top) or averaged across voxels within brain regions as specified with a brain atlas. *Top right frame*: For statistical parametric mapping (SPM), a general linear model (GLM) is specified with a design matrix. The design matrix columns can specify timings of events of interest (such as pain stimuli), convolved with a hemodynamic response function. Additional columns can specify motion artifacts such as head motion along the x,y and z direction and rotations in each direction. The design matrix is regressed against the BOLD signal at each voxel. The result of the GLM is a group-level statistical map of task-induced brain activation. *Bottom frame*: Three network representations showing the relational structure of brain function in terms of functional connectivity (FC). The pairwise correlation between all brain regions from the Schaefer brain atlas (*8*) is shown here in matrix representation (*left*), overlayed on a brain (*middle*) and as a graph (*right*). Notice in the matrix representation that only the lower triangular is shown since correlation is symmetric. The coloring scheme in the matrix and the graph representations show the network to which the brain regions belong. The Schaefer brain atlas and FC-brain overlay were plotted with netplotbrain (*21*). The 3D brain rendering was plotted with MRIcroGL (*22*). The code for the graph plot can be found at https://www.brainnetworkslab.com/coderesources.

Why is FC important? FC could be used to support different types of biomarkers, such as (i) response biomarkers to track the effects of a drug, (ii) predictive biomarkers to help in predicting treatment outcomes for a single patient, (iii) or as a prognostic biomarker to determine the risk of future pain development (*23*). What is the explanatory value of FC? Researchers have argued that FC is not a *mechanism* but can provide rough mechanistic models or approximations for large-scale interactions in the brain (*24, 25*). The rough model sketches provided by FC pathways of the brain can further offer constraints to models of causal pathways estimated with effective connectivity (*26*). This can explain how interactions at the macro-scale support neurocognitive processes such as sustained pain. Its utility,

therefore, extends beyond biomarkers to provide explanations for fundamental principles of brain function.

### *2.3. Brain networks in pain*

A network perspective in neuroscience has been around for a long time, saying that functions of the mind are supported by the interaction of elements of the brain. In the late 20th century, Melzack introduced the concept of the neuromatrix to understand pain (*27*). The neuromatrix refers to the dynamic integration between various functionally specialized brain networks. Other essential components of the neuromatrix perspective are that the structural and functional organization of the brain in large part is genetically constrained and experientially malleable.

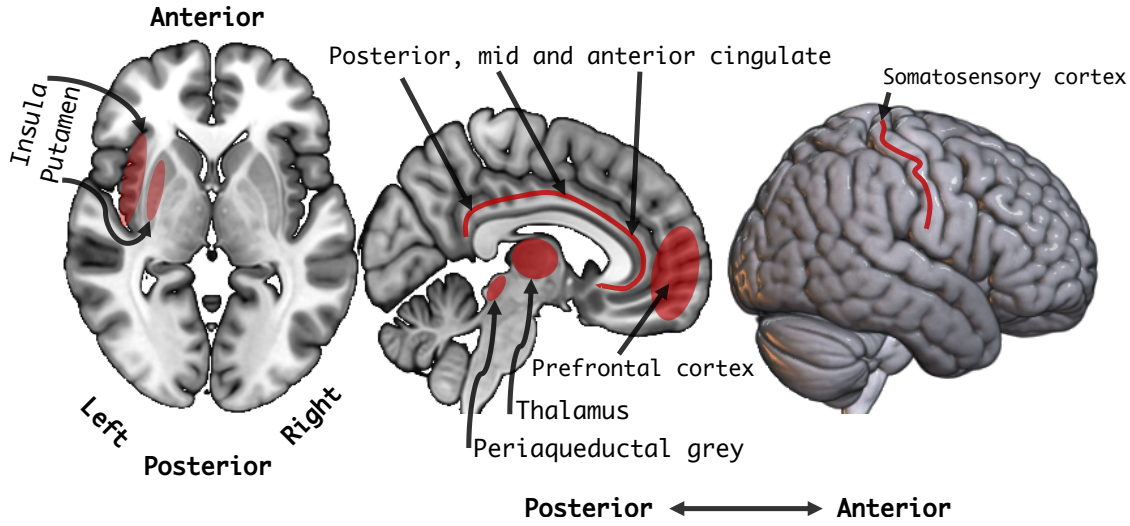


**Figure 2. A schematic of the neurobiological architecture of pain**. The figure highlights anatomical structures that often appear in the pain neuroimaging literature (*28*).

Now, what is the network structure supporting pain? We have some knowledge about the brain regions involved in processing pain (*28, 29*) (**Figure 2**). However, it can be noted that several factors complicate our understanding of pain:

(i) Individual variability: How come different individuals experience pain differently?
(ii) Temporal variability: What are the mechanisms supporting variation in the experience of pain over time?
(iii) Brain-body relationship: What is the relation between activity within the body and the brain?

Is there a single network in the brain that is responsible for pain? Pain is defined in terms of a sensory and an emotional component, according to IASP, and it is distinct from nociception - the neural encoding of noxious stimuli. Furthermore, as mentioned above, the integrated nature of the brain allows for reciprocal influence between cognition and pain perception. For example, there is an interaction between pain and the anticipation of pain, described further in section 2.6. This implies multiple interacting networks for nociception, pain, and cognitive processing of pain (*30*). Furthermore, the anatomical regions depicted in **Figure 2** can be seen as single computing units. For instance, the insular cortex could be represented by a single BOLD signal representing what is going on in the whole structure. However, brain regions are networks themselves integrating information from their substructures (*18*). For example, the insular cortex can be further decomposed along the posterior-anterior axis into the posterior, mid, and anterior insular cortex. These substructures have distinct information-processing roles and have different FC profiles (*31*).

### *2.4. The use of machine learning to find objective markers of pain*

As defined by IASP, pain is a subjective experience. Therefore, subjective pain ratings are the gold standard for assessing pain. There are, however, efforts to define objective markers of pain using machine learning (*32-34*). Machine learning can associate patterns of voxel-wise activations to pain behaviors. This allows for predicting, for example, pain ratings from pain-evoked fMRI data (*35*) or even predicting pain sensitivity from resting-state fMRI data (*36*). The studies are not the final word on the matter, but they can help define the brain regions and the interactions among them that support pain-related phenotypes. In **Study III**, a machine learning-derived pain sensitivity index was used based on patterns of resting-state FC (*36*). **Study I** used the Neurologic Pain Signature (NPS), predicting subjective pain intensity (*35*) as an a priori defined area within which data was analyzed to focus on pain-relevant brain regions and to avoid circular analysis (*37*).

### *2.5. Brain network integration and segregation*

The brain is a dynamic network composed of functionally specialized modules or subnetworks that integrate amongst themselves to support complex functions (*13, 17, 38-41*). Functional specialization refers to neural subnetworks in the brain that are specialized to some domain, such as vision, yet are multipurpose by supporting various neurocognitive functions. Functionally specialized networks can be formed by brain regions that are densely connected amongst themselves and sparsely connected with regions that belong to other networks (*42*). It is well recognized that I/S supports many functions of the brain (*18, 19*), including pain (*43*), as specialized structures in the brain (segregation) inform each other (integration). It is possible that during a task-free state (resting-state) the brain is more modular. On the other hand, there is more integration between networks (or modules) as the brain reconfigures or integrates functionally differentiated brain regions to support tasks engagement (*14, 18*). For instance, in a comparison between innocuous somatosensory stimuli and noxious stimuli, the pattern of FC becomes less modular and more integrated with noxious stimuli (*44*). This finding follows a study that showed that cognitively more demanding tasks are less modular (more integrated) than a simple motor task as well as resting-state (*45*). In addition to integrating spatially distributed brain regions, the brain also requires that the communication between brain regions are orchestrated over time (*17*). The brain in time will be discussed in section 2.8.

### *2.6. Balancing the internal and the external world*

As we actively engage in the world around us, a set of brain regions forming a network - called the salience network - display increased activation. The salience network is anchored in the anterior insula and anterior cingulate cortex (ACC) (*46-48*), two regions that respond to nociceptive input and fear (*49*). The exact functioning of the salience network is unknown, yet it might be responsive to salient features of the world, including the body (*36*). Another network similar to the salience network in its spatial layout is the cingulo-opercular network linked to task-control (*50*). The salience network could also be involved in task control by switching between tasks following changes in saliency (*48*). Another network called the default-mode network (DMN) (*51, 52*) was independently shown by Fransson (*53*) and Fox et al. (*54*) to be anticorrelated to the salience network, such that, somewhat simplified, when activity in the one goes up, the activity in the other goes down. While the salience network activates when external events demand our attention, the DMN show increases intra-network connectivity during spontaneous, internally directed thought processes (*51, 52*). The interplay between the two networks could be interpreted as alternations between spontaneous thoughts and mind-wandering to moments of externally-focused attention (*54*).

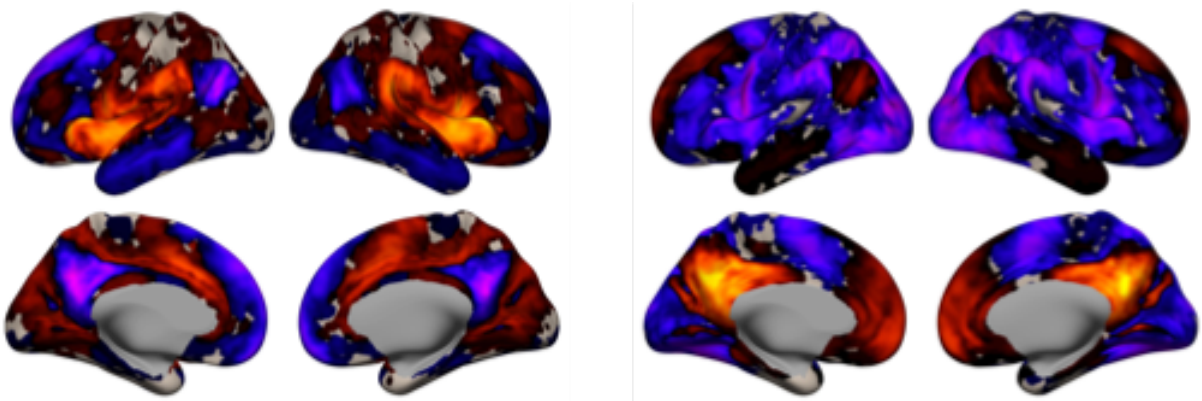

**Figure 3. Group-level seed-to-voxel resting-state FC**. Brain maps of FC with seeds/sources at the right insular cortex (left frame) and the posterior cingulate cortex (PCC) (right frame) correlated with all the voxels of the rest of the brain. The left and right maps resemble the salience network and DMN, as commonly depicted in the literature. Notice also how the maps are anticorrelated. The figure is generated using data from **Study III**.

Why is the DMN and the salience network important in pain neuroimaging? The salience network includes anatomical regions often associated with pain, namely the insula and the anterior cingulate. Yet, it seems to support functions other than pain. For instance, it has been hypothesized to be a defensive system independent of the type of threat (*55, 56*). Furthermore, as the name suggests, the salience network could process salient features in the environment (*46, 47*), and nociceptive stimuli would be a particularly salient feature (*56*). Moreover, brain activity changes within the DMN have been demonstrated in several chronic pain conditions, such as low back pain (53) and fibromyalgia (*57*). Often, altered connectivity is reported between the DMN and salience network in chronic pain (*58, 59*). However, DMN is associated with several neurological and psychiatric conditions (*60*). The psychological interpretation of the DMN is that it is involved in remembering and planning, in self-referential processes (*51*). However, it is unclear what the connection between DMN and salience network during pain (or in chronic pain) means – if it is an alteration in any of these processes, or alternatively merely reflecting the presence of current pain (*57*). The result in **Figure 3** was formed by correlating the right insular cortex with the rest of the brain (left frame). It shows correlation (FC) in the insula, and MCC/ACC, among other regions. The left frame similarly shows the correlation between the posterior cingulate (a major hub in the DMN) and the rest of the brain.

Overall, the distributive nature of the brain means that it can utilize the same networks to support multiple functions (*61*). Such general mechanisms can confound images of brain activation for pain. The most eloquent example was a study with concurrent measurement of brain activity and painful stimuli in patients incapable of feeling pain due to congenital genetic disease, where patients displayed similar activation to healthy participants (*62*). Pain, therefore, could share some of its neural processes with other processes, such as general threat detection (*56*).

### *2.7. Anticipating pain*

Expectations during the time leading up to a painful stimulus involve neurobiological mechanisms tightly linked to those involved in the pain perception itself (*63*). For example, predicted threat increases activation of MCC, and influences its FC with the insula (*64*). Expectations or anticipation are modeled in fear conditioning as cues coupled with aversive stimuli resulting in fear learning (*65*). There is an overlap of the brain regions involved in pain and fear conditioning as measured with fMRI (*66*). **Study IV** is an investigation of the genetic influence on brain activation during fear conditioning, the processes leading up to a noxious stimulus. The same dataset was used in **Study I** to study the genetic influence on nociceptive processing, during the delivery of noxious stimuli. Understanding the anticipation of pain in fear conditioning is important since fear of pain and avoidance is a problem in chronic pain (*67, 68*).

### *2.8. Time-varying functional connectivity*: *measuring fluctuations in FC*

FC describes possible communication pathways in the brain through the patterns of correlation between brain regions across all time points, representing "static" (time-averaged) connectivity. More recently, time-varying functional connectivity (TVFC) has taken place in neuroscience to estimate how correlations between brain regions vary as a function of time. One way to do this is to define a sliding window over the time series, computing a correlation measure at each step to produce a sequence of correlation matrices (**Figure 4**). The result can be used in downstream analyses with temporal network theory (*69, 70*) to investigate the dynamics of brain networks and their relation to pain (*71*).

An incentive of TVFC is that it could provide information over and above that offered by FC. When aggregated over several minutes, FC might reflect structural connectivity, at least partially (*18*). Much research has been conducted on the utility of TVFC. A growing literature shows how it can track fluctuations in cognition and emotion (*72*). It has been demonstrated that TVFC and FC capture different aspects of behavior (*73, 74*). Furthermore, fluctuations in FC are heritable, indicating that flexibility of connections can serve as an intermediate phenotype for behavior (*75*). Genetic influences on brain activation will be the subject of the next section and were the target in **Study I** and **IV**.

In pain neuroimaging, the variability of TVFC is related to behavioral measures and treatment outcomes. The variability of TVFC between brain regions of the antinociceptive network and DMN was associated with participants' tendency to mind-wander away from pain (*76*). FC between DMN and periaqueductal grey (PAG) also predicted pain modulation in chronic low-back pain (*77*). TVFC of the same coupling also indicated a reduction in pain following ketamine treatment (*78*). This shows that dynamics in the brain relate to pain behavior, as derived with BOLD fMRI. The time-resolved estimates can further track how brain regions group together at different time points into tight networks with more connectivity amongst themselves than to other brain regions (*42*). In one study, the insula and ACC grouped together (in a data-driven manner) during pain. Still, with the induction of an opioid analgesic, the ACC displayed flexibility in that it shifted allegiance to another set of brain regions (*79*). This shows the utility of TVFC in capturing principles of dynamic neuronal activity that can be used to track pain behavior.

Findings in TVFC extend investigations of the role of brain network I/S in supporting complex functions. As discussed above, brains alternate between I/S configurations, reflecting alternations in information processing with shifts in external demands (*80, 81*). Temporal profiles of I/S have been demonstrated in cognitively demanding working-memory task (*81*). The goal of **Study II** was to investigate I/S at different temporal scales during varying painful thermal stimulations.

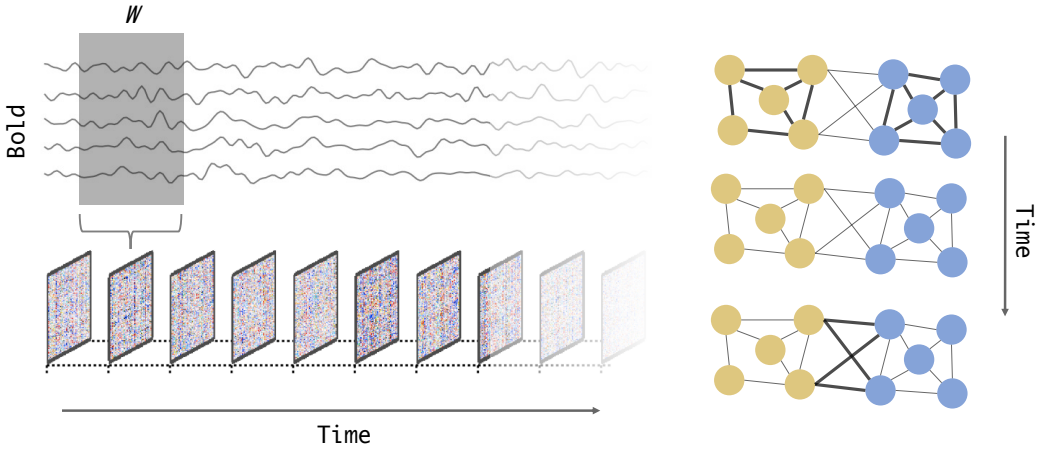


**Figure 4. Measuring time-varying functional connectivity**. *Left*: Pairwise correlation between BOLD time series within a window of size $\omega$ that is moved over the set of time series creates a sequence of correlation matrices. *Right*: Measures from temporal network science can be applied to the sequence to estimate, for example, the degree to which networks are more correlated within networks than between networks for each time point. The circles represent brain regions and the width of the lines or edges connecting the circle represent correlation or functional connectivity estimates. The network configurations change over time. The sequence of matrices (*bottom left*) was plotted with Teneto (*69*).

### *2.9. Genetic influence on nociceptive processing*

Melzack's neuromatrix theory of pain emphasized the interaction of brain networks in generating pain experiences. In addition to brain dynamics, the theory also considers the genetic and experiential factors shaping the brain (*27*). There are considerable individual variations in pain perception (*82*). We can be pain sensitive to a greater or lesser extent, and we can differ in how much we avoid or even seek pain. Individual variability such as this is influenced by genetics and experience, shaping the very neural structures and communication patterns of the brain. Furthermore, some individuals are more at risk of developing chronic pain due to genetic factors (*83, 84*). It is possible to infer the degree of genetic influence on pain-related phenotypes. However, instead of relating genes to pain behavior, which are complex multidimensional aspects of subjective experience, it might be simpler to link genes to neuroimaging-derived intermediate brain phenotypes or endophenotypes – mediators between genes and phenotypes (*85-87*). An endophenotype is a measurable component (such as brain activity) between a disease and the genotype (*85*). There is some evidence to support more consistency in gene-brain associations than in gene-behavior associations (*88*).

The classic twin design can be used to infer genetic influences. This is accomplished by considering the degree of correlation between identical twins (monozygotic or MZ) who share all their co-segregating alleles, and non-identical twins (dizygotic, DZ twins) that share half on average. Given that identical twins are more equal in their neural representation of pain than non-identical twins, the degree of genetic influence on the processes generating the neural representation can be inferred. **Figure 5** shows that the influence on the variance of pain-related brain phenotypes can be decomposed into genetic influence (or additive genetics) (A), common environmental influence (C), and non-shared environmental influence or error (E). Considering that A is a hundred percent in MZ twins and fifty percent on average in DZ twins, and with the C and E components equal among MZ and DZ twins, it is then possible to compute the genetic influence on a phenotype.

Neuroimaging studies have assessed the genetic influence on functional brain networks for resting-state brain connectivity and task-evoked FC (*89-96*). However, the genetic influence on brain activity in specific brain regions and FC during nociceptive processing has not been investigated before and was the aim of **Study I,** while **Study IV** explored the genetic influence on brain activation patterns related to fear learning in anticipation of a noxious stimulus.

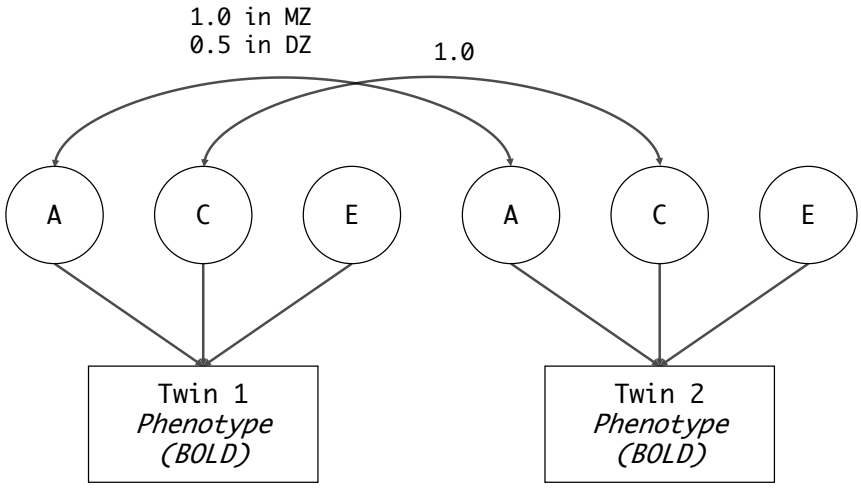


**Figure 5. Estimating the genetic influence on phenotypes**. The influence on a phenotype such as BOLD activation can be decomposed into additive genetics (A), shared environment (C) and unique environmental influences or error (E).

# 3. RESEARCH AIMS

The overall aim of this thesis was to use functional neuroimaging to study the genetic and neural underpinnings of pain both in healthy participants and those with a history of persistent pain. Specifically, the aim of each project was to,

(i) Assess the genetic influence on brain activation and networks associated with nociception
(ii) Characterize the temporal evolution of brain network I/S during pain
(iii) Assess the relationship between peripheral back morphology and brain networks
(iv) Assess the genetic influence on brain activation during fear learning

# 4. METHODOLOGICAL CONSIDERATIONS

The work in this thesis used resting-state and task-based fMRI to study processes involved in pain, nociception, and fear. Below, I describe the preprocessing steps and methods for deriving FC, TVFC, and genetic influence. A description of the participants can be found in **Table I**. An overview of the designs of the different studies can be found in **Table II**, and an overview of fMRI data acquisition and preprocessing steps in **Table III**.

***Table I. Overview of the cohorts***.

| | *Participants* | *N* | *Age* |
|---|---|---|---|
| *Study I, IV* | *Healthy twins* | *246 (148 women)* | *Mean 33.5, SD 10* |
| *Study II* | *Healthy* | *33 (22 women)* | *Mean 27.9, SD 9* |
| *Study III* | *Patient/control* | *23, 23 (12/12 women)* | *Surgery: Mean 31.3, range 29.8-32.9*<br>*Control: Mean 31.6, range 30.1-33.2* |

***Table II. Overview of experimental design***.

| | *(f)MRI data* | *Process* | *Stimuli/Condition* | *Domain* |
|---|---|---|---|---|
| *Study I* | *Task* | *Nociceptive processing* | *Electrical* | *Brain activation & FC* |
| *Study II* | *Task* | *Pain* | *Heat* | *TVFC* |
| *Study III* | *Rest* | *Spontaneous fluctuations* | *NA* | *Whole-brain FC* |
| *Study IV* | *Task* | *Fear learning, anticipation* | *Visual cues, electrical stimuli* | *Brain activation* |

## *4.1. Participants*

**Studies I** & **IV**: The studies included 305 healthy monozygotic (identical) and dizygotic (fraternal) twins, and the final sample included 246. For the final analyses, there were 56 monozygotic pairs (35 female and 21 male) with a mean age of 34 and a standard deviation of 8. There were 67 dizygotic twins (39 female and 28 male) with a mean age of 33 and a standard deviation of 11. Participants not included in the final analysis were removed due to excessive head motion, outliers in the amplitude of brain responses, missing data, or incomplete data collection from both twin pairs. Participants gave written informed consent by the Uppsala Ethical Review Board Guidelines.

**Study II:** The study included 33 healthy participants, with 22 females. The mean age was 27.9, with a standard deviation of 9. The data was downloaded from OpenNeuro, and the details of the participants can be found in the original research paper (*97*). Of the 33 participants, 25 were included in the analysis. The reason for excluding participants was excessive head motion or incomplete data collection. Participants gave informed consent. The study was approved by the Columbia University Institutional Review Board (*97*).

**Study III**: This case-control matched study included 46 participants (24 females). Twenty-three participants had undergone a surgical operation for disc herniation when they were 18 years old or younger (range 17.2 – 17.9 years). Then there were 23 age- and gender-matched controls. The cases were recruited mean 14 years after their surgery (range 8.6 – 20.4 years). Ethical approval was obtained from the Ethical Review Board in Stockholm, and the data was collected in May 2019 to January 2020. Participants gave written and oral consent.

### *4.2. MRI*

**Studies I, III**, **IV***:* Data was acquired using a 3.0 Tesla scanner (Discovery MR750, GE Healthcare) at Karolinska's MR center, Karolinska University Hospital, Stockholm, Sweden. The data in **Study II** was obtained from OpenNeuro (*98*). The data were acquired on a 3.0 Tesla Philips Achieva TX scanner at Columbia University's Program for Imaging in Cognitive Science. **Study I** & **IV** used SPM (Statistical Parametric Mapping) to preprocess the data. These were early studies, and the decision was made later to use fMRIPrep (*99*) for preprocessing, which was done in **Studies II** & **III**. Please refer to the original articles for details concerning specific preprocessing steps. Notice also that **Study III** included an additional analytical procedure that implemented a custom-made preprocessing of the data (described further below) (*36*).

### *4.3. Brain atlases*

The brain data captured in fMRI comes in a four-dimensional package for each participant. The first three dimensions represent the brain at a single time point, and the fourth dimension is time. Each data point is a voxel, a three-dimensional pixel, and a whole-brain image will contain tens of thousands of voxels. Computing the correlation between each voxel would therefore require heavy computational power. Although this is now possible using supercomputers (*100*), I opted for reducing spatial granularity using pre-defined brain atlases. **Studies I** & **II** used the Schaefer brain parcellation with 400 nodes (*8*) assigned to seven Yeo networks (*7*). **Study I** assessed the genetic influence on brain activation, and to restrict the analysis to brain regions known to activate during pain, I could have used activated areas from the study, but this would have resulted in a circular analysis (*37*). Therefore, the study used a binarized NPS (*35*) to constrain the analysis of the genetic contribution to brain activation in brain regions associated with pain. Similarly, **Study IV** used two networks delineated in a meta-analysis (*101*) of fear conditioning to constrain our analysis of the genetic influences on fear conditioning to specific regions a priori and hence reduce the number of multiple comparisons. Notice the similarity between the activation patterns seen in **Study IV** and the results from the meta-analysis.

**Study III** also used the Schafer brain parcellation with 400 brain regions and seven networks (*7, 8*). In addition to this parcellation, a subcortical brain atlas was included (*102*) with 32 brain regions. Additionally, a machine-learning-derived pattern predicting pain sensitivity from resting-state functional connectivity (RPN: resting-state pain susceptibility network) (*36*) was applied to the data, using a brain atlas of 122 brain regions. This analysis also involved custom-made preprocessing of data.

### *4.4. Nuisance regression*

In neuroimaging, the BOLD signal is confounded by in-scanner head motion and physiological effects such as cardiac and respiratory signal. It is essential to implement some form of nuisance regression to attenuate the impact of noise on the BOLD signals (*103*). There is yet no gold-standard way to do this. **Table III** summarizes the confounds that were regressed in each study. In **Study I**, the six motion parameters were included in the design matrix. In the FC part of the study, the six motion parameters and their quadratic effects were included. It was later decided, for **Studies II** & **III**, to additionally include the derivatives of the six motion parameters and their quadratic effects, resulting in 24 parameters (*104*). Additional confounders are signals from white matter (WM) and cerebrospinal fluid (CSF) used in **Study I**, that modestly reduce artifacts (*105*) or the first six principal components of the anatomical CompCor's (Component based noise Correction), which provides signals coming from non-neuronal sources such as WM and CSF (*106*) such as in **Studies II** & **III**.

A particular confounding variable that has sparked controversy is the global signal (GS) (*107*) since it is unclear what exactly is removed in global signal regression (GSR) and that it could be considered a nuisance term as well as that it could contain valuable information (*108*). The GS is the average of the BOLD time series across the whole brain. Studies comparing different approaches to mitigate the effects of head motion show that pipelines that include GSR are most effective in both FC and TVFC (*109, 110*). In **Study II**, I provided results with and without GSR for completeness, as results may vary

depending on whether one chooses to remove the signal or not. GSR was also performed in **Study III**. Additionally, using the GS together with the anatomical CompCor regressors is recommended (*111*).

One can test for a group difference in head motion to ensure that motion artifacts do not influence results. This was done in **Study III** by comparing mean framewise displacement (FD). One could also check the number of time points that exceed some threshold for FD and exclude participants that exceed the threshold for some percentage. This was done in **Study II** & **III**.

The influence of artifacts can be reduced by censoring time points (also known as scrubbing) (*105, 109*). This can be applied to both FC and TVFC. This was done in **Studies II** & **III**. The removed time points were replaced with values estimated with cubic spline interpolation, as done by others (*112*). One drawback of interpolation is that it gives the data synthetic characteristics (*105*). For **Study I**, I used ART-based outlier detection, as implemented in CONN (*113*) by adding additional covariates, with one covariate/regressor for each outlier scan, and then used regression to remove the influence of those scans. A setback is that scrubbing results in an unequal degree of freedom across subjects (*105*).

***Table III: Overview of fMRI data acquisition and preprocessing steps****. Abbreviations: 6aCompCor - the first six principal components of the anatomical CompCor; Friston24 - the six motion parameters, their derivatives and their square; GS - global signal; GS+ refers to global signal with its derivative and their square; FD - Framewise Displacement (FD); HRF - hemodynamic response function; CSF - Cerebrospinal fluid; WM - White matter; ART - Artifact Detection Tool; TR - Time to repetition.*

| | *TR* | *Preprocessing* | *Confound regression* | *Scrubbing* | *Brain atlas* |
|---|---|---|---|---|---|
| *Study I* | *2.4s* | *SPM* | *6 motion parameters (and their quadratic effects), CSF, WM* | *ART* | *Schaefer400_7nets, NPS* |
| *Study II* | *2s* | *fMRIPrep* | *Friston24, 6aCompCor, GS (+ derivative), FD, HRF* | *Cubic spline interpolation* | *Schaefer400_7nets* |
| *Study III* | *2.2s* | *fMRIPrep* | *Friston24, 6aCompCor, GS+,* | *Cubic spline interpolation* | *Schaefer400_7nets, Tian-Subcortical, Oxford-Harvard, RPN* |
| *Study IV* | *2.4s* | *SPM* | *6 motion parameters* | *NA* | *Fullana (101)* |

### *4.5. Assessing genetic contributions to brain activity patterns:* ***Study I, IV***

When computing the genetic influence on brain activation, the phenotypic variance, such as brain responses to nociception, can be decomposed into additive genetic variance, shared environment, and unique environmental variance or error, denoted A, C, and E, respectively (*114*). In the simple Falconer's formula, the factors can be estimated by contrasting MZ twin-pair correlations with DZ twin-pair correlations ($2(MZ_r - DZ_r)$). The A factor can be calculated since MZ twins are genetically identical while DZ twins share half of their co-segregating alleles on average. Contributions of non-shared environment refer to factors that make twins dissimilar. The genetic influence is then estimated as the proportion of phenotypic variance explained by additive genetic effects A and the statistic is usually denoted $h^2$. In **Study I**, the genetic influence on brain activation during nociception was estimated within brain regions defined by the NPS (*35*). The genetic influence on whole-brain FC in response to nociception was assessed with brain regions and networks defined according to the Schaefer atlas (*8*) and Yeo network (*7*). In **Study IV**, the genetic influence on brain activity during fear/safety learning (described further below) was computed within brain regions defined from a previous meta-analysis (*101*). MZ and DZ twins are assumed to share environmental influence (C) to an equal extent. Indeed, C could be higher between MZ twins than in DZ twins. Therefore, the correlation in behavior between the former can be larger than assumed. However, it has been shown that the greater C between MZ twins does not significantly affect estimates of genetic influence (*115*).

A classical fear conditioning paradigm was used for **Study I** and **Study II**. Two virtual characters served as cues (CS), and one cue (CS+) was associated with a brief electrical shock (US) to the left arm with an onset immediately after the offset of the CS. The US was given in eight out of sixteen trials. Another cue (CS-) was never associated with the US. To model fear conditioning (**Study IV**), the brain response to the threat cue (CS+) was compared to the brain response to the safe cue (CS-). Similarly, safety learning was defined as a comparison between the safe cue versus the threat cue. To model nociceptive processing (**Study I**), the activation to the threat cue that was followed by the US ($CS+_{shock}$) (modeling the US as well) was compared to the activation in response to the threat cue when it wasn't followed by the US.

For the functional connectivity part of **Study I**, a weighted region-to-region FC was computed using CONN toolbox (*113*) with a Weighted Least Squares (WLS) linear model with condition-specific boxcar time series convolved with a canonical hemodynamic response function. The boxcar time series represented the onset and duration for $CS+_{shock}$. A separate boxcar time series represented $CS+_{no\ shock}$. Hence, the BOLD time series corresponding to these events were retained, and this was done separately for each of the two events. This allowed for the formation of two connectivity matrices (for each individual) that could be contrasted or subtracted to define nociceptive processing.

In **Study I**, APACE (Accelerated Permutation Inference for the ACE model) was used to compute voxel-wise genetic influence with cluster-level inference (*116*). First-level (individual-level) contrast images from the GLM corresponding to the contrast $CS+_{shock}$ vs. $CS+_{no\ shock}$ (denoted nociceptive processing) was used as input for **Study I**, and first-level contrasts CS+ vs CS- (fear learning) for **Study IV**. The reverse contrast was denoted safety learning in **Study IV**. APACE estimates the A, C, and E components using squared twin pair differences, with ordinary least squares regression with inference using the Likelihood Ratio Test (LRT). Multiple testing correction was performed using permutations (n=1000) to control the FWER (Family Wise Error Rate).

For the FC analysis in **Study I**, individual-level connectivity matrices were formed as described above by subtracting matrices corresponding to $CS+_{no\ shock}$ from $CS+_{shock}$. Using APACE (*116*), the genetic influence was estimated for each edge in the matrices, resulting in new individual-level symmetric matrices of $h^2$. These $h^2$-matrices were entered into a method based on network-based statistics (*117*) by computing the largest connected component of each $h^2$-matrix. This was performed a thousand times with permuted twin identity to create a null distribution – i.e., re-compute $h^2$-matrices following a permutation of twin pairs and then compute the largest connected component. The empirical connected component was then compared to this null distribution. This approach requires a choice of threshold before computing the largest connected component. We tested several thresholds from $h^2 = 0.25$ to 0.32 and found that the component broke at $h^2 = 0.328$. We chose to visualize results for this threshold.

### *4.6. Measuring TVFC: **Study II***

The jackknife correlation (JC) method (*118*) used to compute TVFC (*119*) was implemented in **Study II** to track pain-evoked fluctuations in FC. The JC between two time series *x* and *y* at time point *t* is computed as the Pearson correlation between *x* and *y* except for timepoint *t,* multiplied by negative one to correct for the sign inversion. Specifically,

$$JC_t = -\left(\frac{\sum_i^T (x_i - \bar{x}_i)((y_i - \bar{y}_i)}{\sum_i^T (x_i - \bar{x}_i)^2 \sum_i^T (y_i - \bar{y}_i)^2}\right), \quad i \neq t$$

A normalized JC value of zero at time-point *t* can be interpreted as the mean FC, approximating the static connectivity. Positive values can be interpreted as time-points with larger than usual connectivity and similarly for negative values (*81*). After having derived TVFC, the within-network and between-network temporal strength (*69*) was computed. Then the final measure of I/S was calculated as a difference between the within- and between-network strengths (SID; Segregation and Integration Difference) (*81*). The interpretation given above is then applied to these measures as well. For example, for SID, a value of zero is interpreted as the mean level of I/S across the whole session. In contrast,

positive and negative values denote more segregation or integration than usual. The strength of the connectivity within networks was computed as,

$$D_G^t = \frac{2}{N_1(N_1-1)} \sum_{i,j} A_{i,j}^t \;\; i,j \in G, i \neq j,$$

for a network *G* at time point *t*, with connectivity matrix $A_{i,j}^t$, and number of nodes in the network $N_1$. The strength of the connectivity between two networks was computed as,

$$D_{G1,G2}^t = \frac{1}{N_1 N_2} \sum_{i,j} A_{i,j}^t \quad i \in G_1, j \in G_2,$$

For networks $G_1$ and $G_2$, with $N_1$ and $N_2$ number of nodes, respectively. Using these measures, SID between two networks and for each time point is computed as,

$$SID_{G1,G2}^t = \left( \frac{2}{N_1(N_1-1)} D_{G1}^t - \frac{1}{N_2 N_2} D_{G1,G2}^t \right),$$

The results in **Study II** considered global SID for each network by averaging its I/S across networks.

### *4.7. Summaries of the cartographic profile:* ***Study III***

**Study III** defined weighted region-to-region FC matrices from resting-state fMRI data, using CONN (*113*), defined as Fisher-transformed bivariate correlation coefficient between pairs of brain regions. The resulting matrices were used to compute the cartographic profile using the participation coefficient and the within-module degree z-score (*120*). The participation coefficient, a measure of integration, represents the connectedness of a brain region across networks and is defined as:

$$P_i = 1 - \sum_{s=1}^{N_M} \left( \frac{k_{is}}{k_i} \right)^2$$

where $k_{is}$ is the number of connections of node *i* in network *s* and $k_i$ represents the degree of a node *i*. It is equal to 1 if its connections are uniformly distributed across networks and 0 if confined solely within its network. The module degree z-score, a measure of segregation, represents the degree of connectivity of a node within its network and is defined as:

$$z_i = \frac{k_i - \bar{k}_{s_i}}{\sigma_{k_{s_i}}}$$

where $k_i$ is the degree of node *i* to nodes in its own network $s_i$, $\bar{k}_{s_i}$ is the average degree in network $s_i$, and $\sigma_{k_{s_i}}$ is the standard deviation of the degree in $s_i$. Both measures were then summarized by taking the median and the maximum of each measure and each network, as done previously (*121, 122*).

### *4.8. Resting-state pain susceptibility network (RPN):* ***Study III***

In **Study III**, I also applied a tool developed using machine learning that predicts pain sensitivity from task-free, resting-state fMRI. The question was whether the group that had undergone surgery would display altered pain sensitivity compared to healthy controls since participants showed disc degeneration and some participants reported pain in the lower back. Although this tool is automatic, I describe here that the RPN computes FC using partial correlation across all brain regions to ignore indirect connectivity (*36*). The resulting matrices were used as input to a machine learning protocol that used an elastic net to predict pain sensitivity, resulting in a single score for each participant. The tool was

developed using multiple resting-state datasets. A pain sensitivity score was calculated as a composite of various pain measures acquired outside the scanner. A machine learning protocol learned to predict those scores. The resulting tool can then predict pain sensitivity in new participants (*36*), as was done in our study. Note that we didn't measure pain sensitivity. The predictive network included brain regions from several canonical networks such as the DMN, frontoparietal network, ventral attention network, salience network, somatomotor network, visual network, basal ganglia, mesolimbic network & cerebellum.

### *4.9. Brain, body, and behavior*

**Study III** aimed to relate peripheral measures of disc degeneration to FC measures. Additional to this, we measured gait as participants walked back and forth. Their walking patterns were recorded using the Microsoft® Kinect® for Windows (system v.1, Microsoft, USA), the Microsoft® Software Development Kit (v1.8), and Microsoft® Visual Studio Express 2013 (Microsoft, USA). The rationale behind this is that disc degeneration and pain in the back may have resulted in altered walking patterns in patients, and we wanted to see if this could be detected in terms of gait variables. Additionally, we measured back morphology using a 3T Tesla Scanner and assessed disc degeneration. We related measures of disc degeneration with pain sensitivity predicted from patterns of resting-state FC (*36*).

In **Study IV**, in addition to fMRI, we also measured skin conductance response (SCR), controlled with a BIOPAC system (Goleta, CA) and electrodes placed on the left hand. The SCR measures autonomic responses during fear conditioning. This way, we could compute the genetic correlation between brain responses and autonomic responses, in addition to their respective genetic influence. The genetic correlation indicates the extent to which the same genetic factors contribute to the variance in the two domains. Furthermore, in **Study I**, we estimated the genetic influence on behavioral sensitivity to electrical stimuli.

### *4.10. Statistics*

In **Study IV**, SCR was analyzed to test for conditioning with a t-test to compare CS+ and CS-. We tested for conditioning in the amygdala as well by first averaging the BOLD signal for the left and right amygdala and then implementing a t-test.

**Study II** included thermal stimuli, with five intensities ranging from 44.3° to 48.3° in steps of 1. The two lowest intensities were categorized as "low" and the two highest as "high" thermal intensities. The middle temperature was not included in the analyses. The Supplementary Material in **Study II** included the analyses for all thermal intensities. For TVFC estimated using windows covering a full trial, a permutation test was performed to compare high and low intensities for data averaged over trials. The test was performed for each network, and therefore, the Benjamini-Hochberg procedure was used to control for multiple comparisons (False Discovery Rate, FDR) (*123*). This test was performed for the SID measure and the within-network and between-network temporal strength. At the single time point scale – when TVFC was estimated with a window of a single time point – high and low thermal intensities were compared using a permutation test with cluster-level inference with threshold-free cluster enhancement (TFCE), implementing a one-way analysis of variance. Clusters were identified over time. The test was performed for each brain network. Therefore, the resulting p-values for each time point and all brain networks were also controlled for multiple comparisons using FDR. This was done separately for SID, within-network, and between-network strength. The relationship between the three temporal network measures was tested against the subjective pain ratings using a robust method, implementing skipped Spearman's correlation (*124, 125*). The correlation was estimated after removing bivariate outliers by first computing the robust center of the data using the minimum covariance determinant estimator and then identifying outliers using the box-plot rule by first orthogonally projecting data points onto lines joining each data point to the middle of the data points, and then finally computing Spearman's correlation after removing outliers. With TVFC at the trial time scale, each trial could be associated with the corresponding subjective pain rating. For the single time point scale, since there were seven time points for each trial, we instead correlated each time point with the subjective

rating for that trial. Like previously, p-values were controlled for multiple comparisons using FDR. For the single time point scale, p-values were controlled across time and space (brain networks).

In **Study III**, the summary measures of the cartographic profile were used to compare groups for each network, and each measure, using an independent t-test and controlling for multiple comparisons using FDR. The RPN score was used in an ANCOVA to test for group differences using the RPN as the dependent variable, group as a factor, and results from questionnaires, measures of disc degeneration and gait variables as covariates. As shown in a prior study, groups differed in self-reported health outcomes and disc degeneration (*126*) and those variables were included here. Only the gait variables that were statistically significantly different between groups were included as covariates. However, since several variables were correlated, (Supplementary Material for **Study III**), not all variables were included as covariates. See the manuscript for **Study III** for details about the variables, as well as Lagerbäck, Kastrati et al., (*126*). For the gait variables, linear mixed models were used to compare groups, controlling for multiple comparisons with FDR.

### *4.11. Exploring variations in decisions*

There are several choices that researchers could make in neuroimaging data analysis (*127*). One could explore variations in decisions along the fMRI data analysis pipeline. A small example of this is given in **Study III,** where results are presented for different thresholds used to keep only the strongest links in the connectivity matrices (that were subsequently used to compute summaries of the cartographic profile). An additional methodological choice that could be considered is the choice of similarity measure (e.g., correlation, partial correlation, Euclidean distance) since other measures could result in different FC profiles (*11, 128*). However, this was outside the scope of the current project. Additionally, there is no gold-standard procedure for reducing the influence of confounding variables on static and time-varying FC (*109*). One confounding variable is the influence of the GS on connectivity estimates (*107*). Here, one can produce results with and without the GSR (*107*), as was done in **Study II**. The same could be done with brain atlases since it is unclear how replicable results are across atlases. A caveat of assessing variation in analyses steps is that there will be many results, with possible differences that could be difficult to explain.

### *4.12. Ethical considerations*

In pain neuroimaging, it is essential to ensure that the experimental induction of pain does not lead to any tissue damage. The studies can include thermal pain (**Study II**), electrical stimuli (**Study I/IV**), or other nociceptive stimulation paradigms (*129*). The ethical guidelines for pain research provided by IASP, states that the stimuli must be below that tolerated by the participants and that they can abort their participation at any time. The data from **Study II** was obtained from an online repository, and the authors of that study followed the ethical procedures. For **Study I** and **II**, we followed the procedure by calibrating the stimuli in the MRI before continuing. Participants gave both written and verbal instructions and were told that they could abort at any time, without giving any reason for doing so. There are additional ethical considerations in pain neuroimaging. For example, identified objective markers of pain should not replace subjective reports, for example in court. Objective markers could be used to develop save and effective treatment for pain (*32*).

## 5. RESULTS

**Study I: Genetic influence on nociceptive processing**. The aim was to determine the genetic influence on brain activity and FC during nociceptive processing. In response to the nociceptive stimuli, there was a genetic influence on brain responses in the right (contralateral) postcentral gyrus, right posterior insula, right superior temporal gyrus, right supramarginal gyrus, left postcentral gyrus, left supramarginal gyrus, left posterior insula, left superior temporal gyrus, left ACC, right posterior medial frontal gyrus, and bilateral MCC (**Figure 6**). The study further estimated the genetic influence on FC during nociceptive processing (not shown here). The approach was based on network-based statistics on the genetic-influence-matrix, where each value corresponds to the genetic influence on the FC between two brain regions. The network-based statistics approach was used since it considers the dependency among FC values. The strategy identified a cluster of connections linking all the canonical brain networks. Finally, the results showed a genetic influence on the choice of threshold for the electrical stimuli.

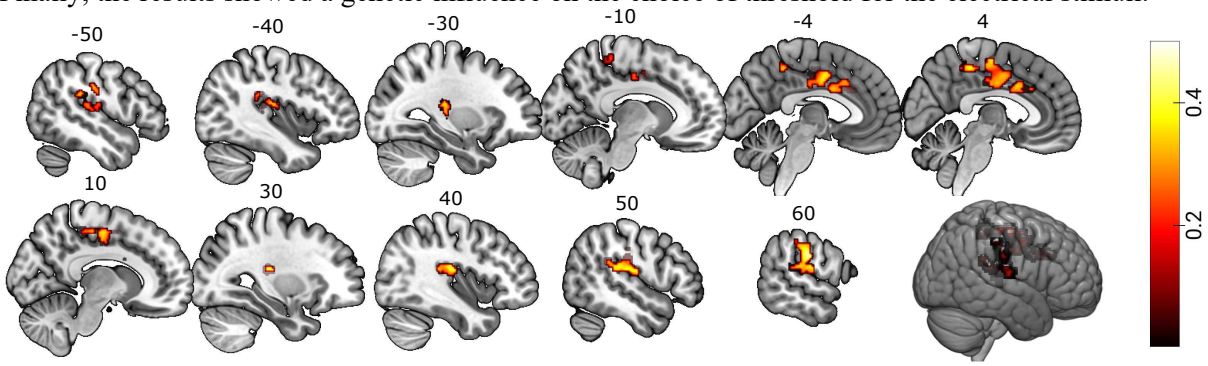


**Figure 6. The main results from Study I, showing the genetic influence $h^2$ on nociceptive processing (*130*).**

**Study II: Time-varying functional connectivity during pain perception**. The study used TVFC in an exploratory study to characterize the temporal evolution of I/S during pain processing. The study used two window sizes to compute TVFC, corresponding to the smallest time-point or a whole trial. The aim was to characterize the temporal I/S of brain networks during high and low thermal intensities. The results showed that when the lower temporal resolution was used, all brain networks showed statistically significant increased integration with higher pain ratings (**Figure 7**). However, these results vanished at the lower temporal resolution for several brain networks (**Figure 8**).

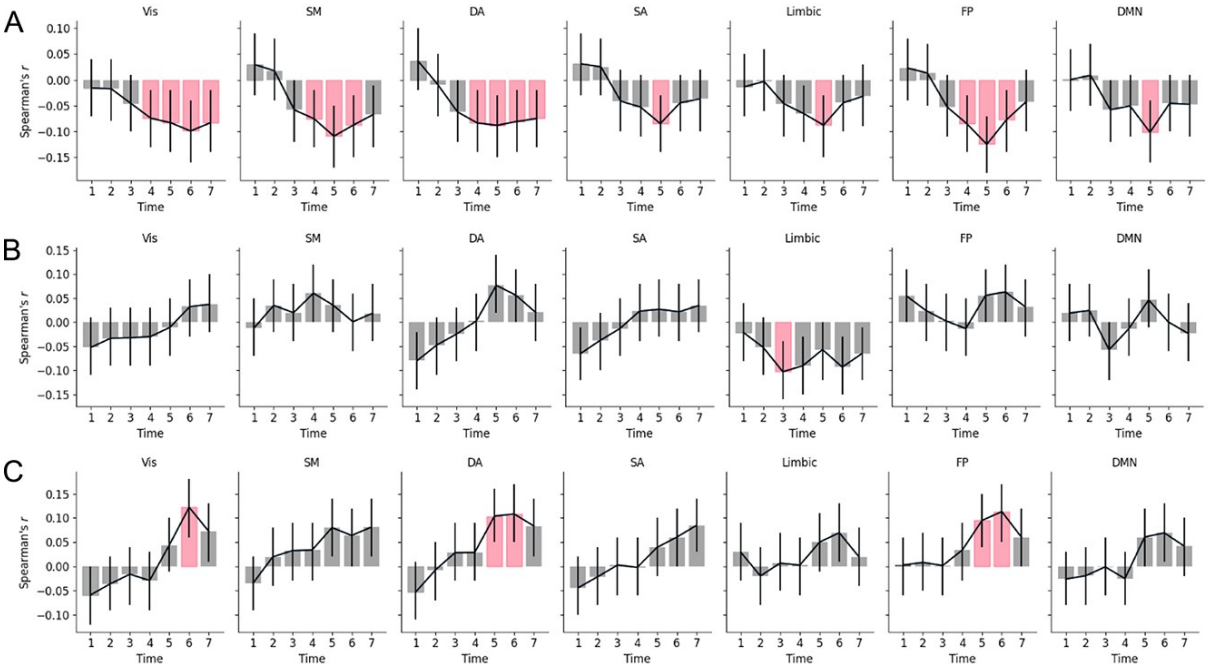


**Figure 7. Result from Study II.** Correlation between subjective ratings of pain and SID computed at the single time point scale. A negative correlation implies increased integration with higher ratings of pain.

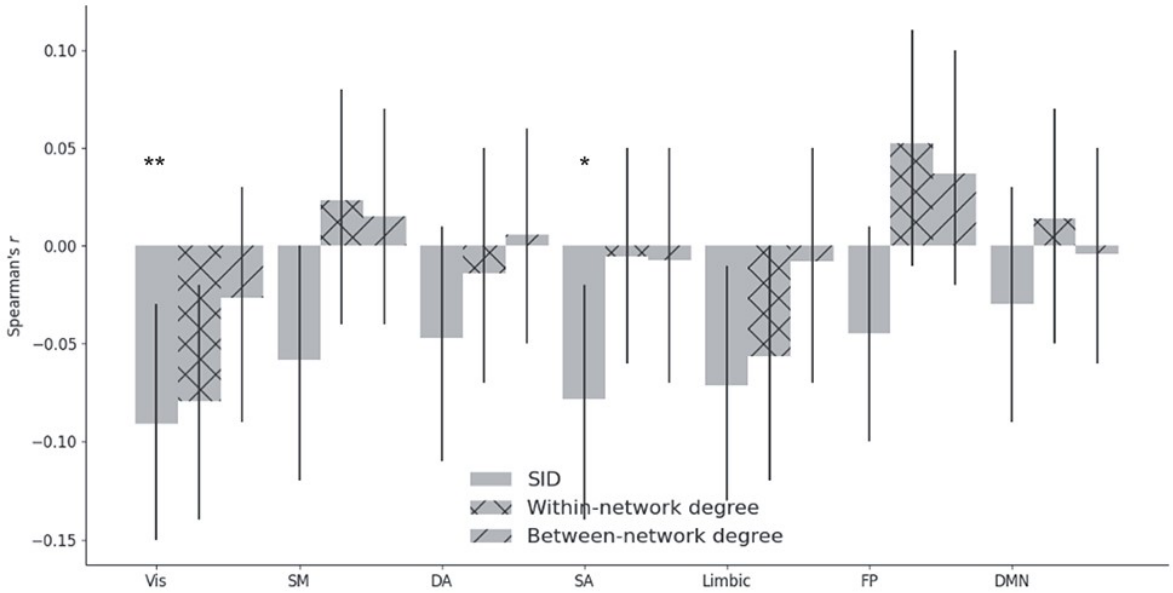


**Figure 8. Result from Study II**. Correlation between subjective ratings of pain and SID at the trial time-scale (i.e., SID estimated from TVFC computed with a window-size covering a whole trial) (*131*). As before, a negative correlation implies increased integration with higher ratings of pain.

**Study III: The brain, the back, and walking patterns following surgery for disc herniation**. In **Study III**, participants had undergone surgery for disc herniation mean 14 years before the study. Outside the scanner, walking patterns were recorded to derive gait variables. Since the images were acquired during resting-state fMRI, we could apply a neurological signature that predicts pain sensitivity from resting-state FC. Additionally, whole-brain FC was estimated to compute measures of I/S (summaries of the cartographic profile). A mean of 14 years since surgery, participants still had degeneration in the lower back and displayed worse patient-reported outcomes compared to matched controls (*126*). Here, the aim was to characterize brain patterns related to disc degeneration. The gait analysis showed a group difference in head angle and trunk angle, with a lower head angle and higher trunk angle for the group that had undergone surgery compared to controls. There was no group difference in pain sensitivity (RPN). However, there was a statistically significant influence of disc degeneration on the RPN score. There were no group differences in brain network I/S measures.

**Study IV: Genetic influence on brain responses during fear and safety learning**. **Study IV** aimed to characterize the genetic influence on brain activation during fear and safety learning. The result in **Study IV** first showed that the activation for the threat cue (CS+ versus CS-) and the activation for the safe cue (CS- versus CS+) were similar to the activations found in a meta-analysis on fear conditioning (*101*). The results further revealed statistically significant genetic influence on brain responses during fear learning in bilateral insula, right putamen, left pallidum, right thalamus, and the PAG (**Figure 9A**). A statistically significant genetic influence during safety learning was found in the bilateral precuneus and contralateral PCC (**Figure 9B**). Moreover, there was a genetic influence on autonomic conditioning to the fear cue compared to the safe cue. A genetic correlation between mean brain activation and SCR was found for safety learning only. Finally, due to its reported importance in fear conditioning (*132-134*), the amygdala was analyzed separately since it was not part of the a priori brain regions used in the main analysis. First, there was a statistically significant response during fear learning in bilateral amygdala. However, there was no statistically significant genetic influence. **Figure 10** shows the activation patterns for fear learning (warm colors) and safety learning (cool colors).

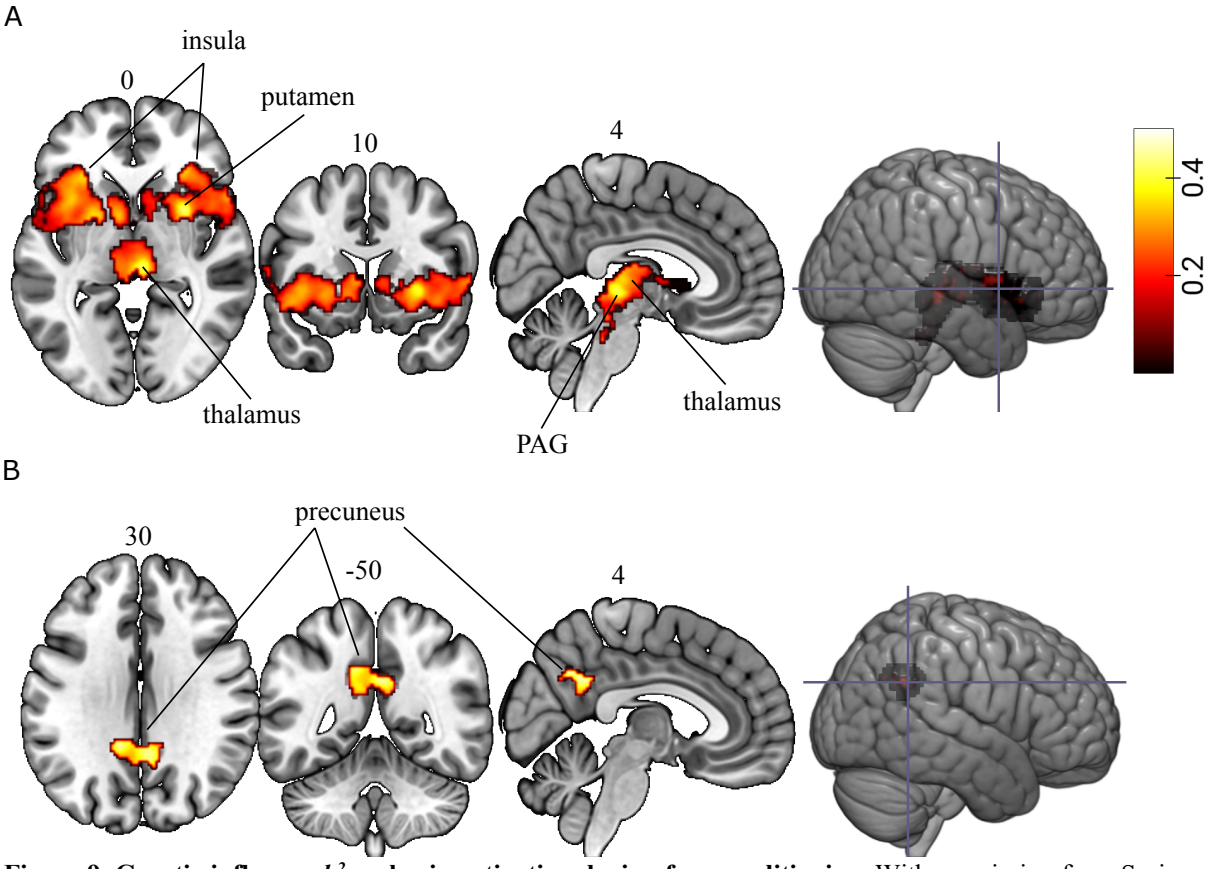


**Figure 9. Genetic influence $h^2$ on brain activation during fear conditioning**. With permission from Springer Nature.

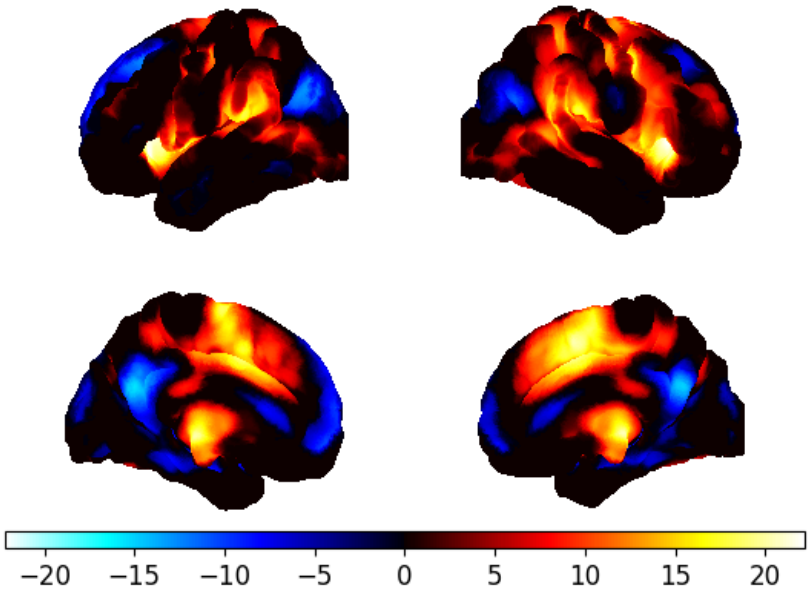


**Figure 10**. **Task-evoked brain activation (statistical parametric map) during fear and safety learning from Study IV**. Warm colors denote the CS+>CS- contrast (classical fear conditioning), and the cool colors refer to the reverse CS->CS+ contrast (safety learning). This result is a modified version from Study IV, with permission from Springer Nature.

# 6. DISCUSSION

Our conscious experience of the world is subjective, and both in psychology and philosophy, the sensation of pain and the feeling of negative mental states are prime examples of *qualia*, or *how mental states feel like* (*135*). A specific neurobiological architecture has been proposed for pain that extends across brain networks (*28*). This architecture is nevertheless embedded in the larger network of the brain and therefore stands in constant dynamic interactions with its various sub-systems (*27*).

This thesis aimed to take a network approach to pain-related processing. Using a unique research project with twins, the aims of **Studies I** and **IV** were to estimate the genetic influence on brain activity and FC in response to a noxious stimulus (**Study I**), as well as to fear learning in events leading up to the noxious event in fear conditioning (**Study IV)**. In **Study II**, using a temporal functional connectivity measure, the aim was to characterize fluctuations in FC during various painful thermal intensities. Specifically, the temporal evolution of brain network I/S was probed. **Study III** assessed the relationship between brains, backs, and behavior. The specific aim was to identify brain-based markers in terms of I/S of brain networks since participants displayed disk degeneration, reported pain, and had worse patient-reported outcomes.

The main findings from **Study I** was a genetic influence on the dorsal posterior insula, MCC, and ACC during nociceptive processing and whole-brain FC. **Study IV** showed a genetic influence on brain activity in the thalamus, the putamen, the PAG, and the anterior insula during fear learning and genetic influence on the precuneus/PCC in response to the safe cue compared to the threat cue. The study also showed a genetic correlation between autonomic responses and mean brain activation during safety learning, indicating the extent to which the same genetic factors influence variance in the two domains. **Study II** found varying temporal profiles of I/S for different brain networks during pain, with a general increase in integration with more pain. The association between pain and integration of all networks was not as evident when TVFC was estimated at a lower temporal resolution. **Study III** showed altered gait and a possible association between pain sensitivity as predicted from resting-state fMRI and disc degeneration yet found no group difference in brain network I/S.

## *6.1. Fear, pain, and salience*

During pain, anticipation, and fear, the activation pattern often shows great overlap (*49, 68*), involving the same large-scale structures such as the insula, MCC, and ACC – suggesting that the same systems are used for different purposes (*136*). However, activation of the posterior insular cortex is often observed with nociceptive processing (*137*). At the same time, the anterior insula is activated both during fear conditioning (*101*) and in pain perception (*28, 35*). In **Study IV**, there was some activation in the posterior insula; however, not in the coordinates found for the left and right insular cortex in **Study I**. **Figure 10** show the activation pattern seen during the threat cue indicating posterior insula activity yet with predominant activity in the anterior insula and MCC.

The patterns of brain activation, anchored in the insular, MCC, and ACC, also resemble the salience network (*47*) or the cinguloopercular network (*50*), see for example the resting-state network depicted in **Figure 3** (left frame). The insular, MCC, and ACC, central in the salience and cinguloopercular networks, are two main components reported in the literature on fear conditioning (*101*) and pain (*28*). It is worth noting that these two structures are often deemed to be two of the most commonly coactivated brain regions in all of neuroimaging (*106, 138*), not only during fear or pain. The salience network has been linked to various functions, such as detecting salient or novel features in the external milieu. It could also be responsible for switching between externally and internally oriented attention, with a unique role assigned to the connectivity between the anterior insula and ACC (*139*). Even though the salience network includes regions involved in pain processing, **Study II** did not reveal a special status to the salience network in terms of I/S during pain. This was the case when measured with a higher temporal resolution. When estimated at a lower temporal resolution, only the integration of the salience network (and the visual network) correlated significantly with subjective pain reports. With the higher temporal resolution, integration of all networks showed some relation to pain to a varying extent and at

varying time points. This is in line with recent research that emphasizes the interplay between classical pain regions and extranociceptive brain regions in pain processing (*140, 141*), extended to the temporal domain in **Study II**.

### *6.2. Genetic influences on brain activity*

**Studies I** and **IV** aimed to estimate the genetic influence on intermediate phenotypes. This is because behaviors are situated further away from the genotype, and it might be simpler to relate genes to neuroimaging-derived phenotypes (*85, 86*). As genes and environment shape phenotypes, we set out to determine the magnitude and spatial locations of genetic influence on brain activity during nociception and fear learning. Twin studies could inform us about the proportion of the variation in brain function that can be attributed to genetics. It could also say something about predispositions to certain phenotypes, for example, sensitivity to pain.

The genetic influence during nociceptive processing included some commonly activated brain regions during nociception and pain. This result resonates with findings from infants with similar nociceptive activity as adults and with diminished expression in the anterior insular cortex (*142*). This is noteworthy because although the electrical stimulation activated the anterior insular cortex (see **Supplementary Figure 2** of **Study I**), there was no statistically significant genetic influence of activity there. Furthermore, in **Study I,** we found a genetic influence on nociceptive thresholds. This is interesting since we saw a genetic influence on posterior insula activity during nociceptive processing, suggesting that cortical response to noxious input is under genetic influence. However, we did not estimate the genetic correlation between nociceptive thresholds and dorsal posterior insula activation. This could have allowed us to see the amount of shared genetic influence (if any) – such that the same genes influence processing of noxious input in the posterior insula that in turn could modify the choice of threshold of the electrical shock. Furthermore, a genetic influence on the anterior insula was found on activation during fear conditioning in **Study IV**. Although there was some activation in the posterior insula, the genetic influence could not be determined since it was not included in the a priori network. It should be noted that we saw activation in the posterior insula during fear learning while there was a deactivation in the posterior insula in the meta-analysis during safety learning (*101*). It is not clear what underlies this difference.

In fear conditioning, cues have acquired an aversive meaning through an association to an aversive stimulus and hence come to elicit conditioned responses on its own. The activation pattern found in **Study IV** showed great similarity to a previous meta-analysis on fear conditioning (*101*). The genetic influence on the brain activation covered several regions, including the insular cortex and the PAG, while safety learning showed a genetic influence on the precuneus/PCC. It is noteworthy that these are the same structures involved in pain processing. The PAG, for instance, is involved in pain modulation (*143*). It is also involved in post-traumatic stress disorder (PTSD), especially its FC to the amygdala (*144*). The findings could have implications for understanding PTSD, which has been shown to have increased brain activation during various forms of conditioning (*145*). Assessment in anxiety disorders have also shown altered activation in response to a safe cue, indicating alterations in inhibitory mechanisms in response to the safe cue (*146*). Furthermore, the mechanisms underlying safety learning may be relevant to chronic pain since previous findings have shown impaired safety learning mechanisms in this group (*67*). **Study IV** also found a genetic correlation between mean brain activation and autonomic responses for safety learning. Since the brain regulates autonomic responses, the genetic control of SCR would act through influences on brain function, which could have implications for interpretations of previous SCR findings on individuals with anxiety (*147*).

The activation that was seen during nociception, fear, and safety learning could be interpreted as activation of the now classical resting-state brain networks: the salience network (during nociception/fear) and default-mode network (during safety learning). Indeed, attention to pain increases salience network activation, while attention away from it is related to DMN FC (*76*). The interaction between attention and pain has been demonstrated in many studies (*76, 148*). This modulation may, in part, be driven through inhibitory signals via the PAG (*76, 149*), seen activated and under genetic

influence during fear learning in **Study IV**. The activation seen during safety learning could be interpreted as DMN resting-state activity (*101*). The statistically significant cluster of genetic influence in the PCC/precuneus region found in **Study IV** would then correspond to a genetic influence of resting-state, DMN hub activity. Indeed, the brain seems to become more resting-state-like or more modular during innocuous stimuli than painful stimuli (*44*) and more segregated with less pain, as seen in **Study II**. Increased DMN activity in **Study IV** corresponded well to the pattern found in a meta-analysis of fear conditioning (*101*) and is supported by other studies. Concerning threat, the increased distance to a tarantula was associated with increased activity in the PCC and anterior orbitomedial prefrontal cortex (*150*). This suggests an increase in DMN brain activation as the distance to a threat is increased. Furthermore, the relative activation of the DMN or insula-centered brain networks could reflect the degree of conscious access to sensory information. For instance, variability in the perception of stimuli can be related to prestimulus baseline brain activity, with a negative correlation between brain activity - including the precuneus/PCC – and subsequent conscious perception of an innocuous somatosensory stimulus (*151*). However, the anterior insula would serve as a gate to conscious perception of sensory information (*152*). Depending on whether a visual cue is threatening or not, one is either made ready to consciously perceive sensory information or assign less importance to external stimuli.

Overall, even if the networks activated during nociception, pain, fear, and safety represent the activity of intrinsic brain networks such as the salience network and DMN, their relative I/S via changes in within-network and between-network connectivity influences the experience, which is in line with previous findings and expanded to the temporal domain in **Study II**. **Studies I** and **IV** showed that activity in central structures within these networks is under genetic influence. Furthermore, in **Study I**, our aim was to move beyond brain activation to capture the genetic influence of connectivity patterns. We found a cluster of connectivity covering all the canonical brain networks used in the study, suggesting a genetic influence on brain integration, affecting mostly connectivity between networks. Previous findings have shown a genetic influence on specific brain networks, such as the DMN (*89*). It has also been shown that genetics influences the strength of the structural connections between brain hubs, suggesting that the links supporting global information integration are under significant genetic control (*153*). With resting-state FC, results show that connectivity within networks (segregation) is genetically influenced while environmental factors influence connectivity between networks (integration) (*94*). The findings from **Study I** demonstrate that brain-wide integration during nociceptive processing is under genetic influence.

### *6.3. Brain network integration and segregation*

What are the mechanisms that control information integration in the brain? Studies on the structural connectome have found evidence for a rich club organization – a network with highly connected and inter-connected hubs (*154*). The rich club in the brain includes the precuneus, PCC, ACC, and the insular cortex (*154*). Further, recent research attempt to define a dynamic functional rich club that is invariant across tasks (*155*) and that should correspond to the global neuronal workspace underlying conscious access or information integration (*156*). Furthermore, recent theories of emotion regard the integration of interoceptive signals and exteroceptive signals as central for emotion (*136, 157*). Although caution should be taken when interpreting resting-state networks since their function is unclear and under debate (*43*), it is tempting to argue for a unified theory whereby the cortical core described above could be a source for integrating the DMN and the salience network – that are specialized yet domain-general – to support interoception and hence to regulate the conscious perception of emotions (*136, 158*). Here, the insula and the MCC/ACC are believed to process information concerning internal and external stimuli relevant to one's capacity to survive and thrive (36) by representing and regulating interoceptive signals (*136, 157*). Under this framework, the signals we observed during **Study I** and **IV** could be related to predictions of interoceptive signals and autonomic control of those signals. However, the magnitude and spatial extent of the genetic influence on predictions of interoceptive signals and autonomic control of those signals under various contexts – aversive or non-aversive – has yet to be determined. To what extent do genetics influence – under different conditions – the perception and regulation of interoceptive signals and profiles of the integration between interoceptive and exteroceptive signals that result in a unique conscious expression of pain and negative affect?

Yet, a subjective experience – such as pain and fear - might give rise to similar bodily reactions in terms of heart rate, blood pressure, respiration, or muscle tension, that are then reflected as similar brain activation patterns, making it difficult to discern the effects of afferent and intrinsic activity using fMRI (*4, 159*). One could compare brain activation patterns to pain and fear controlling for variables such as heart rate and respiration to more clearly reveal where they convergence and divergence.

### *6.4. Strengths and Limitations*

**Study I** aimed to estimate the genetic influence on nociceptive processing that could be relevant in understanding pain processing. One strength of **Study I** (and **Study IV**) is that it used a large cohort collected with fMRI and used a classical twin design. However, if we had collected subjective reports of pain, we could have derived brain responses to pain instead of being restricted to nociception. The results could have been different, for example, displaying a genetic influence on the anterior insula rather than the posterior insula – since the anterior insula is related more to the conscious aspect of pain (*160*). Another concern is that **Study I** used electrical shocks, which may not be the most ecologically valid pain stimuli or may not translate well to a clinical context. Finally, the event-related design of the twin study could have used longer sessions or repeated measures to identify more stable estimates of genetic influence, as it has been shown to be a function of scan length (*75*).

A methodological concern is that we used a group averaged brain parcellation (**Studies I-III**) to define the functional connectome. However, this assumes that functional brain regions do not change over time or are the same between individuals or across tasks (*161*). For example, functional brain regions can be observed within a single individual that is not observed in the group-level parcellation (*162*). It has also been shown that the shape, size, and location of brain regions are predictive of individual behavior (*163*). The variability of functional brain regions and the use of group averaged parcellations might lead to brain-behavior relationships being averaged out. Furthermore, **Study II** estimated TVFC and the degree of I/S over time yet assigned brain regions to networks that did not vary. Another approach could have been to use temporal community detection whereby networks may grow, shrink, split and merge (*42, 164*). A strength of the study was that it was obtained online, allowing others to verify the results.

### *6.5. Points of perspectives*

The findings from **Study I** and **IV** showed a genetic influence on processes relevant to pain, such as nociceptive processes and anticipation which could allow for finding endophenotypes for chronic pain – a measurable component between a disease and genotype (*85*). This could provide an understanding of why some individuals are more vulnerable to developing chronic pain. The findings put forth here could support efforts to link complex genetics – underlying chronic pain – to brain function (*43*). A possible extension in future studies implementing a twin design would be to test participants with painful as well as non-painful stimuli to be able to compare activations and network patterns to pain-related fear and fear since it is not clear at present the extent of their neural similarities and differences (*68*), as well as how they differ from pain.

**Study II** revealed that the integration of each of the networks tested was associated with pain reports. However, the temporal profiles of brain network I/S were assessed on a global level by considering a networks' I/S profile with respect to all other networks. The temporal I/S between networks would provide further specificity to our understanding of pain perception. The spatiotemporal integration between some networks could be more relevant for pain, for instance between the DMN and salience network. One could also characterize the nodes that could drive I/S. What are the brain regions that are driving the I/S of brain networks (*122*) in relation to pain? Furthermore, the correlation between integration and subjective pain says nothing about causality. To what extent would the experience of pain be altered by delaying or prolonging the integration of brain networks – for instance by causally probing brain regions? This could be accomplished using computational models to reveal possible causal links between temporal I/S and pain (*165*).

### *6.6. Concluding remarks*

The way pain is experienced may be shaped not only by spatial relations among brain regions but by temporal relations – the way information flow is coordinated over time. Further investigations on how different temporal profiles relate to pain – acute or chronic – may help build biomarkers. Also, the ways we anticipate pain or process nociceptive input may be shaped by genetics. These findings could be used for endophenotypes, to provide an understanding of why some individuals are more vulnerable to developing chronic pain.

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