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Les mécanismes endogènes de modulation de la douleur et leur dysfonction dans le syndrome de l'intestin irritable

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Les mécanismes endogènes de modulation de la douleur et leur dysfonction dans le syndrome de l'intestin irritable

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Résumé

La douleur est une expérience subjective multidimensionnelle accompagnée de réponses physiologiques. Ces dernières sont régulées par des processus cérébraux qui jouent un rôle important dans la modulation spinale et cérébrale de la douleur. Cependant, les mécanismes de cette régulation sont encore mal définis et il est essentiel de bien les comprendre pour mieux traiter la douleur. Les quatre études de cette thèse avaient donc comme objectif de préciser les mécanismes endogènes de modulation de la douleur par la contreirritation (inhibition de la douleur par une autre douleur) et d'investiguer la dysfonction de ces mécanismes chez des femmes souffrant du syndrome de l'intestin irritable (Sii).

Dans un premier temps, un modèle expérimental a été développé pour mesurer l'activité cérébrale en imagerie par résonance magnétique fonctionnelle concurremment à l'enregistrement du réflexe nociceptif de flexion (RIII : index de nociception spinale) et des réponses de conductance électrodermale (SCR : index d'activation sympathique) évoqués par des stimulations électriques douloureuses. La première étude indique que les différences individuelles d'activité cérébrale évoquée par les stimulations électriques dans les cortex orbitofrontal (OFC) et cingulaire sont associées aux différences individuelles de sensibilité à la douleur, de réactivité motrice (RIII) et de réactivité autonomique (SCR) chez des sujets sains. La deuxième étude montre que l'analgésie par contreirritation produite chez des sujets sains est accompagnée de l'inhibition de l'amygdale par OFC et d'une modulation du réflexe RIII par la substance grise périaqueducale (PAG) et le cortex somesthésique primaire (SI). Dans les troisième et quatrième études, il est montré

que la contreirritation ne produit pas d'inhibition significative de la douleur et du réflexe RIII chez les patientes Sii en comparaison aux contrôles. De plus, les résultats indiquent que la sévérité des symptômes psychologiques est associée au déficit de modulation de la douleur et à une hypersensibilité diffuse chez les patientes Sii. Dans l'ensemble, cette thèse précise le rôle de certaines structures cérébrales dans les multiples composantes de la douleur et dans l'analgésie par contreirritation et montre que les patientes Sii présentent une dysfonction des mécanismes spinaux et cérébraux impliqués dans la perception et la modulation de la douleur.

Mots clés : Cerveau, moelle épinière, douleur, syndrome de l'intestin irritable, modulation, contreirritation, réflexe nociceptif, réponse de conductance électrodermale, IRMf, stimulation électrique.

Abstract

Pain is a subjective experience comprising multiple dimensions and is accompanied by physiological responses. These responses are regulated by neural processes that play a crucial role in cerebral and spinal modulation of pain. However, the mechanisms of this regulation are still not clear and a better understanding of these processes is essential in order to treat pain effectively. The four studies of this thesis were intended to define the central mechanisms of endogenous pain modulation by counterirritation (application of two competing noxious stimuli) and to investigate the dysfunction of these mechanisms in female patients with irritable bowel syndrome (IBS).

First, an experimental model was developed in which functional magnetic resonance imaging was used to measure brain activity concurrently to the recording of the nociceptive flexion reflex (RIII: an index of spinal nociceptive processes) and skin conductance responses (SCR: an index of sympathetic activation). The first study indicates that individual differences in shock-evoked brain activity in the orbitofrontal (OFC) and cingulate cortices are associated with individual differences in pain sensitivity, motor reactivity (RIII), and autonomic reactivity (SCR) in healthy volunteers. In the second study, it is shown that counterirritation analgesia produced in healthy volunteers is accompanied by the inhibition of the amygdala by the OFC, and the inhibition of the RIII reflex by the periacqueductal gray matter (PAG) and the primary somatosensory cortex (SI). In the third and fourth studies, pain and RIII reflex were not significantly modulated by counterirritation in patients with IBS in comparison to healthy controls. Furthermore,

the severity of psychological symptoms was associated with pain modulation deficits and diffuse hypersensitivity in IBS patients. Together, the results of these studies clarify the functions of pain-related activity in specific brain structures and the mechanisms underlying counterirritation analgesia. Moreover, it is concluded that patients with IBS show a dysfunction of cerebral and spinal processes involved in both the perception and modulation of pain.

Key words: Brain, spinal cord, pain, irritable bowel syndrome, pain modulation, counterirritation, nociceptive flexion reflex, skin conductance, fMRI, electrical stimulation.

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Liste des abréviations

ACC…….Anterior cingulate cortex - Cortex cingulaire antérieur

ACTH……..Hormone corticotropique

aINS.....Anterior insula –Insula antérieure

AMY…….Amygdala – Amygdale

ANCOVA….Analysis of covariance – Analyse de covariance

ANOVA…....Analysis of variance - Analyse de variance

AR……....Autoregressive model – Modèle d'autorégression

BOLD……...Blood-oxygen-level-dependant

CRF……..Facteur de relâche corticotropique

CSF……..Cerebrospinal fluid –Liquide cérébrospinal

DCM.....Dorsocaudal medulla – Moelle allongée dorsocaudale

dlPFC……...Dorsolateral prefrontal cortex – Cortex préfrontal dorsolateral

DNIC.....Diffuse noxious inhibitory controls – Contrôles inhibiteurs diffus nociceptifs

DRt.....Dorsal reticular nucleus - Noyau réticulaire dorsale

EEG.....Électroencéphalographie

EMG.....Électromyographie

fMRI/IRMf.…Functionnal magnetic resonance imaging/Imagerie par résonance magnétique fonctionnelle

FWHM……..Full-width-half-maxima

GI……..Gastrointestinal - Gastrointestinale

Hrf…….Hemodynamic response function – Fonction de la réponse hémodynamique

IBS/Sii.....…Irritable bowel syndrome – Syndrome de l'intestin irritable

ICA……Independant component analysis – Analyse par composantes indépendantes

INS……Insula

MCC……..Midcingulate cortex – Cortex cingulaire moyen

mPFC…….Medial prefrontal cortex - Cortex préfrontal médian

MRI……Magnetic resonance imaging – Imagerie par résonance magnétique

NFR……...Nociceptive flexion reflex – Réflexe nociceptif de flexion

ns……Not significant - Non significatif

OFC……...Orbitofrontal cortex - Cortex orbitofrontal

PAG……...Periaqueductal gray - Substance grise périaqueducale

PCC……Posterior cingulate cortex - Cortex cingulaire postérieur

PCL……....Paracentral lobule - Lobule paracentral

PCS……...Pain catastrophizing scale – Échelle de réaction catastrophique à la douleur

PET……Positron emision tomography

PFC……Prefrontal cortex - Cortex préfrontal

pgACC……..Pregenual anterior cigulate cortex

PHG……...Parahippocampal gyrus - Gyrus parahippocampal

pINS……...Posterior insula – Insula postérieure

PPC……Posterior parietal cortex

PrCG……..…Precentral gyrus – Gyrus précentral

Pre-SMA.....Pre-supplementary motor area – Aire motrice pré-supplémentaire

RIII.....Réflexe nociceptif de flexion

RF.....Radiofrequency - Radiofréquence

RSC……...Retrosplenial cortex – Cortex rétrosplénial

RVM.....Rostral ventromedial medulla – Moelle rostrale ventromédiale

SI……...Primary somatosensory cortex - Cortex somesthésique primaire

SII……..Second somatosensory cortex – Cortex somesthésique secondaire

sACC.....Subgenual anterior cingulate cortex

SCL.....Symptoms-check-list

SCR……...Skin conductance response – Réponse de conductance électrodermale

SD.....Standard deviation – Déviation standard

SDT.....Sensory decision theory – Théorie de la détection de signal

SEM……..Standard error of the mean – Erreur standard de la moyenne

sICA……..spatial independant component analysis – Analyse par composantes indépendantes spatiales

SMA……..Supplementary motor area - aire motrice supplémentaire

SPM……..Statistical parametric mapping

SRD……..Subnucleus reticularis dorsalis

TE……..Echo time – Temps d'écho

Thal……...Thalamus

TR……..Repetition time - Temps de répétition

TRPV1……..Transient receptor potential channel, type vanilloid 1 – Canal cationique à récepteur de potentiel transitoire, type vanilloïde 1

TTL…….Transistor-transistor logic

VAS……Visual analogue scale – Échelle visuelle analogue

VPL…… noyau ventroposterolateral du thalamus

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Avant-propos

Au début de mon doctorat, je désirais étudier la douleur viscérale chronique avec des méthodes d'imagerie et d'électrophysiologie humaine. À ce moment, il n'y avait aucune étude sur les mécanismes de modulation de la douleur dans le syndrome de l'intestin irritable. Par contre, les études sur la fibromyalgie suggéraient un déficit des mécanismes endogènes de modulation de la douleur pour expliquer l'hypersensibilité diffuse caractérisant ce syndrome. Il apparaissait très probable que ce déficit puisse également affecter les patients souffrant du syndrome de l'intestin irritable (Sii). C'est donc cette raison qui a motivé nos projets sur le Sii avec les Drs Bouin et Poitras.

Parallèlement, mon cher directeur (Dr Rainville) voulait étudier les mécanismes de modulation descendante en mesurant le réflexe RIII en IRMf. Étant donné la pertinence de ce projet pour les études avec les patients Sii, nous avons « rapidement » mis au point ce modèle expérimental avec l'optique d'étudier les dysfonctions des mécanismes endogènes de modulation de la douleur chez les patients Sii. C'est ce qui explique les études d'imagerie de cette thèse.

Une autre partie du travail que j'ai réalisé pendant mon doctorat concerne la mise au point de certaines méthodes d'imagerie fonctionnelle de la moelle épinière. Toute une équipe a travaillé sur ce projet incluant des membres de notre laboratoire et d'autres laboratoires (Drs Gilles Beaudoin, Habib Benali, Julien Doyon, Rick Hoge et Serge Rossignol). Ces méthodes auraient grandement rehaussé nos études, mais les défis techniques trop importants

dans le cadre d'un doctorat en sciences neurologiques ne m'ont pas permis de les inclure dans mes projets de thèse. Cependant, nous avons réalisé une étude descriptive concernant une problématique majeure en imagerie fonctionnelle de la moelle épinière, soit le bruit physiologique. L'article décrivant ces résultats est retrouvé en annexe étant donné qu'il s'intégrait difficilement au thème principal du présent ouvrage.

INTRODUCTION

INTRODUCTION

Dans la présente section, l'expérience subjective de la douleur et les mécanismes centraux sous-jacents à sa perception seront définis. Les mécanismes endogènes de modulation de la douleur qui sous-tendent l'analgésie par contreirritation seront ensuite discutés. Enfin, les mécanismes centraux de la douleur pathologique du syndrome de l'intestin irritable qui ont été proposés jusqu'à maintenant seront présentés.

L'expérience de la douleur

La douleur est une expérience subjective universelle que nous vivons à maintes reprises au cours de notre vie. Elle se caractérise par de multiples composantes qui soulignent sa complexité. Lorsque la douleur est perçue, la qualité de la sensation, son intensité, sa localisation et sa durée peuvent être décrites (composante sensoridiscriminative). De plus, l'aspect aversif et le sentiment désagréable de la sensation incitent à réagir à la source de douleur (composante motivoaffective). La sensation et ses conséquences sont également évaluées dans le contexte de notre expérience passée (composante cognitive). Enfin, ces processus psychologiques sont associés à des réponses physiologiques et comportementales. La douleur se distingue ainsi de la nociception, qui se définie plutôt par l'activation de récepteurs périphériques par des stimuli dommageables pour les tissus, ou qui deviendraient dommageables s'ils perduraient, et la transmission vers le cerveau des potentiels d'action associés. Évidemment, la

nociception et la douleur sont étroitement liées. Cependant, bien que la nociception mène habituellement à la perception de la douleur et que la douleur dépende généralement de la transmission d'information nociceptive, une dissociation des deux processus est possible dans certaines conditions.

Le substrat anatomique de la douleur

Suite à l'activation des nocicepteurs, les influx nociceptifs sont relayés à la corne dorsale de la moelle épinière par les fibres nerveuses A δ et C[1]. L'information nociceptive emprunte ensuite de nombreuses voies entre la moelle épinière et le cerveau, où elle active de multiples régions. Parallèlement, des projections descendantes du cerveau vers la corne dorsale peuvent inhiber ou faciliter la transmission nociceptive spinale, constituant ainsi les systèmes de modulation descendante de la douleur. Il existe également des connexions entre différentes structures cérébrales permettant l'intégration des diverses composantes de la douleur et sa modulation. Vu une telle complexité, la présente section ne se veut donc pas une revue exhaustive de toutes ces voies nerveuses. Nous décrirons plutôt les principales structures et voies impliquées dans la perception et la régulation endogène de la douleur (voir Figure 1), ainsi que les fonctions associées qui sont pertinentes pour les études de la présente thèse.

[1] Classiquement, on associe les nocicepteurs aux fibres Aδ et C, mais il est reconnu qu'une proportion significative de nocicepteurs (jusqu'à 65 % pour certaines espèces) est associée à des fibres Aβ (voir Djouhri and Lawson, 2004 pour une revue).

Voies ascendantes

On attribue classiquement les composantes sensoridiscriminative et motivoaffective de la douleur aux systèmes latéral et médian, qui sont constitués de deux voies ascendantes principales. La voie spinothalamique, associée au système latéral, achemine l'information nociceptive des membres et du tronc, de la corne dorsale de la moelle épinière vers plusieurs noyaux thalamiques, incluant les noyaux ventropostérolateral, centrolatéral, médiodorsal et le complexe postérieur (Mehler, 1962; Mehler, 1974). La voie spinoréticulaire, associée au système médian, achemine quant à elle les afférences nociceptives de la moelle épinière vers des structures du tronc cérébral, incluant la moelle allongée rostrale ventromédiale (RVM) (Willis and Coggeshall, 2004) et le noyau réticulaire dorsal (DRt)[2] de la moelle allongée[3] caudale (McMahon and Wall, 1985; Lima, 1990). Quoique la voie spinoréticulaire soit souvent présentée comme une voie importante dans l'activation de structures corticales par la douleur (Brodal P., 2004; Gray, 2005), elle transmettrait plutôt les afférences nociceptives spinales au tronc cérébral, où est intégrée l'information ascendante et descendante impliquée dans la nociception et la douleur (Willis and Coggeshall, 2004). En effet, l'information nociceptive de seulement quelques rares neurones spinoréticulaires rejoindraient le thalamus directement (Blomqvist and Berkley, 1992). Ceci n'exclut toutefois pas,

[2] Le noyau réticulaire dorsal est décrit chez le rat et est aussi appelé subnucleus reticularis dorsalis. Une zone fonctionnelle semblable est décrite chez le singe dans la formation réticulaire de la moelle allongée caudale (Villanueva et al. 1990). Chez l'humain, cette zone correspond à la localisation de la formation réticulaire parvocellulaire et à la partie dorsale du noyau réticulaire central de la moelle allongée.

[3] La terminologie anatomique de la présente thèse suit la nomenclature de la terminologia anatomica (1998). C'est pourquoi, entre autres, nous ferons référence à la moelle allongée plutôt qu'au bulbe rachidien.

en continuité à la voie spinoréticulaire, l'existence de projections réticulothalamiques, de la formation réticulaire du tronc cérébral vers les noyaux intralaminaires du thalamus (incluant les noyaux centromédian et parafasciculaire), qui forment le système médian de la douleur (Willis and Coggeshall, 2004).

Du thalamus, l'information nociceptive est ensuite relayée au cortex somesthésique primaire (SI) et secondaire (SII) (système latéral), ainsi qu'au cortex cingulaire antérieur (ACC) (système médian) et insulaire (INS). D'autres structures corticales sont indirectement activées par des projections corticocorticales, incluant l'aire motrice supplémentaire (SMA), le gyrus précentral (PrCG), le cortex cingulaire postérieur (PCC), le cortex pariétal postérieur (PPC) et le cortex préfrontal (PFC) (Price, 2000). La perception de la douleur repose donc sur un vaste réseau de structures, dont certaines sont préférentiellement[4] impliquées dans l'une ou l'autre des composantes de la douleur. En outre, SI et SII sont plutôt impliqués dans la dimension sensoridiscriminative (Bushnell et al., 1999; Ploner et al., 1999; Coghill et al., 1999a; Hofbauer et al., 2001; Chen et al., 2002; Kakigi et al., 2004; Forss et al., 2005) alors que ACC est plutôt impliqué dans la dimension affective (Rainville et al., 1997; Tolle et al., 1999; Fulbright et al., 2001). Quant à l'insula, elle est impliquée dans une vaste gamme de fonctions associées à la douleur. Elle joue un rôle autant dans les aspects sensoridiscriminatifs que motivoaffectifs de la douleur (Berthier et al., 1988; Brooks et al., 2005; Baumgartner et al., 2006) mais également dans des fonctions plus élaborées

[4] Quoique certaines régions soient plus spécifiquement impliquées dans une dimension de la douleur, elles peuvent également contribuer à d'autres composantes et en aucun cas les fonctions mentionnées ici ne sont exclusives.

comme la représentation de la condition physiologique du corps (intéroception) (Craig, 2002) et la conscience (Craig, 2009). Les cortex SMA et PrCG sont des aires motrices et leur activation lors de la douleur est fort probablement associée au contrôle moteur (Coghill et al., 1999b; Price, 2000). De plus, des études récentes suggèrent que le cortex moteur primaire pourrait également jouer un rôle dans la modulation de la douleur puisque l'application de la stimulation magnétique transcrânienne répétitive sur cette partie du cortex procure des effets analgésiques chez les patients souffrant de douleurs chroniques (Lefaucheur et al., 2006a; Lefaucheur et al., 2006b; Passard et al., 2007; Lefaucheur et al., 2008). Quant au PCC, son activité est associée à l'évaluation de la valence de stimuli externes potentiellement menaçants (Maddock and Buonocore, 1997; Maddock et al., 2003) et le modèle théorique des quatre régions du cortex cingulaire (Vogt, 2005) propose qu'il soit associé à l'orientation visuospatiale et squelettomotrice en réponse à des stimuli nociceptifs et non nociceptifs. Le PPC a quant à lui été impliqué dans les processus attentionnels (Peyron et al., 1999) et mnésiques (Albanese et al., 2007) de la douleur. Il a aussi une fonction d'intégration de l'information nociceptive et contextuelle afin d'élaborer la perception de la menace à l'intégrité physique (Price, 2000). Finalement, l'activation du PFC lors de stimulations douloureuses est sous-jacente, entre autres, à la réflexion sur les conséquences de la douleur, à la souffrance associée (parfois appelée affect secondaire de la douleur) et la planification des réponses comportementales (Price, 2000).

Aux voies spinothalamique et spinoréticulaire s'ajoutent plusieurs voies parallèles. La voie spinomésencéphalique (Menetrey et al., 1982) a des projections vers quelques structures de la partie rostrale du tronc cérébral, dont la substance grise périaqueducale (PAG), qui joue un rôle fort important dans la régulation de la douleur (Reynolds, 1969; Basbaum and Fields, 1984) et la régulation des fonctions cardiovasculaires (Behbehani, 1995). Les voies spinoparabrachioamygdalienne et spinoamygdalienne permettent quant à elles une activation directe de l'amygdale par les afférences nociceptives (Bernard and Besson, 1990; Cliffer et al., 1991). L'amygdale est une structure limbique impliquée dans la peur et l'anxiété (Kalin et al., 2004; Phelps and LeDoux, 2005) et joue un rôle dans la composante affective de la douleur et la régulation des réponses autonomiques associées (Neugebauer et al., 2004). La voie spinotélencéphalique inclut des projections vers le cortex cingulaire périgénual et le cortex orbitofrontal (OFC) (Cliffer et al., 1991). Ces structures jouent un rôle très important dans les émotions, la prise de décision (Rolls and Grabenhorst, 2008; Rolls, 2008) et il est probable que leur activation pendant des stimulations douloureuses soit associée à la composante motivoaffective de la douleur et à l'évaluation de la valeur punitive du stimulus nociceptif (Rolls and Grabenhors, 2008). OFC est également impliqué dans l'analgésie produite par certaines interventions cognitives (Petrovic and Ingvar, 2002; Petrovic et al., 2002; Petrovic et al., 2005).

Puisque nous aborderons la douleur viscérale, il est utile de mentionner l'existence de la voie postsynaptique des colonnes dorsales (Uddenberg, 1968). Quoique la voie spinothalamique achemine des afférences viscérales nociceptives

(Al-Chaer et al., 1999), la nociception viscérale dépend surtout de la voie postsynaptique des colonnes dorsales (Hirshberg et al., 1996; Al-Chaer et al., 1996a; Al-Chaer et al., 1996b; Al-Chaer et al., 1998; Willis et al., 1999; Al-Chaer et al., 1999; Wang et al., 1999; Nauta et al., 2000; Palecek et al., 2002). Cette dernière achemine l'information nociceptive viscérale de la moelle épinière vers les noyaux graciles et cunéiformes situés dans la portion caudale de la moelle allongée, qui est ensuite relayée au noyau ventropostérolatéral du thalamus pour finalement atteindre le cortex.

Les voies ascendantes nociceptives sont principalement croisées, mais elles comportent également un contingent de fibres ipsilatérales (Willis and Coggeshall, 2004). Ainsi, le patron d'activation cérébral observé lors de stimulations douloureuses est principalement controlatéral, mais certaines structures montrent aussi une activation bilatérale (Coghill et al., 1999b). Les connexions corticocorticales et sous-corticales renforcent également l'activation bilatérale pour certaines régions (Price et al., 2006a; Nieuwenhuys et al., 2008).

Voies descendantes

Les voies descendantes impliquées dans la modulation de la douleur proviennent de structures corticales, sous-corticales et du tronc cérébral, et se terminent dans la corne dorsale de la moelle épinière. Le système de modulation descendante de la douleur sans doute le plus étudié est constitué de la PAG et de la RVM. Une stimulation électrique de la PAG procure une profonde analgésie, autant chez l'animal que chez l'humain (Reynolds, 1969; Hosobuchi, 1980; Mayer, 1984; Baskin et al., 1986). De plus, la stimulation de la RVM ou son activation par

la PAG peut inhiber ou faciliter la douleur par des projections vers la corne dorsale de la moelle épinière (Fields and Basbaum, 1978; Basbaum and Fields, 1984; Fields et al., 1991).

Des projections descendantes ont également été décrites entre le DRt et la corne dorsale de la moelle épinière du rat (Bernard et al., 1990), suggérant un rôle modulateur du DRt dans la transmission nociceptive spinale. Cette hypothèse a d'ailleurs été confirmée par des études chez le rat (Bouhassira et al., 1992b; Villanueva et al., 1996b). Une étude réalisée chez des patients avec lésions neurologiques suggère également que ces mécanismes seraient très similaires chez l'humain (DeBroucker T. et al., 1990) (voir section sur *la contreirritation*).

Certaines régions corticales peuvent également moduler la transmission nociceptive spinale en modulant l'activité 1) de structures corticales qui projettent vers la moelle épinière 2) de structures du tronc cérébral qui projettent vers la moelle épinière ou 3) des neurones de la corne dorsale de la moelle épinière directement. Par exemple, il a été montré qu'une stimulation électrique de OFC produit une inhibition de la transmission nociceptive spinale et que cet effet dépend de l'activation du système PAG-RVM (Hutchison et al., 1996; Zhang et al., 1997). En revanche, une stimulation électrique de ACC produit une facilitation de la nociception spinale par l'activation de DRt (Zhang et al., 2005). L'insula antérieure peut quant à elle moduler la douleur de façon bidirectionnelle en activant l'amygdale et d'autres structures du cortex ou du tronc cérébral (Jasmin et al., 2003; Jasmin et al., 2004). Quant aux projections corticospinales, une voie originant de SI permet l'inhibition des neurones de la corne dorsale de la moelle

épinière (Yezierski et al., 1983; Cheema et al., 1984; Ralston and Ralston, 1985; Casale et al., 1988).

Les réflexes nociceptifs

En plus de réponses comportementales évoquées par la douleur, des réponses réflexes peuvent également survenir dans certaines conditions. Un exemple commun est un réflexe moteur, le réflexe nociceptif de flexion, qui permet le retrait d'un membre d'une source nociceptive avant même la perception de la douleur. Les mécanismes médullaires du réflexe de flexion ont été mis en évidence par Sherrington dans une étude très exhaustive chez le chat (Sherrington, 1910). Chez l'humain, il est possible de produire le réflexe de flexion expérimentalement, généralement par une stimulation électrique ou un laser (Sandrini, 2005). Le réflexe de flexion est un réflexe spinal polysynaptique impliquant plusieurs segments médullaires et plusieurs muscles et articulations. Lorsque produit par une stimulation électrique du nerf sural, il comporte une composante avec une latence autour de 90 ms qui reflète le recrutement des fibres Aδ (groupe III). Certains auteurs ont ainsi désigné ce réflexe « RIII » en référence au groupe des afférences impliquées (Hugon, 1973). D'un point de vue expérimental, le RIII est d'un intérêt particulier pour l'étude de la douleur puisque son seuil correspond généralement assez bien au seuil de perception de la douleur (Willer, 1977). Cependant, la production du RIII, en plus de l'activation des fibres nociceptives, implique toujours l'activation de fibres cutanées non nociceptives. Les stimulations produiisant le RIII ainsi que les

réponses périphériques et centrales associées ne peuvent donc pas être présumées comme spécifiquement nociceptives. De plus, certaines conditions peuvent entraîner une dissociation entre l'amplitude du RIII et la perception de la douleur (Danziger et al., 1998a; Bouhassira et al., 2003; Terkelsen et al., 2004; French et al., 2005; Defrin et al., 2007). Ceci souligne d'ailleurs que le réflexe RIII n'est en aucun cas une mesure absolue de l'expérience subjective de douleur, mais bien un indice de la transmission nociceptive spinale.

Les stimulations nociceptives produisent également des réflexes autonomiques qui modulent l'activité des viscères par les voies sympathiques et parasympathiques. Un réflexe sympathique d'intérêt dans l'étude de la douleur est la réponse de conductance électrodermale (SCR), soit la production de sueur dans la paume de la main et la plante du pied. Un des rôles importants de la peau est de réguler la température corporelle, qu'elle accomplit entre autres par la production de sueur par les glandes sudoripares eccrines. Ces glandes sont retrouvées sur tout le corps, mais une concentration plus importante est retrouvée dans la paume de la main et la plante du pied (Edelberg, 1972). Ces glandes sont particulièrement influencées par des changements de l'état mental ou des stimuli émotionnels (comme la douleur), et seraient aussi impliquées dans certaines fonctions motrices comme la préhension (Smith et al., 1997). La production de sueur en réponse à des stimuli émotionnels ou douloureux peut être mesurée par des électrodes de surface, qui indiquent la capacité de la peau à conduire un courant, d'où l'appellation *réponse de conductance électrodermale.* Les SCR reflètent des changements de l'activité du système nerveux

sympathique, puisque les glandes sudoripares eccrines ne reçoivent aucune innervation parasympathique (Dawson et al., 2007).

Dans la présente thèse, les réflexes nociceptifs ont été utilisés comme index de la transmission nociceptive spinale (réflexe RIII) et comme un indice de l'orientation sensorimotrice et de la composante affective de la douleur (SCR). De plus, l'implémentation de ces mesures en imagerie par résonance magnétique fonctionnelle (IRMf) nous a permis d'étudier les réponses cérébrales associées aux réflexes nociceptifs afin de déterminer, parmi les régions cérébrales activées par la douleur, lesquelles sont impliquées dans la régulation sensorimotrice et autonomique.

La contreirritation

La contreirritation est une intervention connue depuis des siècles et désigne l'inhibition d'une douleur par une deuxième douleur appliquée sur un autre endroit du corps. Elle est utilisée depuis l'Antiquité pour traiter la douleur et elle est à la base de certaines approches thérapeutiques, comme l'électrothérapie ou la moxibustion (Willer et al., 1999). Ses mécanismes ont été largement étudiés et sont relativement bien compris chez l'animal. Quoiqu'ils semblent assez similaires, ils ne sont pas encore complètement élucidés chez l'humain. Les études 1 et 2 de la présente thèse avaient pour objectif de développer un modèle expérimental permettant d'examiner les mécanismes sous-jacents à l'analgésie induite par la contreirritation chez l'humain. Une brève revue de ces mécanismes chez l'animal et chez l'humain sera présentée

dans cette section et nous insisterons sur les éléments qui sont encore méconnus.

Mécanismes de la contreirritation chez l'animal

La première expérience investiguant les mécanismes de la contreirritation a mis en évidence l'inhibition spécifique des neurones à convergence par des stimulations nociceptives appliquées sur l'étendue de la surface du corps, extérieure à leurs champs récepteurs (Le Bars et al., 1979). Ces mécanismes de modulation ont ainsi été nommés « contrôles inhibiteurs diffus nociceptifs » (DNIC) puisqu'ils affectent les neurones à convergence de tous les niveaux (diffus) et parce qu'il ne sont déclenchés que par des stimulations intenses (nociceptives). Par la suite, plusieurs expériences ont précisé l'origine de cette modulation. Il a ainsi été montré que l'inhibition induite par le DNIC dépendait de structures supraspinales puisque les animaux ayant une lésion complète de la moelle épinière cervicale ne présentaient pas d'inhibition (Cadden et al., 1983). De plus, il a été montré que le DNIC dépendait d'une voie ascendante empruntant le cordon ventrolatéral et d'une voie descendante empruntant le cordon dorsolatéral de la moelle épinière (Villanueva et al., 1986a; Villanueva et al., 1986b). D'autres expériences ont précisé que la moelle allongée caudale (en particulier le DRt) jouait un rôle primordial dans le DNIC (Bouhassira et al., 1992b) alors qu'une lésion de la PAG, du noyau cunéiforme mésencéphalique, de l'aire parabrachiale, du locus coeruleus ou de la RVM ne modifiait pas l'action inhibitrice de la contreirritation

(Bouhassira et al., 1990; Bouhassira et al., 1992a; Bouhassira et al., 1992c; Bouhassira et al., 1993a). Cependant, il est intéressant de noter que la PAG peut moduler le DNIC. En effet, l'administration d'une faible dose de morphine à des rats ayant une lésion de la PAG n'affecte pas le DNIC alors que chez des rats sains, le DNIC est bloqué par la morphine (Bouhassira et al., 1992c). D'autres expériences ont également montré le même effet de la morphine sur le réflexe nociceptif de flexion induit par la stimulation des fibres C chez le rat (Falinower et al., 1994). Chez l'animal, il semble donc assez clair que l'effet analgésique de la contreirritation dépend du DNIC, au moins en partie, qui repose lui-même sur une boucle spinobulbospinale. Cependant, cette boucle modulatrice n'explique pas complètement l'effet inhibiteur de la contreirritation sur la nociception spinale (Bouhassira et al., 1992b) et d'autres voies constituées de structures corticales ou sous-corticales sont susceptibles d'être impliquées dans l'analgésie induite par la contreirritation.

Mécanismes de la contreirritation chez l'humain

Expérimentalement, l'effet analgésique de la contreirritation a été montré dans plusieurs études chez l'humain (Pertovaara et al., 1982a; Jungkunz et al., 1983; Chen et al., 1985; Talbot et al., 1987). Dans ces études, l'application soutenue d'un stimulus douloureux (ischémie ou froid) atténuait la perception d'un autre stimulus douloureux (électrique ou thermique). Dans l'étude de Chen et al., l'amplitude des potentiels évoqués par la stimulation électrique (les composantes nociceptives spécifiquement) était aussi inhibée par la

contreirritation, corroborant les évaluations subjectives de douleur. Des études électrophysiologiques dans lesquelles le réflexe RIII était enregistré parallèlement aux évaluations subjectives de douleur ont également permis de préciser les mécanismes centraux de la contreirritation chez l'humain. Cependant, les résultats des différentes études montrent certaines contradictions que nous allons maintenant préciser. La première de ces études a montré que la contreirritation provoquée par une stimulation électrique douloureuse du nerf ulnaire produisait une inhibition des évaluations subjectives de douleur évoquée par la stimulation électrique du nerf sural, alors que le réflexe RIII produit par cette même stimulation n'était pas modulé (Willer et al., 1979). Considérant le réflexe RIII comme un index de la transmission nociceptive spinale, les auteurs ont ainsi conclu que l'effet analgésique de la contreirritation impliquait des mécanismes supraspinaux. Ils ont également proposé que la dissociation entre la perception de la douleur et le réflexe moteur permette de préserver le rôle protecteur du réflexe RIII (retrait du membre de la source douloureuse) tout en prévenant une perception douloureuse excessive qui pourrait engendrer des réactions autonomiques néfastes pour l'organisme. Dans une étude ultérieure, la contreirritation provoquée par une stimulation thermique nociceptive (chaud douloureux) a produit des résultats contradictoires. En effet, l'inhibition de la perception de la douleur évoquée par la stimulation électrique du nerf sural était accompagnée d'une inhibition du réflexe RIII (Willer et al., 1984a). Les auteurs ont alors suggéré que l'analgésie par contreirritation dépendait plutôt de l'inhibition de la

transmission nociceptive spinale, au moins en partie. Cette interprétation a été confirmée par une étude subséquente dans laquelle des patients tétraplégiques avec lésion complète de la moelle épinière ne montraient pas d'inhibition du réflexe RIII pendant la contreirritation (provoquée par une stimulation électrique des 4^{e} et 5^{e} doigts) en comparaison aux sujets sains (Roby-Brami et al., 1987). D'autres études réalisées chez des patients avec des lésions neurologiques à différents niveaux du névraxe (hémisection de la moelle épinière, moelle allongée, thalamus) ont permis de préciser que l'effet analgésique et l'inhibition du réflexe RIII par la contreirritation dépendait en partie d'une boucle nerveuse incluant la voie spinoréticulaire, la partie caudale de la moelle allongée ainsi que le cordon dorsolatéral de la moelle épinière, et excluant la voie spinothalamique (DeBroucker T. et al., 1990; Bouhassira et al., 1993b). Ces résultats sont donc cohérents avec l'existence des contrôles inhibiteurs diffus nociceptifs (DNIC), tels que décrits chez le rat (LeBars D. et al., 1992; Villanueva and LeBars D., 1995; Bouhassira and Danziger, 2006).

La dissociation entre l'amplitude du réflexe RIII et la perception de la douleur observée dans la première étude demeure inexpliquée et contraire aux autres expériences. Une étude plus récente a cependant montré cette dissociation lorsque la contreirritation était appliquée sur une région du corps dont les afférences projettent sur les mêmes segments que le nerf sural (Terkelsen et al., 2001). Ceci suggère une interaction possible entre les effets inhibiteurs des contrôles descendants et les afférences nociceptives segmentaires du stimulus de contreirritation mais ne peut expliquer les résultats

de l'étude de Willer et al. dans laquelle la contreirritation était hétérosegmentaire (Willer, 1979). Une autre possibilité serait que l'effet analgésique de la contreirritation ne repose pas seulement sur des mécanismes de régulation descendante, mais qu'il dépende également de mécanismes supraspinaux indépendants de la modulation du réflexe RIIII. Le modèle expérimental mis au point et utilisé dans cette thèse a permis d'étudier cette question, que nous discuterons en détail à la lumière de nos résultats.

Puisque le deuxième volet de cette thèse concerne la douleur pathologique du syndrome de l'intestin irritable, il est utile de mentionner les quelques études utilisant la contreirritation pour mettre en évidence un déficit de modulation de la douleur dans certains syndromes douloureux. En outre, il a été montré que des patients atteints de fibromyalgie présentent un déficit de modulation de la douleur lors de la contreirritation (Kosek and Hansson, 1997; Lautenbacher and Rollman, 1997). Des patients souffrant de migraines ou de céphalées de tension chroniques présentent également un déficit de modulation de la douleur ainsi qu'un déficit de modulation des processus nociceptifs spinaux (Pielsticker et al., 2005; Sandrini et al., 2006). Concernant les études avec des patientes Sii, elles seront discutées dans la prochaine section.

Le syndrome de l'intestin irritable

Le syndrome de l'intestin irritable (Sii) est défini comme un trouble digestif fonctionnel, sans lésion pathologique détectable, comportant à la fois des douleurs abdominales et des changements dans le transit gastrointestinal (Drossman, 2006). En plus de ces symptômes, l'exclusion d'autres conditions gastroentérologiques est essentielle pour confirmer le diagnostic. En outre, les maladies intestinales inflammatoires et la maladie coeliaque doivent être exclues par endoscopie et par des tests sérologiques (Mayer, 2008). Le Sii est un problème de santé majeur puisqu'il touche 10 à 15 % de la population (Drossman et al., 2002), affecte la qualité de vie de façon importante (Poitras et al., 2002a; Spiegel et al., 2004) et entraîne des coûts très élevés (Hulisz, 2004). Quoique le Sii soit d'abord et avant tout un trouble gastroentérologique, les études de la présente thèse concernent uniquement les éléments pathologiques de la douleur et visent à mieux comprendre l'implication des mécanismes centraux de la douleur dans le Sii. Malgré de nombreuses études dans les dernières années pour tenter de mieux comprendre la pathophysiologie du Sii, les mécanismes précis des douleurs abdominales sont encore mal définis.

L'hypersensibilité chez les patients Sii

L'hypersensibilité viscérale chez les patients Sii a été rapportée pour la première fois par Ritchie lors de distensions avec un ballon rectal (Ritchie, 1973). Dans cette étude, la douleur était présente chez une plus grande proportion de patients Sii que de volontaires sains, pour les mêmes volumes de

distension. De plus, la tension de la paroi intestinale nécessaire pour produire une douleur chez les contrôles était beaucoup plus élevée que chez les patients Sii. Par la suite, malgré une étude contradictoire (Latimer et al., 1979), plusieurs études ont confirmé ces résultats (Whitehead et al., 1990; Bradette et al., 1994; Mayer and Gebhart, 1994; Mertz et al., 1995; Naliboff et al., 1997; Slater et al., 1997; Verne et al., 2001; Bouin et al., 2002). L'hypersensibilité viscérale est même considérée par certains comme un marqueur biologique du Sii (Mertz et al., 1995) étant donné sa prévalence de 90 à 94 % chez ces patients (Mertz et al., 1995; Bouin et al., 2002; Poitras et al., 2002b). De plus, le test du barostat permet de discriminer les patients Sii sur la base de l'hypersensibilité viscérale avec une efficacité de plus de 86 % (Bouin et al., 2002). L'idée que l'hypersensibilité viscérale soit une des étiologies principales de la douleur abdominale rapportée par les patients Sii est également cohérente avec les études montrant qu'elle est réversible par une application de lidocaïne dans le rectum (Verne et al., 2003b) et que cette intervention est un traitement efficace pour diminuer les douleurs abdominales des patients Sii (Verne et al., 2005). Cependant, certains auteurs associent l'hypersensibilité à des facteurs psychologiques (Whitehead and Palsson, 1998) et il a même été suggéré que l'hypersensibilité viscérale ne serait que le reflet d'une tendance à rapporter plus de douleur plutôt qu'une augmentation de la transmission nociceptive (Dorn et al., 2007). Toutefois, les résultats sont contradictoires à une étude électrophysiologique (Coffin et al., 2004) et sont questionables en raison de la

méthodologie utilisée. Cette question a d'ailleurs été spécifiquement abordée dans l'étude 4 de cette thèse.

Afin de déterminer si les patients Sii présentaient une altération diffuse de la sensibilité à la douleur ou plutôt une altération sélective de la sensibilité viscérale, la sensibilité somatique des patients Sii a été évaluée dans plusieurs études depuis une vingtaine d'années. Dans la première étude, les patients Sii présentaient une plus grande tolérance à la douleur évoquée par des stimulations électriques appliquées sur l'avant-bras (Cook et al., 1987). Par la suite, des études ont confirmé la spécificité de l'hypersensibilité à la sensibilité viscérale (Whitehead et al., 1990; Zighelboim et al., 1995; Accarino et al., 1995; Chang et al., 2000; Iovino et al., 2006.), alors que d'autres ont montré que les patients Sii présentaient une hypersensibilité diffuse, touchant également la sensibilité somatique (Verne et al., 2001; Verne et al., 2003b; Rodrigues et al., 2005; Caldarella et al., 2006; Wilder-Smith and Robert-Yap, 2007; Moshiree et al., 2007). Ces résultats contradictoires pourraient s'expliquer par des considérations méthodologiques, mais comme nous le suggérerons plus loin, elles peuvent aussi refléter l'hétérogénéité importante de l'étiologie et de la pathophysiologie de ce syndrome.

Déficit de modulation de la douleur chez les patients Sii

Ce n'est que tout récemment que l'altération des mécanismes de modulation de la douleur a été proposée comme mécanisme physiopathologique de la douleur chez des patientes Sii (Wilder-Smith et al., 2004; Song et al., 2006; Wilder-Smith and Robert-Yap, 2007). Dans ces études,

la contreirritation a été utilisée pour moduler la douleur rectale évoquée par des distensions et les patientes Sii montraient une moindre inhibition de la douleur rectale en comparaison aux contrôles. Cela suggère que les mécanismes de contrôle descendant de la douleur, en particulier le DNIC, soient altérés chez ces patientes. En appui au déficit de modulation descendante de la douleur, il a été montré que la transmission nociceptive spinale (indexée par le réflexe RIII) n'est pas inhibée par une distension rectale de 3 minutes chez des patientes Sii, alors que les contrôles montrent une réponse biphasique avec facilitation dans la première minute de distension, suivie d'une inhibition par la suite (Coffin et al., 2004). Cependant, les liens entre ces déficits de modulation de la douleur, l'hypersensibilité et les facteurs psychologiques n'ont pas encore été étudiés spécifiquement. Les études 3 et 4 de cette thèse ont examiné si le déficit de modulation de la douleur affecte également la sensibilité somatique chez les patientes Sii et ont testé sa relation avec l'hypersensibilité diffuse, la modulation descendante de la nociception spinale et les symptômes psychologiques.

Présentation des études de la thèse

Dans le premier article de cette thèse, une validation de notre modèle expérimental de douleur en résonance magnétique fonctionnelle sera présentée. Dans cet article, nous discuterons également des différences individuelles d'activité cérébrale évoquée par des stimulations nociceptives et de leur association aux différences individuelles de perception de la douleur, de réactivité motrice et de

réactivité autonomique. De plus, nous discuterons les mécanismes associés aux fluctuations de l'amplitude du réflexe RIII et des SCR. Dans la deuxième étude de la thèse, ce modèle expérimental a été utilisé pour déterminer les mécanismes centraux de la contreirritation. L'article 2 décrira les mécanismes cérébraux et cérébrospinaux de la modulation de la douleur et du réflexe RIII par la contreirritation. Dans le troisième article, des méthodes psychophysiques et psychométriques ont été utilisées pour étudier la relation entre l'hypersensibilité diffuse retrouvée chez les patientes souffrant du syndrome de l'intestin irritable, l'altération des mécanismes de modulation de la douleur et la sévérité des symptômes psychologiques. Dans le quatrième article de la thèse, la mesure du réflexe RIII a été utilisée dans un paradigme de contreirritation pour déterminer si l'altération des mécanismes de modulation de la douleur était reliée à une altération de la modulation de la nociception spinale.

ARTICLES

Article 1 : Dissection of perceptual, motor and autonomic components of brain activity evoked by noxious stimulation

Dissection of perceptual, motor and autonomic components of brain activity evoked by noxious stimulation

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ABSTRACT

In the past two decades, functional brain imaging has considerably advanced our knowledge of cerebral pain processing. However, many important links are still missing in our understanding of pain-related brain activity in relation to regulation of peripheral physiological responses. In this fMRI study, we investigated the cerebral correlates of pain perception, motor (RIII reflex) and autonomic (skin conductance response (SCR)) responses evoked by noxious electrical stimulation. *Individual differences* in shock-related activity in cingulate, orbitofrontal and parahippocampal regions specifically predicted pain sensitivity. Moreover, specific orbitofrontal and cingulate areas showed strong positive associations with *individual differences* in motor reactivity but negative associations with autonomic reactivity. In addition, *trial-to-trial fluctuations* of RIII reflex and SCR were proportional to *trial-to-trial fluctuations* of shock-evoked activity in subgenual cingulate cortex (RIII), anterior insula (SCR) and midcingulate cortex (SCR and RIII). Together, these results demonstrate that *individual differences* in perceptual, motor, and autonomic components of the pain experience reflect robust individual differences in brain activity. Furthermore, the associations between *trial-to-trial fluctuations* of pain responses provide direct evidence of brain regulation of peripheral motor and autonomic responses. This experimental model will be useful to investigate cerebral dysfunction of nociceptive and pain processes in clinical populations with chronic pain.

INTRODUCTION

In the past two decades, functional brain imaging has considerably advanced our knowledge of cerebral pain processing. For instance, it was shown that the primary somatosensory cortex (SI) is involved in the sensori-discriminative component of pain (Bushnell et al., 1999; Ploner et al., 1999; Coghill et al., 1999b; Hofbauer et al., 2001) and the anterior cingulate cortex (ACC) in encoding pain affect (Rainville et al., 1997). Other regions such as the prefrontal (PFC) and orbitofrontal (OFC) cortices were shown to be related to cognitive and emotional aspects of pain (Price, 2000; Rolls et al., 2003). The insula (INS) from its posterior division to its more anterior division was shown to cover a broad spectrum of functions related to pain, from perception to interoception and awareness (Craig, 2002; Brooks et al., 2005; Craig, 2009). It was also demonstrated that individual differences in pain sensitivity is related to activity in SI, ACC and PFC (Coghill et al., 2003). In spite of these advances however, many important links are still missing in our understanding of pain-related brain activity in relation to the regulation of peripheral motor and autonomic responses.

As for motor responses, pain is associated with the spinally mediated nociceptive flexion reflex (RIII), which provides a protective mechanism by triggering the withdrawal of a limb from a source of nociceptive input (Sherrington, 1910). This reflex has been widely used as an index of spinal nociception in numerous psychophysiological studies (Boureau et al., 1978; Willer et al., 1979; Bouhassira et al., 1994; Rhudy et al., 2005) (see Sandrini et

al., 2005 for review). However, the relation between this nociceptive motor response and brain activity has not been investigated directly.

Although the appropriate well-controlled experimental conditions allow the generation of a relatively stable RIII reflex, between-subject variability (motor reactivity) and intra-subject variability (trial-to-trial fluctuations) are observed in every study. This variability may reflect basic individual differences in spinal sensorimotor processes and spontaneous fluctuations, respectively. In turn, this variability is expected to affect the corresponding cerebral responses through multiple ascending pathways. Additionally, cerebral processes affecting spinal activity through descending modulatory pathways contribute to fluctuations in the gain of the spinal response by acting on the sensory and/or motor component of the reflex (Mayer and Price, 1976; Fields and Heinricher, 1989; Villanueva and LeBars D., 1995; Villanueva et al., 1996a). Investigating the cerebral representation of spinal nociceptive processes may significantly contribute to our understanding of neurophysiological pain processes in humans.

In addition to motor responses, noxious stimulation evokes autonomic activity associated with the affective dimension of the pain experience (Rainville et al., 2005). Accordingly, the noxious electrical stimulation used to elicit the RIII reflex also elicits a robust skin conductance response (SCR) which is sensitive to the psychological context (Rhudy et al., 2007b; Rhudy et al., 2007c), confirming the regulatory influence of brain structures (Dawson et al., 2000). SCR amplitude also varies both between subjects (autonomic reactivity) and

within subjects (trial-to-trial fluctuations) and this variability may reflect the activity of brain structures involved in the regulation of orienting and arousal. Identifying these mechanisms must be achieved to improve our understanding of the brain regulation of autonomic functions related to acute pain.

In the present study, pain-related brain activity was examined using fMRI while the RIII reflex and the SCR were elicited using painful electrical stimulation. We specifically examined 1) *Individual differences* in pain perception, motor reactivity and autonomic reactivity and 2) *trial-to-trial fluctuations* of RIII and SCR. We hypothesized that pain sensitivity, and motor reactivity and autonomic reactivity would involve partly separable brain networks. We further postulated that the variability in brain responses to noxious stimuli partly reflects the gain of spinal sensorimotor responses (RIII reflex) and autonomic regulatory processes (SCR).

METHODS

Participants

Fourteen healthy volunteers participated in the study (three males and eleven females; all right-handed except for one subject; mean age 26.9 years; SD, 4.7). Data from 3 participants were excluded because of missing SCR data so a total of eleven subjects were included for all analyses. All participants were familiarized with the stimuli and the pain evaluation procedure in a MRI simulator before the scanning sessions to insure that they would tolerate the experimental procedures. The Research Ethics Board of the “Centre de

recherche de l'Institut de gériatrie de Montréal" approved the study. All participants gave written informed consent and received a compensation of 50$ for the brain imaging session.

Stimulation paradigm

Transcutaneous electrical stimulation was delivered with a Grass S48 square pulse stimulator (Astro-Med Inc., West Warwick, RI, USA) connected to a custom made constant current stimulus isolation unit. The stimulator was equipped with a custom made RF filter preventing artefacts in fMRI data. The stimulation consisted in a train of 10 pulses over 30 ms, delivered on degreased skin over the retro-maleolar path of the right sural nerve by means of a pair of 1 cm^2 custom made surface electrodes (inter-electrode distance: 2 cm). Individual RIII reflex threshold was determined using the staircase method Willer, 1977. Stimulus level determination (see below) and RIII reflex threshold were performed after positioning the participant in the scanner and before the acquisition of functional images.

In each functional scan, 40 stimuli were delivered with a pseudo-random ISI of 6, 9, 12 or 15s. The electrical stimuli were synchronized to the image acquisition with a script running in E-Prime (Psychology Software Tools, Inc., Pittsburgh, PA, USA). Stimulus intensity was set to 150% (mean ± SD: 15.3 ± 4.5 mA) of the RIII reflex threshold to evoke a robust RIII response, which was felt as moderately painful. This method insured that the stimulus was adjusted individually to the subjects' threshold and that variability in the amplitude of the

RIII reflex reflected differences in the *gain* of the spinal nociceptive processes at suprathreshold levels. An additional scan was performed at a lower stimulus intensity (120% of RIII reflex threshold) to confirm the validity of the physiological measurements obtained during fMRI acquisition, as described in the *Supplementary material* (Figures S1 and S2).

Pain evaluations

A visual analog scale (VAS) was used to evaluate the pain induced by the electrical stimulation at the end of each functional run only. This was done in order to avoid contamination of physiological responses (especially SCR) by evaluative processes. Participants were shown the VAS on a computer monitor back-projected onto a screen and viewed on a mirror placed on the head coil in front of the participant's eyes. The VAS was placed horizontally and included the verbal anchors "no pain" and "worst pain imaginable" at the left and right extremities, respectively (Price et al., 1994). Participants used a MRI-compatible response key to move a cursor on the VAS. All ratings were converted linearly to a 0-100 scale. These ratings served as individual pain sensitivity and were used in the multiple regression analysis of shock-evoked brain activity (see below).

fMRI acquisition

Imaging data was acquired at "Unité de Neuroimagerie Fonctionnelle" of the "Centre de recherche de l'Institut de gériatrie de Montréal" on a 3T Siemens

Trio scanner (Munich, Germany) using a CP head coil. The head of the participant was stabilized in a comfortable position using a vacuum bag. Participants were instructed to refrain as much as possible from moving throughout the imaging session and were given earplugs to reduce the noise from the scanner. The anatomical scans were T1-weighted high-resolution scans [repetition time (TR): 13 ms; echo time (TE): 4.92 ms; flip angle: 25°; field of view: 256 mm; voxel size: 1 x 1 x 1.1 mm]. The functional scans were collected using a blood oxygen level-dependent (BOLD) protocol with a T2*-weighted gradient echo-planar imaging sequence (TR: 3.0 s with an inter-volume delay of 500 ms; TE: 30 ms; flip angle: 90°; 64 x 64 matrix; 130 volume acquisitions. Electrical stimuli were always administered during the inter-volume delay (*see supplementary material* - Figure S1), thereby avoiding the potential contamination of fMRI images by shock-induced artefacts and of EMG recordings by RF-pulse artefacts. The scanning planes were oriented parallel to the anterior-posterior commissure line and covered the entire brain from the vertex of the cortex to the first segments of the spinal cord (41 contiguous 5-mm-thick slices; voxel size, 3.44 x 3.44 x 5 mm).

Physiological measurements and analysis

Electromyographic (EMG) activity of the right (ipsilateral) biceps femoris and SCR at the sole of the left foot was recorded with MRI-compatible Ag-AgCl surface electrodes (Type EL-508) using Biopac MP150 system (Biopac systems Inc., Goleta, CA, USA). Custom made RF filters were used for the recording of

physiological measures to prevent introducing artefact in the fMRI data. EMG activity was amplified, band pass filtered (100-500 Hz), digitized and sampled at 1000 Hz. SCR was band pass filtered (0.05-1 Hz), sampled at 1000 Hz and temporally smoothed (0.5 sec.). EMG and SCR data were analysed using Aqcknowledge 3.8 (Biopac systems Inc., Goleta, CA, USA). The raw EMG recordings were filtered off-line (120-130 Hz) and transformed using the root mean square. The resulting signal was integrated between 90-180 ms after the stimulus onset to quantify the RIII reflex to each shock to provide a within-subject regressor in the analysis of *trial-to-trial fluctuations* (see *parametric modulation analyses* below). These values were also averaged over the 40 stimulations of each scan to get the subject's mean response. The mean responses from all subjects were then ranked and determined individual *motor reactivity.* Motor reactivity served as a between-subject regressor in the analysis of *interindividual differences* in pain responses (see *multiple regression analysis* below). It is important to note that stimulus intensity was adjusted to control for individual differences in basic aspects of sensorimotor processing (i.e. RIII threshold). Therefore, motor reactivity reflects the *gain* of spinal sensorimotor processes, i.e. the individual differences in the magnitude of the RIII reflex associated with the 50% increase in stimulus intensity after controlling for interindividual differences in threshold. Consistent with the effectiveness of this control, motor reactivity was completely independent from stimulus intensity across individuals (Pearson's $r^2 < 0.01$, $p = 0.98$).

The onset-to-peak amplitude (within 6 seconds) of the SCR was used to quantify autonomic responses to each shock to provide a within-subject regressor in the analysis of *trial-to-trial fluctuations (see parametric modulation analysis* below). These values were also averaged across all trials to get the subject's mean response. The mean responses for all subjects were then ranked and determined individual *autonomic reactivity*. Autonomic reactivity served as a between-subject regressor in the analysis of *interindividual differences* in pain responses (see *multiple regression analysis* below).

fMRI data analyses

Brain imaging data was analyzed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm/). Pre-processing included slice-time correction and realignment. Anatomical and functional images were then spatially normalized to a standard stereotaxic space using the MNI template. Subsequently, functional images were spatially smoothed using a Gaussian kernel of twice the voxel size (FWHM: 7 x 7 x 10 mm), temporally filtered using a high-pass filter with a cut-off period of 128s, and were corrected for serial autocorrelation using the AR(1) correction implemented in SPM. For each participant, stimulus-related activity was identified with an event-related design by convolving a vector of the onset timings for each stimulus with a canonical hemodynamic response function (hrf). The general linear model was used to model the effects of interest. Group analyses were conducted using random-

effect models with contrast images of individual-subject effects. Three analytic approaches were used.

First, the responses evoked by *noxious stimulation* were assessed by a random-effect one-sample t-test, using images of stimulus-evoked responses from each subject. This analysis provided a non-specific map of cerebral responses to noxious stimulation and confirmed that the short-duration electrical stimulus produced a brain activation pattern consistent with previous pain imaging studies.

Second, in order to investigate the cerebral correlates of *interindividual differences* in pain responses, a *multiple regression analysis* was performed to identify areas activated by *noxious stimulation* and specifically related to pain sensitivity and motor and autonomic reactivity. The random-effect multiple regression model included individual contrast images of *noxious stimulation,* three target regressors for pain sensitivity, motor reactivity and autonomic reactivity, and one covariate of no interest for stimulus intensity (each of those regressors containing one value per subject). The covariate for stimulus intensity was added as a conservative measure to insure that correlations between brain activity and pain responses were not confounded with differences in stimulus intensity (see determination of stimulus intensity above). This analysis allowed the separation of distinct brain processes underlying individual differences in perceptual, motor and autonomic components of the pain experience.

Third, to determine *trial-to-trial fluctuations* in shock-evoked brain activity that specifically covaried with *trial-to-trial fluctuations* in RIII reflex or SCR amplitude, covariance analyses were performed based on the *parametric modulation* of shock-evoked responses. The individual design matrix used here included vectors of onset timings for each stimulus and included two trial-to-trial modulation parameters: one for the RIII reflex amplitude and another one for SCR amplitude (each of those regressors containing 40 values, i.e. one per stimulus). Contrast images were then generated to determine brain activity specifically associated with the RIII reflex (RIII Vs SCR) or SCR amplitude (SCR Vs RIII). These individual contrast-images where then used in the group analyses using random-effect one-sample t-test models. This revealed patterns of brain responses specifically associated with trial-to-trial fluctuations of the RIII reflex or SCR, and consistently found across subjects (i.e. random-effect).

In all analysis models tested, a directed search was first conducted over brain areas receiving nociceptive afferents and/or involved in the regulation of nociceptive processes (seeApkarian et al., 2005). We hypothesized that some of the structures previously shown to respond to acute noxious stimulation, namely the thalamus, primary and second somatosensory cortices (SI-foot area and SII), the insula (INS) and the mid and anterior cingulate cortex (MCC and ACC), would display responses covarying with RIII and/or SCR, consistent with the activation of the multiple pathways ascending from the spinal cord to various brain structures (primarily through the spinothalamocortical pathway; Treede et al., 1999). The RIII reflex amplitude was further expected to predict cerebral

activation within brain networks previously suggested to be involved in the cerebro-spinal regulation of nociceptive activity, including the thalamus, sensorimotor cortices, cingulate cortex, INS, orbitofrontal cortex (OFC), parahippocampal gyrus (PHG), amygdala, and brainstem (see studies on pain modulation in (Apkarian et al., 2005). We further hypothesized that brain areas more closely involved in emotion and autonomic regulation (Critchley, 2005) would be associated more specifically with SCR activity. Target structures included ACC, INS, medial prefrontal cortex (mPFC), OFC, amygdala, hypothalamus and brainstem nuclei such as the periacqueductal gray matter (PAG). Finally, based on a previous study (Coghill et al., 2003), we expected that pain sensitivity would correlate at least in part with SI, cingulate and prefrontal cortices. A threshold of p-corrected < 0.05 was used to search for significant activation in those structures, corresponding to a directed-search volume estimated at 109.58 resels (resel size estimated using the effective FWHM; one-tail tests; Bonferonni-corrected for multiple comparisons based on the Random Field Theory Worsley et al., 1992). This corrected p-value applied to directed searches corresponds to an uncorrected p < 0.0009. Activation maps were also examined using a more permissive criterion (p-uncorrected < 0.005) to minimize the risk of type II error. This tested the activation of brain structures previously shown to be involved in pain and/or autonomic regulation. A global search was also performed over all gray matter volume to identify additional peaks of activations outside the directed-search areas, using a threshold of p < 0.05, Bonferonni-corrected for multiple comparisons over a search volume

estimated at 626.38 resels for the whole brain gray matter and based on the Random Field Theory (Worsley et al., 1992). This corrected p-value corresponds to an uncorrected p = 0.00016. A cluster threshold of three voxels was applied to all analyses. The results from the global search of *noxious stimulation* contrast (analysis 1) are not presented in this study because it has been reported in many other studies and it is out of scope of the present article. However, results from global searches of other analyses (2 and 3) are presented in the *supplementary material in* Table S1.

RESULTS

Cerebral activation evoked by noxious electrical stimulation

Noxious shocks evoked BOLD activation in left contralateral SI (foot area), in bilateral thalamus, SII, INS, cingulate cortex, prefrontal cortex (PFC), amygdala and pons, and in the contralateral midbrain (Figure 1 A-C and Table 1). A decrease in BOLD signal was also found in the contralateral mPFC (Figure 1D and Table 1). These results are generally consistent with previous pain imaging studies (Apkarian et al., 2005).

Individual differences in pain sensitivity, motor reactivity and autonomic reactivity

The multiple regression analysis revealed that individual differences in shock-evoked brain activity in some structures specifically predicted pain sensitivity, motor reactivity or autonomic reactivity. In the directed search, pain

sensitivity was positively correlated to brain responses in midline ACC, contralateral pregenual ACC, ipsilateral mPFC/OFC and bilateral PHG (see Figure 2A and Table 2a). A representative correlation plot between ACC activity and pain sensitivity is shown in Figure 2A. Motor reactivity was positively correlated to brain responses in midline ACC (slightly posterior and dorsal to the peak related to pain sensitivity), midline MCC and the right ipsilateral OFC (see Figure 2B and Table 2b). The robust correlation between the activity of those structures and motor reactivity is illustrated by correlation plots in Figure 2B. In sharp contrast, no positive correlation was found for autonomic reactivity, which was *negatively* correlated to BOLD-responses in midline MCC (slightly medial and ventral to the positive peak for motor reactivity), bilateral OFC, and the midbrain in an area consistent with the location of the PAG (see Figure 2C and Table 2c). Also note the correlation plots for the activity of those structures and autonomic reactivity illustrated in Figure 2C. The peak of right OFC is at the exact same location as the OFC peak positively correlated to motor reactivity suggesting a robust cross-over interaction between autonomic and motor regulation in that area (see Figure 3 for a 3-Dimensional plot,). Importantly, motor and autonomic reactivity were not significantly correlated ($r=0.25$; $p=0.45$), as some subjects showed high autonomic reactivity together with low or high motor reactivity (also shown in 3-Dimensional plot). A multiple regression analysis confirmed the highly significant contribution of both motor (positive slope) and autonomic (negative slope) reactivity to the prediction of OFC activation ($R^2 = 0.93$), as shown in Figure 3.

Trial-to-trial fluctuations in the amplitude of the RIII reflex and SCR

The parametric modulation analysis revealed that trial-to-trial fluctuations in shock-evoked brain activity of some structures covaried with trial-to-trial fluctuations in the amplitude of RIII reflex and SCR. In the analysis contrasting RIII- and SCR-related brain activity, the amplitude of the RIII reflex covaried more strongly with brain responses in the subgenual ACC (sACC) bilaterally, and the right ipsilateral MCC (see Figure 4A and Table 3a). In contrasts, the SCR amplitude covaried more strongly with responses in the left contralateral MCC and right ipsilateral anterior insula (aINS) (see Figure 4B and Table 3b).

DISCUSSION

This is the first study which *concurrently* investigates the perceptual, motor and autonomic components of brain activity evoked by noxious stimulation in humans. Robust individual differences in shock-related brain activity were specifically associated with differences in pain sensitivity, motor reactivity or autonomic reactivity. On the other hand, individual patterns of motor and autonomic reactivity reflected brain activity within a common area of the orbitofrontal cortex. In addition, trial-to-trial fluctuations in shock-related activity in cingulate regions and aINS were significantly associated with trial-to-trial fluctuations in RIII and SCR amplitude. We suggest that brain activity related to within- and between-subject variability in motor and autonomic responses/reactivity reflects variations in ascending nociceptive processes and in regulation of spinal and cerebral sensorimotor functions. In contrast, the

specific correlation of pain sensitivity with cortical regions but not with thalamic or brainstem structures is consistent with the idea that pain sensitivity relies on cortical elaboration of afferent information.

Individual differences in shock-evoked brain activity

Pain sensitivity

Individual differences in pain perception can reflect differences in the recruitment of peripheral afferents and the gain of nociceptive transmission in the spinal cord and some brain structures. In the present study, these factors were taken into account in a multiple regression model controlling for stimulus intensity and the gain of spinal nociceptive transmission (indexed by motor reactivity). This allowed identifying brain regions specifically correlated to pain sensitivity, which included ACC, pgACC, mPFC/OFC and PHG, but not thalamic or brainstem regions. This is consistent with the cortical elaboration of afferent information, as demonstrated in a previous study, in which activation of ACC but not thalamus was related to pain sensitivity (Coghill et al., 2003). The correlation of pain sensitivity with stronger activation of ACC is consistent with increased negative affect associated with pain (Rainville et al., 1997; Vogt, 2005). In addition, our results show that activation of bilateral PHG and mPFC/OFC also contributes to individual differences in pain perception, suggesting a role for pain-related anxiety and emotional processes in pain sensitivity (Bechara et al., 2000; Ploghaus et al., 2001). This is also consistent with known anatomical connections between PHG and mPFC/OFC (Morecraft et al., 1992; Carmichael

and Price, 1995; Kondo et al., 2005). The present results are in general agreement with a previous study on pain sensitivity (Coghill et al., 2003) and both studies strongly suggest that pain sensitivity is most likely attributable to cognitive processes.

Motor and autonomic reactivity

Cerebral processes related to motor or autonomic reactivity independent from stimulus intensity and pain perception were isolated from brain activity evoked by noxious stimulation. The results indicate that individual differences in OFC and ACC activity are positively correlated to motor reactivity. Interestingly, OFC and ACC are often co-activated during the evaluation of punishers that can lead to a change in behavior (Kringelbach, 2005). Consistent with these findings, ACC and OFC are directly or indirectly connected to motor structures and are involved in the regulation of motor functions (Paus, 2001; Fuster, 2001; Vogt, 2005). In this respect, we suggest that the correlation of OFC and ACC activity with motor reactivity reflects evaluative processes of the punishing value of noxious electrical stimulation. In addition, we propose that ACC and OFC processes would follow and add to the “coarse” spinally mediated limb withdrawal in order to elaborate a finer motor behaviour in response to the source of nociceptive input (noxious stimulation). This idea is consistent with the role of ACC and the prefrontal cortex in goal-oriented cognitive processes and behaviour (Vogt and Sikes, 2000; Fuster, 2001).

Another interesting finding is the *negative* correlation found between autonomic reactivity and the activity of OFC and the PAG. In a previous study, activation of OFC has been correlated with autonomic responses evoked by symbolic rewards and punishments (Critchley, 2000), consistent with the role of the prefrontal cortex in emotional behaviour (Damasio, 1996). In addition, the negative correlation of both the OFC and the PAG with autonomic reactivity is consistent with anatomical pathways between these structures (Hadjipavlou et al., 2006) and potentially involved in the regulation of autonomic functions. More specifically, release of serotonin in the dorsolateral PAG produces an inhibition of sympatho-excitation and associated emotional responses (Johnson et al., 2004), a mechanisms that could mediate the down-regulation of autonomic responses (e.g. similar to that observed during the extinction of aversive conditioning; Liberzon and Sripada, 2008).

Unexpectedly, opposite correlations were found between OFC activity and motor and autonomic reactivity. However, a multiple regression clarified this complex interaction. Indeed, although high motor reactivity and low autonomic reactivity were not necessarily found in the same subjects (see the distribution of individual subjects in Figure 3), both output channels explained almost all variance in OFC responses to noxious stimulation (R^2=0.93). This opposite pattern observed between motor and autonomic reactivity in OFC may partly explain the apparently conflicting literature on autonomic reactivity and motor behaviour. For instance, it is generally expected that increased motor reactivity during emotion (e.g. stronger facial expression or startle) be associated with

increased autonomic responses; however, individual differences in the dominant response channel are also common and have been discussed in relation to personality traits by emotion psychologists (e.g. externalizers Vs intervalizers; Jones, 1935; Zuckerman et al., 1981). Therefore, there might be a variety of associations and dissociations between motor and autonomic reactivity that are evoked differently across experimental designs or subjects. Based on the present findings, we propose that these differences may reflect changes in activity within the OFC, a structure suggested to plays a pivotal role in the coordinated regulation of motor and autonomic responses.

Trial-to-trial fluctuations

In the present study, activity in the sACC was specifically related to the fluctuations in RIII reflex amplitude. Activation in this area may reflect the ongoing and fluctuating interactions between emotion and pain processes which have been previously shown to modulate the RIII reflex (Rhudy et al., 2005). Accordingly, activation of sACC has been associated with negative emotional states (Devinsky et al., 1995; George et al., 1995; Vogt, 2005) and has projections to the amygdala, the PAG and other brainstem nuclei (Devinsky et al., 1995; Chiba et al., 2001; Kuipers et al., 2006). Therefore, we suggest that sACC is the origin of some of the feedback pathways regulating spinal nociceptive processes. Interestingly, we have found that sACC in involved in the facilitation of the RIII reflex during negative emotions (unpublished observations).

In parallel, SCR fluctuations were more specifically related to shock-evoked activity in aINS. This is consistent with a previous fMRI study with SCR measures in which the activity of the aINS/inferior frontal gyrus covaried with SCR amplitude (Critchley et al., 2000b). It is also consistent with the role of the insula in pain processing, emotions and autonomic regulation (Augustine, 1996; Craig, 2002; Critchley, 2005). Therefore, the activity of aINS during noxious stimulation in the present study most likely reflects the regulation of SCR that is associated with the motivo-affective component of acute pain.

Sub-regions of the MCC were also correlated to RIII and SCR trial-to-trial fluctuations. The SCR typically reflects a non specific orientation component evoked by noxious stimulation, a function critically involved in the regulation of sensorimotor processes. Orientation processes are also influenced by the strength of sensory activity indexed here by the amplitude of spinal nociceptive responses (RIII). Accordingly, MCC was recently involved in fast attentional orientation and motor responses to pain (Frot et al., 2008). There is also abundant evidence of direct connections between MCC and motor neurones and interneurons of the spinal cord (Dum and Strick, 1996; Picard and Strick, 1996). Therefore, the present results are consistent with the role of MCC in orientation to the noxious stimulation for sensorimotor regulation, as demonstrated in previous studies (Devinsky et al., 1995; Paus, 2001; Vogt, 2005). Together, the results from trial-to-trial fluctuations analyses show that motor and autonomic responses to noxious stimulation rely on partly distinct brain processes.

Limitations and future directions

An important limitation of fMRI methods is the difficulty to disentangle brain activity reflecting afferent from efferent activity. Therefore, interpretation the present results should be limited to the potential implication of some brain structures in the regulation of somatovisceral and sensorimotor responses. Furthermore, the associations reported here with motor and autonomic responses and reactivity may reflect ongoing fluctuations in emotional or cognitive states and/or individual differences in personality traits. Future studies should address these questions by examining how psychological variables affect brain activity evoked by noxious stimulation in structures involved in the regulation of somatovisceral and sensorimotor responses. More specifically, the present results warrants further investigations to test whether RIII reflex facilitation by negative affect or their inhibition by various analgesic procedure is related to activity in ACC, sACC, OFC and other regions suggested to be involved in the regulation of nociceptive responses.

CONCLUSION

The present study provides the first concurrent evaluation of perceptual, motor and autonomic components of brain activity evoked by noxious stimulation in humans. We have shown that individual differences in these components of the pain experience reflect robust *individual differences* in specific brain processes. Furthermore, the dissociation between motor and autonomic processes associated with OFC activity underscores the complex

and pivotal role of this structure in pain-related processing. Moreover, our results support the idea that pain sensitivity relies on cortical elaboration of afferent information. In addition, the correlation of *trial-to-trial fluctuations* of pain responses with specific brain structures provides direct evidence of distinct pain-related regulatory processes of motor and autonomic responses. This experimental model will be useful to investigate cerebral dysfunction of pain processes in patients with chronic pain.

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Table 1. Brain activation peaks* evoked by the noxious painful stimulation in the directed search

Brain area	BA**	Side***	t =	x,y,z****
Postcentral gyrus (SI-putative foot area)	1-3/5	L	5.95	-3, -45, 70
Parietal operculum (SII)	40	L	7.95	-58, -21, 25
	40	R	9.66	65, -24, 25
Insula (INS)	13	L	8.36	-37, 0, 10
	13	R	6.19	38, 0, 5
Cingulate cortex				
Perigenual (pgACC)	24/32	L	6.21	-3, 31, 5
	24/32	R	4.62	7, 34, 5
Anterior (ACC)	32	L	7.58	-7, 14, 40
	32	R	9.38	10, 17, 30
Mid (MCC)	24	L	5.26	-3, -14, 45
	24	R	5.21	7, -7, 40
Posterior (PCC)	23	L	8.90	-3, -38, 15
	23	R	7.31	10, -38, 10
Prefrontal cortex				
dorsolateral (dlPFC)	10	L	4.80	-28, 48, 30
	10	R	5.53	38, 48, 30
Amygdala	----	L	4.48	-24, 0, -20
	----	R	5.84	27,-3, -20
Thalamus	----	L	8.81	-7, -21, 0
	----	R	6.31	10, -20, 5
Midbrain	----	L	6.27	-7, -21, -20
Pons	----	L	5.43	-7, -28, -35
	----	R	4.90	7, -28, -25
Medial prefrontal cortex (mPFC)	11/32	L	-4.48	-7, 38, -15

Note: Peaks of activity thresholded at $p<0.05$ corrected for multiple comparisons for the search volume, using the Random Field Theory, minimum 3 voxels per cluster (see mehtods).

* only one peak (max. T-value) per region of interest is reported

** BA: putative Brodmann area

*** R: right ipsilateral side; L: left contralateral side

**** Coordinates are reported in MNI space

Table 2. Brain activation peaks in the directed-search of responses related specifically to pain sensitivity, motor reactivity or autonomic reactivity

Brain area	BA	Side*	t =	x,y,z**
a. Brain regions specifically related to pain sensitivity				
Cingulate cortex				
Anterior (ACC)	24	----	5.36	-3,14,20
pregenual (pgACC)	24/32	L	4.07***	-10,41,0
Medial prefrontal cortex (mPFC)/orbitofrontal cortex (OFC)	11	R	4.34***	7,34,-20
Parahippocampal gyrus (PHG)	28	L	4.15***	-17,-17,-20
	28	R	4.35***	31,-14,-25
b. Brain regions specifically related to motor reactivity				
Cingulate cortex				
Anterior (ACC)	24	----	6.08	3, 7, 40
Mid (MCC)	23/24	----	7.07	-3, -10, 45
Orbitofrontal cortex (OFC)	11	R	16.22	28, 34, -20
c. Brain regions specifically related to autonomic reactivity				
Midcingulate cortex (MCC)	23/24	----	-5.54	0, -10, 35
Orbitofrontal cortex (OFC)	11	R	-11.10	28, 34, -20
	11	L	-8.06	-7, 41, -20
Midbrain (PAG)	----	----	-4.30***	-3, -38, -10

Note: Peaks of activity thresholded at p<0.05 (one-tail) corrected for multiple comparisons for the search volume, using the Random Field Theory, minimum 3 voxels per cluster.

* R: right ipsilateral side; L: left contralateral side

** Coordinates are reported in MNI space

*** p<0.005 uncorrected

Table 3. Brain activation peaks in the directed-search of responses related specifically to RIII reflex or SCR amplitude

a. Brain regions related to RIII reflex amplitude

Brain area	BA	Side*	t =	x,y,z**
Cingulate cortex				
subgenual (sACC)	32	R	5.31	10, 31, -15
	32	L	4.39	-3, 28, -10
Mid (MCC)	23/24	L	4.03***	-3, -14, 25

b. Brain regions related to SCR amplitude

	BA	Side*	t =	x,y,z**
Insula (INS)	13	R	4.13***	28, 17, 10
Cingulate cortex				
Mid (MCC)	32	L	4.78	-14, 0, 50

Note: Peaks of activity thresholded at $p<0.05$ (one-tail) corrected for multiple comparisons for the search volume, using the Random Field Theory,minimum 3 voxels per cluster (see methods).

* R: right ipsilateral side; L: left contralateral side

** Coordinates are reported in MNI space

*** $p<0.005$ uncorrected

FIGURE LEGENDS

Figure 1: Brain activation related *to noxious stimulation.* Event-related analysis of shock-evoked responses in the directed search. The typical pattern of pain-related activation was found, including SI, SII, ACC and INS (A-C). Additional peaks are shown in the precentral gyrus (PCG), posterior, mid and perigenual cingulate cortex (PCC, MCC and pgACC respectively), dorsolateral prefrontal cortex (dlPFC), thalamus, midbrain and pons. Deactivation was also observed in the medial prefrontal cortex (mPFC) (D). Peak t-values are reported in Table 1.

Figure 2: Brain regions related to individual differences in *pain sensitivity, motor and autonomic reactivity*. (A) Stimulus-evoked brain responses specifically related to pain sensitivity after removing variance related to motor reactivity, autonomic reactivity, and stimulus intensity. (B) Stimulus-evoked brain responses specifically related to motor reactivity after removing variance related to autonomic reactivity, stimulus intensity and pain ratings. (C) Stimulus-evoked brain responses specifically related to autonomic reactivity after removing variance related to motor reactivity, stimulus intensity and pain ratings. Scatter plots in B and C illustrate the corresponding correlations. Peak t-values are reported in Tables 2.

Figure 3: Relation between orbitofrontal cortex activity and motor and autonomic reactivity across subjects. The 3-D linear fit illustrates the positive correlation between OFC activity and motor reactivity (x vs z) and the negative correlation

between OFC activity and autonomic reactivity (y vs z). Also note that motor and autonomic reactivity do not correlate significantly as some subjects showed low autonomic reactivity along with low or high motor reactivity. A multiple regression analysis model including the RIII and the SCR to predict OFC activity confirmed this highly significant effect (multiple R^2 = 0.93; p<.001).

Figure 4: Brain activity associated with *trial-to-trial fluctuations in the RIII reflex and SCR amplitude*. (A) Regions specifically related to RIII reflex amplitude after removing SCR-related variance. (C) Regions specifically related to SCR amplitude after removing RIII-related variance. Peak t-values are reported in Table 3.

Figure 1

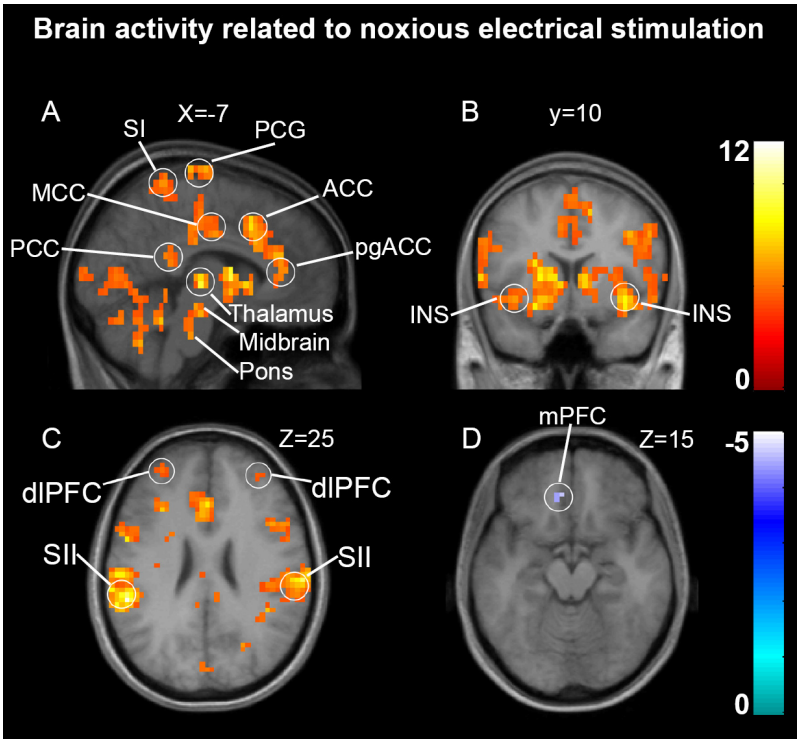


Figure 2

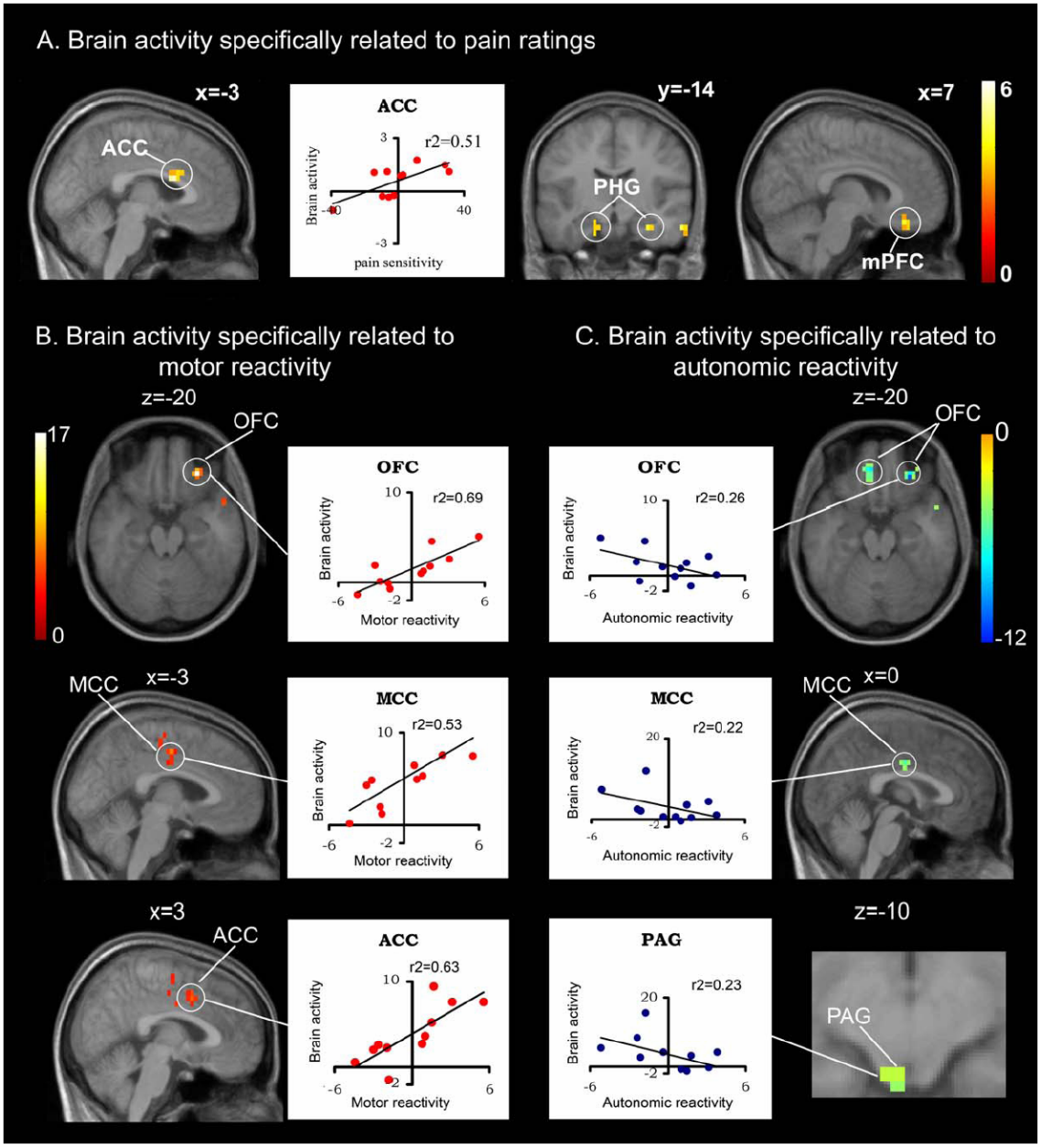

A. Brain activity specifically related to pain ratings
x=-3
ACC
ACC
r2=0.51
Brain activity
pain sensitivity
y=-14
PHG
x=7
mPFC
6
0
B. Brain activity specifically related to motor reactivity
z=-20
OFC
17
0
OFC
r2=0.69
Motor reactivity
C. Brain activity specifically related to autonomic reactivity
z=-20
OFC
0
-12
OFC
r2=0.26
Autonomic reactivity
MCC
x=-3
MCC
r2=0.53
Motor reactivity
MCC
x=0
MCC
r2=0.22
Autonomic reactivity
x=3
ACC
ACC
r2=0.63
Motor reactivity
PAG
r2=0.23
Autonomic reactivity
z=-10
PAG

Figure 3

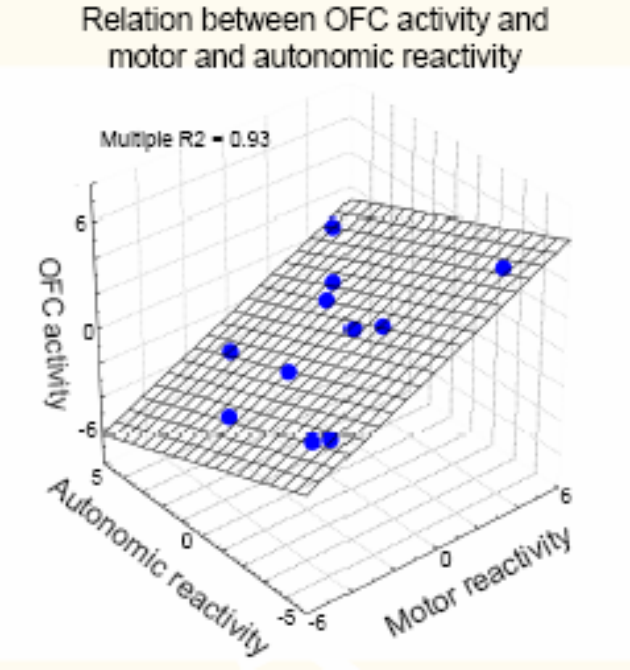
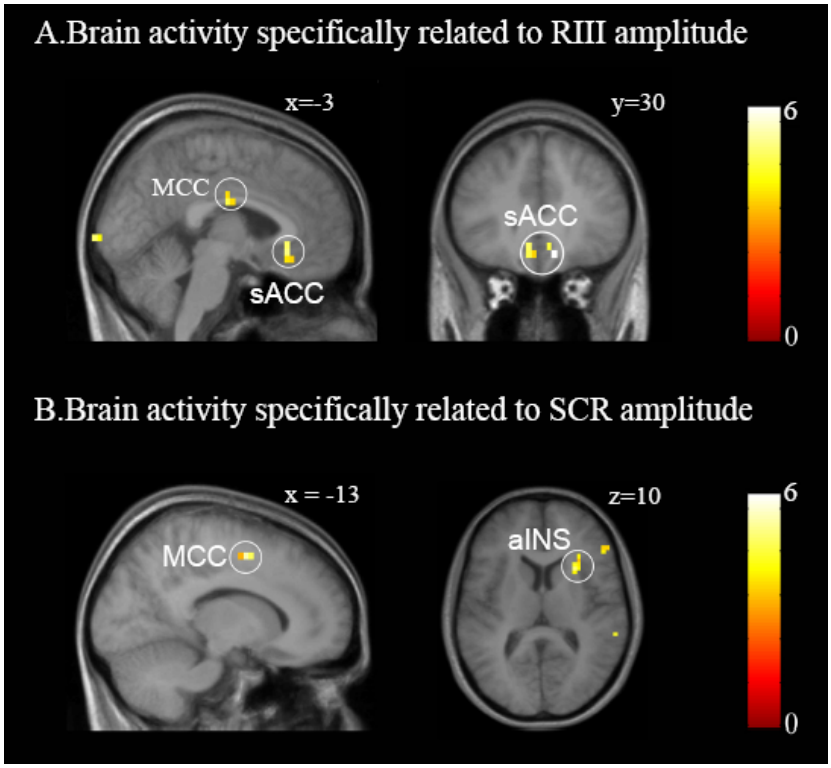


Figure 4



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SUPPLEMENTARY MATERIAL

Physiological signals such as EMG are contaminated by strong electromagnetic noise in the MRI environment. However, recent studies have shown that it is possible to combine fMRI with EMG (Dimitrova et al., 2003; Dimitrova et al., 2004), with SCR (Critchley et al., 2000a; Nagai et al., 2004), and with somatosensory-evoked potentials (Christmann et al., 2007). In the present study, the RIII reflex and associated SCR evoked by noxious electrical stimulation were measured concomitantly with the acquisition of fMRI time series. This experimental design allowed direct correlation of physiological measures and brain activity. Both the RIII reflex and SCR were consistent with previous psychophysiological studies of pain (Sandrini et al., 2005; Rhudy et al., 2007a).

Validation of RIII and SCR measures in the fMRI environment

To demonstrate the validity of physiological recordings in an environment with such a strong electromagnetic noise, the measure of a RIII reflex response during image acquisition is shown in Figure S1. The electrical stimulation was delivered between two successive volumes during the TR delay (S1A-B). The EMG of the biceps femoris was recorded (S1C) and filtered online for a clear visualization of the RIII reflex (S1D). The filtered signal was transformed off-line to the root mean square (S1E) for quantification of the RIII reflex amplitude (integration between 90-180ms post-stimulus). For all participants, the mean threshold was 10.4 ± 3.7 mA, which is consistent with previous studies Sandrini

et al., 2005). Also note the bottom trace of the recordings at the top of Figure S1A, representing the SCR in response to the electrical stimulation.

In addition to the stimulus used in the experimental study (adjusted to 150% of the RIII threshold; 15.3 ± 4.5 mA; see main text), an additional stimulus intensity was applied in a separate scan to validate the physiological measurements. This additional level was adjusted to 120% of the RIII reflex threshold (12.5 ± 4.4 mA) and was slightly painful. The difference in pain ratings, mean RIII reflex and SCR amplitude between intensities were evaluated by means of two-tail paired t-tests. Statistical analyses were done in SPSS (SPSS Inc., Chicago, U.S.A.) with significance thresholds set to $p < 0.05$. The pain ratings (S2A), the mean amplitude of the RIII reflex (2B) and the mean amplitude of SCR significantly increased from level 1 to level 2 ($p<0.01$, $p<0.05$ and $p<0.01$ respectively). This confirmed the validity of the physiological measurements.

Within- and between-subject variability

Within- and between subjects variability of pain rating, RIII reflex amplitude and SCR amplitude is illustrated for the experimental level (150% of RIII reflex threshold) in Figure S3A-C). Each bar of the histograms (A-C) represents a subject and error bars are the standard error of the mean across the 40 trials. As can be notes, there are no error bars for pain ratings (S3A) since they were collected once, at the end of the scans (see Methods in main text). The values of each histogram bar (subject) were used as regressors in the multiple regression analysis of individual differences in pain perception, motor

reactivity and autonomic reactivity. The 40 values which standard error is illustrated by error bars were used as regressors in the parametric modulation analysis of trial-to-trial fluctuations.

Brain activity found in global searches

In the global search, additional peaks were related to motor reactivity in the right PCG, right postcentral gyrus (outside the foot area), and the temporal and occipital cortex (see Table S1). A significant negative peak was also found in the left caudate nucleus, consistent with the up-regulation of motor responses. Two additional peaks in the occipital cortex negatively correlated to autonomic reactivity in the global search (Table S1).

Table S1. Additional peaks (global search) for brain responses related specifically to motor and autonomic reactivity

	a. Specifically related to motor reactivity			
Brain regions	BA	Side*	t =	x,y,z**
Frontal lobe				
Precentral gyrus	4	R	7.63	38, -21, 40
Parietal lobe				
Postcentral gyrus	3	R	5.69	52, -17, 55
Temporal lobe				
Transverse temporal gyrus	41	L	6.30	-45, -28, 10
Uncus	38	R	7.06	-31, 3, -35
Occipital lobe				
Precuneus	19	R	6.29	17, -86, 40
Caudate nucleus	----	L	-7.58	-10, 10, 15
	b. Specifically related to autonomic reactivity			
Occipital lobe				
Middle occipital gyrus	18	L	-9.55	-31, -96, 5
Cuneus	19	L	-6.49	-21, -100, 20

Note: Peaks of activity thresholded at $p<0.05$ (one-tail) corrected for multiple comparisons for the search volume, using the Random Field Theory, minimum 3 voxels per cluster.
* R: right ipsilateral side; L: left contralateral side
** Coordinates are reported in MNI space

LEGENDS OF SUPPLEMENTARY FIGURES

Figure S1: *Acquisition of the RIII reflex and SCR in the fMRI scanner.* The top panel includes 5-channel recording of a 12-sec epoch where the acquisition of three complete fMRI volumes is shown by the pattern of MRI-related noise (A). During that epoch, two stimuli (top trace) were delivered during the delay of 500 ms between the fMRI volumes (time = 3s and 9s). A representative SCR is shown in the bottom channel. The lower panel (B-E) shows an enlargement of a 1s epoch including the TR-delay during which the fMRI acquisition is interrupted, an electrical stimulation is delivered and the RIII reflex is measured. The first channel (B) represents the recording of the stimulus to determine its timing and intensity. The second channel (C) shows the raw EMG of the biceps femoris. After filtering (D), the RIII reflex is clearly identified with an onset latency of 90 ms. The quantification is done from the filtered EMG transformed to root mean square (E), taking the integral between 90-180 ms post-stimulus.

Figure S2: *Responses to the two noxious stimulus intensities*. Intensities were adjusted to 120% and 150% of RIII reflex threshold. These two stimulation intensities produced significantly different pain ratings (A) RIII reflex amplitude (B) and SCR amplitude (C). *p<0.05, **p<0.01, ***p<0.001.

Figure S3: Within- and between-subject variability in pain responses. A) pain ratings B) RIII reflex amplitude C) SCR amplitude. Histogram bars represent the

eleven subjects. Error bars represent the standard error of the mean (SEM) of the 40 trials.

Figure S1

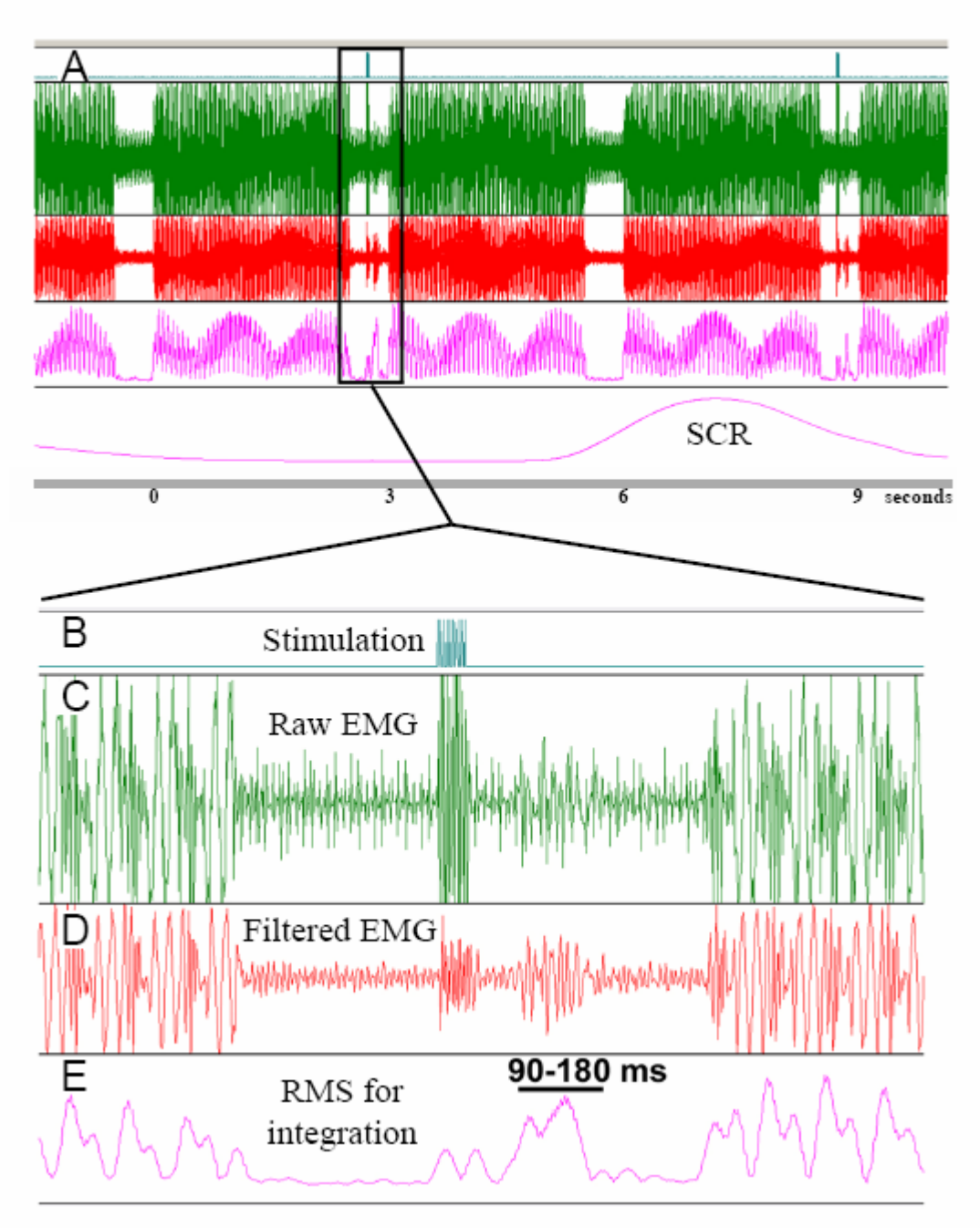


Figure S2

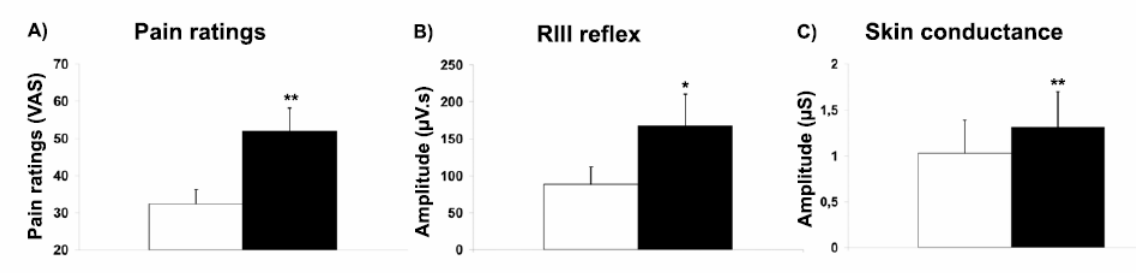
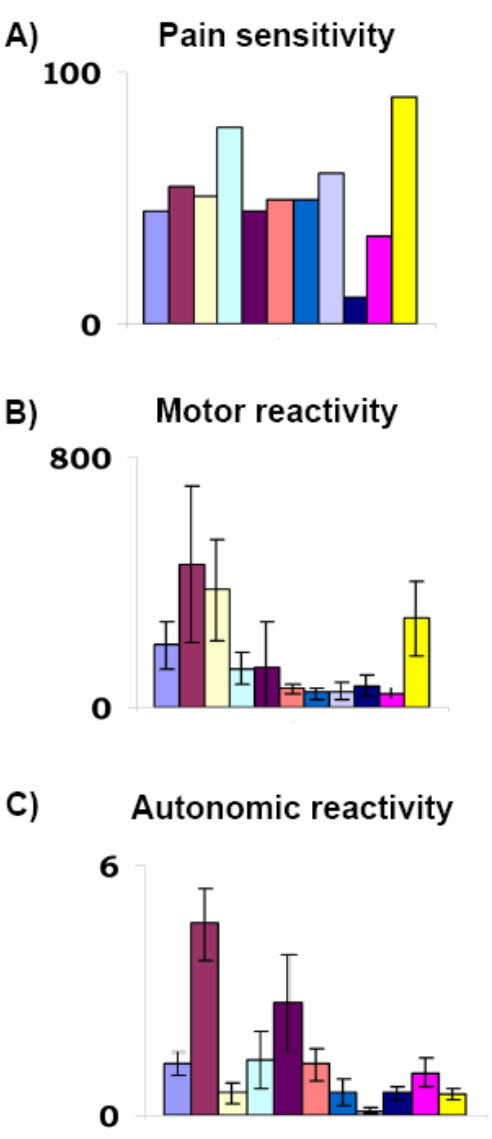


Figure S3



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Article 2: Cerebral and cerebrospinal processes underlying counterirritation analgesia

Cerebral and cerebrospinal processes underlying counterirritation analgesia

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Short title: Neural processes of counterirritation analgesia

Abstract

Pain is a complex experience involving extensive interactions between brain and spinal cord processes. Various interventions that modulate pain, such as the application of a competing noxious stimulus (counterirritation), are thought to involve cerebrospinal regulation. However, no study has yet shown a direct link between brain and spinal cord activity during counterirritation analgesia in humans. In the present study, cerebral and cerebrospinal mechanisms underlying the modulation of pain and the nociceptive withdrawal reflex (RIII) by counterirritation were investigated using fMRI. Acute painful electrical stimulation was admistered to evoke the RIII reflex before, during and after counterirritation induced by the immersion of the left contralateral foot in cold water. As expected, counterirritation produced robust pain inhibition during counterirriation and residual analgesia during the recovery period. In contrast, RIII reflex amplitude was significantly decreased by counterirritation only in a subset of subjects. Modulatory effects of counterirritation on pain perception and spinal nociception were paralleled by decreased shock-evoked activity in pain-related areas that were specifically related to analgesia (primary somatosensory cortex (SI), anterior cingulate and amygdala) or to RIII modulation (supplementary motor area and orbitofrontal cortex (OFC)). Moreover, sustained activation of brain regions involved in cortical modulation of pain (OFC) and descending modulation (SI and periacqueductal gray matter) predicted analgesia and RIII modulation respectively. The partial dissociation found across individuals between the modulation of pain and the RIII provides

evidence for the implication of at least two distinct neural mechanisms underlying the effects of counterirritation.

Introduction

Pain is a complex experience involving extensive interactions between brain and spinal cord processes. Four decades ago, cerebrospinal interactions were already at the core of the leading pain theory (Melzack and Casey, 1968). Although our understanding of pain and pain modulation mechanisms has greatly advanced since then, no study has yet shown direct links between brain and spinal cord activity during interventions that modulate pain in humans.

Counterirritation by the application of two competing noxious stimuli has been known for centuries as a means to induce analgesia. Brain release of endogenous opioids in pain-related areas during sustained pain (Zubieta et al., 2001) is a potential mechanism that may explain how acute pain is typically reduced during counterirritation. Besides, psychophysiological studies have shown that spinal nociceptive processes, indexed by the nociceptive flexion reflex (RIII reflex), can be inhibited by counterirritation (Willer et al., 1999). This effect is thought to relie on diffuse noxious inhibitory controls (DNIC), a spinobulbospinal loop including the caudal medulla (DeBroucker T. et al., 1990). However, some studies found no correlation between RIII and pain modulation as counterirritation analgesia may occur without RIII reflex inhibition (Willer et al., 1979; Terkelsen et al., 2001; Bouhassira et al., 2003). This emphasizes that

changes in pain and RIII reflex amplitude may be dissociated and are most likely regulated by partly distinct mechanisms.

Apart from the caudal medulla, several brainstem structures such as the periaqueductal gray matter (PAG) and the rostral ventromedial medulla (RVM) are involved in descending modulation of pain (Millan, 2002). For example, electrical stimulation of the PAG produces generalized analgesia mediated at least in part by inhibitory pathways affecting spinal nociceptive processes (Hosobuchi, 1980; Mayer, 1984). The recruitment of the PAG by different cognitive interventions (Petrovic et al., 2002; Tracey et al., 2002a; Valet et al., 2004; Bingel et al., 2006) may reflect the activation of similar pathways. At the cortical level, the primary somatosensory cortex (SI) with its projections to the dorsal horn of the spinal cord (Cheema et al., 1984; Ralston and Ralston, 1985; Casale et al., 1988) can inhibit nociceptive spinothalamic cells (Yezierski et al., 1983). In addition, imaging studies have suggested a role of the anterior cingulate cortex (ACC) and the orbitofrontal cortex (OFC) in pain modulation by cognitive interventions (Derbyshire et al., 1998; Peyron et al., 1999; Rainville et al., 1999b; Faymonville et al., 2000; Petrovic et al., 2000; Frankenstein et al., 2001; Petrovic and Ingvar, 2002; Bantick et al., 2002; Valet et al., 2004; Bingel et al., 2006). Therefore, all these structures may contribute to the effects of counterirritation on pain and RIII reflex responses.

The aim of the present study was to elucidate cerebral and cerebrospinal mechanisms of pain and RIII reflex modulation by counterirritation with combined fMRI and electrophysiological methods. Acute painful electrical

stimulation evoking a RIII reflex were administered before, during and after counterirritation induced by the immersion of the left contralateral foot in cold water. We hypothesised that shock pain, RIII reflex amplitude and shock-evoked brain activity would decrease as a result of the activation of cerebral and cerebrospinal inhibitory processes induced by the counterirritation stimulus. Furthermore, we investigated whether these modulatory effects reflected the sustained activation of brain structures previously involved in descending modulation of pain and RIII reflex (SI, ACC, aINS, OFC, amygdala, PAG, the pons, the RVM and the caudal medulla).

Methods

Subjects

Twelve healthy volunteers participated in the study (two males and ten females; mean age 26.7 years; SD, 4.7). Before the scanning day, subjects were familiarized with the painful stimulations and the pain rating scale in a simulator room. Each participant performed one functional scan and one high-resolution anatomical scan. The Research Ethics Board of the “Centre de recherche de l’Institut de gériatrie de Montréal” approved the study. All subjects gave written informed consent and received a compensation of 50$ for their time and commitment to the study.

Painful Electrical stimulation

Transcutaneous electrical stimulation was delivered with a Grass S48 square pulse stimulator (Astro-Med Inc., West Warwick, RI, USA) through a constant-current stimulus-isolation unit and a RF filter, preventing artefacts in fMRI data. The stimulation consisted in a 30 ms train of 10 X 1 ms pulse, delivered on degreased skin over the retro-maleolar path of the right sural nerve using a pair of surface electrodes (1 cm^2, inter-electrode distance 2cm). The individual RIII reflex threshold was determined using the staircase method (Willer, 1977), immediately after positioning the subject in the scanner and before the acquisition of functional images. The stimulation intensity was adjusted to 120% of the threshold (mean ± SD: 13.3 ± 4.6 mA), and remained constant through the duration of the experiment.

Experimental paradigm

The functional scan included 35 electrical stimuli administered with an inter-stimulus interval of 12 seconds. The first five stimuli at the beginning of the scan (one-minute pre-baseline) controlled for the rapid habituation effect observed occasionally on the first few trials of a series of RIII measurements. The subsequent 30 stimuli were distributed equally in three conditions: baseline (n=10), counterirritation (n=10) and recovery (n=10). Counterirritation was produced by placing the subject's left contralateral foot in a MR-compatible container filled with ice and cold water for 2 minutes (temperature adjusted to +4°C immediately before the beginning of the scan).

Pain ratings

A visual analog scale (VAS) was used to evaluate the pain induced by the electrical stimulation and the counterirritation stimulus. Participants were shown the VAS on a computer monitor back-projected onto a screen and viewed on a mirror placed on the head coil in front of the participant's eyes. The VAS was placed horizontally and included the verbal anchors "no pain" and "worst pain imaginable" at the left and right extremities, respectively (Price et al., 1994). Participants used a MRI-compatible response key to move a cursor on the VAS. Subjects rated the pain produced by the electrical shocks after each block of stimuli performed in the three conditions. At the end of the scan, subjects also rated the pain produced by the counterirritation stimulus. All ratings were converted linearly to a 0-100 scale. The evaluation of pain modulation by counterirritation was done with a two-tail repeated-measures ANOVA. Analgesia during counterirritation and recovery was also calculated for each subject by subtracting ratings from that obtained during baseline. These values were ranked (from 1 to 11, 11 being the strongest analgesia) and served as a subject regressor coding for individual differences in analgesia in fMRI analyses (see below).

fMRI acquisition

Imaging data was acquired at "Unité de Neuroimagerie Fonctionnelle" of the "Centre de recherche de l'Institut de gériatrie de Montréal" on a 3T Siemens Trio scanner (Munich, Germany) using a CP head coil. The head of the subject

was stabilized in a comfortable position using a vacuum bag. Subjects were instructed to refrain as much as possible from moving throughout the imaging session and were given earplugs to reduce the noise from the scanner. The anatomical scan was a T1-weighted high-resolution scan [repetition time (TR): 13 ms; echo time (TE): 4.92 ms; flip angle: 25°; field of view: 256 mm; voxel size: 1 X 1 X 1.1 mm]. The functional scan was collected using a blood oxygen level-dependent (BOLD) protocol with a T2*-weighted gradient echo-planar imaging sequence (TR: 3.0 s with an inter-volume delay of 500 ms; TE: 30 ms; flip angle: 90°; 64 X 64 matrix; 150 volume acquisitions). Electrical stimulation was always administered during the inter-volume delay, thereby avoiding the potential contamination of fMRI images by shock-induced artefacts and EMG recordings by RF-pulse artefacts. The scanning planes were oriented parallel to the anterior-posterior commissure line and covered the entire brain from the vertex of the cortex to the first segments of the spinal cord (41 contiguous 5-mm-thick slices; voxel size, 3.44 X 3.44 X 5 mm).

RIII reflex recording and analyses

Electromyographic (EMG) activity of the right (ipsilateral) biceps femoris was recorded with MRI-compatible Ag-AgCl surface electrodes (Type EL-508) using Biopac MP150 system (Biopac systems Inc., Goleta, CA, USA). Custom-made RF filters were used for the recording of physiological measures to prevent introducing artefact in the fMRI data. Electromyographic (EMG) activity was amplified, band pass filtered (100-500 Hz), digitized and sampled at 1000

Hz. EMG data was analysed using Acqknowledge 3.8 (Biopac systems Inc., Goleta, CA, USA). The raw EMG recordings were filtered off-line (120-130 Hz) and transformed using the root mean square. The resulting signal was integrated between 90-180 ms after the stimulus onset to quantify RIII reflex amplitude to each shock. These values were averaged for each condition (10 stimulations each) to assess RIII reflex modulation by counterirritation with a two-tail Friedman's ANOVA for dependant samples. The values were also normalized within each run using a z-transformation and averaged for each condition to calculate RIII modulation for each subject. This was done by subtracting the normalized RIII amplitude during counterirritation and recovery from that obtained during baseline. These values were ranked (from 1 to 11, 11 being the strongest RIII inhibition) and served as a subject regressor coding for individual differences in RIII-modulation in fMRI analyses (see below).

fMRI data analyses

Brain imaging data was analyzed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm/). Pre-processing included slice-time correction and realignment for movement correction. Due to movement artefacts in the functional images, one subject was excluded from all analyses. Anatomical and functional images were spatially normalized to a standard stereotaxic space using the MNI template. Subsequently, functional images were spatially smoothed using a Gaussian kernel of twice the voxel size (FWHM: 7 x 7 x 10 mm), temporally filtered using a high-pass filter with a cut-off period of 240s, and

were corrected for serial autocorrelation using the AR(1) correction implemented in SPM.

The effects of interest were assessed using the general linear model. Individual contrasts were generated using a mixed-design model. The two-minute foot immersion was modeled as a 2-min block, while he 35 shocks were assigned to 4 conditions: pre-baseline (5), baseline (10), counterirritation (10) and recovery (10). These vectors were convolved with a canonical hemodynamic response function (hrf). In addition, movement parameters from the realignment procedure were entered as six regressors of no interest to remove potential movement-related variance/artefacts. This model allowed assessing 1) shock-evoked activity (baseline shocks, 10 stimulations), 2) modulation of shock-evoked activity during counterirritation (counterirritation shocks vs baseline shocks), 3) modulation of shock-evoked activity during recovery (recovery shocks vs baseline shocks) and 4) sustained activity evoked by the two-minute foot immersion. For the group analyses, these individual contrasts were combined in random-effect models with the one-sample t-test function.

Regression analyses of analgesic effects and RIII modulation

The relation between brain activity and changes in pain and RIII reflex during counterirritation and recovery was further tested using multiple-regression models examining the magnitude of the modulation of *shock-evoked activity during counterirritation* (contrast 2) and *during recovery* (contrast 3).

These models included subject-regressors corresponding to the magnitude of analgesia and RIII modulation (see sections on *Pain ratings* and *RIII reflex recording and analyses*). Subsequently, in order to test the relation between sustained activity evoked by the foot immersion in cold water and analgesia and RIII reflex inhibition, individual contrasts for *sustained activity evoked by the two-minute foot immersion* (contrast 4) were entered in a multiple-regression random-effect model with regressors for analgesia and RIII modulation. These analyses were performed with multiple regression models in order to assess brain activity that is more specifically related to analgesia or RIII modulation. Pearson's correlation coefficients were also determined for each significant peak related to analgesia or RIII inhibition.

In a subsequent analysis, Pearson's correlations were performed to test the relation between sustained counterirritation activity and shock-evoked activity. Parameter estimates in brain regions activated by counterirritation and specifically related to analgesia were correlated to parameter estimates of brain regions showing decreased shock-evoked activity specifically related to analgesia. In the same way, parameter estimates of brain regions activated by counterirritation and specifically related to RIII inhibition were correlated to parameter estimates of brain regions showing decreased shock-evoked activity specifically related to RIII inhibition. These correlations were thresholded at $p<0.05$ Bonferroni-corrected for multiple comparisons.

Coactivation analyses were also performed to assess the brain networks involved in modulation of pain and RIII reflex by counterirritation. Considering

that sustained activity produced by counterirritation in OFC was the strongest peak related to analgesia and considering that OFC has been previously involved in analgesia, it was selected as the seed region for analgesia coactivations. For RIII modulation. the PAG area correlated to RIII modulation was selected as the seed regions considering its well documented role in descending modulation of pain. The time courses of OFC and PAG (6mm sphere) were extracted for the 150 volumes in each individual model. These time courses were then used in two separate individual models including only the time course of the OFC or PAG as regressor. Individual contrasts for OFC-related and PAG-related coactivations were then generated. Subsequently, these individual contrasts were used in two separate one-sample t-test random-effect models for group analyses. These models lead to statistical maps of networks associated with the analgesia-related peak in OFC and with the RIII-modulation peak in the PAG.

In all analyses models tested, a directed search was conducted over brain areas receiving nociceptive afferents and/or involved in the regulation of nociceptive processes (see Apkarian et al., 2005). We hypothesized that structures previously shown to respond to acute noxious stimulation, namely the thalamus, primary and second somatosensory cortices (SI-foot area and SII), the supplementary motor area (SMA), the anterior and posterior insula (aINS and pINS), the mid and anterior cingulate cortex (MCC and ACC), the amygdala and parahippocampal gyrus (PHG), the orbitofrontal cortex and the prefrontal cortex (PFC), would show decreased shock-evoked activity during

counterirritation. We also hypothesized that individual differences in analgesia and RIII reflex modulation would be associated with the activity of brain structures previously involved in modulation of nociceptive activity, including the PAG (Petrovic et al., 2002; Tracey et al., 2002a; Valet et al., 2004; Bingel et al., 2006; Fairhurst et al., 2007), SI, SII and the motor cortex (Yezierski et al., 1983; Wise et al., 2007; Passard et al., 2007), cingulate cortex (Faymonville et al., 2000; Frankenstein et al., 2001; Valet et al., 2004; deCharms et al., 2005; Bingel et al., 2006,) anterior insula (Wise et al., 2007), entorhinal cortex (Ploghaus et al., 2001), the orbitofrontal cortex (OFC) (Petrovic and Ingvar, 2002) and the amygdala (Zubieta et al., 2001). The size of this search volume was used to account for multiple-comparison in determining the statistical threshold.

A threshold of p-corrected < 0.05 was applied to search for significant activation in target structures, corresponding to a directed-search volume estimated at 109.58 resels (resel size estimated using the effective FWHM; one-tail tests; Bonferonni-corrected for multiple comparisons based on the Random Field Theory (Worsley et al., 1992)). This corrected p-value applied to directed searches corresponds to an uncorrected $p < 0.0009$. Activation maps were also examined using a more permissive criterion (p-uncorrected < 0.005) to minimize the risk of type II error. This tested the activation of brain structures previously shown to be involved in pain and/or pain modulation.

Results

Pain and RIII modulation

The sustained counterirritation stimulus produced strong pain (mean ± SEM : 70.3 ± 4.2) and produced a robust analgesia on acute pain with baseline shock-pain reduced by about 50% during counterirritation (baseline: 43.8 ± 5.8; counterirritation: 22.4 ± 5.1, $p < 0.01$; see Figure 1). Furthermore, analgesia was partly maintained during the recovery period, as shock pain remained slightly but significantly diminished relative to baseline (37.6 ± 6.6 vs 43.8 ± 5.8, $p < 0.05$, see Figure 1).

On the other hand, the decrease in RIII reflex amplitude during counterirritation and recovery compared to baseline did not reach significance in the group analysis (baseline: 162.9 ± 34.6 μV, counterirritation: 129.2 ± 19.4 μV, and recovery: 129.5 ± 24.2 μV, p's >0.1, see Figure 2A). Moreover, the changes in RIII reflex and pain produced by counterirritation were not significantly related (rho=0.16, p=0.63). However, analyses of individual subjects data revealed that a subgroup of 4 subjects presented a robust and significant decrease in RIII reflex amplitude during counterirritation (see Figure 2B). In the other subjects, RIII reflex amplitude remained unchanged or increased slightly. Individual examples are presented in Figure 2 showing one subject in whom RIII reflex was significantly inhibited by counterirritation (2C) and one subject showing no significant modulation (2D).

Decreased shock-evoked brain activity during counterirritation

Baseline electric shocks evoked robust activation in several pain-related areas including bilateral thalamus, SI, the precentral gyrus (PrCG), SII, pINS and aINS; contralateral PFC; ipsilateral posterior cingulate cortex (PCC); and midline ACC, MCC and SMA (see Figure 3 and Table 1). This event-related response was significantly decreased during counterirritation in bilateral thalamus, PrCG, and SMA; contralateral SI, SII, pINS and PFC; ipsilateral ACC, aINS and PHG; and midline SI, ACC and MCC (Figure 4A and Table 2). This confirmed that brain responses to acute painful stimuli were consistently decreased during counterirritation. Significant decrease in shock-evoked activity during recovery is shown in Figure S2A and table S1 (see *supplementary materials*).

Changes in shock-evoked responses were further examined using multiple regressions. These analyses revealed that subjects reporting stronger counterirritation analgesia displayed larger decreases in shock-evoked responses in contralateral SI, PCC and AMY; ipsilateral PFC and OFC; and midline ACC and MCC was specifically related to counterirritation analgesia (Figure 4B and Table 3a). On the other hand, the changes in the RIII were associated with the magnitude of the modulation of the shock-evoked responses in contralateral SMA, aINS, PFC and OFC and midline SMA was specifically related to RIII modulation (Figure 4C and Table 3b). Some of these correlations are illustrated with scatter plots in Figure 4B and 4C respectively. Changes in

shock-evoked responses during recovery and specifically related to analgesia or RIII modulation are shown in Figure S2B-C and table S2 (see *supplementary materials*).

Sustained brain activity evoked by the counterirritation stimulus

Counterirritation induced sustained activation in common pain-related areas including SI, SII, the cingulate cortex, the INS and the PFC (Figure 5A and Table 4). In addition, strong activation was found in the midbrain and the pons, consistent with the recruitment of the PAG and the reticular formation during tonic pain (Figure 5A and Table 4). Regression analyses revealed that sustained activity in ipsilateral OFC and midline mPFC was specifically related to counterirritation analgesia (Figure 5B and Table 5a) On the other hand, midline SI, PCC and midbrain (consistent with the location of the PAG) and contralateral SMA were specifically related to RIII inhibition (Figure 5C and Table 5b). Interestingly, analgesia-related activity in OFC strongly predicted inhibition of shock-evoked activity in AMY (Figure 6). Other correlations are shown in Table S3 (see *supplementary material*).

Coactivation analyses

The analgesia-related peak in OFC was coactivated with a brain network including subregions of the cingulate cortex (PCC, ACC and sACC), the aINS, AMY and PHG, the mPFC and OFC (see Figure 7A and table 6A). On the other hand, structures coactivated with the RIII-modulation peak in the PAG included

SI, PCL, SMA and pre-SMA, ACC, PCC, PHG, PFC, the thalamus, pons and medulla (RVM) (see Figure 7B and table 6B).

Discussion

This is the first study to examine the effects of counterirritation on pain perception and RIII reflex amplitude concurrently with BOLD measures of brain activity. As expected, counterirritation produced strong analgesia that persisted during the recovery period. On the other hand, RIII reflex amplitude was decreased by counterirritation but this effect reached statistical significance only at the subject level in a subset of participants. The modulatory effects of counterirritation were paralleled by decreased shock-evoked activity in several pain-related brain areas. Moreover, the counterirritation stimulus produced sustained activation in brain areas involved in cortical modulation of pain and descending modulation of spinal nociception. Furthermore, coactivation analyses revealed that distinct brain networks were related to analgesia or RIII modulation. Together these results suggest that counterirritation produces analgesia and RIII modulation through partly distinct mechanisms.

Modulation of pain and RIII reflex by counterirritation

In the present study, shock pain at the right ankle was strongly inhibited by approximately 50% during the immersion of the left foot in painfully cold water. Moreover, the analgesic effects of counterirritation partly persisted over the counterirritation period as pain ratings increased during recovery but did not

reach the baseline level. This indicates that analgesia cannot be explained only by distraction and habituation. These results are consistent with previous studies on counterirritation using ischemic pain and cold pain (Pertovaara et al., 1982b; Jungkunz et al., 1983; Willer et al., 1984a; Chen et al., 1985; Talbot et al., 1987).

In contrast, decreased RIII reflex amplitude reached statistical significance only in a subset of participants, in spite of the robust analgesia. This indicates a sharp dissociation between RIII reflex amplitude and pain perception. This is in contrast with some studies showing that counterirritation analgesia is accompanied by RIII reflex inhibition (Willer et al., 1984a; Roby-Brami et al., 1987; Willer et al., 1989; Danziger et al., 1998b). On the other hand, it is consistent with another study showing a dissociation of pain and RIII with segmental counterirritation (Terkelsen et al., 2001). This raises the possibility that competing segmental facilitatory processes may act on the circuitry of the RIII reflex. However, this is unlikely as dissociation between pain and RIII has also been observed with heterosegmental counterirritation (Willer et al., 1979). Therefore, the sensorimotor dissociation found in the present study most likely reflects cerebral or cerebrospinal processes. This issue could be addressed as the present study was precisely designed to investigate cerebral and cerebrospinal regulation of pain and RIII.

Effects of counterirritation on event-related brain activity

Counterirritation analgesia was accompanied by a parallel inhibition of shock-evoked activity in several pain-related brain areas. Most notably, changes in SI, ACC, PFC and amygdala activity were specifically related to the extent of analgesia. This is consistent with the modulation of sensory and affective dimensions of pain (Rainville et al., 1997; Coghill et al., 1999b; Hofbauer et al., 2001; Buchel et al., 2002; Bornhovd et al., 2002). Moreover, the inhibition of ACC, PFC and amygdala by counterirritation is in agreement with the release of endogenous μ-opiods in those structures during sustained pain (Zubieta et al., 2001). Interestingly, it was shown that the activation of DNIC by counterirritation depends on opioidergic mechanisms (Willer et al., 1990; LeBars D. et al., 1992). The present results therefore suggest that endogenous opioids may modulate pain-related activity in ACC, PFC and amygdala in addition to spinal nociceptive processes.

Changes in shock-evoked activity were also related to RIII modulation in pain-related areas, including SMA and OFC. The modulation of these regions is consistent with their role in motor regulation and behaviours (Picard and Strick, 1996; Paus, 2001; Fuster, 2001; Kringelbach, 2005), suggesting that the magnitude of the spinal sensorimotor output evoked by noxious stimulation predicts the extent of cortical motor regulation in SMA and OFC. Additionally, OFC is involved in reward and punishment representations (O'Doherty et al., 2001; Kringelbach and Rolls, 2004) and changes in shock-evoked activity in

OFC may also represent modulation of the punishing value (indexed by RIII amplitude) of electric shocks during counterirritation.

Sustained effects of counterirritation on brain activity

In addition to inhibition of shock-evoked activity, the counterirritation stimulus produced sustained activation of pain-related areas including SI, ACC, aINS, PFC and OFC, as well as the midbrain (PAG area) and the pons. This is consistent with the recruitment of processes involved in cortical modulation of pain (Oleson et al., 1980; Faymonville et al., 2000; Petrovic and Ingvar, 2002; Bingel et al., 2006) and descending modulation of spinal nociceptive processes (Yezierski et al., 1983; Zhang et al., 1997; Jasmin et al., 2003). Importantly, sustained activity in left OFC was specifically related to the extent of analgesia. Moreover, sustained OFC activation strongly covaried with the inhibition of shock-evoked activity in the amygdala. This is consistent with the anatomical connexion between OFC and amygdala (Kringelbach and Rolls, 2004) and the release of endogenous opiods during sustained pain (Zubieta et al., 2001). Therefore, we suggest that counterirritation analgesia may depend in part on the activation of OFC that could possibly recruit local opioidergic circuitry in the amygdala.

Another possibility is that OFC activation may be related to reward or punishment processes. Indeed, OFC is part of a reward circuitry activated by noxious stimuli (Becerra et al., 2001) and in the representation of rewards and punishers (Kringelbach and Rolls, 2004). Thus, the activation of OFC may

represent monitoring of analgesia reward value. However, considering that the peak in OFC is lateral, it is more conistent with monitoring of the punishing value of the foot immersion (Kringelbach and Rolls, 2004). Accordingly, OFC was coactivated with a network including other subregions of the OFC, the mPFC, ACC and the amygdala, that have been involved in pain or other negative affects (Rainville et al., 1997; Bechara et al., 2000; Vogt, 2005; LaBar and Cabeza, 2006; Rolls and Grabenhorst, 2008; Seymour and Dolan, 2008).

Compared to analgesia, RIII reflex modulation was related to the activity of partly different regions. This is consistent with the sensorimotor dissociation found in the present and in previous reports (Willer et al., 1979; Terkelsen et al., 2001). This suggests that pain perception and spinal nociceptive processes are regulated, at least in part, by different neural mechanisms. Accordingly, RIII modulation was specifically related to sustained activation of the PAG, consistent with its role in descending modulation (Basbaum and Fields, 1984; Millan, 2002). Furthermore, the coactivation analysis revealed that PAG activity covaried with RVM activity, consistent with their anatomical connexions and their important role in descending modulation (Basbaum and Fields, 1984; Morgan et al., 2008). However, although the PAG can modulate counterirritation effects (Bouhassira et al., 1992c), it is not directly involved in DNIC (Bouhassira et al., 1990). Instead, DNIC is thought to mediate counterirritation effects by the recruitment of the caudal medulla (DeBroucker T. et al., 1990; Bouhassira et al., 1992b). Nevertheless, the caudal medulla was not activated in the present study. Significant modulation of RIII in only a subgroup of participants may have

contributed to this negative result. Another possibility is that fMRI methods used in this study may not have been sensitive enough to detect its activation.

In addition to the PAG, RIII modulation was related to sustained activity in SI, consistent with inhibitory corticospinal projections (Yezierski et al., 1983; Cheema et al., 1984; Ralston and Ralston, 1985; Casale et al., 1988). Another possibility is that SI contributes to descending modulation of nociceptive activity through projections to the PAG (Bragin et al., 1984). .

Limitations and future directions

One limitation of this study is the lack of a clear counterirritation effect on RIII modulation. Although some subjects did show a significant decrease of RIII reflex amplitude, the group effect was not significant. This has been reported previously but most studies have shown a clear inhibition of RIII by counterirritation. Therefore, the correlations between RIII reflex modulation and brain activity in the present study should be interpreted with caution, as they may reflect processes other than those related to counterirritation. Future imaging studies with a larger sample may help to clarify this ambiguity and to contrast differences in brain activity between subgroups of subjects showing inhibition or facilitation of the reflex.

As for technical limitations, the acquisition parameters used in this study aimed at imaging the whole brain. In this respect, they were not optimal for brainstem imaging. This may explain, in part, why we could not find activation in the caudal medulla. Future studies using adapted acquisitions for brainstem

imaging (Dunckley et al., 2005) and analyses methods to correct for physiological noise (Perlbarg et al., 2007; Brooks et al., 2008) may help to increase the sensitivity to detect brainstem activations.

Conclusion

In summary, the present study shows that modulatory effects of counterirritation on pain perception and spinal nociception are accompanied by decreased shock-evoked activity in pain-related areas and sustained activation in brain areas involved in cortical modulation of pain. It was shown that OFC strongly inhibited the amygdala and SI and the PAG modulated spinal nociceptive processes. These results provide evidence for distinct cerebral and cerebrospinal mechanisms underlying the modulation of pain and RIII reflex by counterirritation.

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Table 1. Shock-evoked activity

Brain area	BA*	Side**	t =	x,y,z***
Somatosensory cortex				
Postcentral gyrus (SI)	1-3	L	11.71	-14,-48,75
	1-3	R	5.66	7,-48,70
Parietal operculum (SII)	40	L	6.80	-55,-28,20
	40	R	5.43	55,-28,15
Motor cortex				
Precentral gyrus	4	L	13.80	-10,-28,80
	4	L	9.79	-7,-38,70
	4	R	5.37	10,-31,70
Supplementary motor area (SMA)	6	---	6.73	3,3,70
Cingulate cortex				
Anterior (ACC)	32	---	6.32	3,21,35
Mid (MCC)	24	---	5.47	-3,-3,45
Posterior (PCC)	23	R	4.45	7,-17,30
	23	R	5.06	7,-28,30
Insula				
posterior (pINS)	---	L	9.58	-31,-24,20
	---	R	4.66	45,-31,15
anterior (aINS)	---	L	4.22	-45,3,0
	---	R	7.29	34,14,0
Prefrontal cortex (PFC)				
Middle frontal gyrus	9	L	5.68	-31,34,25
Thalamus	----	L	4.87	-17,-10,15
	----	R	5.32	14,-14,15

Note: Peaks of activity thresholded at $p<0.05$ corrected for multiple comparisons for the search volume using the Random Field Theory, minimum 3 voxels per cluster (see mehtods). * BA: putative Brodmann area. ** L: left controlateral; R: right ispilateral. *** Coordinates are reported in MNI space.

Table 2. Brain regions in which shock-evoked activity was decreased during counterirritation.

Directed search

Brain area	BA*	Side**	t =	x,y,z***
Somatosensory cortex				
Postcentral gyrus (SI)	1-3	L	4.73	-7,-48,70
	1-3	---	4.33	3,-48,75
Parietal operculum (SII)	40	L	6.08	-58,-28,15
Motor cortex				
Precentral gyrus (PCG)	4	L	6.35	-7,-31,70
	4	R	6.00	14,-31,80
Supplementary motor area (SMA)	6	L	6.83	-17,0,75
	6	R	5.15	7,3,65
	6	R	5.02	14,0,75
Cingulate cortex				
Anterior (ACC)	32	---	4.28	3,17,35
	24	R	5.34	7,3,40
	24	R	5.27	7,10,35
Mid (MCC)	24	---	4.99	-3,-3,45
Insula				
posterior (pINS)	----	L	7.58	-31,-24,10
anterior (aINS)	----	R	5.63	45,7,5
Parahippocampal gyrus (PHG)	34	R	5.93	17,7,-20
Prefrontal cortex (PFC)	9	L	6.65	-31,28,25
Thalamus	----	L	7.92	-14,-10,10
	----	R	4.68	10,-7,10

Note: Peaks of activity thresholded at $p<0.05$ corrected for multiple comparisons for the search volume, using the Random Field Theory, minimum 3 voxels per cluster (see mehtods). * BA: putative Brodmann area. ** R: right ipsilateral side; L: left contralateral side. *** Coordinates are reported in MNI space.

Table 3. Brain regions in which changes in shock-evoked responses during counterirritation were specifically related to analgesia or RIII modulation

Directed search

a. Specifically related to analgesia

Brain area	BA*	Side**	t =	r^2	x,y,z***
Postcentral gyrus (SI)	1-3	L	5.08	0.47	-14,-41,80
Cingulate cortex					
Anterior (ACC)	32	----	4.39	0.49	3,21,40
Mid (MCC)	24	----	4.24	0.59	3,-17,30
Posterior (PCC)	31	L	4.09	0.55	-14,-45,35
	31	L	5.17	0.49	-7,-31,30
Prefrontal cortex (PFC)					
Middle frontal gyrus	9/46	R	5.20	0.29	41,41,25
	10	R	7.20§	0.72	24,52,-5
Orbitofrontal cortex (OFC)	11	R	4.94§	0.55	31,48,-10
Amygdala (AMY)	----	L	4.74	0.49	-21,0,-20
	----	L	3.51	0.29	-17,-7,-15

b. Specifically related to RIII modulation

	BA*	Side**	t =	r^2	x,y,z***
Supplementary motor area (SMA)	6	L	5.67§	0.33	-7,0,65
	6	----	4.99	0.42	0,-14,75
Anterior Insula (aINS)	----	L	3.83	0.39	-38,17,0
Prefrontal cortex (PFC)					
Middle frontal gyrus	10	L	4.56	0.20	-45,41,15
Orbitofrontal cortex (OFC)	11	L	4.40	0.47	-24,38,-10

Note: Peaks of activity thresholded at p<0.005 uncorrected * BA: putative Brodmann area. ** R: right ipsilateral side; L: left contralateral side. *** Coordinates are reported in MNI space. § p<0.05 corrected for multiple comparisons for the search volume, using the Random Field Theory, minimum 3 voxels per cluster.

Table 4. Brain regions recruited by the counterirritation stimulus

Directed search

Brain area	BA*	Side**	t =	x,y,z***
Postcentral gyrus	1-3	L	11.26	-21,-31,55
	1-3	R	5.91	7,-41,65
	1-3	---	6.27	0,-45,65
Paracentral lobule	5	L	9.40	-10,-34,60
Parietal operculum (SII)	40	L	6.34	-58,-28,20
	40	R	7.79	65,-28,20
Motor cortex				
Precentral gyrus (PCG)	4	L	5.92	-7,-24,70
	4	R	6.25	14,-28,65
Supplementary motor area (SMA)	6	L	4.59	-10,-3,65
	6	---	7.48	-3,-10,70
	6	---	6.15	0,-14,60
Cingulate cortex				
Anterior (ACC)	32	---	4.79	-3,14,35
	32	---	5.39	3,20,30
	24	R	7.17	14,3,40
	24	---	5.50	-3,3,40
Mid (MCC)	24	---	6.03	-3,-10,45
	24	R	6.46	7,-10,40
Posterior (PCC)	31	L	8.81	-14,-31,40
Insula				
anterior (aINS)	---	L	5.40	-38,3,5
	---	L	5.29	-38,10,-10
	---	R	6.32	38,7,0
posterior (pINS)	---	R	5.61	34,-14,20
Parahippocampal gyrus (PHG)	36	L	8.95	-31,-31,-15
Hippocampus	---	L	5.57	-31,-21,-10
Prefrontal cortex (PFC)				
Medial frontal gyrus	10	L	6.82	-14,52,10
	---	---	---	---
Superior frontal gyrus	10	L	5.94	-34,48,25
	10	R	5.76	28,41,30
	10	R	5.97	34,55,20
Orbitofrontal cortex (OFC)	11	R	4.92	21,34,-15
Thalamus	----	R	4.41	7,-17,15
	----	L	4.98	-7-20,5
	----	R	6.46	10,-3,10
	----	L	4.48	-10,-3,10
Midbrain	----	----	5.53	-3,-21,-20
Pons	----	L	8.81	-7,-38,-35
	----	R	7.01	7,-38,-35

Note: Peaks of activity thresholded at $p<0.05$ corrected for multiple comparisons for the search volume, using the Random Field Theory, minimum 3 voxels per cluster (see mehtods). * BA: putative Brodmann area . ** R: right ipsilateral side; L: left contralateral side. *** Coordinates are reported in MNI space.

Table 5. Sustained activity evoked by counterirritation and specifically related to analgesia or RIII inhibition

Directed search	a. Regions specifically related to analgesia				
Brain area	BA*	Side**	t =	r^2	x,y,z***
Medial prefrontal cortex (mPFC)	10	----	3.44	0.35	0,65,10
Orbitofrontal cortex (OFC)	11/47	L	5.26§	0.65	-28,31,-20
	b. Regions specifically related to RIII inhibition				
	BA*	Side**	t =	r^2	x,y,z***
Somatosensory cortex (SI)	1-3	----	3.41	0.31	0,-41,60
Supplementary motor area (SMA)	6	R	4.26	0.65	7,-10,75
Posterior cingulate cortex (PCC)	23	----	3.75	0.37	0,-24,30
Midbrain (PAG)	----	----	3.74	0.36	-3,-31,-5
Midbrain (lateral)	----	L	3.78	0.39	-10,-28,-15

Note: Peaks of activity thresholded at $p<0.005$. § $p<0.05$ corrected for multiple comparisons for the search volume, using the Random Field Theory, minimum 3 voxels per cluster. * BA: putative Brodmann area. ** R: right ipsilateral side; L: left contralateral side. *** Coordinates are reported in MNI space.

Table 6. Brain networks associated with the analgesia-related peak in OFC and the RIII-modulation peak in the PAG.

Directed search	a. regions coactivated with analgesia-related OFC				b. regions coactivated withRIII-modulation peak in the PAG			
Brain area	BA*	Side**	t =	x,y,z***	BA*	Side**	t =	x,y,z***
Postcentral gyrus (SI)	----	----	----	----	1-3	R	9.29	3,-48,70
Paracentral lobule (PCL)	----	----	----	----	5	R	7.83	-3,-41,50
Motor cortex								
Supplementary motor area (SMA)	----	----	----	----	6	----	10.67	0,-10,70
Pre-Supplementary motor area (pre-SMA)	----	----	----	----	6/8	----	11.08	0,17,65
Cingulate cortex								
Anterior (ACC)	24/32	L	8.29	-7,10,40	32	R	6.22	10,7,45
	32	L	5.65	-7,21,35	32	----	8.11	-3,38,20
	24/32	R	7.81	10,21,30	----	----	----	----
	32	----	6.61	0,31,25	----	----	----	----
Subgenual ACC (sACC)	32	----	8.10	-3,24,-10	----	----	----	----
Posterior (PCC)	23	----	6.73	0,-31,25	23	L	6.29	-10,-24,40
	----	----	----	----	31	R	6.23	-7,-48,30
Retrosplenial cortex (RSC)	----	----	----	----	30	----	10.05	-3,-45,10
Anterior Insula (aINS)	----	L	6.78	-45,7,-5	----	----	----	----
Parahippocampal gyrus (PHG)	37	L	5.90	-24,-41,-10	35	R	8.12	21,-17,-30
	36	R	6.10	34,-10,-20	35	R	6.97	17,-31,-10
Amygdala/parahippocampal gyrus (AMY/PHG)	----	L	6.70	-31,-3,-20	----	----	----	----
Prefrontal cortex (PFC)								
Medial frontal gyrus	10/32	L	9.36	-7,45,-15	9	----	9.23	-3,45,30
	----	----	----	----	9	L	11.23	-10,52,25
	----	----	----	----	10	R	11.12	7,62,15
Inferior frontal gyrus	47	L	8.56	-45,21,-15	9	L	11.80	-55,7,30
	47	L	7.86	-41,14,-10	46	L	7.84	-45,45,10
	46	L	6.42	-52,34,15	46	R	8.27	52,41,10
Middle frontal gyrus	10	L	6.50	-45,45,15	46	L	8.23	-41,34,25
	46	R	7.43	48,17,25	9	R	9.00	52,7,40
	10	R	6.94	31,45,25	9	R	7.40	31,34,33
	10	R	6.31	38,48,0	10	R	7.27	45,52,5
	----	----	----	----	10	R	5.89	41,58,0
Superior frontal gyrus	9	R	6.61	38,34,35	10	R	10.21	31,62,20
Orbitofrontal cortex (OFC)	11	R	10.30	28,34,-15	----	----	----	----
	11	R	9.97	21,21,-15	----	----	----	----
	11	R	7.78	17,34,-20	----	----	----	----
Thalamus	----	----	----	----	----	L	7.06	-3,-14,10
	----	----	----	----	----	L	6.86	-17,-21,15
	----	----	----	----	----	R	8.20	7,-14,-10
Pons	----	----	----	----	----	L	6.91	-7,-24,-35
	----	----	----	----	----	R	6.13	10,-28,-40
Pons/Medulla (RVM)	----	----	----	----	----	----	8.15	-3,-24,-45

Note: Peaks of activity thresholded at $p<0.05$ corrected for multiple comparisons for the search volume, using the random-field theory, minimum 3 voxels per cluster.
* BA: putative Brodmann area . ** R: right ipsilateral side; L: left contralateral side. *** Coordinates are reported in MNI space.

Figure legends

Figure 1 : Repeated-measures ANOVA for shock-pain ratings for each condition (baseline, immersion, recovery; 10 stimulations per condition). The counterirritation stimulus produced strong analgesia that partially persisted during the recovery period. *p<0.05; **p<0.01.

Figure 2 : Friedman repeated-measures ANOVA for RIII reflex amplitude averaged for each condition (baseline, immersion, recovery; 10 stimulations per condition). A) The counterirritation stimulus produced a decrease of RIII reflex amplitude during and after counterirritation but the effect was not significant (p>0.1). B) Individual differences in RIII reflex modulation during counterirritation. Two subgroups are evident showing significant inhibition of RIII (grey triangles) and no inhibition (black dots). C) and D) Individual examples of RIII reflex modulation: arrows indicate the beginning and the end of counterirritation. In the first subject, RIII reflex amplitude was decreased during counterirritation and slowly recovered to reach the baseline level at the end of the session (C). In the other subject, RIII reflex amplitude slightly increased during counterirritation and decreased to a stable level during recovery (D).

Figure 3: Group analysis for shock-evoked activity induced by the 10 stimulations during the baseline condition. See Table 1 for peak T-values. ACC: anterior cingulate cortex; MCC: midcingulate cortex; PrCG: pre-central gyrus;

PCL: paracentral lobule; pINS: posterior insula; SI: primary somatosensory cortex; SII: second somatosensory cortex; SMA: supplementary motor area.

Figure 4: A: Contrast of shock-evoked activity induced by the 10 stimulations during baseline vs counterirritation. See Table 2 for peak T-values. B-C: Multiple regression analysis between decreased shock-evoked activity during counterirritation and B) analgesia and C) RIII modulation. Some of the correlations are illustrated with scatter plots. See Table 3a and 3b respectively for peak T-values. ACC: anterior cingulate cortex; AMY: amygdala; pINS: posterior insula; MCC: midcingulate cortex; OFC: orbitofrontal cortex; PCC: posterior cingulate cortex; PFC: prefrontal cortex; PrCG: pre-central gyrus; SI: primary somatosensory cortex; SMA: supplementary motor area.

Figure 5: A: Sustained activity evoked by the 2-minute left foot immersion. See Table 4 for peak T-values. B-C: Multiple regression analysis between sustained activity and B) analgesia and C) RIII inhibition. See Table 5a and 5b respectively for peak T-values. ACC: anterior cingulate cortex; INS: insula; MCC: midcingulate cortex; mPFC: medial prefrontal cortex; OFC: orbitofrontal cortex; pACC: pregenual anterior cingulate cortex; PAG: periacqueductal gray matter; PCC: posterior cingulate cortex; PCL: paracentral lobule; PrCG: pre-central gyrus; PPC: posterior parietal cortex; SI: primary somatosensory cortex; SII: second somatosensory cortex; SMA: supplementary motor area.

Figure 6: Inhibition of shock-evoked activity in amygdala by OFC. Counterirritation produced a robust and sustained activation of lateral OFC ipsilateral to the foot immersion that was strongly correlated to analgesia (top brain slice). Counterirritation also produced a robust inhibition of shock-evoked activity in the left amygdala, contralateral to the shock (bottom brain slice). The activity of these two structures was strongly associated as illustrated by the scatter plot on the right.

Figure 7: Functional connectivity analysis. A) Brain regions coactivated with the analgesia-related peak in OFC. B) Brain regions coactivated with the RIII-modulation peak in the PAG. The inset is an enlargement of the rostral ventromedial medulla (RVM) area. See table 6a and 6b respectively for peak T-values. ACC: anterior cingulate cortex; aINS: anterior insula; Amy: amygdala; mPFC: medial prefrontal cortex; OFC: orbitofrontal cortex; PAG: periacqueductal gray matter; PCL: paracentral lobule; PHG: parahippocampal gyrus; PPC: posterior parietal cortex; pre-SMA: pre-supplementary motor area; RSC: retrosplenial cortex; RVM: rostral ventromedial medulla; SI: primary somatosensory cortex; SMA: supplementary motor area.

Figure 1

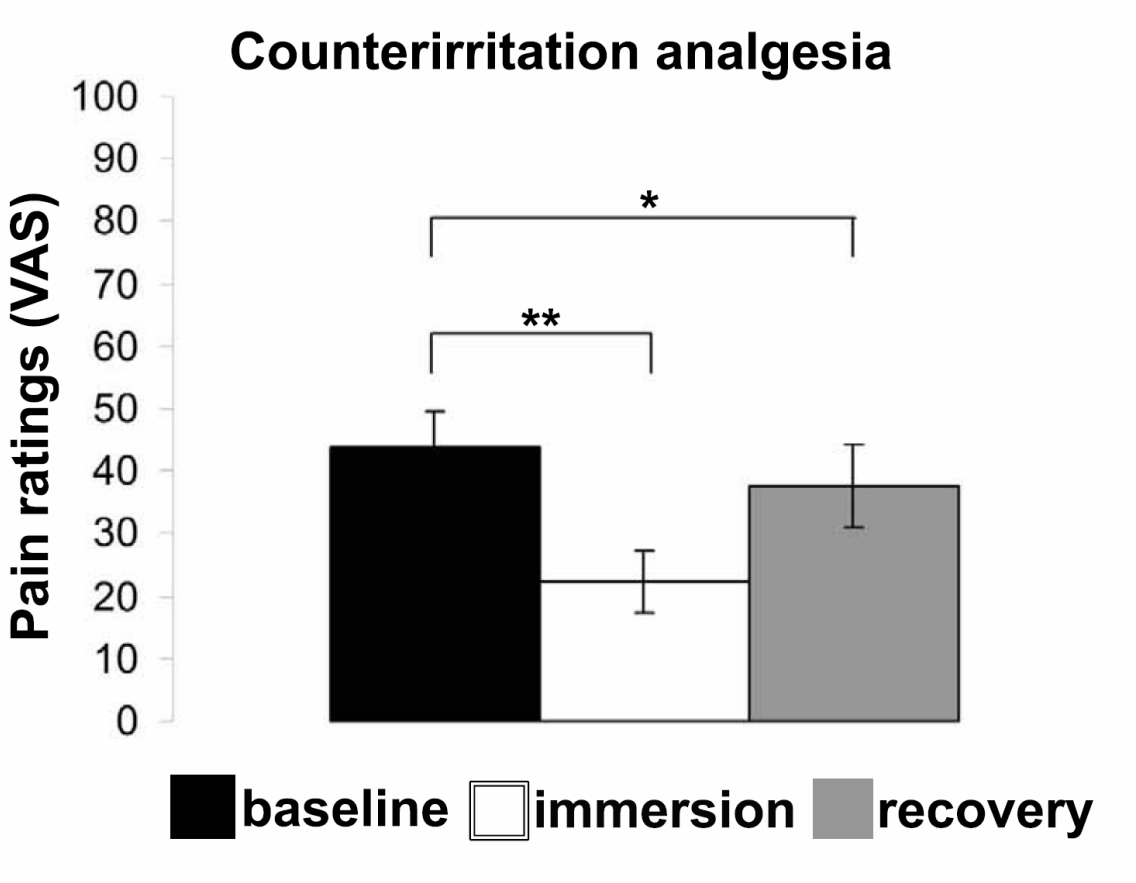

Counterirritation analgesia
Pain ratings (VAS)
100
90
80
70
60
50
40
30
20
10
0
*
**
baseline
immersion
recovery

Figure 2

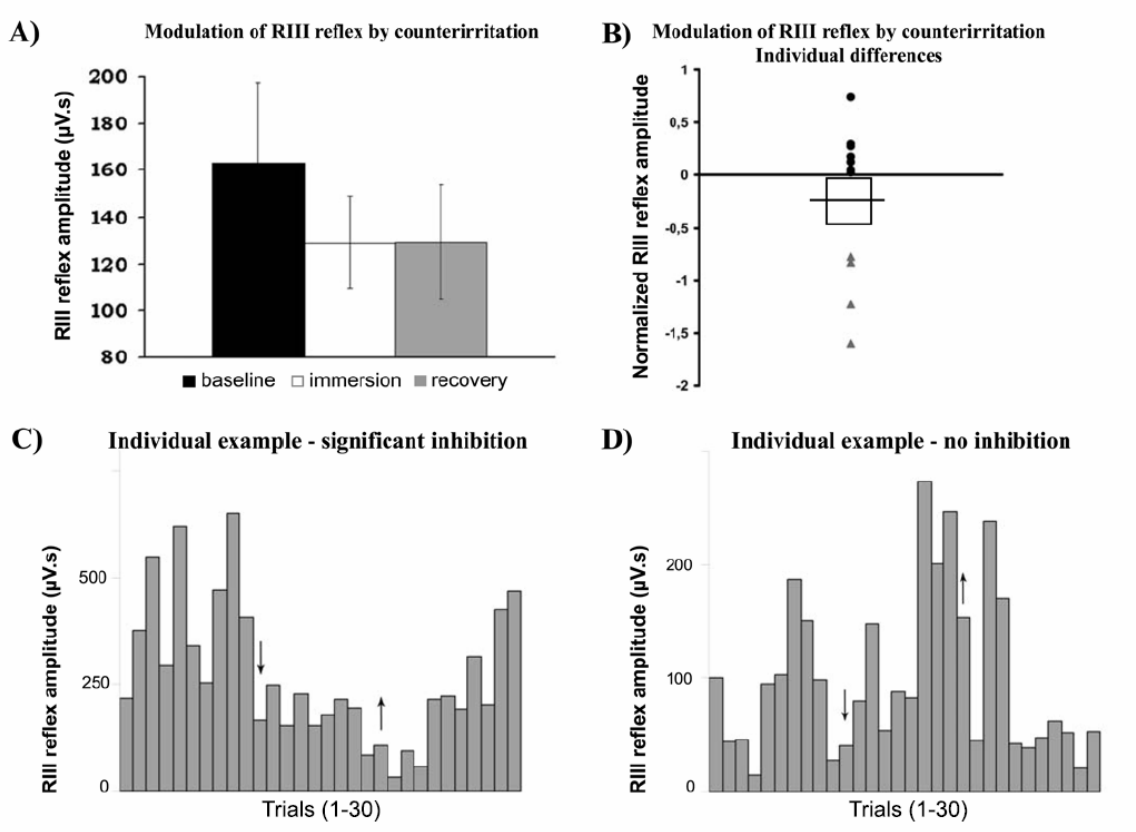


Figure 3

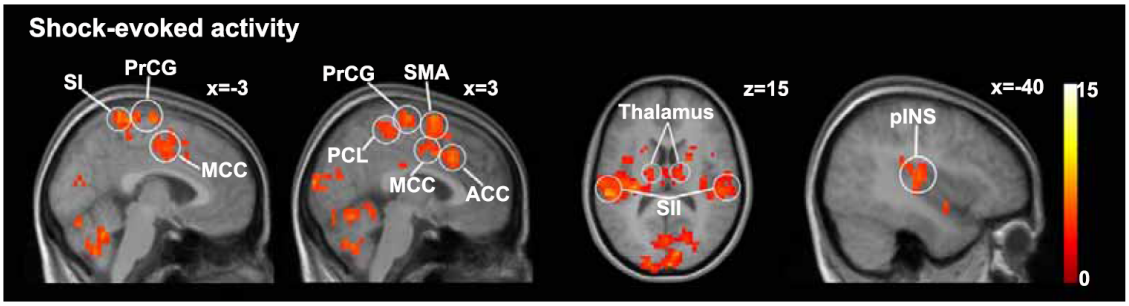


Figure 4

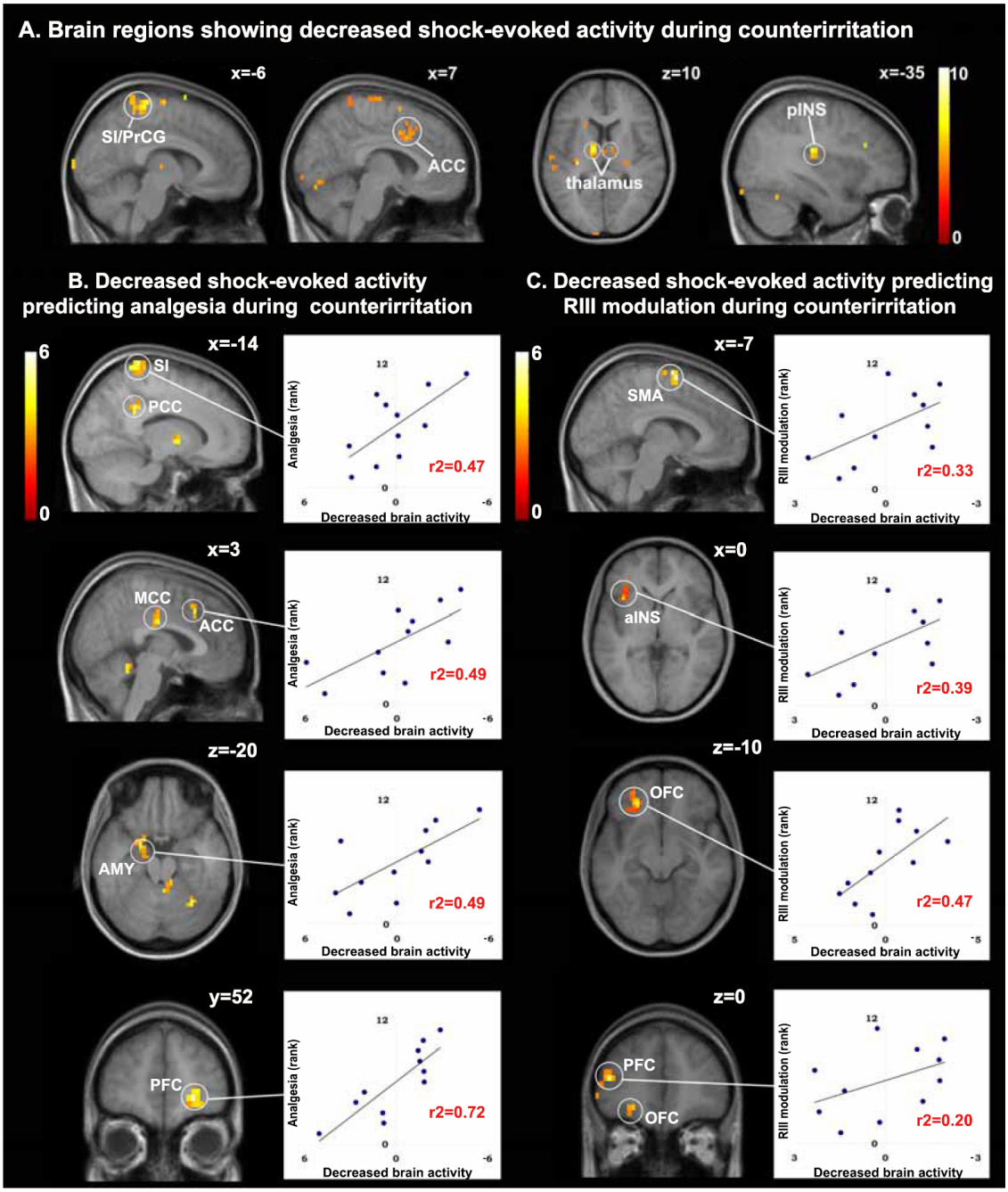

A. Brain regions showing decreased shock-evoked activity during counterirritation
x=-6
x=7
z=10
x=-35
10
0
SI/PrCG
ACC
thalamus
pINS
B. Decreased shock-evoked activity predicting analgesia during counterirritation
C. Decreased shock-evoked activity predicting RIII modulation during counterirritation
6
0
x=-14
SI
PCC
Analgesia (rank)
r2=0.47
Decreased brain activity
x=-7
SMA
RIII modulation (rank)
r2=0.33
x=3
MCC
ACC
r2=0.49
x=0
aINS
r2=0.39
z=-20
AMY
r2=0.49
z=-10
OFC
r2=0.47
y=52
PFC
r2=0.72
z=0
PFC
OFC
r2=0.20

Figure 5

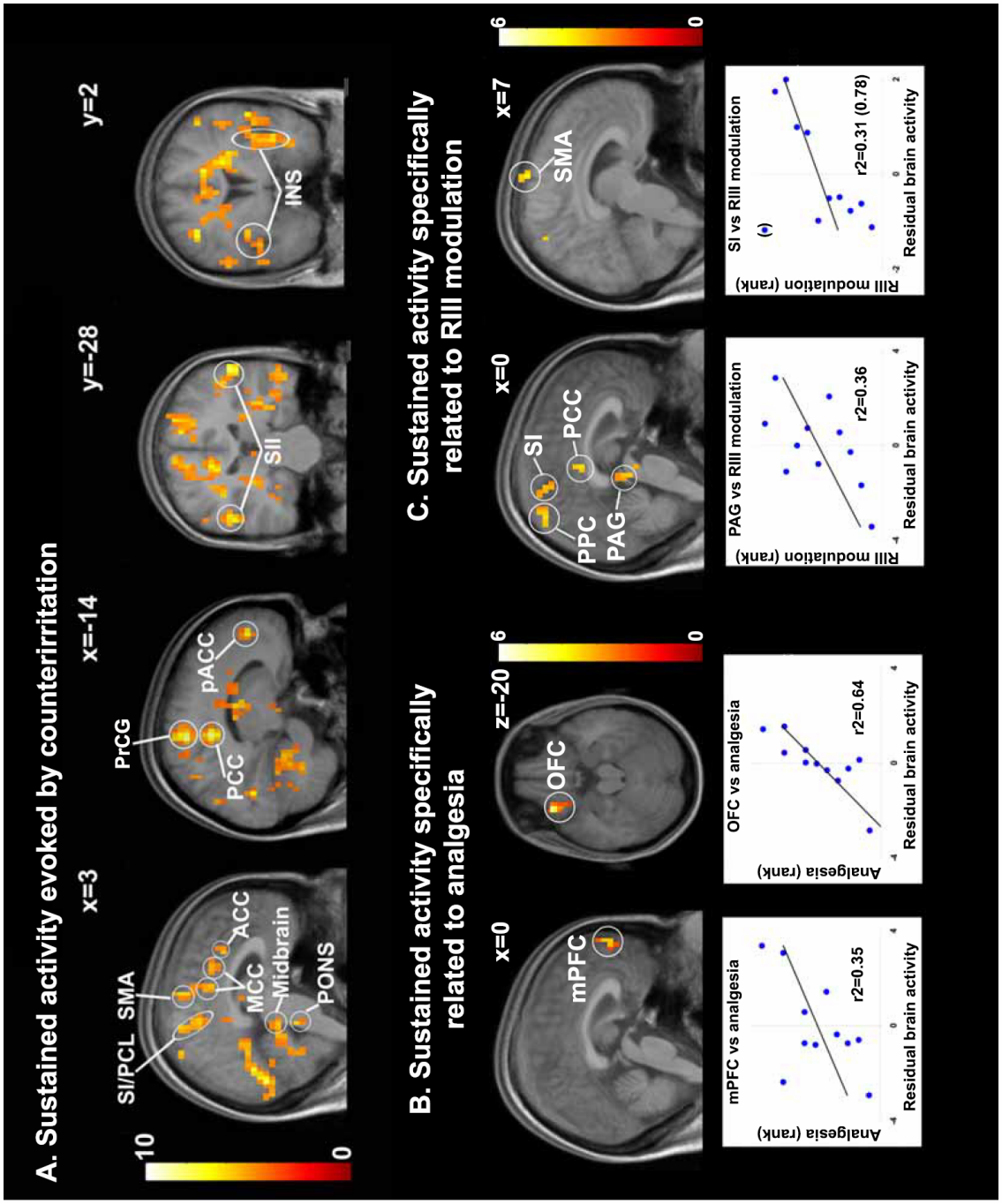

A. Sustained activity evoked by counterirritation
x=3
x=-14
y=-28
y=2
SI/PCL
SMA
MCC
ACC
Midbrain
PONS
PrCG
PCC
pACC
SII
INS
10
0
B. Sustained activity specifically related to analgesia
z=-20
x=0
mPFC
OFC
6
0
mPFC vs analgesia
r2=0.35
Analgesia (rank)
Residual brain activity
OFC vs analgesia
r2=0.64
Analgesia (rank)
Residual brain activity
C. Sustained activity specifically related to RIII modulation
x=7
x=0
SMA
SI
PCC
PPC
PAG
6
0
SI vs RIII modulation
r2=0.31 (0.78)
RIII modulation (rank)
Residual brain activity
PAG vs RIII modulation
r2=0.36
RIII modulation (rank)
Residual brain activity

Figure 6

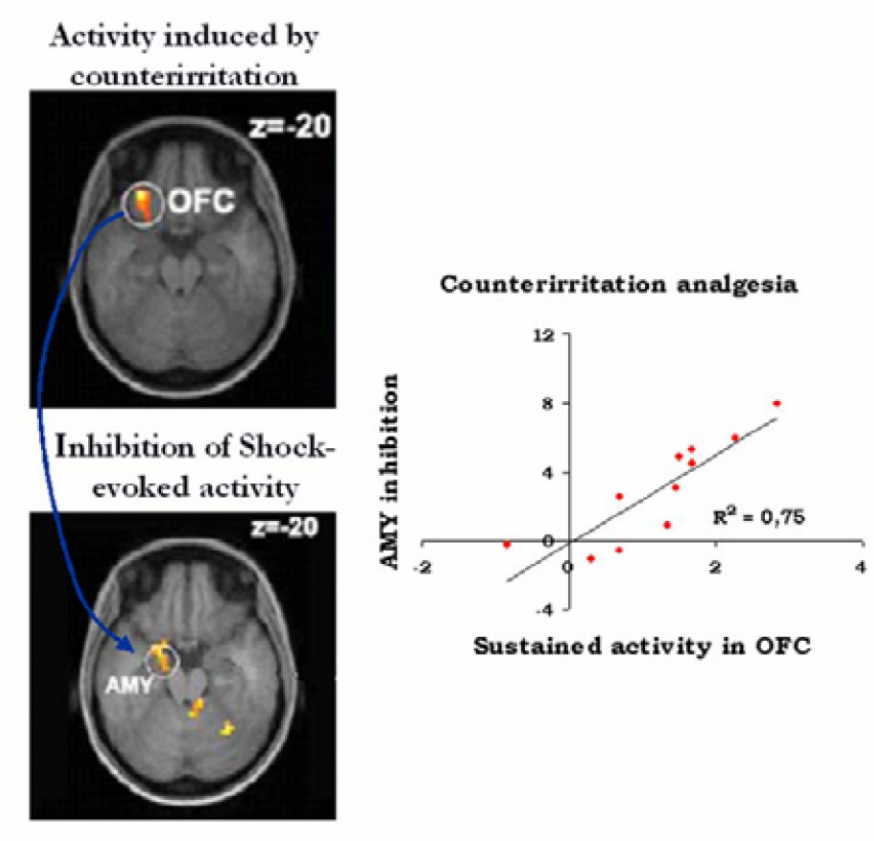
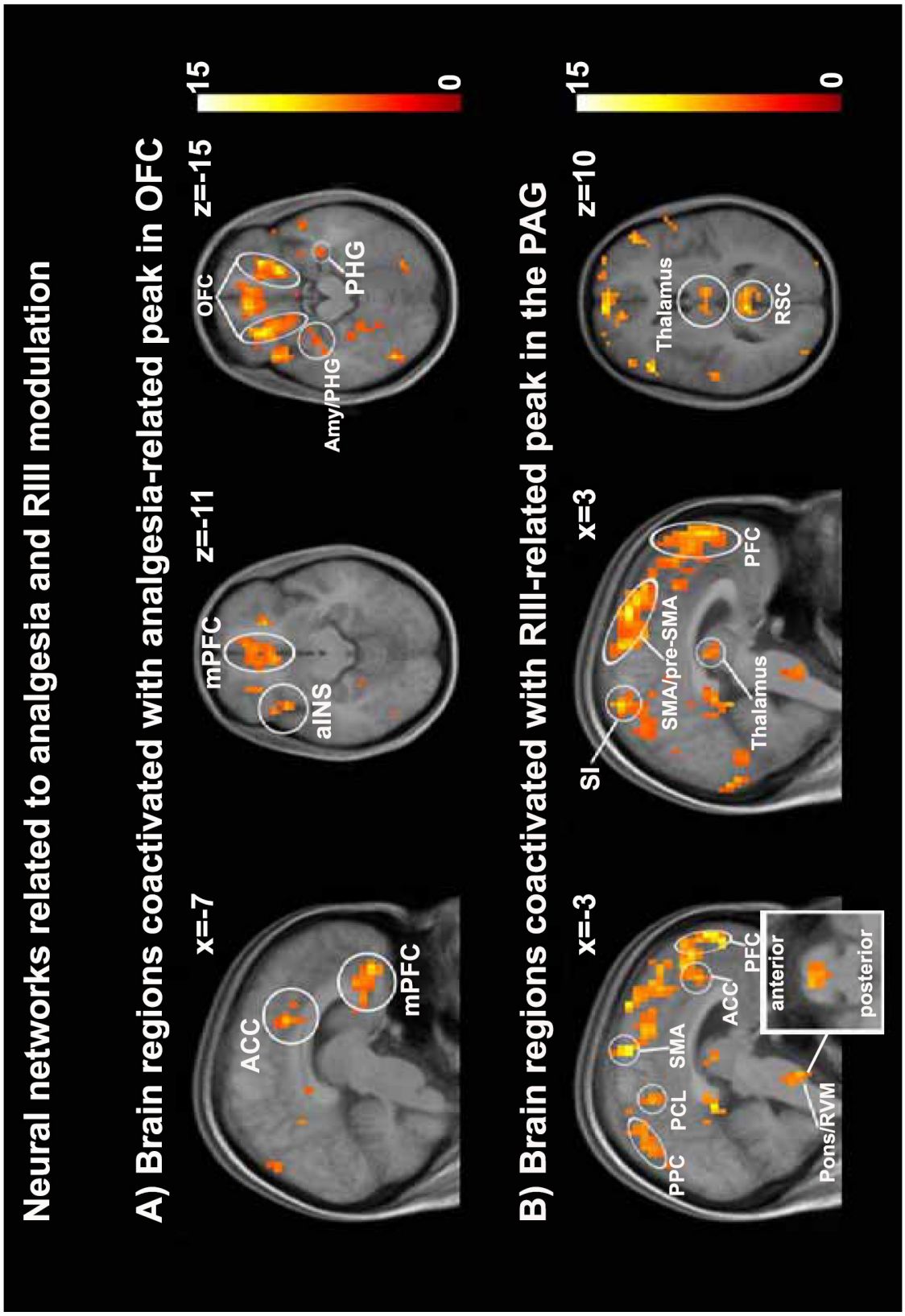

Activity induced by counterirritation
z=-20
OFC
Inhibition of Shock-evoked activity
z=-20
AMY
Counterirritation analgesia
AMY inhibition
Sustained activity in OFC
R^2 = 0,75
12
8
4
0
-4
-2
0
2
4

Figure 7


Neural networks related to analgesia and RIII modulation
A) Brain regions coactivated with analgesia-related peak in OFC
x=-7
ACC
mPFC
z=-11
mPFC
aINS
z=-15
OFC
PHG
Amy/PHG
15
0
B) Brain regions coactivated with RIII-related peak in the PAG
x=-3
PPC
PCL
SMA
ACC
PFC
anterior
posterior
Pons/RVM
x=3
SI
SMA/pre-SMA
Thalamus
PFC
z=10
Thalamus
RSC
15
0

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Supplementary material

Cerebral and cerebrospinal processes underlying counterirritation analgesia

Decreased shock-evoked activity during the recovery period

Shock-evoked activity remained lower than baseline in the recovery period in bilateral PFC, contralateral PrCG, ipsilateral pons and midline PrCG, SMA, ACC and pons (Figure S1A and Table S1). Regression analyses revealed that changes in shock-evoked activity in bilateral pINS and contralateral ACC and PCC was specifically related to analgesia during recovery (Figure S1B and Table S2a). On the other hand, changes in shock-evoked activity in ipsilateral PrCG and OFC, contralateral PHG and midline ACC was specifically related to RIII modulation (Figure S1C and Table S2b). Some of these correlations are illustrated with scatter plots in Figure S1B and S1C respectively. These results indicate that counterirritation effects during the recovery period are paralleled by changes in brain activity and emphasize that counterirritation effects cannot be explained only by distraction.

Interactions between counterirritation activity and shock-evoked activity

During counterirritation, sustained activity related to analgesia in the pons, ACC, mPFC, and OFC predicted the decrease in shock-activity related to analgesia in PCC, PFC and AMY (Table S3). On the other hand, sustained activity related to RIII modulation in PAG, SMA and MCC predicted the decrease of shock-evoked activity related to RIII modulation in SMA and OFC

(Table S3). These results clearly show a functional relation between sustained activity induced by counterirritation and changes in shock-evoked activity.

Table S1. Brain regions in which shock-evoked activity decreased during the recovery period

Directed search				
Brain area	BA*	Side**	t =	x,y,z***
Precentral gyrus (PCG)	4	L	7.17§	-14,-31,70
	4	----	3.73	3,-31,65
Supplementary motor area (SMA)	6	----	5.36§	3,7,70
Anterior cingulate cortex (ACC)	32	----	5.36§	3,21,30
	32	----	4.40	3,21,40
Prefrontal cortex (PFC)				
Superior frontal gyrus	9	L	4.08	-21,52,40
	9	R	3.84	24,52,35
Pons	----	----	3.54	-3.-38,-25
	----	R	4.65	7,-38,-25

Note: Peaks of activity thresholded at p<0.005 uncorrected. * BA: putative Brodmann area. ** R: right ipsilateral side; L: left contralateral side. *** Coordinates are reported in MNI space. § p<0.05 corrected for multiple comparisons for the search volume, using the RandomField Theory, minimum 3 voxels per cluster (see mehtods)

Table S2. Brain regions in which changes in shock-evoked responses during counterirritation were specifically related to analgesia or RIII modulation

Directed search

a. Specifically related to analgesia

Brain area	BA*	Side**	t =	r^2	x,y,z***
Cingulate cortex					
Anterior (ACC)	24	L	4.07	0.45	-14,14,35
Posterior (PCC)	31	L	4.36	0.64	-17,-45,30
Insula					
posterior (pINS)	----	L	7.54§	0.67	-41,-10,15
	----	R	4.76	0.57	45,-10,10
	----	R	5.35	0.61	41,0,5

b. Specifically related to RIII modulaiton

	BA*	Side**	t =	r^2	x,y,z***
Precentral gyrus (PCG)	4	R	4.00	0.48	10,-38,80
Anterior Cingulate Cortex (ACC)	32	----	3.82	0.41	0,17,35
Parahippocampal gyrus (PHG)	36	L	4.28	0.58	-24,-21,-30
Orbitofrontal cortex (OFC)	11	R	4.12	0.44	21,21,-20

Note: Peaks of activity thresholded at $p<0.005$ uncorrected * BA: putative Brodmann area. ** R: right ipsilateral side; L: left contralateral side. *** Coordinates are reported in MNI space. § $p<0.05$ corrected for multiple comparisons for the search volume, using the Random Field Theory, minimum 3 voxels per cluster.

Table S3. Counterirritation activity that predicts shock-evoked activity

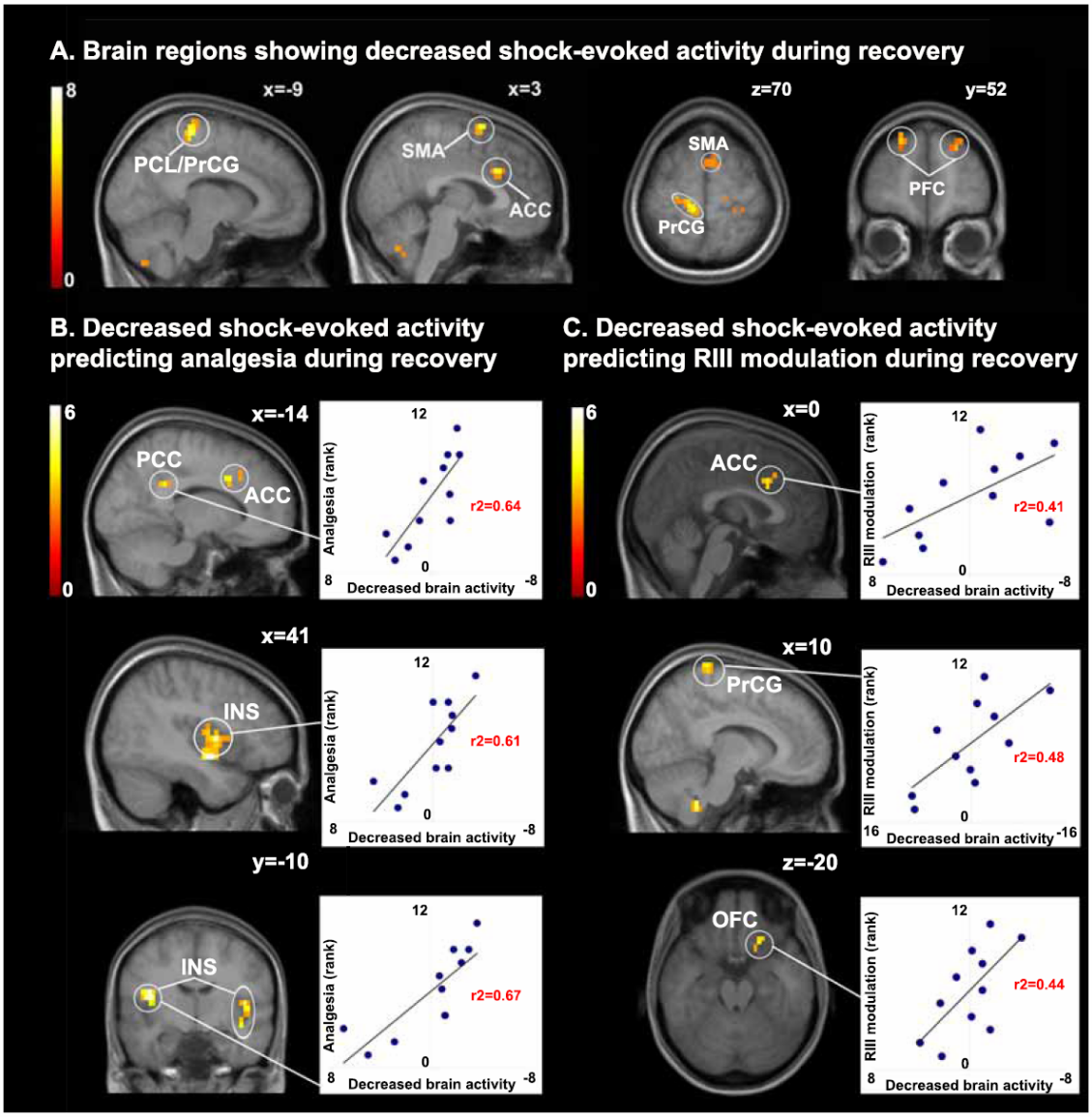
Pearson's r^2	Decreased shock-evoked activity				
	Related to analgesia				
Sustained activity	PCC	PCC	PFC	PFC	AMY
A) Related to analgesia	(-14,-45,35)	(-7,-31,30)	(41,41,25)	(24,52,-5)	(-21,0,-20)
ACC (-14,21,20)			0.64		
mPFC (0,62,5)		0.73	0.65	0.65	
OFC (-31,31,-20)	0.80			0.63	0.75
Pons (-7,-34,-25)		0.64			

Sustained activity	Decreased shock-evoked activity		
B) Related to RIII modulatio	*Related to RIII modulation*		
	SMA (-7,0,65)	SMA (0,-14,75)	OFC (-24,38,-10)
SMA (7,-7,75)	0.70	0.70	
MCC (0,-24,-30)			0.65
PAG (-3,-31,-5)		0.62	

Legend of supplementary materiel figure

Figure S1: A: Contrast of shock-evoked activity induced by the 10 stimulations during baseline vs recovery. See Table S1 for peak T-values. B-C: Multiple regression analysis between decreased shock-evoked activity during recovery and B) analgesia and C) RIII modulation. Some of the correlations are illustrated with scatter plots. See Table S2a and S2b respectively for peak T-values. ACC: anterior cingulate cortex; INS: insula; OFC: orbitofrontal cortex; PCC: posterior cingulate cortex; PCL: paracentral lobule; PFC: prefrontal cortex; PrCG: pre-central gyrus; SMA: supplementary motor area.

Figure S1


A. Brain regions showing decreased shock-evoked activity during recovery
x=-9
x=3
z=70
y=52
PCL/PrCG
SMA
ACC
SMA
PrCG
PFC
B. Decreased shock-evoked activity predicting analgesia during recovery
C. Decreased shock-evoked activity predicting RIII modulation during recovery
x=-14
PCC
ACC
Analgesia (rank)
r2=0.64
Decreased brain activity
x=0
ACC
RIII modulation (rank)
r2=0.41
Decreased brain activity
x=41
INS
Analgesia (rank)
r2=0.61
Decreased brain activity
x=10
PrCG
RIII modulation (rank)
r2=0.48
Decreased brain activity
y=-10
INS
Analgesia (rank)
r2=0.67
Decreased brain activity
z=-20
OFC
RIII modulation (rank)
r2=0.44
Decreased brain activity

Article 3: Widespread hypersensitivity in IBS is related to altered pain inhibition and severity of psychological symptoms.

Widespread hypersensitivity is related to altered pain inhibition processes in irritable bowel syndrome.

Short title*:* Altered pain inhibition processes in IBS

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Abstract

The mechanisms of chronic pain in irritable bowel syndrome (IBS) have been widely investigated but remain unclear. The present study investigated the relation between visceral hypersensitivity, cutaneous thermal sensitivity, and central pain mechanisms. Rectal sensitivity was assessed with a barostat, and forearm and calf sensitivity with a contact thermode. Central mechanisms were assessed by counterirritaiton using sustained cold pain to the hand and painful electric shocks to the ankle. Psychological symptoms were also assessed, using questionnaires. Female volunteers with diarrhea-predominent IBS (n=27) and healthy controls (n=25) participated in the study. IBS patients had lower rectal and calf pain thresholds compared to controls (p's<0.05). IBS patients also reported more pain than controls for rectal distensions, and heat pain on the calf and forearm (all p's<0.001). Cold pain inhibited shock pain in controls but not IBS patients (controls: -13.5 ± 5.3 vs IBS: +1.9 ± 10.5; p<0.01). Importantly, visceral hypersensitivity was correlated to both cutaneous thermal hypersensitivity and pain inhibition deficits. Furthermore, covariance analyses indicated that psychological factors accounted for group differences in visceral hypersensitivity and pain inhibition deficits. In conclusion, this study confirms the relation between altered pain inhibition processes and widespread hypersensitivity in IBS. The present results also suggests that psychological symptoms and altered pain processing in IBS patients may reflect at least in part, common underlying mechanisms.

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder including abdominal pain and bowel dysfunction (Thompson et al., 1999). Several studies have shown that patients with IBS demonstrate increased rectal perception, and central or peripheral sensitization is considered as a plausible physiopathological mechanism in many of these patients (Ritchie, 1973; Mayer and Gebhart, 1994; Mertz et al., 1995; Naliboff et al., 1997; Houghton, 1999; Bouin et al., 2002). Somatic hypersensitivity has also been documented in various studies using a variety of experimental pain tests such as foot or hand immersion in circulating hot water (Verne et al., 2001; Dunphy et al., 2003; Moshiree et al., 2007), hand immersion in cold water (Bouin et al., 2001b), contact heat stimulations applied with a thermode (Rodrigues et al., 2005), or electrical stimulation of the skin, subcutis and muscle layers of the abdomen, upper and lower limb (Caldarella et al., 2006). However, contradictory results showing no somatic hypersensitivity in IBS patients have been reported in other studies using electrocutaneous stimulations (Cook et al., 1987; Iovino et al., 2006), hand immersion in cold water (Whitehead et al., 1990; Zighelboim et al., 1995), transmucosal and transcutaneous electrical stimulation (Accarino et al., 1995) or pressure applied on tender points (Chang et al., 2000). These discrepancies could be related to methodological issues but also to heterogeneity of IBS symptomatology and pathophysiology.

Recent studies were designed to investigate the pathophysiology of pain in IBS. Both peripheral and central mechanisms have been suggested to explain hypersensitivity associated with IBS (Price et al., 2006b; Azpiroz et al., 2007). Spinal sensitization has been proposed to explain both visceral hypersensitivity and the secondary hyperalgesia found in lumbosacral dermatomes, consistent with the viscerosomatic convergence on spinal neurons (Verne et al., 2001). In support of this hypothesis, intra-rectal application of lidocaine in IBS patients was shown to reduce both rectal and thermal cutaneous hypersensitivity in the lower limb. This also implies a role for peripheral afferents in maintaining hypersensitivity (Verne et al., 2003b).

On the other hand, thermal hypersensitivity was also reported in body areas remote from dermatomes in which viscerosomatic convergence might be involved (Verne et al., 2001; Bouin et al., 2001b; Dunphy et al., 2003; Rodrigues et al., 2005; Caldarella et al., 2006; Moshiree et al., 2007). This widespread hypersensitivity has been suggested to reflect an alteration of descending pain modulation mechanisms (Coffin et al., 2004; Wilder-Smith et al., 2004; Song et al., 2006; Wilder-Smith and Robert-Yap, 2007). In these studies, counterirritation was used to activate diffuse noxious inhibitory control (DNIC) mechanisms (LeBars D. et al., 1992). IBS patients displayed decreased inhibition of pain (Wilder-Smith et al., 2004; Song et al., 2006; Wilder-Smith and Robert-Yap, 2007) and facilitation of spinal nociception (Coffin et al., 2004) compared to healthy controls, suggesting a deficit in cerebrospinal modulatory mechanism in IBS patients. However, the profile of pain sensitivity reported in

the patients participating in the studies mentioned above (Wilder-Smith et al., 2004; Song et al., 2006; Wilder-Smith and Robert-Yap, 2007) did not show unequivocal evidence of visceral hypersensitivity in quantitative sensory tests. Furthermore, the correlation between the deficit in inhibitory processes and both somatic and visceral pain sensitivity was not tested directly such that different patients might independently show deficits in any of those tests. Although visceral hypersensitivity, somatic hypersensitivity and altered pain modulation mechanisms could explain pain symptoms in IBS patients, the relation between these deficits remains unclear.

The aim of the present study was to clarify the putative role of central pain mechanisms in IBS. Visceral sensitivity to rectal distensions, somatic sensitivity to cutaneous heat stimulation and modulation of acute pain by the cold pressor test were examined in diarrhoea-predominant IBS patients. Correlation analyses were performed to assess whether visceral hypersensitivity was associated with thermal hypersensitivity and pain modulation deficits. We hypothesized that IBS patients would show visceral and thermal hypersensitivity related to a decrease in pain inhibition during counterirritation, as evidence for an alteration in central pain-regulatory mechanisms.

Methods

Participants

The Research Ethics Board of St-Luc Hospital approved the study. The potential effect of menstrual cycle on pain sensitivity and pain modulation was

controlled by testing all participants in the follicular phase. Participants were instructed to eat and drink as usual on the day of the experiment. All participants gave written informed consent acknowledging their right to withdraw from all or some of the experiments without prejudice and received a compensation of 50$ for their time and commitment to the study.

Controls: Twenty-five healthy women (mean age: 29.0 ± 1.5) volunteered to participate in the study. Recruitment was done by advertisement in the hospital and the university campus. Participants were included if they had normal bowel habits and no known GI disease, and were excluded for: 1) taking any medication within 2 weeks prior to the experiment and 2) having a history of GI symptoms, acute or chronic illness or a psychiatric disorder, as reported in a questionnaire on bowel symptoms and general health history.

Patients: Twenty-seven women (mean age: 34 ± 2.1 years) with diarrhoea-predominant IBS were recruited in the study by advertisement and from referrals to the gastroenterology unit of Hôpital St-Luc in Montreal (Qc, Canada). All these patients were evaluated by one gastroenterologist (MB) experienced in the evaluation of IBS. Normal physical examination, normal colonoscopy with biopsy, exclusion of organic diseases and symptoms of IBS based on Rome III criteria were required to make the diagnosis of IBS. Patients were excluded if they presented other chronic pain syndromes (e.g. fibromyalgia), psychiatric disorders, or used medication that could alter pain perception and modulation in the 2 weeks prior to the experiment.

Procedure

The study consisted in three quantitative sensory experiments performed on the same day: 1) Visceral sensitivity 2) thermal sensitivity and 3) pain modulation by counterirritation. The total duration of sensitivity testing was approximately 40 minutes performed in a single 2-hours session with pauses of at least 15 minutes between tests. Experiment 1 was tested in all 27 IBS patients and 19 controls (some controls only volunteered for the cutaneous tests). Experiment 2 included all 27 IBS patients and all 25 controls but the experiment was interrupted in one control participant who could not tolerate the heat stimulation. The paradigm used in Experiment 3 was always performed last to avoid long-lasting after-effects induced by the sustained cold pressor pain. This experiment was also modified after the beginning of the study because many patients did not tolerate this test (possibly as a result of their hypersensitivity). As a result, a subgroup of 15 IBS patients and 22 controls was tested uniformly with the modified paradigm, as described below. Four additional patients and four controls were further excluded from experiment 3 because they could not tolerate the electrical stimulation. Results of Experiment 3 are therefore based on a subset of 11 IBS and 18 controls.

Visceral sensitivity testing

A barostat bag (thin-wall polyethylene) was attached tightly at both ends to a single-lumen silastic tube (external diameter = 18 French). The lumen of the tube was located within the barostat bag, and the open-end of the tube was

connected to the output of the bellows chamber. The maximal capacity of the bag was 600 mL with a maximal length of 11 cm, resulting in a spherical bag shape. Before placement in the rectum, the barostat bag was checked for air leaks and maintained at 40 mmHg of pressure for 10 minutes. Within the range of volumes used in the study, the barostat bag compliance was considered as infinite.

Distensions were performed with an electronic barostat (J & G electronics, Toronto, Canada) including a built-in computer system programmed to perform automatic distensions with fixed time lags and bag pressure increments. The volume of air inside the bag was determined electronically by the computer from the known excursion of the bellows within the reservoir system. The distension protocol was adapted from previously described procedures for phasic rectal distensions (Whitehead and Delvaux, 1997). After the application of Sodium Phosphates Enema (Fleet-enema; Johnson & Johnson, Toronto, Canada) to empty the rectum, the barostat bag was inserted with the distal attachment site close to the anal canal. The tube was then secured in its proper position with a piece of adhesive. Subjects were comfortably reclined in a supine position on a padded table. Instructions about the nature of the distensions protocol and the ratings of sensations were given to the participants and the examiner remained in the room. Participants had no visual or auditory cues to anticipate the intensity of distensions. Distensions were performed automatically by the computer following the ascending method of limit [25] (10 to 50 mmHg; successive stimuli at 5 mmHg increments). The

volume of air inside the bag was determined by the computer to reach and maintain the desired pressure. Phasic distensions of 30s were delivered with an inter-stimulus interval of 30s, during which the balloon was completely deflated. Each of the 9 distension pressures was delivered only once. During the distension sequence, participants indicated the threshold for the first sensation, pain and unpleasantness and they rated the intensity of pain and unpleasantness at the end of each stimulus. When unpleasantness or pain threshold was not attained, a value of 55 mm Hg was assigned as threshold. It was emphasized that participants could ask the experimenter to interrupt the procedure at any time and that the barostat bag could deflate instantaneously at any time.

Somatic sensitivity testing

Somatic sensitivity was assessed with a $9cm^2$ contact thermode (MEDOC TSA-2001) applied on the right forearm and calf using the ascending method of limit (42-50°C with increments of 0.5°C). Phasic stimulation of 30s was delivered with an inter-stimulus interval of 30s, during which the probe was moved to the other limb (alternating between the anterior aspect of the forearm and the lateral aspect of the calf). Each of the 18 heat stimulation intensity was delivered only once on each limb. Also, the probe was placed on 3 slightly different areas to avoid sensitization (each area of the forearm or calf was stimulated only 6 times at the most, including non painful stimuli). The lateral aspect of the calf was chosen to test for hypersensitivity related to viscerosomatic convergence of

primary afferents from the rectum and lumbosacral dermatomes. The forearm area was chosen as a site remote from lumbosacral dermatomes to test for diffuse somatic hypersensitivity.

Counterirritation paradigm

Participants were comfortably seated in a reclining chair with a knee flexion of approximately 120°. Transcutaneous electrical stimulation was delivered with a Grass S48 stimulator (Astro-Med Inc., West Warwick, RI, USA) isolated with a custom made constant current stimulus isolation unit. The stimulation consisted in a 30 ms train of 10 X 1 ms pulse, delivered on degreased skin over the retro-maleolar path of the left sural nerve by means of a pair of custom made surface electrodes (1 cm^2, inter-electrode distance of 2 cm). Electromyographic (EMG) activity of the short head of the biceps femoris was recorded with a pair of surface electrodes (Type EL-508, Biopac systems Inc., Goleta, CA, USA). The EMG signal was amplified 1000 times, band pass filtered (100-500 Hz), sampled at 1000 Hz (MP150, Biopac systems Inc., Goleta, CA, USA) and stored on a personal computer for off-line analyses. The intensity of the electrical stimulation was adjusted individually at 120% of the RIII-reflex threshold using the staircase method (Willer, 1977). These stimuli were designed to assess the nociceptive flexion reflex (NFR or RIII-reflex). However, because some subjects did not tolerate the stimulus intensity required to reach 120% of the reflex threshold, shock-pain data was obtained using shock intensity corresponding to moderate pain at baseline (i.e. below 120% of

the RIII-threshold). Results of this experiment are therefore limited to ratings of shock-pain and cold-pain.

The brief painful electrical stimulation was delivered every 6 seconds for 8 minutes. After 3 minutes, counterirritation was produced for 2 minutes by immersion of the contralateral hand in cold water adjusted to +4°C just before immersion (cold pressor test). After counterirritation, the hand was removed from water and the shocks continued for 3 minutes to assess recovery. Shock-pain was rated at the end of each of the 3 conditions: (1) before immersion, (2) during immersion and (3) recovery. Cold pain ratings were collected at the end of the recovery period.

Pain and unpleasantness ratings

Participants were asked to rate pain intensity and unpleasantness for each visceral distension and cutaneous heat stimulus in Experiments 1 and 2. Unpleasantness was not limited to pain-evoked unpleasantness but also to the non painful discomfort that the stimulus produced. Pain intensity and unpleasantness were evaluated with two separate visual/numerical analogue scales (VAS) comprising numerical anchors for no pain/unpleasantness (0), light pain/unpleasantness (25), moderate pain/unpleasantness (50), strong pain/ unpleasantness (75) and extreme pain/unpleasantness (100) (Rainville et al., 1992). The sequence of ascending intensities was performed up to the limit of the experiment (50 mmHg or 50°C) or was discontinued when the participants reached their pain tolerance level. In that case, a VAS score of 100 was given to

the subsequent stimulation levels. First, mean pain ratings for all intensities were compared between groups. Then, the group differences were assessed for each intensity level with the Fisher post-hoc analysis.

Psychological assessment

The following three questionnaires were administered before the pain tests to examine their possible modulating effect of pain sensitivity: the pain catastrophizing scale (Sullivan et al., 1995), the Beck Depression inventory (Beck et al., 1996) and the SCL-90 symptom check list for psychological distress (Derogatis, 1994). In addition, the St-Luc Gastrointestinal (GI) Index was used to assess severity of GI symptoms (Poitras et al., 2002a; Poitras et al., 2002b).

Data analysis

All results are expressed as mean ± SEM. Statistical analyses were done in Statistica v6.0 (Statsoft Inc., Tulsa, OK, U.S.A.) with significance thresholds set to $p < 0.05$. Visceral sensitivity, somatic sensitivity, pain modulation and psychological factors were assessed with t-tests, ANOVA, ANCOVA, multiple regressions and Pearson's correlations as needed. For correlation analyses including visceral and thermal hypersensitivity, the ratings at the distension pressure of 50 mm Hg and at a temperature of 44.5°C were used as the group difference was the most important at those levels.

Results

Groups Characteristics

Characteristics of control subjects and IBS patients are reported in Table 1. The groups were not statistically different for age ($p=0.056$), height ($p=0.51$) or weight ($p=0.45$). The severity of GI symptoms was moderate in IBS patients and significantly higher than in controls ($p<0.001$). In addition, IBS patients reported a significantly higher level of pain catastrophizing compared to controls ($p<0.05$), although this level of catastrophizing is within the normal range (Sullivan, 2005) (clinical threshold=30). Moreover, IBS patients reported more symptoms of depression compared to controls ($p<0.05$), indicative of mild depressive symptoms (Beck et al., 1996) (mild depression score: 10-16). Furthermore, IBS patients reported more psychological distress compared to controls ($p<0.001$) although this level of distress is within normal range (Mayer et al., 1975) (clinical threshold=60).

Visceral hypersensitivity in IBS patients

Results on rectal distension thresholds are reported in Figure 1A. There was no significant difference for the first perception threshold between IBS patients and healthy controls (20.7 ± 0.8 mmHg vs 22.4 ± 1.2 mmHg, $p=0.24$). There was also no significant difference for the discomfort threshold between IBS patients and healthy controls (25.0 ± 1.1 mmHg vs 26.6 ± 2.0 mmHg, $p=0.46$). In contrast, the pain threshold was significantly lower in IBS patients

than in controls (35.6 ± 2.1 mmHg vs 46.1 ± 2.4 mmHg, p<0.01), indicating visceral hypersensitivity.

Thermal hypersensitivity in IBS patients

Results on somatic pain thresholds and tolerance are reported in Figure 1B. The analyses of variance testing the group effect across sites confirmed a significant main effect of group for both threshold (F=7.32, p<0.01) and tolerance (F=12.0, p<0.001). Sites did not differ significantly (threshold: F=0.36, p=0.55; tolerance: F=1.6, p=0.21) and the interaction between groups and sites did not approach significance (threshold F=0.06, p=0.80 ; tolerance: F=0.1, p=0.72). Planned pair-wise contrasts confirmed that calf pain threshold was significantly lower in IBS patients than in controls (44.2 ± 0.4 °C vs 45.3 ± 0.4 °C, p<0.05). Calf pain tolerance was also significantly lower in IBS patients than in controls (47.1 ± 0.2 °C vs 47.8 ± 0.1 p<0.05). As for forearm sensitivity, pain tolerance was significantly lower in IBS patients than in controls (47.4 ± 0.2 °C vs 47.9 ± 0.2 °C, p<0.05) but the difference in pain threshold was marginally significant (IBS patients: 44.1 ± 0.4 °C; Controls: 45.0 ± 0.4 °C, p=0.085). However, the absolute group difference in thresholds was similar at the two sites (calf: 1.1°C; forearm: 0.9°C) and the factorial ANOVA revealed no interaction between groups and the site of stimulation (see above). We also verified that the group difference in thermal sensitivity was not accounted for by visceral sensitivity testing performed before thermal sensitivity testing in all IBS patients but not all controls. Excluding controls that did not participate to visceral sensitivity testing did not change any of the results described above. Therefore,

these results are consistent with robust widespread thermal hypersensitivity in IBS patients.

Pain and unpleasantness ratings

Pain and unpleasantness ratings for rectal distensions and cutaneous heat stimulations were compared between groups to assess suprathreshold sensitivity. Overall, for all distension pressures taken together, IBS patients reported significantly more pain ($F(1,387)=34.3$, $p<0.001$) and unpleasantness ($F(1,387)=25.8$, $p<0.001$) compared to controls (see Figure 2A-B). Furthermore, IBS patients significantly reported more pain than controls for distensions of 35 mmHg (23.5 ± 5.3 vs 7.2 ± 3.8; $p<0.05$), 40 mmHg (34.3 ± 6.0 vs 11.8 ± 5.5; $p<0.001$), 45 mmHg (43.9 ± 6.8 vs 15.5 ± 6.1; $p<0.001$), and 50 mmHg (51.8 ± 7.1 vs 19.7 ± 7.2; $p<0.001$), as revealed by the Fisher post-hoc analysis. These results confirm visceral hypersensitivity in IBS patients. As for cutaneous heat stimulation, for all stimulation intensities taken together, IBS patients reported more calf pain ($F(1, 816)=45.1$, $p<0.001$) and unpleasantness ($F(1,816)=26.4$, $p<0.001$) and more forearm pain ($F(1,816)=48.9$, $p<0.001$) and unpleasantness ($F(1,816)=36.6$, $p<0.001$) compared to controls, confirming widespread thermal hypersensitivity in IBS patients (see Figure 2C-F).

Pain modulation by counterirritation

The evaluation of pain modulation by counterirritation was performed by comparing shock-pain intensity and unpleasantness before, during and after immersion of the hand in cold water (see Figure 3A-B). The control group

showed a significant inhibition of shock-pain intensity and unpleasantness during counterirritation compared to baseline (intensity: 28.4 ± 4.7 vs 41.9 ± 6.4, $p<0.001$; unpleasantness VAS: 37.7 ± 5.2 vs 51.7 ± 6.3, $p<0.001$). In contrast, IBS patients showed no significant modulation of shock-pain intensity and unpleasantness during counterirritation compared to baseline (intensity VAS: 51.9 ± 10.5 vs 50.0 ± 7.5, $p=0.67$; unpleasantness VAS: 55.1 ± 8.7 vs 59.1 ± 5.5, $p =0.36$). Furthermore, this modulation of shock-pain intensity and unpleasantness during counterirritation was significantly different between IBS and controls (modulation of intensity VAS: -13.5 ± 5.3 vs +1.9 ± 10.5, $p<0.01$; unpleasantness VAS: -14.0 ± 5.3 vs -4.0 ± 8.8, $p<0.05$ (one-tail). This clearly shows that the decrease in pain normally produced by counterirritation is not observed in this group of IBS patients.

Interestingly, the intensity of the pain produced by the counterirritation stimulus predicted the degree of inhibition of shock-pain intensity ($r^2= 0.27$, $p<0.05$ and unpleasantness ($r^2=0.38$, $p<0.01$) in controls. (see Figure 3C). Conversely, the intensity of the pain produced by the counterirritation stimulus was associated with a non-significant facilitation of shock-pain intensity ($r^2=0.09$, $p=0.37$) and unpleasantness ($r^2=0.14$; $p=0.26$) in IBS patients (Figure 3D). This emphasizes the alteration of pain inhibition processes affecting somatic sensitivity in IBS patients.

Effect of psychological symptoms on pain processing

Table 1 shows that IBS patients scored higher on scales of psychological symptoms. In order to assess the contribution of these psychological factors to pain sensitivity in IBS patients, IBS patients and controls were compared on pain sensitivity measures after accounting for differences in psychological scores (PCS, Beck and SCL-90) using covariance. After controlling for psychological symptoms severity, visceral sensitivity to rectal distensions (threshold and suprathreshold) and pain modulation by counterirritation were not different between groups (p's > 0.05; Table 2). In contrast, thermal sensitivity remained significantly different between groups. This suggests that visceral hypersensitivity and the deficit in pain inhibition shown in IBS patients reflect, at least partly, the effect of psychological factors including catastrophizing, depression and psychological distress on pain processing. To confirm these results, multiple regression models were used to test the correlation between psychological symptoms (catastrophizing, depression and psychological distress) and pain sensitivity. As expected, psychological symptoms were significantly related to visceral pain thresholds (r^2=0.24;p<0.01), visceral hypersensitivity (r^2=0.24;p<0.01) and pain inhibition deficits (r^2=0.;39p<0.01), but not to calf pain thresholds (r^2=0.05;p<0.53), calf pain tolerance (r^2=0.08;p<0.25) and forearm pain tolerance (r^2=0.14;p=0.07). This strengthens the possibility that psychological factors and altered pain processing may reflect common underlying mechanisms in the central nervous system.

Correlation analyses

Pearson's correlations were further performed in order to relate visceral sensitivity to thermal sensitivity, pain inhibition deficits, GI and psychological symptoms in the group of IBS patients separately. Table 3 and Figure 4 summarize the results. Overall, decreased visceral pain threshold was associated with decreased thermal pain threshold of calf and forearm, cold pressor pain sensitivity and with alteration of unpleasantness inhibition during counterirritation. Increased visceral pain during suprathreshold distensions was associated with these same predictors in addition to depression (Beck) and psychological distress (SCL-90). Also, visceral pain thresholds were strongly associated with suprathreshold pain ratings. These results indicate that thermal hypersensitivity, pain inhibition deficits, depression symptoms and psychological distress are predictors of visceral hypersensitivity. Unexpectedly, the severity of GI symptoms did not correlate with visceral nor thermal hypersensitivity. These results confirmed that hypersensitivity and pain inhibition deficits are related to each other and to psychological symptoms but indicate that these pain-related symptoms are not associated with more severe GI symptoms in this group of IBS patients.

Discussion

In the present study, visceral sensitivity was assessed in relation to thermal sensitivity and pain inhibition produced by the cold pressor test, in a group of female patients with diarrhea-predominent IBS and a group of healthy

controls. In addition to visceral and thermal cutaneous hypersensitivity, IBS patients showed a clear deficit of somatic pain inhibition. Importantly, the deficit of pain inhibition was significantly associated with visceral and thermal hypersensitivity. Patients also reported more psychological symptoms compared to controls and the severity of these symptoms was related to visceral hypersensitivity and pain inhibition deficits. This is the first study to show a relation between altered pain inhibition processes and visceral and thermal hypersensitivity in irritable bowel syndrome. The present results also demonstrate that psychological symptoms and altered pain processing in IBS patients, including visceral hypersensitivity and deficits in pain inhibition, may reflect at least in part, common underlying mechanisms.

Widespread hypersensitivity

In the present study, IBS patients showed clear visceral hypersensitivity, with threshold and suprathreshold distensions, consistent with several studies (Ritchie, 1973; Mertz et al., 1995; Accarino et al., 1995; Verne et al., 2001; Bouin et al., 2002; Dunphy et al., 2003; Moshiree et al., 2007; Azpiroz et al., 2007). In addition, widespread thermal hypersensitivity was demonstrated with cutaneous heat stimulation at threshold and suprathreshold temperatures, consistent with a previous study using similar methods (Rodrigues et al., 2005). An important question concerning the mechanisms of hypersensitivity in IBS is whether somatic hypersensitivity depends on viscerosomatic convergence or whether it extends uniformly over all areas of the body. In a study using

cutaneous heat stimulation, a group of 9 IBS patients had uniform cutaneous heat hypersensitivity independent of stimulus location or intensity (Rodrigues et al., 2005). However, this conclusion was based on suprathreshold ratings only. In this context, the use of suprathreshold pain ratings vs pain thresholds may lead to different conclusions. Accordingly, the present results indicate that suprathreshold stimuli are rated as more painful in IBS patients compared to controls, but the group difference for forearm pain threshold was marginally significant. Although the difference between forearm and calf pain thresholds was not statistically significant in IBS patients, this somewhat weaker hypersensitivity at the forearm is consistent with topographically organized central hyperexcitability, as shown in animal models of persistent pain (Mao et al., 1995). It is also consistent with the more pronounced thermal hypersensitivity in lumbosacral dermatomes shown in a study using hot water immersion of the foot and hand (Verne et al., 2001). In addition, it is in agreement with another study showing skin hypersensitivity to electrical stimulation, present in areas of pain referral but not in control sites (Caldarella et al., 2006). Taken together, the results of these studies and ours indicate that somatic hypersensitivity in IBS patients involves different regions of the body but may be more readily detected in dermatomes which afferents converge on spinal neurons receiving visceral afferents. These results also emphasize that somatic sensitivity should be assessed with more than one pain measure (pain threshold, suprathreshold pain ratings, tolerance threshold) and on different sites of the body. These methodological issues in addition to differences in the

specific modality tested (thermal, electrical, mechanical), the severity of psychological symptoms and heterogeneity of IBS symptomatology and pathophysiology may explain why some previous studies using electrocutaneous stimulations (Cook et al., 1987; Iovino et al., 2006), hand immersion in cold water (Whitehead et al., 1990; Zighelboim et al., 1995), transmucosal and transcutaneous electrical stimulation (Accarino et al., 1995) or pressure applied on tender points (Chang et al., 2000) reported no somatic hypersensitivity in IBS patients.

Deficit of pain inhibition processes

Previous studies have shown that visceral pain inhibition induced by the cold pressor test is altered in IBS patients (Wilder-Smith et al., 2004; Song et al., 2006; Wilder-Smith and Robert-Yap, 2007) and it was suggested that altered pain processing in IBS patients is related to modification of cerebro-spinal modulatory processes (Coffin et al., 2004). In the present study, control subjects showed a strong inhibition of shock-pain during cold counterirritation. This effect was also stronger for subjects reporting more cold pressor pain. This is consistent with the recruitment of diffuse noxious inhibitory controls proportionally to the intensity of the counterirritation stimulus (Willer et al., 1989). Conversely, IBS patients showed no significant shock-pain inhibition during the cold pressor test, indicating a clear alteration of pain modulation processes. This is consistent with the studies mentioned above (Coffin et al., 2004; Wilder-Smith et al., 2004; Song et al., 2006; Wilder-Smith and Robert-

Yap, 2007) and shows that not only visceral but also somatic pain inhibition is altered in IBS patients. An important finding is that this pain inhibition deficit was significantly related to visceral and thermal hypersensitivity. Although the present experimental design does not allow the demonstration of a causal relation, this suggests that in addition to spinal sensitization (Verne et al., 2003b) and psychological factors (Whitehead and Palsson, 1998; Mayer and Collins, 2002), altered pain modulation processes may lead to visceral and somatic hypersensitivity.

Contribution of psychological symptoms to altered pain processing

Increased psychological symptoms have been reported in IBS patients (Whitehead et al., 1980; Creed and Guthrie, 1987; Kumar et al., 1990; Folks and Kinney, 1992; Caudell, 1994; Poitras et al., 2002a) and experimental manipulation of the psychological state was shown to modulate sensory thresholds to visceral distension (Ford et al., 1995; Accarino et al., 1997; Dickhaus et al., 2003; Posserud et al., 2004). Therefore, the relative contribution of these factors has to be taken into account in the interpretation of quantitative pain tests. The present results confirm that IBS patients have more psychological symptoms than healthy controls. Importantly, the large group differences in visceral sensitivity and pain inhibition during the cold pressor test were not significant after controlling for the severity of these psychological symptoms. This does not invalidate the findings on visceral hypersensitivity and pain inhibition deficits in IBS patients but shows that at least part of the group

difference relies on common factors underlying psychological symptoms. This is consistent with the role of psychological symptoms in visceral and cutaneous hypersensitivity previously suggested (Whitehead and Palsson, 1998; Dunphy et al., 2003; Moshiree et al., 2007). In addition, it is well known that psychological factors such as negative affects can enhance pain perception and trigger pain facilitation processes (Meagher et al., 2001; Rhudy et al., 2005; Vogt, 2005). In light of these findings, we propose that chronic visceral hypersensitivity in IBS patients leads to the development and/or the reinforcement of psychological symptoms, which in turn leads to enhanced pain facilitation processes. We further suggest that these pain facilitation processes would be recruited during visceral distension testing, therefore contributing to visceral hypersensitivity observed in a majority of IBS patients. Pain facilitation processes would also be recruited during the cold pressor test and mask the effects of diffuse noxious inhibitory controls, therefore contributing to the shock-pain inhibition deficit observed in IBS patients. These hypothetical mechanisms are not exclusive but rather add to peripheral and central sensory dysfunctions that have been proposed by others (Price et al., 2006b), by integrating them within a bio-psychological model of pain processing.

As a demonstration of the complexity of the pathophysiological processes involved, the thermal hypersensitivity observed in this study was not significantly related to psychological symptoms in IBS patients. This implies that hypersensitivity may develop at least partly independently from psychological

symptoms and that psychological symptoms are insufficient to explain every aspect of altered pain processing in IBS.

Limitations and future directions

One limitation of the study is the relatively small number of patients included, especially in the third experiment. Although the difference in counterirritation analgesia between groups is very robust, it is difficult to determine if this pain inhibition deficit is representative of the IBS population. Although the relation between pain inhibition deficits, hypersensitivity and psychological symptoms is very clear, further investigations with larger samples are needed to confirm this relation. Also, our results apply to female IBS-D patients only. Although this is an important limitation, the recruitment of a subgroup of female IBS patients was done to limit heterogeneity. In addition, previous studies suggest that altered central pain modulation processes may generalize to constipation-predominant and alternating IBS (Coffin et al., 2004; Wilder-Smith et al., 2004). Future studies are needed to confirm the generalization of our results.

Additional studies with concomitant measures of brain activity are also required. Although some brain imaging studies have documented differences in brain activity during visceral and cutaneous pain perception or modulation in IBS (Verne et al., 2003a; Song et al., 2006; Berman et al., 2008), future research must further examine the central correlates of the hyperalgesic responses, particularly in relation to the potential abnormality in cerebral and spinal

inhibitory processes. These studies will help assess whether pain inhibition deficits are related to the activity of brain structures such as the medulla, the periacqueductal gray matter or the cingulate cortex and whether abnormal cerebro-spinal regulation is associated with psychological symptoms.

In conclusion, the results of the present study suggest widespread hypersensitivity in IBS patients, affecting visceral as well as thermal cutaneous sensitivity. Importantly, this study demonstrates that this widespread hypersensitivity is related to pain inhibition deficits. In addition, psychological symptoms were shown to contribute to these sensory dysfunctions and may be involved in pain modulation processes that are related to chronic pain. Future studies should clarify the interactions between cerebro-spinal processes involved in pain processing and sensory dysfunctions observed in IBS patients. Understanding these mechanisms may lead to development of better pharmacological and non pharmacological treatments for pain management in IBS patients.

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Table 1: Characteristics of participants

	Age (y.o.)	Height (m.)	Weight (Kg.)	GI Symptoms Severity	Pain catastrophizing	Depression (Beck)	Psychological Distress
IBS (n=27)	34±1.5	1.65±0.01	67.2±2.8	63.6±4.9***	20.1±2.7*	11.1±1.9*	57.3±1.8***
Controls (n=25)	34±1.5	1.65±0.01	64.5±2.1	8.1±2.4	11.6±2.0	4.9±1.4	44.1±2.3

Table 2. Effect of psychological symptoms on pain processing

IBS vs controls	Without covariance t (p-value)	With Psy covariance F (p-value)
Visceral pain threshold	3.26 (0.002)	***2.23 (0.14)***
Visceral hyperalgesia	3.23 (0.002)	***3.18 (0.08)***
Calf pain threshold	2.20 (0.03)	4.99 (0.03)
Forearm pain tolerance	2.28 (0.03)	4.45 (0.04)
Calf pain tolerance	2.61 (0.01)	6.73 (0.01)
Pain modulation by counterirritation	2.80 (0.009)	***1.41 (0.25)***

Table 3. Correlation analyses of sensitivity disturbance in IBS patients

Pearson-r^2	Visceral pain threshold	Visceral hyperalgesia
Visceral pain threshold (n =27)	-	0.76***
Forearm pain threshold (n =27)	0.22*	0.16*
Calf pain threshold (n =27)	0.23*	0.19*
Forearm hyperalgesia (n =27)	0.22*	0.27**
Calf hyperalgesia (n =27)	0.19*	0.20*
Cold pressor pain sensitivity (n =11)	0.18*	0.20*
Pain modulation by counterirritation – intensity (n =11)	0.27	0.19
Pain modulation by counterirritation – unpleasantness (n =11)	0.53*	0.37*
IBS symptoms severity (n =27)	0.01	0.01
Pain catastrophising scale (n =27)	0.08	0.08
Beck Depression Inventory (n =27)	0.06	0.19*
SCL-90 global severity index (n =27)	0.11	0.23*

Figure legends

Figure 1. Visceral and thermal hypersensitivity in IBS patients. A) Perception and discomfort thresholds were not different between groups but IBS patients showed a lower pain threshold compared to controls. B) IBS patients showed lower calf pain thresholds and lower calf and forearm pain tolerance for cutaneous heat stimulation. Forearm pain threshold was also lower in IBS patients although the effect was not statistically significant. **p<0.01; *p<0.05; n.s. not significant.

Figure 2. Stimulus-response curves for visceral and thermal sensitivity. IBS patients showed increased pain intensity and unpleasantness compared to controls for rectal distensions and cutaneous heat stimulation of calf and forearm.

Figure 3. Altered pain inhibition in IBS patients. Counterirritation produced a profound inhibition of pain intensity and unpleasantness in controls (A-B). In contrast, IBS patients did not show significant inhibition of either pain intensity or unpleasantness during counterirritation (A-B). Stronger immersion pain was related to stronger shock-pain inhibition during the cold pressor test in controls (C). In contrast, IBS patients showed a trend towards facilitation of shock-pain with stronger immersion pain (D). ***p<0.001 compared to baseline; # p<0.05, § p<0.01 between groups.

Figure 4. Thermal hypersensitivity and pain inhibition deficits predict visceral hypersensitivity in IBS patients. A) Forearm pain ratings: pain intensity reported at 44.5°C in IBS patients; Visceral pain ratings: pain intensity reported at 50 mmHG in IBS patients. B) Unpleasantness modulation: difference of unpleasantness ratings between baseline and during the cold pressor test.

Figure 1

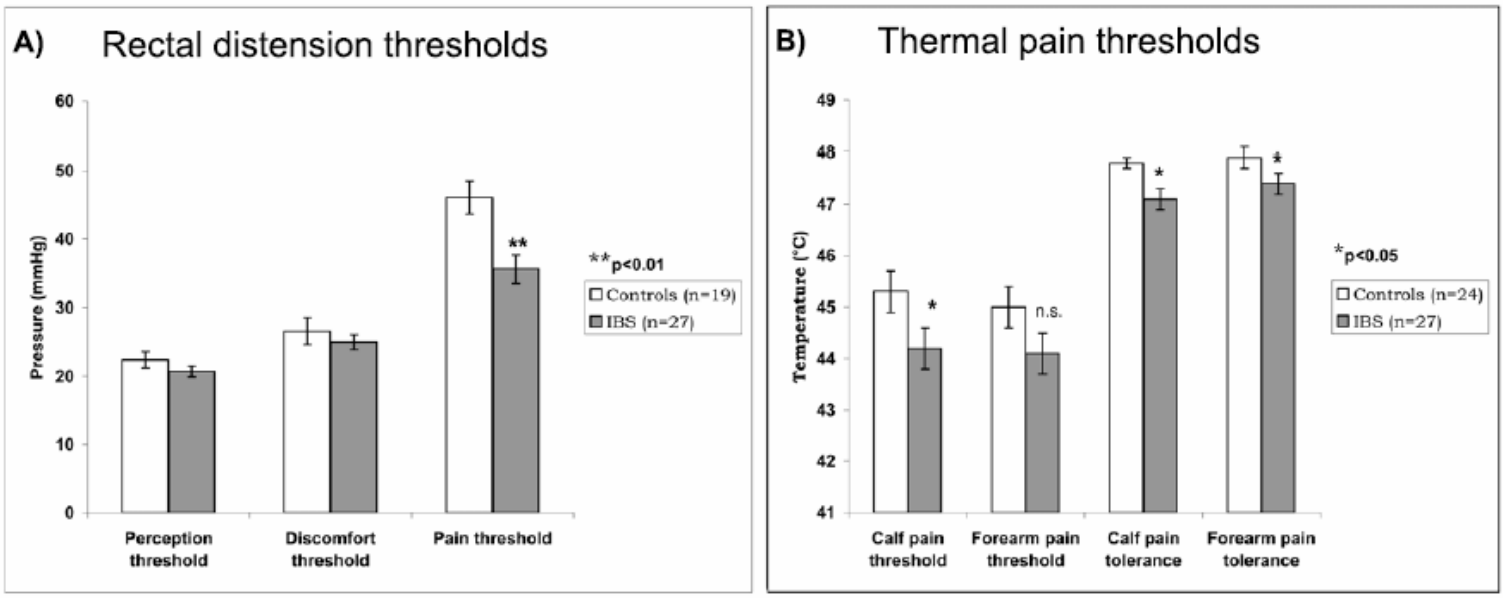


Figure 2

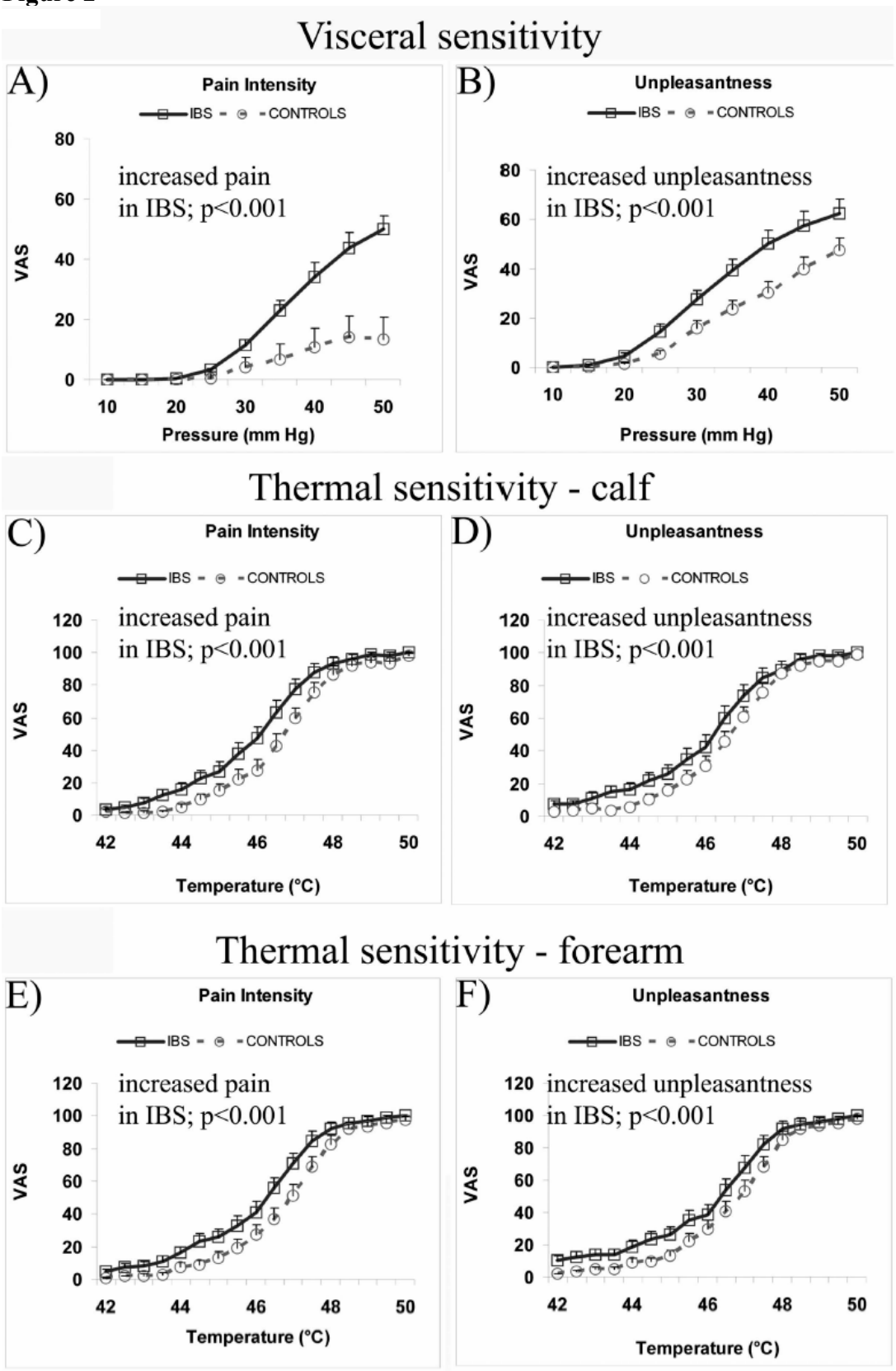

Visceral sensitivity
A)
Pain Intensity
IBS
CONTROLS
increased pain
in IBS; p<0.001
VAS
Pressure (mm Hg)
B)
Unpleasantness
IBS
CONTROLS
increased unpleasantness
in IBS; p<0.001
VAS
Pressure (mm Hg)
Thermal sensitivity - calf
C)
Pain Intensity
IBS
CONTROLS
increased pain
in IBS; p<0.001
VAS
Temperature (°C)
D)
Unpleasantness
IBS
CONTROLS
increased unpleasantness
in IBS; p<0.001
VAS
Temperature (°C)
Thermal sensitivity - forearm
E)
Pain Intensity
IBS
CONTROLS
increased pain
in IBS; p<0.001
VAS
Temperature (°C)
F)
Unpleasantness
IBS
CONTROLS
increased unpleasantness
in IBS; p<0.001
VAS
Temperature (°C)

Figure 3

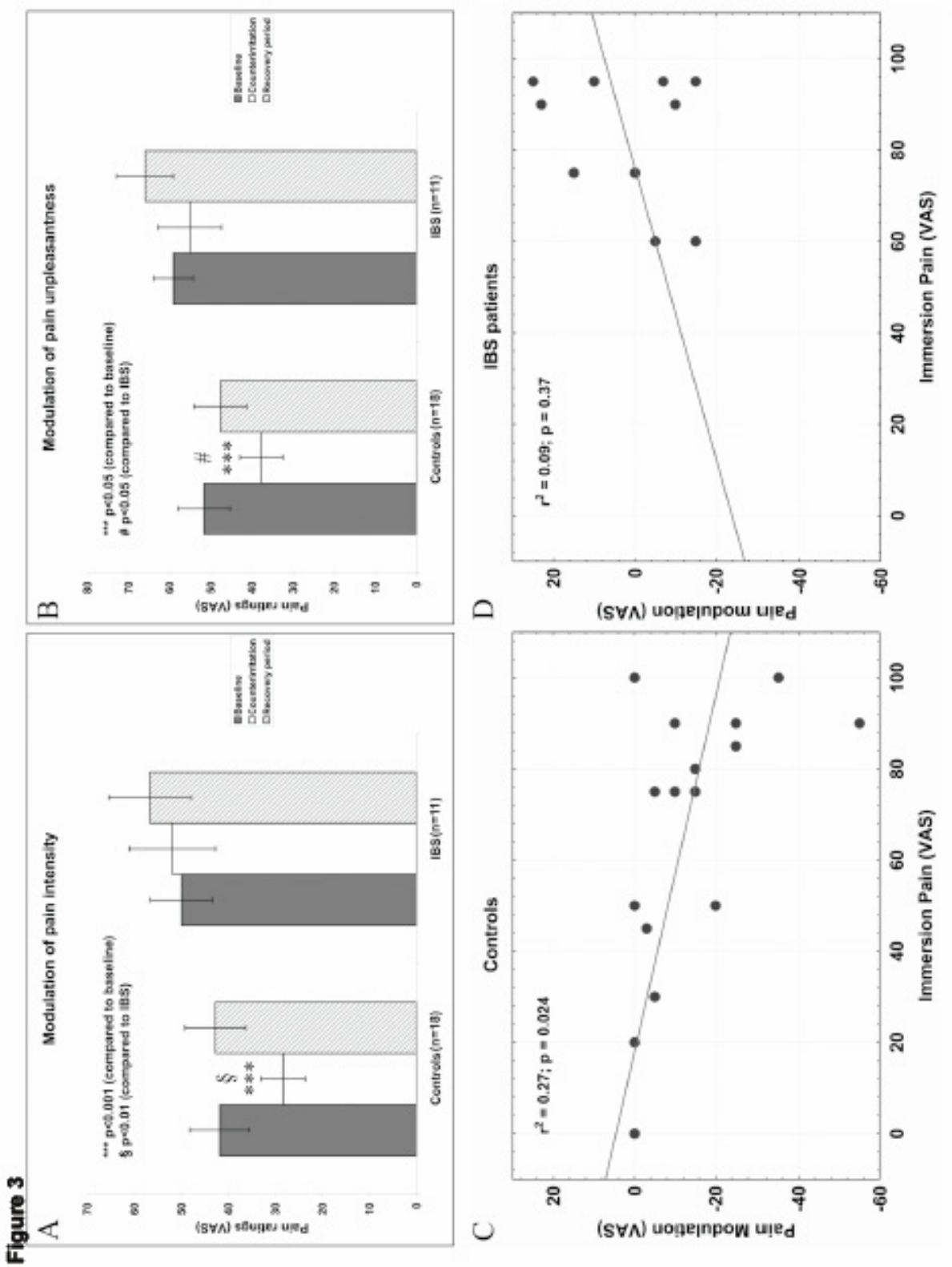
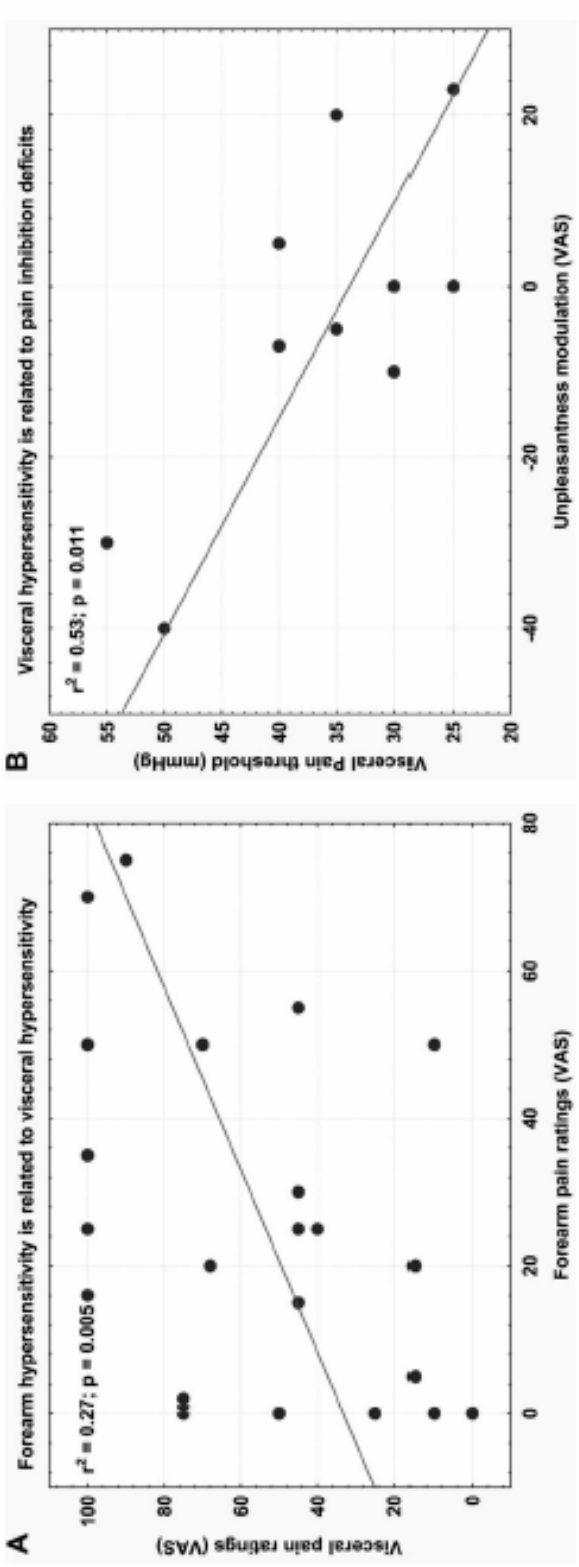

A
Modulation of pain intensity
*** p<0.001 (compared to baseline)
§ p<0.01 (compared to IBS)
Pain ratings (VAS)
Baseline
Counterirritation
Recovery period
Controls (n=18)
IBS (n=11)
B
Modulation of pain unpleasantness
*** p<0.05 (compared to baseline)
# p<0.05 (compared to IBS)
C
Controls
r² = 0.27; p = 0.024
Pain Modulation (VAS)
Immersion Pain (VAS)
D
IBS patients
r² = 0.09; p = 0.37

Figure 4


A
Forearm hypersensitivity is related to visceral hypersensitivity
r^2 = 0.27; p = 0.005
Visceral pain ratings (VAS)
Forearm pain ratings (VAS)
B
Visceral hypersensitivity is related to pain inhibition deficits
r^2 = 0.53; p = 0.011
Visceral Pain threshold (mmHg)
Unpleasantness modulation (VAS)

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Article 4: Decreased pain inhibition in irritable bowel syndrome is associated with altered descending modulation and pain catastrophizing

Decreased pain inhibition in irritable bowel syndrome is associated with altered descending modulation and pain catastrophizing.

Short title*:* Modulation of pain and spinal nociception in IBS

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ABSTRACT

Objective: The aim of the present study was to assess the contribution of spinal as well as supra-spinal processes to pain inhibition deficits in IBS.

Participants: 14 female patients with diarrhoea-predominant IBS (IBS-D) and 14 healthy female volunteers were recruited in the study.

Interventions and outcome measures: Acute pain and the nociceptive flexion reflex (RIII reflex) were evoked by transcutaneous electrical stimulation applied to the right sural nerve and modulated by heterosegmental counterirritation produced by sustained cold pain applied on the left forearm. Psychological symptoms were assessed with questionnaires.

Results: Shock-pain decreased significantly during counterirritation in controls (mean ± SEM: 39.1 ± 5.0 vs 50.5 ± 5.4, $p<0.001$) but not in IBS (41.3 ± 5.2 vs 39.6 ± 4.6, $p=0.64$). Similarly, RIII reflex amplitude decreased during counterirritation in controls ($p<0.003$) but not in IBS patients ($p>0.4$). Furthermore, pain-related anxiety increased during counterirritation in IBS patients (37.8 ± 6.5 vs 27.8 ± 4.7, $p<0.01$) but not in controls 34.4 ± 6.3 vs 35.5 ± 5.9, $p=0.76$). Interestingly, counterirritation analgesia was correlated positively with the RIII-reflex inhibition ($r=0.39$, $p<0.05$) and negatively with pain-related anxiety ($r=0.61$, $p<0.001$). In addition, individual differences in counterirritation analgesia were predicted independently by the modulation of the RIII responses and by pain catastrophizing, the latter factor accounting for pain-related anxiety.

Conclusion: These results demonstrate that pain inhibition deficit in female IBS-D patients involves two potentially separable mechanisms reflecting (1)

altered descending modulation and (2) higher-order brain mechanisms underlying the regulation of pain and affect.

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder involving abdominal pain and bowel dysfunction (Thompson et al., 1999). In addition, several studies have shown that patients with IBS have visceral (Ritchie, 1973; Mayer and Gebhart, 1994; Mertz et al., 1995; Naliboff et al., 1997; Houghton, 1999; Bouin et al., 2002) and somatic (Verne et al., 2001; Bouin et al., 2001b; Dunphy et al., 2003; Rodrigues et al., 2005; Caldarella et al., 2006; Moshiree et al., 2007) hypersensitivity. Peripheral and central neural mechanisms have been proposed to explain hypersensitivity (Price et al., 2006b; Azpiroz et al., 2007) but the underlying pathophysiology is still unclear.

Abnormal activity within the diffuse noxious inhibitory controls (DNIC) (LeBars D. et al., 1992; Villanueva and LeBars D., 1995) may contribute to pain symptoms in IBS patients. Accordingly, counterirritation analgesia, a phenomenon thought to reflect the activation of DNIC, is reduced in IBS (Wilder-Smith et al., 2004; Song et al., 2006; Wilder-Smith and Robert-Yap, 2007). Also consistent with DNIC alteration, a tonic rectal distension does not produce the inhibition of the lower limb nociceptive withdrawal reflex (RIII-reflex) in IBS patients (Coffin et al., 2004). However, in this study, convergence of sural (inducing RIII reflex) and rectal afferents on spinal neurons may have contributed to the lack of RIII reflex inhibition, as shown with homosegmental

counterirritation (Terkelsen et al., 2001). Therefore, it is not known whether heterosegmental stimulation would also fail to inhibit the RIII reflex in IBS patients, consistent with the hypothesized deficit in DNIC.

In addition to DNIC, analgesia induced in the counterirritation paradigm may further involve cerebral self-regulatory mechanisms (e.g. cerebral u-opioid activation) normally triggered by tonic pain stimulation (Zubieta et al., 2001). These mechanisms have also been involved in anticipatory modulatory processes (Petrovic et al., 2005; Scott et al., 2007) (reviewed in Benedetti et al., 2005) as well as affective states (Zubieta et al., 2003; Kennedy et al., 2006). It is therefore plausible that abnormal activity within these higher-order brain systems may contribute to both abnormal pain regulation and higher levels of anxiety and depression typically reported in chronic painful conditions (Ribeiro et al., 2005), including IBS (Whitehead et al., 1980; Creed and Guthrie, 1987; Kumar et al., 1990; Folks and Kinney, 1992; Caudell, 1994). In turn, these higher-order cognitive and affective processes may also influence cerebro-spinal descending regulation (Rhudy et al., 2005; Goffaux et al., 2007) thereby affecting spinal nociceptive responses. A recent functional imaging study has demonstrated that IBS patients show an abnormal anticipatory response to a cued visceral stimulus (Berman et al., 2008). Importantly, this effect involved brainstem regions associated with cerebro-spinal regulation of pain and it was significantly associated with increased anxiety and increased stimulus-evoked brain response (Berman et al., 2008). This is consistent with the possibility that

pain-related anxiety and the corresponding higher-order brain processes may contribute to the decrease in pain inhibition in IBS.

The aim of the present study was to determine whether pain inhibition deficits are related to an altered descending modulation of spinal nociceptive processes in IBS. To that end, we examined the modulatory effect of heterosegmental counterirritation induced by sustained cold-pain on the forearm on the pain, anxiety and RIII reflexes evoked by electric shocks applied to the sural nerve. We hypothesized that IBS patients would show less counterirritation analgesia and RIII-reflex inhibition and we examined the possibility that this deficit relates to psychological factors, including increased pain-related anxiety.

METHODS

The study was approved by Research Ethics Board of "Centre de recherche de l'Institut Universitaire de Gériatrie de Montréal". All participants gave written informed consent acknowledging their right to withdraw from the experiment without prejudice and received a compensation of 25$ for their time and commitment to the study. The study consisted in a 90-minute session including the determination of thresholds (pain and RIII), the evaluation of pain and RIII reflex modulation by counterirritation, and a brief psychological assessment using three questionnaires.

Participants

Patients: Fourteen women with diarrhoea-predominant IBS (IBS-D) were recruited by advertisement and from referrals to the gastroenterology unit of "Hôpital St-Luc" in Montreal (Qc, Canada). All patients were evaluated by a gastroenterologist and co-investigator (MB) experienced in the evaluation of IBS. Normal physical examination, normal colonoscopy with biopsy, exclusion of organic diseases and symptoms were required to make the diagnosis of IBS based on Rome III criteria (Drossman 2006). Patients were excluded if they presented other chronic pain syndromes including fibromyalgia, psychiatric disorders, or used medication that could alter pain perception in the 2 weeks prior to the experiment.

Controls: Fourteen healthy women volunteered to participate in the study. Recruitment was done by advertisement at St-Luc hospital and on the campus of the University of Montreal. Participants were included if they had normal bowel habits and no known GI disease and were excluded for: 1) taking any medication within 2 weeks prior to the experiment and 2) having a history of GI symptoms, acute or chronic illness or a psychiatric disorder, as reported in a questionnaire on bowel symptoms and general health history.

Painful electrical stimulation

Transcutaneous electrical stimulation (30 ms train of 10 X 1 ms pulse) was delivered with a Grass S48 stimulator (Astro-Med Inc., USA) isolated with a

custom made constant current stimulus isolation unit. Stimulation was applied on degreased skin over the retro-maleolar path of the right sural nerve by means of a pair of custom-made surface electrodes (1cm^2; inter-electrode distance of 2 cm). The intensity of the stimulation was adjusted individually at 120% of the RIII-reflex threshold using the staircase method (Willer, 1977) and remained constant throughout the duration of the experiment.

Counterirritation paradigm

Participants laid comfortably in a supine position with a knee flexion of approximately 120°. Two series (control and experimental) of thirty-five electrical stimuli were delivered with an inter-stimulus interval of 12 seconds. The first five stimuli at the beginning of each series (pre-baseline) controlled for the rapid habituation effect occasionally observed in some subjects and were excluded from all analyses. In the control series, the following 30 stimuli were delivered without counterirritation to assess the stability of the RIII reflex. This series was always administered first because of the potentially sustained inhibitory effects of counterirritation. In the experimental series, 30 stimuli were administered in three successive conditions: baseline (n=10), counterirritation (n=10) and recovery (n=10). Counterirritation was induced by applying an ice pack on the subject's left contralateral forearm for 2 minutes. This flexible bag (15 X 20 cm) filled with 500 ml of frozen gel could be wrapped around the forearm and covered most of its anterior surface.

Rating of pain and pain-related anxiety

A visual analog scale (VAS) was shown on a computer monitor to evaluate pain and pain-related anxiety induced by the electrical stimulation. The VAS was placed horizontally and included the verbal anchors “no pain” and “worst pain imaginable” or “no anxiety” and “worst anxiety imaginable” at the left and right extremities, respectively (Price et al., 1994). Participants used a response key to move a cursor on the VAS and rated pain and pain-related anxiety produced by the electrical shocks after each block of stimuli performed in the three conditions of the counterirritation paradigm. Subjects also rated the pain and anxiety produced by the counterirritation stimulus after the end of the recovery block. For the control series, shock-pain and pain-related anxiety were rated at the end of the task. All ratings were converted linearly to a 0-100 scale.

RIII reflex measure and analysis

Electromyographic (EMG) activity of the short head of the biceps femoris was recorded with a pair of surface electrodes (EL-508, Biopac systems, USA). The EMG signal was amplified 1000 times, band pass filtered (100-500 Hz), sampled at 1000 Hz (Biopac systems Inc., USA) and stored on a personal computer for off-line analyses. The raw EMG recordings were transformed using the root mean square. The resulting signal was integrated between 90-180 ms after the stimulus onset to quantify RIII reflex amplitude to each shock. These values were normalized within each series using a z-transformation. A careful examination of the data led to the exclusion of one or two outlier

responses/artefacts per series (> 2 SD), representing 4.6% of the reflexes in controls and 4.4% in IBS patients. The RIII responses were averaged in 6 successive blocks of 5 trials for each series (one-minute time bins). Each minute of counterirritation and recovery periods were compared to the two minutes of baseline. For the control series, blocks 3 to 6 were compared to the two minutes of blocks 1-2. One patient did not perform the control series but her counterirritation data were included and analysed. One control subject did not have a stable RIII reflex and was excluded from RIII analyses.

Psychological assessment

The following three questionnaires were administered before the pain tests to examine their possible modulating effect on counterirritation analgesia: the pain catastrophizing scale (Sullivan et al., 1995), the Beck Depression inventory (Beck et al., 1996) and the state and trait anxiety inventory (Spielberger, 1985).

Statistical analyses

All results are expressed as mean ± SEM. Statistical analyses were done in Statistica v6.0 (Statsoft Inc., U.S.A.) with significance thresholds set to $p < 0.05$ (two-tailed). Modulation of pain, anxiety and RIII reflex were indexed by the difference between the counterirritation or the recovery blocks and the baseline measures. Group differences in psychological variables were assessed with t-tests. Pain, anxiety and RIII modulation within and between groups was assessed with the general linear model. Covariance analyses were used when

appropriate to control for the potential confounding effect of baseline differences between groups. Associations between variables of interest were tested using Pearson correlation.

RESULTS

Groups Characteristics

Characteristics of the IBS patients and control subjects are reported in Table 1. The groups were comparable on age, height, and weight (all p's > 0.75). However, IBS patients reported significantly higher levels of pain catastrophizing, depression, and state and trait anxiety, compared to controls (all p's<0.01).

Baseline RIII and Pain Responses

Baseline values for pain and RIII thresholds are reported in Table 2. Shock-pain threshold was not significantly different between groups (p=0.81). The RIII-reflex threshold was slightly lower in IBS patients than in controls but this effect did not reach statistical significance (p=0.08). Similarly, baseline ratings of shock-pain were slightly lower in IBS patients, possibly because the shock intensity applied (120% of RIII-threshold) was also slightly lower in patients, but the group contrast did not reach significance (p=0.12). There was no indication of habituation or sensitization in pain and pain-related anxiety ratings in the control participants or IBS patients between the control and the experimental series (pain: p=0.47 and anxiety: p=0.73 in controls and pain: p=0.75 and anxiety: p=0.76 in IBS patients). Similarly, the RIII reflex remained

stable throughout the control series in control participants and IBS patients (p=0.37 and p=0.11, respectively).

Effects of Counterirritation

The counterirritation stimulus induced similar levels of pain (p=0.96) and pain-related anxiety (p=0.62) in the two groups. The effect of counterirritation on shock-pain intensity was assessed by comparing ratings before, during and after the application of counterirritation (see Figure 1 and Table 2). The control group showed a robust and significant decrease in shock-pain during counterirritation compared to baseline ($p<0.001$). Furthermore, analgesia was partly maintained during the recovery period, as shock-pain remained significantly lower relative to baseline ($p<0.05$). In contrast, IBS patients showed no significant modulation of shock-pain during counterirritation compared to baseline (p=0.64). Shock-pain in IBS patients was also similar during the recovery period compared to baseline (p=0.53). In addition, modulation of shock-pain during counterirritation was significantly different between groups ($p<0.01$). The modulation of shock-pain during the recovery period was also significantly different between groups ($p<0.05$). After controlling for differences in baseline pain ratings (ANCOVA), this pain inhibition deficit remained significant during counterirritation ($p<0.05$) and approached significance during recovery (p=0.08). These effects indicate an absence of counterirritation analgesia in IBS patients.

The effect of counterirritation was also assessed on pain-related anxiety (see Figure 2 and Table 2). The control group showed a slight decrease in pain-

related anxiety compared to baseline although this change was not significant during counterirritation ($p=0.76$) and only approached significance during the recovery period ($p=0.06$). In contrast, IBS patients showed a significant *increase* in pain-related anxiety compared to baseline, specifically during counterirritation ($p<0.01$) but not during the recovery period ($p=0.74$). In addition, the increase in pain-related anxiety during counterirritation was significantly larger in IBS patients than controls ($p<0.05$). A covariance analysis confirmed that this group difference in the modulation of anxiety by counterirritation remained significant ($p<0.05$) after controlling for the slight (and ns) group difference in anxiety observed at baseline (see Table 2). A correlation performed across all subjects revealed a significant relation between the increase in pain-related anxiety and the decrease in counterirritation analgesia ($r=0.61$, $p<0.001$). Furthermore, after controlling for individual differences in the modulation of pain-related anxiety (ANCOVA), group differences in counterirritation analgesia were no longer significant ($p=0.31$).

Modulation of spinal nociception by counterirritation

Counterirritation had a significant effect on RIII reflex amplitude in the control group ($p<0.003$) (see Figure 3). Planned contrasts revealed that RIII reflex amplitude was significantly decreased compared to baseline during the second minute of counterirritation (time 4 min; $p=0.022$) and during the first ($p=0.004$) and second ($p=0.019$) minutes of recovery (times 5-6 min). In IBS patients, RIII reflex amplitude was not significantly modulated by counterirritation ($p=0.42$). In addition, correlation analyses performed across all

subjects indicated that the reduction in RIII reflex amplitude predicted the magnitude of counterirritation analgesia ($r=0.39$, $p<0.05$) but did not correlate to pain-related anxiety ($r=0.19$, $p=0.33$).

Effects of Psychological Symptoms on Pain Processing

Pearson's correlations performed across all subjects showed that the magnitude of counterirritation analgesia was significantly and inversely related to pain catastrophizing ($r=-0.61$, $p<0.001$) and trait anxiety ($r=-0.41$, $p<0.05$). Similar but non-significant effects were also observed with depressive symptoms ($r=-0.37$, $p=0.055$) and state anxiety ($r=-0.26$, $p<0.18$). In contrast, RIII reflex modulation during counterirritation was not related to psychological scores (all p's > 0.5). Furthermore, covariance analyses revealed that pain inhibition by counterirritation was not significantly different between groups after controlling for catastrophizing ($p=0.36$), depressive symptoms ($p=0.09$) and trait anxiety ($p=0.12$), but remained significant after controlling for state anxiety ($p=0.04$).

Predictors of pain inhibition deficits

Multiple regression analysis indicated that psychological symptoms predicted analgesia (multiple $r^2=0.67$, $p=0.004$) with pain catastrophizing explaining most of the effect (Pain Catastrophizing: $p=0.009$; Beck, state and trait anxiety: all p's > 0.05). A stepwise multiple regression analysis was further performed to evaluate the potential independent contribution of the physiological (RIII modulation) and psychological (pain-related anxiety and catastrophizing)

variables that correlated with counterirritation analgesia (see Figure 4). Both RIII reflex changes (r^2=0.18, p=0.03) and pain catastrophizing (r^2=0.25, p=0.01) significantly predicted analgesia (multiple r^2=0.38, p=0.034). On the other hand, pain-related anxiety was associated with pain catastrophizing (r^2=0.25, p<0.01) but it did not contribute independently to counterirritation analgesia (p=0.19).

DISCUSSION

In the present study, IBS patients showed a clear deficit in modulation of pain and RIII reflex. In addition, the group difference in pain inhibition was not significant after controlling for pain catastrophizing, which also accounted for pain-related anxiety. Interestingly, RIII reflex modulation and pain catastrophizing predicted analgesia independently. These results suggest that pain inhibition deficits in IBS patients are associated with altered descending modulation and with higher-order brain mechanisms involved in the regulation of pain and affect.

Effect of counterirritation on pain ratings and spinal nociceptive processes

In the present study, counterirritation induced an inhibition of shock pain in control subjects, consistent with previous studies (Willer et al., 1984a; Willer et al., 1984b; Talbot et al., 1987; Willer et al., 1989; LeBars D. et al., 1992; Willer et al., 1999). In contrast, counterirritation did not produce a significant change in shock-pain ratings in IBS patients. These results add to previous

studies in which rectal pain inhibition by counterirritation was decreased in IBS patients (Wilder-Smith et al., 2004; Song et al., 2006; Wilder-Smith and Robert-Yap, 2007) by showing that impairment of central pain inhibition affects somatic as well as visceral pain in IBS. Two distinct mechanisms that could explain decreased pain inhibition in IBS patients are considered.

Firstly, the IBS deficit in pain inhibition during counterirritation could be caused by the observed decreased inhibition of spinal nociceptive transmission (RIII reflex). Accordingly, decreased RIII reflex inhibition was associated with decreased pain inhibition. Considering that counterirritation analgesia depends at least in part on descending inhibitory pathways (Willer et al., 1984a; Willer et al., 1989; LeBars D. et al., 1992; Willer et al., 1999; Bouhassira and Danziger, 2006), this suggests that altered descending inhibition leads to decreased inhibition of spinal nociceptive transmission, which in turn contributes to decreased counterirritation analgesia in IBS patients. In accordance with this interpretation, alteration of spinal modulation was previously found in IBS patients in response to rectal distension (Coffin et al., 2004). The lack of RIII reflex inhibition in IBS patients observed in that study could reflect altered descending inhibition or sustained homosegmental facilitation of spinal nociceptive transmission. Indeed, when rectal distensions are performed, spinal convergence of rectal and sural afferents may contribute to the facilitation of the RIII reflex (Coffin et al., 2004) that exceeds the inhibitory effect normally associated with counterirritation. In the present study, heterosegmental (forearm) counterirritation produced RIII inhibition in controls only but neither

control subjects nor IBS patients displayed a facilitation of the RIII reflex. The present results therefore support the hypothesis that the deficit in pain and RIII inhibition in IBS reflects a decrease in descending inhibitory processes.

An important implication of our results is that pain perception is related to spinal nociceptive processes in IBS. This is in contrast to the interpretation of a recent study suggesting that increased colonic pain perception reflects a tendency of IBS patients to report more pain (report bias) rather than increased neurosensory sensitivity (Dorn et al., 2007) In that study, the sensory decision theory (SDT) was used to discriminate between physiological and psychological components of pain. The use of SDT indices has been severely criticized in the context of pain assessment (Rollman, 1977; Rollman, 1979) as true changes in basic sensory processes (e.g. hyperalgesia) may be misinterpreted as report biases. Here, the physiological response to the noxious stimulus was indexed with the RIII reflex and the results indicate that changes in pain ratings correlated with changes in spinal nociceptive transmission. We suggest that increased pain ratings in IBS patients reflect, at least in part, an increased spinal nociceptive transmission and not only on a tendency to report more pain.

Secondly, another mechanism that may account for the pain inhibition deficits observed in IBS patients is increased pain-related anxiety. Interestingly, pain-related anxiety contributed to the group difference in pain inhibition strongly suggesting a role for pain-related anxiety in the lack of counterirritation analgesia in IBS patients. Such effect may reflect the engagement of higher-order brain networks involved in negative affect. This interpretation is supported

by the role of anxiety and anticipation in the facilitation of pain perception (Sawamoto et al., 2000; Ploghaus et al., 2001; Simpson, Jr. et al., 2001; Porro et al., 2002; Porro et al., 2003; Wager et al., 2004; Song et al., 2006; Fairhurst et al., 2007). It is also consistent with a recent study showing that increased negative affect in IBS (including anxiety) was associated with a reduced activation of corticolimbic structures (Berman et al., 2008). Considering that these structures have been associated with pain modulation (Petrovic and Ingvar, 2002; Lieberman et al., 2004; Petrovic et al., 2005; Bingel et al., 2006; Wiech et al., 2006), increased pain-related anxiety in the present group of IBS patients could decrease analgesia through decreased activation of analgesia-related brain structures. Alternatively, pain-related anxiety may alter pain inhibition processes by increasing spinal nociception, as suggested by the facilitation of RIII reflex amplitude by negative affect (Rhudy et al., 2005). However, pain-related anxiety was not significantly related to RIII reflex modulation in the present study suggesting that it may interfere with pain processing at supraspinal levels, independently from the deficit in descending inhibition.

Contribution of psychological symptoms to altered pain processing

It is well known that psychological factors such as negative affect can enhance pain perception and trigger pain facilitation processes (Meagher et al., 2001; Rhudy et al., 2005; Vogt, 2005). In previous studies, IBS patients were found to have more psychological symptoms (Whitehead et al., 1980; Creed

and Guthrie, 1987; Kumar et al., 1990; Folks and Kinney, 1992; Caudell, 1994; Poitras et al., 2002a) and these symptoms have been associated with increased pain perception (Dunphy et al., 2003; Posserud et al., 2004; Posserud et al., 2007ings. We cannot exclude the possibility that IBS patients display a weaker attentional bias towards the counterirritation stimulus, leading to a reduced inhibitory effect. However, this appears unlikely in view of the comparable cold pain reported in the two groups. In addition, persistent analgesia during the recovery period in control pa). In the present study, pain catastrophizing contributed to the lack of counterirritation analgesia in IBS patients and also accounted for pain-related anxiety effects on analgesia. The fact that both pain-related anxiety and pain catastrophizing were independent from the group effect in RIII reflex modulation indicates a distinct contribution of supraspinal processes to the pain inhibition deficit.

Limitations and future directions

In addition to affective processes, attention may also contribute to counterirritation analgesia. For instance, counterirritation pain may drag attention away from shock pain to various degrees in controls and IBS patients, which may explain part of the group differences in shock-pain rat rticipants can hardly be explained by attentional factors.

Another mechanism that should be addressed in future studies is the potential contribution of a putative opioidergic dysfunction in pain inhibition deficits in IBS. Sustained painful stimulation used in counterirritation paradigms

has been shown to produce a release of endogenous μ-opioids in pain-related brain structures, that correlates with the modulation of pain perception (Zubieta et al., 2001). Alteration of μ-opiod receptors activation by decreased release of μ-opioids (Lembo et al., 2000) or by decreased μ-opioid receptors availability could thus explain the lack of counterirritation analgesia showed in the present group of IBS patients. Furthermore, psychological variables have been associated with endogenous opioid activity (Zubieta et al., 2006). This potential neurochemical explanation may provide a more direct physiological link between psychological factors and pain modulation deficit.

Finally, the selection of female IBS-D patients only in the present study does not allow the generalization of our results male patients and to other types of IBS (constipation-predominant or alternating).

In conclusion, the results of the present study show that increased pain perception in IBS patients relies at least in part on altered descending inhibition and pain catastrophizing, consistent with alteration of both spinal and higher-order brain mechanisms involved in the regulation of pain. Importantly, this implies that increased pain perception in IBS patients does not simply reflect a tendency to report more pain but also reflects increased spinal nociceptive processes.

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Table 1. Characteristics of participants

	Age (y.o.)	Height (m.)	Weight (Kg.)	Pain catastrophising	Depression	State anxiety	Trait anxiety
IBS (n=14)	30 ± 1.9	1,65 ± 0.02	61.8 ± 2.6	22.0 ± 2.1**	12.3 ± 1.8**	40.6 ± 2.7**	46.3 ± 2.8**
Controls (n=14)	32 ± 0.9	1,66 ± 0.02	60.9 ± 2.3	12.0 ± 2.2	4.8 ± 1.5	31.9 ± 2.5	33.5 ± 2.7

** significant group difference p<0.01

Table 2. Pain responses

	Thresholds		Counterirritation experiment						Cold Pain	
			Baseline		Counterirritation		Recovery			
	Shock-pain	RIII reflex	Pain	Anxiety	Pain	Anxiety	Pain	Anxiety	Pain	Anxiety
Controls	7.9 ± 0.7 mA	11.1 ± 1.0 mA	50.5 ± 5.4	35.5 ± 5.9	39.1 ± 5.0*** #	34.4 ± 6.3	43.9 ± 5.1* ¶	30.1 ± 5.4	53.1 ± 4.1	38.1 ± 5.3
IBS patients	8.2 ± 1.1 mA	8.7 ± 1.0 mA	39.6 ± 4.6	27.8 ± 4.7	41.3 ± 5.2	37.8 ± 6.5** ¶	41.3 ± 5.2	28.6 ± 5.4	52.7 ± 7.1	42.6 ± 7.8

***p<0.05 compared to baseline; **p<0.01 compared to baseline; ***p<0.001 compared to baseline;# p<0.01 between groups; ¶ p<0.05 between groups.**

FIGURE LEGENDS

Figure 1. Effect of counterirritation on pain ratings. Controls reported lower pain ratings (mean ± SEM) during counterirritation and recovery compared to baseline. IBS patients showed no significant pain modulation. Modulation of pain ratings during counterirritation was also different between groups. *p<0.05, ***p<0.001 compared to baseline.

Figure 2. Effect of counterirritation on pain-related anxiety. Controls showed no significant modulation of pain-related anxiety (mean ± SEM). IBS patients showed increased pain-related anxiety during counterirritation compared to baseline. Modulation of pain-related anxiety was different between groups. **p<0.01 compared to baseline.

Figure 3. Effect of counterirritation on RIII reflex amplitude. RIII reflex amplitude was averaged at one-minute intervals. RIII reflex amplitude (mean ± SEM) is shown for controls (CTL) and IBS patients. Compared to baseline (1st and 2nd min), counterirritation applied during the 3rd and 4th minutes (horizontal bar) produced a significant decrease of RIII reflex amplitude in controls but not in IBS patients. This analgesic effect observed in controls persisted during recovery (5th and 6th min). *p<0.05 **p<0.01, n.s. not significant.

Figure 4. Predictors of pain inhibition deficits in IBS.

Spinal modulation (RIII reflex), pain-related anxiety and pain catastrophizing were all correlated to the pain inhibition deficit (simple correlation; results in parentheses). However, spinal modulation and pain catastrophizing were the only two independent predictors as pain catastrophizing accounted for pain-related anxiety effect on pain inhibition (multiple regression; results in bold).

Figure 1

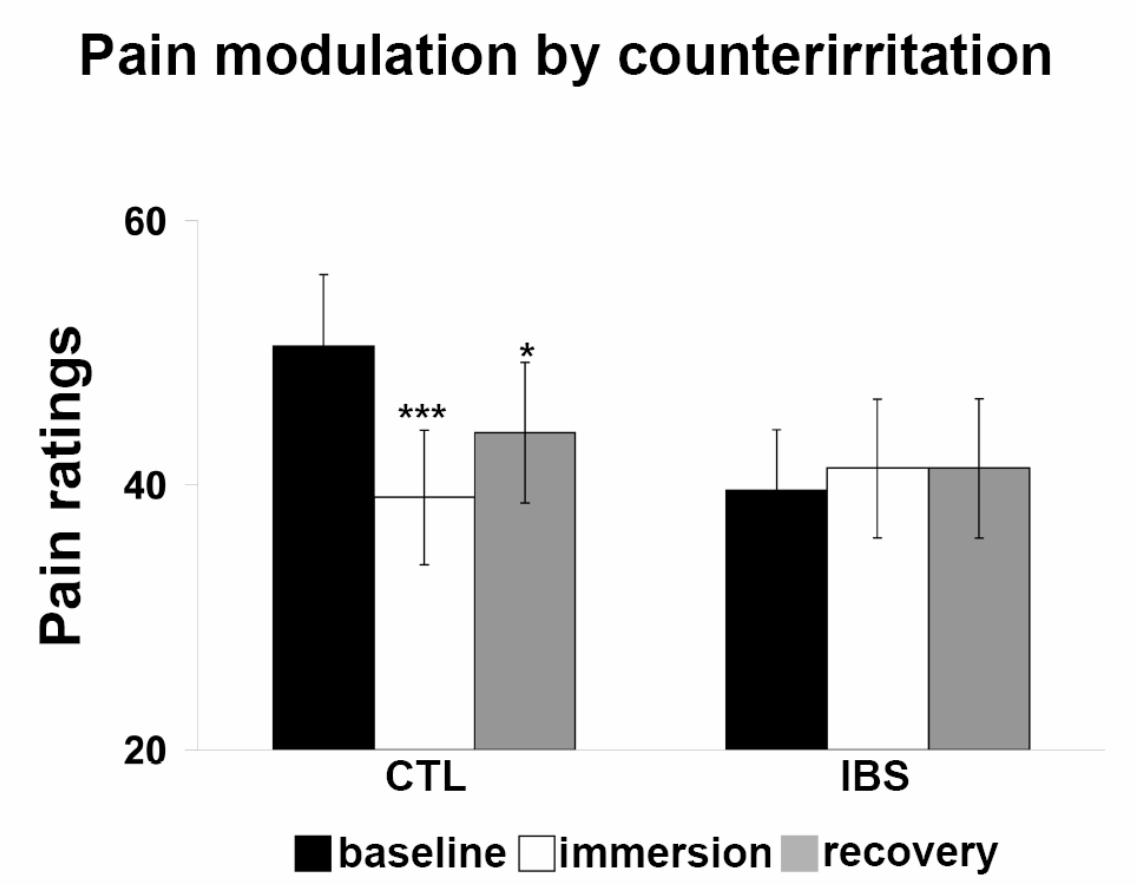


Figure 2

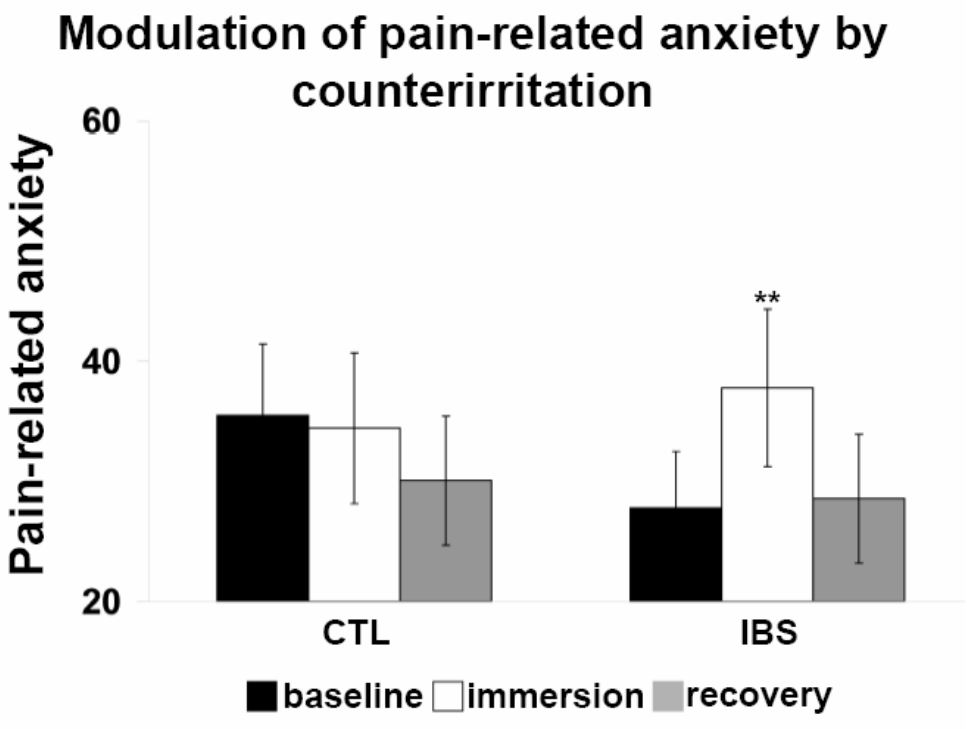


Figure 3

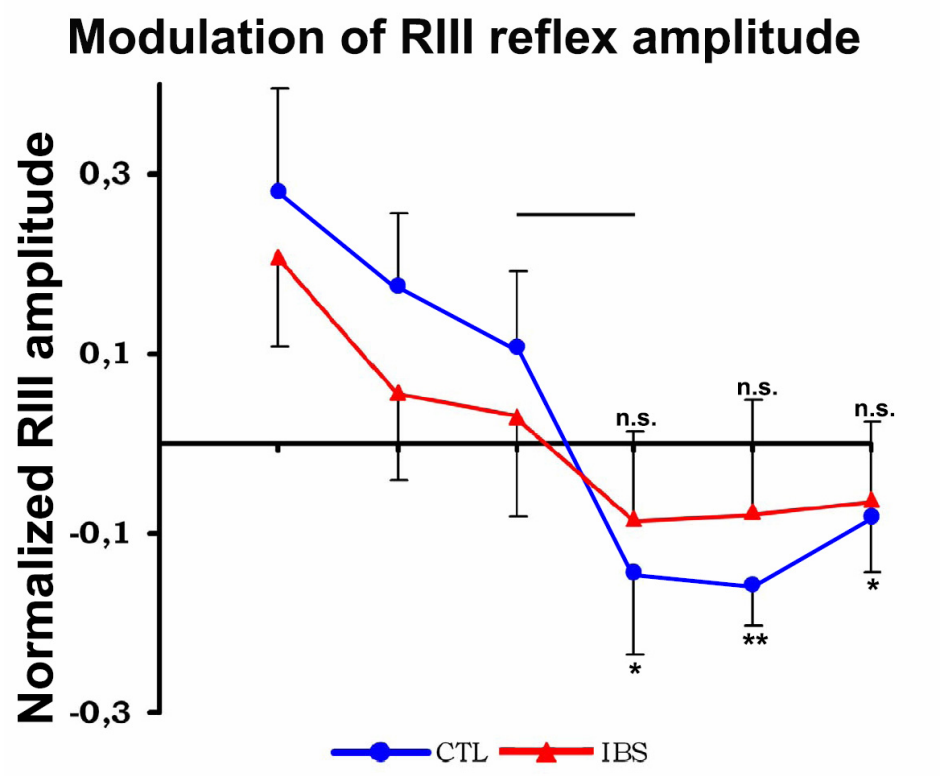
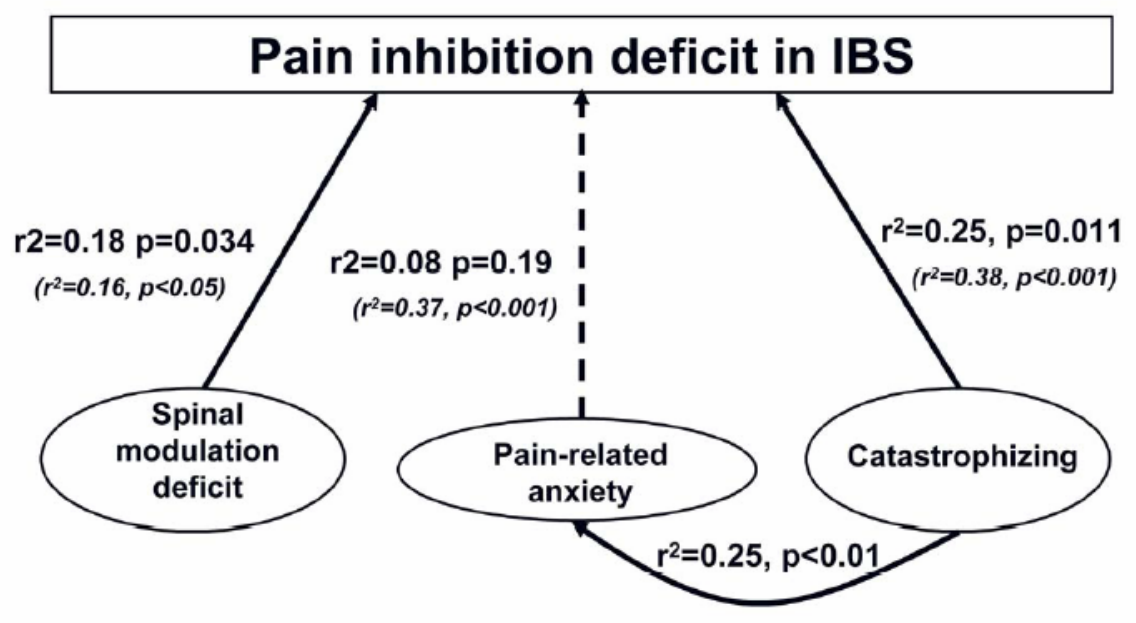


Figure 4


Pain inhibition deficit in IBS
r2=0.18 p=0.034
(r²=0.16, p<0.05)
r2=0.08 p=0.19
(r²=0.37, p<0.001)
r²=0.25, p=0.011
(r²=0.38, p<0.001)
Spinal modulation deficit
Pain-related anxiety
Catastrophizing
r²=0.25, p<0.01

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DISCUSSION GÉNÉRALE

DISCUSSION

Au cours de cette dernière section, nous discuterons les résultats principaux de cette thèse dans deux volets, soit les mécanismes de perception de la douleur et de sa modulation par contreirritation, ainsi que la dysfonction de ces mécanismes dans le syndrome de l'intestin irritable. Finalement, nous proposerons une hypothèse physiopathologique de l'hypersensibilité et du déficit de modulation de la douleur observés dans le syndrome de l'intestin irritable.

Perception et modulation de la douleur

Ségrégation de l'activité nociceptive cérébrale

La première étude de cette thèse avait pour objectif de développer un modèle expérimental permettant de mesurer simultanément l'activité spinale et cérébrale lors de la douleur. Ainsi, la mesure du réflexe RIII dans l'environnement de la résonance magnétique a été mise au point et nous avons montré que les différences individuelles dans l'activité cérébrale évoquée par les stimulations électriques étaient spécifiquement associées aux différences individuelles des différentes composantes de la douleur. Par exemple, l'activité de OFC et de la partie dorsocaudale de ACC était plus spécifiquement associée à la réactivité motrice. L'activation de cette région de ACC est compatible avec les processus de régulation de l'aire cingulaire motrice rostrale (Paus, 2001). Cette région de ACC est constituée de neurones ayant des projections

corticospinales vers les motoneurones de la moelle épinière (Dum and Strick, 1991) et est directement impliquée dans la motricité (Paus, 2001).

En contrepartie, les différences individuelles dans l'activité de ACC (rostralement et ventralement au foyer d'activation précédent), de mPFC et de PHG, mais pas du thalamus ou du tronc cérébral, étaient plus spécifiquement associées aux différences individuelles de sensibilité à la douleur. Ceci est cohérent avec l'idée que les différences individuelles de sensibilité à la douleur sont indépendantes de l'activité thalamique et reposent plutôt sur l'élaboration corticale de l'information nociceptive (Coghill et al., 2003). De plus, l'activation de cette régions de ACC est compatible avec l'encodage de la composante affective de la douleur (Rainville et al., 1997), et l'activation de mPFC et de PHG est compatible avec des processus reliés à l'anxiété (Ploghaus et al., 2001; Simpson, Jr. et al., 2001). Ainsi, puisque l'ensemble de ces régions frontolimbiques activées par la stimulation électrique sont plus spécifiquement associées à la régulation des réponses motrices ou la perception de la douleur, il est proposé que leur activation différentielle pourrait expliquer la dissociation sensorimotrice observée dans plusieurs études (Willer et al., 1979; Danziger et al., 1998a; Terkelsen et al., 2004; French et al., 2005; Defrin et al., 2007). Cette flexibilité de la régulation motrice relativement à la perception de douleur pourrait permettre des réponses comportementales mieux adaptées au contexte. Par exemple, lorsque l'on marche sur un objet causant une lésion sous le pied, le retrait du membre inférieur évoqué de façon réflexe doit être suivi d'une réponse motrice adaptée à l'environnement et au contexte. Cette

réponse adaptée pourrait alors dépendre en partie de régions activées par la douleur et impliquées dans le contrôle moteur, en l'occurrence ACC et OFC. Ceci est compatible avec le rôle de ACC et OFC dans l'évaluation de la valeur punitive d'un stimulus qui peut mener à un changement comportemental (Kringelbach, 2005) et leur rôle dans les comportements et les actions planifiés (Vogt and Sikes, 2000; Fuster, 2001). Afin de préciser si cette activité est spécifique à des réflexes associés à de la douleur ou si elle peut être produite par n'importe quel mouvement réflexe (douloureux ou pas), il serait intéressant d'examiner les réponses cérébrales associées à la production du réflexe H.

Un lien intéressant entre les deux premières études est l'association entre l'amplitude du RIII (réactivité motrice et modulation par contreirritation) et l'activité de OFC. Dans les deux cas, les différences individuelles dans l'amplitude du RIII étaient associées aux différences individuelles dans l'activité de OFC (controlatérale à la stimulation électrique pour la modulation du RIII pendant la contreirritation et ipsilatérale à la stimulation électrique pour la réactivité motrice et la modulation du RIII pendant la récupération). Le fait de retrouver cette relation dans des régions similaires de OFC dans deux expériences différentes renforce l'dée que cette partie du cortex est impliquée dans la régulation motrice (Fuster, 2001; Kringelbach, 2005).

Par ailleurs, on doit noter une limite inhérente à ce modèle expérimental, soit le recrutement de fibres non nociceptives par les stimulations électriques. En effet, la production du RIII est toujours accompagnée de l'activation des fibres Aβ qui ont un seuil plus bas que les fibres Aδ. Ainsi le patron d'activation

cérébral évoqué par les stimulations électriques nociceptive n'est pas nécessairement spécifique à la douleur.

Analgésie par contreirritation

En plus de préciser le rôle spécifique des structures activées par la douleur, le développement de notre modèle expérimental avait comme objectif de montrer le lien entre l'activité cérébrale, la modulation de la douleur, et la modulation du réflexe RIII. Quoique plusieurs interventions puissent moduler la douleur et le réflexe RIII, un paradigme de contreirritation a été utilisé en premier lieu, étant donné l'amplitude de ses effets analgésiques rapportée dans la littérature (Willer et al., 1999), et l'utilité de cette intervention pour tester les mécanismes endogènes de modulation de la douleur chez des populations cliniques (Lautenbacher and Rollman, 1997; Bouhassira et al., 2003; Wilder-Smith et al., 2004; Sandrini et al., 2006; Song et al., 2006).

Bien que les effets de la contreirritation soient connus depuis les temps anciens, seulement deux études ont investigué l'activité cérébrale associée (Wilder-Smith et al., 2004; Song et al., 2006). Dans ces études, des distensions rectales produisaient une douleur viscérale et l'immersion d'un pied dans l'eau froide servait de stimulus de contreirritation. Cependant, les distensions et la contreirritation étaient appliquées simultanément et étaient de même durée, ce qui est problématique pour l'évaluation de l'activité cérébrale. Avec ce type de paradigme expérimental, il n'est pas possible de bien isoler l'activité associée à chacun des stimuli pendant la contreirritation et surtout, l'effet de l'activité

cérébrale évoquée par la contreirritation sur celle évoquée par les distensions rectales. Étant donné ces limites méthodologiques, d'autres études étaient nécessaires pour élucider les mécanismes centraux de la contreirritation.

Une question qui a été peu investiguée dans les études sur la contreirritation est la contribution des processus attentionnels à l'analgésie. Le fait d'appliquer deux stimuli simultanément implique des mécanismes d'attention partagée. Des études sur la modulation attentionelle de la douleur suggèrent que l'attention et la distraction peuvent moduler la douleur et les réponses physiologiques associées (voir plus bas). Il est donc possible qu'une partie de l'effet analgésique de la contreirritation repose sur des processus attentionnels. Une seule étude a investigué spécifiquement l'effet d'une douleur thermique soutenue sur une douleur aigue induite par des stimulations électrique brèves en manipulant l'attention (Lautenbacher et al, 2007). Dans cette étude, il a été montré que la distraction avait un effet très faible sur la douleur et n'affectait pas l'analgésie par contreirritation. Il semble donc que l'analgésie par contreirritation dépende principalement des mécanismes analgésique préalablement décris (Bouhassira and Danziger, 2006).

Dans nos études sur la contreirritation, nous avons utilisé un paradigme classique incluant l'application répétée d'un premier stimulus douloureux de courte durée et l'application soutenue d'un stimulus de contreirritation. Ce paradigme expérimental permet d'isoler l'activité cérébrale associée à chacun des stimuli et de tester l'effet de la contreirritation sur l'activité cérébrale évoquée par le premier stimulus douloureux. Le niveau d'attention n'a pas été

manipulé mais les sujets avec comme instruction de porter attention au stimulation électrique afin de les évaluer à la fin de la contreirritation. L'étude 2 indique que les stimulations électriques brèves produisent le patron d'activation cérébrale cohérent avec d'autres études d'imagerie cérébrale de la douleur (Apkarian, 2005). De plus, la contreirritation produit une activation robuste et soutenue de régions impliquées dans la perception et la modulation descendante de la douleur (Yezierski et al., 1983; Basbaum and Fields, 1984; Millan, 2002; Apkarian, 2005). Nous avons également montré avec une analyse de régression multiple que l'analgésie par contreirritation était accompagnée de deux mécanismes principaux, soit l'inhibition de l'activité évoquée par le choc dans l'amygdale par l'activation soutenue de OFC, et la modulation du réflexe RIII par l'activation soutenue de SI et de la PAG (voir Figure 2 et 3). Puisque ces corrélations étaient spécifiques à l'analgésie ou à la modulation du RIII[5], ces résultats suggèrent que la contreirritation repose sur au moins deux mécanismes indépendants, agissant plutôt sur l'activité cérébrale ou plutôt sur l'activité spinale. Nous allons maintenant insister sur le rôle de deux structures importantes dans la modulation de la douleur, soit OFC et la PAG, afin d'explorer les similitudes entre les mécanismes de la contreirritation et ceux d'autres interventions modulant la douleur.

[5] La régression multiple permet de tester la corrélation entre chaque covariable (analgésie et modulation du RIII) et le signal BOLD (activité cérébrale) de façon indépendante. En effet, lorsque l'analgésie est corrélée à l'activité cérébrale, la variance de la modulation du RIII est enlevée par covariance, et vice-versa.

Cortex orbitofrontal (OFC)

Le cortex orbitofrontal joue un rôle important dans les émotions. Il est plus particulièrement impliqué dans la représentation des récompenses et des punitions qui déterminent la prise de décisions et les comportements (Kringelbach and Rolls, 2004; Rolls and Grabenhorst, 2008). Ses connexions réciproques avec plusieurs structures impliquées dans la douleur, donc ACC (Kringelbach and Rolls, 2004) et l'amygdale (Kita and Kitai, 1990; Carmichael and Price, 1995), en font également un acteur potentiel dans plusieurs processus nociceptifs.

En lien avec la présente thèse, il a été proposé que OFC et l'amygdale soient impliqué dans la modulation cognitive de la douleur (Petrovic and Ingvar, 2002). En accord avec cette idée, il a été montré que OFC est activé lors de la modulation de la douleur par l'attention (Petrovic et al., 2000; Bantick et al., 2002), par l'hypnose (Rainville et al., 1999a) et lors de l'analgésie placebo induite par des attentes (Petrovic et al., 2005). D'autres part, OFC est également impliqué dans la réinterprétation et la suppression des émotions négatives (Elliott et al., 2000; Goldin et al., 2008). Un des mécanismes par lequel OFC accomplit cette fonction est l'inhibition de l'activité de l'amygdale (Ochsner et al., 2002; Schaefer et al., 2002; Ochsner et al., 2004; Phan et al., 2005; Goldin et al., 2008). Considérant que l'analgésie par contreirritation produit une activation soutenue de OFC et une inhibition de l'activité évoquée par le choc dans l'amygdale, il est proposé que la contreirritation partage des mécanismes communs à plusieurs interventions modulant la douleur. Il est

également proposé que l'inhibition de l'amygdale par OFC puisse représenter une suppression de la composante émotionnelle de la douleur.

Un autre mécanisme à considérer dans l'analgésie par contreirritation en lien avec l'activité de OFC est la modulation descendante de la douleur. Considérant les projections anatomiques entre OFC et la PAG et le rôle de cette connexion dans l'inhibition de la nociception spinale (Zhang et al., 1997), il est probable que l'activation de OFC ait produit l'analgésie par contreirritation en modulant le réflexe RIII. Cependant, nos résultats ne supportent pas cette possibilité puisque l'activité soutenue de OFC n'était pas corrélée à la modulation du RIII ni à la PAG. En contrepartie, ces résultats appuient l'idée que l'analgésie produite par l'activation de OFC lors de la contreirritation est d'origine supraspinale.

Enfin, un des mécanismes les plus probables pouvant sous-tendre l'analgésie par contreirritation est le recrutement par OFC de circuits opioïdergiques locaux dans l'amygdale. Ceci est compatible avec une étude en tomographie par émissions de positons (PET) dans laquelle une douleur soutenue (injection d'une solution saline hypertonique dans le muscle masséter) provoquait une diminution de la disponibilité des récepteurs opioïdes de type μ dans plusieurs régions cérébrales dont l'amygdale (Zubieta et al., 2001). Dans cette étude, les différences individuelles dans la relâche d'opioïdes endogènes étaient également associées aux évaluations subjectives de douleur. Ceci est cohérent avec l'association entre les différences individuelles d'analgésie par contreirritation et l'inhibition de l'amygdale que nous avons observée.

Substance grise périaqueducale

La PAG est une structure communément activée lors de la modulation de la douleur. Dans les études d'imagerie chez l'humain, son activation a été associée à l'anticipation de la douleur (Fairhurst et al., 2007), à l'analgésie placebo (Petrovic et al., 2002; Bingel et al., 2006) et à la modulation attentionnelle de la douleur (Tracey et al., 2002b; Valet et al., 2004). Le fait que la PAG soit fortement impliquée dans la modulation descendante suggère que son action modulatrice dépendrait au moins en partie de la modulation de la nociception spinale (Millan, 2002). L'étude 2 a permis de tester cette hypothèse en examinant l'activité de la PAG pendant la contreirritation. Les résultats indiquent que l'activité de la PAG est associée à la modulation du RIII et à l'activité de la RVM. Ces résultats sont donc cohérents avec la modulation des processus nociceptifs spinaux par les projections descendantes du système PAG-RVM (Basbaum and Fields, 1984). Quoique nous n'ayons pas observé l'activation de la moelle allongée caudale, ces résultats sont également en accord avec l'idée que la PAG peut moduler l'inhibition descendante associée à la contreirritation (Bouhassira et al., 1992c). La variabilité interindividuelle dans la modulation du RIII est cependant une limite importante à l'interprétation des résultats et soulève la possibilité d'une interaction entre des processus facilitateurs et inhibiteurs de la PAG, qui pourraient engendrer la dissociation RIII/douleur. Cette dissociation sensorimotrice pourrait s'expliquer par des mécanismes qui produisent l'activation du système PAG-RVM et l'analgésie mais qui ont un effet antagoniste sur la nociception spinale. Cette possibilité est

compatible avec l'existence de sous populations de neurones dans la RVM qui peuvent faciliter (cellules ON) ou inhiber (cellules OFF) la nociception spinale (Fields et al., 1991). Dans la prochaine section, nous allons donc considérer que l'attention pourrait contribuer à l'analgésie par contreirritation tout en facilitant le réflexe RIII.

Analgésie par la contreirritation : une modulation attentionnelle ?

Chez le singe, il a été montré que l'activité des neurones de la corne dorsale est inhibée lorsque l'animal détourne son attention de la douleur, alors qu'elle est facilitée lorsque l'animal doit accomplir une tâche de discrimination de stimuli thermiques douloureux (Bushnell et al., 1984). En accord avec ces résultats, des études psychophysiologiques chez l'humain ont montré qu'une tâche attentionnelle induit l'analgésie et une inhibition du réflexe RIII (Bathien and Hugelin, 1969; Bathien, 1971; Willer et al., 1979).

En contrepartie, d'autres études ont montré une dissociation entre la modulation de la douleur et les réflexes nociceptifs. En l'occurrence, le RIII et le réflexe de clignement (blink reflex) ne sont pas modulés ou sont facilités pendant l'analgésie induite par une tâche sollicitant l'attention (Terkelsen et al., 2004; McIntyre et al., 2006; Edwards et al., 2006; Koh and Drummond, 2006; Edwards et al., 2007). Les différences entre les études peuvent possiblement s'expliquer par le type de tâche impliqué et le niveau d'attention requis pour l'exécuter (Bathien, 1971). Néanmoins, l'ensemble de ces études suggère que dans certaines conditions, le RIII est facilité pendant l'analgésie induite par des

processus attentionnels. Ceci est compatible avec les résultats de l'étude 2 montrant une dissociation entre la modulation de la douleur et du RIII. Un mécanisme pouvant expliquer la facilitation du RIII pendant ces tâches attentionnelles et pendant la contreirritation, malgré l'analgésie, est l'augmentation de l'excitabilité des motoneurones par des voies descendantes indépendamment de la transmission nociceptive spinale et de la perception de la douleur. Cette hypothèse est supportée par les études montrant une facilitation du réflexe H par différentes tâches sollicitant l'attention (Bathien and Hugelin, 1969; Bathien, 1971). Ainsi, chez certains sujets et dans certaines conditions, la contreirritation pourrait produire l'analgésie sans produire une inhibition significative du RIII.

Tel que discuté précédemment, une des sources potentielles de cette dissociation sensorimotrice est la PAG, étant donnée son activation dans les études d'imagerie sur la modulation attentionnelle de la douleur (Tracey et al., 2002b; Valet et al., 2004) et son rôle dans la modulation descendante (Millan, 2002). Il est donc proposé que chez certains sujets, la contreirritation soit accompagnée d'une modulation attentionnelle plus importante. Ensemble les processus de la contreirritation et de l'attention produiraient une inhibition de la douleur, accompagnée de l'activation du système PAG – RVM. Cependant, leur effet net sur le RIII dépendrait de l'équilibre entre les effets facilitateurs de la modulation attentionnelle et les effets inhibiteurs de la contreirritation. Tel que proposé précédemment, cette idée est cohérente avec la présence de cellules ON et OFF dans la RVM, qui seraient activées par la distraction et la

contreirritation respectivement. Ces mécanismes demeurent cependant spéculatifs et des études ultérieures contrastant l'effet de l'attention et de la contreirritation seront nécessaires pour tester cette hypothèse.

Contrairement à l'étude 2, l'analgésie produite par la contreirritation chez les sujets sains était associée à une inhibition significative du RIII dans l'étude 4. Une possibilité pouvant expliquer cette différence est le fait que la contreirritation était produite par l'application d'un coussin froid sur l'avant-bras, qui était moins douloureux et possiblement moins distrayant que l'immersion du pied dans l'eau froide.

Analgésie par contreirritation : un effet placebo?

L'effet placebo est déterminé par le contexte psychosocial et influence les bénéfices d'une intervention. C'est un phénomène psychobiololgique qui repose sur plusieurs mécanismes, incluant les attentes d'un bénéfice associé à une intervention et le conditionnement à ce bénéfice (Benedetti et al., 2005). Dans le cas particulier de la modulation de la douleur, il a été montré que les attentes d'analgésie constituent un facteur fondamental de l'effet placebo (Benedetti et al., 1999; Amanzio and Benedetti, 1999; Colloca and Benedetti, 2005). Comme toute intervention comporte un effet placebo, serait-il possible que les attentes d'analgésie puissent moduler les effets de la contreirritation? C'est du moins ce que suggère une étude récente sur les attentes d'analgésie (Goffaux et al., 2007). Dans cette étude, les effets de la contreirritation par l'immersion de la main dans l'eau froide sur la douleur, le RIII et les potentiels évoqués par une stimulation du nerf sural ont été modulés par des attentes

d'analgésie ou d'hyperalgésie. Les résultats indiquent que la contreirritation produit une diminution de la douleur et de l'amplitude du RIII et des potentiels évoqués chez les sujets du groupe avec attentes d'analgésie. En contrepartie, les effets inhibiteurs de la contreirritation étaient bloqués chez les sujets du groupe avec attentes d'hyperalgésie. Ceci indique une modulation des effets de la contreirritation par les attentes. Dans nos études sur la contreirritation, aucune attente n'a été induite. Cependant, considérant que les sujets puissent avoir des attentes en fonction de leurs croyances, il n'est pas exclu que les attentes aient contribuées en partie aux effets observés dans nos études.

Dysfonction des mécanismes de perception et de modulation de la douleur dans le syndrome de l'intestin irritable

L'hypersensibilité viscérale est bien documentée dans le Sii et est considérée comme un mécanisme physiopathologique de la douleur abdominale chez la plupart des patients (Ritchie, 1973; Mayer and Gebhart, 1994; Mertz et al., 1995; Naliboff et al., 1997; Houghton, 1999; Bouin et al., 2002). Malgré l'hétérogénéité des symptômes et de l'étiologie du Sii, une proportion importante de patients présentent également une hypersensibilité somatique (Verne et al., 2001; Bouin et al., 2001b; Rodrigues et al., 2005; Caldarella et al., 2006; Moshiree et al., 2007). Les résultats de la présente thèse s'ajoutent à ceux de ces études et montrent une corrélation positive entre l'hypersensibilité somatique et l'hypersensibilité viscérale. Cette association peut s'expliquer par au

moins trois mécanismes n'étant pas mutuellement exclusifs, que nous allons maintenant considérer.

D'abord, l'hypersensibilité somatique pourrait être secondaire à l'hypersensibilité viscérale. Cette possibilité est déjà appuyée par des études antérieures chez des patientes Sii et un modèle animal d'hypersensibilité viscérale (Verne et al., 2003b; Zhou et al., 2008). Dans l'étude de Verne et al., il a été montré que l'application d'une gelée de lidocaïne dans le rectum diminuait l'hypersensibilité viscérale ainsi que l'hypersensibilité somatique secondaire, telle que mis en évidence par l'immersion d'un pied dans l'eau chaude (47°C) (Verne et al., 2003b). Cette étude suggère que l'hypersensibilité viscérale dépend d'une sensibilisation spinale, que l'hypersensibilité somatique est secondaire à cette sensibilisation et qu'elle est maintenue par une activité soutenue des afférences nociceptives rectales (Verne et al., 2003b). Cependant, ces résultats ne peuvent expliquer l'hypersensibilité diffuse retrouvée chez une grande proportion des patients Sii, qui inclue des dermatomes pour lesquels une convergence viscérosomatique entre le tube digestif et la peau est improbable (Rodrigues et al., 2005).

D'autre part, l'association entre l'hypersensibilité rectale et cutanée pourrait dépendre de l'altération d'un mécanisme commun à la sensibilité viscérale et somatique. Une possibilité intéressante est une dysfonction des mécanismes de modulation descendante de la douleur (Coffin et al., 2004; Wilder-Smith et al., 2004; Song et al., 2006;Wilder-Smith and Robert-Yap, 2007). En accord avec cette idée, l'étude 3 montre un déficit d'inhibition de la douleur somatique chez les

patientes Sii lors de la contreirritation. De plus, ce déficit était associé à l'hypersensibilité viscérale et somatique, confirmant sa contribution à l'hypersensibilité diffuse. L'étude 4 suggère également que l'hypersensibilité diffuse pourrait dépendre de la modulation descendante puisque le déficit de modulation de la douleur était associé à un déficit de modulation spinale.

Les symptômes psychologiques généralement plus élevés chez les patientes Sii pourraient également affecter la sensibilité à la douleur de façon non sélective, contribuant à l'hypersensibilité viscérale et somatique. L'association entre l'hypersensibilité viscérale et les facteurs psychologiques est documentée (Whitehead and Palsson, 1998; Posserud et al., 2007; Moshiree et al., 2007) et il a été montré que des manipulations expérimentales de l'état psychologique peuvent moduler les seuils sensitifs lors de distensions rectales (Ford et al., 1995; Accarino et al., 1997; Dickhaus et al., 2003; Posserud et al., 2004). L'étude 3 est en accord avec ces résultats et indiquent que la sévérité des symptômes psychologiques est associée autant à l'hypersensibilité viscérale qu'à l'hypersensibilité somatique. Les expériences de contreirritation des études 3 et 4 montrent également que les symptômes psychologiques contribuent au déficit d'inhibition de la douleur. Il semble toutefois que les symptômes psychologiques reposent sur des processus supraspinaux puisque qu'ils ne sont pas associés à l'inhibition du réflexe RIII (étude 4).

À la lumière de ces résultats, il est proposé que l'hypersensibilité diffuse observée chez plusieurs patients Sii dépende d'une combinaison de facteurs incluant la sensibilisation périphérique et spinale, un déficit de modulation spinale

et supraspinale de la douleur et des facteurs psychologiques. Dans la prochaine section, une hypothèse physiopathologique plus complète du Sii sera proposée en tenant compte d'autres mécanismes qui n'ont pas été abordés dans nos études.

Hypothèse physiopathologique de la douleur dans le Sii

Afin d'intégrer l'ensemble des résultats de cette thèse et ceux d'autres études, une hypothèse physiopathologique de la douleur du syndrome de l'intestin irritable sera maintenant proposée. Les mécanismes que nous allons considérer sont 1) la sensibilisation périphérique 2) l'augmentation des afférences périphériques exprimant le canal TRPV1 3) la sensibilisation spinale 4) une élaboration corticale plus importante de l'information nociceptives 5) des déficits de modulation de la douleur et 6) le stress et l'augmentation du facteur de relâche corticotropique (CRF). La figure 4 illustre l'ensemble des mécanismes proposés que nous allons détailler ici.

1) *Sensibilisation des nocicepteurs mécaniques et des nocicepteurs silencieux*

L'inflammation peut mener à l'hypersensibilité viscérale en sensibilisant les afférences périphériques par une relâche de médiateurs inflammatoires (Woolf and Salter, 2000; Costigan and Woolf, 2000). Ces derniers abaissent le seuil de transduction des afférences nociceptives et activent les nocicepteurs silencieux (dont la transduction n'est possible qu'après l'inflammation). Quoique le Sii ne soit pas catégorisée comme une maladie inflammatoire, environ un tiers des patients développent la maladie suite à une infection ou à une inflammation du tractus

digestif (Spiller and Campbell, 2006). Ainsi, il est reconnu que la sensibilisation périphérique postinfectieuse contribue à l'hypersensibilité viscérale chez au moins une partie des patients Sii (Anand et al., 2007). En accord avec ce mécanisme, l'irritation de la muqueuse rectale par une application de glycérol entraîne une hypersensibilité chez des sujets sains (Bouin et al., 2001a). De plus, l'application de lidocaïne dans le rectum diminue l'hypersensibilité viscérale chez les patients IBS, fort probablement en limitant la transduction nociceptive (Verne et al., 2003).

2) *Augmentation des afférences nociceptives exprimant le canal TRPV1* :

Récemment, l'augmentation de l'expression du canal cationique TRPV1 par les afférences rectales a été associée à la douleur abdominale chez les patients IBS (Akbar et al., 2008). Ce canal cationique retrouvé sur les nocicepteurs facilite la transduction nociceptive et son expression est augmentée lors de l'inflammation de la muqueuse intestinale (Yiangou et al., 2001). Dans le cas du Sii, l'expression du canal TRPV1 serait augmentée malgré l'absence de changements inflammatoires (Chan et al., 2003). Cependant, une étude récente a montré que les patients Sii auraient bel et bien faible réponse inflammatoire associée à l'augmentation des canaux TRPV1 (Akbar et al., 2008). Ainsi, ce mécanisme est fort probablement lié à la sensibilisation périphérique décrite plus haut, chez une partie des patients.

3) *Sensibilisation spinale*

La sensibilisation des neurones de la corne dorsale peut survenir suite à l'augmentation prolongée de la transmission nociceptives en provenance de la périphérie. Comme nous l'avons mentionné plus haut, la sensibilisation périphérique serait retrouvée chez plusieurs patients Sii. Ainsi, elle pourrait mener à la sensibilisation des neurones de la corne dorsale, dont les réponses serait ainsi amplifiées (Woolf and Salter, 2000). Cette amplification de la transmission nociceptive dans la corne dorsale de la moelle épinière s'ajouterait aux mécanismes décrits précédemment pour contribuer à l'hypersensibilité viscérale (Price et al., 2006b). Cette interprétation est appuyée par une étude ayant testé spécifiquement cette hypothèse (Verne et al., 2003b). Dans cette étude, l'hyperalgésie secondaire retrouvée au niveau du membre inférieur chez les patients Sii était diminuée par l'application de lidocaïne dans le rectum. Une étude électrophysiologique utilisant le réflexe RIII supporte également ces résultats, montrant que la transmission nociceptive spinale est augmentée chez les patients Sii lors de distensions rectales (Coffin et al., 2004).

4) *Augmentation de l'élaboration corticale de l'information nociceptive*

Tel que mentionné précédemment, les patients Sii présentent plus de symptômes psychologiques que les sujets sains. Ces facteurs psychologiques sont associés au déficit d'inhibition de la douleur des patientes Sii et semblent dépendre de mécanismes supraspinaux (études 3 et 4 de cette thèse). Les facteurs psychologiques pourraient donc altérer les fonctions de certaines régions

cérébrales impliquées dans la douleur. En l'occurrence, l'anxiété et la réaction catastrophique à la douleur sont associées à l'activité de ACC et de PFC (Davidson, 2002; Gracely et al., 2004; Seminowicz and Davis, 2006). De plus, l'exacerbation de la douleur par l'anxiété est associée à l'activité de PHG (Ploghaus et al., 2001). Puisque ces régions sont associées aux différences individuelles de sensibilité à la douleur (voir étude 1 et Cogill, 2003), Il est probable que les facteurs psychologiques influencent l'élaboration corticale de l'information nociceptive chez les patients Sii. En accord avec cette possibilité, les études d'imagerie cérébrale chez les patientes Sii rapportent de plus fortes réponses cérébrales à des stimuli viscéraux ou somatiques dans ACC (Mertz et al., 2000; Chang et al., 2003; Mayer et al., 2005; Berman et al., 2008) et PFC (Silverman et al., 1997; Mayer et al., 2005). Il est donc probable qu'une élaboration corticale plus importante de l'information nociceptive s'ajoute à l'augmentation des afférences nociceptives (sensibilisation périphérique et spinale) mais cette hypothèse devra être testée spécifiquement. Une bonne façon d'aborder cette question serait par la mesure de l'activité cérébrale combinée à l'enregistrement du RIII comme dans l'étude 1, ce qui permet de contrôler pour l'intensité de la stimulation et la quantité d'information nociceptive transmise au cerveau.

5) *Déficit de modulation de la douleur*

Le déficit de modulation de la douleur est une hypothèse intéressante pour expliquer l'hypersensibilité du Sii. Certains patients montrent une hypersensibilité diffuse et nous avons montré dans l'étude 3 que cette dernière est corrélée au

déficit d'inhibition de la douleur. Nous avons également confirmé que les mécanismes de modulation descendante sont impliqués dans ce déficit (étude 4). Ces études s'ajoutent à quatre autres études qui suggèrent un déficit de modulation supraspinale ou spinale de la douleur (Coffin et al., 2004; Wilder-Smith et al., 2004; Song et al., 2006; Wilder-Smith and Robert-Yap, 2007). Il est probable que le déficit de modulation de la douleur dépende de mécanismes opioidergiques. En appui à cette hypothèse, le fentanyl, un opioïde agissant préférentiellement sur les récepteurs μ, produit un effet analgésique plus important chez les patientes Sii que chez des sujets sains lors de distensions rectales (Lembo et al., 2000). Ceci suggère que la relâche d'opioïdes endogènes est diminuée chez les patientes Sii. Une étude récente a également montré que l'anticipation d'une distension rectale produit l'inhibition de la partie dorsale du pont, qui est associée à une activation de OFC et ACC pendant les distension (Berman, 2008). Chez les patientes Sii, l'inhibition préparatoire du pont était diminuée et cette diminution était associée 1) aux affects négatifs 2) à une plus forte activation de ACC caudal pendant les distensions et 3) à l'absence d'activation significative de OFC et ACC rostral pendant les distensions (Berman et al., 2008). Tel que proposé par les auteurs, ces résultats suggèrent une altération de l'inhibition descendante recrutée par OFC et ACC et une facilitation de la transmission nociceptive par le système noradrénergique du pont.

6) *Stress et augmentation du facteur de relâche corticotropique (CRF)*

Un mécanisme que nous n'avons pas considéré dans nos études est le rôle du stress et du CRF dans la douleur du Sii. Le stress engendre la production de CRF par l'hypothalamus qui agît sur des récepteurs centraux et périphériques (Tache and Brunnhuber, 2008). Le lien entre le stress et le Sii repose sur l'action du CRF sur la motilité gastrointestinale (Fukudo et al., 1998) et sa contribution à l'hypersensibilité viscérale (Tache et al., 2004). Les patients Sii seraient par ailleurs plus réactifs au stress (Whitehead et al., 1992) qui est reconnu comme un facteur important dans le Sii. Le CRF pourrait agir par l'axe hypothalamo-pituitaire mais il peut également agir sur des récepteurs périphériques du tractus digestif. Le stress et l'augmentation du CRF pourraient être indépendants des 5 mécanismes précédents, mais l'action du CRF sur le cerveau, sur le tube digestif et sur la douleur est probablement partiellement associée aux mécanismes proposés ci-haut.

Conclusion

En résumé, la présente thèse a permis de préciser le rôle de plusieurs structures cérébrales dans les multiples composantes de la douleur et dans l'analgésie par contreirritation chez des sujets sains. Il a été montré que les différences individuelles d'activité cérébrale évoquée par les stimulations électriques dans les cortex orbitofrontal et cingulaire sont associées aux différences individuelles de sensibilité à la douleur, de réactivité motrice et de réactivité autonomique. De plus, il a été montré que l'analgésie par contreirritation

est accompagnée de l'inhibition de l'amygdale par le cortex orbitofrontal et d'une modulation du réflexe RIII par la substance grise périaqueducale et le cortex somesthésique primaire. Chez les femmes souffrant du syndrome de l'intestin irritable, les facteurs psychologiques ont été identifiés comme un des facteurs contribuant à l'hypersensibilité diffuse et au déficit de modulation de la douleur. De plus, il a été démontré que le déficit de modulation de la douleur reposait en partie sur l'altération de la modulation descendante et des mécanismes supraspinaux associés à l'anxiété et la réaction catastrophique à la douleur. Il sera maintenant intéressant de déterminer si ces altérations de la perception et de la modulation de la douleur chez les patientes Sii reflètent une dysfonction des mécanismes que nous avons décrits en imagerie cérébrale chez les sujets sains.

FIGURES

Légendes des Figures

Figure 1 : Principales voies nociceptives. 1. Voie spinothalamique. 2. Voie spinoréticulaire. 3. Voie spinomésenscéphalique. 4. Voie spinoamygdalienne. 5. Voie spinoparabrachioamygdalienne. 6. Voie spinotélencéphalique. 7. Voie postsynaptique des colonnes dorsales. 8. Voie projetant de la formation réticulaire dorsocaudale de la moelle allongée vers la corne dorsale. 9. Voie projetant formation réticulaire vers la corne dorsale. ACC : cortex cingulaire antérieur; AMY : amygdale; DCM : moelle allongée dorsocaudale; FRP : formation réticulaire pontique; INS : insula; NC : noyau cunéiforme; NG : noyau gracile; OFC : cortex orbitofrontal; PAG : substance grise périaqueducale; PB : noyau parabrachiale; PCC : cortex cingulaire postérieur; PFC : cortex préfrontal; pgACC : cortex cigulaire antérieur prégénual; PPC : cortex pariétal postérieur; PrCG : gyrus précentral; RVM : moelle allongée ventromédiale rostrale SI : cortex somesthésique primaire; SII : cortex somesthésique secondaire; SMA : aire motrice supplémentaire.

Figure 2 : Mécanismes supraspinaux de la contreirritation. Le cortex orbitofrontal (OFC) gauche (ipsilatéral à la contreirritation) est activé de façon soutenue et est associé à l'inhibition de l'activité évoquée par le choc dans l'amygdale (AMY) lors de la contreirritation. À droite, le graphique illustrant corrélation des différences individuelles.

Figure 3 : Mécanismes cérébrospinaux de la contreirritation. La substance grise périaqueducale et le cortex somesthésique primaire (SI) sont activée de façon soutenue et sont associé à l'inhibition du réflexe RIII pendant la contreirritation. À droite, les graphiques illustrant les corrélations respectives.

Figure 4 : Hypothèse physiopathologique du Sii. De nombreux facteurs contribuent à l'augmentation de la perception de la douleur chez les patients Sii. 1. La sensibilisation des nocicepteurs mécaniques et des nocicepteurs silencieux augmente la transduction nociceptive. 2. L'augmentation des afférences exprimant le canal TRPV1 contribue également à l'augmentation de la transduction nociceptive. 3. La sensibilisation des neurones de la corne dorsale augmente leurs réponses aux afférences périphériques et la transmission nociceptive spinale. 4. L'élaboration corticale de l'information nociceptive est amplifiée et augmente la sensibilité à la douleur. 5. La modulation de la douleur par les mécanismes supraspinaux et par les mécanismes cérébrospinaux est altérée et contribue à l'augmentation de la transmission nociceptive et à la sensibilisation centrale. 6. Le stress provoque l'activation de la voie du CRF qui affecte la motilité gastrointestinale et la sensibilité viscérale.

Figure 1

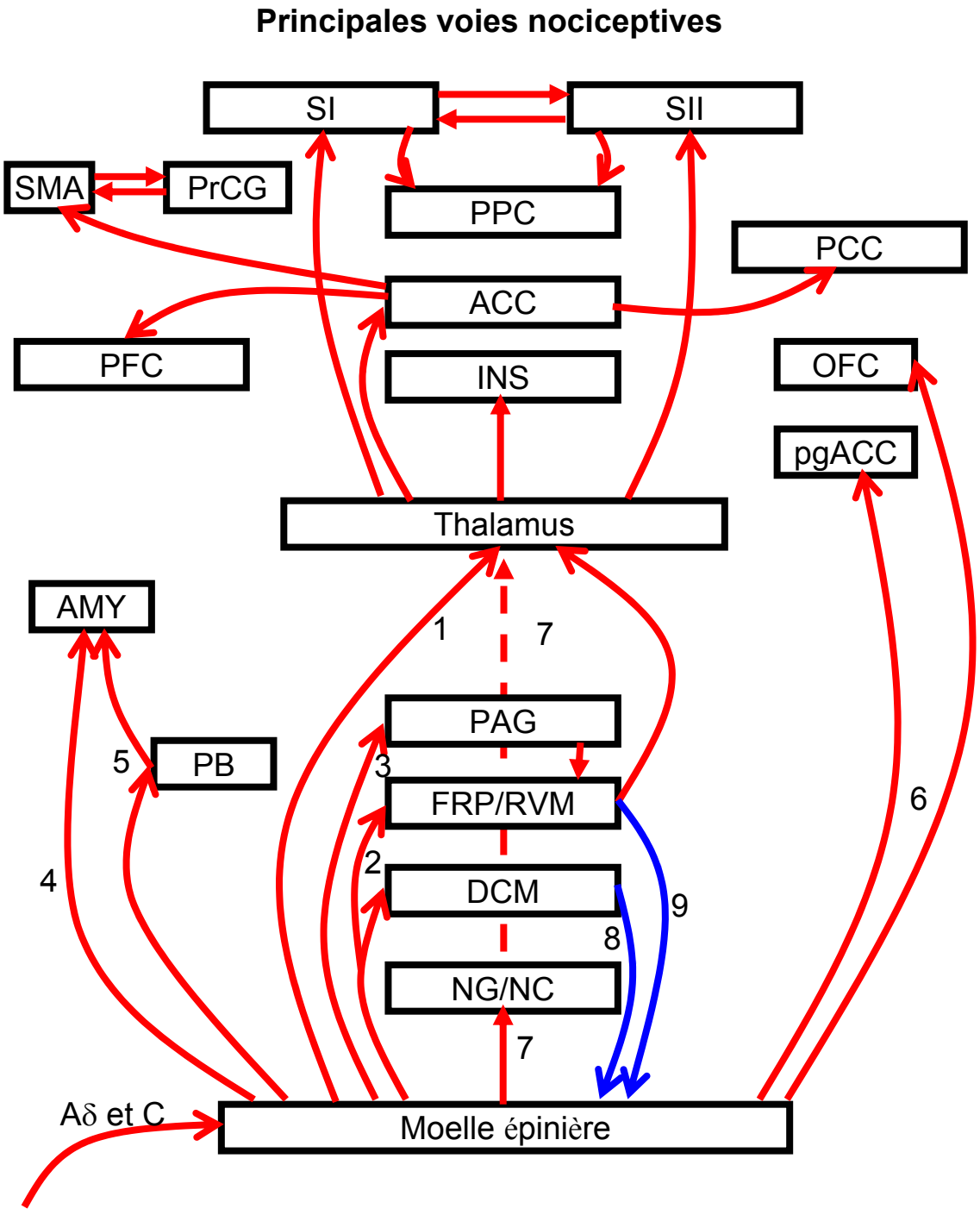
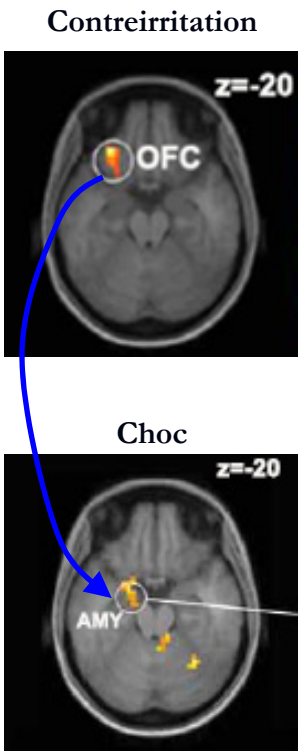

Principales voies nociceptives
SI
SII
SMA
PrCG
PPC
PCC
ACC
PFC
INS
OFC
pgACC
Thalamus
AMY
1
7
PAG
5
PB
3
FRP/RVM
6
4
2
DCM
9
8
NG/NC
7
Aδ et C
Moelle épinière

Figure 2

Mécanismes supraspinaux de l'analgésie par contreirritation



Le cortex orbitofrontal est activé par la contreirritation et inhibe l'activité évoquée par le choc dans l'amygdale

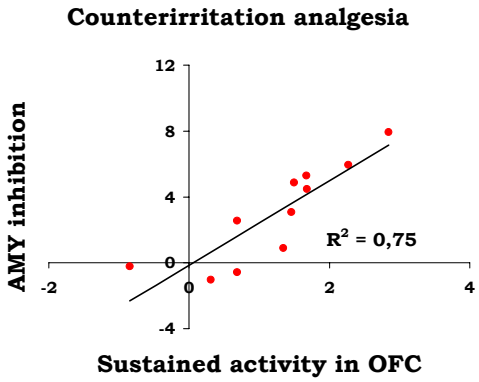


Figure 3

Mécanismes cérébrospinaux de l'analgésie par contreirritation

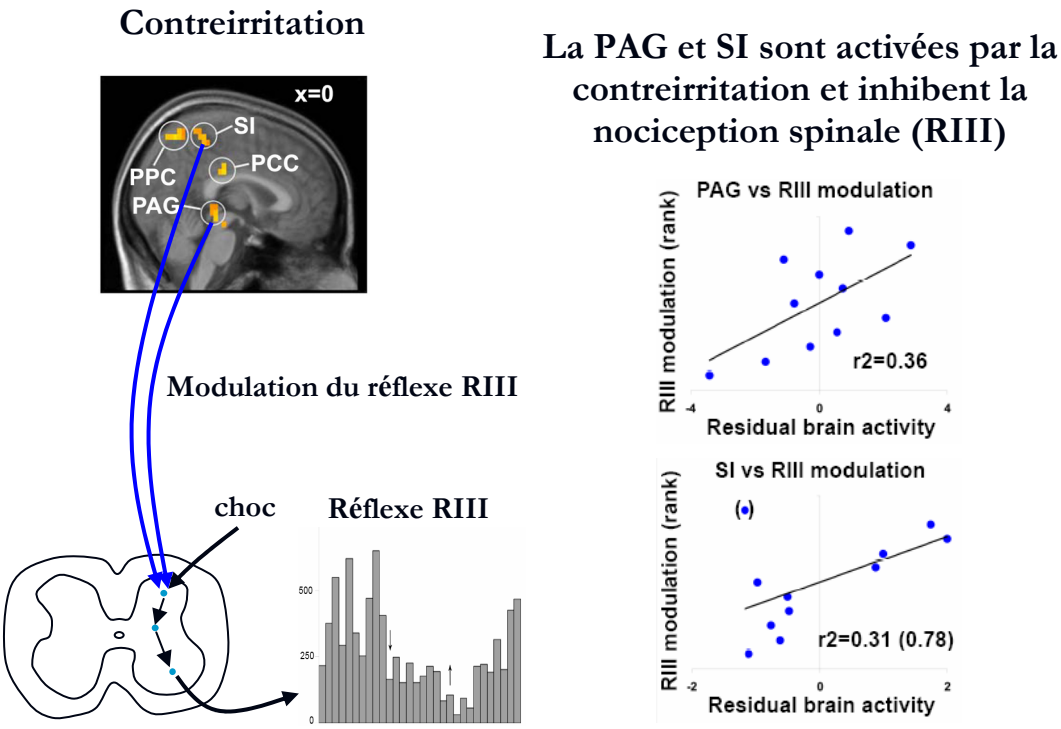
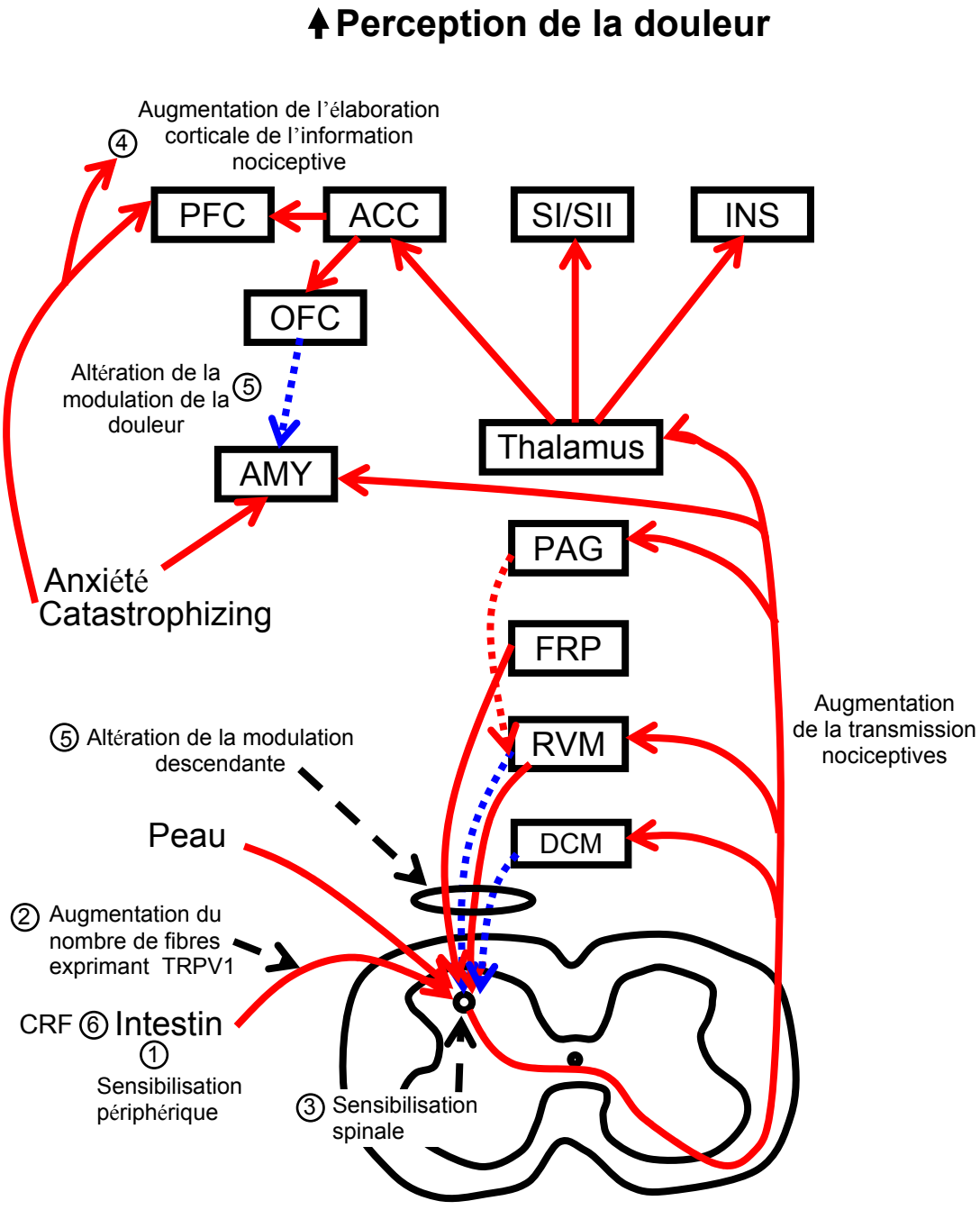


Figure 4

Hypothèse physiopathologique du Sii



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ANNEXES

Article 5 : Characterization of cardiac-related noise in fMRI of the cervical spinal cord

Characterization of cardiac-related noise in fMRI of the cervical spinal cord

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ABSTRACT

Resonance magnetic imaging (MRI) has recently been applied to study spinal cord function in humans. However, spinal fMRI encounters major technical challenges with cardiac noise being considered a major source of noise. The present study relied on echo-planar imaging of the cervical cord at short-TR (TR=250ms; TE=40ms; flip=45°), combined with plethysmographic recordings to characterize the spatio-temporal properties of cardiac-induced signal changes in spinal fMRI. Frequency-based analyses examining signal change at the cardiac frequency confirmed mean fluctuations of about 10% (relative to the mean signal) in the spinal cord and surrounding CSF, with maximal responses reaching up to 66% in some voxels. A spatial independent component analysis (sICA) confirmed that cardiac noise is an important source of variance in spinal fMRI with several components showing a response coherent with the cardiac frequency spectrum. The time-course of the main cardiac components approximated a sinusoidal function tightly coupled to the cardiac systole with at least one component showing a comparable temporal profile across runs and subjects. Spatially, both the frequency-domain analysis and the sICA demonstrated cardiac noise distributed irregularly along the full rostro-caudal extent of the segments scanned with peaks concentrated in the ventral part of the lateral slices in all scans and subjects, consistent with the major channels of CSF-flow. These results confirm that cardiac-induced changes are a significant source of noise likely to affect the detection of spinal BOLD responses. Most importantly, the complex spatio-temporal structure of cardiac noise is unlikely to

be accounted for adequately by ad-hoc linear methods, especially in data acquired using long-TR (i.e. aliasing the cardiac frequency). However, the reliable spatio-temporal distribution of cardiac noise across scanning runs and within subjects may provide a valid means to identify and extract cardiac noise based on sICA methods.

INTRODUCTION

The development of functional magnetic resonance imaging (fMRI) methods has been outstanding but significant sources of noise strongly limit their application to study spinal cord activity. Spinal cord activation has been reported with BOLD fMRI in humans (Yoshizawa et al., 1996; Stroman et al., 1999; Backes et al., 2001; Madi et al., 2001; Stroman and Ryner, 2001; Komisaruk et al., 2002; Stracke et al., 2005; Govers et al., 2007; Maieron et al., 2007), but no standard protocol has been established and the intra and inter-subject reliability dramatically needs to be improved (Giove et al., 2004). The relatively poor reliability of the effects reported in most of the available studies could be explained, at least in part, by a poor control of physiological noise. A few spinal fMRI studies were designed to control for cardiac noise (Backes et al., 2001; Stroman, 2006) artefacts. However, these methods need to be improved and significant advances require a better characterization of physiological noise.

There are at least two ways by which cardiac activity may affect fMRI signal at the spinal level. The first one involves direct effect induced by the

dilation of arteries following the systole. Cerebral fMRI signal has been found to vary considerably across the cardiac cycle, especially along the major brain vessels (Dagli et al., 1999). At the cervical level, the arterial inflow comes from branches of the vertebral arteries to one anterior spinal artery and two posterior spinal arteries which run along the rostro-caudal axis of the ventral and dorsal cord, respectively. Pulsatile activity within those vessels may induce cardiac-related signal change that may contaminate fMRI signal within the ventral and dorsal horn of the spinal cord.

The second possible source of cardiac noise in fMRI signal is indirect, through the movement of the CSF. A transient increase in intracranial pressure is observed following the systole leading to a rostro-caudal displacement of CSF from the intracranial subarachnoid space into the subarachnoid space of the spinal cord (O'connell, 1943; du Boulay, 1966; Alperin et al., 1996; Schroth and Klose, 1992a). During the diastole, the CSF flows back in the caudo-rostral direction (Henry-Feugeas et al., 1993). This flux and reflux of CSF flows at approximately 3 cm/s but can reach a velocity as high as 7 cm/s at the cervical cord level (Henry-Feugeas et al., 1993; Watabe et al., 1999). This CSF flow is further organised spatially along four channels of the spinal subarachnoid space: one ventral, one dorsal and two lateral (left and right) channels (Henry-Feugeas et al., 1993). An MRI study using a cine-phase-contrast pulse sequence demonstrated that CSF flow is not continuous from the upper cervical to the thoracic level and is not uniform in the different channels (Henry-Feugeas et al., 2000). More specifically, the velocity is lower in the narrow dorsal CSF

channel, and the dorsal and ventral channels are interrupted in part by the curvature of the spine (cervical lordosis). This leads to a flow from the anterior and posterior channels into the lateral channels, from the midcervical level to the thoracic level. Thus, the cervical CSF pulsation is spatially heterogeneous and varies according to the location within the spinal canal. This CSF flow is likely to produce significant changes in fMRI signal that must be characterized before an adequate correction method can be developed and applied effectively.

Previous studies have looked at cardiac-induced signal change in brain fMRI (Hu et al., 1995; Dagli et al., 1999) but, as in the vast majority of fMRI studies, the fMRI acquisition rate was much lower than the heart rate, therefore aliasing the fundamental frequency of the cardiac cycle. A retrospective gating method has been applied to estimate and remove the signal change induced by the cardiac cycle; however, although this method may prove to be effective in some circumstances, it does not allow for a precise characterization of the cardiac noise and it may partly remove variance in the signal of interest because of aliasing. Another strategy is to increase the sampling rate of fMRI images to avoid aliasing. Images can then be filtered temporally to estimate the variance within the bandwidth of cardiac frequency and generate a spatial map of the related signal change. In the present study we first applied such an approach to fMRI at the spinal level, allowing quantification of the cardiac fluctuations.

A second analysis strategy based on independent component analysis (ICA) can be used to identify cardiac components in spinal fMRI data. ICA

allows for the extraction of a phenomenon from a mixture of signals (Comon, 1994) and is a validated approach to identify and characterize physiological noise in brain-related physiological data in EEG recordings (Srivastava et al., 2005). Spatial ICA (sICA) has been applied to identify and correct for structured noise in brain fMRI data (Perlbarg et al., 2007). This method may contribute to the characterization of cardiac noise from spinal fMRI data.

The primary goal of this study was to quantify the cardiac-related signal change in spinal fMRI and describe its spatio-temporal characteristics. In a first analysis, a filtering procedure was used to isolate cardiac variance in the images and quantify the signal change. In the second analysis, the most important cardiac components in the spinal fMRI time series were identified by sICA and characterized in relation to the cardiac systole. Using both methods, we provide spatial maps of the cardiac-related signal change.

METHODS

Spinal imaging

A total of 15 fMRI scans were performed in four healthy volunteers familiar with the MRI acquisition procedure as part of developmental tests to assess the feasibility of spinal fMRI. Images were acquired on a 1.5T Siemens Avanto MR scanner. All subjects performed 4 scanning runs (390s each) except one in whom the last scan was discontinued due to technical problems. Subjects were positioned to limit head movement and reduce neck lordosis and were requested not to move. A sagittal localizer was first acquired to determine

the area to cover in the functional scans and a coronal localizer was acquired to allow slice positioning within the spinal cord. The subject's neck was carefully positioned and repositioned if necessary after the localizers. The second of three sagittal slices was centered on the mid-sagittal plane of the spinal cord and the two lateral slices covered the lateral aspects of the spinal cord including dorsal and ventral horn regions (thickness = 2 mm; gap = 0.4 mm). Slices were positioned to cover the vertebral body of C3 to T1-T2 (Fig. 1). FMRI data were acquired using a gradient-echo EPI sequence with a TE = 40 ms, a matrix of 64 X 64, and a Field of View = 120 mm (in plane resolution = 1.9 mm). A short TR of 250 ms was used to sample the signal changes across the cardiac cycle adequately. The flip angle was adjusted accordingly to 45°. Gradient spoiling, consisting in 2 diffusion gradients in opposite phase, was used to suppress spin-memory effects (steady-state free precession) due to the short-TR acquisition and potentially affected by cardiac activity. Although the present study was not designed to detect spinal activity, a motor task consisting in 6 blocks of 30s of isometric fist clenching was performed. This insured that the assessment of cardiac-related noise was performed in conditions similar to those normally tested in BOLD-activation studies. Ad-hoc analysis of task-related activation based on the general linear model (SPM2) and correcting for multiple comparisons using an appropriate statistical criterion (e.g. Bonferroni correction based on random field theory) did not yield significant effects.

Physiological monitoring

Heart rate was monitored continuously using a plethysmograph (Nonin model 8600FO) with the sensor attached to the subject's left index finger. During the functional scans, a TTL pulse for each fMRI volume and the continuous plethysmographic data were sampled at 1000 Hz and recorded on a MP150 system (Biopac Systems, Goleta, CA 93117). This allowed us to determine precisely the acquisition time of each volume relative to the cardiac systole (i.e. post-systole delay).

Data analysis

Data analysis is outlined as follow. FMRI data analysis was done in parallel using two separate procedures as described in Fig. 2. Mosaic DICOM files were saved into Analyze format. In the first procedure, masks for each run were generated to include the spinal cord and CSF. Next, the images were filtered to extract, quantify and localize variance in the range of cardiac frequency. In the second procedure, sICA was applied to extract 30 independent components from unmasked data. Linear regression between the power spectra of independent components and that of the corresponding plethysmographic recordings allowed selecting sICA components showing time course coherent with cardiac activity. Spatial maps of those cardiac components were generated. The temporal relationship between the time course of the cardiac components and the plethysmographic recording was further examined by plotting the signal change against the post-systole delay of each volume

acquisition. Details on each of these analysis steps are described in the following subsections.

Subject motion and slice timing

Motion and slice-timing correction are part of standard fMRI analysis. However, the impact of cardiac noise is expected to be widespread, inducing variation in a large number of voxels, so the estimation of the motion-correction matrix might include these variations. Moreover, realignment has been shown to affect the integrity of the data (Freire and Mangin, 2001) and may affect the estimate of cardiac-related signal changes (Cohen-Adad et al., 2007). In the present data set, motion was less than 1 mm and 0.2 degree along the three axes in all runs (rigid-body transformation; SPM realign). Since the aim of the present study was to characterize the cardiac-related signal, realignment was not applied to preserve the integrity of the signal as much as possible.

The maximum inter-slice time interval was only 160 ms (between the first and third slice acquired). No slice-timing correction was applied as variations occurring within the acquisition of a volume would not hinder our ability to characterize the magnitude and location of cardiac-related noise. This also preserves the integrity of the data from the interpolation process artefacts inherent to slice-timing correction (Calhoun et al., 2000). It may introduce a phase shift in the spatio-temporal pattern of cardiac-related signal fluctuations between slices. However, sICA is designed to capture effects that are spatially distributed (including between slices). Moreover, this would represent a rostro-

caudal shift (between outermost slices) of 5 mm at the most for a CSF velocity of 3cm/s (Henry-Feugeas et al., 1993; Watabe et al., 1999).

Design of masks

A mask containing the spinal cord and the surrounding cerebrospinal fluid (CSF) was created using the mean functional image of each run. The design of the mask was based on the following automatic procedure: the volume was normalized then smoothed using a Gaussian filter (FWHM = 11 mm). Next, the smoothed volume was made binary using a threshold according to the intensity histogram. Mathematical morphology was used to eliminate isolated voxels (opening with a 5-voxels disk). The created mask was then multiplied by a coarse mask containing the spinal cord and surrounding CSF to eliminate unwanted structures, such as large arteries, in which cardiac variance was present. The output masks were examined and validated by an expert after the automatic procedure. A separate mask of the spinal cord with surrounding CSF was created for each run.

Cardiac data processing and fMRI data filtering

A fast Fourier transformation was performed on each plethysmographic recording, resampled from 1000Hz to 4 Hz to have a sampling identical to fMRI time-series (TR of 250 ms = 4 Hz). Based on the power spectrum of the plethysmographic recording, a band pass Butterworth filter was designed as follows: (i) the frequency centre of the filter was set to the observed modal

frequency and (ii) an ad hoc spectral window of 0.3 Hz was used to account for intra-recording cardiac frequency variations (Thomas et al., 2002). We verified that most of the cardiac spectrum was contained within that window. A constant spectral window was used in order to avoid biased intra-subject quantification of standard deviation after filtering the fMRI series.

Quantification and localization of cardiac variance

A baseline was defined for each run as the mean of all voxels intensity within the mask. All fMRI series were then filtered in the time domain with the run-specific Butterworth filter described above to extract variance within the observed cardiac frequency band. The magnitude of signal change in the range of cardiac frequency was quantified (%) in relation to the mean signal in the unfiltered fMRI time-series. Since Nyquist frequency was more than twice the maximum of the cardiac spectrum, there was no aliasing of the fundamental frequency of the cardiac signal. The standard deviation of every time course was estimated and normalized to the baseline (Dagli et al., 1999). Thus, a voxel-by-voxel analysis of the standard deviation of MRI signal within the range of cardiac frequency was performed for each run and spatial maps of percent signal-change were generated.

Spatial ICA decomposition and selection of cardiac-related components

In a subsequent analysis (Fig. 2; right panel), each scan was analysed separately with a spatial ICA decomposition (sICA © 2006 Inserm U678 V.

Perlbarg) to isolate the independent components from the raw fMRI data (McKeown et al., 1998). It was assumed that most of the cardiac variance would be captured within the first 30 components. Using a fast Fourier transformation, the power spectrum of each component was extracted and compared with the power spectrum of concurrent plethysmographic recordings using a linear regression analysis. This frequency–domain coherence analysis allows for the identification of putative cardiac components independent from potential phase-shifts between the cardiac systole and the time courses of the sICA components. For each scan, correlation coefficients reached statistical significance for at least one component (Pearson-r ; $p < 0.05$, adjusted using the Bonferroni-correction based on the number of components tested). The spatial structure of these cardiac components was then examined.

Temporal characterization in relation to the cardiac systole

To further characterize the signal change of putative cardiac components in relation to the cardiac activity, the normalized signal of each identified component was plotted against the post-systole delay, as determined by the cardiac and image acquisition recordings. Based on combined ECG and plethysmographic recordings obtained outside of the scanner, the cardiac systole was estimated to occur about 400 ms before the finger pulse. This interval was used to approximate the true post-systole delay. This analysis provided (1) a confirmation that the coherence analysis correctly identified signal-change time-locked to the cardiac systole and (2) a description of the

temporal characteristics of cardiac noise. It also allowed for a comparison of cardiac noise with patterns of CSF flow velocity described in previous studies (Henry-Feugeas et al., 1993; Watabe et al., 1999).

RESULTS

Heart rate activity was calculated from the plethysmographic recording obtained in all scans. The mean heart rate across all runs and subjects was 63.6 ± 5.4 bpm. The mean standard deviation (intra-run variance) for all runs and subjects was 6.4 ± 2.5 bpm.

Quantification of signal change at the cardiac frequency

The first question addressed concerned the magnitude of the cardiac-related signal change. The quantification was done on the temporally filtered images (bandwidth of 0.3 Hz centered on the modal frequency). The value of signal intensity for the voxels included in the mask (see methods) was calculated and the results for each of the three slices are presented separately for each scanning run in Table 1. Considering the mean voxel value, the signal change was similar across slices, runs and subjects (around 10%). However, based on peak values (the maximum variation in a voxel), some voxels presented very large signal change. For instance, a peak of 66% was found in subject 1, run 2 (see Table 1, peak_s3). Interestingly, the most extreme variations are found in the two outermost slices. Indeed, the maximum signal change in the middle slice did not exceed 20% whereas the outermost slices

presented variations between 22% and 66%. This distribution is illustrated in the spatial maps of standard deviation (Figs 3-5).

Localization of signal change at cardiac frequencies

The filtered signal in one slice for one typical functional run was overlaid on an anatomical image and is presented in Fig. 3. The peak variations are found at the interface between the CSF and the spinal cord, with maxima reaching as high as 48% signal change in this representative example. Importantly, this effect was not limited to isolated voxels but rather extended to groups of voxels along the rostro-caudal axis. To better illustrate the effect, the three slices for all runs in one subject are presented in Fig. 4. The prominent characteristic is the larger signal variations at the anterior CSF/spinal cord interface, especially in the two outermost slices.

Intra- and inter-session reliability

To examine the reliability of the effect described above, intra- and inter-session comparisons are illustrated. The intra-session comparison across runs is shown for one subject in Fig. 4. Note that similar results were obtained in all subjects. The four runs acquired in that subject clearly show that the signal change at cardiac frequency is very stable both in terms of localization and magnitude. The peaks are located at the same position and the pattern across slices is preserved. The range of mean and peak signal change is also comparable across runs (see Table 1).

To evaluate the inter-session reliability of the signal change within the range of cardiac frequency, one run from each subject is shown in Fig. 5. Some inter-individual differences were expected but the comparison of the four subjects indicates that the spatial distribution of the most affected voxels and their range of signal change was relatively comparable (see Table 1 for quantitative results). Larger effects were found again on the rostro-caudal axis along the CSF/spinal cord border. For all subjects, the anterior CSF/spinal cord interface in the two outermost slices was more affected by the extreme signal variations, confirming the results from one subject presented in Fig. 4. Subject 4 displayed the most pronounced cervical lordosis and presented additional peak signal variations in the dorsal CSF/spinal cord interface and in the mid-saggital slice. This subject also displayed the largest peak signal change in the middle slice compared to the other subjects, and the smallest differences across slices (see Table 1). This suggests a relatively more important spreading of cardiac noise that might be associated with the sharper lordosis.

Spatial ICA decomposition and identification of cardiac components

In the second analytical approach (see left panel in Fig. 2), the first 30 independent components (ranked by eigenvalues) were extracted from each volumetric time-series using sICA. The mean variance explained by these first 30 components across all runs and subjects was 17.8 ± 1.5 % of the total variance. Individual component over the 30th explained less than 0.25% of the total variance. Among the selected components, one to four cardiac

components were identified based on the significant coherence with the cardiac frequency spectrum extracted from the plethysmographic recordings. The mean variance explained by the selected cardiac components within a run was 4.0 ± 1.2 % of the total variance. In all runs, all significant cardiac components were found within the first 7 sICA components (ranked by eigenvalue). The first cardiac component was always found within the first three sICA components and in 11/15 cases the first two sICA components were related to cardiac activity. This confirmed that at least part of the cardiac noise has a stable spatio-temporal structure that can be captured by sICA.

A spatial map of z-scores assessing the contribution of each voxel to the time course of each independent component was used to localize cardiac noise. One representative example is presented in Fig. 6. Overall, the spatial distribution of the cardiac noise was highly consistent with the results from the frequency-based analysis. Here again, the anterior CSF/spinal cord border in the two outermost slices was more affected by the extreme signal variations. To look at temporal characteristics of the cardiac components, the normalized signal of the cardiac components was plotted against the post-systole delay for each volume acquisition. An example of this plot for one cardiac component is shown at the bottom right of Fig. 6. Each point represents a volume with the associated signal value of the corresponding component plotted against the post-systole delay. For that component, there was a clear sinusoidal pattern describing the signal change time-locked to the cardiac systole.

Pattern of signal change relative to the cardiac systole

All cardiac components identified from the output of sICA by the coherence analysis were examined in the time-domain. The normalized signal for each cardiac component was plotted against the post-systole delay. To allow for an intra- and inter-session comparison, the results are presented together for all runs in Fig. 7. The plot of each component was fitted with a 10th degree polynomial in order to provide an accurate summary of each time-course with minimal constraints.

In addition to showing a clear temporal relation with the cardiac cycle, all significant components are spatially distributed along the rostro-caudal axis at the CSF/spinal cord border (as shown in the representative example in Fig. 6). There is also a remarkable stability in the spatio-temporal profile of several cardiac components identified across runs within the same subject (Fig. 7). For example in subject 1, two cardiac components with a very similar temporal profile were found across the 4 runs. For all subjects, at least one component could be readily identified that followed the same temporal pattern across all runs. One cardiac component also displayed a very similar temporal profile across subjects and runs (see red lines in Fig. 7) except for subject 3 runs 1-2. The signal change for that component has its minimum and maximum around 200 ms and 700 ms post-systole, respectively.

DISCUSSION

Cardiac-related noise is widely recognized in spinal fMRI (Giove et al., 2004; Stroman, 2005; Stroman, 2006; Figley and Stroman, 2007; Brooks et al., 2008). However, until recently (Brooks et al., 2008), there was no study in which a method was used to appropriately isolate and remove cardiac noise from the functional data. In the current study, a spatio-temporal characterization of cardiac noise in cervical spinal fMRI is provided. Results indicate that functional images of the cervical spinal cord are significantly affected by very large signal variations time-locked to the cardiac systole. Those variations are very consistent across runs and, to a certain degree, across subjects. The characteristics of the signal fluctuations are compatible with mechanisms involving pulsating CSF flow induced by cyclic changes in intra-cranial pressure produced by cardiovascular activity.

Quantification of cardiac-induced signal change

It was previously suggested that cardiac noise contributes to deterioration of the signal in spinal fMRI (Giove et al., 2004; Brooks et al., 2008) but no study had yet characterized its magnitude. In the present study, a band pass filter was applied to the fMRI time series to estimate the magnitude of the signal changes occurring at cardiac frequencies. The results confirmed the hypothesis that cardiac noise is very significant in cervical spinal fMRI with signal changes of 10.4% in average and up to 66% in some voxels. As a comparison, cardiac-induced noise is thought to induce signal changes in the order of 1-8% along the

major brain vessels in cerebral fMRI (Dagli et al., 1999). Considering that the BOLD changes due to neuronal activity are expected to be considerably smaller (Giove et al., 2004), it appears indispensable to control for cardiac noise to avoid deterioration of spinal signal and improve the detection of valid functional activations.

Source and localization of cardiac effects

The two potential sources of cardiac-related noise postulated are the systole-induced pulsation in spinal arteries and CSF pulsation in the subarachnoid space. According to the spatial distribution of the cardiac-related effects observed, a CSF-pulsation effect appears more likely. Indeed, the middle slice of our acquisitions included the ventral fissure of the spinal cord where the anterior spinal artery lies and where cardiac-related effects were weakest relative to the two lateral slices. This result contrasts with the effect reported in brain fMRI where cardiac noise appears to be concentrated along major brain vessels (Dagli et al., 1999). This difference may reflect the smaller diameter of spinal compared to brain arteries. Cardiac noise in spinal fMRI may therefore be largely secondary to CSF flow induced by variations in intracerebral pressure due to cerebral arterial inflow.

The CSF flow in the spinal subarachnoid space induced by cardiac-related changes has been characterized in studies using flow-sensitive MRI sequences (Schroth and Klose, 1992a; Henry-Feugeas et al., 1993; Henry-Feugeas et al., 2000; Friese et al., 2004), and is the most plausible source of

signal variation in the present study. The most extreme variations are found in the two outermost slices of the volume, at the ventral aspect of the CSF/spinal cord interface. This is highly consistent with the ventro-lateral inflow of CSF due to anatomical interruptions of the subarachnoid space (Henry-Feugeas et al., 1993), as described in the introduction. Assuming that increasing CSF velocities induce a greater artefact in the MRI signal, these differences are also compatible with the earlier description of CSF flow velocity in the different channels of the spinal subarachnoid space.

Additional sources of structured noise might also contribute to the present results. Future studies should include additional lateral slices that are not expected to contain CSF flow in order to confirm the proposed interpretation and rule out some other forms of structured field disturbance. Furthermore, a recent study by Figley and Stroman (2007) suggests that cardiac-related noise might involve motion of the spinal cord, primarily in the ventral-dorsal direction. However, this motion is likely to be secondary to CSF flow within the spinal canal and such motion would be expected to affect signal at the CSF-cord interface in all three sagittal slices acquired in the present study. In contrast, we observed cardiac-related signal fluctuations primarily in the two lateral slices and much less in the mid-sagittal plane so our results likely reflect more than cord motion. In any case, Figley and Stroman (2007) and the present results demonstrate the usefulness of principal component analysis and sICA to characterize physiological noise in spinal fMRI data (also see Brooks et al., 2008).

Temporal pattern of cardiac effects

One of the cardiac component common to most runs (red lines in Fig. 7) displayed a temporal profile highly compatible with cardiac-induced CSF pulsation. Indeed, signal changes peaked around 200 ms and 700 ms, consistent with previous studies on CSF-velocity changes (Henry-Feugeas et al., 1993; Henry-Feugeas et al., 2000; Friese et al., 2004). Importantly, more than one cardiac component was found in most runs. Those multiple components varied in phase relative to the cardiac systole and were distributed along the rostro-caudal axis of the spinal cord. This further suggests a broad spatio-temporal distribution of cardiac noise. Several sICA components were out of phase by a delay approximating, or larger than, the TR (250ms) as shown in Fig. 7. Those separate components are unlikely to reflect a single cardiac-related wave of signal change captured at different time points in different slices, depending on a systematic shift potentially introduced by the slice-time delay (160ms between slice 1 and 3). This illustrates the complexity of the cardiac noise and the potential difficulties in the attempts of modelling it a priori in spinal fMRI analyses.

Intra- and inter-session reliability

The reliability of the cardiac-related signal fluctuations identified with the temporally filtered images was assessed by qualitatively comparing the results across runs and subjects. Based on anatomical landmarks visible on the images (disks and vertebral bodies), there is a remarkable stability across runs in the

same subject (see Fig. 4 and Table 1). The peaks were generally in the same segment and the magnitude of the signal change was similar. This within-subject stability has an important impact on future developments of spinal fMRI. This suggests that cardiac noise correction in long-TR data may be based on cardiac-related information derived from separate scanning runs TR (see below).

As for inter-individual reliability, differences were expected in the signal magnitude and distribution. For instance, anatomical interruption of the subarachnoid space and the differences in the dynamic of CSF flow is likely to vary across subjects (Henry-Feugeas et al., 1993; Watabe et al., 1999; Henry-Feugeas et al., 2000). Nevertheless, as shown in Fig. 5, the spatial pattern is relatively similar across the four subjects. These comparisons confirm that the cardiac-related noise identified is present in all subjects at the CSF/spinal cord interface.

The reliability of the cardiac-related signal fluctuations was also evaluated based on the cardiac components identified using sICA. A plot of the signal change relative to the post-systole delay showed that for each subject, there was a stable component across runs (Fig. 7). This has an important implication for the correction of the cardiac noise: the identification of subject-specific spatial patterns of cardiac noise with a single short TR acquisition should be valid for a fMRI acquisition session involving several scans. Interestingly, at least one component was present for most of the runs across all subjects,

suggesting a common phenomenon. These results further demonstrate the reliability of sICA in extracting cardiac variance from spinal fMRI time-series.

Limitations of the study

The present study was designed to characterize the cardiac noise in spinal fMRI and the proposed methodology relied on two general approaches that have some limitations. The quantification of the systole-related signal changes was based on filtered images. Although most cardiac variance was preserved within the window of 0.3 Hz, the analysis might have underestimated the importance of signal changes. On the other hand, the bandwidth selected for the filter might include phenomenon other than cardiac in origin. This would bring an overestimation of the signal changes. Nevertheless, the spatial distribution of the variance argues for a cardiac source involving CSF pulsation. In any case, while the quantification procedure might include some error, results clearly confirmed that the relative magnitude of cardiac noise is significant relative to the magnitude of signal changes typically associated with BOLD-related effects.

Another factor not addressed in the present study is the additional contribution of other sources of physiological noise. Although the present study did not address the potential artefacts created by respiratory movements, their impact on spinal fMRI have to be considered. Apart from gross movement and susceptibility artefacts created by the expansion of the thorax, respiration may also affect the CSF flow and contribute to physiological noise (Schroth and

Klose, 1992b; Friese et al., 2004). Further research is needed to characterize respiratory-related noise and validate a method to control for it in spinal fMRI. The methodology proposed in the present study could be applied to this question by simply adding a concurrent monitoring of respiratory activity.

Another limitation of the present study is related to the acquisition parameters. The short-TR acquisition used here is well suited for cardiac noise characterization but is not optimally sensitive to T2* effects and BOLD signal changes due to neuronal activity; i.e the lower flip angle used in short-TR acquisition considerably reduces signal (Huettel et al., 2004). On the other hand, long-TR acquisitions typically used to detect BOLD effects do not allow for an unequivocal identification and removal of cardiac noise because of aliasing the cardiac frequency. At long TR, aliasing of the cardiac frequency may contaminate task-related signal changes and introduce a confounding factor that could contribute to false positive activations. In that respect, short-TR acquisition can provide a compelling account of cardiac signal changes as demonstrated in the present study. An important consideration for short-TR acquisitions however is the disturbance of the steady-state free processing. This phenomenon can occur in gradient-echo EPI acquisitions when TR<T2 (especially in the CSF) and causes voxel-wise temporal variation or additional noise (Zhao et al., 2000). The present study did not address the characterization of that potential source of noise. However, that additional source of noise was suppressed by the gradient-spoiling used here, as described in the methods.

Further research may provide additional characterization of SSFP disturbances by comparing acquisitions with or without gradient spoiling in spinal fMRI.

CONCLUSION

There is yet no consensus on a strategy to control physiological noise in spinal fMRI. The present results demonstrate that the large cardiac-related signal-change in spinal fMRI is characterized by complex spatio-temporal patterns which were stable across runs and relatively comparable across subjects. Linear modelling of the cardiac noise (e.g. Stroman, 2006) may be a difficult task in view of the observed nonlinear effects. In contrast, ICA-based methods similar to the second analytical approach used here, have recently provided encouraging results (Brooks et al., 2008). This research further emphasize the importance of developing and validating more efficient strategies to control for cardiac, and other, sources of physiological noise.

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Table 1

subj	run	mean_s1	mean_s2	mean_s3	peak_s1	peak_s2	peak_s3	sd_s1	sd_s2	sd_s3
1	1	10.8	10.7	10.3	36.5	15.8	61.7	4.9	5.1	5.4
	2	11.2	11.2	10.8	41.9	15.6	66.2	5.8	6.0	6.3
	3	11.1	11.2	10.7	39.5	16.4	58.1	5.6	5.8	6.1
	4	10.9	10.9	10.5	37.6	13.6	47.2	5.2	5.4	5.7
mean		**11.0**	**11.0**	**10.6**	**38.9**	**15.4**	**58.3**	**5.4**	**5.6**	**5.9**
2	1	11.0	10.8	10.3	33.9	12.5	49.3	4.5	4.9	5.1
	2	10.4	10.5	9.9	48.0	13.2	48.4	5.7	6.0	6.1
	3	10.2	10.3	9.8	38.6	11.7	47.8	5.0	5.3	5.4
	4	11.4	11.6	10.8	47.1	13.0	52.4	5.3	5.6	5.9
mean		**10.7**	**10.8**	**10.2**	**41.9**	**12.6**	**49.5**	**5.1**	**5.4**	**5.6**
3	1	10.7	10.5	10.4	24.2	15.1	25.9	2.8	3.2	3.3
	2	10.2	10.1	10.0	22.4	14.1	21.5	2.4	2.8	2.9
	3	10.8	10.5	10.4	25.8	14.2	24.2	2.8	3.4	3.5
	4	10.6	10.4	10.3	24.9	13.2	25.3	2.7	3.2	3.4
Mean		**10.6**	**10.4**	**10.3**	**24.3**	**14.1**	**24.2**	**2.7**	**3.1**	**3.3**
4	1	10.5	10.8	10.1	30.4	19.6	31.4	3.5	3.6	4.2
	2	9.5	9.7	8.9	24.3	17.9	22.1	2.4	2.6	3.7
	3	9.9	10.1	9.5	23.6	19.6	21.1	2.5	2.7	3.8
Mean		**10.0**	**10.2**	**9.5**	**26.1**	**19.0**	**24.9**	**2.8**	**3.0**	**3.9**

FIGURE LEGENDS

Figure 1: Anatomical image in the sagittal plane showing the field of view of fMRI time series acquisitions.

Figure 2: Data processing. Two procedures were used to quantify the cardiac-related signal change in spinal fMRI and to describe its spatio-temporal characteristics. On the left, raw images are band pass filtered based on the

target cardiac frequency band determined by the power spectrum of the plethysmographic recordings. The resulting mean image allows quantification and localization of the noise at cardiac frequencies. On the right, sICA is applied to extract the first 30 independent components from the raw data. The cardiac components are determined by a coherence analysis between the power spectra of the plethysmographic recordings and the components. The resulting cardiac components allow for temporal characterization and localization of the cardiac noise.

Figure 3: Signal change within the cardiac frequency range overlaid on the T1 anatomical image in one subject. The color scale indicates the percent signal change relative to the mean value of all voxels included in the mask (see methods). The highest peaks are found in the CSF but note that decreasing the threshold also shows signal changes within the spinal cord.

Figure 4: Illustration of the cardiac-induced signal change in one subject. Columns represent each of the four runs with the vertical colour scale indicating the percent signal change (relative to the mean value of all voxels included in the mask). Each column is composed of three images representing the three slices of the volume from left (top) to right (bottom). The maps of percent signal-change are overlaid on the mean functional images. Vertebral bodies are easily identified in the images (see C3 and T1 bodies in the upper right image).

Figure 5: Comparison of the cardiac-induced signal changes in four subjects for one run. Columns represent each of the four subjects with a vertical scale indicating the percent signal change (relative to the mean value of all voxels included in the mask). Each column is composed of three images representing the three slices of the volume from left (top) to right (bottom). The maps of percent signal-change are overlaid on the mean functional images.

Figure 6: Spatial map of variance for one sICA component displaying a cardiac-coherent power spectrum. The left and middle slices are presented at the top left and right, respectively, and the right slice at the bottom left. The graph represents the time course of the normalized signal for that component plotted against the post-systole delay. Each point in the graph corresponds to one time point (each run = 390s * 4Hz = 1560 data points).

Figure 7: Cardiac-induced signal change in relation to the post-systole delay for all runs. In all runs, the cardiac-related effect was decomposed into several independent components coherent with the cardiac power spectrum and demonstrating responses time-locked to the cardiac systole. One component displaying a consistent temporal pattern (red line) was found in most runs (except in subjects 3, runs 1-2),. Also note the variable phase-shift of the different components within runs.

Figure 1

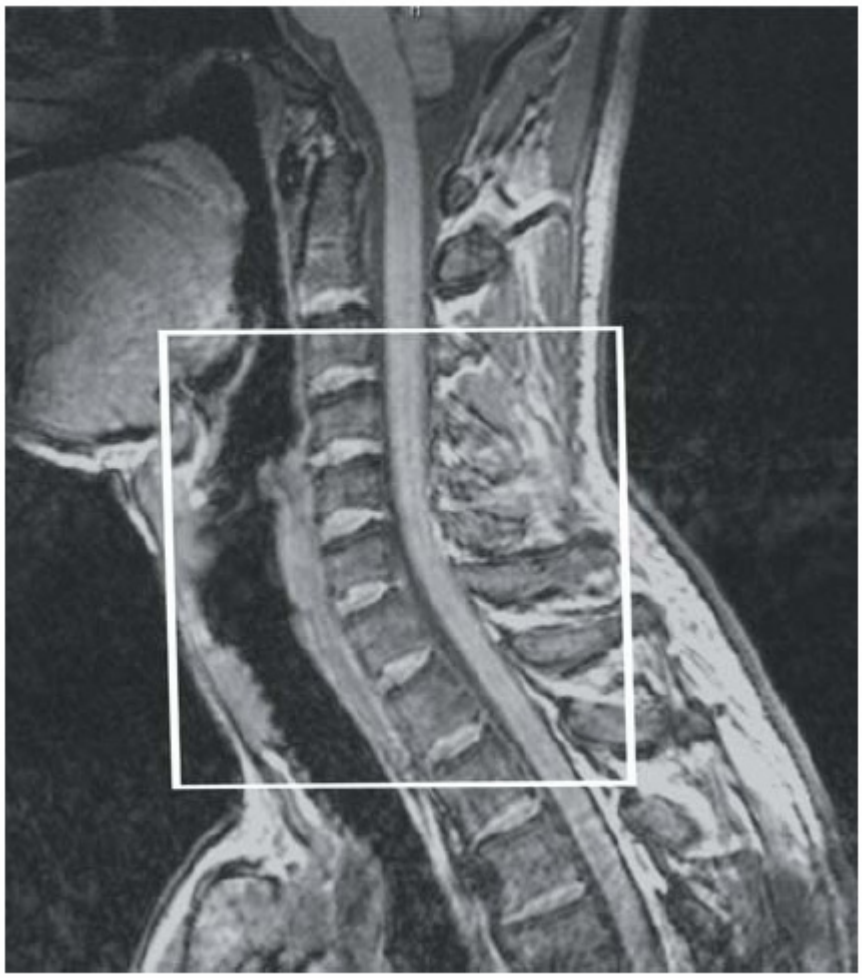


Figure 2

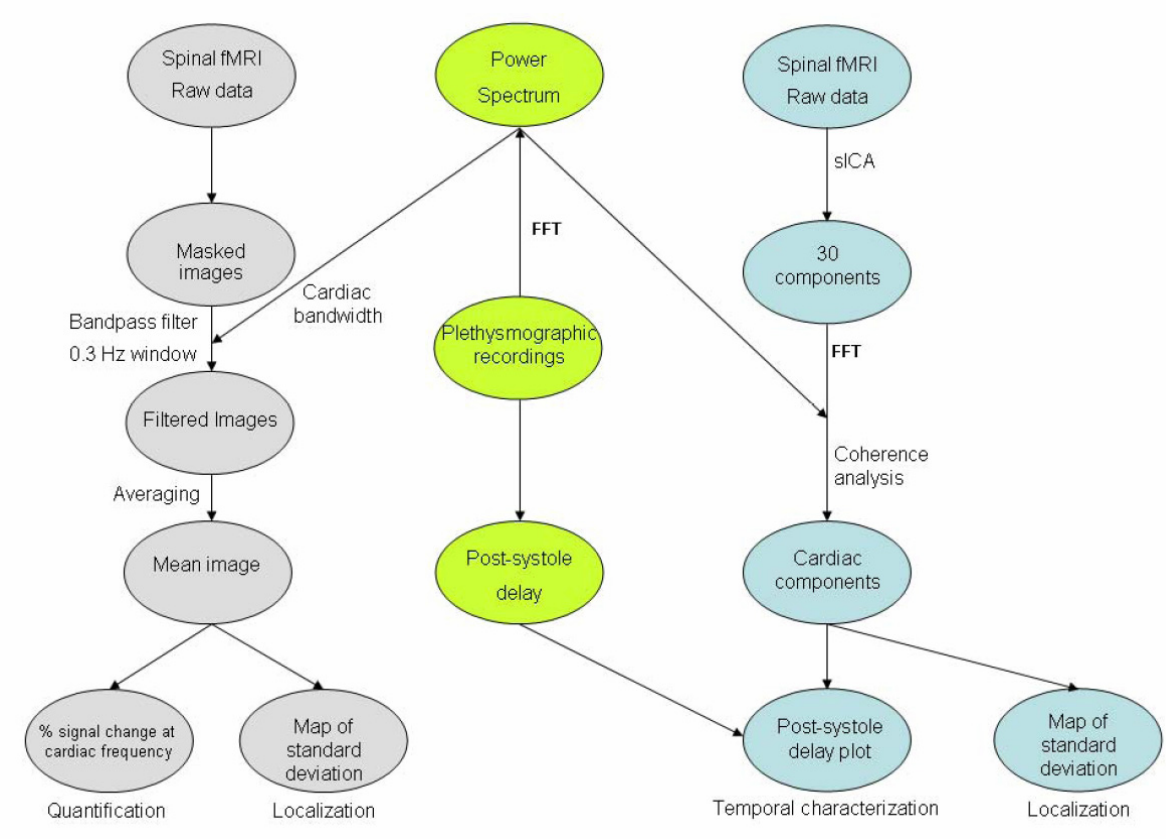


Figure 3

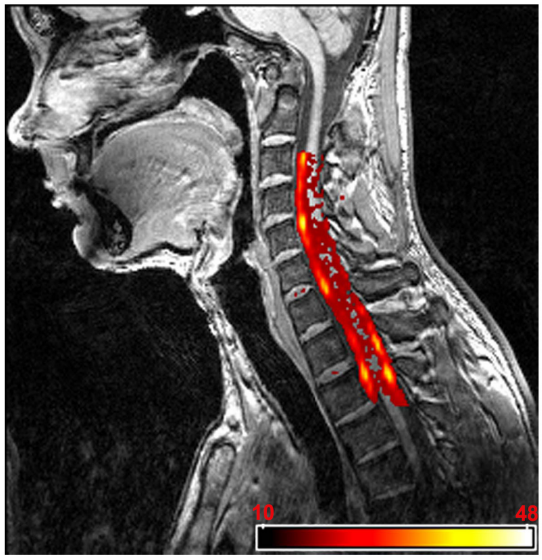


Figure 4

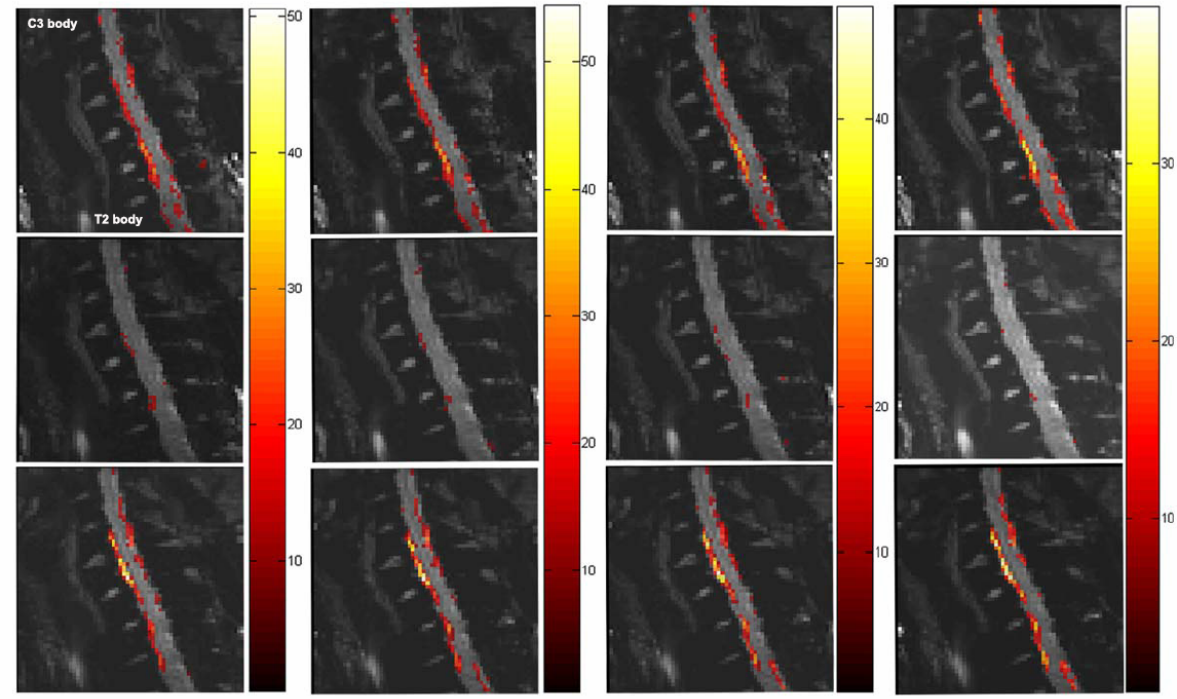


Figure 5

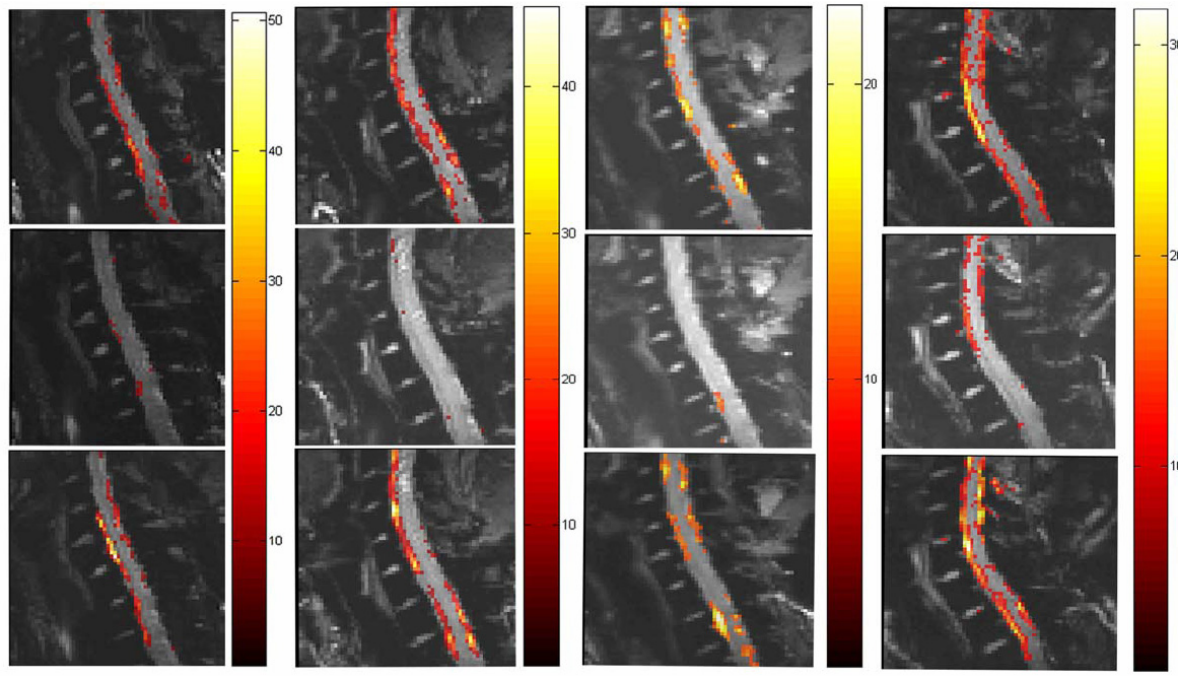


Figure 6

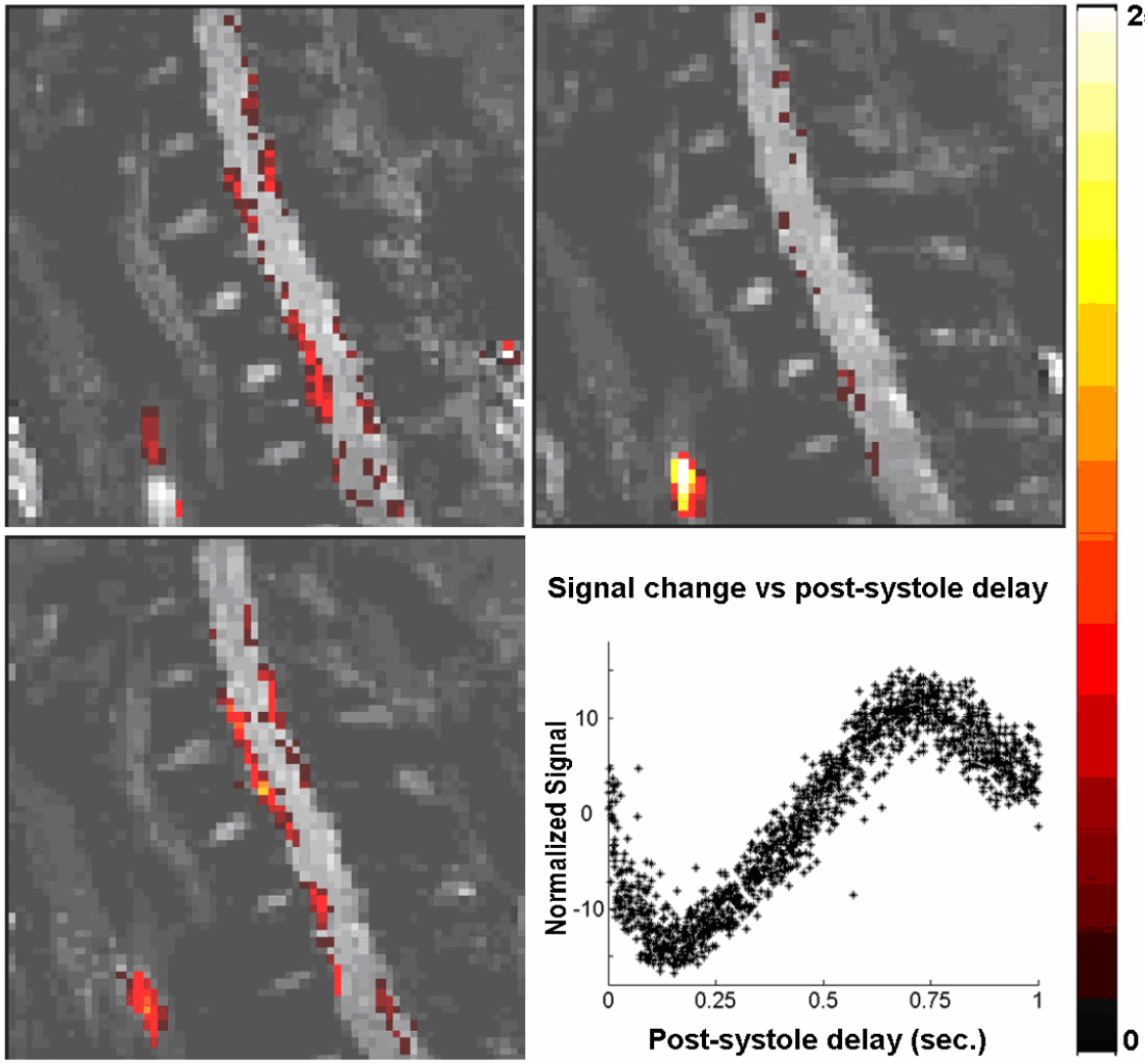
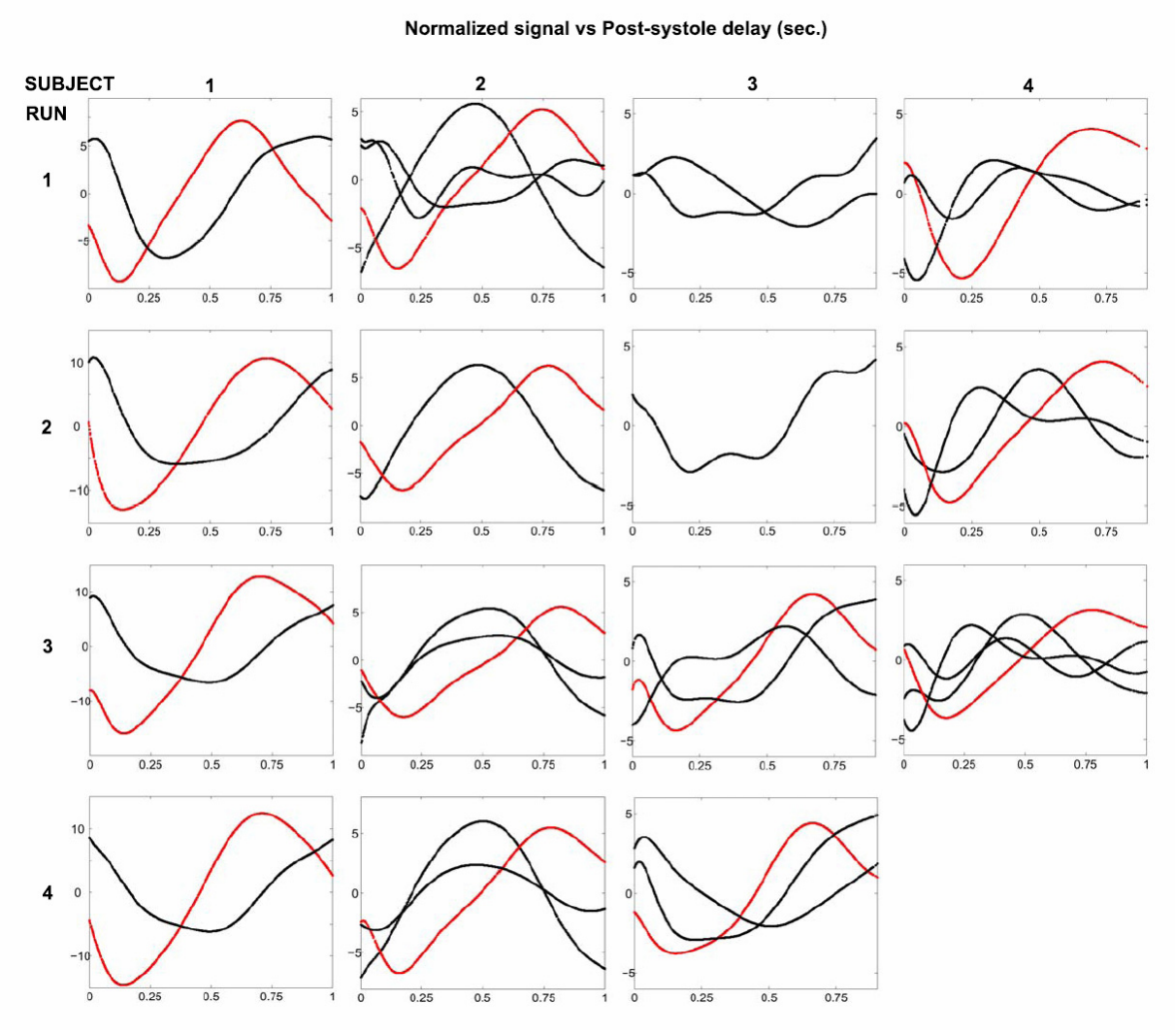

Signal change vs post-systole delay
Normalized Signal
10
0
-10
0
0.25
0.5
0.75
1
Post-systole delay (sec.)
0

Figure 7


Normalized signal vs Post-systole delay (sec.)
SUBJECT
RUN
1
2
3
4

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