INSOMNIA DISORDER IS ASSOCIATED WITH INCREASED AMYGDALA REACTIVITY

Insomnia Disorder is Associated with Increased Amygdala Reactivity to Insomnia-Related Stimuli

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Study Objectives: Alterations in emotional reactivity may play a key role in the pathophysiology of insomnia disorder (ID). However, only few supporting experimental data are currently available. We evaluated in a hypothesis-driven design whether patients with ID present altered amygdala responses to emotional stimuli related and unrelated to the experience of insomnia and, because of chronic hyperarousal, less habituation of amygdala responses.

Design: Case-control study.

Setting: Departments of Psychiatry and Psychotherapy and of Radiology of the University of Freiburg Medical Center.

Participants: There were 22 patients with ID (15 females; 7 males; age 40.7 ± 12.6 y) and 38 healthy good sleepers (HGS, 21 females; 17 males; age 39.6 ± 8.9 y).

Interventions: N/A.

Measurements and Results: In a functional magnetic resonance imaging session, five different blocks of pictures with varying emotional arousal, valence, and content (insomnia-relatedness) were presented. Pictures were presented twice to test for habituation processes. Results showed that patients with ID, compared to HGS, presented heightened amygdala responses to insomnia-related stimuli. Moreover, habituation of amygdala responses was observed only in HGS, but not in patients with ID who showed a mixed pattern of amygdala responses to the second presentation of the stimuli.

Conclusions: The results provide evidence for an insomnia-related emotional bias in patients with insomnia disorder. Cognitive behavior treatment for the disorder could benefit from strategies dealing with the emotional charge associated with the disorder. Further studies should clarify the role of insomnia disorder with respect to habituation of amygdala responses.

Keywords: amygdala, emotional arousal, emotional bias, emotional reactivity, emotional valence, habituation, insomnia disorder, International Affective Picture System

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INTRODUCTION

Insomnia disorder (ID) is a prevalent and costly health condition.[1,2] Its prevalence rates are particularly high in psychiatric samples and it has been indicated as a risk factor for sick leave from work[3] or mental health problems, as for example affective disorders[4] and suicidal behavior.[5] Despite its considerable socioeconomic impact, the pathophysiology of the disorder is still poorly understood. In particular, theoretical models of ID[6–9] underlined increased emotional arousal as a main factor involved in the pathophysiology of the disorder and in its close relationship with psychopathology, yet experimental evidence is scarce.

Indirect evidence for an association between ID and emotional alterations derives from the sleep deprivation literature, which shows a crucial role of sleep, and specifically of rapid eye movement (REM) sleep, for adaptive emotional functioning.[10] Although ID is associated with polysomnographic alterations, including a reduction in REM sleep,[11,12] this condition is not comparable to sleep deprivation, as the amount of sleep loss per night is typically only moderate in ID and, in its chronic form, may involve physiological habituation. For this reason, emotional processes should be directly investigated in patients with ID.

Studies using self-report measures found that patients with ID report more negative emotions than good sleepers.[9] In addition, two studies evaluated emotional reactivity in ID through psychophysiological measurements.[13,14] In both studies, facial electromyography (EMG) was recorded while pictures with different valence (negative, positive and neutral) and different content (sleep related and nonsleep related) were presented. Both studies found that individuals with ID show reduced activation of the corrugator muscle when exposed to sleep related stimuli with a positive valence (i.e., pictures of people in bed at night calmly sleeping). These findings suggest a "craving" response in people with ID for pictures showing good sleep. Summarizing the evidence, ID may be associated with altered emotional reactivity, principally evident for sleep related information. Consistently, the attentional system of patients with ID has been described as particularly sensitive to sleep related information.[15–18] However, there is still a great deal of work to be done to clarify the nature of emotional processes in ID.

Some limitations of these previous studies should be considered. First, the two investigations that used psychophysiological measures included only undergraduate students with ID.

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Therefore, the results may not generalize to other insomnia populations, such as older adults or treatment-seeking individuals. Second, the emotion-eliciting stimuli that were used in these studies were not matched for the dimension of arousal, that is, stimuli with negative valence had higher levels of arousal than neutral and positive stimuli. This was particularly evident for sleep related stimuli with positive valence, which were associated with very low levels of arousal. Thus, the effects of valence and arousal on emotional reactivity could not be entirely separated in these previous studies.

Neuroimaging techniques allow direct measurement of emotion-related brain activation. From a neurobiological point of view, subcortical areas, including the amygdala, which interacts with the prefrontal cortex to mediate emotion processing,[19] are functionally altered in psychopathology.[20] For example, patients with depression present with increased amygdala activation in response to negative stimuli, especially if related to sadness.[21] Moreover, heightened amygdala reactivity to emotional stimuli was observed in patients with social anxiety,[22] posttraumatic stress,[23] and borderline personality disorders.[24] In contrast, a meta-analysis of studies conducting facial emotional processing tasks in patients with schizophrenia found a decreased activation of the amygdala compared with healthy controls.[25] Alterations in amygdala reactivity seem associated with impairments in emotional functioning and may be a shared phenomenon in psychopathology. So far, surprisingly few studies have used functional magnetic resonance imaging (fMRI) to investigate patients with ID in comparison with healthy good sleepers (HGS).[26] The results of these studies suggest that ID is associated with a hypoactivation of task-related brain activity.[27–29] However, at present, fMRI has not been used to investigate emotional processes in ID. In light of the aforementioned research on emotional reactivity in ID, an association between the disorder and altered amygdala reactivity to negative emotional stimuli can be hypothesized. Findings showing reduced REM sleep duration in ID[11,12] further encourage the evaluation of possible emotional alterations in these patients, because of the role of this sleep stage in modulating emotional responses.[10,30]

In order to examine emotional processes in ID, we evaluated brain reactivity, and especially amygdala responses, to pictures eliciting negative emotions related or unrelated to the experience of insomnia in a group of patients with ID compared to that of HGS. The main aim of the current study was to evaluate whether patients with ID and HGS differ with respect to amygdala activation in response to stimuli that vary along: (1) the arousal dimension; (2) the valence dimension; and (3) the content dimension (insomnia-relatedness). In order to separate the effects of valence and arousal on emotional reactivity, four different types of stimuli were used in a block-design fMRI experiment: (1) neutral noninsomnia-related stimuli with moderate arousal levels; (2) negative noninsomnia-related stimuli with moderate arousal levels; (3) negative noninsomnia-related stimuli with high arousal levels; and (4) negative insomnia-related stimuli with moderate arousal levels. Positive stimuli were not used in the current study to limit conditions to a manageable number. A fifth group of stimuli was used as a control condition (neutral noninsomnia-related stimuli with low arousal levels). The effect of arousal was investigated by comparing stimulus types 2 and 3; the effect of valence by comparing stimulus types 1 and 2; and the effect of content (insomnia-relatedness) by comparing stimulus types 2 and 4.

A secondary aim of this study was to evaluate the ability of patients with ID to habituate their emotional responses. In healthy individuals, amygdala responses habituate rapidly.[31] However, as ID is characterized by chronically increased levels of psychophysiological hyperarousal, we hypothesized that patients with ID show an impairment of amygdala habituation. To test this hypothesis, each stimulus was presented twice and amygdala reactivity was separately analyzed for first and second appearances of the stimuli.

METHODS AND MATERIALS

Participants

Twenty-two patients with ID (15 females; 7 males; age 40.7 ± 12.6 y) and 38 HGS (21 females; 17 males; age 39.6 ± 8.9 y) completed the study. This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the European Union Programme Commission and by the local ethical committee. All participants gave written informed consent prior to inclusion in the study and received a reimbursement for their participation.

Screening

A total of 29 patients with ID were referred to our sleep disorders unit by their primary care provider and were interested in study participation, and 71 HGS were recruited through local advertisements. An experienced psychiatrist conducted a clinical interview to exclude any history of psychiatry and sleep disorders (including sleep related breathing, hypersomnia, parasomnia, sleep related movement, and circadian rhythm sleep disorders). Moreover, current shift work was excluded. All potential participants underwent a standard physical examination, including electrocardiogram (EKG), electroencephalogram (EEG), and routine blood work (blood cell count, and liver, renal, and thyroid function) to exclude those with relevant medical conditions. All participants had to be right-handed as assessed with the Edinburgh Handedness Inventory[32] and free of any psychoactive medication for at least 2 w prior to and during the study participation. In addition, MRI contraindications such as metallic devices in the body or pregnancy were exclusionary criteria. All participants refrained from alcohol, caffeine, and daytime naps during the recording days. After the screening, the sample included 22 patients with ID and 48 HGS according to Research Diagnostic Criteria.[33] Of these, eight HGS were excluded after the polysomnographic recordings (see next paragraphs). An additional two HGS were excluded after the MRI session, because of incidental findings.

Questionnaires

For descriptive purposes, all participants filled in questionnaires measuring sleep, insomnia, and psychiatric characteristics. Specifically, the following self-report measures were used:

Insomnia Severity Index (ISI)[34]

A brief questionnaire of seven items designed to evaluate the severity of the nighttime and daytime symptoms of insomnia in the last 2 w prior to compilation.

***Pittsburgh Sleep Quality Index* (PSQI)[35]**

A widely used sleep questionnaire designed to assess different dimensions of sleep quality and quantity in the last month.

***Brief version of the Dysfunctional Beliefs and Attitudes about Sleep Scale* (DBAS-16)[36]**

A 16-item questionnaire measuring dysfunctional cognitions, as unrealistic beliefs, faulty expectations, and excessive worry with respect to sleep.

***Glasgow Sleep Effort Scale* (GSES)[37]**

A seven-item measure of need for control over sleep, performance anxiety about sleep, and trying too hard to sleep.

***Pre-Sleep Arousal Scale* (PSAS)[38]**

A 16-item questionnaire assessing the phenomenology of presleep and nocturnal awake times. The questionnaire includes two subscales related to somatic arousal and to cognitive arousal.

***Epworth Sleepiness Scale* (ESS)[39]**

An eight-item questionnaire designed to assess the subject's general level of sleepiness during the day. Participants are asked to rate the probability of falling asleep in a variety of situations.

***Beck Depression Inventory* (BDI)[40]**

A widely used 21-item questionnaire measuring the severity of symptoms of depression.

***State-Trait Anxiety Inventory* (STAI)[41]**

A widely used 40-item questionnaire measuring the levels of anxiety. The questionnaire includes two subscales related to general levels of anxiety (trait version) and the levels of anxiety at the moment of the compilation (state version). For descriptive purposes, only the trait version was used.

Polysomnography

All participants slept for 2 consecutive nights in our sleep laboratory with standard polysomnography (PSG) recordings to exclude any other occult sleep disorder pathology. Specifically, participants with a periodic limb movements in sleep (PLMS) arousal index or a sleep apnea index per total sleep time (TST) of more than 5.0/h were not included in the study. The first night served for adaptation and the second night as baseline. Sleep was recorded on 24-channel Sagura EEG-polysomnographs for 8 h from "lights out" (22:00 to 23:00) until "lights on" (06:00 to 07:00). All recordings included EEG (C3-A2; C4-A1), electrooculogram (horizontal and vertical), and EMG (submental), and were scored visually by experienced raters according to the American Academy of Sleep Medicine criteria.[42] All participants were screened for apnea and PLMS by monitoring abdominal and thoracic effort, nasal airflow, oxymetry, and bilateral tibialis anterior EMG. The sleep parameters measured are described in Table S1 (supplemental material).

fMRI Emotional Task

In a fMRI session, patients with ID and HGS watched negative and neutral pictures with different arousal levels and related and unrelated to the experience of insomnia.

Stimuli

In total, 80 pictures were presented using the Presentation software (www.neurobs.com). Sixty-five pictures were taken from the International Affective Picture System (IAPS[43]) (see endnote A). Stimuli of the IAPS are validated based on the dimensions of valence (the emotional content of the picture: negative, neutral, and positive) and arousal (the emotional intensity of the picture: low to high). With respect to valence, the scale ranges from 1 (nonpleasant) to 9 (very pleasant). With respect to arousal, the scale ranges from 1 (low arousal) to 9 (high arousal). The other 15 pictures were not taken from the IAPS, but self-made or found in the Internet and validated on an independent sample prior to the beginning of the study. The validation study was conducted on a sample of 30 subjects (18 females, 12 males; mean age ± standard deviation: 24.0 ± 3.2 y; 18 good sleepers and 12 poor sleepers based on ISI[34] scores) at the Department of Psychology of the University of Rome "La Sapienza." Following the description of the validation of the noninsomnia-related stimuli reported in the IAPS manual,[43] participants were asked to rate valence and arousal of the pictures on the aforementioned two nine-point scales. There are two reasons why we validated 15 additional pictures: (1) to add 10 insomnia-related stimuli, a category not included in the IAPS; and (2) to add five neutral noninsomnia-related stimuli with low arousal levels because the IAPS database does not include enough stimuli for this category.

In total, we used 40 "neutral stimuli with low arousal levels" as a control condition (Neut_low; mean ± standard deviation: valence = 5.39 ± 0.32; arousal = 3.38 ± 0.46). Moreover, to compare groups with respect to amygdala activation in response to stimuli that vary along the arousal, the valence, and the content (insomnia-relatedness) dimensions we used: 10 "neutral stimuli with moderate arousal levels" (Neut_moderate; valence = 5.58 ± 0.35; arousal = 4.33 ± 0.38); 10 "negative stimuli with moderate arousal levels" (Neg_moderate; valence = 3.18 ± 0.54; arousal = 4.35 ± 0.28); 10 "negative stimuli with high arousal levels" (Neg_high; valence = 2.83 ± 0.55; arousal = 5.78 ± 0.11); and 10 "insomnia-related negative stimuli with moderate arousal levels" (Sleep_Neg_moderate; valence = 3.02 ± 0.45; arousal = 4.49 ± 0.42). For insomnia-related negative stimuli we used pictures of individuals lying awake in bed at night and being evidently frustrated. In appendix 1 (supplemental material), the 10 insomnia-related stimuli are presented together with their valence and arousal scores.

The valence values of the three "negative" groups of stimuli and the arousal values of the three groups with "moderate arousal level" stimuli were not significantly different, as shown in Figure S1 (supplemental material). The stimuli were all pictures of persons and the categories were matched for size and brightness. In Figure 1, the design of the task is presented as well as an example of a stimulus for each category.

Procedure

The fMRI session was conducted at the Department of Radiology of the University of Freiburg Medical Center. The appointment for this was always given after the PSG assessment, in most participants 2-4 w later. As a previous study showed a diurnal pattern of symptoms in insomnia,[44] the fMRI experiment was conducted always at the same time of the day, namely starting between 18:00 and 20:00 and lasting about 2 h.

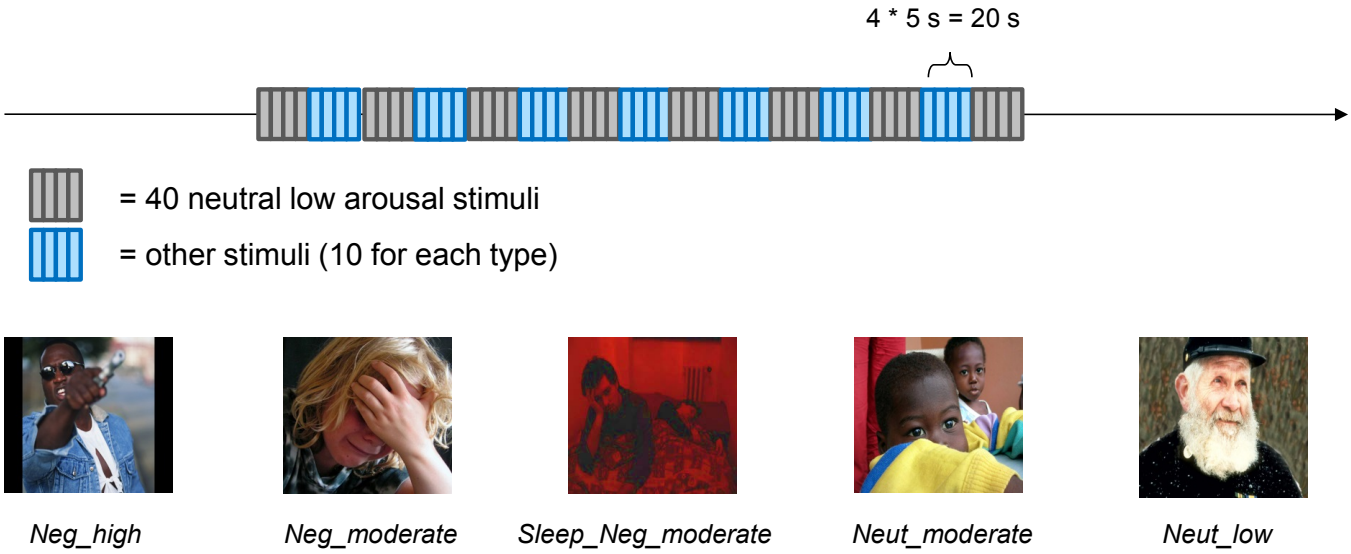

Figure 1—Task design and an example stimulus for each category. Pictures were presented in an functional magnetic resonance imaging block design. Each block included four pictures of the same type of stimuli lasting 5 sec each. Stimuli were presented twice in order to test habituation processes of amygdala responses. Within each category, the second presentation of the pictures started after all pictures were presented for a first time. The Neut_low blocks always preceded all other blocks types. Apart from these constraints, the presentation of the pictures and the sequences of blocks were randomized.

Structural brain imaging results of this sample obtained by analyzing T1-weighted MRI data were published previously.[45] Before the beginning of the task, participants filled in the state version of the STAI[41] and the Stanford Sleepiness Scale[46] to control for state levels of anxiety and sleepiness. The duration of the emotional task was about 13 min (see endnote B). An fMRI block design was used. Each block included four pictures of the same type of stimuli, lasting 5 sec each. Stimuli were presented twice in order to test habituation processes of amygdala responses. Within each category, the second presentation of the pictures started after all pictures were presented for a first time. The Neut_low blocks always preceded all other block types. Apart from these constraints, the presentation of the pictures and the sequences of blocks were randomized. In order to ensure the attention of the participants during the MRI session, a recognition task was announced at the beginning of the session and performed at the end of it (see appendix 2, supplemental material).

MRI Data Acquisition

Blood oxygen level dependent contrast-based fMRI was acquired using a 3 Tesla MRI Scanner (Magnetom TIM-Trio, Siemens, Erlangen, Germany). One run of 330 axial, single-shot, gradient-recalled, echoplanar-image, whole-brain volumes was acquired with the following parameters: volume acquisition time = 2,490 ms; echo time = 30 ms; number of slices = 40; voxel size = 3 × 3 × 3 mm^3. T1 data were acquired using a magnetization-prepared rapid gradient-echo imaging (MPRAGE) sequence (repetition time –TR- 2.2 s; echo time –TE- 2.6 ms; 160 sagittal slices of 256 × 256 voxels, 1.0 × 1.0 × 1.0 mm^3).[47] All scans were inspected for the absence of pathological findings by a neurologist.

Data Analysis

Groups were compared with respect to age, Body mass index (BMI in kg/m^2), education levels, questionnaires scores, and PSG variables using independent t-tests. A chi-square test was used to compare groups with respect to sex.

fMRI Emotional Task

Preprocessing and statistical analysis of brain images were performed using the Analysis of Functional NeuroImages (AFNI) software.[48] Preprocessing included slice timing, smoothing with a full width at half maximum of 9 mm and filtering with a 1/128-Hz high-pass filter. Anatomical scans were normalized on the Montreal Neurological Institute single-subject brain template and coregistered with the functional data. The coregistration resulted in a resampling of the functional data to 1 × 1 × 1 mm^3 voxels. The quality of the normalization and coregistration was visually verified. For each individual (within-subject analyses), a whole-brain multiple linear regression analysis was performed to analyze brain activation associated with the first appearance of each stimulus of the five categories (Neut_low; Neut_moderate, Neg_moderate, Neg_high, and Sleep_Neg_moderate). The corresponding five experimental regressors were convolved with a standard hemodynamic response function. In addition, the six motion estimates resulting from the image realignment were used as covariates.

Because of the low-frequency noise in the signal, the analyses in fMRI studies can be restricted only to comparisons between conditions that are close together in time.[49] For this reason, after conducting individual analyses, the Neut_low stimuli were used as a control category in the second-level group analyses (between-subject analyses) and the other stimuli categories were compared with it: the differences between the beta values of each category and the one of the Neut_low category was calculated, in order to obtain the following contrasts for group comparisons:

- Contrast 1 = Neut_moderate – Neut_low = C1_Neut_moderate;
- Contrast 2 = Neg_moderate – Neut_low = C2_Neg_moderate;

- Contrast 3 = Neg_high – Neut_low = C3_Neg_high;
- Contrast 4 = Sleep_Neg_moderate – Neut_low = C4_Sleep_Neg_moderate.

Analysis Focusing on the Amygdala

Functional data were analyzed with individual amygdala masks created by using the FreeSurfer software (http://surfer.nmr.mgh.harvard.edu/). For each contrast, the mean value of all voxels in the amygdala both on the left and the right sides of the brain were calculated. Afterward, considering only the first appearance of the stimuli, a mixed design factorial analysis of variance (ANOVA) Group (patients with ID vs HGS) × Contrast (C1_Neut_moderate; C2_Neg_moderate; C3_Neg_high; C4_Sleep_Neg_moderate) × Side of the amygdala (Left versus Right) was performed. This analysis procedure was hypothesis-based and outlined in a European grant application before the conduction of the experiment (see Acknowledgements). BDI scores were used as a covariate in this analysis to control for the influence of depression. Planned comparisons were conducted to evaluate group differences with respect to the effects of arousal, valence, and content (insomnia-relatedness) in the following way:

1. The effect of arousal was analyzed by comparing C3_Neg_high with C2_Neg_moderate;
2. The effect of valence was analyzed by comparing C1_Neut_moderate with C2_Neg_moderate;
3. The effect of insomnia-relatedness was analyzed by comparing C4_Sleep_Neg_moderate with C2_Neg_moderate.

For analyzing habituation processes, a second mixed design factorial ANOVA Group × Contrast × Appearance (first versus second appearance of the stimuli) was carried out with BDI scores and the factor "Side" as covariates. For each contrast (C1_Neut_moderate, C2_Neg_moderate, C3_Neg_high, and C4_Sleep_Neg_moderate) and group, planned comparisons were used to compare amygdala reactivity to the first and the second presentation of the stimuli.

Explorative Voxelwise Analysis

A mixed design factorial ANOVA Group × Contrast was performed, considering the first appearance of each stimulus. Choosing a whole-brain P value of 0.05, we considered a cluster-size threshold of 766 voxels to be significant with a voxel-threshold of P = 0.001 (determined by Monte Carlo analysis).

Table 1—Sample characteristics.

	Patients	Controls	t/χ^2	P
Sex [F/M]	15/7	21/17	0.97	0.325
Age [y]	40.7 ± 12.6	39.6 ± 8.9	0.39	0.695
BMI [kg/m²]	22.8 ± 2.2	23.2 ± 3.4	-0.53	0.596
Education	3.6 ± 0.9	4.1 ± 0.9	-2.21	**0.031**
ISI	15.2 ± 3.9	2.3 ± 2.3	16.07	**< 0.001**
PSQI	10.7 ± 3.1	3.9 ± 1.9	10.69	**< 0.001**
STAI-trait	39.0 ± 8.3	32.8 ± 7.3	3.01	**0.004**
BDI	8.0 ± 4.6	3.5 ± 3.3	4.51	**< 0.001**
BDI w/o sleep-related	5.0 ± 4.0	3.0 ± 2.9	2.34	**0.023**
DBAS-16	4.3 ± 1.6	2.3 ± 1.1	5.86	**< 0.001**
GSES	5.9 ± 2.7	1.2 ± 1.5	8.74	**< 0.001**
ESS	8.0 ± 4.5	6.7 ± 3.9	1.15	0.254
PSAS-cognitive	17.9 ± 6.4	13.4 ± 4.2	3.25	**0.002**
PSAS-somatic	11.1 ± 4.1	10.2 ± 2.9	1.00	0.320
PSG data (baseline night)				
TST [min]	389.3 ± 52.9	416.3 ± 24.0	-2.71	**0.009**
SEI [%]	81.2 ± 11.1	86.7 ± 5.0	-2.65	**0.010**
SOL [min]	18.7 ± 15.5	17.8 ± 16.5	0.20	0.839
NA	28.8 ± 11.3	35.4 ± 13.7	-1.91	*0.061*
WASO [min]	58.5 ± 46.9	41.6 ± 16.8	2.02	**0.048**
WAKE [% SPT]	12.8 ± 10.0	9.0 ± 3.5	2.14	**0.037**
S1 [% SPT]	7.8 ± 3.6	8.7 ± 4.5	-0.80	0.425
S2 [% SPT]	51.7 ± 10.1	53.8 ± 5.9	-1.05	0.298
SWS [% SPT]	11.2 ± 9.6	8.9 ± 7.2	1.09	0.281
REM [% SPT]	16.4 ± 6.0	19.5 ± 3.8	-2.45	**0.017**

Results are presented either as frequencies (x/y: sex) or as means ± standard deviations (all other variables). Significant results are evidenced in bold. Marginally significant results are evidenced in italics. BDI, Beck Depression Inventory; BDI w/o sleep-related, Beck Depression Inventory without sleep-related items; BMI, body mass index; DBAS-16, Brief version of the Dysfunctional Beliefs and Attitudes Scale; Education: the German Education System was considered: 1 = less than 9 y, 2 = 9-10 y, 3 = 10-12 y, 4 = 12-13 y, 5 = more than 13 y; ESS, Epworth Sleepiness Scale; GSES, Glasgow Sleep Effort Scale; ISI, Insomnia Severity Index; NA, number of awakenings; PSAS-cognitive, Presleep Arousal Scale-subscale measuring cognitive arousal; PSAS-somatic, Presleep Arousal Scale-subscale measuring somatic arousal; PSG, polysomnography; PSQI, Pittsburgh Sleep Quality Index; REM, time spent in rapid eye movement sleep; S1, time spent in stage 1 sleep; S2, time spent in stage 2 sleep; SEI, sleep efficiency index; SOL, sleep onset latency; SPT, sleep period time; STAI-trait, State Trait Anxiety Inventory-trait version; SWS, time spent in slow wave sleep; TST, total sleep time; WAKE, time spent awake during the night; WASO, wake after sleep onset.

RESULTS

The sample characteristics are summarized in Table 1. Patients with ID reported an average duration of the disorder of 10.3 ± 10.9 y (range: 1–30 y). Eleven patients reported both sleep onset and sleep maintenance problems, seven only sleep maintenance problems, three only sleep onset problems, and one only nonrestorative sleep.

State Anxiety and Sleepiness

No group difference was found regarding state levels of anxiety (ID patients: 33.4 ± 8.1; HGS: 33.8 ± 6.4; t = -0.21, P = 0.83) and sleepiness (ID patients: 2.1 ± 1.1; HGS: 2.2 ± 0.9; t = -0.18, P = 0.85) before the beginning of the MRI session.

Analysis Focusing on the Amygdala

Considering the mixed design factorial ANOVA Group × Contrast × Side for the first appearance of each picture with BDI scores as covariate, a significant main effect of the factor Contrast ($F_{(3,406)} = 18.66$; $P < 0.01$) and a significant interaction

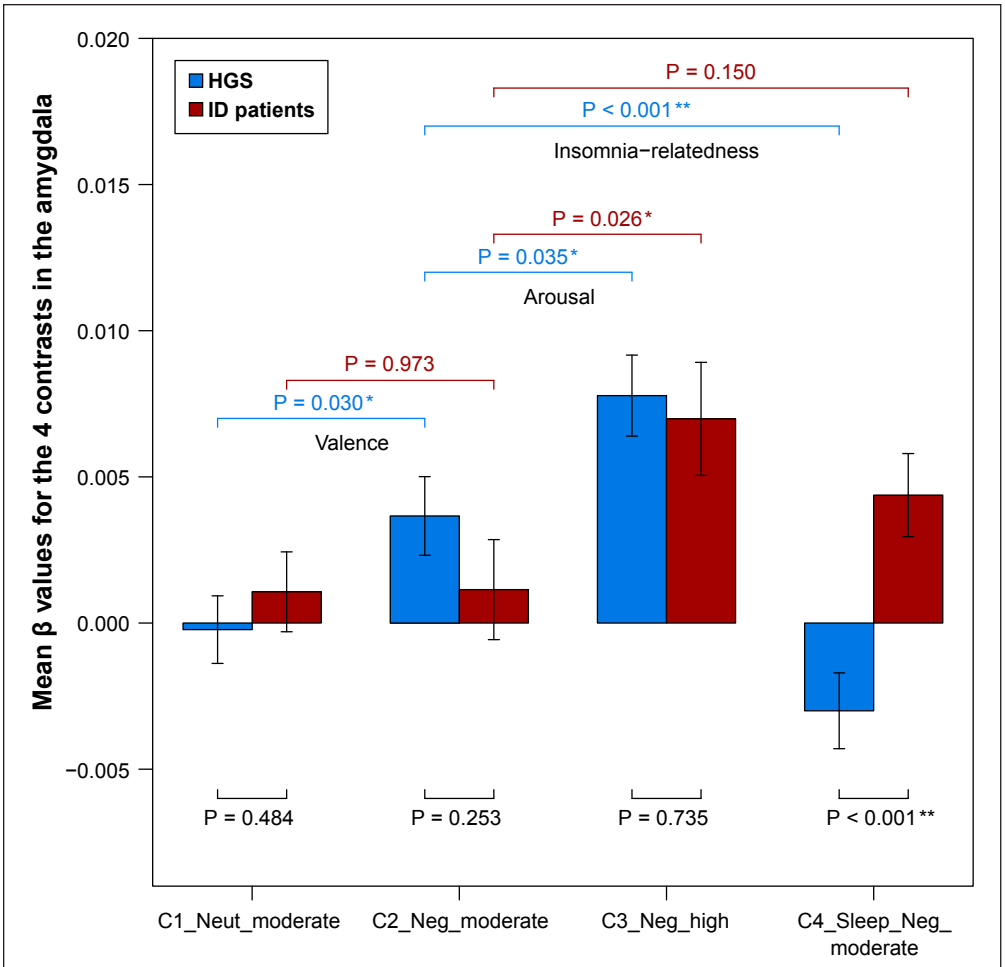

Figure 2—Amygdala reactivity (means ± standard error) to the four Contrasts (C1_Neut_moderate; C2_Neg_ moderate; C3_Neg_high; C4_Sleep_Neg_moderate) in patients with insomnia disorder (ID) and in heavy good sleepers (HGS) with respect to the first appearance of each stimulus. * P < 0.05; ** P < 0.001.

Group × Contrast ($F_{(3,406)}$ = 6.41; P = 0.01) were found. Planned comparisons were conducted with BDI scores and the factor Side as covariates. Figure 2 summarizes the results.

Effect of the Arousal Dimension: C3_Neg_high Versus C2_Neg_ moderate

We performed a 2 × 2 ANOVA Group × Contrast (C3 versus C2) in order to test whether patients and controls differ with respect to amygdala activation in response to stimuli that vary along the arousal dimension. Results evidenced a significant effect of the factor Contrast ($F_{(1,177)}$ = 19.39; P < 0.01). Following up this result, dependent t-tests showed an increased amygdala activation in response to negative stimuli with high arousal levels compared to negative stimuli with moderate arousal levels in both groups (patients with ID: $t_{(64)}$ = 3.38; P < 0.01; HGS: $t_{(112)}$ = 2.98; P < 0.01). The interaction Group × Contrast was not significant ($F_{(1,177)}$ = 0.59; P = 0.44).

Effect of the Valence Dimension: C1_Neut_moderate Versus C2_Neg_moderate

The 2 × 2 ANOVA Group × Contrast (C1 versus C2) conducted to investigate whether patients and controls differ with respect to amygdala activation in response to stimuli that vary along the valence dimension, evidenced a significant effect for the factor Contrast ($F_{(1,177)}$ = 5.02; P = 0.03). Dependent t-tests in each group comparing C1 and C2 revealed different pathways for HGS and patients with ID; more specifically, HGS responded with increased amygdala activation to negative stimuli compared to neutral stimuli with similar levels of arousal ($t_{(112)}$ = - 2.56; P = 0.01), whereas patients with ID did not present different amygdala responses to these two types of stimuli ($t_{(64)}$ = - 0.05; P = 0.96). However, no significant interaction Group × Contrast was observed ($F_{(1,177)}$ = 2.73; P = 0.10).

Effect of the Content (Insomnia-Relatedness) Dimension: C4_Sleep_Neg Versus C2_Neg_ moderate

The 2 × 2 ANOVA Group × Contrast (C2 versus C4) performed to test whether patients and controls differ with respect to amygdala activation in response to stimuli that vary along the content (insomnia-relatedness) dimension evidenced a main effect of the factor Contrast ($F_{(1,177)}$ = 8.07; P = 0.01) and a significant interaction Group × Contrast ($F_{(1,177)}$ = 19.93; P < 0.01). Dependent t-tests revealed different results in the two groups: patients with ID reacted with increased amygdala activation to insomnia-related stimuli compared to noninsomnia-related stimuli ($t_{(64)}$ = 2.10; P = 0.03). Differently, HGS responded with increased amygdala activation to noninsomnia-related stimuli compared to insomnia-related stimuli ($t_{(112)}$ = - 4.64; P < 0.01).

Habituation Effects: Second Appearance Versus First Appearance of the Stimuli

Considering the mixed design factorial ANOVA Group × Contrast × Appearance, conducted with BDI scores and the factor Side as covariates, we observed a significant main effect of the factor Contrast ($F_{(3,885)}$ = 7.26; P < 0.01), a significant main effect of the factor Appearance ($F_{(1,885)}$ = 8.22; P < 0.01), a significant interaction Group × Contrast ($F_{(3,885)}$ = 5.13; P < 0.01), a significant interaction Contrast × Appearance ($F_{(3,885)}$ = 7.20; P < 0.01), and a significant interaction Group × Contrast × Appearance ($F_{(3,885)}$ = 2.98; P = 0.03). Dependent t-tests showed similar amygdala activation in response to the first and the second appearance of the pictures in HGS with respect to C1_Neut_moderate ($t_{(112)}$ = 0.76; P = 0.45), C2_Neg_moderate ($t_{(112)}$ = -0.96; P = 0.34), and C4_Sleep_Neg ($t_{(112)}$ = 0.96; P = 0.34). Instead, a reduction of reactivity was noted in HGS considering C3_Neg_high ($t_{(112)}$ = -3.80; P < 0.01), an effect that was also found in patients with ID ($t_{(64)}$ = -3.26; P < 0.01). With respect to C1_Neut_moderate,

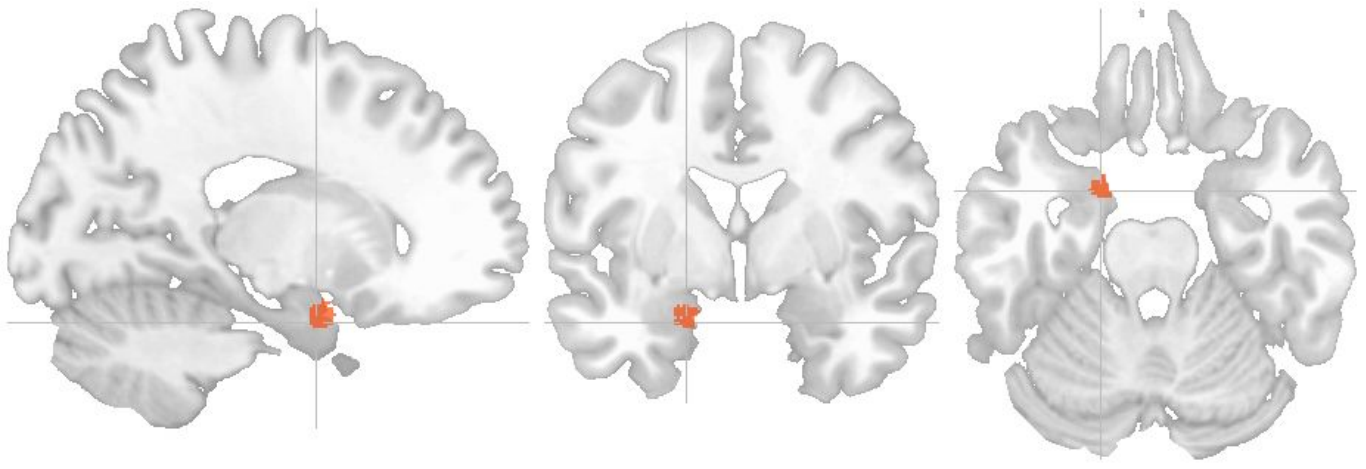

Figure 3—Voxelwise explorative Group × Contrast analyses (performed using the Analysis of Functional NeuroImages program *GroupAna*), conducted for the first appearance of each stimulus, revealed a cluster of activation in the left amygdala (orange areas).

patients with ID presented increased amygdala activation when seeing the pictures for the second time ($t_{(64)} = 2.55$; $P = 0.01$). In contrast, with respect to C2_Neg_moderate and C4_Sleep_Neg, patients with ID presented amygdala deactivation to the second appearance of the stimuli compared to the first appearance (C2: $t_{(64)} = -2.00$; $P = 0.05$; C4: $t_{(64)} = -3.6$, $P < 0.01$). Of note, there was a significant increase of the between-subject variance in amygdala reactivity during the second presentation of the stimuli (variance during first appearance = $1.2*10^{-4}$; variance during second appearance = $1.8*10^{-4}$; $P < 0.01$). Figure S2 (supplemental material) shows the comparison between first and second appearance of the stimuli for each contrast and in each group. In order to better understand the habituation processes, we conducted a further analysis in which we controlled for time-dependent effects on amygdala reactivity by multiplying the five regressors of interest (Neut_moderate; Neg_moderate; Neg_high; Sleep_Neg_moderate and Neut_low for the full duration of the task) with a linearly decreasing function, thus representing time × stimulus type interactions. This model accounted for gradual monotonic decreases of amygdala responses over time.[50] The results of this analysis were very similar to those found in the primary analysis evaluating amygdala reactivity with respect to the first appearance of the stimuli. The only difference that we could observe was a significant interaction Group × Contrast for C1_Neut_moderate versus C2_Neg_moderate ($F_{(1,177)} = 12.88$; $P < 0.001$) that was evident in the additional analysis. Dependent t-tests comparing C2 and C1 in each group revealed that HGS responded with increased amygdala activation to negative stimuli compared to neutral stimuli ($t_{(112)} = 3.80$; $P < 0.001$), whereas patients with ID did not present different amygdala responses to these two types of stimuli ($t_{(64)} = -1.64$; $P = 0.11$). This result is indicative for a possible association between ID and impaired emotional responses to the valence dimension of the stimuli. Detailed results of this analysis are presented in appendix 3 (supplemental material).

Explorative Voxelwise Analysis

Considering the first appearance of each stimulus, regarding the Interaction Group × Contrast, no significant clusters of voxels were found (larger than 766 voxels). Nevertheless, using a more liberal minimal cluster-size threshold of 100 voxels, the analysis revealed a cluster in the left amygdala (volume: 262 voxels; coordinates of the centre of mass: x = 17.7; y = -1.3; z = -21.9). The results are presented in Figure 3. No further *post hoc* tests or analyses of habituation effects were carried out, as the amygdala reactivity has been investigated in detail in the hypothesis-driven part of the results section.

Considering the main effect of the Group, no significant clusters of voxels were found neither considering the cluster-size threshold nor considering a minimum cluster-size of 100 voxels. With respect to the main effect of the Contrast, significant clusters corresponding to areas related to sensory-perceptual (e.g., occipital) and emotional (e.g., amygdala) processes were found and the results related are shown in Figure S3 and summarized in Table S2 (supplemental material).

Explorative Correlation Analysis

Based on the aforementioned results, we further conducted explorative correlation analyses between PSG parameters and amygdala reactivity to the four contrasts, first considering the entire sample together and then considering separately the group of patients with ID and the group of HGS. The correlations were performed separately for left and right amygdala reactivity to the first appearance of the stimuli. Results considering all participants together evidenced a negative association between TST, sleep efficiency index, and the bilateral activation of the amygdala with respect to C3_Neg_high, as well as a positive association with SOL duration and the bilateral activation of the amygdala with respect to C3_Neg_high. These results were also evident for the group of HGS. With respect to SOL duration, the same negative association was also noted in HGS with respect to C2_Neg_moderate. Only in the group with ID, a negative association was evidenced between amygdala reactivity with respect to C3_Neg_high and the duration of both SWS and REM sleep. Additionally, no correlations were found between insomnia duration and amygdala reactivity in any of the four predefined contrasts. These results are presented in Table 2.

DISCUSSION

The results of this study indicate that patients with ID, compared to HGS: (1) show no impairment in amygdala reactivity to different arousal levels; (2) present impaired responses for stimuli varying along the valence dimension; and (3) show heightened amygdala responses to insomnia-related stimuli. Of note, these results were found although depression was statistically controlled for. Considering the arousal dimension, both patients with ID and HGS showed the expected increased amygdala reactivity to negative stimuli with high arousal levels compared to negative stimuli with similar valence levels but lower arousal intensity. Thus, amygdala reactivity to varying arousal levels does not seem to be impaired in patients with ID. Considering the valence dimension, HGS responded with increased amygdala reactivity to negative stimuli compared to neutral stimuli with similar arousal levels, as expected. On the contrary, patients with ID did not present different amygdala reactivity to these two types of stimuli. This may indicate that ID is associated with impaired sensibility to the valence of pictorial stimuli. The attentional and emotional focus on insomnia-related stimuli that characterize patients with ID may be associated with a reduction of sensibility to noninsomnia-related stimuli with negative valence, unless these stimuli induce high levels of emotional arousal. Although this may be an interesting aspect of emotional processes in ID, further studies should replicate this finding and investigate it in more detail.

Considering the content, we found that patients with ID and HGS responded very differently to insomnia-related stimuli. Specifically, although the patient group responded with heightened amygdala reactivity to negative stimuli related with the experience of insomnia compared to negative stimuli with similar arousal levels but noninsomnia-related, HGS had the opposite pattern. The experience of some nights of stress-related poor sleep is common and very likely accompanied by negative feelings of discomfort. In most individuals, sleep returns to normal when the stressor is resolved. In contrast, in patients with ID, the experience of poor sleep *per se* may be associated with negative emotional arousal. In this case, a vicious circle may develop: poor sleep leads to increased emotional arousal, which, in turn, leads again to worse sleep. This excessive emotional charge associated with the sleep difficulties may be a key pathophysiological factor of the disorder. The first theoretical model suggesting an association between altered emotional processes and insomnia was presented by Kales et al. in 1976.[51] According to this model, the predisposition to internalize psychological conflicts leads to heightened emotional arousal, which in turn causes physiological hyperarousal and renders the individual unable to sleep, experiencing difficulties in sleep initiation or maintenance. As a consequence, patients with ID develop a conditioned fear of sleeplessness that contributes to the maintenance of the disorder. Our data support this model and encourage its reinterpretation based on a modern conceptualization of insomnia and advances in our understanding of the neurobiology of emotional processes. A recent meta-analysis analyzed neural correlates of discrete emotions, such as happiness, sadness, anger, fear, and disgust.[52] Increased amygdala activation, mainly on the left side, was especially associated with the exposition to fearful stimuli. In our study, increased amygdala reactivity in patients with ID seemed peculiar to the exposition to emotional negative stimuli related to the experience of insomnia. As already observed by Kales and co-authors in 1976,[51] from a psychological perspective, the emotional hyperarousal experienced by patients with ID may be described by feelings of fear linked to the sleep environment, as, for example, fear of fatigue on the next day.[53]

A further aim of the current study was to test whether emotional responses in patients with ID rapidly habituate or, as a result of chronically increased psychophysiological hyperarousal, these patients maintain heightened physiological reactivity when the same emotional stimuli are presented more than once. Surprisingly, patients with ID showed increased amygdala activation during the second presentation of the neutral pictures with moderate arousal levels, habituation for negative pictures with high levels of arousal, and deactivation for both insomnia-related and noninsomnia-related negative stimuli with moderate levels of arousal. Various explanations of these results can be advanced. The first possibility is that the results may be explained by a time-dependent gradual monotonic effect on amygdala activation across the scanning session. Nevertheless, the results of an additional analysis that controlled for time-dependent gradual effects were very similar to those of the analysis for the first appearance of each picture, suggesting that the two factors "time on task" and "appearance" cannot be well distinguished in our design. A second possible interpretation is based on the observation of increased variance in amygdala reactivity during the second appearance of the stimuli, which may indicate a reduced validity of the results for this part of the experiment. In particular, it could be possible that the second appearance of nonemotional stimuli (i.e., the neutral stimuli) induced an intrinsic focus of attention in patients with ID, and thus, amygdala activation caused by the ruminative nature of the disorder. Two further possible explanations may be more challenging. First, previous neuroimaging studies reported that patients with schizophrenia responded with increased amygdala activation to the first presentation of emotional stimuli and with compensatory reduced amygdala activation during the second one.[54,55] Similarly, patients with ID may have reacted with an amygdala deactivation to the second appearance of the emotional stimuli (i.e., the nonneutral stimuli). If replicated, this phenomenon may be an important part of the characteristic emotional reactivity pattern in ID. Second, insomnia-related pictures may have lost their amygdala-activating properties during the course of the experiment because of their presentation among other pictures. Thus, insomnia-related pictures may have been associated with neutral stimuli resulting in a reinterpretation of the pictures as not being harmful (i.e., not leading to a bad night or to negative feelings). The same process, but in a reverse direction, may have occurred for the noninsomnia-related stimuli with neutral valence. Over the course of the experiment they may have been associated with insomnia-related stimuli resulting in increased amygdala reactivity.

We have recently proposed that a moderate REM sleep deprivation in ID may be associated with emotional alterations.[30] The correlation analyses of the current study suggest a relationship between several indicators of poor sleep and increased amygdala activation to negative stimuli both in ID and HGS. Specifically, the amygdala activation in response to negative stimuli correlated negatively with TST, sleep efficiency, SWS,

Table 2—Explorative correlation analyses between polysomnographic parameters and amygdala activation and between duration of insomnia disorder and amygdala activation with respect to the four contrasts and the first appearance of the stimuli.

Left amygdala	All participants		ID patients		HGS	
TST [min]	**r**	**P**	**r**	**P**	**r**	**P**
C1_Neut_moderate	0.01	0.910	-0.06	0.795	0.10	0.535
C2_Neg_moderate	-0.03	0.834	-0.04	0.867	-0.12	0.459
C3_Neg_high	**-0.27**	**0.036**	-0.31	0.163	*-0.31*	*0.055*
C4_Sleep_Neg_moderate	-0.15	0.255	0.02	0.923	-0.06	0.712
SEI [%]						
C1_Neut_moderate	0.02	0.884	-0.07	0.752	0.13	0.426
C2_Neg_moderate	-0.04	0.735	-0.04	0.865	-0.17	0.317
C3_Neg_high	**-0.28**	**0.029**	-0.31	0.161	**-0.34**	**0.035**
C4_Sleep_Neg_moderate	-0.16	0.217	0.02	0.917	-0.10	0.532
SOL [min]						
C1_Neut_moderate	0.05	0.724	0.02	0.945	0.06	0.702
C2_Neg_moderate	0.23	0.082	0.12	0.589	*0.29*	*0.077*
C3_Neg_high	**0.42**	**0.001**	0.35	0.114	**0.48**	**0.002**
C4_Sleep_Neg_moderate	0.07	0.607	0.18	0.424	0.01	0.964
NA						
C1_Neut_moderate	0.15	0.257	0.20	0.361	0.12	0.455
C2_Neg_moderate	-0.07	0.602	-0.25	0.256	-0.03	0.867
C3_Neg_high	-0.06	0.646	-0.34	0.125	0.08	0.612
C4_Sleep_Neg_moderate	-0.19	0.143	-0.29	0.192	-0.03	0.858
WASO [min]						
C1_Neut_moderate	-0.07	0.621	-0.00	0.996	-0.19	0.262
C2_Neg_moderate	-0.11	0.412	-0.16	0.468	0.02	0.884
C3_Neg_high	-0.04	0.759	-0.05	0.822	-0.00	0.987
C4_Sleep_Neg_moderate	0.04	0.775	-0.18	0.436	0.04	0.835
WAKE [% SPT]						
C1_Neut_moderate	-0.06	0.632	0.00	0.999	-0.19	0.266
C2_Neg_moderate	-0.10	0.452	-0.15	0.494	0.04	0.804
C3_Neg_high	-0.01	0.955	-0.02	0.917	0.05	0.746
C4_Sleep_Neg_moderate	0.04	0.743	-0.17	0.448	0.03	0.868
S1 [% SPT]						
C1_Neut_moderate	-0.01	0.953	0.11	0.627	-0.07	0.697
C2_Neg_moderate	0.01	0.965	0.31	0.154	-0.16	0.349
C3_Neg_high	-0.01	0.936	-0.32	0.153	0.16	0.336
C4_Sleep_Neg_moderate	0.04	0.752	0.11	0.611	0.08	0.617
S2 [% SPT]						
C1_Neut_moderate	0.09	0.491	0.00	0.998	0.19	0.266
C2_Neg_moderate	0.14	0.301	0.27	0.218	-0.02	0.901
C3_Neg_high	-0.08	0.533	-0.02	0.929	-0.17	0.299
C4_Sleep_Neg_moderate	-0.14	0.277	-0.25	0.255	0.03	0.871
SWS [% SPT]						
C1_Neut_moderate	-0.03	0.826	-0.00	0.986	-0.05	0.783
C2_Neg_moderate	-0.07	0.613	-0.18	0.426	0.05	0.785
C3_Neg_high	0.19	0.155	*0.42*	*0.052*	-0.02	0.887
C4_Sleep_Neg_moderate	0.20	0.127	0.33	0.135	0.05	0.772
REM [% SPT]						
C1_Neut_moderate	0.00	0.976	-0.06	0.806	0.05	0.757
C2_Neg_moderate	0.03	0.819	-0.11	0.635	0.09	0.597
C3_Neg_high	-0.16	0.228	*-0.40*	*0.067*	0.07	0.656
C4_Sleep_Neg_moderate	-0.20	0.120	0.12	0.600	-0.26	0.112
ID_duration						
C1_Neut_moderate			-0.13	0.575		
C2_Neg_moderate			0.05	0.813		
C3_Neg_high			-0.29	0.194		
C4_Sleep_Neg_moderate			-0.09	0.701		

Right amygdala	All participants		ID patients		HGS	
TST [min]	**r**	**P**	**r**	**P**	**r**	**P**
C1_Neut_moderate	-0.11	0.414	-0.22	0.334	0.07	0.697
C2_Neg_moderate	-0.05	0.697	-0.03	0.891	-0.17	0.301
C3_Neg_high	*-0.23*	*0.077*	-0.24	0.283	**-0.33**	**0.043**
C4_Sleep_Neg_moderate	-0.14	0.289	-0.01	0.978	-0.13	0.433
SEI [%]						
C1_Neut_moderate	-0.10	0.436	-0.23	0.307	0.09	0.595
C2_Neg_moderate	-0.07	0.572	-0.03	0.898	-0.23	0.160
C3_Neg_high	*-0.24*	*0.065*	-0.24	0.274	**-0.35**	**0.031**
C4_Sleep_Neg_moderate	-0.15	0.263	-0.00	0.993	-0.16	0.339
SOL [min]						
C1_Neut_moderate	0.08	0.533	0.11	0.639	0.07	0.684
C2_Neg_moderate	*0.25*	*0.056*	0.11	0.620	**0.32**	**0.050**
C3_Neg_high	**0.36**	**0.005**	0.29	0.197	**0.39**	**0.015**
C4_Sleep_Neg_moderate	0.13	0.322	0.28	0.211	0.06	0.700
NA						
C1_Neut_moderate	0.06	0.631	0.02	0.917	0.13	0.438
C2_Neg_moderate	-0.11	0.384	-0.37	0.086	-0.04	0.793
C3_Neg_high	0.03	0.796	-0.15	0.502	0.10	0.554
C4_Sleep_Neg_moderate	-0.14	0.295	-0.33	0.131	0.01	0.947
WASO [min]						
C1_Neut_moderate	0.06	0.657	0.17	0.448	-0.15	0.375
C2_Neg_moderate	-0.09	0.517	-0.18	0.414	0.09	0.610
C3_Neg_high	0.03	0.849	0.02	0.912	0.06	0.724
C4_Sleep_Neg_moderate	0.02	0.897	-0.14	0.540	0.04	0.823
WAKE [% SPT]						
C1_Neut_moderate	0.06	0.630	0.17	0.437	-0.15	0.380
C2_Neg_moderate	-0.08	0.565	-0.17	0.444	0.10	0.543
C3_Neg_high	0.05	0.707	0.04	0.846	0.11	0.525
C4_Sleep_Neg_moderate	0.02	0.854	-0.13	0.567	0.04	0.826
S1 [% SPT]						
C1_Neut_moderate	0.06	0.673	0.06	0.793	0.08	0.652
C2_Neg_moderate	0.01	0.965	0.22	0.324	-0.10	0.560
C3_Neg_high	0.04	0.763	-0.11	0.628	0.09	0.576
C4_Sleep_Neg_moderate	0.14	0.273	0.15	0.500	0.19	0.266
S2 [% SPT]						
C1_Neut_moderate	0.01	0.958	-0.11	0.620	0.14	0.416
C2_Neg_moderate	0.11	0.394	0.27	0.219	-0.04	0.799
C3_Neg_high	-0.09	0.503	0.06	0.796	-0.23	0.168
C4_Sleep_Neg_moderate	-0.09	0.487	-0.10	0.672	-0.04	0.810
SWS [% SPT]						
C1_Neut_moderate	-0.03	0.791	0.04	0.857	-0.12	0.490
C2_Neg_moderate	-0.03	0.815	-0.06	0.774	0.01	0.932
C3_Neg_high	0.11	0.423	0.20	0.366	0.06	0.737
C4_Sleep_Neg_moderate	0.09	0.474	0.17	0.440	-0.00	0.990
REM [% SPT]						
C1_Neut_moderate	-0.09	0.515	-0.20	0.383	0.06	0.740
C2_Neg_moderate	-0.03	0.845	-0.20	0.369	0.06	0.740
C3_Neg_high	-0.14	0.295	*-0.42*	*0.052*	0.04	0.825
C4_Sleep_Neg_moderate	-0.17	0.196	0.01	0.966	-0.19	0.255
ID_duration						
C1_Neut_moderate			-0.09	0.676		
C2_Neg_moderate			0.08	0.737		
C3_Neg_high			-0.15	0.501		
C4_Sleep_Neg_moderate			-0.10	0.663		

Significant results are evidenced in bold. Marginally significant results (between P > 0.05 and P < 0.08) are evidenced in italics. Polysomnography data refer to baseline night. HGS, healthy good sleepers; ID_duration, duration of insomnia disorder; NA, number of awakenings; REM, time spent in rapid eye movement sleep; S1, time spent in stage 1 sleep; S2: time spent in stage 2 sleep; SEI, sleep efficiency index; SOL, sleep onset latency; SPT, sleep period time; SWS, time spent in slow wave sleep; TST, total sleep time; WAKE, time spent awake during the night; WASO, wake after sleep onset.

and REM sleep, and positively with SOL. Of note, the correlations with sleep architecture variables (SWS and REM sleep) were found only in the group with ID. These findings further support a key role of sleep for emotional balance.[10] However, the interpretation of these data is limited by the fact that the assessment of PSG parameters was not closely related in time to the recording of brain activity. Accordingly, these explorative findings warrant further investigation.

Limitations

The current study is the first investigation to evaluate the neurobiological correlates of emotional processes in insomnia disorder. As such, replication is needed to reach more stable conclusions. Subjective ratings of the stimuli were not collected during the experimental session; however, this type of data might have deepened the interpretation of the results. In addition to this, we used an emotional task that did not require active reactions. Further studies may use tasks involving active participation which, in turn, may reduce the variability in amygdala reactivity during the repetitive presentation of emotional stimuli.

Clinical Implications

Considering clinical implications, the treatment of ID could benefit from the addition of strategies targeting the emotional experience linked to the sleep environment, such as the feeling of fear or discomfort when trying to fall asleep. As an example, Harvey and Payne[56] found that a positive imagery task was an effective intervention strategy in patients with ID.

CONCLUSIONS

Our results support the association between ID and altered emotional reactivity to insomnia-related stimuli. This is only the third study so far evaluating emotional reactivity in ID combining physiological and psychological methods and the first using neuroimaging techniques. Thus, further research is needed to understand the nature of emotional processes in ID. Nevertheless, the findings of this study, together with the results of the two previous psychophysiological studies,[13,14] suggest that ID is characterized by an insomnia-related emotional bias, which may constitute an important maintaining and worsening factor in the disorder. This insomnia-related emotional bias may be associated with an inability to adopt effective emotion regulation strategies in response to feared events, e.g., a night of insomnia. The construct of emotion regulation is receiving increased interest in psychopathology, but so far no study evaluated possible difficulties in the modulation of emotional responses in patients with ID. In the current study, we could observe different emotional responses in patients and controls independently of a possible effect of depression, which was statistically controlled in the analyses. As both ID and difficulties in emotion regulation have been indicated as precursors of many mental disorders, the investigation of their mutual influence may be relevant for elucidating common mechanisms in mental disorders.

ENDNOTES

A. The number of the IAPS stimuli selected for this study are: Neut_low: 2028, 2038, 2102, 2190, 2191, 2214, 2305, 2345, 2385, 2393, 2394, 2396, 2435, 2485, 2487, 2495, 2499, 2500, 2506, 2513, 2516, 2518, 2579, 2580, 2620, 2840, 2850, 2870, 2890, 4571, 5875, 7493, 7550, 8311, 9070; Neut_moderate: 2005, 2025, 2220, 2372, 2575, 2616, 2635, 4274, 4534, 8241; Neg_moderate: 2100, 2276, 2278, 2455, 2490, 2718, 2753, 6010, 9041, 9220; Neg_high: 26, 91, 2703, 3022, 3180, 6213, 6244, 6571, 8230, 9160, 9429.

B. Participants were involved in another study aiming at investigating the neurobiology of sleep-related attentional bias in patients with ID (preliminary results: Spiegelhalder et al. Brain reactivity to sleep-related cues in patients with primary insomnia. Journal of Sleep Research 2010; Volume 19, Issue Supplement 2, Page 160). The entire duration of the MRI session was circa an hour.

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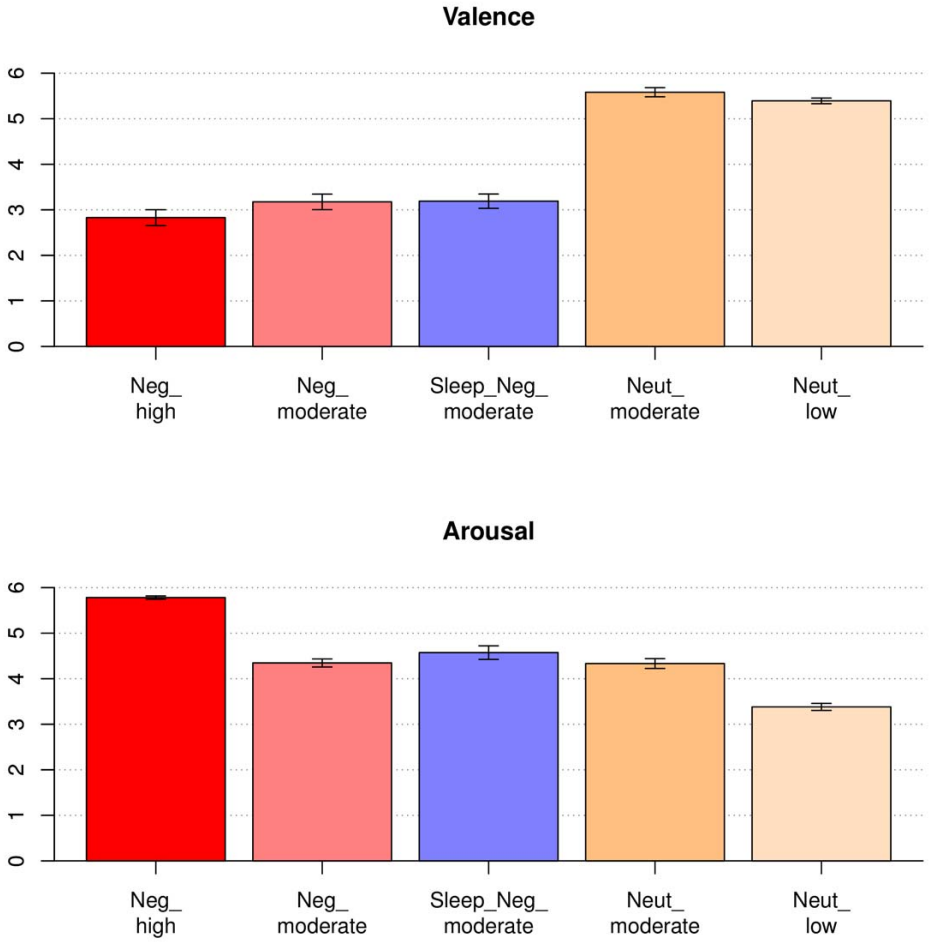


Figure S1—Stimuli match for the dimension of valence and arousal. As shown by the top graph, the stimuli Neg_high, Neg_moderate and Sleep_Neg_moderate are not significantly different with respect to negative valance, whereas the stimuli Neut_moderate and Neut_low are not significantly different with respect to neutral valence. As shown by the bottom graph, the stimuli Neg_moderate, Sleep_Neg_moderate and Neut_moderate are not significantly different with respect to arousal levels, whereas Neg_high is significantly more arousing and Neut_low is significantly less arousing as compared to the other categories. Moderate levels of arousal were chosen because, although insomnia-related stimuli can be of negative valence, they hardly reach high levels of arousal. In order to have comparable categories of stimuli with the insomnia-related stimuli, we included a group of negative noninsomnia-related stimuli and a group of noninsomnia-related neutral-stimuli matched for arousal levels with the negative insomnia-related stimuli.

Table S1—List of the sleep parameters measured in the present study.

Sleep Parameter	Abbreviation	Description
Total sleep time	TST	The total time spent asleep during the recording night.
Sleep efficiency	SEI	Ratio of TST to time in bed × 100%.
Sleep onset latency	SOL	Time from lights out until sleep onset (defined as first epoch of stage 2).
Wake after sleep onset	WASO	The difference between sleep period time (SPT; time from sleep until final awakening) and TST.
Number of awakenings	NA	The total number of awakenings during the recording night.
Percentages of stage 1, stage 2, slow wave, sleep, REM sleep and wake	S1, S2, SWS, REM, WAKE	Amount of each stage as percentage of SPT.

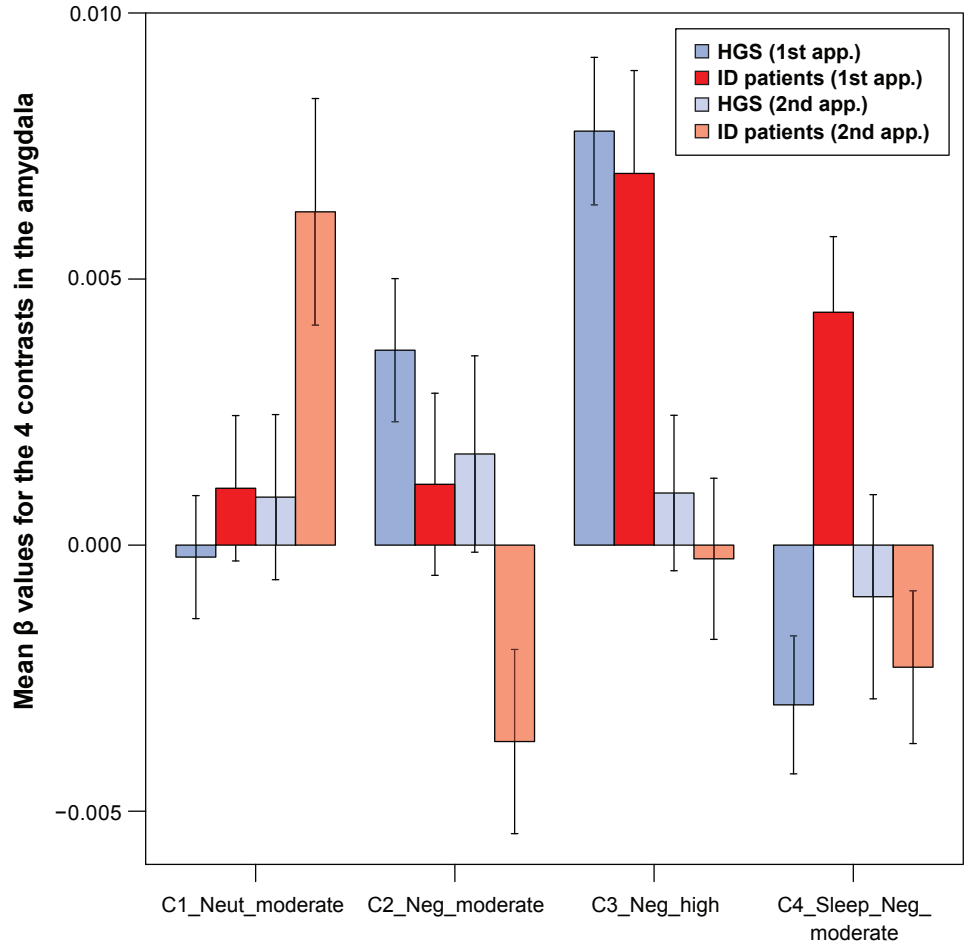


Figure S2—Test for habituation of amygdala responses: Comparison of amygdala reactivity (means ± standard error) to the four Contrasts (C1_Neut_moderate; C2_Neg_moderate; C3_Neg_high; C4_Sleep_Neg_moderate) in patients with insomnia disorder and in heavy good sleepers HGS for first versus second appearance of each stimulus. 1st app = first appearance of the stimuli; 2nd app = second appearance of the stimuli.

Table S2—Voxelwise explorative analysis considering the main effect of the factor Contrast with respect to the first appearance of each stimulus: coordinates for significant clusters found applying the considered cluster-size threshold = 766 voxels.

Volume	CM RL	CM AP	CM IS	min RL	max RL	min AP	max AP	min IS	max IS
147295	-2.6	73.1	-0.2	-68.5	66.5	21.8	104.8	-50.2	49.8
19336	0.7	16.5	-11.6	-43.5	44.5	-7.2	45.8	-35.2	6.8
4004	-1.1	-55.2	33.2	-15.5	10.5	-69.2	-44.2	17.8	48.8
3790	-33.2	-46.2	29.3	-42.5	-25.5	-57.2	-36.2	15.8	45.8
3587	60.1	27.0	32.6	49.5	67.5	15.8	37.8	19.8	46.8
3570	-49.3	-26.3	15.1	-59.5	-33.5	-42.2	-11.2	-16.2	39.8
3008	0.5	-55.3	-14.2	-9.5	10.5	-69.2	-33.2	-21.2	-6.2
2723	-55.9	-1.5	-17.2	-65.5	-45.5	-14.2	14.8	-25.2	-9.2
2309	37.4	-21.3	13.1	25.5	45.5	-40.2	-6.2	0.8	26.8
2123	-3.4	4.4	6.4	-17.5	10.5	-10.2	14.8	-0.2	13.8
2008	46.6	-13.4	-24.9	34.5	60.5	-22.2	-3.2	-35.2	-16.2
1367	-36.5	-17.4	10.3	-47.5	-24.5	-29.2	-5.2	0.8	18.8

- CM RL = Centre of mass for the cluster in the right-left direction
- CM AP = Centre of mass for the cluster in the anterior-posterior direction
- CM IS = Centre of mass for the cluster in the inferior-superior direction
- min RL – max RL = Bounding box for the cluster min and max coordinates in the right-left direction
- min AP – max AP = Bounding box for the cluster min and max coordinates in the anterior-posterior direction
- min IS – max IS = Bounding box for the cluster min and max coordinates in the anterior-posterior direction

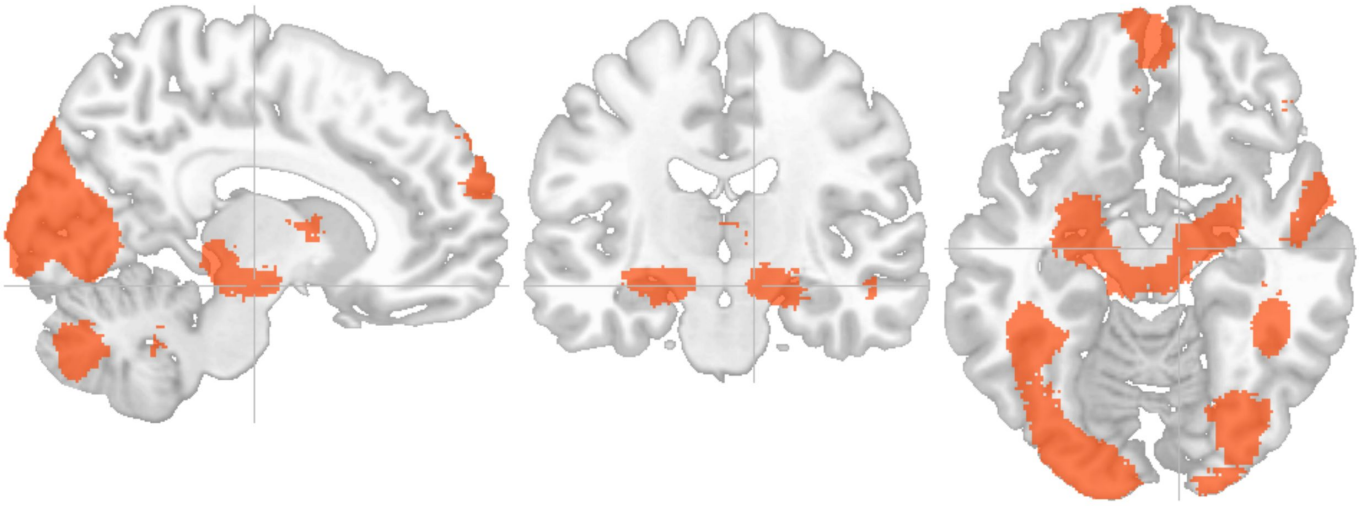
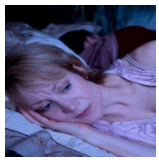
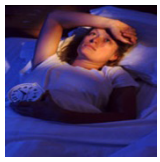


Figure S3—Voxelwise explorative analyses considering the main factor Contrast (analysis performed using the Analysis of Functional NeuroImages program *GroupAna*) with respect to the first appearance of each stimulus. Significant clusters of voxels corresponding to areas related to sensory-perceptual (e.g., occipital) and emotional (e.g., amygdala) processes were evidenced.

APPENDIX 1: INSOMNIA-RELATED STIMULI

	Valence = 3.9 Arousal = 2.9		Valence = 2.6 Arousal = 5.1
	Valence = 2.6 Arousal = 4.8		Valence = 2.7 Arousal = 5.0
	Valence = 3.3 Arousal = 4.1		Valence = 3.1 Arousal = 5.1
	Valence = 3.5 Arousal = 4.0		Valence = 2.5 Arousal = 5.1
	Valence = 2.9 Arousal = 4.3		Valence = 3.1 Arousal = 4.5

APPENDIX 2: RECOGNITION TASK

Procedure

An old/new recognition task was programmed using the Presentation software. This task included the above described "old" Neut_moderate, Neg_moderate, Neg_high and Sleep_Neg_moderate stimuli as well as 80 "new" stimuli of these categories matched for valence and arousal to the old stimuli. The presentation of each picture lasted for 1 second. Participants indicated with two buttons whether they had already seen the picture in the MRI scanner or not.

Data-analysis

T-tests were performed to evaluate group differences with respect to: a) the percentages of the correct answers given for each of the 8 conditions presented (old Neut_moderate, Neg_moderate, Neg_high and Sleep_Neg_moderate; and new Neut_moderate, Neg_moderate, Neg_high and Sleep_Neg_moderate); b) the mean reaction time with respect to each of the 8 conditions presented.

Results

With respect to all old stimuli and the new Neg_high stimuli, both groups performed high with very few wrong recognitions and no significant between-group differences were found (old: Neut_moderate: patients with ID = 0.9 ± 0.1 vs HGS = 0.9 ± 0.2; Neg_moderate: patients with ID = 0.9 ± 0.2 vs HGS = 0.9 ± 0.1; Neg_high: patients with ID = 0.9 ± 0.1 vs HGS = 0.9 ± 0.1; Sleep_Neg_moderate: patients with ID = 0.9 ± 0.1 vs HGS = 0.9 ± 0.2; new Neg_high: patients with ID = 0.8 ± 0.2 vs HGS = 0.9 ± 0.1).

Patients with ID performed slightly worse as compared to HGS in the recognition of the new stimuli of the categories Neg_moderate (patients with ID = 0.7 ± 0.2 vs HGS = 0.9 ± 0.1), Neut_matched (patients with ID = 0.8 ± 0.2 vs HGS = 0.9 ± 0.1), and Sleep_Neg_moderate (patients with ID = 0.5 ± 0.2 vs HGS = 0.6 ± 0.2). No group differences were found with respect to reaction times.

APPENDIX 3: TIME-DEPENDENT EFFECTS ON AMYGDALA REACTIVITY

In order to better understand the habituation processes, we conducted a further analysis in which we controlled for time-dependent effects on amygdala reactivity by multiplying the 5 regressors of interest (Neut_moderate; Neg_moderate; Neg_high; Sleep_Neg_moderate and Neut_low for the full duration of the task) with a linearly decreasing function, thus representing time × stimulus type interactions. This model accounted for gradual monotonic decreases of amygdala responses over time.

The following steps of the analysis were identical to the ones of the primary analysis presented in the manuscript (analysis of the first presentation of the stimuli): Four contrasts were used:

- Contrast 1 = Neut_moderate – Neut_low = C1_Neut_moderate;
- Contrast 2 = Neg_moderate – Neut_low = C2_Neg_moderate;
- Contrast 3 = Neg_high – Neut_low = C3_Neg_high;
- Contrast 4 = Sleep_Neg_moderate – Neut_low = C4_Sleep_Neg_moderate.

A mixed design factorial ANOVA Group (patients with ID vs HGS) × Contrast (C1_Neut_moderate; C2_Neg_moderate; C3_Neg_high; C4_Sleep_Neg_moderate) × Side of the amygdala (left vs right) was performed with BDI scores as a covariate. Three planned comparisons were conducted to evaluate group differences with respect to the effects of arousal, valence, and content (insomnia-relatedness):

1. The effect of arousal was analyzed by comparing C3_Neg_high with C2_Neg_moderate;
2. The effect of valence was analyzed by comparing C1_Neut_moderate with C2_Neg_moderate;
3. The effect of insomnia-relatedness was analyzed by comparing C4_Sleep_Neg_moderate with C2_Neg_moderate.

The results of this analysis are presented in Figure S4. In the ANOVA, we found a significant main effect of the factor Contrast ($F_{(3,406)} = 16.58$; $P < 0.001$) and a significant interaction Group × Contrast ($F_{(3,406)} = 9.91$; $P < 0.001$).

With respect to the planned comparisons, the 2 × 2 ANOVA Group × Contrast (C2_Neg_moderate vs C3_Neg_high; investigation of the arousal dimension) showed a significant effect of the factor Contrast ($F_{(1,177)} = 14.57$; $P < 0.001$). Dependent t-tests showed an increased amygdala activation in response to negative stimuli with high arousal levels compared to negative stimuli with moderate arousal levels in both groups (patients with ID: $t_{(64)} = 3.41$; $P = 0.001$; HGS: $t_{(112)} = 2.27$; $P = 0.025$). The interaction Group × Contrast was not significant ($F_{(1,177)} = 1.33$; $P = 0.25$).

The 2 × 2 ANOVA Group × Contrast (C2_Neg_moderate vs C1_Neut_moderate; investigation of the valence dimension) showed a significant effect of the factor Contrast ($F_{(1,177)} = 5.76$;

P = 0.017) and a significant interaction Group × Contrast ($F_{(1,177)} = 12.88$; $P < 0.001$). Dependent t-tests comparing C2 and C1 in each group revealed that HGS responded with increased amygdala activation to negative stimuli compared to neutral stimuli ($t_{(112)} = 3.80$; $P < 0.001$), while patients with ID did not present different amygdala responses to these two types of stimuli ($t_{(64)} = -1.64$; $P = 0.11$).

The 2 × 2 ANOVA Group × Contrast (C2_Neg_moderate vs C4_Sleep_Neg_moderate; investigation of the content dimension) revealed a main effect of the factor Contrast ($F_{(1,177)} = 11.85$; $P < 0.001$) and a significant interaction Group × Contrast ($F_{(1,177)} = 29.40$; $P < 0.001$). Dependent t-tests showed that patients with ID reacted with increased amygdala activation to insomnia-related stimuli in comparison with the reactivity to non-insomnia-related stimuli ($t_{(64)} = 2.54$; $P = 0.013$), while HGS responded with increased amygdala activation to non-insomnia-related stimuli compared to insomnia-related stimuli ($t_{(112)} = -5.64$; $P < 0.001$).

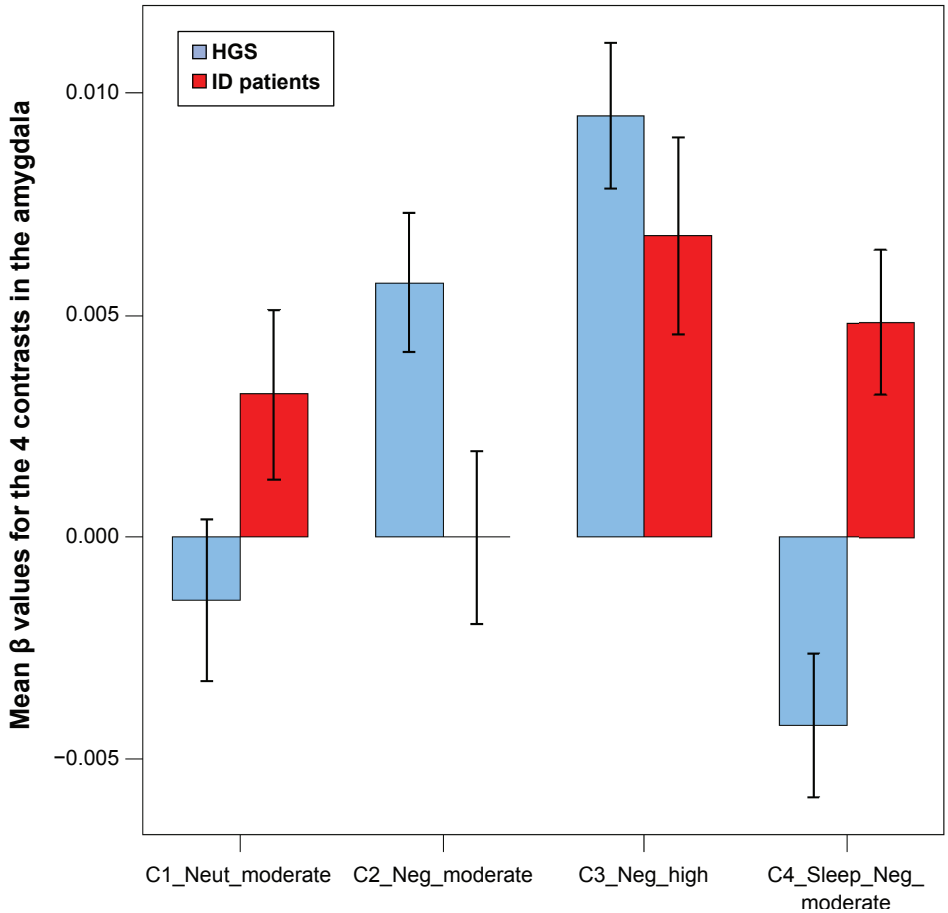


Figure S4—Amygdala reactivity (means ± standard error) to the 4 Contrasts (C1_Neut_moderate; C2_Neg_moderate; C3_Neg_high; C4_Sleep_Neg_moderate) in patients with ID and in HGS with respect to the full duration of the task.