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Influence du sommeil sur l'analgésie placebo

par
Danièle Laverdure-Dupont

Département de Physiologie
Faculté de Médecine

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Présentée par :
Danièle Laverdure-Dupont

a été évaluée par un jury composé des personnes suivantes :

Vincent Castellucci
président-rapporteur

Gilles Lavigne
directeur de recherche

Pierre Rainville et Jacques Montplaisir
codirecteur de recherche

Julie Carrier
membre de jury

Gary Rollman
examinateur externe

Résumé

La douleur est une expérience subjective multidimensionnelle pouvant être modulée par plusieurs facteurs cognitifs. L'impact des attentes liées à une expérience douloureuse imminente a été largement étudié dans un contexte d'analgésie placebo et il a été suggéré que la présence d'attentes conscientes de soulagement est nécessaire à la production d'une réduction de douleur. Par ailleurs, certaines études cliniques ont observé une amélioration thérapeutique après l'administration d'un placebo, même lors du sommeil, ce qui suggère qu'un effet placebo peut être rencontré même en absence d'attentes explicites de soulagement. La première étude de cette thèse vise donc à examiner si une réduction de douleur, ainsi qu'une diminution des perturbations du sommeil associées à des stimulations nociceptives expérimentales, peuvent être rencontrées suite à l'induction d'attentes de soulagement nocturne. Les résultats démontrent qu'une réduction de douleur et des perturbations du sommeil a effectivement été rapportée rétrospectivement suite à l'application d'un placebo, De plus, le traitement placebo semble moduler la réactivité à la douleur expérimentale durant le sommeil, en fonction des stades de sommeil dans lesquelles les stimulations sont présentées.

Bien que le développement d'une analgésie placebo repose sur la génération d'attentes de soulagement, il semble que l'exposition préalable à un traitement efficace augmente l'ampleur de l'effet, ce qui suggère que des phénomènes d'apprentissage associatif puissent également être impliqués dans la genèse de ces effets. Comme un rôle du sommeil à été montré dans l'apprentissage et la

mémorisation de plusieurs aptitudes, l'objectif de la seconde étude était d'examiner la possibilité que la présence d'un épisode de sommeil entre l'induction et l'évaluation d'un effet placebo puisse renforcer l'intégration des attentes et par conséquent, favoriser la production d'effets dépendants des attentes. Les résultats ont effectivement montré que le sommeil augmente l'association entre les attentes et le soulagement, et que celles-ci semblent liées à la durée relative de sommeil REM mesurée suite à l'induction. Dans l'ensemble cette thèse démontre que le sommeil peut influencer la production d'une analgésie placebo, et ce, à plusieurs niveaux.

Mots clés : Analgésie placebo, sommeil, attentes, sommeil REM, conditionnement.

Abstract

Pain is a multidimensional experience which can be modulated by many cognitive factors. The impact of expectations associated with an impending painful experience was largely studied in the context of placebo analgesia, and it was suggested that the presence of conscious relief expectation was necessary for the production of pain reduction. On the other hand, some clinical studies have observed therapeutic improvements following the administration of a placebo, even during sleep, which suggest that a placebo effect can be seen even in the absence of explicit relief expectation. The first study of this thesis aims at examining if a pain reduction, as well as a decrease in sleep disturbances associated with the experimental pain stimuli, can be seen following the induction of night-time expectations. The results indeed showed a significant reduction in pain and associated sleep disturbances evaluated retrospectively following the application of a placebo. In addition, placebo treatment appears to modulate responses to experimental pain during sleep, in a manner dependent of the sleep stage in which they are presented.

Although the development of placebo analgesia relies on the production of relief expectations, previous exposure to efficient treatment appears to augment the magnitude of the effect. This suggests that associative learning processes might also be implicated in the genesis of these effects. As a role of sleep was shown in the learning of different aptitudes, the objective of the second study was to examine whether the presence of a sleep episode between the placebo induction and the evaluation of the effect can reinforce the integration of expectations and

consequently, favour the production of expectation-dependent effects. The results show that sleep increases the association between expectation and relief, and that the level of expectation appears to be linked to the relative duration of REM sleep measured after the induction. Globally, this thesis demonstrates that sleep can influence placebo analgesia at many levels.

Key words : Placebo analgesia, sleep, expectation, REM sleep, conditioning.

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ACC	Anterior cingulate cortex – Cortex cingulaire antérieur
ANOVA	Analyse de variance
BAI	Beck Anxiety Inventory
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram
INT	Intensity - Intensité
IRMf	Imagerie par résonance magnétique fonctionnelle
KSS	Karolinska sleepiness scale
LOT-R	Life Orientation Test – Revised
PAG	Periaqueducale gray matter – substance grise périaqueducale
PCS	Pain catastrophizing scale
PFC	Prefrontal cortex – Cortex préfrontal
PVT	Psychomotor vigilance task
REM	Rapid eye movement
RVM	Rostral ventromedial medulla – moëlle rostrale ventromédiale
SEM	Standard error of mean – Erreur standard de la moyenne
SWS	Slow wave sleep – sommeil à ondes lentes
TEP	Topographie par émission de positrons
UNP	Unpleasantness – Désagrément
VAS	Visual analogue scale – Échelle visuelle analogue

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INTRODUCTION

INTRODUCTION

Dans la présente section, les substrats psychophysiques et neuroanatomiques de l'expérience douloureuse ainsi que les principaux mécanismes cognitifs de modulation seront présentés. La section suivante portera spécifiquement sur la modulation de la perception douloureuse suite à l'administration d'un placebo, avec une attention particulière aux mécanismes psychologiques sous-jacents. Les effecteurs neurochimiques et neuroanatomiques de l'analgésie placebo ainsi que certains protocoles d'induction seront également discutés. En troisième lieu, les caractéristiques générales du sommeil seront décrites, avec une emphase particulière sur les fonctions cognitives lui étant attribué. Finalement, les objectifs et hypothèses de la présente thèse seront exposés.

La douleur

Selon l'Association Internationale pour l'Étude de la Douleur (IASP), la douleur est décrite comme étant une expérience sensorielle et émotionnelle désagréable, associée à une lésion tissulaire réelle ou potentielle (Merskey 1986). Contrairement à la nociception, faisant référence aux mécanismes de transmission et d'intégration des stimuli nociceptifs, la douleur peut être décrite dans plusieurs dimensions. La composante sensoridiscriminative de la douleur est liée à certaines propriétés physiques telles la qualité de la sensation, son intensité et sa localisation. La composante motivoaffective reflète l'ensemble des réactions émotionnelles résultant de l'aspect aversif et désagréable de la douleur, et incite une réponse

comportementale afin de mettre fin au stimulus qui cause cette sensation. De plus, la composante cognitive de la sensation douloureuse permet son évaluation et sa réinterprétation à travers les expériences individuelles passées. En contextes cliniques et expérimentaux, il est possible de quantifier subjectivement l'expérience douloureuse à travers ses composantes sensorielles et affectives par l'utilisation d'échelles visuelles analogues validées (VAS) (Price et al. 1983). Ces mesures psychophysiques proportionnelles simples fournissent des évaluations fiables permettant de comparer les niveaux de douleur à travers divers groupes de sujets ou de patients ainsi que les niveaux de douleur recueillis d'un seul individu.

L'expérience douloureuse peut également être décrite grâce aux techniques d'imagerie cérébrale, telles la tomographie par émission de positrons (TEP) et l'imagerie par résonnance magnétique fonctionnelle (IRMf). Ces méthodes reposent sur l'utilisation de marqueurs du niveau d'activité neuronal issus des variations régionales du flux sanguin et du taux d'oxygénation cérébral. Suite à l'administration de stimuli nociceptifs, les réponses hémodynamiques enregistrées par ces techniques ont permis d'identifier certaines régions cérébrales dont l'activité est couramment associée à la perception de douleur. Les cortex somatosensoriel primaire et secondaire, les régions insulaires, le cortex cingulaire antérieur (ACC), le thalamus ainsi que le cortex préfrontal (PFC) représentent les régions les plus souvent activées durant le traitement de l'information douloureuse (Peyron et al. 2000; Apkarian et al. 2005). Bien que la plupart de ces régions participent à plusieurs aspects de la douleur, certaines structures sont préférentiellement impliquées dans diverses composantes de l'expérience douloureuse. En outre, le système latéral, composé des cortex

somatosensoriels primaire et secondaire traitent préférentiellement la dimension sensoridiscriminative (Bushnell et al. 1999; Coghill et al. 1999; Forss et al. 2005), alors que le système médian, incluant l'ACC, semble plutôt impliqué dans la dimension affective (Rainville et al. 1997; Tolle et al. 1999). En effet, l'activation des portions centrales et postérieures de l'ACC a montré une relation significative positive avec les évaluations du désagrément de la douleur. De plus, les régions centrales de l'ACC semblent également impliquées dans le traitement de la dimension cognitive de l'expérience douloureuse, en particulier celle associée aux processus attentionnels soutenus et transitoires (Peyron et al. 1999; Tolle et al. 1999). De son côté, l'insula est généralement impliquée dans l'intégration somatosensorielle d'une vaste gamme de stimuli (Coghill et al. 1994; Mauguiere et al. 1997; Iadarola et al. 1998; Baron et al. 1999; Frot and Mauguiere 1999; Small et al. 1999), mais semble présenter un rôle spécifique dans l'encodage de l'intensité de stimuli thermiques douloureux (Coghill et al. 1999; Peyron et al. 1999). Pour sa part, le PFC, dont les fonctions attentionnelles et exécutives sont bien connues, collabore sans doute à la réévaluation cognitive secondaire de la douleur et de ses conséquences ainsi qu'à la planification de réponses comportementales (Peyron et al. 2000; Price 2000).

La modulation de la douleur

En parallèle aux principales voies ascendantes qui acheminent les influx nociceptifs de la corne dorsale de la moelle épinière vers le thalamus (voie spinothalamique), le tronc cérébral (voie spinoréticulaire) et le mésencéphale (voie

spinomésencéphalique), un système descendant de modulation permet la régulation de la perception douloureuse (Willis and Westlund 1997; Fields 2000; Tracey and Mantyh 2007). L'une des voies de modulation les mieux connues comprend le réseau décrit entre la substance grise périaqueducale (PAG), la moëlle rostrale ventromédiale (RVM) et la moëlle épinière. La stimulation électrique ainsi que l'action locale d'opioïdes au niveau de l'une ou l'autre de ces régions du tronc cérébral déclenchent une profonde analgésie par l'inhibition des neurones de projection nociceptifs au niveau spinal (Depaulis et al. 1987; Oliveras and Besson 1988; Heinricher et al. 1992; Heinricher et al. 1994; Vaughan and Christie 1997; Vaughan et al. 1997). Puisque que la PAG ainsi que la RVM contiennent des neurones excitateurs et inhibiteurs, l'activation de ce système peut conduire, selon les circonstances, à une facilitation ou à une inhibition de la sensation douloureuse (Tracey and Mantyh 2007; Morgan et al. 2008).

Plusieurs régions corticales et sous-corticales, telles les lobes frontaux, l'ACC, l'insula, l'amygdale et l'hypothalamus convergent vers le réseau PAG-RVM-moëlle épinière et influencent la perception douloureuse (Tracey and Mantyh 2007). Une étude récente a effectivement confirmé la présence de connections anatomiques reliant plusieurs structures frontales et limbiques à la PAG du tronc cérébral (Hadjipavlou et al. 2006). Ainsi, il semble que la modulation de la douleur par certains facteurs attentionnels, émotionnels et cognitifs puisse provenir du cortex frontal et du système limbique pour ensuite agir sur les structures effectrices du tronc cérébral (Fields 2000). Par exemple, grâce à l'utilisation de paradigmes de distraction, Valet et collaborateurs ont démontré l'influence des régions fronto-

cingulaires sur la modulation attentionnelle de la douleur par l'entremise d'une action sur la PAG (Valet et al. 2004). De manière similaire, la modulation expérimentale de la douleur par les émotions semble impliquer, entre autres, l'ACC, l'insula et l'amygdale et conduire à une modification de l'activité de la PAG (Wiech and Tracey 2009). De plus, suite à l'induction d'une analgésie associée à l'administration d'un agent opioïdergique ou d'un placebo, une corrélation significative a été démontrée entre l'activation de l'ACC et du tronc cérébral (Petrovic et al. 2002). Une relation similaire fut aussi établie durant la phase anticipatoire de la douleur entre l'activité du PFC et de la PAG ainsi qu'avec les évaluations subjectives d'analgésie (Wager et al. 2004).

En activant ces mécanismes endogènes de régulation, plusieurs facteurs cognitifs peuvent moduler l'expérience subjective de la douleur. De façon générale, le détournement de l'attention du stimulus douloureux conduit à une réduction de l'intensité et du désagrément de la douleur perçue (Miron et al. 1989; Bushnell et al. 1999; Villemure and Bushnell 2002). Quoiqu'il soit parfois difficile de départager les effets émotionnels des effets attentionnels, la manipulation de l'humeur ou de l'état émotionnel par des stimuli plaisants engendre le plus souvent une réduction de l'expérience douloureuse (Cogan et al. 1987; Zelman et al. 1991; de Wied and Verbaten 2001; Meagher et al. 2001). Suite à une exposition à des éléments à valence négative, l'effet contraire est aussi noté, mais de façon moins constante (Zelman et al. 1991; Weisenberg et al. 1998; de Wied and Verbaten 2001; Meagher et al. 2001). Contrairement aux effets liées à la modulation de la douleur par l'attention, il semble que les manipulations destinées à l'altération de l'état émotionnel affectent

sélectivement la dimension affective de la douleur (Zelman et al. 1991; Villemure and Bushnell 2002). Le niveau d'anxiété entretenu par un individu est aussi reconnu pour son effet d'amplification de la douleur (Weisenberg et al. 1984; al Absi and Rokke 1991; Rhudy and Meagher 2000), et cette relation est aussi vraie durant la période d'anticipation de la douleur, lorsque les attentes sont associées à une incertitude quant à la nature de l'évènement anticipé (Ploghaus et al. 2001; Ploghaus et al. 2003). Dans cette condition, des attentes incertaines de douleur vont engendrer un état anxieux qui aura pour effet d'augmenter la sensibilité à la douleur. À l'opposé, lorsque l'évènement aversif est anticipé de manière certaine, l'état émotionnel engendré s'apparente à la peur, laquelle entraine généralement une hypoalgésie (Blanchard et al. 1993; Rhudy and Meagher 2000; Ploghaus et al. 2003).

L'analgésie placebo

Les effets placebo sont rencontrés lorsqu'un changement physiologique ou psychologique positif survient suite à l'administration d'une substance inerte ou d'une procédure simulée. Quant à elle, l'analgésie placebo représente spécifiquement une diminution de douleur en réponse à un traitement inerte. Puisque les placebos ne contiennent pas d'éléments actifs proprement dit, leur effet est généralement considéré comme non-spécifique. Par ailleurs, les effets placebo peuvent engendrer l'amélioration d'une multitude de symptômes en fonction des divers mécanismes sous-jacents et présentent vraisemblablement des effets spécifiques. En plus d'être rencontrés suite à l'administration d'un agent inerte, les effets placebo peuvent également contribuer aux effets thérapeutiques associés à une multitude de

traitements actifs. Il semble donc qu'une partie de l'amélioration des symptômes suite à l'administration d'un agent actif puisse dépendre de l'effet placebo. Ce concept fut étudié grâce à l'utilisation d'un paradigme dans lequel le même traitement pharmacologique est administré à un individu à sa vue, par un professionnel de santé ou à son insu, par une pompe intraveineuse contrôlée par un ordinateur (Gracely et al. 1983; Price 2001; Colloca et al. 2004). La comparaison de l'ampleur de l'effet thérapeutique dans ces circonstances différentes permet vraisemblablement d'évaluer la part de l'effet liée spécifiquement à la conscience du sujet du moment de l'administration du traitement. Conceptuellement, ces observations suggèrent que l'étude de l'effet placebo repose principalement sur le contexte psychosocial associé à l'administration d'un agent thérapeutique actif ou non, et que le développement de ce contexte repose sur divers facteurs associés, entre autres, à l'environnement thérapeutique, aux praticiens et aux modes d'administration.

La présence de plusieurs facteurs confondants doit être prise en compte afin de permettre l'identification des réponses placebo réelles. L'un d'eux est associé à la fluctuation spontanée des symptômes pouvant être observée en absence de toute intervention. Également, l'effet de régression à la moyenne est un concept statistique par lequel les évaluations élevées des symptômes ont tendance à redescendre spontanément vers les valeurs moyennes. La variation spontanée de l'intensité des symptômes peut être contrôlée en comparant ces changements à ceux observés dans un groupe n'ayant subit aucune intervention, et qui s'apparente à l'histoire naturelle de la condition. En connaissant ainsi le décours de base de l'intensité des symptômes,

il devient alors possible d'identifier les variations qui sont liées spécifiquement à l'effet placebo. D'autres facteurs peuvent biaiser l'identification des effets thérapeutiques, particulièrement lorsque l'évaluation des symptômes repose principalement sur des mesures subjectives. Dans ce cas, il arrive parfois que l'estimation des symptômes soit influencée par le désir du patient à satisfaire les attentes éventuelles du thérapeute. De plus, la difficulté d'évaluer correctement certains symptômes, par l'introduction de distorsions mnésiques par exemple, peut également influencer l'ampleur de l'effet thérapeutique mesuré. Ceci a été en effet rencontré lors de l'estimation de l'analgésie placebo en contexte expérimental, alors que l'effet analgésique basé sur les mesures rétrospectives de douleur était supérieur à celui calculé à partir des valeurs concurrentes (Price et al. 1999).

Mécanismes psychologiques de l'analgésie placebo

Plusieurs facteurs cognitifs et émotionnels ont été proposés comme médiateurs des effets placebo en général et de l'analgésie placebo en particulier. Notamment, le développement d'attentes de soulagement suite à l'administration d'un traitement a été fréquemment associé à l'analgésie placebo. Une des premières études à démontrer une corrélation significative entre l'anticipation de réduction de douleur et l'analgésie effectivement perçue a été menée par Montgomery et Kirsch (Montgomery and Kirsch 1997). Dans cette étude, une douleur aigüe était induite par l'administration d'un courant électrique au niveau du bras de volontaires sains. Après l'identification de l'intensité de stimulation nécessaire à l'obtention d'un niveau stable de douleur, les sujets étaient soumis à une manipulation expérimentale dans

laquelle l'intensité des stimulations était subrepticement réduite suite à l'application d'une crème topique décrite comme analgésique. Avant l'administration des essais suivants, les sujets devaient estimer prospectivement le niveau de douleur anticipé. Lors du bloc d'essais subséquent, une réduction significative de douleur a été mesurée, malgré le rétablissement de l'intensité des stimulations au niveau de base initial. De plus, cette réduction de douleur perçue lors des stimulations était directement proportionnelle au niveau de douleur attendue. Suite à cette démonstration, plusieurs études ont subséquemment confirmé cette relation et ont proposé un rôle causal du niveau d'attente sur l'analgésie placebo (Amanzio and Benedetti 1999; Price et al. 1999; Benedetti et al. 2003; Wager et al. 2004; Scott et al. 2007).

Le conditionnement classique, basé sur l'association répétée d'indices contextuels et de changements du niveau de douleur, est un autre facteur fréquemment mis de l'avant pour expliquer les réponses analgésiques placebo. En effet, suite à une exposition à un traitement efficace, la ré-exposition ultérieure à un agent neutre empruntant les mêmes caractéristiques physiques peut déclencher une réponse thérapeutique conditionnée. Plusieurs études intéressées à l'analgésie placebo ont effectivement isolé la contribution des processus de conditionnement et ont noté qu'une exposition préalable à un agent analgésique efficace engendrait une réponse placebo supérieure à celle observée suite à une induction par de simples suggestions verbales de soulagement (Amanzio and Benedetti 1999; Benedetti et al. 2003; Colloca and Benedetti 2006; Colloca et al. 2008). Par ailleurs, comme l'administration de suggestions verbales opposées est suffisante pour renverser les

effets liés au conditionnement sensoriel, il semble que ceux-ci soient dépendants de la génération d'attentes de soulagement (Montgomery and Kirsch 1997; Benedetti et al. 2003). Ainsi, la pré-exposition à un traitement efficace semble amplifier les effets analgésiques placebo indirectement, par l'entremise d'un renforcement explicit des attentes.

Certaines variations de l'état émotionnel peuvent aussi participer au développement des réponses placebo. Particulièrement en contexte clinique, le désir de soulagement semble agir de concert avec les attentes de soulagement pour engendrer une amélioration thérapeutique (Vase et al. 2003; 2005). Ainsi, selon le modèle de Price et Barrell, un désir de soulagement élevé dans un contexte de traitement de douleur serait associé à une myriade d'émotions négatives (par exemple la rumination pouvant entrainer un effet inverse, soit une hyperalgésie), et par conséquent, une réduction du désir de soulagement pourrait contribuer à l'analgésie placebo par une diminution de l'intensité de ces émotions (Price and Barrell 1984). Par contre, en contexte expérimental, alors que la motivation de soulagement est relativement faible, l'impact du désir de soulagement sur l'analgésie placebo semble significativement réduite (Price et al. 1999).

Le niveau d'anxiété des sujets est une autre composante émotionnelle pouvant avoir un effet sur la perception douloureuse suite à l'administration d'un placebo. En théorie, celle-ci pourrait engendrer une réduction d'anxiété, laquelle serait ensuite associée à une diminution de la dimension affective de la douleur (Benedetti and Amanzio 1997). Dans certains cas, une diminution d'anxiété situationnelle associée à la réponse placebo a effectivement été enregistrée (Morton et al. 2009; Morton et al.

2010), mais il est difficile d'établir un lien de causalité entre ces deux phénomènes. D'autres parts, suite à l'induction d'une réponse émotionnelle placebo par la création d'attentes de réduction d'anxiété et un pré-conditionnement avec un agent anxiolytique, une étude d'imagerie fonctionnelle a permit d'observer la similitude des réseaux d'activation d'une réponse placebo émotionnelle et analgésique (Petrovic et al. 2002; Petrovic et al. 2005). Ces résultats suggèrent donc que l'analgésie placebo peut reposer sur l'activation de processus généraux de modulation émotionnelle également impliqués dans la régulation des états anxieux.

Mécanismes neurophysiologiques de l'analgésie placebo

Au niveau neuroanatomique, des études d'imagerie cérébrale ont permit d'identifier certaines régions corticales activées suite à l'induction d'une analgésie placebo. Parmi les plus connues, les études de Wager ont montré, suite à l'administration d'un placebo, une réduction de l'activité de certaines régions impliquées dans le traitement de l'information douloureuse, dont le cortex cingulaire antérieur, l'insula et le thalamus, et ce, de manière proportionnelle à la réduction subjective de douleur (Wager et al. 2004). Des résultats similaires montrant une diminution de l'activité du thalamus, des cortex insulaires et somatosensoriels secondaires ont aussi été notés suite à l'induction d'un effet placebo dans une cohorte de patients atteints de douleur chronique (Price et al. 2007). Durant la phase anticipatoire de la douleur, le groupe de Wager a également démontré un rapprochement entre l'activité des cortex préfrontal dorsolatéral et orbitofrontal et le niveau d'attente de soulagement. En plus d'être corrélée avec l'ampleur de

l'analgésie placebo, l'activité de ces régions était également associée à l'activation d'une région importante impliquée dans la modulation de la douleur, la substance grise périaqueducale. En fait, l'augmentation de la connectivité fonctionnelle entre l'ACC et la PAG mésencéphalique suite à l'administration d'un placebo représente l'un des résultats les plus constamment rapportés, suggérant fortement le recrutement de mécanismes inhibiteurs descendants lors de l'analgésie placebo (Petrovic et al. 2002; Wager et al. 2004; Bingel et al. 2006; Wager et al. 2007).

Au niveau moléculaire, l'implication du système opioïdergique endogène a depuis longtemps été proposée. En 1978, Levine et collaborateurs ont montré qu'une réduction de douleur post-opératoire pouvait être engendrée suite à un traitement placebo couplé à des suggestions verbales d'analgésie, et que celle-ci pouvait être complètement bloquée par l'administration d'un antagoniste des récepteurs opioïdes, le naloxone (Levine et al. 1978). Plusieurs études ont subséquemment confirmé ces observations (Benedetti 1996; Amanzio and Benedetti 1999; Eippert et al. 2009). De plus, des résultats d'imagerie moléculaire permettant de mesurer la disponibilité des récepteurs opioïdes mu *in vivo* ont fournit une démonstration directe de la libération endogène d'endorphines. L'activation localisée du système opioïdergique associée à l'analgésie placebo a été observée au niveau de la partie rostrale de l'ACC, du cortex préfrontal dorsolatéral, de l'insula, du noyau accumbens, de l'amygdale ainsi que de la PAG (Zubieta et al. 2005; Wager et al. 2007). Par ailleurs, le développement d'une analgésie placebo peut aussi, dans certains cas, reposer sur des mécanismes non-opioïdergiques. Par exemple, lorsqu'une analgésie placebo est induite suite à un pré-conditionnement avec un agent anti-inflammatoire non-stéroïdien, celle-ci est

insensible à une application de naloxone (Amanzio and Benedetti 1999). De plus, il est important de noter l'implication éventuelle du système dopaminergique, puisqu'il a été montré que la libération de dopamine au niveau du noyau accumbens était positivement corrélée avec les attentes d'analgésie et était prédictive du niveau d'analgésie perçue ainsi que de l'ampleur de la libération endogène d'opioïdes au niveau de plusieurs régions impliquées dans la modulation de la douleur (Scott et al. 2007; 2008). Ces résultats suggèrent que l'activation des circuits de récompense en réponse à l'attente de soulagement pourrait entrainer l'induction de réponses effectrices représentées par le recrutement du système opioïdergique.

Protocole d'induction de l'analgésie placebo

La clarification des mécanismes psychologiques associés à la genèse des effets analgésiques placebo a permit de développer des protocoles expérimentaux d'induction efficaces. Plusieurs groupes de recherche ont effectivement utilisé des suggestions verbales d'analgésie standardisées ainsi que des paradigmes de conditionnement sensoriels conduisant à des niveaux significatifs de réduction de douleur (Price et al. 1999; Petrovic et al. 2002; Wager et al. 2004; Bingel et al. 2006; Wager et al. 2007). La méthode d'induction de l'analgésie placebo décrite dans la présente thèse a elle aussi été inspirée de tels modèles. Ainsi, suite à l'application d'une crème topique inerte décrite comme un agent ayant été démontré efficace pour diminuer la douleur lors d'études préliminaires dans d'autres universités (Price et al. 1999), les sujets étaient soumis à une manipulation dans laquelle la température du stimulus thermique administré au niveau du site placebo était subrepticement réduite

comparativement au site control. Afin de s'assurer de l'équivalence des deux conditions, la même crème était aussi appliquée au niveau du site control, mais étant cette fois décrite comme un composé neutre présent uniquement pour contrôler les effets non-spécifiques. Après cette phase de conditionnent, des stimuli thermiques douloureux d'intensité équivalente étaient administrés au niveau des sites control et placebo, et la différence des évaluations de douleurs issues de ceux-ci permettait de quantifier l'ampleur de l'analgésie placebo de chaque sujet. Les résultats, présentés subséquemment, démontrent des effets significatifs de réduction de douleur.

Le sommeil

Le sommeil est un état physiologique hétérogène associé à un niveau de vigilance altéré. Selon ses patrons caractéristiques d'activité cérébrale, de mouvements oculaires et de tonus musculaire, le sommeil peut généralement être divisé en périodes cycliques de sommeil REM (*Rapid Eye Movement*) et non-REM (Rechtschaffen and Kales 1968). Le sommeil non-REM est également subdivisé en stades 1 à 4, avec les stades 3 et 4 collectivement connus sous le terme de sommeil lent profond (SWS; *Slow Wave Sleep*). Le SWS est définit en fonction de la prévalence d'oscillations corticales synchronisées à basse fréquence et du niveau global relativement faible d'activité métabolique cérébral (Maquet et al. 1997). Il est de plus caractérisé par une profonde déconnection du milieu environnant et une inertie de sommeil élevée. À l'opposé, le sommeil REM est associé à une activité neuronale intense représentée par une activité électrique désynchronisée à fréquence rapide, à la présence de saccades oculaires ainsi qu'à une importante hypotonie

musculaire (Maquet et al. 1996). Aussi, les rêves s'apparentent principalement à ce stade. Chez de jeunes sujets en santé, une période normale de sommeil de 7,5 h est généralement composée de 45-55% de stade 2, de 13-23% de SWS et de 20-25% de sommeil REM (Kryger et al. 2005).

Neuroanatomie du sommeil

En plus de présenter d'importantes différences au niveau global, les niveaux locaux d'activité neuronale varient grandement en fonction des stades de sommeil. Ainsi, en combinant des mesures de flux sanguin régional cérébral à des enregistrements polysomnographiques chez des sujets endormis, plusieurs études ont examiné les patrons d'activation localisés spécifiques au sommeil lent profond (SWS) et au sommeil REM (Maquet et al. 1996; Braun et al. 1997; Maquet et al. 1997; Nofzinger et al. 1997; Maquet 2000; Buchsbaum et al. 2001). Lors du SWS, la plupart des régions cérébrales présentent un niveau d'activation moindre que lors de l'éveil, avec une désactivation plus prononcée au niveau des régions pontiques et mésencéphaliques du tronc cérébral, du thalamus, des noyaux gris centraux, des cortex cingulaire antérieur, orbitofrontal et préfrontal ainsi que de l'aspect médian du lobe temporal (Braun et al. 1997; Maquet et al. 1997; Maquet 2000; Buchsbaum et al. 2001). De plus, ce stade de sommeil est caractérisé par une diminution importante de la connectivité fonctionnelle des diverses régions corticales entre-elles ainsi qu'avec le thalamus, ce qui pourrait expliquer l'importante baisse de conscience rencontrée en sommeil lent profond (Massimini et al. 2005). À l'opposé, durant le sommeil REM, des activations métaboliques significatives ont été enregistrées au niveau du

tegmentum pontique, du thalamus, de la formation hippocampique, de l'operculum pariétal ainsi que de plusieurs structures limbiques médianes, dont les cortex cingulaire antérieur et orbitrofrontal médian et les complexes amygdaliens bilatéraux (Maquet et al. 1996; Braun et al. 1997; Nofzinger et al. 1997; Maquet 2000; Buchsbaum et al. 2001). Certaines désactivations ont également été notées durant ce stade, particulièrement au niveau des cortex préfrontal dorsolatéral, pariétal et cingulaire postérieur (Maquet et al. 1996; Maquet 2000). Le caractère émotionnel et hautement associatif des rêves issus de ce stade peut sans doute s'expliquer par le profil d'activation spécifique au sommeil REM.

Fonctions cognitives du sommeil : Apprentissage et mémoire

En établissant une corrélation entre les progrès d'apprentissage et certains paramètres de sommeil mesurés dans la nuit suivant l'entrainement ou par l'utilisation de paradigmes de déprivation totale ou partielle de sommeil, plusieurs études ont démontré un rôle bénéfique du sommeil dans le développement d'aptitudes et la rétention d'informations. Classiquement, plusieurs études se sont intéressées à l'apprentissage de tâches procédurales simples, telle la capacité de discrimination de textures visuelles Ces résultats ont globalement montrés une augmentation de performance dépendante du sommeil et proportionnelle à la quantité de SWS et de sommeil REM mesurée dans la nuit subséquente à la période d'entrainement ainsi qu'une abolition de cette amélioration suite à une déprivation de l'un ou l'autre de ces stades (Karni et al. 1994; Gais et al. 2000; Stickgold et al. 2000a; Stickgold et al. 2000b; Mednick et al. 2003). Les tâches liées à la mémoire

déclarative comme l'apprentissage de paires de mots ont également montré un certain niveau de dépendance au sommeil, particulièrement lorsque celles-ci présentaient une valence émotionnelle (Empson and Clarke 1970; Chernik 1972; Lewin and Glaubman 1975; Meienberg 1977; Plihal and Born 1997; Gais et al. 2002; Gais and Born 2004).

Les tâches cognitives complexes contenant une règle d'exécution implicite, telles la tâche de la tour d'Hanoi ainsi que les tâches arithmétiques probabilistiques, semblent quant à elles, bénéficier d'une période riche en sommeil REM (Conway and Smith 1994; Smith 1995; 1996; Wagner et al. 2004; Rauchs et al. 2005; Stickgold 2005). Il semble effectivement que ce stade de sommeil soit particulièrement impliqué dans l'intégration de nouvelles informations à l'intérieur d'un réseau existant d'éléments sémantiquement liés, ce qui favoriserait l'identification de principes généraux pouvant être appliqués aux évènements futurs. La nature hyper-associative du sommeil REM a aussi été mise en évidence lors d'études montrant une capacité accrue à la résolution d'anagrammes ainsi qu'à l'utilisation d'amorces immédiatement suite à un réveil en sommeil REM comparativement aux autres stades (Stickgold et al. 1999; Walker et al. 2002; Cai et al. 2009). Il semble ainsi que par son profil étendu d'activation corticale, le sommeil REM pourrait faciliter l'intégration d'éléments cognitifs complexes afin de faciliter leur interprétation dans des contextes nouveaux. De plus, l'une des hypothèses pouvant expliquer cette intégration associative dépendante du sommeil, repose sur la réactivation, lors des périodes subséquentes de sommeil, des réseaux neuronaux activés pendant l'encodage. Effectivement, il a été suggéré que la réactivation corticale des épisodes

récents d'éveil pouvait favoriser le renforcement des connections synaptiques à l'intérieur de la nouvelle représentation mnésique ou avec des représentations anciennes (Maquet et al. 2000; Peigneux et al. 2003; Peigneux et al. 2004; Stickgold 2007). En plus de permettre la stabilisation des traces mnésiques, cette réactivation pourrait engendrer une restructuration conduisant à l'intégration des nouveaux engrammes à l'intérieur de réseaux associés.

Traitement de l'information sensorielle durant le sommeil

En plus de réactiver l'information recueillie à l'éveil, le cerveau peut également traiter les afférences sensorielles durant le sommeil. Par exemple, la capacité de détecter un stimulus auditif présenté durant le sommeil à été démontrée par l'enregistrement de potentiels évoqués (Cote et al. 2001; Takahara et al. 2006). Certaines études ont aussi montré qu'en plus d'une simple détection, certains facteurs, tels le niveau d'attention ou la valence émotionnelle, pouvaient modifier le traitement de certaines afférences sensorielles même durant le sommeil (Langford et al. 1974; Portas et al. 2000; Takahara et al. 2006). Par exemple, il a été observé que l'ampleur des potentiels évoqués par des stimuli auditifs déviants pendant le sommeil pouvait être augmentée, lorsqu'avant l'endormissement, il était demandé aux sujets de discriminer l'intensité des stimuli (Takahara et al. 2006). Ces effets n'étaient observés que lors de la période de sommeil REM. Bien que la capacité de discriminer la déviance de stimuli auditifs en sommeil REM a également été observée dans d'autres études (Bastuji et al. 1995; Pratt et al. 1999; Cote et al. 2001), il semble que la possibilité d'identifier les éléments auditif saliens puisse également se retrouver en

SWS (Portas et al. 2000). L'ensemble de ces résultats suggère donc que certains mécanismes aptes à moduler les afférences sensorielles demeurent effectifs durant le sommeil.

Certaines études se sont également penchées sur le traitement des afférences somatosensorielles durant le sommeil (Lavigne et al. 2000; Bentley et al. 2003; Lavigne et al. 2004; Daya and Bentley 2009). De façon générale, l'intensité de stimulation requise pour engendrer un éveil en SWS et en sommeil REM est plus élevée qu'en stade 2. Ainsi, suite à une stimulation nociceptive engendrée par une infusion intramusculaire de salin hypertonique ou par une stimulation thermique aigue, une réaction d'éveil, caractérisée par un changement abrupt du tracé encéphalographique de plus de 10 secondes, est engendrée dans environ 40 à 50 % des cas lors d'une stimulation en stade 2, dans environ 20 à 30 % des cas lors d'une stimulation en SWS, et dans environ 30 à 45 % des cas lors d'une stimulation en sommeil REM (Lavigne et al. 2000; Lavigne et al. 2004). À notre connaissance, aucune étude n'a été spécifiquement conduite afin d'examiner si la modulation de la perception douloureuse par des facteurs cognitifs était possible durant le sommeil.

Effets placebo rencontrés pendant le sommeil

Bien que la production d'un effet placebo semble souvent reposer sur la génération d'attentes conscientes de soulagement, l'amélioration significative de symptômes cliniques a déjà été observée durant le sommeil (Ziegler et al. 2001; Breuer et al. 2006; Partinen et al. 2006). Certaines études examinant l'effet de traitements contre la douleur, ont effectivement observé des effets bénéfiques sur les

variables associées aux perturbations de sommeil liées à la douleur ainsi que sur d'autres mesures de la qualité du sommeil dans le groupe placebo (Dogra et al. 2005; Richter et al. 2005; Breuer et al. 2006; Gabis et al. 2009). Par contre, comme ces améliorations étaient établies à partir d'une comparaison avec les mesures de références observées avant l'administration du traitement, et non en rapport avec un groupe illustrant l'histoire naturelle de la condition et servant à contrôler la fluctuation spontanée des symptômes, il est difficile de confirmer la présence d'un effet placebo réel. Par conséquent, la possibilité qu'une réponse analgésique placebo puisse être produire chez des sujets endormis reste à confirmer.

Objectifs et présentation des études expérimentales

Le premier objectif de la présente thèse, illustré dans la première étude expérimentale, est donc de déterminer si la conscience, associée à l'état d'éveil d'un individu, est nécessaire à la production d'une analgésie placebo. Ainsi, suite à la génération d'attentes explicites de réduction de douleur aigue par l'application d'une crème topique, l'ampleur des perturbations du sommeil engendrées par des stimulations nociceptives nocturnes à été comparée avec celle engendrée lors d'une nuit contrôle, en absence d'attentes de soulagement. Une diminution des perturbations nocturnes liées à la douleur expérimentale durant la nuit lors de laquelle le placebo a été appliqué témoignerait de la présence d'une analgésie placebo malgré l'absence de conscience de l'individu.

Le deuxième objectif, associé au deuxième article, consiste à déterminer si la présence d'un épisode de sommeil après l'induction d'attentes de soulagement, contribue à la consolidation de l'analgésie placebo ainsi qu'à l'association entre les attentes et la réduction de douleur. Pour ce faire, une analgésie placebo a été induite en soirée, par des suggestions verbales couplées à un conditionnement sensoriel, et a été testée 12 h plus tard, après un épisode de sommeil. L'ampleur de l'effet ainsi que l'effet médiateur des attentes sur la réponse placebo a par la suite été comparé avec ceux rencontrés chez un groupe en absence de période de sommeil. Puisque la présence d'un épisode de sommeil consolide l'apprentissage d'une variété d'aptitudes, il est possible que celui-ci favorise l'intégration des attentes de soulagement et renforce leur association avec le niveau d'analgésie placebo mesuré.

ARTICLES DE RECHERCHE

Article 1

Can placebo analgesia occur during sleep?

Danièle Laverdure-Dupont[1,2], Pierre Rainville[3], Jacques Montplaisir[2], Gilles Lavigne[2,3]

1. Department of Physiology, Faculty of Medicine, University de Montréal, Montréal, Canada
2. Centre d'étude du Sommeil et des Rythmes Biologiques, Hôpital du Sacré-Cœur, Montréal, Canada
3. Department of Stomatology and Oral Health, Faculty of Dental Medicine, Université de Montréal, Montréal, Canada

Corresponding author:
Danièle Laverdure-Dupont
Centre d'étude du Sommeil et des Rythmes Biologiques
Hôpital du Sacré-Cœur de Montréal
5400 boul. Gouin Ouest
Montréal, Québec, Canada
H4J 1C5
Tel: 514-338-2222, ext. 3745
Fax: 514-338-2694
[redacted]

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ABSTRACT

Placebo analgesia relies at least partly on conscious expectations and thus may require an individual to be awake to allow its generation. This experimental study examined whether placebo effects induced before sleep modulate acute heat pain and associated sleep disturbances.

Methods: Expectations of analgesia were manipulated in 9 healthy volunteers by verbal suggestion and sensory conditioning using a topical cream said to be either analgesic (placebo) or inert (control). After a habituation night, standard polysomnography (EEG, EMG, EOG, heart rate) was used to assess sleep disturbances induced by phasic thermal stimuli applied to the subject's forearm in two nights, immediately following the application of the placebo or the control cream. Expected and experienced nocturnal pain, anxiety and sleep disturbances were assessed using VAS's. Sleep stages and sleep arousal induced by each thermal stimulus (3-10 sec micro-arousal and awakening) were scored by an experienced technician blind to the treatment conditions.

Results and Interpretation: Subjects reported that the nocturnal stimulations induced significantly less pain ($p=0.01$), less sleep disturbance ($p=0.01$), and less anxiety ($p=0.04$) in the placebo night. Consistently, the placebo treatment also produced a significant reduction in arousal induced by the stimulations administered during REM (83% in placebo Vs 93% in control; $p=0.03$). This implies a down-regulation of physiological responses to the noxious input, consistent with the generalization of placebo effects during sleep. However, the placebo treatment was also associated

with *more* arousal induced by the noxious stimuli during slow-wave-sleep (stages 3&4) (89% in placebo Vs 75% in control; p=0.02). This suggests that effects of the noxious sensation may override placebo-induced expectations in these sleep stages. Furthermore, during the control night, expectation of stronger pain was correlated with a higher percentage of stages 3&4 (r=0.74, p=0.02). This suggests that expectation of stronger pain in the control night might have triggered a deepening of sleep as a protective mechanism to maintain SWS continuity.

Conclusions: These results demonstrate that placebo manipulations performed prior to sleep can modulate the responsiveness to nocturnal pain, but that these effects differ according to the sleep stage. We suggest that the pattern of sleep-disturbances reflects the relative influence of sensory inputs and expectation-related top-down processes across sleep stages.

INTRODUCTION

The experience of pain can be modulated by centralized top-down processes related to attention, emotions and cognitive factors (Tracey and Mantyh 2007). In wake individuals, the development of expectations associated with the anticipation of a positive treatment outcome is sufficient to trigger an alleviation of symptoms (Vase et al. 2005; Goffaux et al. 2009; Kong et al. 2009). Placebo analgesia is a very well studied model of expectation-mediated effects which often relies on verbal suggestions of relief and conditioning procedures (Price et al. 1999; Petrovic et al.

2002; Wager et al. 2004; Bingel et al. 2006; Wager et al. 2007b). Although pre-exposure to an effective pain reducing agent can enhance the magnitude of analgesia, opposite verbal suggestion is sufficient to reverse this effect (Benedetti et al. 2003). This implies that conscious expectation takes precedence over unconscious conditioning processes and that pre-conditioning might indirectly promote placebo analgesia by further consolidating positive expectations. Under these assumptions, conscious awareness associated with wake may be required for the development and expression of placebo analgesic responses.

However, clinically significant improvements following the administration of a placebo have been reported during sleep and in sleep-associated pathologies (Ziegler et al. 2001; Breuer et al. 2006; Partinen et al. 2006). In fact, substantial placebo responses reported in clinical trials have warranted the publication of meta-analytic reviews of placebo effects encountered in diseases such as insomnia and restless leg syndrome (McCall et al. 2003; Perlis et al. 2005; Belanger et al. 2007; Fulda and Wetter 2008). In the field of pain, a number of placebo-controlled clinical trials have also observed improvements in the placebo group relative to the pre-treatment baseline in self reports of pain and pain-related sleep interferences or in other subjective measures of sleep quality (Dogra et al. 2005; Richter et al. 2005; Breuer et al. 2006; Gabis et al. 2009). Moreover, a recent clinical study of fibromyalgia patients showed highly significant correlation between reduced pain after placebo treatment and improvements in sleep quality, consistent with the possibility that placebo analgesia may be generalized during sleep (Russell et al.

2009). However, these clinical studies were not designed specifically to assess the effect of a placebo treatment against a no treatment condition so that the inferences on placebo mechanisms are limited.

Although expectation of relief appears to produce positive effects during sleep, no experimental study has yet investigated the development of placebo responses during sleep. In the current study, a well-establish model of placebo induction (Price et al. 1999; Petrovic et al. 2002; Wager et al. 2004; Bingel et al. 2006; Wager et al. 2007b) was used in healthy subjects to explore whether placebo analgesia induced before sleep could modulate acute heat pain and associated sleep disturbances. Expectations of pain relief associated with an inert topical cream (placebo treatment) were generated by suggestions of analgesia and conditioning procedures in a pre-testing phase in wake participants (Laverdure-Dupont et al. 2009). Sleep was then monitored on two experimental nights in each participant immediately after the application of the placebo treatment (placebo night) or an inert cream said to be ineffective (control night). Nocturnal noxious stimulations induced by contact heat pain were then applied throughout the various sleep stages. In addition to subjective evaluations of pain and related sleep disturbance reported in the mornings, the arousal responses provoked by each of the stimuli were assessed, blind to the treatment conditions. The profile of stimuli-associated arousals was then compared between the placebo and control nights to evaluate whether the presence of the placebo treatment and associated relief expectation could indeed reduce participant's responsiveness to acute pain during sleep.

METHOD

1. Subjects

Eleven participants were recruited from University of Montreal campus. One participant was excluded due to unreliable pain reports in the calibration phase (i.e. did not show a monotonic stimulus-response function to increasing stimulus temperature). Another participant was excluded due to equipment failure. The final sample included 9 healthy right-handed volunteers aged between 21 and 26 years old (22.8 ± 0.6 years; 5 women and 4 men) with no history of chronic pain or sleep problems. In addition, participants reported no neurological or psychiatric disorders and were free of medication except for contraceptive pills in 3 women. Participants were also selected on the basis of their regular sleep-wake cycle (7-8 hours per night, with bedtime between 10:00 PM and 12:00 AM), which they were asked to maintain during the entire course of the study. They were also asked to abstain from alcohol and caffeine 24 h before and during each of the 3 testing sessions.

The study was conducted at the Centre for the Study of Sleep and Biological Rhythms at Sacré-Coeur Hospital (Montréal, Québec, Canada) where participants were greeted by the experimenter wearing a white lab coat. Subjects were told that the aim of the study was to investigate the effect of an analgesic cream on pain perception and pain-related sleep disturbances. The cream was described as topical cream that had proven effective in reducing pain in preliminary studies at other universities (Price et al. 1999). The cream was also described as having a long-lasting action (± 8 hrs), but subjects were told that its exact composition would only be

revealed at the end of the study. The experiment was approved by the Hospital Ethics Committee and all participants signed a written consent form and were debriefed at the end of the study.

2. Pain stimulations and experimental trials

Stimulation protocol and experimental procedures were adapted from our previous work (Lavigne et al. 2000; Laverdure-Dupont et al. 2009). Contact heat pain was induced from a baseline temperature of 32°C by a Peltier-based computerized thermal stimulator with a 3 cm^2 probe (NeuroSensory Analyser TSA-II; Medoc Ltd.). Stimulations were applied on the ventral forearm in three different blocks as described below (calibration, conditioning and placebo test block; see Figure 1). Each 17-sec stimulus (5-sec ramps and 7-sec plateau) were separated by 60 s and preceded by a 5 s auditory count-down in the wake conditions. In the calibration blocks (6 in total), a sequence of ascending stimulations (increments of 1°C) were delivered to each arm starting from 41°C to the subject's tolerance or to a maximum of 50°C. Calibration blocks were used to determine the moderately painful temperature to be used for each individual in the conditioning and testing phases of the study and to assess possible changes in baseline pain sensitivity (pain threshold) across the three testing sessions.

In the experimental nights, nociceptive stimulations were administered with the contact probe stably attached to the subject's forearm with surgical mesh and a

flexible band. Stimulation sites were marked to confirm that the thermal probe had not moved during the night. To avoid alerting participants of incoming stimulations, the apparatus was placed in an adjacent room and linked to the probe via a long cable passing through an opening in the wall.

3. Experimental design and procedure

The within-subject design involved 3 separate overnight sessions, between about 8:00 PM and 9:00 AM (Figure 1). The first and second sessions were always on consecutive nights, and the third session was scheduled a week later. In each session, a calibration block was performed in the evening (8:00PM) and the following morning (8:00 AM). The first session served as a habituation night to acclimatize participants to the sleep laboratory and experimental equipment and to initiate placebo conditioning. The order of the control and placebo conditions applied in the second and third sessions was counterbalanced between subjects. Finally, in the last morning of the study (end of third session), a placebo test block was administered to all subjects.

3.1. Habituation session

In the evening of this first session (9:00 PM), immediately following the calibration block, a conditioning block was performed with stimulations on both the control and placebo sites. At the beginning of this first conditioning block, the same

inert cream was applied on the control and placebo sites. However, the cream applied to the placebo site was presented as a topical analgesic, while the one applied on the control site was described as an inert compound used to control for non-specific effects of the vehicle. The placebo condition was assigned to the dominant forearm in half of the subjects. A series of 8 stimulations were then administered on each site. The intensity of stimulations applied to the control site was adjusted based on the first calibration block in order to produce a moderate level of pain intensity (40-60/100 VAS intensity units). On the treated site (placebo site), the intensity of stimulation was surreptitiously decreased by 2°C. This manipulation was used to provide a positive experience of analgesia and has been shown to increase subject's expectation of pain relief (Montgomery and Kirsch 1997; Price et al. 1999; Laverdure-Dupont et al. 2009). Following this first placebo conditioning block, subjects were then prepared for polysomnographic recording with standard electroencephalographic, electrooculographic and electromyographic settings. Lights were turned off at 11:00 PM, no thermal stimulus was applied during this first night, and subjects were awakened at 7:00 AM. A calibration block was performed at 8:00 AM.

3.2. Experimental sessions

The control and placebo nights also included polysomnographic recordings between 11:00 PM and 7:00 AM (Figure 1). In the evening of the control night (10:45 PM), only the neutral cream described as inert was applied on the control site

followed by 8 stimuli at a moderate level of pain intensity. In the evening of the placebo night (10:45 PM), the same cream was applied on the placebo site with the suggestion that it will generate a long-lasting analgesia. Eight stimuli were then delivered on the placebo site at a level which was surreptitiously reduced by 2°C compared to the one applied on the control site. Lights were turned off at 11:00 PM.

Nocturnal stimulations of equal intensity, corresponding to moderate pain in wake subjects, were applied in both the control and placebo nights. Stimulations were delivered pseudo-randomly over stage 2, slow wave and REM sleep, in an attempt to obtain comparable number of trials in each stage in both the placebo and control nights. The first stimulus was always delivered after the first stable period of SWS was attained in order to preserve sleep continuity in the following hours (Lavigne et al. 2004). Following that, stimuli were administered only after a sleep stage was stable for at least 2 minutes and with an inter-stimulus interval of at least 2 minutes. Subjects were awaken at 7:00 AM and a calibration block was performed at 8:00 AM. Stimuli were always applied to an adjacent site to prevent sensitization.

3.3. Placebo test

Following the calibration block in the morning of the third session, a placebo test block was conducted in the wakeful state. The placebo and the control cream were reapplied to the subjects' forearm using exactly the same procedure and suggestions as in the first conditioning block of session 1. A total of 5 stimulations

were then applied on both the placebo and the control sites using stimuli adjusted to the same moderate-pain intensity level and used on the control site in the conditioning block and across the two testing nights.

4. Dependent variables

4.1. Pain ratings

Subjective ratings of pain intensity and unpleasantness were acquired using a 15 cm mechanical visual analog scale (VAS) (Price al. 1983) translated in French, and were then linearly converted to a numerical value from 0 to 100. The sensory VAS was anchored at the left and right by the respective descriptors ‘no pain sensation’ and ‘most intense pain imaginable’. Likewise, the affective VAS was anchored by the descriptors ‘not at all unpleasant’ and ‘most unpleasant imaginable’. These 2 scales were also used to record expected and retrospective pain evaluations.

Expected pain. Expected pain intensity and unpleasantness were recorded at the beginning of the first conditioning block and placebo test block of the last morning by asking: ‘What do you expect the pain intensity/unpleasantness to be without/with the analgesic cream?’

Concurrent pain. During the calibration blocks, subjects were simply asked to rate the pain intensity they felt immediately after each trial. Immediately after each stimulus delivered in the 3 conditioning blocks and placebo test block, participants were asked to rate the intensity and the unpleasantness of the pain felt.

Remembered pain. Approximately 2 min after the completion of the first conditioning and placebo test block, the overall pain intensity and unpleasantness experienced on the control and placebo sites were rated: 'Retrospectively, what was the overall pain intensity/unpleasantness felt without/with the analgesic cream?'

4.2. Night expectation ratings

Just before light were turned off in the control and placebo nights, subjects were asked to prospectively evaluate their forthcoming nocturnal experience in term of expected pain, pain-related sleep disturbances and anxiety. They were asked the following questions: 'What do you expect the pain intensity/unpleasantness to be during the night?'; 'To what extent do you expect the experimental pain to disturb your sleep?'; 'If you think of the upcoming night, what is your level of anxiety?' Measures of expected pain were collected on the sensory and affective VAS described above and similar scales were used for expected sleep disturbances and anticipatory anxiety.

4.3. Morning retrospective ratings

After subjects were gradually awakened in the morning of the control and placebo nights, they were asked to retrospectively assess their nocturnal experience using the same scales. They were asked the following questions: 'What was the intensity/unpleasantness of the pain felt during the night?'; 'To what extent did the

experimental pain disturb your sleep?'; 'How many stimulations do you remember perceiving during the night?'; 'What was your level of anxiety during the night?'

4.4. Polysomnographic variables

Electrode placement followed the International 10-20 System (Munday 2005) and were positioned at Cz, Fz and Oz, with linked earlobes (A1 + A2) used as a reference. In addition to on-line scoring during the nights, sleep stage identification was confirmed off-line according to the guidelines of Rechtschaffen and Kales (Rechtschaffen and Kales 1968) by an experienced technician blind to the experimental conditions. The following variables were extracted for each of the 3 nights. Sleep latency represents the time in minutes from light off to the first sleep episode, sleep duration (min) correspond to the total sleep time and sleep efficiency (%) was calculated by dividing the duration of sleep by the sleep period (overall duration from sleep onset to final awakening). The overall number of awakening also included those related to the nocturnal experimental stimulation. The relative duration of stage 2 sleep, slow wave sleep (SWS; stages 3 and 4) and REM sleep, expressed as the percentage of time spent in each stage, was calculated by dividing the total amount in minutes by the total sleep time. REM sleep latency is the delay in minutes between sleep onset and the first REM sleep episode.

For each of the nocturnal stimulations, arousal responses were identified by an experienced technician blind to the experimental condition. An absence of

reaction was recorded when no change in EEG, ECG or EMG was apparent. Arousal responses consisted in micro-arousals reflecting an abrupt shift in the alpha, theta and/or delta EEG waves lasting between 3 and 10 s, and awakenings when the shift lasted more than 10 s (American Sleep Disorders Association, 1992; Lavigne et al. 2000; Lavigne et al. 2004). In addition to these criteria, stimulus-evoked responses had to occur within a 15 sec window starting from stimulation onset (Lavigne et al. 2000).

4.5. Vigilance, subjective sleepiness and questionnaires

Participant's vigilance was assessed objectively in the evening and morning of each of the 3 sessions. Reaction times were extracted from a psychomotor vigilance task (PVT) lasting 10 min and the average was compiled for each of the 6 tests. The Karolinska Sleepiness Scale was used to evaluate subjective sleepiness in the evening and morning of each session. Additionally, during the first session, all subjects filled the Pain Catastrophizing Scale (PCS), the Life Orientation Test-Revised (LOT-R; a scale of dispositional optimism-pessimism) and the Beck Anxiety Inventory (BAI).

5. Statistical analysis

Data are presented as mean ± SEM. The heat pain threshold obtained in the calibration blocks was analysed using an ANOVA for repeated measures with

Huynh-Feldt correction in a 3 (session) x 2 (time-of-day) x 2 (right and left arms) model. The effects of the conditioning and placebo test were explored with a paired T-test between the control and placebo sites for each of the dependent variables; expected, concurrent and retrospective pain ratings. The effect of expectations on concurrent and remembered placebo analgesia was evaluated with Pearson's correlation. In addition, subjective evaluations of expected and retrospective pain, pain-related sleep disturbance and anxiety during the control and placebo nights were also compared using a paired T-test. The sleep parameters extracted from the 3 night of polysomnographic recordings were analysed with an ANOVA for repeated measures and their relationship with nocturnal expectations were assessed with Pearson's correlation coefficient. Partial correlation analyses were also used to partial out the effects of anxiety. The total number of stimulation per night and for each stage was pooled across all subjects (Lavigne et al. 2000) and was compared using a paired T-test across conditions. The proportion of experimentally provoked arousal responses were compared between the control and placebo nights with a Yates corrected Chi-square for each sleep stage. The correlation between the various questionnaires and placebo measures and sleep parameters were calculated using the non-parametric Spearman test with a threshold set at α=0.05 (2-tailed), after Bonferroni correction for multiple comparisons. Finally, the mean reaction times extracted from the PVT data were entered into a 3 (session) x 2 (time-of-day) ANOVA for repeated measures and the evening and morning KSS evaluations were compared between the 3 nights with the non-parametric Friedman test. All statistical

analyses were performed with SPSS 16.0 except for the Yates corrected Chi squares, which were calculated in Statistica 6.1.

RESULTS

Pain sensitivity (calibration blocks)

In the evening and morning of each of the 3 sessions, all subjects were submitted to a calibration block aimed at quantifying individual pain sensitivity. The overall average heat pain threshold was 44.9 ± 0.5°C, with no significant difference between right and left forearms (45.0 ± 0.6 vs 44.8 ± 0.5°C; $F(1,7)=0.44$, $p=0.53$) nor between calibration blocks performed in the evening and in the morning (44.8 ± 0.6 vs 45.0 ± 0.5°C; $F(1,7)=0.36$, $p=0.57$). Subjects tended to be slightly more sensitive to heat pain in the habituation session (session 1: 44.3 ± 0.7°C, session 2: 45.1 ± 0.5°C, session 3: 45.3 ± 0.5°C; $F(2,14)=3.67$, $p=0.08$), but no significant variation was found between the control and placebo nights performed in the second or third sessions (main effect of experimental night order: $F(1,7)=0.04$, $p=0.85$; interaction between session and night order: $F(2,14)=0.68$, $p=0.52$). The only other significant interaction observed was between the time of testing (evening or morning) and laterality, which show that the difference in pain threshold between the right and left arm appears to be greater in the evening calibration blocks ($F(1,7) = 11.79$, $p=0.011$). This effect, however, should not influence our measures of pain relief, because the attribution of the control and placebo sites were randomly balanced between subjects

and when this factor was taken into account, the interaction was no longer significant ($F(1,7) = 0.42$, $p=0.54$).

Effect of conditioning

The stimulation temperature required to induce moderate pain in the control condition of the conditioning block was estimated from the pain intensity scores recorded in the first calibration block. The average temperature delivered to the control and placebo sites were 48.6 ± 0.2°C and 46.6 ± 0.2°C respectively, which corresponded to mean pain intensity ratings of 46.1 ± 4.8 and 26.6 ± 3.8 VAS units, respectively.

Before stimulations were administered in the conditioning block of the habituation night, subjects were asked to rate how much pain they expected to feel on the control and placebo sites. The average expected pain intensity reduction (control – placebo) was 10.33 ± 2.56 VAS units ($T(8)=4.04$, $p=0.004$, paired T-test) and the average expected pain unpleasantness reduction was 10.33 ± 3.02 VAS units ($T(8)=3.42$, $p=0.009$, paired T-test). The actual relief experienced during the conditioning manipulation was 14.28 ± 3.12 VAS units for intensity ($T(8)=4.58$, $p=0.002$, paired T-test) and 15.25 ± 3.69 VAS units for unpleasantness ($T(8)=4.13$, $p=0.003$, paired T-test). Moreover, overall pain evaluations were also acquired after the completion of the pain trials, and average remembered relief was evaluated at 18.22 ± 3.10 VAS pain intensity units ($T(8)=5.88$, $p < 0.001$, Paired T-test) and 23.89

± 4.46 VAS pain unpleasantness units (T(8)= 5.35, p=0.001, Paired T-test). These results show that the subjects did initially expect pain reductions by the placebo cream and that the conditioning manipulations produced a positive experience of analgesia.

In addition to the conditioning manipulations performed in the habituation night, subjects were exposed to 8 stimulations just before lights were turned off in the control and placebo nights. The intensity of stimulation corresponded to the levels used on the control and placebo sites in the conditioning block of the first session and generated an average pain intensity of 39.94 ± 5.52 VAS units in the control night and 16.72 ± 6.46 VAS units in the placebo night (T(8)=4.60, p=0.002, Paired T-test). Similar results were recorded for pain unpleasantness with an average of 34.56 ± 5.57 and 14.60 ± 6.06 VAS units in the control and placebo nights respectively (T(8)=4.66, p=0.002). This additional manipulation was performed just before sleep in order to reinforce the participant's expectation of relief induced by the placebo cream.

Experimental nights: Expectations

After the end of the conditioning pain trials of the control and placebo nights, subjects were asked to evaluate how much pain they expected to feel during the night and to what extent they anticipated this pain to disturb their sleep. The results are illustrated in Figure 2 and show that, in the placebo night, subjects expected to

experience a lower level of nocturnal pain intensity (T(8)=4.85, p=0.001) and unpleasantness (T(8)=3.58, p=0.007), and less sleep disturbance induced by the nocturnal nociceptive stimulations (T(8)=3.12, p=0.014). In addition, participants reported less anticipatory anxiety in the placebo than in the control evening (T(8)=3.66, p=0.006).

Experimental nights: Retrospective evaluations

In the morning of the control and placebo nights, participants were asked to rate their night-time experience retrospectively (Figure 3). They evaluated the pain felt during the placebo night to be smaller than during the control night (intensity: T(8)=3.13, p=0.014; unpleasantness: T(8)=3.42, p=0.009). Furthermore, their subjective assessments of sleep disturbances caused by the nociceptive stimulations was significantly different between the placebo and control nights (respectively 17.22 ± 5.65 VAS units vs 35.44 ± 4.63, T(8)=3.21, p=0.012), and subjects reported less anxiety during the placebo night compared to the control night (T(8)=2.44, p=0.041). In addition, subjects reported remembering fewer stimulation in the morning of the placebo night in contrast with the control night (placebo night: 3.11 ± 0.80; control night: 4.28 ± 0.69; T(8)=2.48, p=0.038). Because the sequence of experimental nights was randomized (5 subjects underwent the control night first and 4 subjects had the placebo night first), the order effect was assessed and resulted in no significant differences for all the retrospective variables measured (main effect of order and interaction between treatment and order: all p's > 0.1).

Experimental nights: Sleep arousals responses

During the control and placebo nights, nociceptive stimulations of the same intensity were delivered on the control or placebo site. The average temperature used was 48.6 ± 0.2°C and corresponded to the intensity used on the control site during the conditioning blocks. Across all subjects, the number of stimuli was distributed equally between the conditions and resulted in a total of 350 stimulations administered in the control nights and 347 stimulations delivered in the placebo nights. The distribution of all the nocturnal stimuli administered is displayed in Table 1 and confirms that a comparable number of stimulations were given across the sleep stages in the placebo and control nights (Paired T-test between total stimulation per night and per sleep stages, all p's > 0.49). This allowed for a balanced assessment of the placebo effect on sleep perturbations across all sleep stages.

The rate of arousal responses induced by the noxious stimuli in the placebo condition was compared to the rate of the control condition for each sleep stage separately. The proportion of arousal responses produced by the stimuli in stage 2 sleep was comparable in the placebo and control nights (reaction vs no reaction: Yates corrected χ^2 (1) =0.09, p=0.77), but significant differences were found during REM sleep and SWS. During REM sleep, 20 stimulations out of a total of 119 (17%) went unnoticed (i.e. no arousal response) in the placebo night, compared to only 8 out of 115 (7%) in the control night (Yates corrected χ^2 (1) =4.49, p=0.034). In other words, the placebo treatment produced a significant reduction in arousal responses induced by the stimulations administered during REM (83% in placebo Vs 93% in

control), which is consistent with the diminution of pain-related sleep disturbance reported subjectively in the morning of the placebo session. This reduction in responsiveness during REM in the placebo night was primarily the result of a decrease in the proportion of awakenings (31% of stimuli in the placebo condition and 41% in the control) while the proportion of micro-arousal response was comparable across conditions (52% in both).

In contrast, only 11 stimulations out of a total of 98 (11%) delivered in SWS went unnoticed (i.e. no arousal reaction) during the placebo night, compared to 26 out of 103 (25%) in the control night (Yates corrected χ^2 (1)=5.67, p=0.017). This means that during SWS, the subjects displayed *more* arousal responses to the noxious stimuli in the placebo condition (89% in placebo Vs 75% in control). This reflected placebo-related increases, or control-related decreases, in both awakenings (27% in placebo and 18% in control) and micro-arousals (62% in placebo and 56% in control). Taken together, these results indicate that the responsiveness to nociceptive nocturnal stimuli is differentially modulated by a placebo treatment as a function of the sleep stage in which the stimuli are presented.

Polysomnographic recordings

The sleep parameters extracted from the polysomnographic recordings of the habituation, control and placebo nights are presented in Table 2. Overall, there were no significant differences between the 3 nights in sleep latency, sleep duration, sleep

efficiency, REM sleep latency and percentages of stages 2, slow wave and REM sleep (all p's > 0.05). One outlier displayed longer sleep latency (larger than the group mean + 2 standard deviations) with a correspondingly shorter sleep duration and poorer sleep efficiency (less than the group average – 2 standard deviations) during the control night and was therefore removed from the analysis of these variables. In contrast to the other sleep parameters, the overall number of awakening show a significant effect of night ($F(2,16)=11.39$, $p=0.001$), with an increased number during both the control and placebo nights compared to the habituation night (night 1 vs 2: $p=0.025$; night 1 vs 3: $p=0.006$; night 2 vs 3: $p=0.467$). Taken together, these results suggest that the general sleep profile was comparable between the 3 nights recorded, but that the number of awakening during the control and placebo nights was larger than in the habituation night.

To evaluate the impact of expectations on the architecture of the forthcoming sleep period, exploratory correlations analyses were performed with the sleep parameters extracted from the 2 experimental nights. Although no significant association were observed for stage 2 sleep, the relative duration of SWS and REM sleep were associated with various measures of expected pain and anxiety in both nights. Specifically, during the control night, expected pain intensity was *positively* correlated with the percent of SWS ($r = 0.74$, $p=0.022$) and *negatively* associated with percent REM sleep ($r = -0.78$, $p=0.013$), suggesting a shift from REM to SWS, in subjects expecting more pain during the control night. A similar negative relation was also found between expected pain unpleasantness and percent REM sleep (r = -

0.80, p=0.010). Because prospective anxiety was also found to be positively associated with the relative duration of SWS (r = 0.75, p=0.021), the removal of its contribution by partial correlation analysis show that the relation between SWS and expected pain intensity is linked to anxiety effects. On the other hand, the association between expected pain and REM sleep percent could not be explained entirely by anxiety (partial correlation removing the effect on anxiety; pain intensity: r = -0.68, p=0.065; pain unpleasantness: r = -0.73, p=0.042).

During the placebo night, only prospective anxiety was positively associated with the relative duration of SWS (r = 0.82, p=0.006) and negatively associated with REM sleep percent (r = -0.82, p=0.007). As a whole, these results suggest that the high level of pain anticipated during the control night, and perhaps the associated anxiety, caused a shift from REM to SWS sleep, whereas in the placebo night, this effect was not linked to expected pain, but rather to the level of anxiety.

Placebo analgesia measured during wake

For all participants, placebo analgesia was assessed during the last morning of the experimental protocol and mean pain ratings for the control and placebo site are displayed in Figure 4. The analysis revealed that, during this last experimental block, subjects still expected to experience less pain on the placebo site compared to the control site (expected pain intensity relief: 12.00 ± 1.72 VAS units, T(8) = 6.99, p<0.001; expected pain unpleasantness relief: 11.00 ± 2.74 VAS units, T(8) = 4.01,

p=0.004). In addition, a modest but statistically significant reduction in pain intensity was recorded during concurrent evaluations (3.64 ± 1.35, T(8) = 2.70, p=0.027) and a trend was measured for pain unpleasantness reduction (2.33 ± 1.29, T(8) = 1.80, p=0.109) . After the completion of the pain trials, retrospective evaluations of the pain experienced confirmed the presence of a significant analgesic effect of the placebo cream (remembered pain intensity relief: 7.56 ± 2.54 VAS units, T(8) = 2.98, p=0.018; remembered pain unpleasantness relief: 9.00 ± 3.21 VAS units, T(8) = 2.80, p=0.023). When these analgesic effects were paralleled with the subjective evaluations of pain relief during the placebo night relative to the control night, no significant correlations were found either for the expected or actual pain reductions. This implies that anticipated and experienced analgesia in the wake state was not a predictor of the subjective pain relief reported during sleep.

As placebo effects are linked to relief expectation (Montgomery and Kirsch 1997; Price et al. 1999; Benedetti et al. 2003), correlation analyses were performed between expected relief and the measures of placebo analgesia in the awake placebo test. Expected pain intensity reduction was moderately correlated with the reductions in concurrent pain intensity (r = 0.59, p=0.098) and significantly associated with remembered pain intensity relief (r = 0.68, p=0.046). A similar trend was observed for expected pain unpleasantness diminutions (correlation with concurrent relief: r = 0.55, p=0.124; correlation with remembered relief: r = 0.60, p=0.089). These results are consistent with previous reports that relief expectation is a mediator of placebo analgesic effects during wakefulness.

Questionnaires

To evaluate the influence of personality traits on placebo responding and sleep architecture, the Pain Catastrophizing Scale (PCS), the Life Orientation Test-Revised (LOT-R) and the Beck Anxiety Inventory (BAI) were administered to all participants. Using a non-parametric approach, correlation analysis show no significant effect of these dispositional variables on any of the subjective measures of placebo responding, including morning evaluations of nocturnal pain, pain-related sleep disturbance and anxiety in the control and placebo nights, as well as on the measures of placebo analgesia during wake reported in the last morning of the study (all p's >0.05, Spearman). On the other hand, pain catastrophizing scores were positively correlated with the expected pain intensity (rho = 0.83, p=0.006) as well as with the expected pain-related sleep disturbance during the placebo night (rho = 0.68, p=0.046). When those personality traits were correlated with the sleep parameters of the 3 nights of recording, results show that, in the control night, higher optimism scores on the LOT-R was correlated with shorter sleep latency (rho = -0.72, p=0.029) and higher pain catastrophizing (PCS) was associated with shorter REM sleep latency (rho = -0.74, p=0.022). However, the only relationship that remained significant after correction for multiple comparisons at a threshold of $p \leq 0.017$ was the positive association between PCS and the expected pain intensity during the placebo night. Finally, the relative duration of stage 2 sleep, SWS and REM sleep in each of the 3 nights was not significantly modulated by those dispositional variables (all p's > 0.2, Spearman).

Vigilance and sleepiness

In the evening and morning of each session, a psychomotor vigilance task was used to objectively assess vigilance. There was no significant effect of session ($F(2,10) = 2.44$, $p=0.14$; 2 missing data) or time of testing ($F(1,5) = 0.001$, $p=0.98$; 2 missing data) in mean reaction time. Finally, the Karolinska Sleepiness Scale was used to evaluate subjective sleepiness, and show comparable results between the data recorded in the 3 evening sessions (χ_r^2 (2)= 1.19, p=0.553, Friedman) as well as the ones originating from the morning measures (χ_r^2 (2)= 2.70, p=0.26, Friedman).

DISCUSSION

Placebo-induced analgesic effects have been repeatedly measured in wake individuals, but little is known about their occurrence during sleep. In this study, two experimental nights were used to investigate whether the presence of a long-lasting placebo analgesic cream and associated pain relief expectation could modulate subjects' responses to nociceptive stimuli during sleep. Similar to our previous work (Laverdure-Dupont et al. 2009), verbal suggestions of analgesia and sensory conditioning were used to create belief into the effectiveness of a topical cream while subjects were awake. The pain and related sleep disturbances expected to be experienced during the night were monitored in the evening of the experimental nights, and show that subjects did expect to experience less pain and sleep disturbances in the placebo night compared to the control night.

After having administered a comparable number of nociceptive stimulations at the same intensity throughout the 2 nights, subjective evaluations of nocturnal pain and sleep quality were recorded upon awakening. The results show that subjects reported having experienced less pain and pain-related sleep disturbances in the night in which the placebo cream was applied. In the morning of the placebo nights, subjects also indicated having perceived less stimulation. Anxiety was also found to be modulated by the treatment, as participants were less anxious during the placebo night. The findings that subjective measures of pain and pain-related sleep disturbance can be improved by the administration of a placebo are consistent with reports of clinical trials showing some improvements in pain ratings and related sleep problems in placebo-treated patients (Dogra et al. 2005; Richter et al. 2005; Breuer et al. 2006; Gabis et al. 2009). Furthermore, a recent study among individuals with fibromyalgia provided evidence that changes in pain after placebo administration were highly correlated with measures of sleep quality and sleep disturbance (Russell et al. 2009), suggesting that the effects of placebo analgesia could be generalised during sleep.

In line with the reduction of pain and pain-related sleep disturbances reported subjectively, placebo treatment also produced a significant reduction in arousal responses induced by the stimulations administered during REM sleep (83% in placebo Vs 93% in control). This suggest that, at least during this sleep stage, the top-down mechanisms associated with placebo responses could still be functional, even in the absence of conscious awareness. In wake individuals, one of the most

consistent finding implicated in the generation of placebo analgesic effects involves the activation of the rostral anterior cingulate cortex and its increased connectivity with the mesencephalic periaqueductal gray (Petrovic et al. 2002; Wager et al. 2004; Bingel et al. 2006; Wager et al. 2007a). The amygdala as also been implicated in placebo-dependent pain modulation through its action on brainstem structures (Bingel et al. 2006; Wager et al. 2007a; Zubieta and Stohler 2009). Interestingly, during REM sleep, pronounced activation in limbic structures, such as the amygdaloid complexes bilaterally and the anterior cingulate cortex (Brodmann area 24) (Maquet et al. 1996; Buchsbaum et al. 2001) has been reported. In addition, the hippocampal formation, thalamic nuclei and most brainstem neurons, especially those located in the pontine tegmentum and dorsal mesencephalon show important activation during this sleep stage (Maquet et al. 1996; Braun et al. 1997; Nofzinger et al. 1997; Siegel 2005). It therefore appears that the neuronal network underlying placebo analgesia in wake individuals is markedly activated during REM sleep which could underlie the maintenance of placebo-dependent pain modulation during this sleep stage.

Another potential explanation for the occurrence of placebo analgesia specifically during REM sleep might be linked to the increased potential for the processing of external stimuli during this stage. Indeed, studies using auditory evoked potentials have demonstrated that the specific electrophysiological features associated with the detection of salient or deviant stimuli during wake could also be observed during sleep, but exclusively in REM sleep (Bastuji et al. 1995; Pratt et al.

1999; Cote et al. 2001). Moreover, additional findings suggest that voluntary attention engaged by pre-sleep instructions as well as manipulations of participants' motivation by using the threat of electric shock can modulate the amplitude of the potentials evoked by deviant stimuli or the accuracy of the behavioural response specifically during REM sleep (Williams 1967; Takahara et al. 2006). Taken together, these findings suggest a continued engagement of cognitive processes able to modulate incoming sensory information during paradoxical sleep.

Unexpected results, however, were observed during SWS. The proportion of arousal related to the noxious stimulations administered in this stage was indeed increased in the placebo night (89% in placebo Vs 75% in control), which suggests that subjects were less responsive to the stimuli during SWS in the control night. In addition, during the control night, high expectation of nocturnal pain was associated with more SWS. This suggests that expectation of stronger pain in the control night might have triggered a deepening of sleep as a protective mechanism to maintain SWS continuity. In sum, it appears that during SWS, the anticipation of high nocturnal pain in the control night took precedence over placebo-induced expectation.

Brain imaging studies have demonstrated a marked decrease in global cerebral blood flow during SWS, predominantly in thalamic nuclei, brainstem, basal forebrain, anterior cingulate and orbito-frontal cortices (Braun et al. 1997; Maquet et al. 1997; Buchsbaum et al. 2001). In addition, fronto-parietal association cortices are relatively deactivated (Braun et al. 1997; Kajimura et al. 1999). This suggests that,

while the first relay of afferent sensory signals may remain active during this sleep stage, higher-order processing of sensory inputs may be limited. Consistent with this, electrophysiological recordings indicate that the marked thalamic inhibition observed during SWS prevents sensory afferent inputs to be further processed through thalamocortical pathways and associative cortical areas (Steriade 1993). Furthermore, a breakdown in cortical connectivity during non-REM sleep has been suggested to limit the brain's ability to fully integrate afferent information thought the concerted action of specialized regions (Massimini et al. 2005). Taken together, these observations suggest that the possibility to engage pain modulatory systems during SWS may be limited. This further implies that the anticipation of stronger pain in the control condition may have induced a deepening of sleep, thereby increasing the gating of noxious inputs and reducing the number of arousal responses.

In the last morning of the experiment, placebo analgesia was evaluated during wakefulness. At this point, subjects still expected to experience pain relief following placebo administration, to a level comparable to the one reported in the conditioning block of the first evening, which implies that expectations remained relatively stable across the whole study. However, expectations of nocturnal pain relief by placebo (Vs control; Figure 2) were larger compared to the wake measures (Figure 4), possibly reflecting the effect of other factors that influenced the anticipated experience. Indeed, it is likely that those measures not only captured the expected pain reduction associated with placebo treatment, but also the ways in which experimental acute pain could be experienced during sleep. These different

perspectives might explain why the expectations of pain relief during wake and sleep diverged and, consequently, why the placebo-dependent analgesia assessed during those two states was uncorrelated.

In the current study, personality trait associated with optimism, anxiety and pain catastrophizing were measured to identify potential factors that could modulate the effect of pain and placebo-related analgesia on sleep architecture. The strongest relation observed indicated that high catastrophizers anticipated higher pain intensity in the placebo night. Although pain catastrophizing has not specifically been implicated in placebo responding, other personality traits, such as dispositional optimism and state anxiety were previously reported to be significant predictors of placebo responses (Morton et al. 2009). While the different context of the current study could potentially explain those divergent findings, the use of a larger sample may help to shed light on the importance of those dispositional variables.

In summary, the present report is the first experimental study directly aimed at investigating whether placebo analgesia could occur during sleep. These results demonstrate that placebo manipulations performed prior to sleep can modulate the responsiveness to nocturnal pain, but that these effects differ according to the sleep stage. Consistent with the subjective measures of pain and pain-related sleep disturbances, placebo administration was associated with a reduction in arousal responses during REM sleep. On the other hand, higher anticipated pain during the control night reduced responsiveness to noxious stimuli during SWS and was associated with a deepening of sleep. In conclusion, it appears that placebo-

dependent pain modulatory processes might still be efficient during REM sleep, but that the anticipation of high experimental night pain might be associated with a shift in sleep architecture in favour of SWS.

Table 1: Total number of nociceptive stimulations applied and number of sleep arousal responses produced during each sleep stage in the control and placebo nights across all subjects. The percents of responses observed relative to the number of stimuli administered within each sleep stage and for the entire sleep period (Total) are displayed for each condition in parentheses. SWS: slow wave sleep; REM: rapid eye movement sleep.

	Total Stimulation		Awakening		Micro-arousal		No reaction	
	Control	Placebo	Control	Placebo	Control	Placebo	Control	Placebo
Stage 2	132	130	57 (43.2)	55 (42.3)	69 (52.3)	71 (54.6)	6 (4.5)	4 (3.1)
SWS	103	98	19 (18.4)	26 (26.5)	58 (56.3)	61 (62.2)	26 * (25.2)	11 * (11.2)
REM	115	119	47 (40.9)	37 (31.1)	60 (52.2)	62 (52.1)	8 * (7.0)	20 * (16.8)
Total	350	347	123 (35.1)	118 (34.0)	187 (53.4)	194 (55.9)	40 (11.4)	35 (10.1)

* Significant difference between Placebo and Control; Yates corrected χ^2, $p<0.05$.

Table 2: Mean ± SEM of sleep parameters recorded with polysomnography during the 3 nights. The sequence of the control and placebo analgesia nights was counterbalanced randomly between subjects.

	Habituation night	Control night	Placebo night
Sleep latency (min)	12.5 ± 2.7	20.0 ± 9.6 (10.8 ± 3.2 without outlier)	9.9 ± 3.1
REM sleep latency (min)	102.5 ± 14.8	96.5 ± 18.2	99.0 ± 13.4
Sleep duration (min)	418.8 ± 12.9	385.3 ± 13.4 (397.1 ± 7.3 without outlier)	410.6 ± 11.7
Sleep efficiency (%)	93.1 ± 1.6	89.0 ± 2.1 (90.8 ± 1.2 without outlier)	91.4 ± 1.6
Number of awakenings	34.8 ± 3.5*	45.0 ± 3.4*	50.8 ± 4.4*
Stage 2 (%)	56.8 ± 2.1	53.4 ± 2.5	52.8 ± 1.8
SWS (%)	21.3 ± 2.5	20.8 ± 3.0	21.4 ± 2.4
REM (%)	17.8 ± 2.0	19.9 ± 2.3	21.0 ± 1.5

* Night 1 vs 2: p=0.025; night 1 vs 3: p=0.006; night 2 vs 3: p=0.467.

NB: One outlier (n=1) with values outside the range defined by the mean of the group ± 2 standard deviations was excluded from the analyses reported in this Table.

Figure 1: Experimental design including 3 nights of polysomnographic recordings. The first night served to habituate subjects and contained no nocturnal stimulation. The second and third nights consisted of a control and a placebo night in which nociceptive stimuli of the same intensity were administered. The sequence of the second and third nights was balanced randomly between subjects. A calibration block was included in the evening and morning of each session and a placebo test was performed on the last morning of the study.

Figure 2: Ratings of expected pain, expected pain-related sleep disturbance and prospective anxiety measured in the evening of the control and placebo nights. Int.: Intensity; Unp.: Unpleasantness. Paired T-test; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

Figure 3: Retrospective ratings of pain, pain-related sleep disturbance and anxiety measured in the morning of the control and placebo nights. Int.: Intensity; Unp.: Unpleasantness. Paired T-test; ** $p \leq 0.01$; * $p \leq 0.05$.

Figure 4: Expected, concurrent and retrospective pain intensity and unpleasantness measured on the control and placebo site during the test block in the last experimental morning. Int.: Intensity; Unp.: Unpleasantness. Paired T-test; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$, † $p = 0.109$.

Figure 1

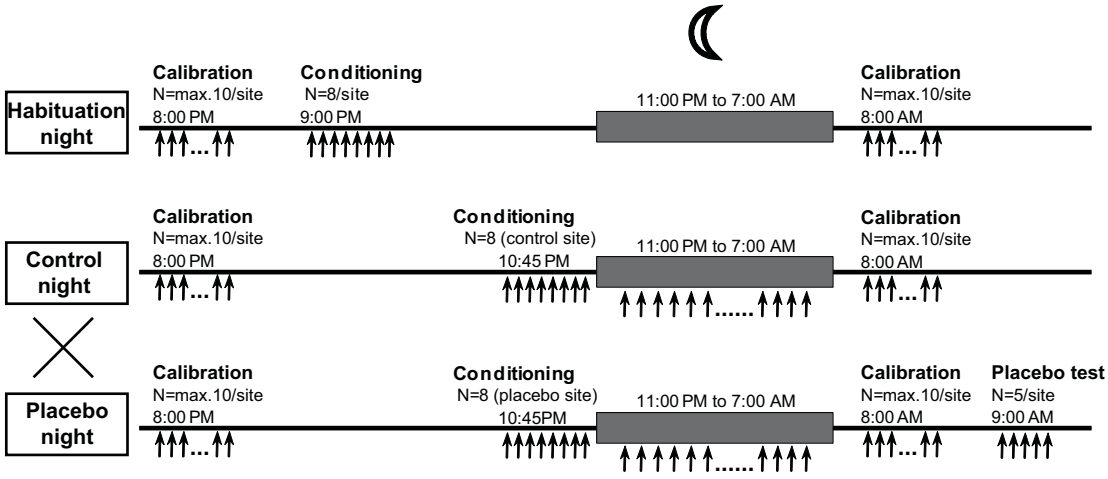


Figure 2

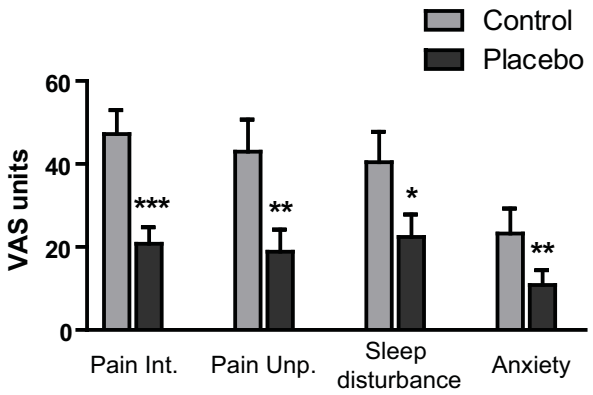


Figure 3

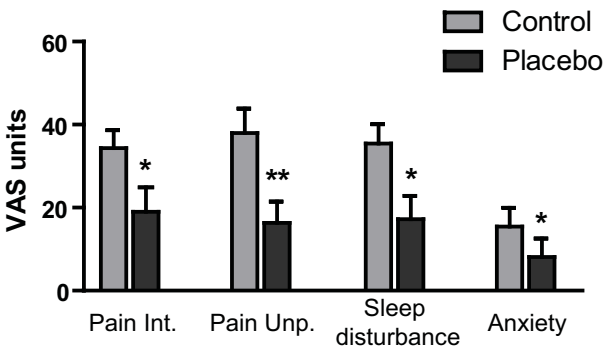
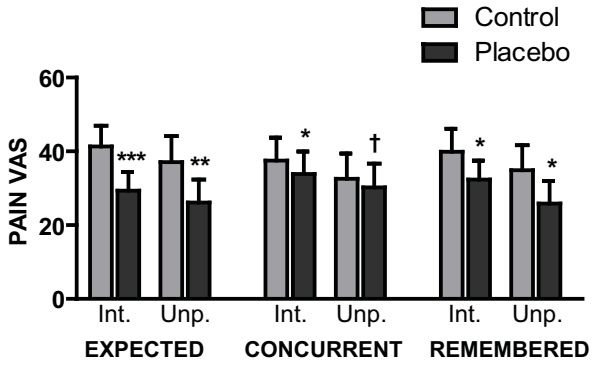


Figure 4



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Article 2

Changes in REM sleep associated with placebo-induced expectations and analgesia.

Danièle Laverdure-Dupont[1,2], Pierre Rainville[3], Jacques Montplaisir[2], Gilles Lavigne[2,3]

1. Department of Physiology, Faculty of Medicine, University de Montréal, Montréal, Canada
2. Centre d'étude du Sommeil et des Rythmes Biologiques, Hôpital du Sacré-Cœur, Montréal, Canada
3. Department of Stomatology and Oral Health, Faculty of Dental Medicine, Université de Montréal, Montréal, Canada

Corresponding author:
Danièle Laverdure-Dupont
Centre d'étude du Sommeil et des Rythmes Biologiques
Hôpital du Sacré-Cœur de Montréal
5400 boul. Gouin Ouest
Montréal, Québec, Canada
H4J 1C5
Tel: 514-338-2222, ext. 3745
Fax: 514-338-2694
[redacted]

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ABSTRACT

The experience of a sensory event is extensively shaped by past experience and expectations. Placebo analgesia, one of the most studied models of expectation-mediated effects, can be induced by suggestion of analgesia and conditioning. The present study examined the possibility that sleep might contribute to the consolidation of new expectations and consequently influence the generation of expectation-mediated placebo effects. Strong expectations of analgesia were generated before sleep by conditioning manipulations wherein the intensity of thermal pain stimulation was surreptitiously reduced following the application of a topical placebo cream. Expectations and placebo analgesic effects were measured the following morning and compared to those of a control daytime group without sleep. Although placebo effects were observed in both groups, correlation analysis suggests that the mediating effect of expectations on placebo responses was strongest in the overnight group. Moreover, following exposure to a convincing analgesia experience, the relative duration of REM sleep *decreased* in subjects showing higher analgesic expectations and placebo responses the next morning. In a third group exposed to less consistent analgesic experiences before sleep, expectations reported in the morning were comparable to other groups. However, expectations were *positively* correlated with REM sleep and didn't emerge as a significant mediator of the analgesic effect. Taken together, these findings show that sleep-related processes may influence the association between expectations and placebo analgesia, and that REM sleep can predict placebo-induced expectations of pain relief. However, equivocal prior

experience with treatments may significantly alter the relationship between relief expectation, REM sleep, and placebo effects.

INTRODUCTION

Expectations originate from the integration of current information with past experience and personal beliefs, and can greatly shape the experience of a particular sensory event (Ploghaus et al., 2003; Koyama et al., 2005). Many pain studies have highlighted the pivotal role of expectation in the establishment of placebo-induced analgesic effects (Montgomery and Kirsch, 1997; Price et al., 1999; Benedetti et al., 2003). A combination of verbal suggestion and sensory conditioning is commonly used to induce placebo analgesia, and is one of the most studied models of expectation-mediated effects (Price et al., 1999; Petrovic et al., 2002; Wager et al., 2004; Bingel et al., 2006; Wager et al., 2007). Because prior exposure to an effective treatment can enhance analgesic responses when compared to suggestion alone (Colloca and Benedetti, 2006; Colloca et al., 2008), placebo effects may result from learning processes involving the consolidation of expectation-related effects. In recent years, the role of sleep in memory reprocessing and learning has been increasingly recognized (Walker and Stickgold, 2004), but its involvement in the consolidation of newly acquired expectations remains unexplored.

Training and sleep deprivation studies have highlighted the differential contribution of separate sleep stages to the acquisition of various types of memories (Walker and Stickgold, 2004; Rauchs et al., 2005). Whereas the learning of simple procedural skills has been linked to stage 2 sleep (Peters et al., 2007), REM sleep

appears to be specifically implicated in the processing of complex cognitive tasks (Smith, 2001) and emotional memories (Stickgold et al., 2001; Wagner et al., 2001). In addition, the consolidation of episodic memories seems to be mainly dependent on REM sleep (Rauchs et al., 2004), and memories encoded while awake have been shown to be reactivated during subsequent sleep periods in both animals (Pavlides and Winson, 1989; Wilson and McNaughton, 1994) and humans (Maquet et al., 2000; Peigneux et al., 2004). This sleep-dependent processing is proposed to allow for a reinterpretation of the meaning of novel information and to facilitate its integration into a network of related memories (Paller and Voss, 2004). Furthermore, sleep appears to promote the mental restructuring involved in the discovery of hidden rules and consequently favor the gain of explicit knowledge that can influence behavior (Wagner et al., 2004).

The objective of the present study was to investigate the possibility that sleep contributes to the integration of new expectations and consequently influences the generation of expectation-mediated placebo effects. In group 1, strong expectations as to the efficacy of a placebo cream were generated prior to sleep by a conditioning manipulation, and expectations and analgesic effects were measured the following morning in a placebo testing block (12 hr post-conditioning). Placebo effects were then compared to those of a control daytime group (group 2) which comprised a 12 hr post-conditioning delay but no sleep episode. An additional night group was included (group 3), with subjects conditioned in the evening and tested both before and after sleep. Individual differences in expectations and analgesic effects reported in groups 1 and 3 were specifically investigated in relation to polysomnographic measures to

test the hypothesis that expectation-mediated placebo effects are associated with changes in sleep architecture.

METHOD

Participants

Thirty-eight healthy volunteers (22 females and 16 males, one left-handed) aged 20–35 years old (23.42 ± 0.47) were recruited on the campus of the *Université de Montréal* and were alternately assigned to three groups. Groups were comparable on mean age (23.7 ± 2.3, 23.2 ± 3.5, 23.5 ± 3.1 years old, for groups 1, 2, and 3) and female/male proportion (7/12, 8/13, and 7/13, for groups 1, 2, and 3, respectively). Subjects reported no history of chronic pain, neurological, psychiatric, or sleep disorders, and no drug or medication consumption at the time of the experiment, except for contraceptive pills in 13 women. All women were tested during their follicular phase. Subjects were asked to abstain from caffeine and alcohol 24 hr before and during the entire course of the study, and reported regular sleep-wake rhythm (7–9 hr of sleep per night, with morning awakening from 7:00 to 9:00 A.M.).

The study took place at the Centre for the Study of Sleep and Biological Rhythms at Sacré-Coeur Hospital, Montreal, where participants were greeted by the experimenter wearing a white lab coat. All instructions to participants followed a standardized script. Subjects were told that this experimental study aimed to evaluate the effect of sleep and circadian phase on the analgesic effects of a cream. The treatment was presented to participants as a topical cream that had proven effective in reducing pain in preliminary studies at other universities (Price et al., 1999).

Participants were told that the exact composition of the cream could be revealed only at the end of the study. The experiment involved no risk for the subjects and was approved by the Hospital Ethics Committee. Participants signed a written consent form and were debriefed at the end of the study.

Pain stimulation and experimental procedure

Two sites of cutaneous stimulation, over each deltoid muscle, were delimitated for each participant. A 3 cm^2 peltier probe (NeuroSensory Analyser TSA-II, Medoc Ltd, Israel) was used to induce contact heat pain (44–50°C) from a baseline temperature of 32°C. The stimulation protocol was similar to that developed by Price and colleagues (Price et al., 1999) and contained 4 different blocks of pain trials: familiarization, calibration, conditioning, and placebo testing. In the familiarization block, subjects were exposed to one trial each of 44, 45, 47, and 49°C stimuli in order to become gradually accustomed to the stimuli. In the calibration block, series of stimulations were delivered to each arm using the ascending method of limits to determine the individually-adjusted temperature to be used during the conditioning and experimental blocks for each participant, as described below.

At the beginning of the conditioning and placebo blocks, the same inert cream was applied to the control and treatment sites identified on each arm. The cream applied to the treated site (placebo site) was described as a topical analgesic, while the cream applied to the control site was described as an inert cream used to control for non-specific effects of the vehicle compound of the analgesic cream. The placebo condition was assigned to the dominant arm in half the subjects. In both the

conditioning and placebo test blocks, successive phasic stimuli were delivered with the temperature rising from baseline at a rate of 4°C/s. and maintained for 5 sec at target intensity. The beginning of each stimulus was preceded by a 5 s. auditory count-down, and successive stimulus onsets were separated by 60 s intervals to minimize the risk of local sensitization.

During the conditioning block, a sequence of 8 stimuli was delivered to both the control and placebo site. Whereas the control site was stimulated at a moderate pain level (50-60/100 on the pain intensity scale) determined individually based on the first calibration block, the temperature of the stimuli applied to the placebo site was surreptitiously decreased by 2°C compared to the control site. This manipulation was intended to provide participants with an unambiguous experience of analgesia. In the placebo testing block, subjects received 5 thermal stimuli of the same predetermined moderate pain stimulation level on each arm.

Experimental design

The 38 participants were divided into three groups. In group 1 (n=12; Fig. 1a), familiarization and calibration blocks were performed at 8:00 P.M. and the conditioning procedure began at 9:00 P.M. Subjects were then prepared for nocturnal polysomnographic recording using standard electroencephalographic, electro-oculographic, and electromyographic measures. Lights were turned off at 11:00 P.M. and subjects were awakened at 7:00 A.M. the next morning. Placebo analgesia was evaluated 2 hr after waking (9:00 A.M.). In group 2 (n=13; Fig. 1b), familiarization and calibration blocks were performed at 8:00 A.M., conditioning manipulation

started at 9:00 A.M., and placebo analgesia was tested in the evening at 9:00 P.M., 12 hr after conditioning, to control for sleep-independent effects associated with the simple passage of time. Subjects in this group were free to carry out their normal daily activities, but were asked not to nap. Group 3 (n=13; Fig. 1c) followed a design similar to group 1, with the addition of an evening placebo test introduced 30 min post-conditioning (placebo test 1) to evaluate placebo effects before sleep. This group was tested again the next morning (placebo test 2), as in group 1. The addition of an evening placebo test in group 3 was used to test for the effect of the variable daytime delay between the conditioning manipulations and the evening placebo test (12 hr in group 2; 30 min in group 3, test 1) and was expected to provide subjects with a more ambiguous experience of analgesia in the evening compared to conditioning alone (group 1). At the conclusion of the study (9:30 A.M. for groups 1 and 3, and 9:30 P.M. for group 2), another calibration block was administered on the control arm of all the participants.

Dependent variables: **A - Pain ratings**

Subjective evaluations of pain intensity and unpleasantness were obtained using a 15 cm mechanical visual analogue scale (Price et al., 1983). Sensory VAS was anchored at the left by the descriptor “no pain sensation” and at the right by the descriptor “most intense pain imaginable.” Similarly, affective VAS was anchored at the left and right by the respective descriptors “not at all unpleasant” and “most unpleasant pain imaginable.” These two scales were also used to rate expected and

remembered pain. All ratings were linearly converted to a numerical value from 0 to 100.

Expected pain. Expected pain intensity and unpleasantness were prospectively acquired at the beginning of the conditioning and testing blocks. Participants were asked, "What do you expect the pain intensity/unpleasantness to be without/with the analgesic cream?"

Concurrent pain. In the conditioning and testing blocks, participants were asked to rate the intensity and unpleasantness of the pain felt immediately after each stimulation.

Remembered pain. Approximately 2 minutes after the end of the placebo testing block, participants were asked to retrospectively rate the overall pain felt on the control and placebo sites: "Retrospectively, what was the overall pain intensity/unpleasantness felt without/with the analgesic cream?"

B- Polysomnographic measures

Sleep stage was monitored using standard polysomnographic methods. Electrodes were positioned at Cz, Fz, and Oz according to the International 10–20 System (Munday, 2005). Linked earlobes (A1 + A2) were used as a reference. Sleep stages were determined according to the guidelines of Rechtschaffen and Kales (Rechtschaffen and Kales, 1968) by experienced technicians blind to the aim of study and group assignment. All polysomnographic traces were reviewed by two independent technicians to extract the following dependent variables. The relative duration of the different sleep stages, expressed as the percent of time spent in each

stage, was calculated by dividing the total amount in minutes by the total sleep time. The number of atonic REM events was obtained from the number of 2-second milliepochs of REM sleep where muscle activity was absent. Sleep latency corresponded to the time between lights off and the beginning of the first sleep episode, and sleep efficiency was calculated by dividing the duration of sleep by the overall duration of the sleep period (including night-time awakenings). REM sleep latency corresponds to the delay between the first sleep event and the first REM sleep episode.

C- Psychomotor vigilance task and questionnaires

To control for potential fluctuations in vigilance and subjective sleepiness, a 10-minute psychomotor vigilance task (PVT) and the Karolinska Sleepiness Scale (KSS) were administered to all subjects at the beginning and end of the study. In addition, the Beck Anxiety Inventory (BAI), the Pain Catastrophizing Scale (PCS) and the Life Orientation Test-Revised (LOT-R) were administered to all subjects.

Statistical analysis

Data are presented as mean ± SEM. The placebo effect for expected, concurrent, and remembered pain intensity and unpleasantness ratings obtained in the placebo test performed 12 hr post-conditioning was analyzed using 3 (groups) x 2 (treatments) ANOVAs. An additional ANOVA was performed to compare the evening placebo response of group 3 (placebo test 1) and group 2 to assess the simple effect of the delay between the conditioning block and the placebo test (group 3 test

1: 30 min; group 2: 12 hr). Analgesia was calculated as the difference (control – placebo) in VAS ratings of intensity and unpleasantness for expected, concurrent, and remembered pain. Placebo analgesia did not vary with the laterality of the placebo test (dominant Vs non-dominant arm; all p's > 0.05) and this factor was not considered further in the analysis. Analgesia scores were used to calculate Pearson-r correlations and estimate explained variance (R^2). Additionally, independent T-tests were used when appropriate, and the non-parametric Kruskal-Wallis test was used to assess score differences in questionnaires. Statistical analyses were performed with SPSS 16.0 and global coincidence tests were carried out with Prism 5.0. The global coincidence test allows the comparison of entire curves (slopes and intercepts) using an F test. To do so, the sum-of-square and degrees of freedom from the best-fit of each curve is added and compared to a combined model where the slopes and intercepts are shared among the data sets. All statistical tests were performed at α = 0.05 and multiple comparisons were accounted for using Bonferroni adjustments. Effect sizes for main effects and interactions (ANOVAs) were calculated using partial eta-squared (η_p^2), and pairwise contrasts were calculated using Cohen's *d* with Hedges-bias correction (Cohen, 1988) .

RESULTS

Effects of Conditioning

As the experimental design involved pain stimuli applied in the morning and evening, two calibration blocks were performed for all 38 subjects in order to assess potential variations in pain with regards to circadian phase. The results show that the

temperature required to induce moderate pain was similar in the morning and evening calibration blocks (48.5°C ± 0.2 vs 48.4°C ± 0.2, $F(1,35)=0.355$, $p=0.56$). In the conditioning block, the stimulus intensities used for each individual were adjusted based on the first calibration block in order to generate moderate pain in control condition (average of 50.7 ± 3.3 VAS units on the pain intensity scale). The overall mean temperatures used in the conditioning block for control and placebo conditions were 48.7°C ± 0.1 and 46.7°C ± 0.1, respectively. Expected pain intensity reductions (control – placebo) induced by the placebo cream before conditioning procedures were comparable across groups (groups 1-2-3: 16.6 ± 3.2 vs. 14.2 ± 2.7 vs. 18.4 ± 3.6 VAS units; $F(2,35)=0.44$, $p=0.65$). Similarly, the three groups reported comparable levels of analgesia in concurrent pain intensity ratings during the conditioning manipulations (groups 1-2-3: 19.0 ± 4.2 vs. 21.8 ± 3.5 vs. 20.2 ± 4.1 VAS units; $F(2,35)=0.13$, $p=0.88$). Comparable results were found for expected pain unpleasantness reductions ($F(2,35)=0.78$, $p=0.47$) and concurrent pain unpleasantness reductions ($F(2,35)=0.10$, $p=0.90$) during the conditioning manipulations. These results indicate that, before any manipulation, all subjects displayed similar initial expectations toward the proposed treatment, and that the conditioning procedures induced a comparable experience of pain relief across the three groups.

Assessment of placebo effects and mediating influence of expectations

For all 38 participants, placebo analgesia was evaluated 12 hr post-conditioning. Expected, concurrent, and remembered pain intensity and

unpleasantness ratings for control and placebo sites are summarized in Figure 2. The results show that, 12 hr post-conditioning, subjects expected a substantial reduction in pain intensity after placebo treatment ($F(1,35)=108.4$, $p< 0.001$, $\eta_p^2 = 0.756$), which was comparable across groups (main effect of group: $F(2,35)=0.54$, $p=0.59$; interaction: $F(2,35)=0.45$, $p=0.64$). Similar results were found for expected pain unpleasantness reductions (Main effect of placebo treatment: $F(1,35)=63.46$, $p< 0.001$, $\eta_p^2 = 0.645$; main effect of group: $F(2,35)=0.01$, $p=0.99$; interaction: $F(2,35)=0.14$, $p=0.87$).

Analysis of variance of concurrent pain intensity during the testing block revealed a main effect of placebo treatment ($F(1,35)=15.68$, $p< 0.001$, $\eta_p^2 = 0.309$), with no significant effect of, or interaction with, groups (main effect of group: $F(2,35)=0.05$, $p=0.95$; interaction: $F(2,35)=0.54$, $p=0.587$). Likewise, the application of a placebo considerably reduced concurrent pain unpleasantness ($F(1,35)=11.55$, $p< 0.002$, $\eta_p^2 = 0.248$), with no effect of, or interaction with, groups (main effect of group: $F(2,35)=0.19$, $p=0.829$; interaction: $F(2,35)=0.36$, $p=0.697$). These effects corresponds to a mean decrease of 13.5% ± 3.9% in concurrent pain intensity ($p=0.001$) and a 14.3% ± 4.5% decrease in concurrent pain unpleasantness ($p=0.003$). Similarly, retrospective pain intensity evaluations revealed significant analgesia induced by the placebo treatment ($F(1,35)=17.97$, $p<0.001$, $\eta_p^2 = 0.339$), with no effect of, or interaction with, groups (main effect of group: $F(2,35)=0.19$, $p=0.829$; interaction: $F(2,35)=0.72$, $p=0.495$). Retrospective pain unpleasantness showed a similar pattern (Main effect of placebo treatment: $F(1,35)=11.37$, $p=0.002$, $\eta_p^2 = 0.245$; main effect of group: $F(2,35)=0.53$, $p=0.592$; interaction: $F(2,35)=0.87$,

p=0.426). This demonstrates that, 12 hr after the conditioning manipulations, a significant placebo effect was observed across all three groups.

In group 3, placebo effects were also measured in the evening, 30 minutes after the completion of the conditioning block. When compared to the placebo effects also measured in the evening for subjects in group 2 but 12 hr after conditioning, no significant differences were noted between groups ($F(1,24)=0.50$, $p=0.49$), and the significant effect of placebo treatment ($F(1,24)=9.64$, $p=0.005$) did not show any interaction with groups ($F(1,24)=0.30$, $p=0.59$). These results indicate that the daytime delay between the conditioning block and the placebo test did not alter the magnitude of placebo response.

Table 1 summarizes the placebo responses measured during the testing block in each group. In group 1, a sleep episode was present between the conditioning procedures and the testing block, and subjects in this group displayed a strong analgesic effect 12 hr after conditioning for both concurrent and remembered relief, with expectations predicting as much as 77% and 74% of pain intensity reductions (R^2). In group 2, the experimental manipulations were carried out during the daytime, and subjects were tested 12 hr post-conditioning without having slept. A strong placebo effect was also reported, but the predictive value of expectations on concurrent and remembered placebo response decreased to 38% and 44%, respectively. These results suggest that sleep may contribute to the consolidation of the mediating effect of expectations on placebo responses. However, because the lack of EEG fitting in the control daytime group 2 might have prevented the reinforcement of expectations by exposure to a more elaborate experimental protocol, the difference

between the initial expectations and the expected reliefs measured 12 hours later was assessed. The results show that expectations in both groups were increased to a similar extend by the experimental manipulations (Main effect: $F(1,23)=8.07$, $p=0.009$; Interaction: $F(1,23)=0.24$, $p=0.63$; Main effect of group: $F(1,23)=0.80$, $p=0.38$), which suggest that the exposure to EEG in group 1 did not further enhance expectations when compared to group 2.

Subjects in Group 3 were tested both before and after sleep, with a moderate level of repeatability ($r=0.52$, $p=0.066$), consistent with previous studies (Wager et al., 2004). The first test was conducted in the evening 30 min after conditioning, and showed the presence of an analgesic effect of placebo treatment, although expectations were only marginally associated with 24% of the concurrent pain intensity reductions. These results are consistent with the effects measured in group 2, and suggest that when placebo analgesia was evaluated in the evening after either a short or long delay without the presence of sleep, expectations accounted for 24% to 38% of the placebo effect observed in concurrent pain intensity reports. These expectation-related responses contrasted with the effects observed in group 1.

The second placebo-test block in group 3 was performed in the morning, 12 hr post-conditioning. The results show a moderate placebo effect in the morning for both concurrent and remembered pain intensity and unpleasantness reductions (Table 1). In addition, placebo-related expectations did not significantly predict changes in concurrent or remembered pain. When compared to subjects in group 1 who only underwent the conditioning block prior to sleep , the global coincidence test revealed that the correlations between expected relief and pain intensity reductions as well as

pain unpleasantness reductions measured in the morning in those two groups were significantly different (concurrent intensity: $F(2,21)=7.02$, $p=0.0046$; remembered intensity: $F(2,21)=9.09$, $p=0.0014$; concurrent unpleasantness: $F(2,21)=7.62$, $p=0.0033$; remembered unpleasantness: $F(2,21)=12.58$, $p=0.0003$). These results indicate that the presence of an evening testing block in addition to the conditioning manipulations prior to sleep hinders the establishment of expectation-mediated analgesic effects the following morning and changes the relationship between expected relief and placebo analgesia.

Control parameters: vigilance, sleep quality and questionnaires

A 10-minute psychomotor vigilance task administered at the beginning and end of the study revealed no significant differences in vigilance between the three groups (main effect of group for mean reaction time: $F(2,34)=1.12$, $p=0.34$), nor between the time of testing ($F(1,34)=0.54$, $p=0.47$; interaction: $F(2,34)=0.04$, $p=0.96$). Subjective sleepiness assessed by the Karolinska Sleepiness Scale showed comparable results at the beginning of the study ($H(2)=0.989$, $p=0.610$; Kruskal Wallis), but group 1 reported less sleepiness at the end of the experiment ($H(2)=6.617$, $p=0.037$; Kruskal Wallis). To verify that sleep quality was comparable between the two night groups, different sleep parameters were assessed, as reported in Table 2. Overall, there were no differences in sleep latency, sleep duration, number of awakenings, or sleep efficiency between group 1 and group 3 (all p's>0.05; Independent T-test). In addition, the 25 participants who slept in the laboratory (groups 1 and 3) spent 57.7 ± 1.6% of their total sleep time in stage 2 sleep, 15.2 ±

1.4% in slow wave sleep, and 20.3 ± 0.9% in REM sleep. A normal 7.5 hr sleep period in young healthy adults generally constitutes 45 to 55% stage 2 sleep, 13 to 23% slow wave sleep, and 20 to 25% REM sleep, with wakefulness accounting for around 5% (Kryger et al., 2005).

Psychological measures of anxiety, catastrophising and optimism were also compiled for each subject. Scores from the Beck Anxiety Inventory, the Pain Catastrophizing Scale or the Life Orientation Test-Revised did not reveal any differences between the 3 groups (Kruskal-Wallis, BAI: $H(2)=0.744$, $p=0.69$; PCS: $H(2)=0.462$, $p=0.79$; LOT-R: $H(2)=2.785$, $p=0.25$). Furthermore, they did not show any significant correlation with any measures of placebo analgesia or sleep variables, either as a whole or within each group (Spearman, all p's > 0.05). These results suggest that the personality traits assessed here did not have a significant mediating effect on either sleep or placebo susceptibility, nor could they explain potential groups differences.

Effect of placebo manipulations on sleep architecture

Comparative analysis of polysomnographic recordings between the 2 night groups revealed that subjects in group 1 displayed a higher proportion of stage 2 sleep then group 3 (61.4% ± 2.0% vs. 54.3% ± 1.9%, $F(1,21)=6.18$, $p=0.021$) and spent a lesser proportion of their total sleep time in REM sleep (18.7% ± 1.5% vs. 21.7% ± 1.1%, $F(1,21)=4.69$, $p=0.042$; Fig. 3). Because the placebo manipulations were designed to alter expectations and placebo responses, their influence on sleep profiles was also analyzed by dividing the groups into placebo responders (n=12) and

non-responders (n=13) based on a 20% decrease in concurrent pain intensity ratings (e.g., (Zubieta et al., 2005), which here corresponded to a large effect size at $d = 0.82$, and divided the experimental groups close to the median. A significant interaction was observed, whereby only placebo-responders in group 1 showed a significant reduction in the percent of total sleep time spent in REM ($F(1,21)= 6.61$, $p=0.018$; Fig. 3). This REM sleep decrease corresponds to a remarkable 30.2-minute difference from an average of 95.5 minutes for the other subgroups. Further analysis revealed that the placebo response influenced REM sleep latency correspondingly, where placebo-responders had significantly longer REM latency than non-responders (125.96 min ± 12.18 min vs. 88.11 min. ± 11.57 min; $F(1,21)=5.08$; $p=0.035$). These results demonstrate that profile differences in evening exposure to the placebo significantly altered sleep architecture, and that the reduction in REM sleep observed in placebo-responders in group 1 is related to extended REM sleep latency.

To further explore the relationship between REM sleep, expectation, and placebo analgesia, we correlated the morning analgesia scores with the percent of REM sleep in all subjects for each of the two night groups (Fig. 4). In group 1, expected, concurrent, and remembered analgesia for both intensity and unpleasantness were negatively correlated with the proportion of REM sleep (trend at $r = -0.521$, $p=0.082$ for concurrent pain intensity relief, all other p's < 0.05; Pearson-r and p-values are reported in Fig 4). In this condition, it appears that subjects who spent a lesser proportion of their total sleep time in REM sleep expected, experienced, and remembered higher treatment-induced relief in the placebo test performed the next morning. Conversely, REM sleep measured in subjects in group 3

was *positively* correlated with the analgesia expected in the evening (intensity: r=0.626, p=0.022; unpleasantness: r=0.604, p=0.029) and morning placebo tests (see Figure 4). However, in this group, changes in REM sleep were not significantly related to either concurrent or remembered placebo analgesia in the morning (see Figure 4), and only marginally associated with concurrent placebo analgesia measured in the preceding evening (intensity: r=0.519, p=0.069; unpleasantness: r=0.525, p=0.065). Similar results were found for both groups when analgesia scores were correlated with the number of atonic REM events (Suppl. Fig. 1). In addition, global coincidence analysis revealed that the correlations established between expected analgesia in the morning and REM sleep in groups 1 and 3 were significantly different (intensity: $F(2,21)=5.21$, $p=0.0145$; unpleasantness: $F(2,21)=8.65$, $p=0.0018$). These findings indicate that the relation between REM sleep and both expectations and placebo analgesia experienced in the morning are critically influenced by prior experience with the treatment in the preceding evening.

DISCUSSION

Effect of sleep on the integration of new expectations

In the present study, we adapted a placebo analgesia induction protocol previously used by others (Price et al., 1999; Petrovic et al., 2002; Wager et al., 2004; Bingel et al., 2006; Wager et al., 2007) to investigate the effect of sleep on the development of expectation-related placebo responses. Suggestion of pain relief and sensory conditioning were used to create analgesic expectations towards a placebo cream, and placebo responses were measured 12 hr post-conditioning. As previously

shown (Montgomery and Kirsch, 1997; Price et al., 1999; Benedetti et al., 2003), expectations were a significant mediator of the analgesic effects, but their relative contribution varied considerably when the post-conditioning delay included a sleep period. In this condition, expectations were tightly correlated with placebo analgesia and predicted as much as 77% of the effect. The acquisition of expectations leading to changes in perception can be viewed in the general framework of episodic memory formation in which new information is acquired, consolidated, and recalled. In the present experiment, expectations of relief induced experimentally were gradually integrated into an existing network of personal beliefs and past experience and were evoked in subsequent testing. During sleep, the reactivation of memory traces of recent waking events have been demonstrated in both animals (Wilson and McNaughton, 1994; Louie and Wilson, 2001) and humans (Maquet et al., 2000; Peigneux et al., 2004), and this process is proposed to consolidate various types of learning. Because placebo effects can be at least partly attributed to learning effects (Voudouris et al., 1990; Colloca et al., 2008), sleep might enhance the integration of complex experiences and reinforce the impact of expectations on subsequent perceptions. Our results suggest that sleep-dependent processing of the information acquired during conditioning enhanced the integration of new expectations, which in turn strongly modulated experienced relief. In addition, because the magnitude of the analgesic effect was not significantly different between groups, our results indicate that sleep might act specifically on the expectation-mediated component of the analgesic effect by strengthening the association between expectations and analgesia,

while other independent factors might also contribute to the overall extent of the effect.

Expectations and REM sleep

Because the mediating role of expectations in placebo analgesia appeared to be facilitated by the presence of a sleep period, changes in the physiological features of post-conditioning sleep could potentially reflect this process. In group 1, the exposure to treatment prior to sleep was manipulated in the conditioning phase in order to generate a convincing experience of analgesia, which translated into a robust expectation-mediated placebo response the next morning. In this condition, reduction in REM sleep was associated with stronger expectations of pain relief and stronger placebo effects the next morning. Consistently, placebo responders specifically exhibited the greatest reduction in REM sleep (and correspondingly longer REM sleep latency) compared to non-responders, and compared to the normal sleep architecture generally observed in young healthy subjects, as previously described in our laboratory and others (Aslan et al., 2002; Shaw et al., 2005). Moreover, the decrease in REM sleep not only reflected individual expectations, but also predicted concurrent pain unpleasantness reductions and remembered relief in the morning placebo test. Previous preliminary data in healthy individuals revealed a relationship between individual variation in REM sleep and central pain-modulatory processes, such that subjects who normally express a greater percentage of REM sleep have heightened central sensitivity to pain through increased supraspinal pain facilitation

(Smith et al., 2005). This is consistent with the present results demonstrating stronger expectation-related pain relief in individuals showing reduced REM sleep.

Sleep architecture changes have been observed in the night following training in a variety of tasks (for a review see (Smith, 1995; Rauchs et al., 2005), but to our knowledge, the learned association between a placebo treatment and analgesia has never been studied in relation to sleep. Nevertheless, the consolidation of episodic memories and the reprocessing of complex cognitive tasks have been previously linked with REM sleep alterations (Smith, 2001; Rauchs et al., 2004). In the current study, when positive expectations were accompanied by a consistent sensory experience prior to sleep (group 1), placebo responders specifically displayed a reduction in the amount of time they spent in REM sleep. Because high expectations likely reflect a concordance between the new episode and previous beliefs, REM sleep reductions could reflect a limited need to seek implicit information and restructuration. Indeed, processes occurring during REM sleep have been proposed to assist neurocognitive searches for novel interpretations (Maquet et al., 2003) and to be involved in the discovery of hidden associative rules (Walker et al., 2002). For example, in a paradigm of implicit sequence learning, REM sleep has been shown to be particularly important for the acquisition of the implicit rule rather than the motor component, which suggests that REM sleep in particular is involved in the consolidation of implicitly acquired complex relationships (Peigneux et al., 2003). In this view, if the new experience is coherent with previously consolidated beliefs, its integration into a network of older related memories could be facilitated, and the requirement for cognitive reappraisal during REM sleep would therefore be

diminished. Conversely, in subjects in group 3, the presence of the conditioning manipulations and a testing block generated a more inconsistent sensory experience prior to sleep. Notwithstanding, high expectations of relief were still observed in some participants, but contrary to the first night group, the results indicate that subjects who anticipated more relief spent a higher percent of their sleep time in REM sleep. In light of the proposed role of REM in the reprocessing of implicit information, this positive relation suggests that REM sleep contributed to the preservation of positive expectations despite the implicit sensorial conflict. Interestingly, in this context, expectations did not emerge as an important mediator of the analgesic effects measured the next morning, and REM sleep did not predict placebo effects. This suggests that, when sensory input is inconsistent, sleep might promote the dissociation of expectations from the observed placebo effect, and that the residual morning placebo response observed in group 3 may reflect a persistent effect independent of both expectations and changes in REM sleep.

Taken together, these findings show that the relative duration of REM sleep can predict expectation-mediated analgesic effects, but that subtle differences in prior experiences can dramatically alter this relationship. The degree of certainty associated with an expectation is another important mediating factor that triggers different emotional states, which can in turn significantly influence pain perception (Ploghaus et al., 2003). Whereas certainty that a particular aversive event is impending reduces pain sensitivity, uncertain expectations may generate anxiety-mediated hyperalgesia (Ploghaus et al., 2001). In the current study, it is possible that the inconsistent sensory inputs delivered to participants before sleep in group 3 may

have generated a mediating emotional state that necessitated further REM-dependent cognitive reprocessing, particularly in subjects displaying high expectations. This is in line with previous findings that the strength and direction of the relationship between waking experiences and REM sleep can be moderated by situational and emotional factors (Germain et al., 2003). However, in the present study, the psychological variables assessed (anxiety, pain catastrophizing, optimism) could not explain the effects observed, suggesting that the mediating emotional states induced by the placebo manipulations were not associated with such traits, but rather with a contextual transient emotional set. Future investigations using direct measures of certainty and anticipatory emotions generated by discordant cognitive information and sensory inputs are needed to better understand the exact mindset of subjects exposed to a placebo treatment and how it influences sleep architecture.

In addition to the effects observed on REM sleep, stage 2 sleep was also altered by the evening placebo manipulations. Along with the decrease in REM sleep, subjects exposed solely to the conditioning block before sleep displayed an increase in stage 2 sleep. Although stage 2 sleep has mainly been associated with the acquisition of simple procedural skills (Peters et al., 2007), its involvement in the consolidation of episodic memories has also been proposed (Rauchs et al., 2005; Fogel and Smith, 2006). In this view, whereas REM sleep might have been implicated in the reprocessing of implicit information associated with the episode, stage 2 sleep could underlie the maintenance of a different aspect of the experience. Alternatively, as stage 2 sleep did not correlate with placebo analgesia measured in the subsequent testing blocks (not reported in detail here), the increase in stage 2

sleep observed in this group might reflect a simple compensatory mechanism that counterbalanced the decrease in REM sleep. As the current experimental manipulations admittedly involved many types of memory systems, the interactions with sleep were likely to be complex, and different sleep stages could have been implicated in the processing of various dimensions of the task.

Clinical relevance

Although the evidence linking placebo responses and sleep architecture is scarce, an interesting study demonstrated that patients with panic disorders responding to either pharmacological or placebo treatments exhibited normalized sleep patterns, characterized by a reduction in the percent of REM sleep (Baker et al., 2003). Because anxiety is believed to be one of the factors involved in placebo effects (Benedetti et al., 2005; Price et al., 2008), the authors postulated that the reduction of REM sleep observed after placebo administration reflected diminished anxiety and that the amount of REM sleep after exposure to a treatment might represent a correlate of therapeutic response. Interestingly, the current investigation, performed in healthy individuals, revealed a very similar reduction in REM sleep in placebo responders to that described by Baker and colleagues (14.9% vs. 14.7%; (Baker et al., 2003). Taken together, these findings suggest that the generation of a transient positive emotional state induced by relief expectation may significantly alter sleep profiles, and that reduction in REM sleep might represent a correlate of expectation-mediated therapeutic responses.

Table 1: Concurrent and remembered placebo effects and correlations between expected relief and concurrent and remembered pain reductions across groups and placebo tests. Cohen's d with Hedges-bias correction was used to evaluate effect size and Pearson's correlation coefficients (r) were established between expected, concurrent, and remembered analgesia scores. Analgesia scores correspond to the difference between pain ratings at the control and placebo stimulation sites.

a) Pain intensity

	N	Concurrent analgesia Cohen's d (CI)	Remembered analgesia Cohen's d (CI)	Expected X Concurrent; Pearson-r	Expected X Remembered; Pearson-r
Group 1 (Overnight delay; morning test)	12	1.16 (0.29 – 2.02)	1.17 (0.31 – 2.04)	0.88 ***	0.86 ***
Group 2 (Daytime delay; evening test)	13	0.93 (0.12 – 1.74)	1.34 (0.49 – 2.19)	0.61 *	0.66 *
Group 3 Test 1 (No delay; evening test)	13	0.80 (0.00 – 1.60)	0.89 (0.08 – 1.69)	0.49 †	0.12
Group 3 Test 2 (Overnight delay; morning test)	13	0.65 (-0.14 – 1.43)	0.54 (-0.24 – 1.32)	-0.23	-0.43

b) Pain unpleasantness

	N	Concurrent analgesia Cohen's d (CI)	Remembered analgesia Cohen's d (CI)	Expected X Concurrent; Pearson-r	Expected X Remembered; Pearson-r
Group 1 (Overnight delay; morning test)	12	0.95 (0.11 – 1.80)	0.93 (0.09 – 1.77)	0.83 ***	0.87 ***
Group 2 (Daytime delay; evening test)	13	0.89 (0.08 – 1.69)	1.29 (0.45 – 2.14)	0.68 **	0.78 **
Group 3 Test 1 (No delay; evening test)	13	0.57 (-0.21 – 1.36)	1.30 (0.45 – 2.14)	0.31	0.05
Group 3 Test 2 (Overnight delay; morning test)	13	0.54 (-0.24 – 1.32)	0.41 (-0.36 – 1.19)	-0.35	-0.49 †

CI: confidence interval. ***p < 0.001, **p < 0.01, *p < 0.05, † p = 0.09.

Table 2: Control sleep parameters for the 25 subjects recorded with polysomnography.

	Sleep latency (min)	Sleep duration (min)	Number of awakenings	Sleep efficiency (%)
Group 1	12.93 ± 4.39	441.91 ± 8.54	33.42 ± 3.06	92.73 ±1.09
Group 3	12.74 ± 2.94	434.56 ± 7.01	26.15 ± 2.38	93.93 ± 1.03

FIGURE LEGEND

Figure 1. Time course of experimental events. Stimuli were given in 4 different experimental blocks: familiarisation, calibration, conditioning, and testing block. (a) Group 1 (night group, n=12) (b) Group 2 (daytime group, no sleep, n=13) (c) Group 3 (night group, two placebo testing blocks, n=13).

Figure 2. Expected, concurrent, and remembered pain intensity (INT) and unpleasantness (UNP) measured 12 hr post-conditioning on control and placebo sites for all 38 subjects. Paired T-test; ***P< 0.001, **P < 0.01.

Figure 3. Sleep architecture of placebo responders and non-responders of groups 1 and 3. Subjects in group 1 displayed higher proportions of stage 2 sleep and smaller proportions of REM sleep. The reduction in REM sleep was specific to placebo responders in group 1. ANOVA, *P < 0.05. Slow Wave Sleep (SWS).

Figure 4. Correlation of expected, concurrent, and remembered analgesia (control-placebo) in pain intensity and unpleasantness with % REM sleep measured in the night prior to the testing block in (a) group 1 and (b) group 3 – test 2 (morning). Pearson's r and corresponding p-values are reported on the graphs.

Suppl. Figure 1. Correlation of expected, concurrent, and remembered analgesia (control-placebo) in pain intensity and unpleasantness with the number of atonic

REM events measured in the night prior to the testing block in (a) group 1 and (b) group 3 – test 2 (morning). Pearson's r and corresponding p-values are reported on the graphs.

Figure 1

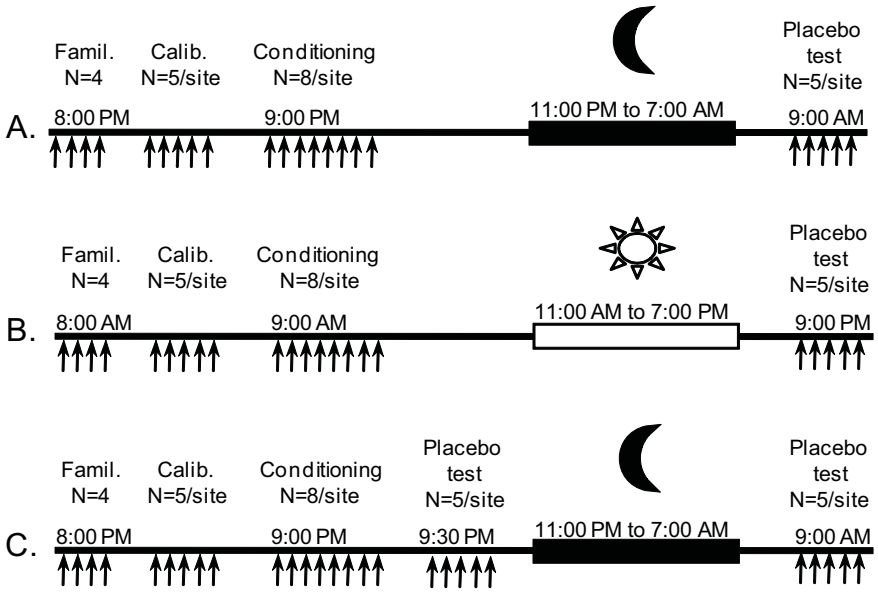


Figure 2

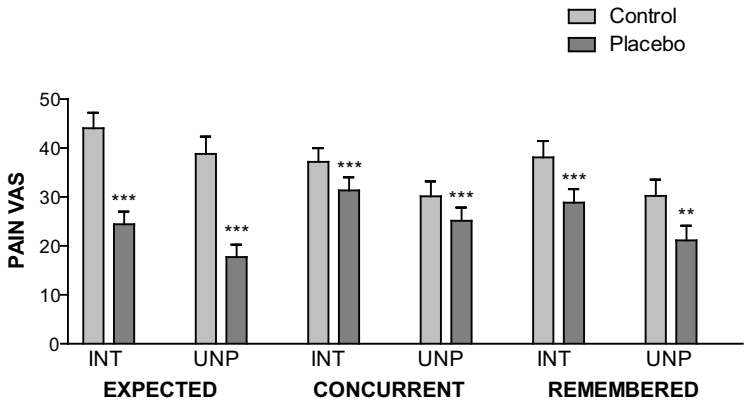


Figure 3

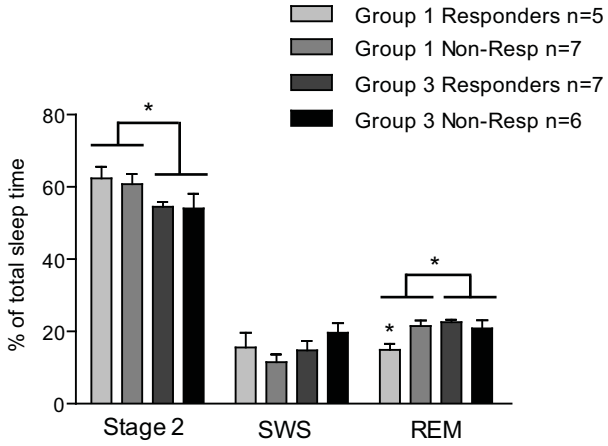


Figure 4

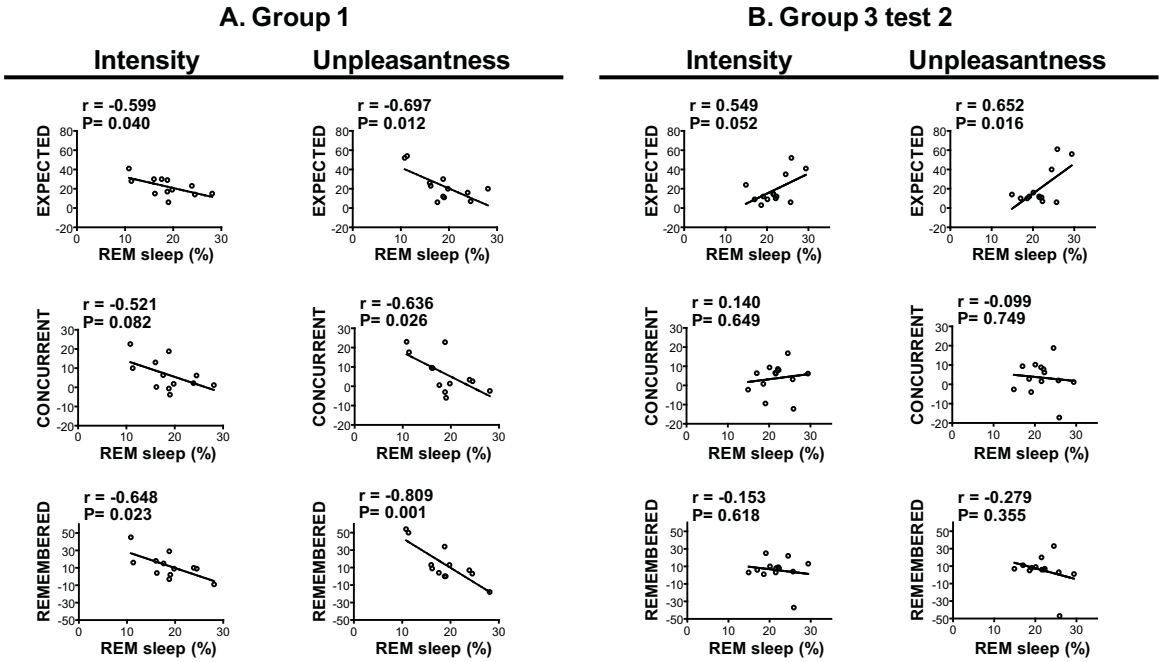
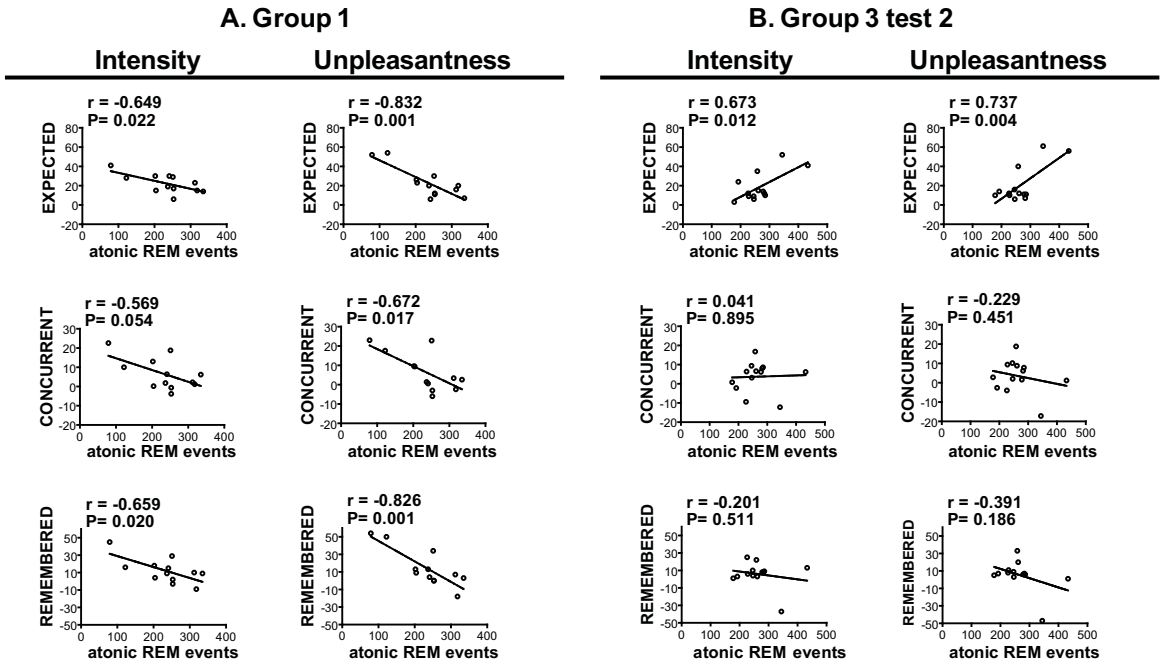


Figure S1


A. Group 1
Intensity
Unpleasantness
B. Group 3 test 2
Intensity
Unpleasantness
EXPECTED
CONCURRENT
REMEMBERED
atonic REM events
r = -0.649
P= 0.022
r = -0.832
P= 0.001
r = 0.673
P= 0.012
r = 0.737
P= 0.004
r = -0.569
P= 0.054
r = -0.672
P= 0.017
r = 0.041
P= 0.895
r = -0.229
P= 0.451
r = -0.659
P= 0.020
r = -0.826
P= 0.001
r = -0.201
P= 0.511
r = -0.391
P= 0.186

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DISCUSSION GÉNÉRALE

DISCUSSION

Démonstration d'un effet placebo à l'éveil

Bien que l'objectif de la première étude expérimentale ait été de déterminer si l'administration d'un placebo peut réduire la douleur chez des volontaires endormis ainsi que les perturbations du sommeil associées, il était important de montrer que les manipulations d'induction placebo ont engendré des attentes de soulagement ainsi qu'une analgésie lors de l'éveil. Ainsi, durant la phase initiale d'induction, les résultats ont montré que les sujets anticipaient une réduction significative de la douleur suite à l'application du placebo. De plus, pendant le block de conditionnement sensoriel, une diminution considérable de douleur a été enregistrée par les évaluations données immédiatement suite aux stimulations ainsi que celles recueillies à la fin du block d'essais. Ces résultats démontrent ainsi que les sujets présentaient des attentes initiales de réduction de douleur associée à la présence du traitement et qu'ils ont ressentit une expérience positive d'analgésie suite aux manipulations sensorielles. Additionnellement, ces attentes de soulagement de douleur se sont maintenues jusqu'au matin de la troisième session, alors que l'ampleur de l'analgésie placebo fut évaluée à l'éveil. Une réduction modeste, mais statistiquement significative a alors été observée grâce aux évaluations concomitantes et rétrospectives de douleur.

Des résultats similaires ont aussi été recueillis dans la deuxième étude présentée. Des attentes de soulagement ainsi que des réductions de douleur significatives ont été enregistrées lors de la phase de conditionnement sensoriel ainsi

que lors des blocks de test permettant d'évaluer l'ampleur de l'analgésie placebo induite. De plus, tel qu'attendu, le niveau de soulagement anticipé s'est avéré être un important médiateur des effets placebo rencontrés. Mis ensemble, ces résultats démontrent que le protocole d'induction adapté d'études précédentes (Price et al. 1999; Petrovic et al. 2002; Wager et al. 2004; Bingel et al. 2006; Wager et al. 2007) a permit l'établissement d'effets analgésiques placebo statistiquement significatifs à partir desquels il a été possible d'aborder les questions spécifiques des études de cette thèse.

Démonstration d'un effet placebo durant le sommeil

En premier lieu, la présence d'un effet placebo rencontré pendant le sommeil a été estimé indirectement par des évaluations subjectives rétrospectives de douleur. Ainsi, après avoir démontré que les sujets anticipaient vraisemblablement une réduction de la douleur nocturne et des perturbations de leur sommeil liées aux stimulations nociceptives, des évaluations rétrospectives recueillies au réveil ont révélées la présence d'effets considérables associés à l'application du traitement. Les sujets ont effectivement rapporté avoir ressenti une douleur moins intense et moins désagréable durant la nuit placebo comparé à la nuit contrôle. Ils ont aussi dit avoir été moins perturbé et moins anxieux et de se souvenir d'un nombre moindre de stimulation lors de la nuit placebo. L'ensemble de ces variables démontre qu'une analgésie placebo a bien été perçue, du moins rétrospectivement, chez des sujets endormis suite à l'application d'un traitement inerte. Certains essais cliniques ont aussi montré une amélioration subjective de la douleur nocturne ainsi que des

perturbations du sommeil suite à l'administration d'un placebo (Dogra et al. 2005; Richter et al. 2005; Breuer et al. 2006; Gabis et al. 2009). Bien que la présence d'une analgésie placebo significative soit difficile à évaluer dans ces études, il semble que la présence d'un placebo puisse modifier la perception de la douleur, et ce, même chez des patients endormis.

En plus d'évaluer la présence d'une analgésie placebo rétrospectivement, les réactions nocturnes associées aux stimulations nociceptives ont aussi été quantifiées directement dans les nuits contrôles et placebo. Malgré que l'intensité des stimulations ait été identique lors de ces deux nuits, des différences significatives ont été rencontrées suite aux stimulations présentées en sommeil REM et en SWS. En fait, les résultats démontrent que la présence du traitement placebo a engendré une diminution significative du taux global de réponses nocturnes en sommeil REM, associée principalement à une réduction du nombre d'éveils provoqués par les stimulations. Cette diminution de réactivité nocturne associée à la présence du placebo concorde avec la diminution des perturbations de sommeil rapportée subjectivement par les sujets au réveil de la nuit placebo. Par contre, durant les périodes de SWS, l'effet inverse a été noté. En effet, une augmentation du taux global de réponses nocturnes a été enregistrée en SWS durant la nuit placebo comparé à la nuit contrôle. Il semble donc que l'utilisation d'un traitement placebo peut modifier le taux de réactivité à la douleur expérimentale durant le sommeil en fonction du stade dans lequel les stimuli nociceptifs sont présentés.

L'anatomie fonctionnelle propre au sommeil REM et au SWS est un facteur pouvant expliquer les différences de réactivité nocturne observées. Durant le sommeil

REM, une importante activation des régions limbiques, telles l'amygdale et le cortex cingulaire antérieur ainsi que de la plupart des neurones du tronc cérébral a été notée (Maquet et al. 1996; Braun et al. 1997; Nofzinger et al. 1997; Buchsbaum et al. 2001; Siegel 2005). Ces régions sont aussi impliquées dans la modulation descendante de la douleur, notamment suite à l'induction d'une analgésie placebo chez des sujets éveillés (Petrovic et al. 2002; Wager et al. 2004; Bingel et al. 2006; Wager et al. 2007; Zubieta and Stohler 2009). Ainsi, il semble que le réseau neuronal associé aux réductions de douleur suite à l'administration d'un placebo est activé de manière importante en sommeil REM, ce qui pourrait expliquer le maintien d'une modulation de la douleur dépendante du placebo pendant le sommeil. À l'opposé, la relative désactivation de plusieurs régions corticales ainsi que la diminution générale de la connectivité fonctionnelle en SWS pourrait empêcher le recrutement des voies de modulation descendantes de la douleur (Braun et al. 1997; Maquet et al. 1997; Kajimura et al. 1999; Buchsbaum et al. 2001; Massimini et al. 2005).

Un autre facteur distinguant les stades de sommeil REM et SWS repose sur le niveau de traitement des informations sensorielles durant ces stades. Certaines études ont effectivement suggéré que la modulation de potentiels évoqués par des stimuli auditifs pendant le sommeil par certains facteurs tels la salience ou la déviance, pouvait être maintenue, particulièrement en sommeil REM (Bastuji et al. 1995; Pratt et al. 1999; Cote et al. 2001; Takahara et al. 2006). De plus, comme certains éléments des épisodes vécus avant l'endormissement peuvent être incorporés dans les rêves (Cipolli et al. 2004), il se peut que la réduction de la douleur ressentie à l'éveil et attribuée à la présence du traitement placebo soit réactivée durant les épisodes de

sommeil REM de la nuit subséquente. Juste avant l'endormissement de la nuit placebo, les sujets étaient effectivement soumis à une phase de conditionnement dans laquelle la température était subrepticement réduite.

Finalement, comme l'inertie de sommeil est plus importante suite aux réveils en stades lents profonds, comparativement à ceux en sommeil REM (Tassi and Muzet 2000), il est possible que, lors de l'évaluation du nombre de stimulation nocturnes perçues, les sujets se rapportaient spécifiquement à celles associées aux réveils en stade de sommeil REM, lesquelles étaient moins nombreuses dans la nuit placebo que dans la nuit contrôle. Ainsi, même si, en SWS, les stimulations nocturnes ont engendré un nombre de perturbations supérieur dans la nuit placebo, il se peut que celles-ci n'aient pas été retenues à cause de l'importante inertie de sommeil rencontrée dans ce stade. De cette façon, les expériences nocturnes les plus aptes à influencer les évaluations subjectives matinales pourraient être celles issues du sommeil REM. De plus, il a aussi été montré que la perception d'une douleur expérimentale est généralement réduite suite à un éveil en sommeil REM comparé aux autres stades (Daya and Bentley 2009).

Effet des attentes sur l'architecture du sommeil

En plus de démontrer la possibilité de moduler la réactivité à la douleur durant le sommeil par un agent placebo, la première étude s'est aussi intéressée à l'impact des attentes sur l'architecture du sommeil. Dans le but de produire des attentes de soulagement dans la nuit placebo, des attentes de douleur ont nécessairement été engendrées dans la nuit contrôle. Bien que les durées relatives du stade 2 de sommeil

n'aient pas montré de changements significatifs, les stades de sommeil REM et SWS ont été influencés par certaines variables d'anticipation dans les deux nuits expérimentales. Ainsi, dans la nuit contrôle, le niveau de douleur attendu s'est révélé être corrélé positivement avec la durée relative de SWS et négativement avec la durée relative de sommeil REM. Ces relations étaient partiellement médiée par le niveau d'anxiété, particulièrement pour le SWS. Il semble donc que, durant la nuit contrôle, l'anticipation d'une douleur nocturne élevée ait été associée à une augmentation de la quantité de sommeil lent profond et une diminution de sommeil REM. Cette augmentation de la représentation des stades de sommeil profond pourrait représenter un mécanisme de protection, permettant de maintenir la continuité du sommeil lorsqu'une douleur expérimentale est anticipée.

Lors de la nuit placebo, les variables associées au SWS ainsi qu'au sommeil REM ont aussi démontré une corrélation significative avec des variables d'anticipation. Par ailleurs, dans ce contexte, l'anxiété et non le niveau de douleur attendu s'est révélé être la variable d'influence. Ces résultats sont sans doute liés au fait que la douleur nocturne anticipée ait été relativement faible à cause de l'effet analgésique attendu. Ainsi, de façon similaire aux relations retrouvées dans la nuit contrôle, un niveau d'anxiété élevé a été associé à une augmentation de la quantité de SWS et une diminution du sommeil REM. Ensemble, ces résultats démontrent que l'anticipation de douleur et le niveau d'anxiété associé peut modifier l'architecture du sommeil. Plusieurs études ont effectivement montré des altérations du sommeil, particulièrement du sommeil REM, suite à l'induction ponctuelle d'anxiété par des épisodes à l'éveil (Baekeland et al. 1968; Koulack et al. 1985; Cartwright and Wood

1991; Reynolds et al. 1993; Buguet et al. 1998; Germain et al. 2003; Gitanjali and Ananth 2003).

La seconde étude présentée dans cette thèse montre aussi des modifications de l'architecture du sommeil en fonction d'attentes, mais celles-ci étant liées à des attentes de soulagement et non à des attentes de douleur nocturne. Dans cette étude, une analgésie placebo était induite par des suggestions verbales et un conditionnement sensoriel en soirée. L'enregistrement polysomnographique de la nuit subséquente a ensuite permit d'extraire certaines variables de sommeil et de les corréler avec les attentes de soulagement verbalisée le lendemain matin, lors du test visant à quantifier l'effet placebo. Les résultats ont montré que, chez un groupe ayant été exposé uniquement à une expérience positive d'analgésie, le niveau d'effet analgésique attendu était corrélé de façon significative avec la durée relative de sommeil REM. Ainsi, chez les sujets qui anticipaient un soulagement élevé de douleur, une diminution notable du sommeil REM était observée. Dans ce contexte, des attentes élevées de soulagement pourraient refléter un haut niveau de concordance entre les nouvelles informations présentées et les croyances individuelles préalables, ce qui nécessiterait vraisemblablement un faible niveau de traitement cognitif associé au sommeil REM. En parallèle avec les résultats de la première étude montrant une relation négative entre les attentes de douleur et la quantité de sommeil REM, il semble que le niveau d'attente lié à l'appréhension d'une douleur nocturne ou à l'efficacité d'un traitement analgésique puisse produire un état émotionnel suffisant pour modifier l'architecture de la période de sommeil à venir.

Effet du sommeil sur l'intégration de nouvelles attentes de soulagement

En plus de démontrer une relation entre les attentes de soulagement, l'analgésie placebo et le sommeil REM, la deuxième étude suggère aussi que la présence d'un épisode de sommeil, par rapport au simple passage du temps, favorise l'association entre les attentes de soulagement et l'ampleur de l'effet analgésique mesuré suite à l'administration d'un placebo. Comme un rôle cognitif du sommeil a souvent été proposé dans l'apprentissage et la mémorisation, et que l'effet placebo peut en partie découler d'un apprentissage associatif, il parait fort probable que le sommeil puisse favoriser l'intégration de nouvelles attentes, et, par conséquent, permettre la production d'effet placebo dépendant des attentes.

Une discussion exhaustive de cette thématique est présentée dans l'article de revue introduit à la suite de la présente section. L'invitation à rédiger cette revue a fait suite à la publication du deuxième article de recherche dans *Journal of Neuroscience*.

Conclusion générale

En résumé, la présente thèse a permit d'explorer la relation pouvant exister entre le sommeil et la production d'une analgésie placebo. Tout d'abord, il a été montré que, suite à des manipulations placebo, la génération d'attente de réduction de douleur durant le sommeil était suffisante pour produire une diminution de la douleur perçue rétrospectivement ainsi qu'une réduction des perturbations du sommeil associées aux stimulations nociceptives. Coïncidemment, durant le sommeil REM, le traitement placebo a engendré une diminution significative des réponses en sommeil suite aux stimulations nociceptives. Ces résultats suggèrent donc que la modulation de la douleur par un placebo demeure possible même en sommeil, en absence du recrutement conscient d'attentes de soulagement. Par ailleurs, la modulation de la douleur durant le sommeil semble dépendre du stade de sommeil dans lequel les stimuli nociceptifs sont administrés, puisqu'en SWS, le traitement placebo était associé à une augmentation de la réactivité nocturne. De plus, l'anticipation de douleur élevée durant la nuit contrôle était corrélée avec une plus forte représentation de SWS, ce qui suggère un approfondissement du sommeil afin de maintenir sa continuité.

Les résultats présentés ont aussi démontré que la présence d'un épisode de sommeil entre l'induction d'une analgésie placebo et son évaluation favorisait l'intégration des attentes de soulagement ainsi que la production d'effets analgésiques dépendants des attentes. De plus, il semble qu'un lien étroit puisse exister entre les attentes de soulagement, l'analgésie placebo et le sommeil REM, et que celui-ci est influencé par l'exposition préalable au traitement placebo. En effet,

alors qu'une expérience convaincante d'analgésie avant l'endormissement est associée avec une réduction du sommeil REM chez les sujets présentant de fortes attentes, une expérience incertaine d'analgésie est associée à une forte proportion de sommeil REM chez les sujets qui anticipent malgré tout un soulagement efficace. Ces résultats suggèrent donc que la durée relative de sommeil REM peut dépendre du niveau de traitement cognitif nécessaire à l'intégration de nouvelles attentes à l'intérieur des croyances individuelles préalables.

Comme les études présentées dans cette thèse sont les premières a s'intéresser spécifiquement au rôle du sommeil dans la production d'une analgésie placebo, plusieurs questions additionnelles peuvent être soulevées par celles-ci. Par exemple, comme le protocole d'induction placebo incluait des suggestions verbales ainsi qu'un conditionnement sensoriel, il serait intéressant de vérifier si une analgésie placebo engendrée suite à des suggestions verbales seules pourrait être mesurée durant le sommeil. Également, l'utilisation d'une déprivation sélective du sommeil REM suite à l'induction d'une analgésie placebo pourrait confirmer son rôle dans l'intégration de nouvelles attentes de soulagement. Considérant la nouveauté des concepts présentés dans cette thèse, une multitude de perspectives de recherche sont envisageables.

ARTICLE DE REVUE

Article 3

Relief expectation and sleep

Danièle Laverdure-Dupont[1,2], Pierre Rainville[3], Jacques Montplaisir[2], Gilles Lavigne[2,3]

1. Department of Physiology, Faculty of Medicine, University de Montréal, Montréal, Canada
2. Centre d'étude du Sommeil et des Rythmes Biologiques, Hôpital du Sacré-Cœur, Montréal, Canada
3. Department of Stomatology and Oral Health, Faculty of Dental Medicine, Université de Montréal, Montréal, Canada

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Corresponding author:
Danièle Laverdure-Dupont
Centre d'étude du Sommeil et des Rythmes Biologiques
Hôpital du Sacré-Cœur de Montréal
5400 boul. Gouin Ouest
Montréal, Québec, Canada
H4J 1C5
Tel: 514-338-2222, ext. 3745
Fax: 514-338-2694
[redacted]

Synopsis

Originally, a role of sleep in learning and memory formation was advocated following the observation of sleep-dependent performance enhancements at simple procedural tasks. With the use of more complex cognitive activities, additional stages of memory processing were proposed to benefit from sleep. In particular, REM sleep has been implicated in the integration of new information into associative networks as well in the abstraction of general rules allowing insightful behaviours. In a recent study, we extended these observations by demonstrating that, compared to wake, sleep can enhance the mediating effect of expectation on placebo-induced analgesia and that the individual amount of REM sleep is correlated with expected relief (Laverdure-Dupont et al. 2009). As placebo responses are adaptive behaviours which likely derive from the learned association between contextual cues and subsequent relief, these results are discussed in relation to the proposed roles of REM sleep in the integrative stages of memory processing. In light of the responsiveness of REM sleep to waking events, its expression is also proposed to reflect the cognitive demand associated with the assimilation of new expectation.

Introduction

Expectations originate from the integration of new information with past experiences and personal beliefs and can significantly shape future perceptions (Ploghaus et al. 2003; Koyama et al. 2005). In a therapeutic context, placebo responses are a common example of expectation-mediated effects which can originate from simple verbal suggestion of relief and pre-exposure to an effective

treatment (Voudouris et al. 1990; Montgomery and Kirsch 1997; Amanzio and Benedetti 1999; Price et al. 1999; De Pascalis et al. 2002; Benedetti et al. 2003). Although different mechanisms, such as response expectancy and classical conditioning, have been proposed to initiate clinical benefits following the administration of a placebo, placebo effects generally appear to rely on the learned association between contextual cues and subsequent relief (Benedetti et al. 2005; Kong et al. 2007; Price et al. 2008). Given the increasing evidence demonstrating a role of sleep in learning and memory (Smith 2001; Stickgold et al. 2001; Walker and Stickgold 2004; Rauchs et al. 2005; Stickgold 2005; Walker and Stickgold 2006), we recently investigated the effect of off-line processing on the consolidation of expectation-mediated effects (Laverdure-Dupont et al. 2009). Our results showed that the presence of a sleep period between the initial exposure to a treatment and subsequent testing can enhance the association between relief expectation and placebo analgesia, and that REM sleep in particular, reflects the individual level of expected relief. By suggesting the involvement of sleep in the processing and integration of context-dependent expectations, these observations extent the range of the potential cognitive functions of sleep.

Considering the novelty of the proposed relation between relief expectation and sleep, the present review will attempt at positing these findings in the context of the existing knowledge related to the cognitive functions of sleep as well as the mechanisms underlying expectation-related effects and placebo analgesia. This discussion is divided in four sections. First, we will briefly review some of the classic findings pointing to an involvement of sleep in learning and memory formation, with

a particular emphasis on complex cognitive tasks and the effects of REM sleep on their integration. Secondly, we will highlight the importance of the learned association between contextual cues and therapeutic outcome in the induction of placebo analgesia through the generation of relief expectation and classical conditioning. Thirdly, we will discuss some of the findings which indicate that sleep can facilitate the integration of new information into a related network and how this could enhance the assimilation of expectations leading to increased effects. Finally, the specific role of REM sleep in expectation processing will be examined in relation to its proposed role in dealing with cognitive demands and anxiety.

1- Cognitive functions of sleep

Although the exact functions of sleep are still being debated, there is little doubt that it is vital for emotional and physical well-being (Haack and Mullington 2005; Siegel 2005; Tononi and Cirelli 2006; Walker and Stickgold 2006). Beyond it putative physiological role, many studies have demonstrated that learning and memory formation benefit from the off-line processing believed to occur during sleep (for review see (Smith 2001; Walker and Stickgold 2004; Rauchs et al. 2005; Walker and Stickgold 2006; Diekelmann and Born 2010). Most often, the involvement of sleep in the acquisition of various skills or knowledge is being studied by correlating learning progress after training with sleep parameters on subsequent nights or by the use of total or partial sleep deprivation (Smith 2001). Using these approaches, very consistent findings have been observed in human studies of procedural memory. Performance gain at a visual texture discrimination task were demonstrated to be

sleep dependent and to correlate with the amount of slow wave and REM sleep measured on the first night after training (Karni et al. 1994; Stickgold et al. 2000a; Stickgold et al. 2000b; Mednick et al. 2003). Furthermore, selective deprivation of either of these sleep stages resulted in the loss of these performance enhancements (Karni et al. 1994; Gais et al. 2000). Similarly, a night of sleep induces an improvement in speed and accuracy at a sequential finger-tapping task that is not seen after an equivalent period of wake (Fischer et al. 2002; Walker et al. 2002a; Korman et al. 2003). On the other hand, early studies using simple declarative tasks such as verbal learning of unrelated word pairs have yield mixed results (Empson and Clarke 1970; Chernik 1972; Lewin and Glaubman 1975; Meienberg 1977; Plihal and Born 1997). Later findings however, did show actual improvements as well as modification of sleep characteristics following learning of a similar task (Gais et al. 2002; Gais and Born 2004). These discrepancies may be linked to the nature of the task, seeing that increased complexity (Empson and Clarke 1970; Tilley and Empson 1978; Kuriyama et al. 2004) or the presence of an emotional dimension embedded in the task enhances its degree of sleep dependency (Wagner et al. 2001; Sterpenich et al. 2009).

Although the attribution of a specific role in memory consolidation to a particular stage might be an overgeneralization, it appears that performance enhancements at procedural motor task could rely mainly on stage 2 sleep (Walker et al. 2002a) while perceptual learning could involve both slow wave and REM sleep (Gais et al. 2000; Stickgold et al. 2000b). On the other hand, declarative memory might benefit the most from slow wave sleep (Gais and Born 2004). In the case of

more complex cognitive tasks, which often involve the discovery and clarification of intricate concealed rules, REM sleep in particular has been implicated in performance improvements (Conway and Smith 1994; Smith 1995; 1996; Walker et al. 2002b; Peigneux et al. 2003). For example, in the tower of Hanoi task, which is a mathematical puzzle involving the displacement of discs on different rods by following specific rules, subject show most improvement after a night of sleep containing REM sleep (Conway and Smith 1994; Smith 1995; 1996; Rauchs et al. 2005; Stickgold 2005). In another study using the serial reaction time task containing a hidden probabilistic rule which defined stimulus sequence, Peigneux and colleagues observed a reactivation during post-training REM sleep reflecting the acquisition of this implicit rule rather than a simple replay associated with the visuomotor component of the task (Peigneux et al. 2003). This suggests that REM sleep is concerned with the reprocessing of high-order aspects of the task to extract contingencies.

The ability of REM sleep to promote complex cognitive processing was further demonstrated by the fact that subjects awaken from REM sleep were able to solve significantly more anagram word puzzles than those awaken from NREM sleep (Walker et al. 2002b). This task allows problem solving by applying semantic knowledge to new contexts and is believed to reflect cognitive flexibility. The hyper-associative nature of this sleep stage was also confirmed by a paradigm of semantic priming, which showed a greater priming effect for weakly related words than for strong primes after REM sleep awakening (Stickgold et al. 1999). More recently, Cai and colleagues used a remote associates test to examine the possibility that exposures

to items prior to sleep would facilitate the formation of new association especially for those primed elements (Cai et al. 2009). Their results indeed showed that sleep improved creative problem solving for items that were primed prior to sleep, but uniquely so after sleep periods that included REM sleep. Taken together these findings suggest that REM sleep facilitate the development of associative networks in order to integrate new information into related mnemonic representations. The capacity of REM sleep to process associative memories and thus promote insightful behaviour in the future may rely on its unique neurophysiological properties characterized by its widespread cortical activation as well as the increased information flow from the neocortex to the hippocampus (Buzsaki 1996; Maquet et al. 1996; Hobson et al. 1998; Stickgold et al. 2001). Under these assumptions, the collaboration of large-scale networks would permit better interpretation of new experiences in the context of pre-existing semantic memory stored within the neocortex (Walker 2009). Furthermore, the pronounced activation of limbic areas during this stage likely explains why REM sleep as been consistently involved in the processing of emotional memories (Stickgold et al. 2001; Wagner et al. 2001; Nishida et al. 2009; Walker 2009).

While the initial post-encoding steps of memory processing, often associated with performance enhancements or resistance to normal decay, seems to depend preferentially on NREM sleep, REM sleep could be involve in subsequent stages of memory integration (Walker and Stickgold 2010). Indeed, REM sleep appears to play an important role in the assimilation of new elements into related networks as well as in the abstraction of general rules that would facilitate better adaptation to events in

the future (Walker and Stickgold 2004; 2010). According to this sequential model of sleep-dependent learning, the various sleep stages would act in concert to support the progression of mnemonic representations from the static consolidation of new episodic items to their integration into networks of related information, perhaps leading to an anatomical reorganization of memory traces (Giuditta et al. 1995; Walker and Stickgold 2004; 2006). This process could ultimately insure the development of a common body of knowledge which would provide a mental structure to generate accurate predictions and associated expectations regarding events in the future. Thus, these REM-dependent integrative stages of memory processing would permit the extraction of general concepts from individual experiences and would represent a true correlate of associative learning.

2- The involvement of associative learning in the development of relief expectation and placebo analgesia

Expectations about a particular event are shaped by past experiences and personal beliefs and can significantly alter the perception of the upcoming experience (Ploghaus et al. 1999; Ploghaus et al. 2003; Koyama et al. 2005; Keltner et al. 2006). These predictive judgments often derived from high contingencies between foretelling cues and associated outcomes and this form of implicit learning enables organisms to detect causal relationships in the environment and to adapt behaviours accordingly (Koyama et al. 2005). By promoting rewarding outcomes and avoiding potential harm, flexible learning processes provide obvious evolutionary advantages. As the cessation of a painful or unpleasant event can be rewarding, motivational

processes are likely involve in the establishment of relief expectations (Seymour et al. 2005; Scott et al. 2007; Schweinhardt et al. 2009). In the case of placebo-induced relief, the activation of dopaminergic neurotransmission in the mesolimbic pathways has been linked with significant therapeutic expectations and effects in the context of both pain and Parkinson's disease (de la Fuente-Fernandez et al. 2001; de la Fuente-Fernandez et al. 2002; de la Fuente-Fernandez et al. 2004; Scott et al. 2007; 2008; Pollo and Benedetti 2009). These findings strongly suggest that motivational processes can drive the learned association between anticipated and actual clinical benefits induce by placebo treatments.

One of the first study to examine the relationship between expectation and relief was conducted by Montgomery and Kirsch more than a decade ago (Montgomery and Kirsch 1997). In this study, transdermal electric current was used to induce acute pain at a baseline level in healthy individuals. Once the intensity of stimulation inducing a stable level of pain was obtained, conditioning trials, in which the strength of the stimulation was surreptitiously reduced in the presence of an inert cream, were performed. Before the next block of trials started, subjects were asked to evaluate how much pain they expected to experience in the upcoming trials. When subjects were unaware of the conditioning manipulations, a marked pain decrease was recorded in the subsequent trials even though the stimuli intensity was restored to the original baseline levels. Furthermore, the actual pain relief experienced by the participants were significantly correlated with the pain reductions anticipated. On the other hand, when subjects were informed that the intensity had been lowered in the conditioning trials, no placebo effect was recorded. These results demonstrated that

conscious expectation is essential for placebo analgesic response to develop and that it can reverse the effects of sensory conditioning.

Other experimental studies were conducted to tease out the relative contribution of expectations and conditioning in the context of placebo effects (Voudouris et al. 1990; Amanzio and Benedetti 1999; Price et al. 1999; De Pascalis et al. 2002; Benedetti et al. 2003). In relation to pain, prior exposure to an effective analgesic agent along with suggestion of pain relief has been shown to induce larger placebo responses than verbal suggestions alone (Amanzio and Benedetti 1999; Benedetti et al. 2003; Colloca and Benedetti 2006; Colloca et al. 2008). This suggests that learning processes initiated from the repeated association between placebo treatment and relief are involved and can play a significant role in the development of placebo analgesic responses (Colloca and Benedetti 2006; Colloca et al. 2008). However, because opposite verbal suggestions are sufficient to completely antagonize the effects of the conditioning procedure by reversing both placebo expectations and responses (Montgomery and Kirsch 1997; Benedetti et al. 2003), the main factor implicated in the generation of placebo analgesic effects appears to dependent on the generation of strong expectations. Thus, it seems likely that conditioning affects the magnitude of placebo analgesia responses indirectly, through the conscious enhancement of anticipated relief.

In other contexts, classical conditioning procedures appear to play a predominant role in the development of placebo effect (Goebel et al. 2002; Benedetti et al. 2003). After a pre-exposition to sumatriptan, a synthetic serotonin agonist which stimulates growth hormone and inhibits cortisol secretion, Benedetti and

colleagues have demonstrated that the administration of a placebo described as the active drug, can produce a significant hormonal placebo response (Benedetti et al. 2003). Interestingly, even after the conditioning procedures, opposite verbal suggestions as to the effects of the compound did not change the outcome, which suggest that the sumatripan-like effects observed after placebo administration were not mediated by expectations but were the direct result of the pre-exposure to the drug. In addition to hormonal placebo effects, the immune system appears to be responsive to placebo administration after pre-exposure to an active drug. Indeed, the repeated association between cyclosporine A and a flavoured drink produced a conditioned immunological response, in which the flavour drink alone induced a suppression of the immune function (Goebel et al. 2002). Because these trained responses do not seem to be voluntarily amendable, they likely arise from unconscious processes linked to Pavlovian conditioning.

These diverse evidences demonstrate that both expectation of relief and classical conditioning are factors that contribute to the emergence of placebo responses, but that their relative contribution varies depending on the context in which they are observed. Whereas expectations best modulate conscious perceptions such as pain and motor performance in Parkinson's disease, conditioning processes are crucial for the development of unconscious physiological placebo responses (Montgomery and Kirsch 1997; Amanzio and Benedetti 1999; de la Fuente-Fernandez et al. 2001; Goebel et al. 2002; Benedetti et al. 2003). Even though these mechanisms differ, a common point emerges from the fact that both these processes rely on the learned association between contextual cues and subsequent relief. While

these associations can be integrated either consciously or unconsciously, placebo effects appears to be a phenomenon that can be learned by the generation of an associative network that links context-dependent expectation with a particular outcome (Benedetti et al. 2003).

3- The effect of sleep on the integration of new expectations

As placebo effect may results from learning processes involving the consolidation of expectation-mediated effects and given the proposed role of sleep in learning and memory, we recently investigated the possibility that sleep contributes to the integration of new expectations leading to the development of placebo responses (Laverdure-Dupont et al. 2009). To do so, we induced placebo analgesic responses in young healthy volunteers by way of a commonly used protocol (Price et al. 1999; Petrovic et al. 2002; Wager et al. 2004; Bingel et al. 2006; Wager et al. 2007), that included verbal suggestions of pain relief and conditioning manipulations wherein the strength of the stimulation on the placebo site was surreptitiously reduced in the presence of an inert topical cream. To examine the effect of sleep compared to the simple passage of time, the cohort of subjects was divided into a night and a daytime group. Subjects assigned to the night group were conditioned in the evening and tested 12 h later, after an 8h sleep period. In opposition, the subjects in the day group were conditioned in the morning and tested 12 h later the same day in the absence of sleep (Figure 1). When the pain ratings from the control and placebo sites of the test block were compared, a significant placebo analgesic effect was observed in both groups. However, in the night group, the analgesia anticipated

just prior to the test block predicted as much as 77% of the concurrent relief, whereas only 38% of the analgesic effects were associated with expected pain reductions in the day group (Figure 1). These results suggest that sleep-dependent processes can enhance the mediating role of relief expectation on placebo analgesia.

The integration of new expectations leading to perceptual changes has, to our knowledge, never been studied in relation to sleep. One issue that makes this investigation difficult is the fact that this type of associative learning involves many types of memory sources. Indeed, while subjects are able to explicitly state their expectation in relation to a particular event, the repeated pre-exposure to efficient treatment during the conditioning manipulations, which has been shown to increase the magnitude of placebo analgesia (Amanzio and Benedetti 1999; Benedetti et al. 2003; Colloca and Benedetti 2006; Colloca et al. 2008), relies on a more implicit form of learning. Thus, both declarative and non-declarative memory systems are most likely sought after during the integration of new expectation linked to clinical benefit. On the other, this is also true during the acquisition of various abilities where many forms of learning co-exist to generate behavioural improvements (Gabrieli 1998; Walker and Stickgold 2004). The acquisition of a language is one example of learning that requires a combination of memory sources, ranging from the formation of declarative representations associated with learned words and grammatical rules, to the development of procedural memory programs associated with the articulation of speech (Fenn et al. 2003). Nevertheless, although the integration of new expectation might represent a high-order cognitive ability compared to other simpler tasks, this might actually promote its dependency to sleep, since more complex tasks

appears to benefit the most from sleep-dependent learning process (Peigneux et al. 2003; Kuriyama et al. 2004).

Although a role for sleep in the assimilation of placebo-associated expectation had not been directly investigated previously, studies of sleep-dependent memory consolidation of other types of learning can help to shed light on the processes underplay. The neuronal replay of recent waking events during sleep is one of the mechanisms proposed to facilitate the retention of memories in both animal (Pavlides and Winson 1989; Wilson and McNaughton 1994; Louie and Wilson 2001) and humans (Maquet et al. 2000; Peigneux et al. 2003; Peigneux et al. 2004). Indeed, it was shown that the cortical neuronal ensembles active during encoding can be reactivated during the subsequent sleep episode. By strengthening synaptic connections within the initial memory structure or with related previously learned material, this process could enhance the mnemonic representation and thus allow its consolidation (Stickgold 2007). In addition to stabilizing newly formed memories, this sleep-dependent reactivation of neuronal networks could promote the restructuring of memory traces to facilitate their integration into a network of older, related memories (Paller and Voss 2004) or could facilitate the extraction of explicit knowledge and insightful behaviour (Wagner et al. 2004).

In the context of placebo treatment, new relief expectations were first generated during the initial exposure to the treatment and were then re-evoked in subsequent testing trials. The presence of a sleep period between the original encoding and delayed recall might have facilitated the incorporation of these new expectations into an existing network of personal beliefs and past experience. In line

with this assumption, our results suggest that sleep-dependent processing, and perhaps replay, of the information acquired during the conditioning manipulations improved the assimilation of new relief expectations by rendering them more proficient in mediating perceived analgesia (Laverdure-Dupont et al. 2009). In addition, because the overall magnitude of the analgesic effect was not significantly larger in the presence of sleep compared to the simple passage of time, sleep-dependent processes appear to act specifically on the association between expectations and analgesia. This is in agreement with the fact that, in some instances, sleep-dependent consolidation can take place although it is not reflected on simple performance gain, but rather on more intricate processes (Atienza et al. 2004). Thus, in the search for sleep's involvement in different skills and abilities, various aspects of a task need to be asses in order to identify the specific components benefiting from sleep.

Classical studies aimed at identifying a role for sleep in learning, especially those involving the acquisition of simple procedural skills or word pairs, focused exclusively on processes of memory enhancement and resistance to normal decay or interference (Karni et al. 1994; Fischer et al. 2002; Walker et al. 2002a; Gais and Born 2004; Ellenbogen et al. 2006). However, in addition to consolidation, memory representations undergo several post-encoding phases of development. After the stabilisation and assimilation of the new memory traces, other integrative stages of memory processing are believed to allow for the extraction of the conceptual meaning linked to the experience which would support the generalization to novel stimuli (Paller and Voss 2004; Stickgold 2007; Walker 2009; Walker and Stickgold

2010). As such, this abstraction would promote the capability to make inference judgments and thus enable the generation of more adaptive behaviours in response to events in the future. In a perceptual learning task of computer-generated spoken language, the effect of sleep on generalized speech recognition was tested by Fenn and colleagues (Fenn et al. 2003). Compared to wake, the presence of a sleep episode between the training and testing sessions significantly improved performance accuracy. Because subjects never hear the same word twice, the perceptual improvements observed were necessarily cause by generalization from phonemes that were presented previously in different words. Another example of sleep-dependent abstraction of general rules is illustrated by the extraction of an hidden concept in the number reduction task (Wagner et al. 2004). During a training session, subjects used a specific addition procedure to solve the problem even though a much simpler rule was concealed in the construct of the task. After a period of wake or sleep, subjects were retested and those who had slept were more than twice as likely to gained insight into the hidden rule. Taken together, these findings demonstrate that, beyond the static consolidation of memory representations, an emerging outlook with regards to sleep-dependent learning processes proposes a dynamic integrative approach involving mental restructuring that leads to the abstraction of general rules potentially useful for dealing with events in the future.

The generation of expectations is a phenomenon that shares commonalities with the extraction of a general meaning allowing increased predictability. Indeed, expectations are associated with the integration of new experiences and prior beliefs leading to the anticipation of forthcoming events and might also benefit from the off-

line processing associated with sleep. In the context of placebo-induced expectations of relief, the initial experience with an effective treatment enables subjects to generate predictions about future exposures, which would instigate the development of explicit expectations. By allowing subjects to sleep between the initial encounter and subsequent experience, off-line processes known to assist in memory assimilation and interpretation may occur and favour the generalization of the perceived relief to future exposures. In other words, sleep might promote the carry-over of the experience encounter during the conditioning manipulations to a subsequent situation. This is in agreement with recent findings in healthy humans which show that, in a context of concurrent conditioned fear to two different stimuli, repeated trials aimed at the extinction of the conditioned response to a particular cue will also induce the extinction of the conditioned response to a similar cue when sleep, but not wake, followed the extinction training (Pace-Schott et al. 2009). The increased generalization of a learned response between similar contexts, such as between the conditioning manipulation and subsequent exposure to a placebo, could be a way by which sleep might promote the development of expectation-dependent placebo effects.

4- The specific role of REM sleep in expectation processing

When sleep is shown to improve some aspects of a task, physiological features of post-training sleep can reflect the performance gain observed. For example, overnight learning gains in speed and accuracy at a sequential finger tapping task were demonstrated to be correlated with the amount of stage 2 sleep

(Walker et al. 2002a). Similarly, the amount of SWS early in the night following practice at a visual texture discrimination task as well as the amount of REM sleep late in that same night were both related to the overnight improvements (Stickgold et al. 2000b). In our study, which proposes a role of sleep in the enhanced mediation of placebo analgesia by relief expectation, REM sleep appeared to be particularly implicated (Laverdure-Dupont et al. 2009). In fact, in the night group exposed to the conditioning manipulations prior to sleep, the relative duration of REM sleep was significantly correlated with the pain relief anticipated on the next day. Thus, subjects with high relief expectation spent a lesser proportion of their total sleep time in REM sleep in the night following the initial exposure with the treatment. In addition to the level of relief expected on the next day, the amount of REM sleep also predicted the actual magnitude of placebo responding measured either concurrently or retrospectively. These results suggest that variations in the relative duration of REM sleep could be associated with the elaboration of expectation-dependent placebo effects. However, because it is subjects who display high anticipated relief and correspondingly high perceived placebo effects who show reduced REM sleep (14,9%; (Laverdure-Dupont et al. 2009)) relative to normal values in healthy individuals (about 20-25%; (Kryger et al. 2005)), it seems that REM sleep might be specially involve in the processing of low anticipated outcome.

Because expectations originate from the integration of current information with past experiences (Ploghaus et al. 2003; Koyama et al. 2005), the level of concordance between new episode and previous beliefs will likely influence the nature of the expectation generated. Thus, in the case of low expectation caused by

conflicting information, the need for mental restructuring and cognitive adaptation could be increased. Conversely, the requirement for reappraisal might be limited when the anticipated effect is more certain, which could then easily be incorporated into a network of related older memories. Previous sleep studies have shown that, in addition to participating in the reprocessing of recent memory traces in human (Maquet et al. 2000; Laureys et al. 2001), REM sleep is particularly involved in the formation of associative networks and in the integration of newly presented information (Stickgold et al. 1999; Stickgold et al. 2001; Walker et al. 2002b; Stickgold 2005; Cai et al. 2009). Furthermore, REM sleep appears to support the consolidation of implicitly acquired complex relationships by enabling the processing and optimization of the high-order information contained in the material to be learned (Peigneux et al. 2003). In the context of placebo analgesia induction, subjects extrapolate future relief from the verbal suggestions and sensorial conditioning trials they were exposed to. However, because various levels of expectation are created just prior to sleep, the cognitive demands imposed on these REM-dependent integrative processes may also vary, and thus underlie the different amount of REM sleep observed in the night following initial exposure to the treatment.

These assumptions are further supported by the fact that the addition of an inconsistent sensory experience prior to sleep completely changed the relationship between relief expectation and REM sleep (Laverdure-Dupont et al. 2009). Indeed, in a second night group exposed to trials where the intensity of the stimuli following placebo administration were reduced and others where they were not, high expectation of relief were still generated in some participants, but were then

associated with a high amount of REM sleep. Because the experience of a relatively ineffective analgesia in some of the trials prior to sleep might have generated an implicit conflict, the cognitive demand and REM-associated mental restructuring may have been increased, particularly in subjects still displaying high expectations in the morning trials. This might explain why, in this experimental group, placebo responders did not display the reduction in REM sleep percent observed in those who were exposed solely to a notable analgesia during the evening conditioning manipulations. Notwithstanding, a significant placebo effect was still present in the group exposed to conflicting sensorial information prior to sleep, but in this context, it was no mediated by relief expectation. These results suggest that while REM sleep contributes to the preservation of positive expectations despite the implicit sensorial conflict, it might also promote their dissociation from the placebo effects measured which would then be driven by other independent factors.

Taken together, these results show that competing past experiences can greatly impact on the relationship established between relief expectations and REM sleep, presumably by increasing the reprocessing needs**.** Another consequence of the evening sensory conflict is to create uncertainty as to the intensity of the pain that could be perceived in future trials. It has been previously shown that in a context where sensorial cues unreliably predict the intensity of the impending pain, the noxious experience is exacerbated by anxiety (Ploghaus et al. 2001). Indeed, during a behavioural conflict, the neural representation of the aversive event is reactivated by the action of the hippocampal formation, which promotes the adaptive response to the worst possible outcome (Ploghaus et al. 2000; Ploghaus et al. 2001; Ploghaus et al.

2003). This process is believed to underlie anxiety-induced hyperalgesia associated with uncertain expectations. Another study have confirmed that uncertain beliefs linked with an impending pain experience leads to a high degree of emotional reactivity and consequently influence the effects of expectation on perceived pain (Brown et al. 2008). Therefore, it is possible that the ambiguity and associated negative emotional mindset generated in subjects exposed to both effective and ineffective treatment before sleep might have required additional cognitive processing and consequently more REM sleep, especially in subjects who conserved a significant expectation of pain relief.

One of the few studies that demonstrated a relationship between pain modulatory process and individual variations in the amount of REM sleep was recently conducted by Smith and colleagues (Smith et al. 2005). In this study, the sleep architecture of sixteen healthy women was recorded on two consecutive nights along with next-day measures of threshold and suprathreshold thermal pain ratings. The thermal stimuli were administered on the forearm of participants by a computer-controlled heating probe and short interstimulus intervals were used to generate temporal summation of pain perception. In addition to thermal pain threshold measures, mean and peak suprathreshold ratings, as well as ratings of painful aftersensations which are believed to represent indices of central pain processing, were collected and correlated with sleep variables. When the values were averaged for the two nights, a positive relationship was found between the relative duration of REM sleep and all of the measures of central pain processing, with this association being much more pronounced for the first compared to the second night of recording.

Because heat pain thresholds were not related significantly to sleep measures, the authors postulated that high amount of REM sleep in some individuals, particularly during the first night of recording, might reflect a heightened central sensitivity to pain. These results are in agreement with the findings that individuals showing high expectation-related analgesia following placebo administration display reduced REM sleep (Laverdure-Dupont et al. 2009), which suggest that REM sleep expression may be closely associated with central mechanisms of pain modulation.

In contexts other than pain, a few studies described the effects of placebo administration on sleep architecture, all of which revealed REM sleep alterations (Hartmann 1968; Hartmann and Cravens 1973; Baker et al. 2003; Suetsugi et al. 2007). Early reports looking at the experimental variation of polysomnographic measures in the context of long-term drug administration have shown that first-time exposure to a placebo can produce either an increase or a decrease in the duration of REM sleep compared to baseline values (Hartmann 1968; Hartmann and Cravens 1973). More recent findings, again comparing with a group not exposed to any intervention, have shown that placebo administration increased the relative duration of REM sleep (Suetsugi et al. 2007). The authors suggested that this effect took place by counteracting the usual first-night effect often seen as an adaptive response to the sleep laboratory environment. This effect is believed to be caused by the physical and psychological discomfort associated with the experimental context and is characterized by a decreased total sleep time, REM sleep and sleep efficiency (Agnew et al. 1966; Toussaint et al. 1995; Le Bon et al. 2001; Goel et al. 2005). The conclusions drawn by Suetsugi and colleagues are however unlikely because the first

night of polysomnographic recording in the drug-free group did not show any reduction in total sleep time, which suggest that no significant first-night effect was even present (Suetsugi et al. 2007). Nonetheless, compared to all other sleep stages, it appears that REM sleep is the one most responsive to placebo administration.

Another study examining the effect of a placebo on sleep measures took into account whether subjects displayed significant therapeutic improvements following administration of the inert substance (Baker et al. 2003). In a clinical trial of patients suffering from panic disorders, more than half of the subjects assigned to the placebo group showed significant reduction of their anxiety levels at the end of the study as well as a normalization of their sleep pattern. In particular, the relative duration of REM sleep differed significantly between placebo responders and non-responders, with a considerable reduction to 14.7% in subjects who experienced improvements following placebo treatment. Because similar effects on sleep were also observed in subjects responding to clonazepam (Baker et al. 2003) and because considerable placebo effects have been reported in the treatment of panic disorders (Hirschfeld 1996; Piercy et al. 1996; Huppert et al. 2004), it is possible that the clinical benefits observed following the active treatment might have also been produced by the placebo effect. Nonetheless, these results raises the intriguing possibility that REM sleep reductions following exposure to a treatment could represent a correlate of therapeutic improvement underlying the generation of a positive emotional state derived from relief expectation. Interestingly, a very similar reduction in REM sleep percent (down to 14.9%) in placebo responders as well as a negative correlation between relief expectation and REM sleep was recorded in our study using healthy

volunteers (Laverdure-Dupont et al. 2009). This further suggests that measures of REM sleep could potentially predict the level of anticipated relief and positive emotions following exposure to a new treatment.

The reduction of anxiety associated with relief expectation is another mechanism assumed to be involved in the development of placebo responses (Benedetti et al. 2005; Price et al. 2008; Morton et al. 2010), and this process might also play a significant role in the induction of concomitant sleep alterations. Indeed, anxiety initiated by stressful waking events can cause sleep alterations, especially with regards to REM sleep (Baekeland et al. 1968; Koulack et al. 1985; Cartwright and Wood 1991; Reynolds et al. 1993; Buguet et al. 1998; Germain et al. 2003; Gitanjali and Ananth 2003). However, the various results collected exhibit some inconsistency as to the directionality of the effects. For example, in the weeks following divorce procedure or spousal bereavement as well as the viewing of stressful films prior to sleep significantly increase REM sleep's expression (Baekeland et al. 1968; Cartwright and Wood 1991; Reynolds et al. 1993). On the other hand, nocturnal cold exposure and the experience of loud noise prior to sleep cause a reduction in the total sleep time spent in REM sleep (Buguet et al. 1998; Gitanjali and Ananth 2003). In addition, as described earlier in experimental contexts, the duration of REM sleep is often decreased during the first adaptation night in the sleep laboratory (Agnew et al. 1966; Toussaint et al. 1995; Le Bon et al. 2001; Goel et al. 2005). Although the effects of acute stress on REM sleep parameters can be moderated by situational and dispositional factors (Germain et al. 2003), these differences are likely due to the highly variable characteristics of the

stressors. Thus it is possible that episodes highly salient in nature, such as divorce or loss of a loved one, might necessitate increased REM sleep-dependent emotional processing, while more neutral or circumscribe events could trigger a simpler adaptive reaction. Under these assumptions, it is possible that the reduction in REM sleep observed in subjects exposed to painful stimuli who will subsequently show significant placebo analgesia reflect an adaptation response that enable the effect to taken place (Laverdure-Dupont et al. 2009).

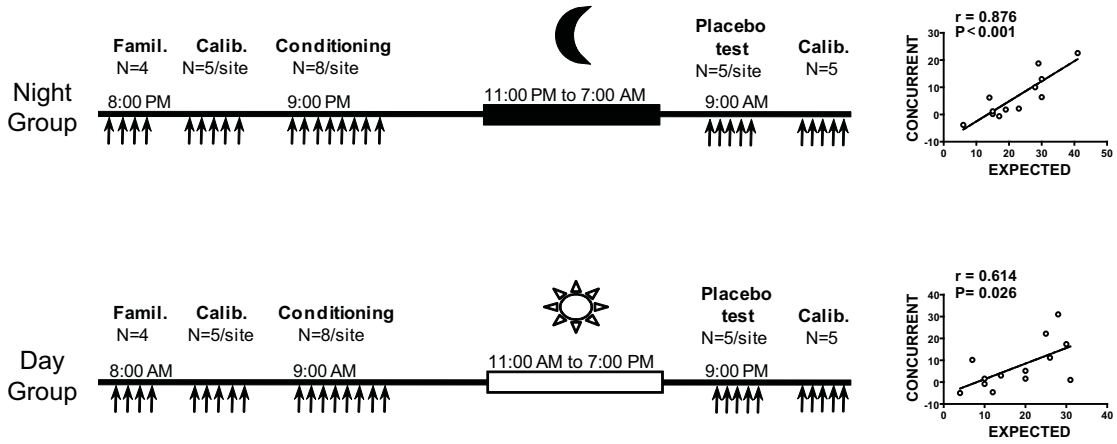
Conclusion

In addition to the treatment of uni-dimensional tasks, sleep-dependent processing is seemingly involve in far more complex activities which require the integration of new information within already established networks as well as the abstraction of generalized concepts that allows adaptive behaviour. The modulation of pain by relief expectation also relies on the assimilation of prior experiences or cues to extrapolate the intensity of impending pain in the future, thus triggering a modulatory response as a process of adaptation to environmental circumstances (Zubieta and Stohler 2009). In light of these similarities, the integration of expectation and associated effects appears to represent another example of learned behaviour that benefit from sleep-dependent memory processing. In particular, variations in the expression of REM sleep might reflect the cognitive demands imposed on an individual in order to assimilate new expectations.

Figure legend

Fig. 1: Time course of experimental events. Stimuli were given in four experimental blocks: familiarization, calibration, conditioning and testing block. In the night group (n = 12), the conditioning manipulations were performed in the evening while the test block was performed in the morning, 12 h later. In the day group (n = 13), the conditioning and test blocks were separated by 12 h of daytime. The grafs illustrate the correlation between expected and concurrent pain intensity reductions (pain ratings on the control site minus pain ratings on the placebo site) measured during the test blocks. Adapted from Laverdure-Dupont et al., 2009.

Figure 1


Night
Group
Famil.
N=4
Calib.
N=5/site
Conditioning
N=8/site
8:00 PM
9:00 PM
11:00 PM to 7:00 AM
Placebo
test
N=5/site
9:00 AM
Calib.
N=5
r = 0.876
P< 0.001
CONCURRENT
EXPECTED
Day
Group
Famil.
N=4
Calib.
N=5/site
Conditioning
N=8/site
8:00 AM
9:00 AM
11:00 AM to 7:00 PM
Placebo
test
N=5/site
9:00 PM
Calib.
N=5
r = 0.614
P= 0.026
CONCURRENT
EXPECTED

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