

## ORIGINAL ARTICLE

# Volume of the amygdala is reduced in patients with narcolepsy – a structural MRI study

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## Abstract

Based on the clinical observation that patients suffering from narcolepsy with cataplexy (NC) have cataplectic attacks when they experience positive emotions, it is therefore hypothesised that the abnormal processing of external emotional input through the limbic system, or motor dysregulation induced by emotions, takes place during these episodes. To date, imaging studies have failed to reveal consistent brain abnormalities in NC patients. Considering the discrepancies in reported structural or functional abnormalities of the hypothalamus, amygdala, and nucleus accumbens, we used the MRI volumetry to determine the volumes of the amygdala and nucleus accumbens in a group of eleven patients with NC (5 males and 6 females, mean age 41.7 years  $\pm$  17.7). This data was compared to an equal number of examinations in healthy volunteers matched for age and gender. We found a decrease in the amygdalar volume of NC patients in both raw ( $p < 0.001$ ) and relative ( $p < 0.01$ ) data sets. The difference in amygdalar volume between healthy volunteers and NC patients was about 17%. In contrast to the amygdala, we did not find any differences in the volumes of nucleus accumbens. In the present MRI volumetric study, we found bilateral gray matter loss in the amygdala only.

## INTRODUCTION

Narcolepsy, a chronic sleep disorder, is characterized by excessive daytime sleepiness, disturbed nocturnal sleep, cataplexy (sudden loss of muscle tone) and other symptoms associated with abnormal rapid-eye movement (REM) sleep, including hypnagogic hallucinations and sleep paralysis (Overeem *et al* 2001).

Emotions or provocative circumstances can modulate the pattern and severity of clinical appearance. Narcolepsy with cataplexy (NC) has been linked to the reduction or absence of hypocretin/orexin neurons in the lateral hypothalamic region (Mignot *et al* 2002; Nishino *et al* 2000; Thannickal *et al* 2000). Hypocretin/orexin containing neurons have widespread projections to multiple structures of the central nervous

system comprising the brainstem nuclei, thalamus, basal forebrain, and the amygdala amongst others (Peyron *et al* 1998; Siegel *et al* 1999). Hypocretin/orexin fibers and receptors are relatively dense within the amygdala. Hypocretin/orexin exerts strong postsynaptic activation on neurons of central and medial subnuclei, thus influencing the major amygdalar output (Bisetti *et al* 2006). It is known that the limbic network, including the ventral striatum, hypothalamus, and amygdala show increased activation during laughter and rapid-eye-movement (REM) sleep (Maquet *et al* 2006). Furthermore, stimulation of the amygdala can increase REM sleep, perhaps via its projections to the laterodorsal nuclei, pedunculo-pontine tegmental nuclei, and nearby regions (Calvo *et al* 1996; Simón-Arceo *et al* 2003).

The clinical observation that NC patients have cataleptic attacks when they experience positive emotions led to the hypothesis that abnormal processing of external emotional input through the limbic system, or motor dysregulation induced by emotions, takes place during these episodes. Although imaging studies did not reveal consistent brain abnormalities in NC patients, a few reports support these hypotheses. Recent fMRI studies showed heightened activity of the amygdala, nucleus accumbens (Schwartz *et al* 2008), and hypothalamus (Reiss *et al* 2008) in NC patients when exposed to humorous stimuli. Interestingly, using brain SPECT subtraction, Hong *et al* (2006) observed hyperperfusion of the amygdala and basal ganglia during cataplexy suggesting that cataplexy is produced by the activation of the amygdalo-cortico-basal ganglia-brainstem circuit. Siegel *et al* showed that the onset of the narcolepsy may be related to the neurodegenerative changes occurring in the amygdala and the basal forebrain (Siegel *et al* 1999). Considering the reported structural and functional brain abnormalities in NC patients (Draganski *et al* 2002; Schwartz *et al* 2008) together with animal models (Gulyani *et al* 2002; Siegel *et al* 1999), and the functional features of basal ganglia circuits, our aim was to measure the volume of the amygdala and nucleus accumbens by means of standard MRI volumetry and to compare it to the volume of amygdala in control subjects.

## MATERIAL AND METHODS

### Subjects

Eleven patients suffering from narcolepsy with cataplexy (5 males and 6 females, mean age 41.7 years  $\pm$  17.7) and eleven healthy volunteers matched for age and gender participated in the study. All subjects provided written informed consent. The research protocol was approved by the local ethics committee. Narcolepsy with cataplexy was diagnosed according to ICSD 2 criteria (American Academy of Sleep Medicine, 2005). All patients had excessive daytime sleepiness and cataplexy; every patient showed at least two sleep onset REM episodes in the Multiple Sleep Latency Test; all patients had HLA-DQB1\*0602 positivity. Nine patients

were taking drugs (tricyclic antidepressants, selective inhibitors of serotonin reuptake, sodium oxybate, and psychostimulants) at time of examination or in the past, whereas two patients did not have any pharmacological treatment for narcolepsy with cataplexy. Mean duration of the disease was 13.6 years (SD  $\pm$  13.7). Hypocretin level in the CSF was not measured.

### MRI segmentation protocol

MRI scans were performed with the Philips Gyroscan NT 15 (1.5T). We acquired T1 weighted images/3D (T1WI/3D) in transverse sections with the following parameters: repetition time (TR) 10.50 ms, time to echo (TE) 3.33 ms, flip angle (FLIP) 20°, thickness of slices (THK/gap) 1.6/0 mm; and T1 weighted image – inversion recovery (T1WI-IR) in transverse section with the following parameters: TR 2000 ms, TE 13 ms, inversion time (TI) 350 ms, flip angle (FLIP) 90°. The MRI scans were reviewed by an experienced neuroradiologist. No structural pathology was present in the NC and control group respectively.

The regions of interest (ROI) for manual volumetry were traced by a mouse-driven cursor and computed by in-house developed software (Rasova *et al* 2005). The delineation of all ROIs was performed in reference to the frontal plane, i.e. the plane orthogonal both to the sagittal plane (determined by the interhemispheric fissure) and the horizontal plane that connects the anterior and posterior commissure. During the delineation of ROIs, all three orthogonal views were available to monitor the correct position of the cursor.

### Amygdalar delineation

The neuroanatomic criteria chosen for amygdalar delineation were adapted from an existing protocol (Brabec *et al* 2010). In coronal MRI images, there are no definitive landmarks to signal the anterior origin of the amygdala. The anterior pole of the amygdala is particularly difficult to differentiate from the surrounding gray matter. Therefore, sections in the sagittal and horizontal planes have to be used in its' definition. The lateral and basal subnuclei are easily distinguished from surrounding structures in the 6–7 lowermost horizontal sections through the amygdala. For the separation of the amygdala from the area praeamygdalaris in more cranial levels, we elected to use sagittal sections. Sagittal sections through the amygdala are also essential for its separation from the hippocampus inferoposteriorly. We labelled the above-mentioned borders in sagittal and horizontal images and projected them onto the coronal view, where they served as definitive landmarks for amygdalar delineation. Once the anterior border of the amygdala and the posterior separation of the amygdala from the hippocampus were performed, the remaining amygdalar ROIs were drawn in the coronal view moving from anterior to posterior (**Figure 1**).

Inferolaterally the amygdala borders the white matter of the temporal lobe (in anterior sections) or the

lateral ventricle (posteriorly). Inferiorly, the amygdala borders the white matter of the temporal lobe anteriorly, moving posteriorly with the alveus of the hippocampus, and finally with the temporal horn of the lateral ventricle. The dorsolateral border is formed by the praeamygdalar area anteriorly and the amygdalo-striatic transitional area posteriorly. The superior boundary in anterior sections is determined by a strip of white matter superior to the area amygdalaris anterior. This white strip continues posteriorly and forms the superior border of the centromedial amygdala. The medial border consists of the brain exterior, with its lowermost aspect at the semiannular sulcus. The uppermost aspect of the medial border extends into the substantia innominata in rostral sections, whereas the optic tract forms the medial border caudally.

#### Delineation of nucleus accumbens

Because the transition between the caudate nucleus and nucleus accumbens is often difficult to determine, the superior border was defined as a line drawn from the most ventral part of the lateral ventricle to the most ventral part of the internal capsule. From this latter point, a vertical line was drawn to define the lateral border with the putamen. The anterior border was defined by the most anterior frontal section in which the putamen appeared (Neto *et al* 2008). The most caudal border was defined by the appearance of the anterior commissure in the midline. The raw volumes were related to the intracranial volumes. The intracranial volume was determined by tracing the margins of the inner table of the skull from a coronal scan at the level of anterior commissure, first present from anterior to posterior. The volumes of the regions of interest were multiplied by 100 for better readability.

The ROI contours were outlined in all brains by a single investigator (J.B.) blind to group status. The intra-individual coefficient of reliability was 0.99 for intracranial volumes measured at the anterior commissure, 0.98 for raw amygdalar volumes, and 0.94 for the volumes of nucleus accumbens.

**Tab. 1.** Absolute and relative volumes of the amygdala, and the nucleus accumbens; all volumes are in  $\text{cm}^3 \pm \text{SD}$ . VAM – raw amygdalar volume; VNA – raw volume of the nucleus accumbens; ICV – intracranial volume at the level of the anterior commissure. Rel-V – relative volume. \* $p < 0.01$ ; \*\* $p < 0.001$ .

	Narcolepsy with cataplexy patients	Controls
$V_{AM}$	$1.078 \pm 0.083^{**}$	$1.295 \pm 0.012^{**}$
$V_{NA}$	$0.366 \pm 0.102$	$0.363 \pm 0.058$
ICV	$116.64 \pm 10.58$	$114.73 \pm 9.56$
Rel $V_{AM}$	$0.930 \pm 0.101^*$	$1.132 \pm 0.107^*$
Rel $V_{NA}$	$0.317 \pm 0.092$	$0.316 \pm 0.043$

#### Statistical analysis

The intergroup differences in raw and relative volumes were compared by the Mann-Whitney U-test. The Wilcoxon sign rank test was used to compare left and right volumes. The relationship between the duration of the disease and the volume of ROIs was compared by Mann Whitney U test. The results at  $p < 0.05$  were considered as statistically significant.

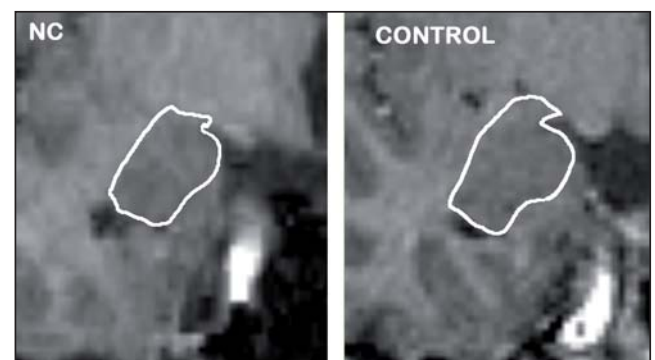
## RESULTS

Using standard volumetric methods, we detected a difference in amygdalar volume only (**Table 1**). No significant right-left differences were obtained for either of the ROIs; therefore the averages of both sides were used in further analysis. We found no relationship between the duration of the disease and the volume of ROIs.

## DISCUSSION

In this study, we found bilateral gray matter loss only in the amygdala. The mean loss of amygdalar volume was 16.8%. This finding is in line with the observations of Siegel *et al* (1999) in canine models. The duration of disease did not affect the amygdalar size in our data set, which is in agreement with the well accepted opinion that narcolepsy is a nonprogressive disease (Siegel *et al* 1999; Sonka *et al* 1991). In contrast to the findings of Draganski *et al* (2002) and of Joo *et al* (2009), we found no changes in the size of nucleus accumbens. This corresponds to the observations made by several other authors (Brenneis *et al* 2005; Kaufmann *et al* 2002; Overeem *et al* 2003). Our study is small in size and CSF hypocretine was not measured. However, to our knowledge this is the first report on manual MRI volumetric analysis in patients with narcolepsy.

Given that the amygdalar nuclei are bidirectionally connected to hypothalamic neurons (Bisetti *et al* 2006), the hypocretin mediated amygdalar dysfunction may affect key features of narcolepsy, such as the emotional triggering of cataplexy (Gulyani *et al* 2002) and emo-



**Fig. 1.** The manual delineation of the amygdala in patient suffering from narcolepsy with cataplexy and control healthy subject.

tional motor behaviour (Khatami *et al* 2007; Mileykovskiy *et al* 2005). The minimal loss of a small neuronal population in the lateral hypothalamus does not have to produce observable structural changes in this region, as has been observed in the reports of several authors (Draganski *et al* 2002; Overeem *et al* 2003). Conversely, the loss of axonal branches and terminations might itself lead to volume changes in target structures, which may explain the resulting amygdalar atrophy. An additional explanation is secondary neuronal loss due to the destruction of specific hypocretin projections (Siegel *et al* 1999; Overeem *et al* 2003).

We did not assess the effect of medication in our study. In previous papers, no effect of medication on the regional cerebral volumes was reported, or the use of medication was not indicated. The effect of stimulants to the cognitive skills and possible structural brain changes can be discussed but in our small sample of NC subjects, the available data are too scarce to derive any reliable conclusion.

It can be assumed that the structural changes of the amygdala occur in reaction to the lack of hypocretin transmission with certain delay. Also, the cataplexy occurs after the sleepiness onset in narcolepsy-cataplexy patients (Guilleminault *et al* 2007). Clinically this structural change has a clinical substrate in the disproportional reaction to emotion resulting in cataplexy. However, the pathological changes in amygdala do not have to be the only part in the pathophysiology of cataplexy. In voxel based morphometry studies (VBM) the reduction of gray matter was observed also in other regions: thalamus, brainstem, limbic prefrontal cortex, and occipital cortex (Kim *et al* 2009).

The involvement of the amygdala has been also supported by the recently described reduced concentration of myo-inositol in the right amygdala, shown by proton resonance spectroscopy (Poryazova *et al* 2009).

All previous MRI studies in NC patients have used exclusively the voxel based morphometry with inconsistent results. No changes of amygdalar volume between the control and NC group was found in none of the studies. Kaufmann *et al* observed only a tendency for bilateral reduction of gray matter in the amygdala (Kaufmann *et al* 2002).

Some authors did not find any structural difference between NC group and controls (Overeem *et al* 2003), others showed the reduction in hypothalamic gray matter (Buskova *et al* 2006; Draganski *et al* 2002), and others showed differences in various neocortical areas (Brenneis *et al* 2005; Kaufmann *et al* 2005). Due to the small sample size, most of the authors have indicated their results as preliminary and plead for more detailed analysis including classical manual segmentation (Brenneis *et al* 2005; Kaufmann *et al* 2005).

VBM is a popular whole brain morphometric assessment tool detecting regional structural changes on a voxel by voxel basis. As a fully automated technique it allows evaluation of large amounts of structural data

in a short time on a routine basis. The measurement can be performed by minimally trained personnel with no special requirements for knowledge and training in neuroanatomy. Furthermore, the assessment of the whole brain precludes observer bias in contrast to manual segmentation.

On the contrary, the VBM may prove less reliable in small sample size and insufficient MRI resolution. Segmentation, registration, and normalization steps can introduce distortions and are a main concern in evaluating VBM performance. The difficulties of interpreting VBM findings are especially significant when small brain structures are concerned (Crum *et al* 2003; Davatzikos *et al* 2001, Senjem *et al* 2005). There is a balance between spatial resolution (improving with a smaller smoothing kernel) and statistical robustness. The statistical robustness is generally optimal with a smoothing kernel between 10–13 mm, which is a large number for small subcortical structures like the amygdala. In studies comparing the VBM with manual segmentation there had been limited overlap between the two techniques, concluding that the VBM cannot replace manual measurement despite its speed and reliability (Kennedy *et al* 2009). Overall, combination of different images may give additional information, but it also introduces additional limitations on accuracy.

It is also important to note that the MRI contrast resolution of small and anatomically complex structures like the amygdala, nucleus accumbens, and hypothalamus may not be optimal and therefore suitable for automated analysis. Some structural changes like more discrete neuronal loss lay beyond the resolution capacities of the modern MRI scanners, including magnetic high field devices. Manual segmentation, although time consuming and cumbersome for use on routine basis, remains a tool hard to replace in the hands of an experienced operator in specific cases.

The challenge for future MRI structural investigations is in the refining of automated methods, for example by combining automated and region of interest approach like fully automated analysis of amygdala or hippocampus provided by Freesurfer or FSL (Fischl *et al* 2002).

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