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Abnormal activity in hypothalamus and amygdala during humour processing in human narcolepsy with cataplexy

Sophie Schwartz,¹ Aurélie Ponz,¹ Rositsa Poryazova,² Esther Werth,² Peter Boesiger,³ Ramin Khatami² and Claudio L. Bassetti²

¹Laboratory for Neurology and Imaging of Cognition, Department of Clinical Neurology & Department of Neurosciences, University Medical Center, Michel-Servet 1, 1211 Geneva, Switzerland, ²Neurologische Klinik und Poliklinik, Zurich University Hospital, Frauenklinikstrasse 26, 8091 Zurich, Switzerland and ³Institute for Biomedical Engineering, University and ETH Zurich, Gloriastrasse 35, 8092 Zurich, Switzerland

Correspondence to: Sophie Schwartz, Neurology and Imaging of Cognition, Department of Neurosciences, University Medical Center, Michel-Servet 1, 1211 Geneva, Switzerland
E-mail: sophie.schwartz@medecine.unige.ch

Narcolepsy with cataplexy (NC) is a complex sleep–wake disorder, which was recently found to be associated with a reduction or loss of hypocretin (HCRT, also called orexin). HCRT is a hypothalamic peptide implicated in the regulation of sleep/wake, motor and feeding functions. Cataplexy refers to episodes of sudden and transient loss of muscle tone triggered by strong, mostly positive emotions, such as hearing or telling jokes. Cataplexy is thought to reflect the recruitment of ponto-medullary mechanisms that normally underlie muscle atonia during REM-sleep. In contrast, the suprapontine brain mechanisms associated with the cataplectic effects of emotions in human narcolepsy with cataplexy remain essentially unknown. Here, we used event-related functional MRI to assess brain activity in 12 NC patients and 12 controls while they watched sequences of humorous pictures. Patients and controls were similar in humour appreciation and activated regions known to contribute to humour processing, including limbic and striatal regions. A direct statistical comparison between patients and controls revealed that humorous pictures elicited reduced hypothalamic response together with enhanced amygdala response in the patients. These results suggest (i) that hypothalamic HCRT activity physiologically modulates the processing of emotional inputs within the amygdala, and (ii) that suprapontine mechanisms of cataplexy involve a dysfunction of hypothalamic–amygdala interactions triggered by positive emotions.

Keywords: narcolepsy with cataplexy; functional MRI; hypocretin/orexin; amygdala; emotion

Abbreviations: HCRT = hypocretin; NC = narcolepsy with cataplexy; REM = rapid eye movement sleep

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Introduction

Narcolepsy with cataplexy (NC) is a disabling sleep–wake disorder, which affects approximately 1 in 2000 individuals. NC is characterized by excessive daytime sleepiness and several manifestations of so-called ‘dissociated’ or isolated rapid eye movement (REM) sleep features, such as muscle atonia (i.e. cataplexy), sleep-paralysis and hallucinations (Baumann and Bassetti, 2005). The pathognomonic symptom of NC is cataplexy, which corresponds to short episodes of muscle tone loss with preserved consciousness triggered by emotions, most often by laughing or joking (Bassetti and Aldrich, 1996; Sturzenegger and Bassetti, 2004). This striking clinical feature suggests a close

interaction between emotions, motor control and NC-related anomalies in the brain. Human narcolepsy has recently been found to be associated with a reduction or loss of a hypothalamic peptide called hypocretin (HCRT, also called orexin; de Lecea *et al.*, 1998; Sakurai *et al.*, 1998; Peyron *et al.*, 2000; Thannickal *et al.*, 2000, 2003). HCRT is implicated not only in sleep/wake regulation, but also in feeding and reward functions (Mignot *et al.*, 2002; Baumann and Bassetti, 2005; Lu *et al.*, 2006).

Cataplexy is explained by an inappropriate intrusion of physiological REM sleep atonia into wakefulness (Broughton *et al.*, 1986; Guilleminault and Gelb, 1995). This assumption is based on electrophysiological studies

and clinical observations that demonstrated areflexia and H-reflex attenuation during muscle atonia in both REM-sleep and cataplexy (Hishikawa *et al.*, 1965; Shimizu *et al.*, 1966; Guilleminault, 1976). Muscle atonia during REM sleep results from an excitation of medullary atonia-generating neurons which in turn inhibit spinal alpha-motoneurons (Siegel *et al.*, 1991). Recent data from pharmacological (Nishino *et al.*, 2000) and neurophysiological (Overeem *et al.*, 1999, 2004) studies have questioned the concept of dissociated REM sleep symptoms and suggest that atonia during cataplexy and REM-sleep may be generated by distinct mechanisms but recruit common descending ponto-medullary-spinal pathways. However, the functional brain anatomy underlying the recruitment of motor-atonia neurons in the brainstem by emotions remains unknown.

Several observations support the possibility of an involvement of the amygdala in cataplexy. First, electrophysiological studies in narcoleptic dogs demonstrated changes of neuronal firing in the amygdala during cataplexy (Gulyani *et al.*, 2002). Second, the amygdala was found to be strongly activated during REM sleep in normal human subjects (Maquet *et al.*, 1996; Maquet and Franck, 1997). Third, neuroimaging, neurophysiological and clinical studies have shown that the amygdala is critically involved in emotional information processing in both animals and humans (see LeDoux, 2000; Zald, 2003; Vuilleumier, 2005). In addition, the discovery of HCRT deficiency in NC suggests that the hypothalamus represents a second main suprapontine brain site whose dysfunction might contribute to cataplexy in NC.

To date, imaging studies failed to reveal consistent brain abnormalities in NC patients. Advanced neuroimaging techniques could not demonstrate any systematic structural or functional change in the hypothalamus and/or the amygdalae. We briefly review these results hereafter.

In recent years, MRI anatomical investigations have used voxel-based morphometry methods that allow statistical comparisons of local tissue composition (cortex, white matter and cerebrospinal fluid) across the whole brain. Voxel-based morphometry studies have produced variable results ranging from no evidence for any structural change in NC patients (Overeem *et al.*, 2003), through to cortical gray matter reduction in frontal brain regions (Brenneis *et al.*, 2005), inferior temporal regions (Kaufmann *et al.*, 2002), as well as hypothalamus (Buskova *et al.*, 2006), cerebellum (vermis), superior temporal gyrus and right nucleus accumbens (Draganski *et al.*, 2002). Some other studies used proton magnetic resonance spectroscopy to assess *in vivo* neuronal loss in the hypothalamus, but found either significant (Lodi *et al.*, 2004) or no (Ellis *et al.*, 1998) such neuronal loss. Similarly, the few available functional imaging studies provided mixed results for measures of baseline cerebral activity in NC patients (PET, Joo *et al.*, 2004; SPECT, Joo *et al.*, 2005), and for measures of brain activity during simple sensory stimulation

(fMRI, Ellis *et al.*, 1999) or during cataplexy attack (SPECT, Hong *et al.*, 2006; Chabas *et al.*, 2007).

The main goal and methods proposed in the present study differ from these previous brain imaging studies. Based on the clinical observation that NC patients often have cataplexy attacks when they experience positive emotions, we hypothesized that the patients may show abnormal processing of external emotional inputs within limbic circuits or, alternatively, increased activation of efferent motor systems (i.e. motor dysregulation induced by emotions, see for example LeDoux, 2000; Moskowitz, 2004). To test this hypothesis, we used a rapid event-related functional MRI (fMRI) paradigm performed on a 3T scanner to compare neural activity elicited by humorous versus neutral pictures in NC patients and healthy volunteers. We predicted that NC patients would show abnormally high fMRI activation in some regions previously reported to respond to humorous stimuli in normal controls, including the hypothalamus, amygdala and ventral striatum (Goel and Dolan, 2001; Mobbs *et al.*, 2003; Moran *et al.*, 2004; Watson *et al.*, 2006, for review see Wild *et al.*, 2003). Our high-resolution fMRI study provides the first assessment of regional brain responses to positive emotions in human narcolepsy.

Methods

Subjects

Twelve drug-free narcoleptic patients with clear-cut cataplexy (based on clinical examination and standard questionnaires; The International Classification of Sleep Disorders, 2005) and 12 healthy volunteers (matched for age, gender and body-mass index) provided written informed consent to participate in an fMRI study which was approved by the local ethics committee (Tables 1 and 2). The patients' Epworth Sleepiness score (ESS; Johns, 1991) was 15.6 ± 4.2 (mean \pm SD; range: 7 to 21), the Ullanlinna Narcolepsy Scale (UNS; Hublin *et al.*, 1994) was 22.4 ± 8.5 (9 to 36), the Swiss narcolepsy scale (SNS; Sturzenegger and Bassetti, 2004) was -42.7 ± 35.4 (-101 to 11) and the Stanford cataplexy scale (Anic-Labat *et al.*, 1999) showed a mean of $74.2 \pm 26.2\%$ (32.5 to 91.7) (see Tables 1 and 2 for detailed scores); patients differed from the controls on all these scales [all $F(1,22) > 38$, $P < 0.001$]. HLA typing was positive for all patients (HLA-DQB1*0602 for 11 patients and DR2 for one patient). CSF-hypocretin assessment was obtained in eight patients and hypocretin was not detectable in six patients and reduced (112.5 ± 7.8) in two patients. In all patients, pharmacological treatments were discontinued for at least 14 days prior to the experimental day and at least five elimination half-life times of the respective substances before entering the experiment. Note that we had to exclude two patients from an initial group of 14 patients because they felt asleep during one scanning run. We also excluded the two controls matching these patients from further analyses. Based on the behavioural responses collected during scanning and on detailed questioning after each scanning run, we can rule out that any of the 12 patients included in the results had a cataplexy attack during scanning.

Table 1 Clinical characteristics of narcoleptic patients and control subjects

	Age (years)	Sex	BMI	HLA DQB1*0602	HCRT (pg/ml)	Cataplexy	Duration of illness (years)	ESS	Stanford (%)	UNS	SNS
Patients^a											
1	22	F	15.8	+	107	+	4	20	32.5	34	−101
2	30	F	28.2	+	<20	+	7	19	91.7	36	−68
3	33	F	24.2	+	<20	+	19	16	91.7	24	−66
4	36	F	33.7	+	na	+	18	10	45.8	9	−29
5	39	F	43	+	na	+	18	15	91.7	23	−68
6	43	F	20.8	+	<20	+	15	19	32.5	18	11
7	19	M	24	+	<20	+	1	21	91.7	20	−7
8	25	M	32.3	+	<20	+	9	7	45.8	11	−37
9	27	M	41.1	DR2 +	na	+	20	16	91.7	21	−28
10	33	M	34.6	+	na	+	27	12	91.7	25	−42
11	39	M	26.2	+	118	+	18	17	91.7	16	6
12	45	M	34.7	+	<20	+	3	15	91.7	32	−83
Controls^b											
1	22	F	19.5	na	na	na	na	8	0.6	4	31
2	30	F	19.4	na	na	na	na	4	0.6	4	27
3	31	F	22.6	na	na	na	na	10	32.5	7	16
4	40	F	39.9	na	na	na	na	3	0.6	2	36
5	42	F	39	na	na	na	na	11	0.6	3	39
6	40	F	23.8	na	na	na	na	5	0.6	3	15
7	25	M	29.7	na	na	na	na	3	0.6	2	33
8	28	M	28	na	na	na	na	5	0.6	4	11
9	31	M	31.9	na	na	na	na	6	0.6	4	21
10	36	M	27.2	na	na	na	na	11	0.6	13	27
11	39	M	24.7	na	na	na	na	6	0.6	8	43
12	42	M	21	na	na	na	na	6	0.6	1	9

^aMean age (\pm SD): 32.6 \pm 8.3.

^bMean age (\pm SD): 33.8 \pm 6.9.

Note: Patients were selected by a team of neurologists with extensive experience in narcolepsy (C.B., E.V., R.K.). They were all off medication for at least 14 days prior to the experimental day. All the patients reported cataplexy episodes following positive or arousing emotions such as laughing (12/12 patients), telling or hearing a joke (10/12), as well as being surprised (10/12), emotionally moved (8/12) or excited (7/12). na = not applicable; HCRT = hypocretin-1 level in CSF (normal values >320 pg/ml; <20 = concentration below detection limit of assay); BMI = body mass index; ESS = Epworth Sleepiness Scale (0–24, higher scores indicate increased self-reported sleepiness cut off score >10); Stanford = Stanford cataplexy questionnaire (probability for clear cut cataplexy >32.5%); UNS = Ullanlinna Narcolepsy Scale (suggestive of narcolepsy if >14); SNS = Swiss-Narcolepsy Scale (suggestive of narcolepsy if <0).

Table 2 Multiple Sleep Latency Test (MSLT) measured in narcoleptic patients

Patients (cf. Table 1)	Mean sleep latency (min)	Naps	SOREM	REM latency (min)
1	3.8	5	3	5.5
2	1	4	4	3.5
3	na	na	na	na
4	5	5	3	11.2
5	na	na	na	na
6	2.1	4	3	3.3
7	1.4	4	4	3
8	1	4	4	1.4
9	1.5	5	5	3.4
10	1.8	5	3	5
11	0.9	4	4	2.1
12	0.3	4	4	1.4

Note: The MSLT was measured across five naps at 2 h intervals, starting between 9 and 10 am. 'Naps' correspond to the number of naps during which the patients felt asleep, and sleep onset REM period (SOREM) is defined as one or more epochs of REM sleep occurring within 15 min of the first 30 s epoch scored as sleep.

Humour judgement paradigm

Stimuli

We selected 100 humorous and 100 corresponding neutral pictures matched for visual complexity and content (objects, characters, animals and actions depicted), as well as for mean luminance. We obtained two additional series of 100 neutral pictures from this initial set of stimuli by removing the humorous element in each humorous picture and an equivalent element in each neutral scene. We could thus create mini-sequences with a first picture that was always neutral followed by a second picture that revealed either a humorous or a neutral element (Fig. 1). Twenty-six subjects who did not take part in the fMRI experiment rated these 200 mini-sequences for humour intensity on a 0 to 3 scale ('neutral' to 'very funny'). For the fMRI experiment, we selected the 39 funniest sequences (mean humour intensity: 2.2) and the 39 neutral sequences matching these funny sequences (all rated neutral). During scanning, the stimuli covered 8 \times 8 degrees of visual angle and were displayed using E-prime software (Psychology Software Tools, Pittsburgh, PA) allowing for precise response recording and synchronization with fMRI acquisition.

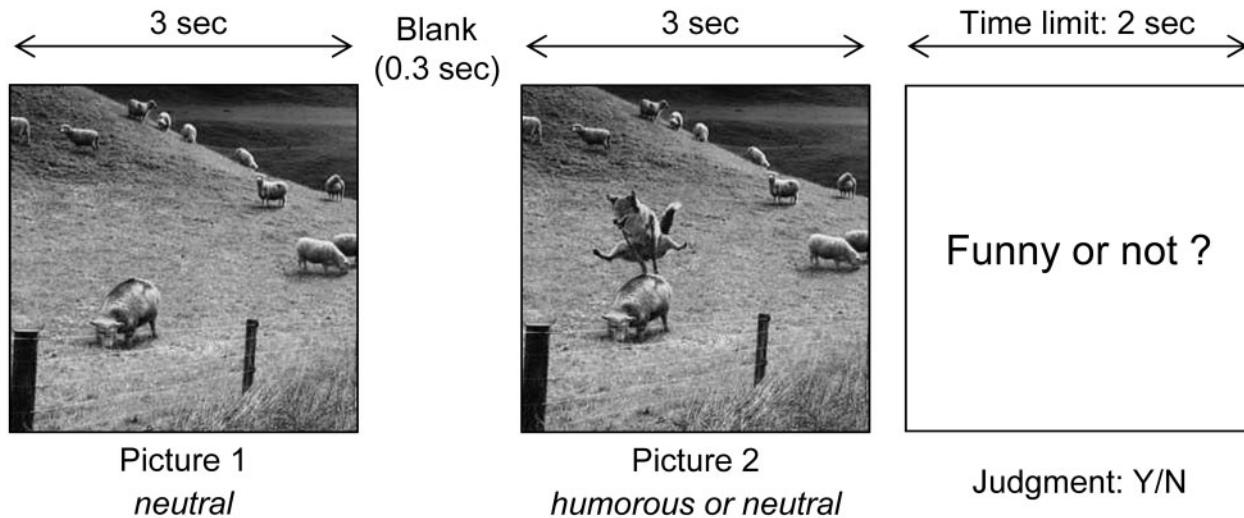


Fig. 1 Stimulus sequence. Each trial was composed of a first neutral scene followed by a second picture revealing a new element either neutral or humorous. Subjects made a humour judgement response after the offset of the second picture. Inter-stimulus interval varied from 8.8 to 15.4 s.

Task

All participants were scanned while they watched 78 humorous or neutral picture-sequences (Fig. 1). On each trial, the participants judged whether they found the sequence funny or not (by pressing one of two keys; optic fibre response pad, Current Design, Philadelphia, PA).

MRI acquisition

Whole-brain event-related fMRI data were acquired on a Philips Intera 3.0-Tesla whole-body system (Philips Medical Systems, Best, NL) equipped with an eight-element head coil array (MRI Devices Corporation, Waukesha WI), using sensitivity-encoded single-shot echo-planar sequence (SENSE-sshEPI). Functional volumes consisted of 36 contiguous axial slices positioned parallel to the AC–PC plane and covering the whole brain with a spatial resolution of $1.8 \times 1.8 \times 3.9 \text{ mm}^3$ (FoV = 220 mm, TE = 35 ms, TR = 2.2 s, SENSE reduction factor $R=2.0$). The scanning parameters were optimized during pilot testing to minimize susceptibility-related signal losses in orbito-frontal cortex and inferior temporal regions. Careful examination of each individual set of data confirmed that there was no signal drop in hypothalamic and amygdala regions reported in the results section. Functional images ($n=390$) were acquired across three scanning runs separated by brief pauses. High-resolution 3-D T1-weighted scan was obtained for anatomical reference and volumetric analyses (voxel-size = $0.9 \times 0.9 \times 0.75 \text{ mm}^3$).

MRI data analysis

Processing and statistical analyses of imaging data were performed with SPM2 (www.fil.ion.ucl.ac.uk). Functional scans were realigned, corrected for slice timing, normalized to the MNI template (resampled voxel size: $3 \times 3 \times 3 \text{ mm}^3$), and spatially smoothed (8 mm Gaussian kernel). Whole-brain statistical analyses were conducted on individual time-series using the general linear model with two main regressors coding for neutral and humorous trials (second pictures in sequence; Fig. 1), as classified by each

participant during fMRI, convolved with a canonical hemodynamic response function. Additional covariates of no interest included onset-times for the first pictures (always neutral), as well as movement parameters from realignment correction to account for residual movement artefacts. Statistical parametric maps were generated from linear contrasts between conditions in each participant. The (humour > neutral) contrast images from each subject were submitted to a second-level group analysis, using a two-way ANOVA treating subjects as a random effect.

In the 'Results' section, we first describe effects found in patients and controls using conjunction analyses to preserve only voxels that were significant in the contributing SPM maps of both populations (Friston *et al.*, 2005). We then report group comparisons performed using exclusive masking to reveal voxels showing significant activation for the contrast (humour > neutral) in one population but no such effect whatsoever in the other population for the exact same contrast. SPM exclusive masks were thresholded at $P < 0.05$, whereas the contrasts to be masked were thresholded at $P < 0.001$. Note that the more liberal the threshold of an exclusive mask, the more conservative is the masking procedure. For the patient group, we performed additional whole-brain second-level correlations analyses between the contrast (humour > neutral) and the main clinical measurements, including age at disease onset, duration of disease, sleepiness and each of the cataplexy scores (see earlier). These analyses allowed us to assess whether the modulation of brain responses to humour might relate to individual clinical characteristics.

Results

Behaviour

During scanning, patients and controls did not differ in the proportion of images judged as humorous (mean percentage \pm SD, 40.06 ± 7.64 and 41.67 ± 6.59 , respectively). As expected from excessive daytime sleepiness in NC, patients were generally slower than control [reaction times: ms \pm SD, 789.04 ± 120.26 , 607.36 ± 121.65 ,

Table 3 Regional brain responses to humour versus neutral ratings

Region	L/R	MNI coordinates			BA	Z-score
		x	y	z		
Main effect for [humour > neutral] contrast						
Group conjunction with controls and patients						
Fusiform cortex	L	-45	-51	-18	37	4.07
	R	48	-63	-15	37	3.97
Insula/Inferior frontal	L	-42	18	-3	47/48	3.47
Middle occipital gyrus	L	-39	-87	6	18/19	3.45
Superior medial frontal	L	-12	39	45	9	3.44
Amygdala	R	18	6	-18	-	3.21*
Amygdala	L	-21	-6	-21	-	3.10*
Group comparison for [humour > neutral] contrast						
Controls > Patients using exclusive masking procedure						
Hypothalamus	R	12	3	-18	-	4.55
Anterior cingulate	L	-9	45	15	32/24	4.54
Orbitofrontal	R	27	12	-21	38	4.00
Lateral occipital	R	42	-78	-6	19	3.91
Orbitofrontal/insula	L	-27	24	-18	38/47	3.88
Inferior frontal gyrus	L	-48	18	15	48	3.84
Anterior cingulate	R	0	18	36	24	3.84
Insula	R	45	18	-12	47	3.83
Medial prefrontal	R	12	60	3	10	3.71
Group comparison for [humour > neutral] contrast						
Patients > Controls using exclusive masking procedure						
Supramarginal gyrus	R	57	-33	27	48/2	4.17
Insula	L	-36	3	-6	48	4.07
Posterior cingulate	L	-9	-45	33	23/26	4.05
Inferior frontal gyrus	R	51	30	3	45	3.94
Fusiform cortex	R	33	-57	-15	37	3.70
	L	-39	-60	-12	37	3.48
Ventral striatum (N. accumbens)	L	-15	9	-3	-	3.52
Amygdala	R	33	3	-21	-	3.14*

Note: Stereotactic coordinates correspond to the standard Montreal Neurological Institute (MNI) brain. Reported regions survived a threshold level of $P < 0.001$ uncorrected, or a threshold of $P < 0.05$ corrected (small volume correction using a 9 mm radius sphere, indicated by an asterisk). Mask threshold for group comparisons was set at a conservative level of $P < 0.05$.

respectively; $F(1,22) = 13.97$, $P < 0.01$], but without any effect of picture-type (neutral, humorous) and no interaction of group by picture-type.

Functional MRI: main effect of humour

Using a conjunction analysis, we first identified brain regions that responded to humorous compared to neutral trials in both patients and controls (Table 3). This analysis revealed activation in limbic (amygdala and insula) and frontal regions known to be recruited by the affective content of humorous inputs and experience (Mobbs *et al.*, 2003; Moran *et al.*, 2004; Watson *et al.*, 2006). Additional activity increase was observed in visual regions, possibly involved in the maintenance and processing of information necessary for the appreciation of humorous pictures (Goel and Dolan, 2001).

Increased brain response to humour in controls

We then tested for regions showing increased fMRI signal during humorous (versus neutral) trials in controls

but not in patients. Controls showed a maximal activity difference in the right hypothalamus (peak at 12x, 3y, -18z), whereas patients did not show any humour-related modulation in the hypothalamus (Fig. 2 and Table 3). This result is consistent with a hypothalamic dysfunction in our patients (Mignot *et al.*, 2002; Baumann and Bassetti, 2005).

Controls also showed increased activity in the anterior cingulate, left anterior insula, orbitofrontal cortex and medial prefrontal cortex, a network of regions previously associated with hedonic experience and autonomic control (Critchley *et al.*, 2004; Kringelbach, 2005), as well as with emotional regulation and learning (Hariri *et al.*, 2003; Phelps *et al.*, 2004).

Increased brain response to humour in NC patients

When compared to controls, NC patients showed increased response to humorous stimuli in the right amygdala (Fig. 3). The patient-selective activation lied in a more lateral and anterior region of the amygdala than the

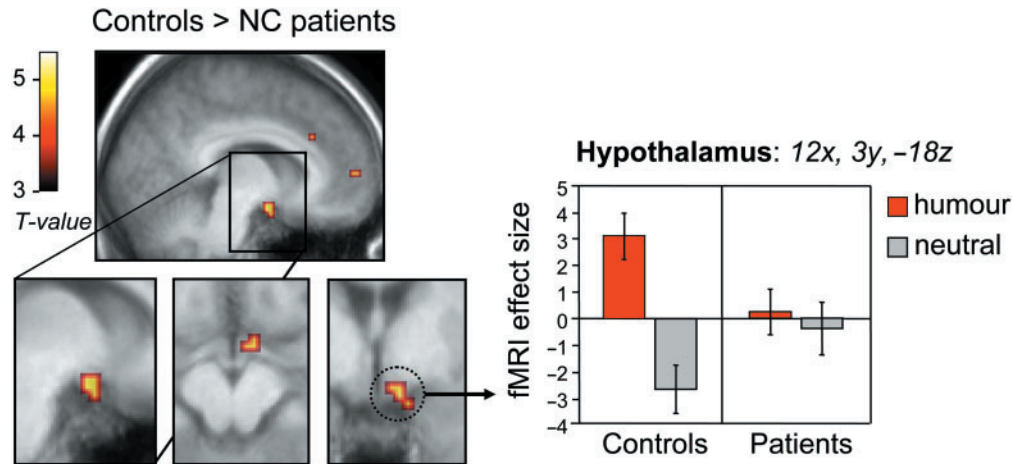


Fig. 2 Increased response to humour in right hypothalamus, medial prefrontal and cingulate cortex for controls relative to NC patients. Parameter estimates extracted from hypothalamic peak illustrate selective activation for humorous stimuli in the controls but not in the patients. Statistical maps are overlaid on mean-normalized T1-structural scan.

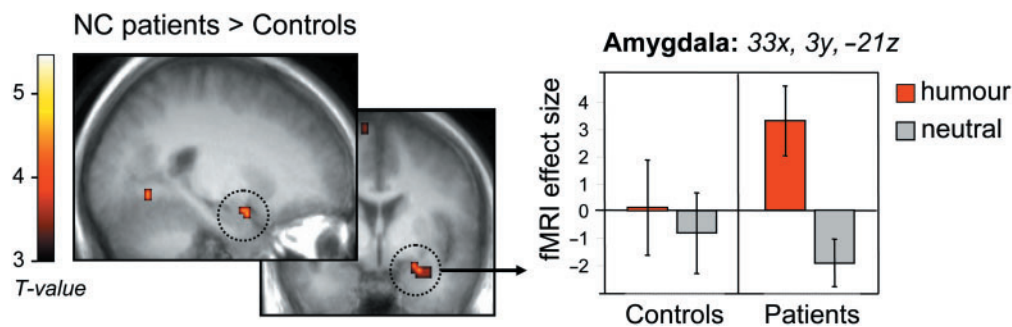


Fig. 3 Increased amygdala response to humour in NC patients compared to controls. Parameter estimate showed increased fMRI signal to humorous sequences in the patients but not in the controls. Statistical maps are overlaid on mean-normalized T1-structural scan.

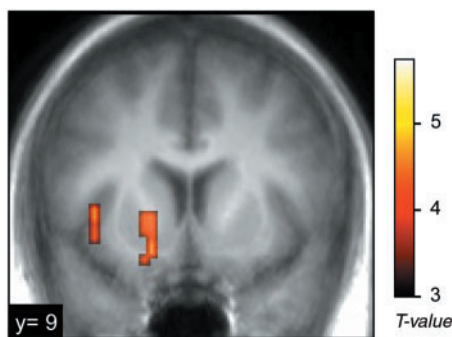


Fig. 4 Increased fMRI signal in left insula and nucleus accumbens in NC-patients (but not in controls). Clusters of activation are overlaid on the mean-normalized T1-weighted structural scan.

activation revealed by the group conjunction. Increased activity in right inferior parietal and in fusiform cortex in the patients might reflect the impact of top-down influences from the amygdala on sensory pathways, which can prioritize the representation of emotional events within attentional and perceptual systems (Vuilleumier, 2005). Humour-selective increases in NC patients were also observed in other regions contributing to the

integration of emotion and reward-related functions (Price, 2005) with more activity in inferior frontal cortex, insula and ventral striatum, including left nucleus accumbens (Fig. 4, Table 3).

Additional whole-brain correlation analyses using individual clinical characteristics of the patients as regressors (see ‘Methods’ section) did not disclose any significant linear relationship with activity levels within these regions.

Discussion

We report here the first event-related fMRI data obtained on NC patients while they experienced positive emotion. Our results reveal that narcolepsy is associated with increased amygdala activity together with reduced medial prefrontal and hypothalamic activity during humour processing.

A recent SPECT study in two patients reported increased neuronal activity in the amygdala during cataplexy attacks triggered by emotions (Hong *et al.*, 2006, for related animal results, see Gulyani *et al.*, 2002). However, another SPECT study did not replicate this finding in one patient

(Chabas *et al.*, 2007). It is noteworthy that, in this second study, the patient's cataplexy attack had not been triggered by any particular emotion. Our results on a group of 12 NC patients do not only add support to, but also go beyond these observations by providing the first demonstration that narcolepsy disease is associated with exaggerated amygdala response to transient humour stimuli—even in the absence of any cataplexy episodes. These findings suggest that elevated amygdala response to positive emotional events might contribute to the pathophysiology of cataplexy.

Neural circuits underlying emotion-triggered cataplexy

All patients included in our study reported joking and laughing as a main trigger of cataplexy attacks. Behaviourally, NC patients and their matched controls exhibited a similar response profile during the humour appreciation task, with the same proportion of stimuli judged funny in both groups and no reaction times difference between funny and neutral stimuli. Thus, the observed differences in brain activation cannot be attributed to some general alteration of perceived affective values or to emotional suppression strategies that the patients might use to protect themselves from cataplexy (note that the latter would rather lead to decrease in limbic activity; see Phan *et al.*, 2005). At the brain level, the present fMRI data reveal that NC patients showed increased brain response to humorous stimuli in several regions associated with emotional and reward processing. We also observed increased activity in attentional and sensory regions, possibly reflecting enhanced perceptual processing of emotional stimuli mediated by direct feedback signals imposed by amygdala on cortical pathways (Vuilleumier, 2005). These findings therefore provide a neural basis for the patients' subjective reports and well-documented clinical observations that positive emotions often trigger abnormal reactions such as cataplectic attacks in NC patients.

While it is generally accepted that connections from the amygdala to the hypothalamus can modulate reflex responses to emotional stimuli (LeDoux, 2000; Sullivan *et al.*, 2004; Price, 2005), our new fMRI results suggest that the hypothalamus might also have modulatory influences on amygdala activity during positive emotions, possibly via direct projections from hypothalamic HCRT neurons to the amygdaloid complex (Peyron *et al.*, 1998; Date *et al.*, 1999; Marcus *et al.*, 2001; Bisetti *et al.*, 2006). Reduced hypothalamic activation and exaggerated amygdala response to humour could be due to loss of hypothalamic HCRT neurons in NC. Another neural circuit possibly mediating a regulatory action of HCRT on affective responses has recently been identified and implicates projections of HCRT onto the ventral tegmental area (VTA; Fadel *et al.*, 2002). These projections might act to increase dopamine (DA) efflux in the prefrontal cortex and increase time spent awake based on

motivational signals (Vittoz and Berridge, 2006; see also Wisor *et al.*, 2001). Critically, activity in prefrontal cortex and anterior cingulate might be involved in the suppression of amygdala response (Hariri *et al.*, 2003), and was found to mediate extinction in conditioning paradigms in both animals and humans (Milad and Quirk, 2002; Phelps *et al.*, 2004). Thus, the reduced hypothalamic and prefrontal activity together with increased amygdala activation in NC patients found in the present study could reflect a dysfunction of HCRT/DA-mediated pathways that usually inhibit amygdala activity, but could lead to an abnormally high amygdala response to positive emotions in narcolepsy.

Other brain regions showed different responses in NC patients and controls. Although animal research has shown that HCRT neurons send widespread projections to the entire CNS (Peyron *et al.*, 1998; Date *et al.*, 1999; Sakurai, 2007), one should remain cautious when interpreting all of these regional changes as primarily reflecting modulations at direct HCRT projection sites. Alternatively, some changes may reflect indirect consequences from abnormal HCRT activity within networks associated with emotion and arousal.

Our human imaging data also show that NC patients have elevated fMRI responses to humour in the left nucleus accumbens, a key component of the mesolimbic reward system known to be involved in humour processing (Mobbs *et al.*, 2003), and which has strong interconnections with the amygdala, prefrontal cortex and thalamus (Price, 2005). Increased activity in the nucleus accumbens could be secondary to increased amygdala activity, or might result from a disruption of direct HCRT modulation on reward systems (Harris *et al.*, 2005; Narita *et al.*, 2006). An effect of HCRT depletion on the behavioural response to reward is well-documented as it has been reported that NC patients rarely become addicted to stimulants (Bassetti and Aldrich, 1996) and HCRT knockout mice show attenuated withdrawal response to morphine (Georgescu *et al.*, 2003).

However, the present fMRI data alone do not allow us to determine the exact role of the striatum in emotional responses to humour in NC, and the possible relations to motor effects associated with cataplexy. Further research is warranted to clarify how HCRT depletion might differentially affect distinct functional divisions of the striatum linked to reward processing (e.g. sensorimotor versus limbic processes; Voorn *et al.*, 2004).

Taken together, our findings provide evidence for an implication of amygdala circuits in the pathophysiology of human narcolepsy and abnormal responses to positive emotions in these patients, as clinically observed during cataplexy. Furthermore, these data support recent proposals suggesting a key role of the human HCRT system in modulating activity in hypothalamus-limbic circuits that are involved in the integration of emotion, reward and sleep processes.

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