

Reduced Amygdala Activity during Aversive Conditioning in Human Narcolepsy

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Narcolepsy with cataplexy is a sleep-wake disorder caused by a loss of hypothalamic hypocretins. Here we assessed the time course of amygdala activation during aversive conditioning in unmedicated patients with narcolepsy. Unlike healthy matched control subjects, narcolepsy patients had no enhancement of amygdala response to conditioned stimuli and no increase in functional coupling between the amygdala and medial prefrontal cortex. These findings suggest that human narcolepsy is accompanied by abnormal emotional learning, and that, in line with animal data, the hypocretin system and the amygdala are involved in this process.

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Narcolepsy with cataplexy (NC) is a sleep-wake disorder that affects about 1 in 2,000 individuals.¹ NC patients experience excessive daytime sleepiness, as well as episodes of muscle atonia (cataplexy) that are typically triggered by positive emotions.^{2,3} Additional symptoms include sleep paralysis, hallucinations, and fragmented nighttime sleep. Narcolepsy is caused by a deficiency in a neuropeptide called hypocretin (Hcrt or orexin), which is produced by a few thousand neurons localized in the perifornical area of the lateral hypothalamus.^{4,5} In addition to its role in sleep-wake functions,⁶ recent animal work has suggested that Hcrt sig-

naling may also contribute to the regulation of emotional processes including stress response and neural plasticity related to addiction.^{7,8} A relevant observation in this context is that human narcolepsy is associated with emotional peculiarities: emotions trigger cataplexy, and most anticataplectic medications are antidepressants; NC patients do not become addicted to stimulant medications; and depression and psychosocial dysfunctions are frequent in NC.² We recently found that NC patients have an exaggerated amygdala response to positive humorous stimuli and no physiological startle potentiation during the presentation of unpleasant stimuli.^{9,10} Here we hypothesized that NC patients would show an abnormal amygdala response also to unpleasant stimuli and/or an abnormal time course of amygdala activity during aversive conditioning.

Subjects and Methods

Experimental Procedures

Fourteen unmedicated narcoleptic patients with clear-cut cataplexy (based on standard questionnaires and clinical examination; Table) and 14 healthy control subjects (matched for age, sex, handedness, and body mass index) gave informed consent to participate in a functional magnetic resonance imaging (fMRI) study approved by the Zurich University Hospital ethics committee. Four patients and one control subject, as well as their corresponding matching subjects, were excluded from the analyses because they fell asleep during one of the scanning runs. Hypocretin levels were measured in seven patients (of the nine remaining patients) and were low or undetectable in all these patients. Patients and control subjects were scanned during three successive fear conditioning (acquisition) runs and two extinction runs. On each trial, one triangle was displayed at the center of the screen and the subjects had to decide as quickly and as accurately as possible whether the triangle was pointing to the left or to the right side (for more details about the task, see Supplementary Methods). Because the subjects were required to respond on each trial, we could check online that the subjects paid attention to each stimulus, which was important considering the patients' increased sleepiness. Triangles could be colored in either blue or yellow. One color (conditioned stimulus, CS+) signaled a possible upcoming aversive unconditioned stimulus (US), which was a brief painful electrical stimulation delivered on one finger on half of the CS+ trials (ie, partial reinforcement conditioning paradigm). The other color was never associated with any other stimulation (non-conditioned stimulus, CS-). The acquisition phase comprised three fMRI runs each including 6 CS+ paired with the US, 10 CS+ alone, and 10 CS-. The acquisition was immediately followed by two extinction runs with 13 CS+ (not paired with the US anymore) and 13 CS-.

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TABLE: Clinical Characteristics of the Narcoleptic Patients and the Healthy Control Subjects

Subjects	Age (yr)	Sex	BMI	HLA DQB1*0602	Hcrt (pg/ml)	Cataplexy	Duration of Illness (yr)	ESS Score	Stanford (%)	UNS	SNS
Patient No. ^a											
1	22	F	15.8	+	107	+	4	20	32.5	34	-101
2	25	F	23.0	+	0	+	8	12	45.8	16	-43
3	33	F	24.2	+	<20	+	19	16	91.7	24	-66
4	39	F	43.0	+	N.A.	+	18	15	91.7	23	-68
5	43	F	20.8	+	<20	+	15	19	32.5	18	11
6	25	M	32.3	+	<20	+	9	7	45.8	11	-37
7	33	M	34.6	+	N.A.	+	27	12	91.7	25	-42
8	39	M	26.2	+	118	+	18	17	91.7	16	6
9	45	M	34.7	+	0	+	3	15	91.7	32	-83
Control Subject No. ^b											
1	22	F	19.5	N.A.	N.A.	N.A.	N.A.	8	0.6	4	31
2	27	F	20.0	N.A.	N.A.	N.A.	N.A.	4	0.6	5	39
3	31	F	22.6	N.A.	N.A.	N.A.	N.A.	10	32.5	7	16
4	42	F	39.0	N.A.	N.A.	N.A.	N.A.	11	0.6	3	39
5	40	F	23.8	N.A.	N.A.	N.A.	N.A.	5	0.6	3	15
6	28	M	28.0	N.A.	N.A.	N.A.	N.A.	5	0.6	4	11
7	36	M	27.2	N.A.	N.A.	N.A.	N.A.	11	0.6	13	27
8	39	M	24.7	N.A.	N.A.	N.A.	N.A.	6	0.6	8	43
9	47	M	32.65	N.A.	N.A.	N.A.	N.A.	11	0.6	8	10

Patients were selected by a team of neurologists with extensive experience in narcolepsy (C.B., E.W., R.K.). All patients were off medication for at least 14 days before the experimental day.

^aMean age \pm standard deviation: 33.78 ± 8.36 years.

^bMean age \pm standard deviation: 34.66 ± 8.15 years.

BMI = body mass index; HLA = human leukocyte antigen; Hcrt = hypocretin-1 level in cerebrospinal fluid (normal values >320 pg/ml; <20 = concentration less than detection limit of assay); ESS = Epworth Sleepiness Scale (0-24, higher scores indicate major self-reported sleepiness); 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); N.A. = not applicable.

Results

During scanning, NC patients and control subjects did not differ in reaction times (mean \pm standard deviation: patients: 636 ± 132 milliseconds; control subjects: 627 ± 142 milliseconds) or in accuracy (mean percentage of hits \pm standard deviation: patients: 94.69 ± 10.32 ; control subjects: 98.74 ± 5.68) on the triangle-orientation task. Comparable behavioral performance in both groups during scanning ensures that any observed difference in regional brain activity might not primarily be driven by differences in attentional levels or task difficulty. It should

be noted that none of the nine patients reported any signs of cataplexy during scanning.

Using statistical parametric mapping of whole-brain fMRI data and random-effects group analyses (see Supplementary Methods), we first identified any region that was more activated during the presentation of the CS+ (with or without accompanying US) than during the presentation of the CS-. This analysis demonstrated that, in both NC patients and control subjects, the painful stimulation activated the so-called pain-matrix, known to be recruited during the processing of noxious stimuli.¹¹ As

shown on Figure 1, this network included the primary and secondary somatosensory cortices, insular regions, the anterior cingulate cortex, as well as the right amygdala (see Fig 1, top left panel). This latter finding evidences normal amygdala response to painful stimulations in NC patients (Fig 2B). By contrast, the right amygdala responded more to the CS+ alone (unpaired to the US) than to the CS- in control subjects, but not in NC patients ([CS+ minus CS-] in control subjects vs patients: $x, y, z = -18, 0, -18; Z = 3.36; p < 0.001$, uncorrected; $p < 0.05$, family-wise error corrected for a sphere of 8mm radius; see Fig 2). This result in control subjects suggests that the visual presentation of the CS+ inherits aversive properties from the US during the acquisition phase and can thus activate the neural circuit associated with fear response.^{12,13} Figure 2C shows the dynamic increase in amygdala response to the conditioned stimulus (CS+ minus CS-) in control subjects over the three blocks of aversive conditioning and the rapid reduction of activity during extinction. No such learning-related increase in amygdala activity was found in NC patients.

We then performed a connectivity analysis (psychophysiological interaction, see Supplementary Methods) testing for changes in the functional coupling between the amygdala and any other brain region during the process-

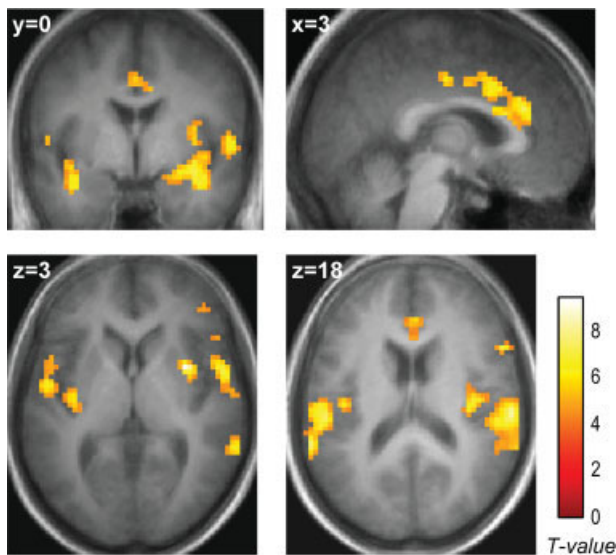


FIGURE 1: Activation of the pain matrix during aversive stimulation. Common activation in control subjects and narcolepsy with cataplexy (NC) patients (contrast: [visual conditioned stimulus (CS+) and CS+ paired with an unconditioned stimulus (US)] > visual nonconditioned stimulus (that was never associated with an US; CS-)) included the bilateral activation of the insula, secondary somatosensory cortices, as well as activation of the cingulate and of the right amygdala. The statistical maps are overlaid on the mean-normalized T1-structural scan of all the subjects; threshold at $p < 0.001$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

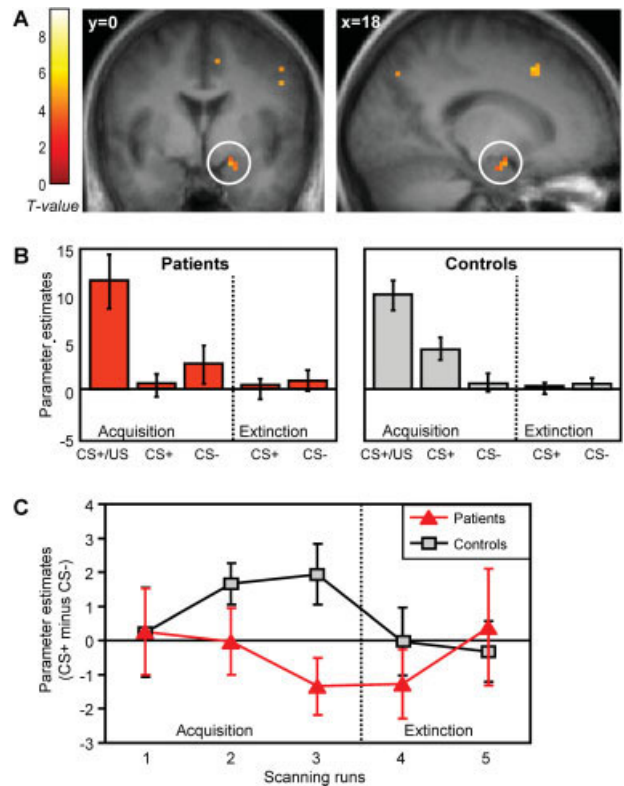


FIGURE 2: Activation of amygdala in control subjects. (A) Whole-brain comparison showing increased activity in the amygdala in control subjects compared with narcolepsy with cataplexy (NC) patients, selectively during the presentation of the conditioned stimulus (contrast: conditioned stimulus [CS+] > nonconditioned stimulus [CS-]). The statistical map is overlaid on the mean-normalized T1-structural scan; threshold at $p < 0.001$. (B) Mean parameter estimates of functional magnetic resonance imaging (fMRI) signal extracted from the amygdala peak ($x, y, z = 18, 0, -18$) for the main conditions during acquisition and extinction showing the expected increased activation level for the CS+ compared with the CS- during the acquisition in control subjects. By contrast, although the patients' amygdala showed a robust response to the unconditioned stimulus (US), there was no indication of a conditioned response to the CS+. (C) Time course of amygdala activity over the three successive blocks of acquisition followed by the two blocks of extinction for the CS+ minus CS- contrast. For the control subjects, the graph shows a progressive increase of activity for CS+ trials relative to CS- trials during the acquisition followed by a rapid reduction during the extinction. NC patients did not show such increase in activity associated with CS+. Note that the decrease of differential CS+ activation during the third block of acquisition is due to the slightly higher activity during the CS-, as suggested on (C). Triangles represent patients; squares represent control subjects. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ing of CS+ trials. In the control subjects, the psychophysiological interaction analysis demonstrated increased negative coupling between the right amygdala and the medial prefrontal cortex (mPFC; $x, y, z = 6, 48, 0; Z = 3.30; p < 0.001$, uncorrected; $p < 0.05$, family-wise error

corrected for a sphere of 8mm radius; see Supplementary Fig 1), suggesting an inverse functional relation between the time course of activity in the mPFC and the amygdala during the acquisition of the conditioned response (CS+). The same analysis on the NC patients did not show any change in the coupling between the amygdala and any other brain region. The result in healthy subjects for this psychophysiological interaction analysis is in line with previous reports indicating mutual regulatory processes between the amygdala and the mPFC.^{14,15}

Discussion

The main finding of this fMRI study is the documentation of an impaired aversive conditioning in human NC. Although the aversive stimulation (painful electrical stimulation) strongly activated the pain matrix (including the amygdala and insula) in both NC patients and control subjects, the patients did not show any increase in amygdala response to the conditioned stimulus alone (CS+), thus suggesting a selective impairment in emotional learning.¹⁶ These results have important clinical and neurobiological implications.

The amygdala dysfunction found in this study during aversive learning fits with our previous report of a blunted startle eye-blink response to unpleasant (aversive) stimuli, a finding that can be seen not only in human NC but also in animals and patients with amygdala lesions or dysfunctions.¹⁰ Together with the observation of an exaggerated response during the processing of humorous pictures,⁹ these data suggest a central role of the amygdala in the pathophysiology of human NC. The high frequency of psychiatric disturbances in these patients and the fact that cataplexy can be triggered by both positive and negative emotions^{2,17} could also reflect a dysfunction of the amygdala. Importantly, because the patients had normal amygdala response to the CS+ when paired with an actual aversive stimulation (unpredictably after 37.5% of the CS+), it is unlikely that reduced amygdala during the presentation of the CS+ alone would be caused by more efficient downregulation of negative emotions in the patients.¹⁸ These clinical features give support to the view that the amygdala is involved not only in fear responses (as traditionally thought) but more generally in the processing of both positive (prosocial) and negative (aversive/defensive) stimuli that may eventually affect reward behaviors.^{19,20}

What is the cause of reduced amygdala activation during aversive conditioning in NC? Our data suggest that both a decreased activation of the mPFC and a hypocretin deficiency may be involved. An increased functional coupling between the amygdala and the mPFC during

aversive conditioning was, in fact, found in control subjects but not in NC patients. Furthermore, the existence of an interaction between amygdala and mPFC during emotional processing was previously demonstrated.¹⁵ In hypocretin-deficient NC patients, the altered interaction between amygdala and mPFC could arise from a decreased hypocretinergic or dopaminergic activity. Indeed, both systems have been suggested to act synergistically in emotional and particularly reward processes.⁷ Urry and colleagues²¹ recently demonstrated that the inverse functional relation between mPFC and amygdala during emotional regulation depends on the hypothalamic-pituitary-adrenal system (and therefore possibly also hypocretins). On the other hand, Marowsky and coauthors²² have shown that a dopaminergic blockade reduces the inhibiting influences of the PFC on the amygdala. A plausible neurobiological interpretation of our findings is that the Hcrt system, through direct or indirect (via PFC) effects, may modulate processes that allow the amygdala to efficiently and rapidly adapt to emotional challenges. Clinically, these data indicate that human NC (and possibly also other disorders associated with hypocretin deficiency²³) should be considered not only as a sleep-wake disorder but also as a condition that can impair emotional learning and regulation.

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Pregnancy Increases the Growth Rates of World Health Organization Grade II Gliomas

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Twelve pregnancies in 11 adult women harboring World Health Organization (WHO) grade II gliomas (GIIgs) prior to pregnancy were reviewed to address whether pregnancy affects tumor growth using a quantitative approach of the radiological velocity of diametric expansion (VDE) on successive magnetic resonance images. VDE was significantly increased during pregnancy as compared to prepregnancy ($p < 0.001$) and to postdelivery ($p = 0.012$) periods. Pregnancy increases the radiological growth rates of GIIgs. An increase in seizure frequency was observed concomitantly in 40% of cases and further oncological treatment was started after delivery in 25% of cases.

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