Hypocretin-deficient narcolepsy patients have abnormal brain activation during humor processing

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Abstract

Study Objectives

To assess brain activation patterns in response to fun-rated and neutral-rated movies we performed functional magnetic resonance imaging (fMRI) during a humor-paradigm in narcolepsy type 1 (NT1) patients with cataplexy (muscle atonia triggered by emotions) and controls.

Methods

The fMRI-humor-paradigm consisted of short movies (25/30 with a humorous punchline; 5/30 without a humorous punchline (but with similar build-up/anticipation)) rated by participants based on their humor experience. We included 41 NT1 patients and 44 controls. Group-level inferences were made using permutation testing.

Results

Permutation testing revealed no group differences in average movie ratings. FMRI analysis found no group differences in brain activations to fun-rated movies. Patients showed significantly higher activations compared to controls during neutral-rated movies; including bilaterally in the thalamus, pallidum, putamen, amygdala, hippocampus, middle temporal gyrus, cerebellum, brainstem and in the left precuneus, supramarginal gyrus and caudate. We found no brain overactivation for patients during movies without a humorous punchline (89.0% neutral-rated).

Group analyses revealed significantly stronger differentiation between fun-rated and neutral-rated movies in controls compared with patients (patients showed no significant differentiation), including bilaterally in the inferior frontal gyrus, thalamus, putamen,

precentral gyrus, lingual gyrus, supramarginal gyrus, occipital areas, temporal areas, cerebellum and in the right hippocampus, postcentral gyrus, pallidum and insula.

Conclusion

Patients showed significantly higher activations in several cortical and subcortical regions during neutral-rated movies, with no differentiation from activations during fun-rated movies. This lower threshold for activating the humor response (even during neutral-rated movies), might represent insight into the mechanisms associated with cataplexy.

Keywords: narcolepsy type 1, fMRI, cataplexy, humor, hypocretin

Statement of Significance

Cataplexy, muscle atonia triggered by emotions, is a defining clinical feature of narcolepsy type 1, but the mechanism underlying cataplexy has not been established. In the present study we explore this by studying humor (a potent trigger of cataplexy) in narcolepsy type 1 patients compared with controls. We show that patients have similar brain activations on functional magnetic resonance imaging (fMRI) during neutral-rated and fun-rated movies. This "overactivation" during neutral-rated movies, containing a potential humorous punchline, might represent a hypersensitivity to potential humorous stimuli. Patients seem to have a lower threshold for activating the humor response, even when they subjectively rate the movie as neutral.

Introduction

Narcolepsy type 1 (NT1) is a disabling, chronic neurological sleep disorder primarily characterized by excessive daytime sleepiness, cataplexy and sleep-onset rapid eye movement (REM)-periods. Patients also experience fragmented night sleep, hypnagogic/hypnopompic hallucinations and sleep paralysis [1-3]. NT1 patients are hypocretin (also called orexin)-deficient due to loss of hypocretin producing hypothalamic neurons [4,5], with projections widely distributed in the brain [6-8]. Hypocretin 1 and 2 (Orexin-A and B) are central regulators of sleep-wake and muscle tonus [9,10]. Autoimmune destruction of hypocretin-producing neurons is hypothesized to be the pathogenesis of NT1 [2], which is further supported by the >10-fold increase of H1N1-vaccine related NT1 cases occurring after the H1N1 flu vaccination campaigns with Pandemrix® in 2009/2010 in several European countries, including Norway [11].

Cataplexy, emotionally triggered involuntary muscle weakness or paralysis during wakefulness, is a defining clinical feature of NT1 [3]. Episodes can typically last from several seconds to several minutes with retained consciousness, usually triggered by strong, positive emotions (for example thinking of, hearing or telling a joke), although various emotions can be triggers. Cataplectic episodes can also be reported by patients to be unrelated to emotions and have no identifiable trigger [2,3]. The frequency of cataplexy episodes varies greatly; while some patients seldom have episodes, others can have more than 20 per day. There is also variation in the degree of muscle atonia involvement in cataplexy, from partial slight hypotonia to a complete inability to move [2]. It has also been reported [12] that some patients can feel cataplexy attacks coming on with warning signs and learn to avoid cataplexy by avoiding triggering situations [12,13].

Three previous functional magnetic resonance imaging (fMRI) studies used humorous pictures [14,15] or movies [16] to study humor-processing and cataplexy in sporadic (non-

vaccinated) NT1 patients. These studies involved a relatively small number of patients (n = 10-21). The largest study [16] acquired with simultaneous electroencephalography (EEG), attempted to elicit cataplexy in patients with a naturalistic paradigm in which the humorous movies were selected in accordance with each patient's humor preferences, while the other two studies [14,15] primarily assessed humor processing in NT1 patients.

Schwarz et al. found lower activation in several areas including the hypothalamus and higher activation in several areas including the amygdala for humorous pictures for NT1 patients compared with controls. Reiss et al. found higher activations for NT1 patients compared with controls when looking at funny cartoons compared to non-funny cartoons in several regions, including; hypothalamus, ventral striatum and right inferior frontal gyrus. Meletti et al. found that laughter was associated with higher activation bilaterally in anterior cingulate gyrus and the motor/premotor cortex, and that cataplexy was associated with higher activation in several areas, including; the amygdala, anterior insula, ventromedial prefrontal cortex, nucleus accumbens, locus coeruleus and the anteromedial pons.

The mechanism underlying cataplexy has not been established [2]. One theory suggests that cataplexy is a form of tonic immobility that can be seen in some animals [17], while another states that cataplexy represents dissociated REM sleep that appears while the person is awake [2,3]. Due to cataplexy possibly representing dissociated REM sleep we were particularly interested in regions that have been implicated in REM sleep and cataplexy/humor (thalamus, amygdala and basal ganglia). Previous studies using fMRI [18-20] and positron emission tomography (PET) [21-24] in healthy individuals with polysomnographic monitoring have revealed higher thalamus activation during REM sleep. Further, the thalamus have been shown to have higher activity in response to humor processing [25], with higher activation in response to cartoons compared with neutral pictures [26,27] and during humor-induced smiling [28].

The amygdala has been reliably associated with humor appreciation in humans [25]. PET [22,23,29] and fMRI [20] studies have also shown amygdala activation during REM sleep in healthy individuals. Increased basal ganglion activation has also been linked to REM sleep [18,20,21] and humor [28,30,31].

Our study focuses on humor processing, as partially explored by previous studies [14,15], but using a larger sample size. The previous studies included ratings of pictures, but only reported results of neutral-rated pictures in relation to fun-rated pictures. Here, we assess brain activation patterns during fun-rated and neutral-rated short movies, including movies with and without a humorous punchline.

To test the hypothesis of abnormal humor processing in NT1, we compared fMRI-based brain activation patterns during presentations of short movies in 41 NT1 patients (31 females, mean age 23.6 years) and a control group of 44 first-degree relatives of NT1 patients (24 females, mean age 19.6 years). We tested for group differences across the whole brain and corrected for non-independence due to familiarity and for multiple comparisons by permutation testing.

Methods

Participants

Table 1 summarizes the demographic and clinical information about the two groups. We have previously reported on 40 patients and 44 controls considered in the current study [32], and nine of the patients have also been reported on in a quality of life study [33]. Participants were recruited from those who were referred for narcolepsy family disease education and counseling courses at the Norwegian Centre of Expertise for Neurodevelopmental Disorders and Hypersomnias (NevSom) during the inclusion period from June 2015 to April 2017. 41 patients with NT1, with disease onset after the H1N1-vaccination in 2009/2010, and 44 first-

degree relatives of NT1 patients were included consecutively. Not all first-degree relatives of NT1 patients were first-degree relatives of patients included in this study, as sometimes patients were excluded, but all these excluded patients had a verified NT1 diagnosis. The disease onset was changed to being before the H1N1 vaccination for three NT1 patients after a thorough evaluation of their medical history and records (3/3 had typical NT1 phenotypes, being hypocretin-deficient, *HLA-DQB1*06:02*-positive with cataplexy, and were therefore kept in the study). Written informed consent was provided by all participants before inclusion, and the Norwegian regional committees for medical and health research ethics (REK) approved our study. The official Norwegian Immunization Registry (SYSVAK) was used to obtain H1N1-vaccination status of patients and first-degree relatives of patients. Pandemrix® was the only vaccine used for H1N1-vaccination in Norway. Two patients who reported having been H1N1-vaccinated in their workplace without being registered in the SYSVAK were also included in the H1N1-vaccinated group.

Fourteen days before their inclusion, all patients had ceased all narcolepsy medication, except for one patient who, due to severe cataplexy, was without narcolepsy medication for only 7 days. Exclusion criteria for patients and first-degree relatives were severe neurological, psychiatric or somatic disorders, previous head injury with loss of consciousness for 10 minutes or 30 minutes amnesia, metallic implants, excessive movement during the magnetic resonance imaging (MRI)-scanning, and neuroradiological findings requiring clinical follow-up. Since NT1 is associated with an increased number of comorbidities [2] we performed the analysis in the full sample including the comorbidities listed below (for patients and first-degree relatives) and in a reduced sample, from which we excluded patients and first-degree relatives with the comorbidities. In the reduced sample we also excluded participants who had to re-watch the movies (mainly due to drowsiness/falling asleep during the first viewing; n = 8) or watch the movies in black and white (due to a technical problem; n = 1), or without

sound (due to a human error; *n* = 2), first-degree relatives who had experienced cataplexy-like episodes (*n* = 6), sleep paralysis (*n = 8*) and hypnagogic hallucinations (*n = 9*).

In the full sample analysis, the following comorbidities were present in the NT1 patients: Asperger syndrome (*n* = 2), attention deficit hyperactivity disorder (ADHD, *n* = 1), migraine (*n* = 4), Tourette syndrome (*n* = 1), anxiety (*n* = 1), depression (*n* = 1), prematurity without severe long-term complications (*n* = 1), kidney disease (*n* = 1), type 2 diabetes (*n* = 1) and hypothyroidism (*n* = 2). We also accepted the following morbidities in the first-degree relatives group for the full sample analysis: attention deficit disorder (ADD, *n* = 2), migraine (*n* = 6), dyslexia (*n* = 4), anxiety (*n* = 1), prematurity without severe long-term complications (*n* = 2) and bipolar type 2 disorder (*n* = 1). Some patients and first-degree relatives had more than one comorbidity.

All patients (41/41) and 61.4% (27/44) of the first-degree relatives were *HLA-DQB1*06:02*- positive. All patients whose hypocretin level was measured (*n* = 40) were hypocretin-deficient (cerebrospinal fluid (CSF) hypocretin-1 level < 110 pg/ml or < 1/3 of the normal mean, as previously reported [34,35]), while one HLA-DQB1*0602-positive patient with typical cataplexy had not yet had this measured. 92.7% (38/41) of all patients and 68.2% (30/44) of first-degree relatives were H1N1-vaccinated. 95.1% (39/41) of all patients reported having had cataplexy episodes. 13.6% (6/44) of the first-degree relatives reported signs of muscle weakness, although they all experienced this rarely, triggered by emotions known to elicit cataplexy: laughter, fun/excitement and surprise. 85.4% (35/41) of patients reported hypnagogic hallucinations and 70.7% (29/41) experienced sleep paralysis. 20.5% (9/44) of first-degree relatives also experienced hypnagogic hallucinations and 18.2% (8/44) had experienced sleep paralysis.

Narcolepsy diagnosis

International Classification of Sleep Disorders (ICSD)-3 criteria were used to establish the NT1 diagnoses [1] given by the experienced neurologist and sleep medicine expert Stine Knudsen.

Patients and first-degree relatives completed clinical consultations, a neurological examination, routine blood samples, actigraphy, polysomnography, the multiple sleep latency test (MSLT) and HLA typing. Participants also took part in semi-structured interviews about narcolepsy and sleep disorders, including a Norwegian translation of the Stanford Sleep Questionnaire [36]. Measurements of CSF hypocretin-1 levels in patients were also obtained (Phoenix Pharmaceutical St. Joseph, MO, USA), slightly modified, and analyzed at the Hormone Laboratory, Oslo University Hospital) [34,35].

After taking into consideration clinical evaluation, polysomnography, MSLT and hypocretin measures, all patients fulfilled the ICSD-3 criteria for narcolepsy. No first-degree relatives met the ICSD-3 criteria for narcolepsy.

Polysomnography recordings

10-14 days of actigraphy (Philips Actiwatch, Respironics Inc., Murrysville, PA, USA) preceded all polysomnography (PSG) recordings. International Classification of Sleep Disorders (ICSD)-3 criteria [1] were used to evaluate all participants with PSG and MSLT. The SOMNOmedics system (SOMNOmedics GmbH, Randersacker, Germany) was used to obtain PSG recordings with the F3-A2, C3-A2, O1-A2, F4-A1, C4-A1 and O2-A1 electrodes, in addition to vertical and horizontal electro-oculography, surface electromyography (EMG) of the submentalis and tibialis anterior muscles, electrocardiography, nasal air flow, thoracic respiratory effort and oxygen saturation. EMG impedance was kept below 10kΩ (preferably 5Ω). All participants had a full-night PSG followed by a 5-naps MSLT the next day, where the

naps (30 minutes) were separated by 2-hour intervals. AASM criteria [1] were applied to the sleep scoring.

FMRI paradigm

Participants watched 30 movies by looking through a mirror attached to a 32-channel headcoil to view an MRI-compatible LCD screen (NNL LCD Monitor®, NordicNeuroLab, Bergen, Norway) behind the scanner. Stimuli were presented using E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA). 25/30 movies included a humorous punchline, while the humorous punchline had been edited out of the remaining five. Movies with and without a humorous punchline varied in duration from 10-20 s (mean 14.4 ± 3.5 s) and from 10-15 s (mean 12.0 ± 2.3 s), respectively. Both types of movie had similar build-up/anticipation that something funny might happen.

Participants rated all movies (with or without a humorous punchline) using an MRI-compatible subject response collection system (ResponseGrip®, NordicNeuroLab, Bergen, Norway) to choose from three "emoji" faces representing neutral, "a little funny", and "funny". A trigger pulse from the scanner synchronized the onset of the experiment to the beginning of the acquisition of a fMRI volume. Participants were instructed that they should wait for the movies to start. The experiment was set up to start with an input from user. However, it was discovered that this input could not be registered due to a technical problem and the experiment was therefore triggered by the scanner instead, changing the start of the experiment to occur one TR-time later, this was then corrected. Therefore, the first 61 participants (27 patients and 34 controls) had their experiment triggered one repetition time (TR)-time (2.25 s) earlier than the final 24 participants (14 patients and 10 controls). This was corrected for in the individual-level fMRI analysis. All subjects were questioned after the scanning about the occurrence of cataplexy.

41 patients and 44 first-degree relatives rated the movies, but one patient and one first-degree relative could not be included in the ratings analysis because they rated all movies as neutral, confirming after the session that they did not think any of the movies were funny. All three rating categories were considered for the analysis of behavioral responses, but the ratings of "a little funny" and "funny" were combined to give a category ("fun") for the fMRI analysis due to a large number of participants in both the patient and control group having few trials being rated as "funny"; 9 patients and 6 controls had zero trials rated as "funny", and 12 patients and 15 controls had only 1-4 trials rated as "funny". Eight participants had to stop the experiment and run it again later, mainly due to feeling drowsy/falling asleep under the first experiment. Due to a technical problem one participant watched the movies in black and white, and due to a human error, two participants viewed the movies without sound.

MRI acquisition and processing

Imaging was conducted on a General Electric Discovery MR750 3T scanner at Oslo University Hospital using a 32-channel head coil. For registration purposes we acquired a T1-weighted scan (duration: 4 min 43 s) with voxel size 1 x 1 x 1 mm; TR: 8.16 ms; echo time (TE): 3.18 ms; flip angle: 12°; 188 sagittal slices. We acquired fMRI data with a T2*-weighted echo-planar imaging sequence (duration: 16 min 19 s) with 430 volumes; 3 mm slice thickness, in-plane resolution: 2.67 x 2.67; TR: 2250 ms; TE: 30 ms; slice gap: 0.5 mm; flip angle: 79°; 43 axial slices.

FMRI data were processed and analyzed with the FMRI Expert Analysis Tool (FEAT) from the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) [37,38]. Individual first-level preprocessing included motion correction with MCFLIRT [39], spatial smoothing (full width at a half-maximum of 5

mm), grand-mean intensity normalization of the whole 4D dataset by a single scaling factor and high-pass temporal filtering (128 s).

We processed T1-weighted data in FreeSurfer (http://surfer.nmr.mgh.harvard.edu) to obtain brain-extracted T1-weighted volumes. The T1 images were further registered to the functional images using FMRIB's Linear Image Registration Tool (FLIRT) [39,40], optimized by boundary-based registration [41], and then nonlinear registration to a standard MNI space was done using FMRIB's Non-linear Image Registration Tool (FNIRT) [42,43].

Statistical analysis

Individual-level general linear models (GLM) were fitted using FILM (FMRIB's Improved Linear Model) modeling the movies (fun/neutral) and rating/response periods as blocks and the interspersed fixation periods as implicit baselines. The design matrix included nuisance regressors for six motion parameters and their derivatives. Temporal derivatives were added to account for regional differences in the timing of the hemodynamic response, e.g., due to differences in acquisition time between slices. Regressors were filtered and convolved with a double-gamma hemodynamic response function before the model fit. Single-subject contrasts were calculated for fun+ (activations in the brain correlating with fun movies vs baseline), fun- (deactivations in the brain correlating with fun movies vs baseline), neutral+ (activations in the brain correlating with neutral movies vs baseline), neutral- (deactivations in the brain correlating with neutral movies vs baseline), fun > neutral, neutral > fun, response+ (activations in the brain correlating with the response vs baseline), response- (deactivations in the brain correlating with the response vs baseline). An additional analysis was performed to compare brain activation during movies without a humorous punchline (89.0% neutral-rated).

Contrast of parameter estimate (COPE)s for each first-level contrast were concatenated in standard space and submitted to group analysis in Permutation Analysis of

Linear Models (PALM) [44,45], testing for group differences between NT1 patients and first-degree relatives, while controlling for age and gender. We corrected for multiple testing by running 5000 permutations and threshold-free cluster enhancement (TFCE) as implemented in PALM. To control for lack of independence (patients and first-degree relatives from the same family, two related patients, and siblings within the first-degree relative group) permutations were constrained between first-degree relatives. Corrected two-tailed values of $p < 0.05$ were considered significant. We also performed a separate analysis for a reduced sample (22 NT1 patients (15 females, mean age 21.5 ± 8.2 years) and 23 controls (11 females, mean age 19.4 ±7.8), in which we excluded all patients and first-degree relatives with comorbidities, as well as all participants who had to re-watch the movies ($n = 8$) or had watched the movies in black and white ($n = 1$) or without sound ($n = 2$), and first-degree relatives who had experienced cataplexy-like episodes ($n = 6$), sleep paralysis ($n = 8$) and hypnagogic hallucinations ($n = 9$). To assess the similarities in the results between the full and reduced samples, spatial correlations were computed between the uncorrected t-statistic maps. Reported brain areas in the results section, except for findings in the cerebellum, were consulted with the atlases Harvard-Oxford Cortical Structural Atlas and Harvard-Oxford Subcortical Structural Atlas as implemented in FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki)[37,38].

For the behavioral responses we coded no rating, neutral, “a little funny” and “funny” as 0, 1, 2 and 3, respectively. We tested for group differences in average movie ratings (for all movies/only rated movies) using PALM [44,45], controlling for age, gender and the number of movies not rated. We ran 5000 permutations, and to control for lack of independence, these were constrained between first-degree relatives. Corrected two-tailed values of $p < 0.05$ were considered significant. We performed chi-square tests of independence to test for the relation between group and rating (neutral, “a little funny” and “funny”), including and excluding the movies without rating.

Data availability

The data are not publicly available due to ethical restrictions as it could compromise the privacy of research participants.

Results

Behavioral responses

One patient reported a cataplectic attack during the scanning, but could not precisely identify during which movie it had occurred. Patients rated an average of (mean± SD) 11.5 ± 5.3 movies as neutral, 14.3 ± 6.6 movies as fun (9.5 ± 4.7 as "a little funny" and 4.9 ± 4.9 as "funny"), and 4.2 ± 6.1 movies had no rating. Controls rated 13.7 ± 6.1 movies as neutral, 15.6 ± 6.3 movies as fun (9.6 ± 3.9 as "a little funny" and 5.9 ± 5.1 as "funny"), and 0.8 ± 1.9 movies had no rating. Considering only rated trials, patients and controls rated an average of 55.5% and 53.2% of movies as fun, respectively. Permutation testing revealed no significant group difference in average movie ratings when considering all movies or only rated movies. The chi square test of independence was not significant when excluding the movies without rating, but it was significant when including these movies.

Brain activations

Main effects and group differences for fun-rated movies in the full and reduced samples

Figure 1 summarizes the results for fun-rated movies in the full sample. Both groups showed significant activations in several areas including bilateral temporal poles, temporo-parietal areas, temporo-occipito-parietal areas, amygdala, hippocampus, thalamus, occipital areas, and

in the right inferior frontal gyrus. Permutation testing revealed no significant group differences between patients and controls. Subsequent sub-analysis of the reduced sample yielded similar results for the main effects of fun, and no significant group differences.

Main effects and group differences for neutral-rated movies in the full and reduced samples

Figures 1 and 2 summarizes the results for neutral-rated movies in the full sample. Both groups showed significant activation in several areas including bilaterally in the thalamus, occipital areas, temporal areas and right inferior frontal gyrus. Patients showed significantly higher activation for neutral-rated movies compared with controls in widespread areas (16 267 voxels), including bilaterally in the thalamus, pallidum, putamen, amygdala, hippocampus, middle temporal gyrus, cerebellum, brainstem and in the left precuneus, supramarginal gyrus and caudate.

Sub-analysis of the reduced sample revealed even more widespread group differences than in the full sample (37 588 voxels), with many areas exhibiting significantly higher activations in patients compared to controls, including bilaterally in amygdala, thalamus, putamen, hippocampus, caudate, pallidum, insula, paracingulate gyrus, cingulate gyrus, middle temporal gyrus, precuneus, precentral gyrus, inferior frontal gyrus, supramarginal gyrus, cerebellum, frontal areas, temporal areas and brainstem (Figure 3). The spatial correlation between the uncorrected t-statistic maps obtained from the group comparisons in the full and reduced samples was 0.82, suggesting a highly similar pattern and direction of effects**.**

Main effects and group differences between fun and neutral in full and reduced samples

Figures 1 and 4 summarizes the results for the fun *vs.* neutral contrast in the full sample. In controls, several areas showed significantly higher activation for fun-rated compared with neutral-rated movies. These included the bilateral temporal poles, amygdala, inferior frontal gyrus, thalamus, putamen and frontalorbital cortex. No areas showed significantly increased activation in neutral-rated movies compared to fun-rated movies in controls. Patients showed no significant differences in brain activation between fun and neutral-rated movies.

Hence, group comparisons for fun > neutral revealed several areas with a significantly higher increase in activation for controls compared with patients (16 604 voxels), including bilaterally in the inferior frontal gyrus, thalamus, putamen, precentral gyrus, lingual gyrus, supramarginal gyrus, occipital areas, temporal areas, cerebellum, and in the right hippocampus, postcentral gyrus, pallidum and insula.

Group comparison in the reduced sample revealed an even more widespread (23 944 voxels) pattern of significantly higher activation in fun > neutral in controls compared with patients, largely overlapping with the previously described areas for the full sample, but also including other areas like bilaterally the hippocampus, the right amygdala and left caudate. The spatial correlation between the uncorrected t-statistic map was 0.76, suggesting a highly similar pattern and direction of effects**.**

Main effect and group differences in movies without a humorous punchline

Figure S1 summarizes the main effect for movies without a humorous punchline (89.0% neutral-rated). Similar brain activations were seen in patients and controls, with activations in several areas, including bilaterally in the thalamus, temporal and occipital areas. We found no significant group differences in activations for movies without a humorous punchline.

Discussion

Theoretical and empirical work has suggested that abnormal humor processing may take place in the brain during fun stimuli and cataplexy in NT1 patients, but previous studies with limited sample sizes [14-16] have not provided a coherent account of the brain activation patterns. Further, they did not explore the brain activation patterns of other parts of humor processing with neutral-rated movies, which might contain a humorous punchline and therefore have the potential to be experienced as funny, despite being rated as neutral, or movies without a humorous punchline.

Our study is the largest fMRI study of humor-processing in NT1 patients to date, and the first to examine well-characterized post-H1N1 NT1 patients. Our main finding is the lack of differentiation in brain activations between fun-rated and neutral-rated movies in patients, in contrast to the differentiation found in controls, due to patients showing "overactivation" during neutral-rated movies. In contrast to previous studies[14,15] we did not find any group differences between patients and controls while watching fun-rated movies.

Three previous fMRI studies have studied humor processing in NT1 patients using humorous pictures [14,15] or movies [16]. Cataplexy attacks were elicited in zero [15], one [14] and 10 [16] patients. The first study [15] compared 12 NT1 patients (all with cataplexy and unmedicated for at least 14 days before the scanning) with 12 healthy controls. Eight patients had reduced or undetectable levels of CSF-hypocretin. The authors reported that patients and controls rated similar proportions of images as funny. Patients compared to controls had lower activation in several regions including the hypothalamus, and higher activation in several regions including the amygdala, to humorous compared to neutral pictures.

The second study [14] included 10 NT1 patients, all with cataplexy and who were unmedicated for at least 5 days before scanning, and 10 healthy controls. Six patients had low levels of hypocretin. Unlike the first study [15], the authors reported that patients rated significantly fewer cartoons as funny compared with the controls. Further they reported that

patients showed increased brain activation compared to controls in several regions including the hypothalamus, the ventral striatum and right inferior frontal gyrus when looking at funny compared with non-funny cartoons (pictures).

The third study [16] took a different approach by focusing on eliciting cataplectic attacks in the scanner. 21 NT1 patients (all, drug-naïve, hypocretin-deficient and with cataplexy) were studied with fMRI acquired with synchronously EEG, while watching funny movies that were tailored to each patient's preference. The study did not include any healthy controls. 10 patients had cataplectic attacks and 16 experienced laughter. Laughter was associated with an increased brain response bilaterally in the anterior cingulate gyrus and the motor/premotor cortex. Cataplexy was associated with increased brain response in several areas, including; the amygdala, anterior insular cortex, ventromedial prefrontal cortex, nucleus accumbens, locus coeruleus and the anteromedial pons.

We found that NT1 patients showed no differences from the control group in their average ratings of the movies, similar to the findings of the study of Schwartz et al.[15] but in contrast to Reiss et al.[14]. Further, we found that several brain regions previously considered associated with humor processing [25] were activated in both patients and controls during fun-rated movies, but in contrast to the other studies [14,15], we found no group differences in brain activations for fun-rated movies. However, interestingly, there were several brain regions with higher activation during neutral-rated movies in patients compared with controls. Importantly, the neutral-rated movies in our study could have a potentially humorous punchline even if they were not subjectively rated as fun. A sub-analysis of five of the 30 movies without a humorous punchline revealed no significant differences in activations between patients and controls. The movies without a humorous punchline (89.0% neutral-rated) are otherwise similar in build-up and the anticipation that something funny might happen (even though it does not), which suggests that the recognition of a humorous punchline plays an important

role in abnormal humor processing in NT1. However, this sub-analysis must be interpreted with caution since there only were five trials without a humorous punchline.

In short, we found that the NT1 patients compared to controls have a normal ability to subjectively rate humorous and neutral movies, but that neutral-rated movies with a potential humorous punchline elicit a brain overactivation in patients that is similar to the activation observed in response to fun-rated movies.

Due to our overall focus on the mechanisms of cataplexy, we were particularly interested in brain regions that have been linked to humor/cataplexy and REM sleep. One theory suggests that cataplexy represents dissociated REM sleep appearing while awake [2,3,10]. An alternative theory suggests that cataplexy is a variant of tonic immobility, which can be seen in animals that are unable to move in situations they experience to be dangerous [17]. However, this type of tonic immobility has not been observed in humans or other primates, and the most potent trigger for cataplexy is usually strong, positive emotions (thinking of, hearing, or telling a joke) [2,17].

Interestingly, in the present study, during neutral-rated movies, the thalamus showed significantly higher activation bilaterally in patients compared with controls. The thalamus has previously been shown to activate in response to humor in several studies [25-28]. Further, the thalamus has repeatedly been shown to have higher activation for REM sleep in fMRI [18-20] and PET [21-24] studies with polysomnographic monitoring.

Putamen, pallidum and caudate also showed significant overactivation in our patients compared with controls during neutral-rated movies, and have also been associated with REM sleep and humor. The basal ganglia show increased activity in PET [21] and fMRI [18,20] studies with PSG recordings during REM sleep. Humor perception has been found to correlate with higher activation in the pallidum and putamen [28], amusing films have been associated with

higher activation in the right putamen and left globus pallidus [30], and a meta-analysis reported that 70% of happiness-induction studies reported activation in the basal ganglia [31].

Among several alternative hypotheses, the "overactivation" during neutral-rated movies could possibly represent some form of defense mechanism against cataplexy with increased attention directed towards the presented stimuli. However, as we expect that the fun-rated movies represent the greatest risk of cataplexy for the patients, it is surprising that we did not observe higher brain activations for fun-rated movies in patients compared to controls. As such, the relative overactivation in response to neutral-rated movies (but still containing a humorous punchline) might reflect hypersensitivity towards ambiguous yet potentially humorous stimuli for the NT1 patients, possibly indicating a lower threshold for activating the humor response (even when a movie is ultimately not perceived/rated as funny).

In the present study, we have used first-degree relatives as controls, and previous studies [46-48] have reported a higher risk of developing narcolepsy in first-degree relatives of narcolepsy patients. However, all controls in our study were objectively ICSD-3-evaluated for a DG47.4 narcolepsy diagnosis, which was excluded in all controls. All controls that had experienced cataplexy-like episodes, sleep paralysis and hypnagogic hallucinations were included in the full sample, but excluded from the sub-analysis of the reduced sample. Additionally, in the reduced sample we excluded patients and first-degree relatives with comorbidities and the participants who had to re-watch the movies or had watched the movies in black and white or without sound.

The results in the reduced sample were similar to those from the full sample, but even more widespread. There were more voxels showing significantly higher activation in patients compared with controls for neutral-rated movies in the reduced sample, as well as more voxels showing significant group difference in the fun > neutral contrast in the reduced sample than in the full sample. The similarity of the findings in the full and reduced samples

was further supported by the spatial correlation between the uncorrected test statistics suggesting very similar patterns and direction of effects. Since the reduced sample have similar, but even more widespread results, the inclusion of all the participants in the full sample might have diminished some of the results in the full sample. However, it is a strength that the results in full and reduced samples support the same conclusions.

Re-watching movies can give reduced activation in several regions, including the amygdala[49]. We did find significantly higher activity in the amygdala in patients compared to controls for neutral-rated movies for the reduced sample, where the eight participants who had to re-watch the movies mainly due to drowsiness/falling asleep were excluded. When comparing fun and neutral-rated movies in the reduced sample, controls showed significantly higher brain activation in the right amygdala compared with patients. The amygdala has been reliably associated with humor appreciation [25] and also with cataplexy[16,50,51]. Interestingly, PET [22,23,29], and fMRI [20] studies have shown amygdala activation during REM sleep in healthy individuals.

In our study, 92.7% of the patients had been H1N1-vaccinated, although the time of disease onset was changed to before the H1N1-vaccinations for three patients after a thorough evaluation of their medical history and records. Few differences have so far been found between sporadic and H1N1-vaccinated narcolepsy, except for a higher frequency of disturbed nocturnal sleep, shorter mean sleep latency [52], a sudden onset of symptoms [11,53], and more frequently sleep-onset REM periods [54]. Post-H1N1 influenza narcolepsy also shows novel genetic associations [55], but so far it seems unlikely that cataplexy mechanisms in H1N1-vaccinated narcolepsy are different from cataplexy in sporadic narcolepsy.

We included two patients with hypocretin deficiency, but without cataplexy, as prospective studies show that a substantial proportion of hypocretin-deficient non-cataplectic patients will later develop cataplexy [56].

In conclusion, we report fMRI-based evidence of abnormal neuronal humor-processing in NT1 patients compared with controls, particularly an overactivation in response to neutral-rated movies in regions previously shown to be associated with humor, cataplexy and REM-sleep, including the thalamus, amygdala, and basal ganglia. Unlike controls, patients showed similar activation during neutral-rated and fun-rated movies, so there is no significant differentiation of fMRI activations between these two states, which might provide insight into the mechanisms associated with cataplexy. This "overactive" brain state during neutral-rated movies (but with a potentially humorous punchline) might represent a risk (hypersensitivity to potential humorous stimuli) for the NT1 patients. NT1 patients seem to have a lower threshold for activating the humor response, even when they subjectively rate the movie as neutral.

Abbreviations

AASM = American Academy of Sleep Medicine; ADD = attention deficit disorder; ADHD = attention deficit hyperactivity disorder; COPE = Contrast of parameter estimate; CSF= cerebrospinal fluid; EEG = electroencephalography; EMG = Electromyography; FEAT = FMRI Expert Analysis Tool; FILM = FMRIB's Improved Linear Model; FLIRT = FMRIB's Linear Image Registration Tool; FNIRT = FMRIB's Non-linear Image Registration Tool; FMRIB = Functional Magnetic Resonance Imaging of the Brain; FOV = field of view; fMRI = functional magnetic resonance imaging; FSL = Functional Magnetic Resonance Imaging of the Brain Software Library; GLM = general linear models; HLA = human leukocyte antigen; ICSD = International Classification of Sleep Disorders; MRI = magnetic resonance imaging; MSLT = multiple sleep latency test; NT1 = narcolepsy type 1; PALM = Permutation Analysis of Linear Models; PET = positron emission tomography; PSG = polysomnography; REM sleep = rapid eye movement sleep; SD = standard deviation; TE = echo time; TR = repetition time; TFCE = threshold-free cluster enhancement

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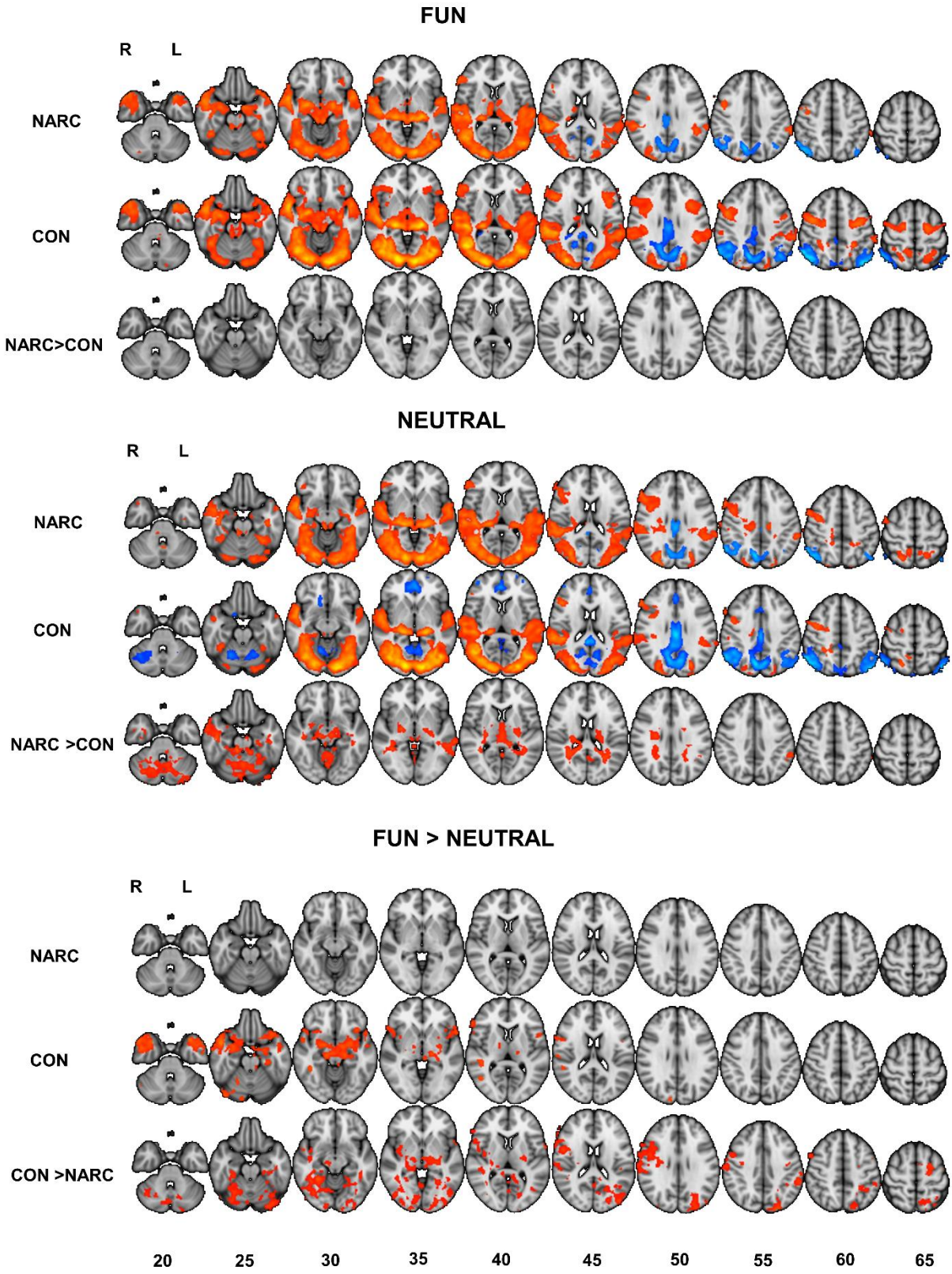
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Figure 1. Main effects of task on brain activation and group comparisons

Summarizing functional magnetic resonance imaging (fMRI) results with main effects and group comparisons. Numbers reflect the z-coordinate in the MNI 2-mm space. Only voxels with a two-tailed value of $p < 0.05$, corrected for multiple comparisons using permutation testing and TFCE (threshold-free cluster enhancement) are shown. Narc: narcolepsy type 1 patients, Con: first-degree relatives (controls). R: right, L: left. Red/Orange: higher activation. Blue: lower activation.

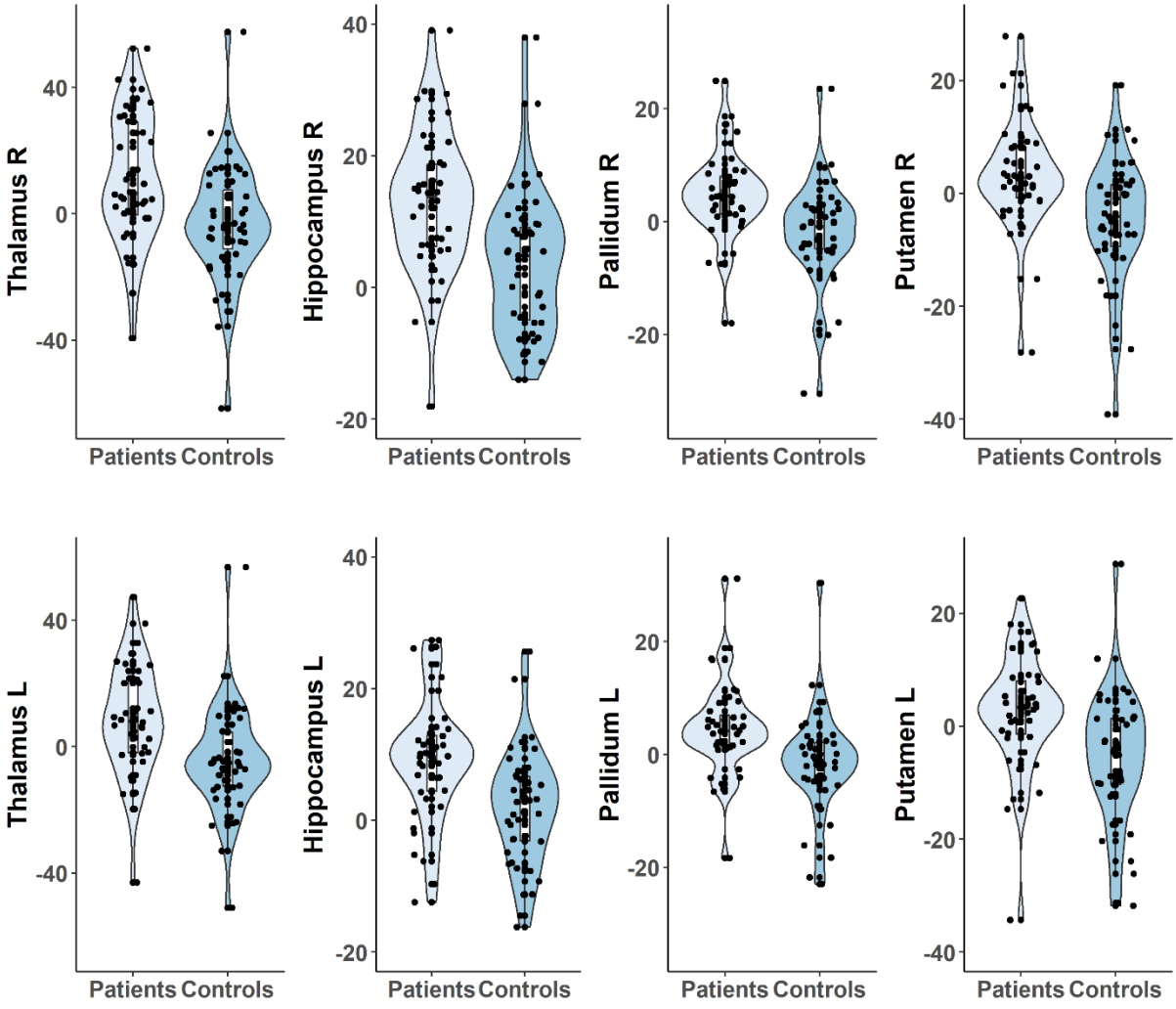

Figure 2. Group-wise distributions of COPE values for neutral+ across select regions of interest

The violin plots show the mean COPEs for voxels with significant group differences (identified by permutation testing) in the first-level contrast neutral+ for regions of interest. The probabilistic atlas, Harvard-Oxford Subcortical Structural Atlas, implemented in FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) [37,38] was used to extract masks (threshold = 10) of regions of interests. COPE: contrast of parameter estimate, FSL: Functional Magnetic Resonance Imaging of the Brain Software Library, Patients: Narcolepsy type 1 patients, Controls: first-degree relatives. R: right, L: left

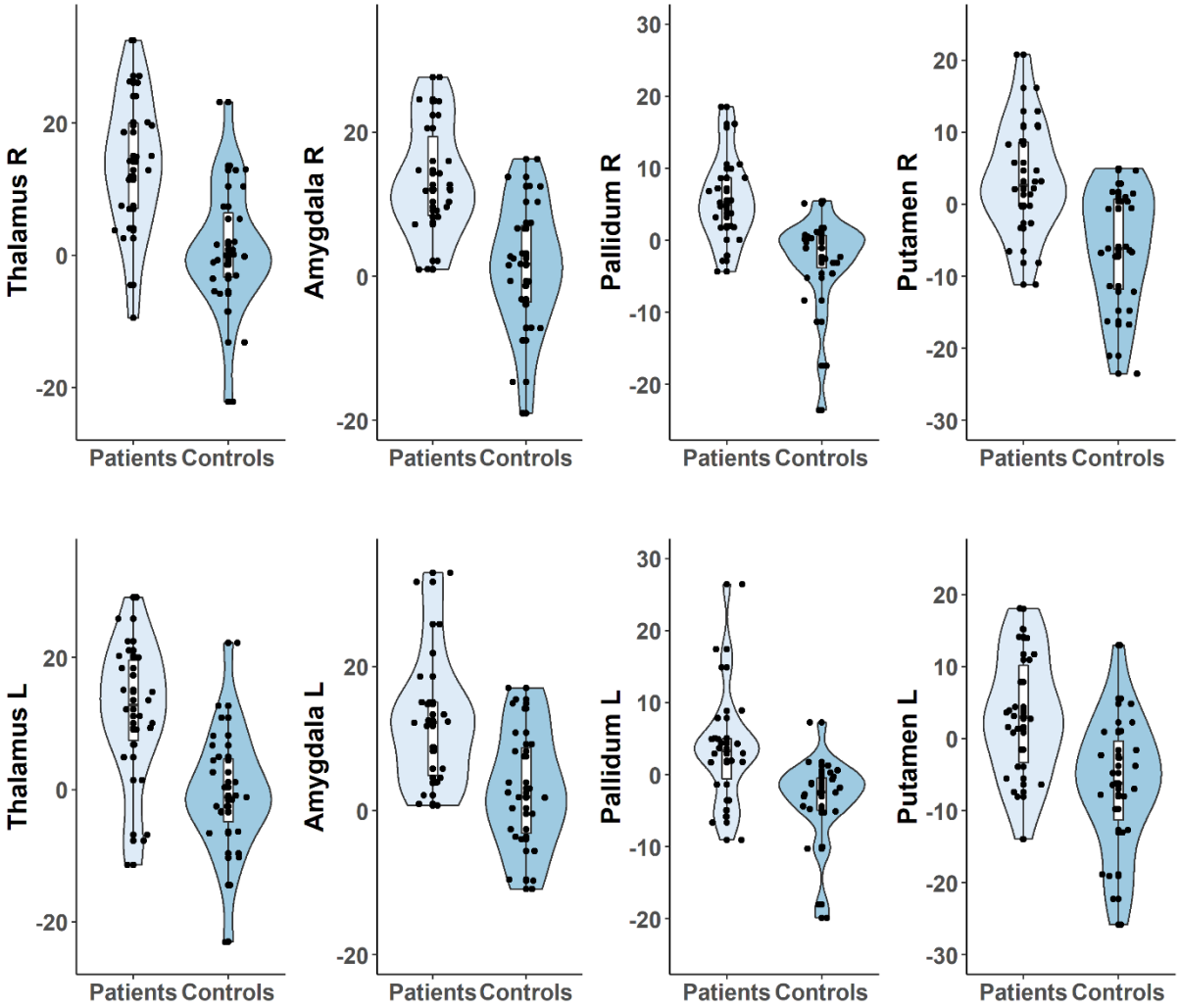

Figure 3. Group-wise distributions of COPE values for neutral+ across select regions of interest in the reduced sample

In the reduced sample of 22 narcolepsy type 1 patients (15 females, mean age 21.5 ± 8.2 years) and 23 controls (11 females, mean age 19.4 ± 7.8), we excluded all patients and first-degree relatives with comorbidity, as well as all participants who had to re-watch the movies or watch the movies in black and white or without sound, and first-degree relatives who had experienced cataplexy-like episodes, sleep paralysis and hypnagogic hallucinations. The violin plots show the mean COPEs for voxels with significant group differences (identified by permutation testing) in the first-level contrast neutral+ for regions of interest in the reduced sample. The probabilistic atlas Harvard-Oxford Subcortical Structural Atlas implemented in FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) [37,38] was used to extract masks (threshold = 10) of regions of interest. COPE: contrast of parameter estimate, FSL: Functional Magnetic Resonance Imaging of the Brain Software Library, Patients: Narcolepsy type 1 patients, Controls: first-degree relatives. R: right, L: left

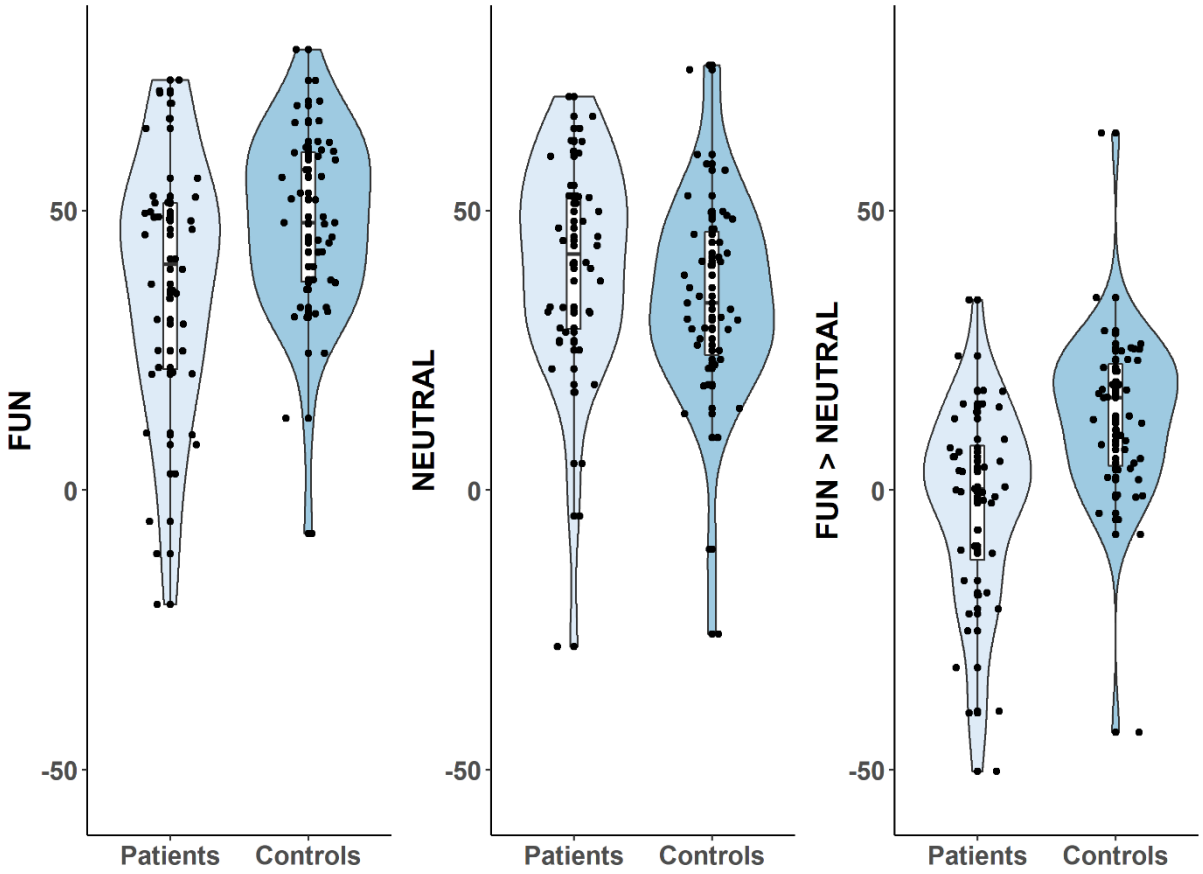

Figure 4. Group-wise distributions of COPE values across all significant voxels

The violin plots show the mean COPEs for the first-level contrasts; fun+, neutral+, fun > neutral, for voxels (16 604 voxels) with a significant group difference in fun > neutral identified by permutation testing. COPE: contrast of parameter estimate, Patients: Narcolepsy type 1 patients, Controls: first-degree relatives.

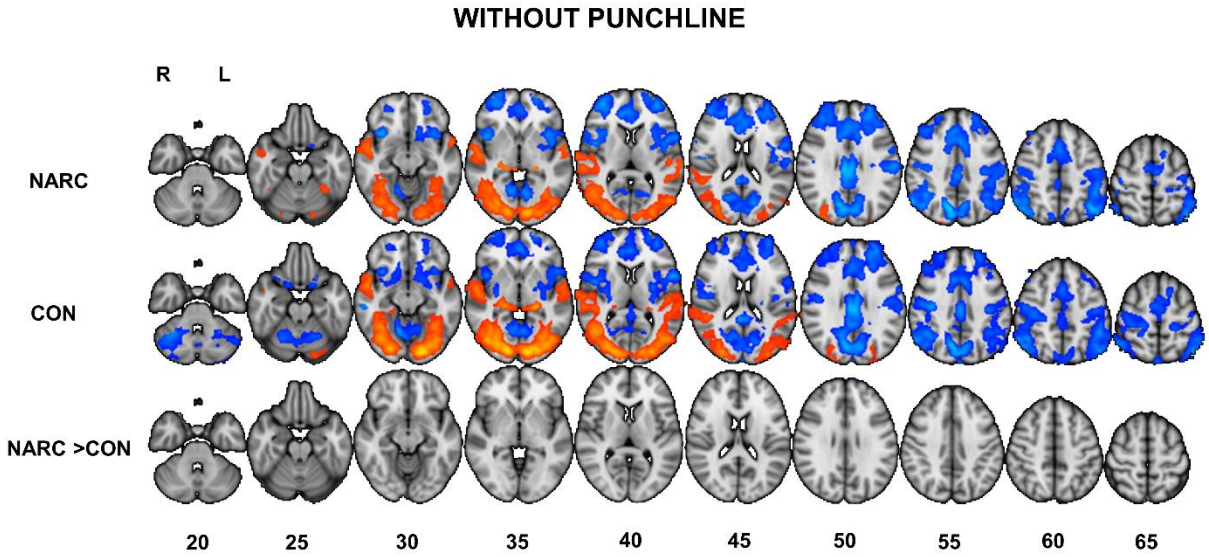

Figure S1. FMRI results for movies without a humorous punchline

Summarizing functional magnetic resonance imaging (fMRI) results with main effects and group comparisons for movies without a humorous punchline. There were no significant differences in activations between narcolepsy type 1 patients and controls. Numbers reflect the z-coordinate in MNI 2-mm space. Only voxels with a two-tailed value of $p < 0.05$, corrected for multiple comparisons using permutation testing and TFCE (threshold-free cluster enhancement), are shown. Narc: narcolepsy type 1 patients, Con: first-degree relatives (controls). R: right, L: left. Red/Orange: higher activation. Blue: lower activation.

Table 1. Demographic and clinical data

	Narcolepsy type 1 (n=41)	First-degree relatives (n=44)
Gender (female), n (%)	31 (75.6)	24 (54.5)
Age (years), mean ± SD	23.6 ± 11.4	19.6 ± 8.4
Age at disease onset (years), mean ± SD	17.7 ± 10.9	N/A
Disease duration (years) mean ± SD	5.9 ± 1.5	N/A
H1N1-vaccinated, n (%)	38 (92.7)	30 (68.2)
Cataplexy, n (%)	39 (95.1)	6 (13.6)*
*HLA-DQB1*06:02*-positivity, n (%)	41 (100)	27 (61.4)
CSF hypocretin-1 ≤ 1/3 of level in normal population	40/40 (1-N/A)	N/A
Hypnagogic hallucinations, n (%)	35 (85.4)	9 (20.5)
Sleep paralysis, n (%)	29 (70.7)	8 (18.2)

N/A: not available, SD: standard deviation, CSF: cerebrospinal fluid. Disease onset was reclassified to being before the H1N1-vaccinations for three narcolepsy type 1 patients after a thorough evaluation of their medical history and records (3/3 were typical narcolepsy type 1 phenotypes, hypocretin-deficient, *HLA-DQB1*06:02*-positive with cataplexy, and so were retained in the study). *First-degree relatives with signs of cataplexy all experienced it rarely, but with triggers known to elicit cataplexy, like laughter, fun, excitement and surprise.