# Positive Correlation Between the Density of Neuropeptide Y Positive Neurons in the Amygdala and Parameters of Self-Reported Anxiety and Depression in Mesiotemporal Lobe Epilepsy Patients

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**Background:** Neuropeptide Y (NPY) has been implicated in depression, anxiety, and memory. Expression of human NPY and the number of NPY-positive neurons in the rodent amygdala correlate with anxiety and stress-related behavior. Increased NPY expression in the epileptic brain is supposed to represent an adaptive mechanism counteracting epilepsy-related hyperexcitability. We attempted to investigate whether NPY-positive neurons in the human amygdala are involved in these processes.

**Methods:** In 34 adult epileptic patients undergoing temporal lobe surgery for seizure control, the density of NPY-positive neurons was assessed in the basal, lateral, and accessory-basal amygdala nuclei. Cell counts were related to self-reported depression, anxiety, quality of life, clinical parameters (onset and duration of epilepsy, seizure frequency), antiepileptic medication, and amygdala and hippocampal magnetic resonance imaging volumetric measures.

**Results:** Densities of NPY-positive basolateral amygdala neurons showed significant positive correlations with depression and anxiety scores, and they were negatively correlated with lamotrigine dosage. In contrast, NPY cell counts showed no relation to clinical factors or amygdalar and hippocampal volumes.

**Conclusions:** The results point to a role of amygdalar NPY in negative emotion and might reflect state processes at least in patients with temporal lobe epilepsy. Correlations with common clinical parameters of epilepsy were not found. The question of a disease-related reduction of the density of NPY-positive amygdalar neurons in temporal lobe epilepsy requires further investigation.

**Key Words:** Amygdala, anxiety, depression, human temporal lobe epilepsy, Neuropeptide Y

Neuropeptide Y (NPY) was isolated a quarter of a century ago (1). Soon thereafter, it was shown that intracerebroventricular injection of NPY induces a long-lasting electroencephalogram synchronization and reversible general reduction of activity (2,3). Since then, NPY has been implicated in a variety of cerebral functions as reactive control of emotional processes (4), ingestion (5), learning and memory (6), or epilepsy-related neuroprotection (7).

In human mesial temporal lobe epilepsy (MTLE), the expression of entorhinal NPY-Y1 receptors was found to be consistently downregulated (8), pointing to an involvement of NPY in epilepsy-related compensatory or dysregulative processes. In the epileptic human hippocampus, downregulation of NPY-Y1 and upregulation of NPY-Y2 receptors together with sprouting of NPY fibers are hypothesized to be part of an endogenous anticonvulsant mechanism (9). In the kainic acid and kindling models of temporal lobe epilepsy (TLE), NPY expression was upregulated in fiber systems of the hippocampus, in cortical areas, and in the amygdala (7), whereas in the pilocarpine model, the number of NPY-expressing interneurons was found to be reduced in the amygdala and neighboring regions (10). In NPY knockout mice, epileptogenic effects of amygdala kindling and different chemical provocants remained generally unchanged, whereas thresholds for pilocarpine- or kainate-induced limbic seizures were lowered and severities were increased, pointing to a more prominent antiepileptic role of NPY in the latter processes (11).

Related to emotion, it was proposed that NPY counteracts corticotropin-releasing factor (CRF)-mediated stress effects to maintain "emotional homeostasis" (12,13), with the amygdala, a brain area intimately involved in mediation and processing of emotion and emotional memory (14), as a central substrate. Consistently, human NPY expression is negatively correlated with trait anxiety and amygdalar and hippocampal activation by threatening stimuli and is positively correlated with pain- and stress-induced activation of the $\mu$-opioid-system (15). In animal studies, intraamygdalar injections of CRF and CRF-agonists had anxiogenic effects, whereas injections of NPY or its agonists were anxiolytic and inhibited anxiogenic effects of CRF (16,17). Furthermore, the number of NPY-containing neurons in the amygdala of rodents correlated inversely with the level of anxiety (18,19). Thus, the amygdala seems to be a key region subject to possible epilepsy-related alterations in NPY expression, which might have an impact both on mnestic and emotional changes in human MTLE. Encephalitic amygdalar atrophy in MTLE patients was associated with an increased incidence of sudden aggressive behavior, whereas preservation of the amygdala contralateral to the side of primary sclerosis was related to an increased incidence of major depression (20,21). The incidence of depression, which is the most frequent comorbidity in MTLE patients,

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depended neither on the side of hippocampal sclerosis nor on side-specific functional deficits, as impaired verbal or nonverbal memory (22). Amygdalar sclerosis in cases without hippocampal sclerosis was associated with milder memory deficits than in cases with hippocampal sclerosis (23).

Parts of the basolateral complex of the amygdala containing γ-aminobutyric acid (GABA)ergic interneurons expressing NPY (24,25) are routinely removed surgically in MTLE patients (26). In these surgical specimens, morphological, ultrastructural, and neurofunctional changes as total volume reduction, cell loss in distinct subareas, and reduction of inhibitory axosomatic synapses were found (27–30). The aim of the present study was to elucidate the role of NPY in emotional processes associated with MTLE. Therefore, we investigated the density of NPY-immunoreactive neurons in the nuclei of the basolateral complex of the amygdala in surgical specimens obtained from patients suffering from intractable MTLE and correlated these with the self-reported emotional status, clinical parameters, and preoperative volumetric measures of mesiotemporal structures.

## Methods and Materials

### Study Population

Analyses were performed on data from 34 patients (18 male, 16 female, ages 18–63 years) receiving unilateral resection of epileptogenic tissue in the mesiotemporal region including lateral, basal, and accessory basal nuclei of the amygdala (30 selective amygdalohippocampectomies, 2 "standard" 2/3 anterior lobectomies, 1 extended lobectomy, 1 functional hemispherectomy). One patient did not receive antiepileptic medication, 4 patients were receiving monotherapy, 29 were receiving polytherapy (17 patients received two, 11 received 3, and 1 received 4 antiepileptic drugs [AEDs]). Mostly prescribed AEDs were levetiracetam (14 patients), lamotrigine (13), carbamazepine (10), oxcarbamazepine (10), clobazam (9), valproate (7), and topiramate (5). All patients were able to fully perform an extended general neuropsychological examination and to answer a set of questionnaires related to emotional state. Informed consent was obtained from all patients for additional histopathological evaluation. All procedures were approved by the ethics committee of the University of Bonn Medical Center. Autopsy tissue was obtained from patients (3 male, 4 female, age range 58–77 years) without cerebral or psychiatric disorders according to clinical history and confirmed by thorough neuropathological examination (postmortem delays 4–30 hours).

### Measures

**Morphological Analyses.** Amygdala specimens were immersion fixed in 4% paraformaldehyde in .1 mol/L phosphate buffer (pH 7.4) for 2–5 days, transferred into a fixative solution containing 4% paraformaldehyde with 1% glutaraldehyde in .1 mol/L phosphate buffer (pH 7.4) for 1 day, transferred back to 4% paraformaldehyde, and stored at 4°C until further use (for approximately 2–3 weeks). Finally, each block was sectioned at 50 μm with a vibratome (Lancer Series 1000, St. Louis, Missouri), and the sections were collected in .1 mol/L phosphate buffer.

Immunocytochemistry of NPY was performed in free floating sections. First, the endogenous peroxidase activity was suppressed (5% methanol, .3% hydrogen peroxide in .1 mol/L phosphate buffer; 10 min), and unspecific binding sites were blocked (10% normal goat serum, 10% bovine serum albumin, .25% Triton-X-100 in .1 mol/L phosphate buffer, 30 min). Thereafter, sections were incubated with the primary antibody at 4°C for 2 days (polyclonal rabbit anti-NPY, 1:300 [Biogenesis, Poole, United Kingdom], 2% normal goat serum, and .25% Triton-X-100 in .1 mol/L phosphate buffer). Immunostaining was visualized by incubating sections with a biotinylated secondary antibody (1:200) for 90 min, the avidin-biotin complex for 1 hour (Vectastain ABC-Kits, Alexis, Grünberg, Germany), and dimaminobenzidinetetrahydrochloride (DAB) as a chromogen (room temperature). Omission of the primary antibody (negative control sections) or blockade of the primary antibody with an excess of NPY (sc-14727 P , Santa Cruz Biotechnology, Santa Cruz, California) but not with peptide YY (sc-47318, Santa Cruz Biotechnology) or pancreatic polypeptide (RP10398, GenScript Corporation, Piscataway, New Jersey) during incubation resulted in lack of staining. Further details of the morphological analyses are provided in Supplement 1.

### Questionnaires

**Depression.** Depressive mood was assessed with the Beck Depression Inventory (BDI), a reliable and widely acknowledged self-rating questionnaire consisting of 21 items (item score range: 0–3). Scores of 11–17 were considered to indicate mild forms of depressive mood disorders, whereas scores larger than 17 were regarded as indicators of clinical depression (31).

**Anxiety.** Anxiety was assessed with the Self-rating Anxiety Scale (SAS) developed by Zung (32) as a self-reporting instrument for anxiety-associated symptoms. The patient answers 20 questions related to the frequency of various symptoms. The SAS total sum scores (range of scale scores: 20–80; cut-off for signs of anxiety: score $> 37$) was used as measure of the actual degree of anxiety.

**Quality of Life.** Health-related quality of life was assessed with a German version of the Quality of Life in Epilepsy Inventory-10 (QOLIE-10) (33), a self-report questionnaire comprising 13 items to self-evaluate disease worry, quality of life, emotional well-being, energy/fatigue, cognitive functioning, medication effects, and social functioning (range of scores: 13–65, cut-off for reduced quality of life: score $> 32$).

### Magnetic Resonance Imaging

All patients were studied with a 1.5-T scanner (Gyroscan ACS-NT; Philips Medical Systems, Best, The Netherlands) with an epilepsy-dedicated magnetic resonance imaging (MRI) protocol that has been reported previously (34). The MRI protocol details are provided in Supplement 2.

### Statistical Analyses

Statistical evaluations were performed with the SPSS 14 software package (SPSS, Chicago, Illinois). Due to the exploratory nature of our investigation, the level of significance was set to .05 (two-tailed tests). To avoid effects of aging or disease duration (years) and severity (number of complex seizures/month) of the epileptic disorder, we used partial correlations (35) to analyze the relations between cell scores, self-rating scores, and other parameters. According to Cohen (36), we interpreted coefficients $.1 \leq r < .3$ as small, $< .5$ as moderate, and $> .5$ as large correlations. Groups of patients differing in clinical categories according to BDI and SAS scores were compared by means of univariate analysis of variance (ANOVA) and post hoc Tukey "Honestly Significantly Different" (HSD)-tests. To estimate medication effects, Pearson correlations of AED dosages with SAS and BDI scores and number of NPY positive cells in the amygdalar subareals were calculated; AEDs showing significant correlations with these parameters were included into partial correlation analyses and were used as ANOVA covariates.