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MULTIMETHODOLOGICAL BRAIN IMAGING STUDIES OF HUMAN EPILEPSY

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To my family

ABSTRACT

Although the pathophysiological mechanisms of human epilepsy are extensively investigated, many questions remain unanswered. One is whether idiopathic generalized epilepsies (IGE) have anatomical substrates. Another is functional integrity of the limbic networks outside the epileptogenic region in mesial temporal lobe epilepsy (MTLE). The present work addresses these important issues by combining several different magnetic resonance (MR) and positron emission tomography (PET) methods.

According to electrophysiological experiments, IGE is associated with abnormal thalamo-cortical volleys, suggesting that both thalamus and cortex could be affected. In Study I MR spectroscopy (MRS) was used in patients with two major IGE syndromes of adulthood - juvenile myoclonic epilepsy (JME) and generalized tonic clonic seizures on isolation (GTCS) to investigate whether the thalamic concentration of Glx (glutamine and glutamate) is altered, and if there are any signs of neuronal damage in this region. The concentration of Glx was significantly elevated in both syndromes, whereas the concentration of the neuronal marker N-acetyl aspartate (NAA) was reduced (Study I).

To exploratively investigate possible anatomical changes also elsewhere in the brain we subsequently employed MR volumetry and voxel based morphometry (Study II) in 19 patients with GTCS and 52 controls. Reduced gray matter fractions were detected in patients in the frontal, parietal, temporal cortex, the thalamus, and cerebellum, along with elevated frontal lobe fractions of cerebrospinal fluid. Furthermore, the structural volumes were reduced in the thalamus, cerebellum, and also the caudate and putamen.

These findings, suggesting a particular affection of motor circuits in human IGE were further evaluated in Study III, which strictly focused on the dopamine (DA) system. Only patients with JME were investigated in these first experiments because our previous studies showed that JME in contrast to GTCS is associated with working memory problems, which like seizures could be attributed to the DA system. Using PET and [^{11}C] PE2I we estimated the binding potential to the DA transporter (DAT) in the substantia nigra/midbrain, as well as the striatum. The nigral/midbrain DAT binding was significantly reduced in relation to controls, whereas the striatal values were normal. Patients also showed impairments in executive and motor functions, with results directly related to the midbrain DAT, and suggesting that the DA system indeed may be involved in the pathophysiology of JME.

Together, the findings in study I-III suggest that IGE is associated with specific cortical and subcortical changes. Their distribution is compatible with the semiology in the two investigated syndromes. The results provide a further argument for a re-evaluation of the current classification and diagnostic criteria for IGE, which currently assumes absence of anatomical substrates.

In study IV cerebral blood flow was measured with PET during passive perception of familiar and unfamiliar odors as a unique tool to investigate how the extrahippocampal limbic structures respond to normal environmental stimuli in mesial temporal lobe epilepsy (MTLE). Although widespread interictal metabolic and receptor changes may exceed the seizure generating area, little is known about functional integrity of the limbic circuits in human MTLE. In controls, both odor types bilaterally activated the amygdala, piriform, insular and cingulate cortex. Familiar odors also activated the right parahippocampus, and the left Brodmann area (BA) 44, 45, and 47. Patients failed to activate the amygdala, piriform and anterior insular cortex on the epileptogenic side. Those with *left* MTLE also could not activate the *left* BA 44, 45 and 47 with familiar odors, which they perceived as less familiar than the controls. Analysis of functional connectivity confirmed these findings including the functional disconnection with the language circuits in left, but not right MTLE.

Imaging of odor perception seems to be a suitable approach to delineate functional disintegration of the limbic networks in MTLE. It shows an altered response in several regions, which may underlay some interictal behavioral problems associated with this condition.

LIST OF PUBLICATIONS

I. Helms G, **Ciumas C**, Kyaga S, Savic I. Increased thalamus levels of glutamate and glutamine (Glx) in patients with idiopathic generalised epilepsy. J Neurol Neurosurg Psychiatry 2006; 77(4): 489-494.

II. **Ciumas C**, Savic I. Structural changes in patients with primary generalized tonic and clonic seizures. Neurology 2006; 67(4): 683-686.

III. **Ciumas C**, Robins Walhin TB, Jucaite A, Lindstrom P, Savic I. Reduced DA transporter binding in patients with juvenile myoclonic epilepsy. Submitted to Neurology, 2007

IV. **Ciumas C**, Lindstrom P, Aoun B, Savic I. Imaging of odor perception delineates functional disintegration of the limbic circuits in mesial temporal lobe epilepsy. Neuroimage, in revision, 2007

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LIST OF ABBREVIATIONS

5-HT_{1A}	5-hydroxytryptamine $_{1A}$ serotonin receptor
AED	Antiepileptic medication
BA	Brodmann area
BOLD	Blood oxygenation level-dependent
CAE	Childhood absence epilepsy
Cr	Creatine
CSF	Cerebral spinal fluid
CT	Corticothalamic fibers
DA	Dopamine
DAT	Dopamine transporter
EEG	Electroencephalography
FAM	Familiar odor
FDG	Fluorodeoxyglucose
FID	Free induction decay
fMRI	Functional magnetic resonance imaging
GABA	Gamma-amino-butyric acid neurotransmitter
Gln	Glutamine
Glu	Glutamate
Glx	Glutamine+glutamate
GTCS	Generalized tonic clonic seizures
IGE	Idiopathic generalized epilepsy
JME	Juvenile myoclonic epilepsy
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MTLE	Mesial temporal lobe epilepsy
NAA	N-acetyl aspartate
nRt	Thalamic reticular nucleus
OFC	Orbitofrontal cortex
PET	Positron emission tomography
ROI	Region of interest
SPM	Statistical parametric mapping
SNR	Signal to noise ratio
SWD	Spike and wave discharges
TC	Thalamocortical fibers
TLE	Temporal lobe epilepsy
TR	Repetition time
uFAM	Unfamiliar odor
VA	Ventral anterior nucleus
VAS	Visual analogue scale
VBM	Voxel based morphometry
VL	Ventral lateral nucleus
VOI	Volume of interest
WM	Working memory

1 INTRODUCTION AND BACKGROUND

1.1 Definition of epilepsy and epileptic seizure

In 2005 the definitions of epilepsy and epileptic seizure were revised by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE) The following was proposed: *Epilepsy* is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by neurobiological, cognitive, psychological, and social consequences of this condition. *Epileptic seizure* is characterized by transient abnormal excessive or synchronous neuronal activity in the brain associated with specific signs of behavioral changes (Fisher, van Emde Boas et al. 2005).

1.2 General classification of epilepsies

This thesis includes studies of generalized (Study I, II and III) and partial (Study IV) epilepsies. Two major classifications of the ILAE are currently employed in the studies of epilepsies:
a) The classification of epileptic seizures (1981), which is based entirely on electrophysiological and behavioral features of the epileptic ictal event, and classifies epileptic seizures into partial, generalized and unclassified epileptic seizures. The partial seizures can be simple partial, complex partial or partial evolving to secondary generalization.
b) The classification of epileptic syndromes (1989), divides epilepsies into idiopathic (without any detectable brain lesions) and symptomatic or cryptogenic epilepsies (in which a lesion is detected or presumably exists). An epileptic syndrome is characterized by a cluster of signs and symptoms occurring together (1989). The classification includes not only syndromes (that might have common etiology, as of yet undiscovered, or with common etiology that will be revised in the next classification), and diseases. Thus, idiopathic generalized epilepsies (IGE) are defined as forms of generalized epilepsies in which all seizures are initially generalized, with a typical EEG pattern of generalized, bilateral, synchronous, and symmetrical discharges with age related onset, a predominantly genetic etiology and variable phenotypes (study I, II, III) (1989). Symptomatic epilepsies are characterized by a specific cerebral pathological substrate (genetic or acquired) that generates epileptic seizures. Localization related epilepsies of interest for study IV was mesial temporal lobe epilepsy (MTLE).

Throughout the studies included in this thesis we used the 1989 classification. One of the aims was to investigate the specific pathophysiological features of the separate syndromes, as a continuation from previous work by our group (Savic, Osterman et al. 2004).

The 1981 and 1989 classifications are generally accepted. However, many other proposals for re-evaluation of classification and definitions have been proposed over the years. Since the previous definitions of *epilepsy* and *epileptic seizure* were dissatisfactory (debates in January 2003 issue of *Epilepsia*), a final common agreement upon the definitions was not made until 2005 (Fisher, van Emde Boas et al. 2005). An ILAE Task Force has considered a revision of the classification (Engel 2001), but due

to several controversies no change will occur in the near future (Wolf 2003). In the re-evaluation of the classification efforts are made to take several new aspects into account: the neuronal substrate for seizures, the various response to antiepileptic medication, the multivariety of pathophysiological mechanisms, the involvement and affection of networks not primarily generating seizures, the pattern of EEG etc. To allow such a classification, much work remains to be done. In IGE, the neuroanatomic, neurophysiological and neurochemical substrates need to be identified. This has to a much larger extent been elucidated in partial epilepsy, but important information is still missing as to how the neuronal circuits involved in the generation and expression of partial seizures respond to normal external stimuli. The studies included in this thesis shed light on some aspects of anatomy and neurochemistry in IGE (Paper I-III), and on the functional integration of the limbic circuits in mesial temporal lobe epilepsy (MTLE), (Paper IV).

1.3 Idiopathic generalized epilepsy

Juvenile myoclonic epilepsy (JME), childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE) and epilepsy with generalized tonic clonic seizures only (GTCS) are the most common syndromes of idiopathic generalized epilepsy (IGE). They differ in their predominant seizure types, but are considered to share several clinical features. According to the 1989 classification of epileptic syndromes the diagnosis of IGE requires absence of pathology in routine neuroradiology (CT scan and MRI). The seizures are generally well controlled by antiepileptic medication. One important common feature for all IGE syndromes is that seizures are initially generalized, with generalized, synchronous, and symmetrical epileptiform activity (1989).

In our studies we have focused on GTCS and JME. About 26% of all patients with IGE are diagnosed with JME (Janz 1997). JME onset is at adolescence with myoclonic seizures that typically occur after awakening and are triggered by sleep deprivation and fatigue. Typical seizure phenomena are: myoclonic jerks of the arms and sporadic generalized tonic–clonic seizures, occurring in approximately 90% of patients (Browne and Holmes 2004). About 30% of the patients also experience absences. Onset is usually in adolescence, at the age of 10 to 23 years, with a peak around 15 years (Loiseau and Duche 1990). There is no gender preference. Patients with JME respond well to AED medication.

Patients with GTCS have generalized tonic clonic seizures only, accompanied by loss of consciousness. Primary GTCS have a usual onset during childhood or adolescence, but they may appear even later. They occur without prior warning or aura and involve the whole brain. Seizures in primary GTCS have motor manifestations - tonic extensions of the limbs lasting ~20–40 s, followed by a clonic phase of rhythmic jerking of the extremities for ~30–50 s. The majority of patients with GTCS report seizures at random, although some experienced them most frequently on awakening. Like most of the IGE syndromes, sleep deprivation and alcohol are triggering factors. Myoclonic seizures and absences do not occur. Patients with GTCS only are, as recently suggested, regarded as a separate population, (Reutens and Berkovic 1995; Andermann and Berkovic 2001; Unterberger, Trinka et al. 2001). They are classified as belonging to the group of ‘other idiopathic generalized epilepsies’ (1989), and will here be denoted as patients with GTCS, as opposed to those with JME.

The pattern of abnormal oscillatory activity seen in IGE is the spike and wave discharge (SWD). Many questions concerning SWD remain unanswered. Which networks are important for the generation of SWD, and how can these abnormal oscillations be explained? Which structures define these networks and how can the function be modulated in these structures? How do the other networks get engaged into the widespread process of IG seizures? Are there specific chemical, anatomical and physiological substrates of idiopathic generalized seizures? Are the seizures truly generalized seizures, and are all the neurons engaged evenly? Although the information is sparse, some cellular and molecular mechanisms of SWD generation have been elucidated by animal models, and lately it has become possible to investigate these mechanisms in patients with IGE.

We add information regarding the pathophysiology of IGE in the studies I-III. SWD involve numerous cortical and subcortical structures, including the brainstem and basal ganglia. However, most studies of SWD generation have demonstrated a crucial role for the highly interconnected circuitry of the cortex and thalamus. Presently known anatomical substrates of these abnormal networks will be further discussed.

1.3.1 Neuroanatomical findings

1.3.1.1 The thalamus and the cerebral cortex in IGE

To date, the information on the pathophysiology of IGE is derived mostly from neurophysiological studies. These studies indicate a major involvement of thalamo-cortical circuits.

Sensory excitatory input to the cortex is transmitted via the thalamic relay neurons, with the exception of olfaction. The term "relay neurons" might suggest a simple bypass for the sensory activity, but it becomes increasingly clear that these neurons are members of a complete circuit that involves ascending, descending and recurrent sets of neuronal connections. Richly and reciprocally connected with cortical structures, thalamic nuclei function both to relay and modulate the transmission of information throughout the brain.

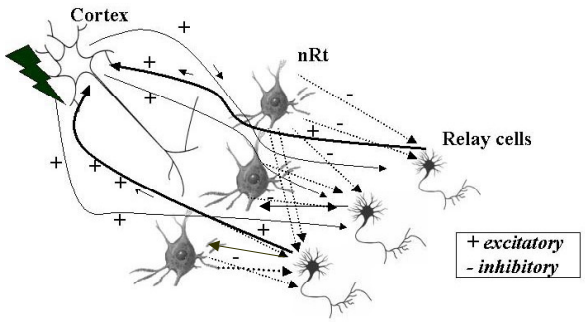


Figure 1. Cortical and thalamic connection. Activation of nRt inhibits thalamo-cortical relay neurons, which generate action potentials that excite the nRt.

These thalamocortical (TC) cells send and receive excitatory (glutamatergic) projections to and from the cortex. The cortex and the thalamic relay contain inhibitory interneurons, but the main inhibitory input is received from a nucleus located in the thalamus – the thalamic reticular nucleus (nRt). The nRt is a collection of GABAergic neurons situated in the bundles of corticothalamic (CT) and TC fibers that course between the thalamus and cerebral cortex (Figure 1). These neurons are innervated by

axon collaterals from TC cells as well as from CT fibers and give rise to a dense innervation of particular regions of thalamic nuclei (McCormick 1992).

Cortico-thalamic monosynaptic connections deliver the excitatory input to the thalamic relay cells. The inhibitory input arrives either via the GABAergic nuclei in the nRt or from GABA interneurons within the thalamic relay nuclei (Steriade, Jones et al. 1997). The constant phasic activity of the nRt excites the cortical cells that in turn re-excite the nRt. Thus, the nRt forms a feed-forward pathway for inhibition from the cerebral cortex to the thalamus, and a feedback pathway for inhibition from the thalamus to the cerebral cortex.

Under certain circumstances spindles may transform into paroxysmal activity with ~3 Hz SWD complexes, detected in the absence seizures. It seems that synchronous spindle oscillations of the nRt are the results of excitatory inputs to the nRt from the cortex (Bal and McCormick 1993; Destexhe, Contreras et al. 1994). Based on cellular studies and computer modeling, it has been hypothesized that this discharge is triggered by the inhibition of $GABA_A$-mediated circuits and depends critically on the activation of thalamic $GABA_B$ receptor-mediated inhibitory postsynaptic potentials for its initiation (McCormick and Contreras 2001). The pacemaker role of the nRt was demonstrated by the persistence of spindle rhythms after isolation of nRt connections to the dorsal thalamus and the cortex (Steriade, Domich et al. 1987). One of the essential factors in the initiation of SWD is the diffuse hyperexcitability of the cortex. Another is the enhancement and synchronization of the TC and CT circuitry (Ferster, Chung et al. 1996; Wehr and Zador 2003). Strong inhibition might predispose to the strong bilateral hypersynchronization that is characteristic for SWD. The hyperexcited cortex, due to prolonged membrane depolarization (Avoli and Gloor 1982), gives rise to continuous neuronal burst firing. With this in mind, the mechanism of seizures is suggested to be the following; cortical cells, the nRt cells and thalamo-cortical relay cells are in a permanent hyperpolarized state. This makes them prone to fire due to initiation of prolonged membrane depolarization. The cortex initiates the paroxysmal oscillation, since the firing starts earlier in the cortex than in the thalamus (Avoli, Gloor et al. 1983), with a secondary engagement of the thalamus and a further reinforcement of neuronal firing. Phasic bursting in a few nRt cells induce bursts in thalamo-cortical relay cells that through CT projections further re-excite the nRt.

The variability of studies suggesting initiation of SWD after triggering the cortex (Gloor, Quesney et al. 1977), the thalamus (Castro-Alamancos 2000), or the brainstem (Cesa-Bianchi, Mancia et al. 1967; Voiculescu, Ungher et al. 1971) propose that there might be multiple onset regions. However, it is at present evident, that both the cortex and the thalamus are essential to maintain the SWD in the brain (Avoli 1990).

1.3.1.2 Proposed theories for IGE generation

There are several theories proposed to explain the pathophysiology of generalized epilepsy (discussed below). Unfortunately, there are no good animal models of IGE, other than that of absence epilepsy. The findings from the animal model studies of absence epilepsy have been generalized to all IGEs, since these epilepsies share several features.

Jasper and Kershman (1941) proposed a subcortical origin for seizures, based on electroencephalographic data from patients with absence seizures (Jasper and

Kershman 1941). Penfield recommended that the seizures should be called centrencephalic, and proposed *the theory of centrencephalic origin* (Penfield 1952).

Later, Gloor suggested that the cortex initiates the SWD. He proposed *the corticoreticular theory* as a result of recording bilateral SWD, observed after intracarotid injection of pentylentetrazol (a proconvulsive drug) in patients with generalized seizures (Gloor 1968). Gloor assigned an essential role in the genesis of discharges to the reticular system of the thalamus, the brainstem and the cortex.

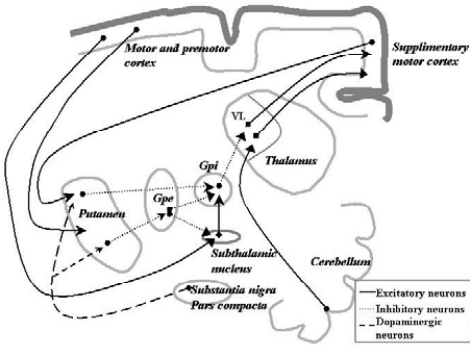
Niedermeyer and Bancaud further evaluated the cortical involvement in IGE, and proposed *the cortical theory* (Niedermeyer 1969; Bancaud, Talairach et al. 1974). The authors found a regional origin of generalized seizures, concluding that SWD seizures might be a consequence of synchronized neuronal discharges in the frontal or mesiofrontal areas, with a diffuse spread over both hemispheres (Bancaud, Talairach et al. 1974) .

Finally, Meeren et al. (2002) found discrete cortical generators by studying rat models of spontaneous absence-like seizures. The primary discharge appears to have a frontal origin, projecting to the thalamus where the excitation is reinforced, allowing the two structures to act together as a unifying oscillatory network (Meeren, Pijn et al. 2002).

Even though IGE are classified as epilepsies with a generalized involvement of the brain during seizures, it is wise to consider the brain sensitive to changes within certain networks (Blumenfeld 2003), with a consecutive engagement of the rest of the brain due to interconnected networks. If the key structures in generation of seizures in IGE could be identified, this could enable a targeted medication to control the epileptogenic process. Presently, the cortical and thalamic roles in SWD are well established. It is, however, not determined in which regions the SWDs are generated. Besides the cortical and thalamic involvement in IGE, several other subcortical structures have been identified to play an important role in seizure modulation, propagation and occurrence.

1.3.1.3 Motor circuits

All IGE syndromes are characterized by the presence of SWD, with different clinical manifestations. GTCS often occur in all IGE syndromes, and most frequently on awakening (1989).



Knowing the semiology of the seizures, it is of interest to investigate the cortical and subcortical structures involved in motor control, focusing mainly on *behavioral* features of seizures in patients with GTCS only. It is well known that the motor cortex integrates inputs from the premotor cortex (precentral gyrus) and the thalamus.

Figure 2. Motor pathway

Patients with IGE exhibit bilateral reductions in both $GABA_A$- and $GABA_B$-mediated inhibition within the motor cortex, implying increased motor cortex excitability in IGE (Badawy, Curatolo et al. 2007). These reductions also emphasize the involvement of motor circuits in GTCS. The motor pathway from the cortex to the ventral lateral nucleus of the thalamus is excitatory (Afifi and Bergman 2005). The thalamus mediates motor functions via specific connections from the ventral anterior and ventral lateral (VL) nuclei to the motor cortex, the basal ganglia, and the cerebellum (Figure 2).

Whereas the motor cortex sends excitatory output to the thalamus, the basal ganglia, and the cerebellum, the connection from the basal ganglia to the thalamus is mainly inhibitory (Purves and Williams 2001). Such an organization of the excitatory and inhibitory neuronal connections provides a substrate for a vicious circle in which seizure activity may lead to excitotoxic lesions. This suggests that the excitotoxic lesions are localized in the specific networks supporting seizures, rather than distributed to the areas particularly susceptible to diffuse hypoxia or diffuse excitotoxicity. The cortico-thalamic loop connects the cortex to the thalamus through the basal ganglia, particularly through the caudate and putamen (Figure 2).

Apart from the cortex and the thalamus, which are believed to have a leading role in the generalization of primary seizures, other structures might have an important role in the modulation or spread of seizures. Proctor and Gale divided the brain into areas based on their epileptic activity (trigger, target, pathway, gating input). The basal ganglia were attributed to the gating structures, which influence seizure activity (Gale 1992; Pedley Timothy and Engel 1998). The general role of the basal ganglia has been reviewed over the years. The functional and dynamic organization of the basal ganglia could have a role in generalized seizures through their input-output relationships. Recent clinical and experimental findings have demonstrated that the basal ganglia affect seizure expression as a propagational pathway or as a remote inhibitory control circuit for seizure discharges. Experimental data from a rat model of absence epilepsy demonstrates that the basal ganglia participates in the propagation of oscillatory cortical activity during SWD (Slaght, Paz et al. 2002). This is also complemented by the theory that some of the basal ganglia circuits influence the epileptic seizure occurrence (Gale 1992; Deransart, Vercueil et al. 1998). The striatum (the caudate and the putamen), in particular, is involved in controlling the cortical excitability and regulation of neuronal synchronization in widespread cortical regions (Slaght, Paz et al. 2002). The striatum has also been implicated in the spread (Henry, Mazziotta et al. 1990; Dupont, Semah et al. 1998), modulation (Dematteis, Kahane et al. 2003), and in persistence of seizures (Deransart and Depaulis 2002).

1.3.1.4 Current findings of anatomical substrate in IGE

It is postulated that IGE is not associated with specific morphological changes, nor tissue pathology (1989). However, this concept is contradicted by reports concerning migrational disturbances (Meencke and Janz 1984; Meencke and Veith 1992), cerebral and cerebellar distortions (Savic, Seitz et al. 1998), and diffuse cortical structural changes in IGE. Studies using volumetric MRI demonstrate a significant increase in volume of the cortical gray matter in the mesial frontal lobe in IGE patients (Woermann, Sisodiya et al. 1998; Woermann, Free et al. 1999; Betting, Mory et al. 2006).

The level of thalamic involvement in IGE has been studied extensively using various techniques. The thalamic volume is reported to be altered in mixed IGE populations

(Savic, Pauli et al. 1994; Chan, Briellmann et al. 2006). However, additional studies report normal thalamic volumes in IGE (Bernasconi, Bernasconi et al. 2003; Natsume, Bernasconi et al. 2003; Seeck, Dreifuss et al. 2005). The most abundant metabolite visible with MRS in the healthy human brain is N-acetyl aspartate (NAA). It is present almost exclusively in the nervous system (Birken and Oldendorf 1989), and the decrease of NAA is often associated with neuronal damage, neuronal loss, or altered mitochondrial metabolism (Petroff, Pleban et al. 1995). Using magnetic resonance spectroscopy (MRS), our group found a reduced concentration of NAA in the prefrontal cortex in JME patients when compared to healthy controls. This finding is supposedly due to neuronal lesions in the prefrontal cortex (Savic, Lekvall et al. 2000). Subsequent comparison of NAA in GTCS and JME patients show reduced prefrontal NAA concentration in JME but not in GTCS (Savic, Osterman et al. 2004). Patients with GTCS on the other hand had reduced NAA concentrations in the thalamus compared to healthy controls. These changes could be syndrome-specific, with localized neuroanatomical changes. NAA levels were also reduced in a mixed population of IGE. A negative correlation between the thalamic NAA level and the duration of epilepsy has been reported (Bernasconi, Bernasconi et al. 2003). A decrease in thalamic NAA/Cr (creatine) was observed in 10 JME patients (Mory, Li et al. 2003).

In both JME and GTCS patients, hyperexcitability and excitotoxicity related alterations have been demonstrated. These alterations could be induced by glutamate excess in the thalamus. In Study I, these patients have been investigated by analyzing the combined levels of glutamate (Glu) and glutamine (Gln) (denoted as Glx = Glu + Gln) in parallel with levels of NAA. Given the behavioral manifestation of seizures in GTCS, not only the thalamus, but also subcortical areas known to be involved in motor pathways, were analyzed using structural volumetry and voxel based morphometry, in an attempt to determine which structures might be altered in this subtype of IGE.

1.3.2 Neurophysiological findings

The standard EEG waves observed in the brain have well-defined frequencies. Examples are the reverberating cortico-thalamic circuits with the frequencies α 8-13 Hz, $\beta > 13$, $\delta < 4$ Hz, and θ 4-7 Hz. However, there is a common EEG pattern in IGE, the SWD, which are the best-known abnormal oscillations in IGE. SWD consist of intense, high frequency neuronal firing during the spike phase, alternating with relative inactivity of neuronal networks during the resulting slow wave. Interictal EEG recordings in JME are characterized by bilateral, symmetric, synchronous, and diffuse polyspike- and slow-wave complexes with a frequency of 4-6 Hz. During myoclonias (frequency 6 to 16 Hz), polyspikes lead to higher voltage recruiting patterns. During absences in JME patients, the frequencies 4 to 6 Hz decrease to 3 Hz and develop into polyspike- or SWD activity. The majority of the patients are photosensitive.

In GTCS, especially in its clonic phase, the seizures are accompanied by a polyspike-and-wave EEG pattern that usually evolves in a decreasing pattern, with decreasing frequency and rhythmicity during the course of the convulsive episode. It is often accompanied by photosensitivity. However, sometimes the pattern of EEG observed in IGE can have a localized site similar to the pattern observed in localization related epilepsy. Focal EEG have been described in JME patients (Usui, Kotagal et al. 2005), who also had asymmetric jerks of the limbs. It seems that the correct diagnostic criteria would be video EEG monitoring of these patients and reevaluation of patients' ictal and interictal EEG recordings (Usui, Kotagal et al. 2005). This is quite common in IGE patients that do not respond well to AED medication. The reason for some patients

responding differently to AED might be hidden in the variable genetic background controlling the response to AED, in particular ion channel properties and neurotransmitter regulation.

1.3.3 Major neurotransmitter systems and IGE

Epilepsy is believed to originate from an inhibitory-excitatory imbalance.
Glutamate (Glu) is the major excitatory neurotransmitter in the brain. Excess of Glu in the synaptic cleft is known to have an excitotoxic effect. Therefore, Glu is postulated to have a leading role in excitotoxicity. Previous studies demonstrate elevated levels of Glu in the epileptic brain (Ingram, Tessler et al. 2000; Savic, Thomas et al. 2000). Epileptogenic activity is associated with an increase of Glu and an impaired clearance of Glu in epileptogenic tissue (Petroff, Errante et al. 2002). Glu released by neurons is reuptaken by surrounding astrocytes and then converted into glutamine (Brookes 2000). Glutamine (Gln) is synthesized only in astrocytes *(Figure 3).* The Gln that is reuptaken by neurons replenish the Glu stores lost during neurotransmission. Glu-Gln cycling reflects one aspect of the metabolic interaction between neurons and glia.

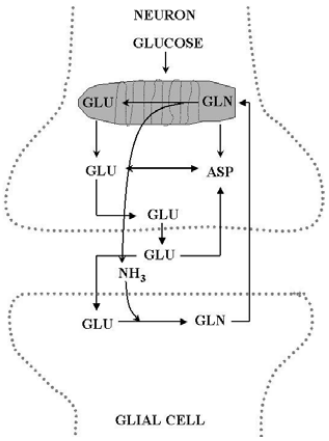


Figure 3. Glutamate-Glutamine turnover

Perturbance of excitatory mediator turnover may play a significant role in the generation of seizures. For instance, low rates of neuron–glia cycling are caused by glial dysfunction, resulting in a down-regulation of Gln synthetase. Partial failure of Gln synthesis results in increased glial Glu concentrations, which could contribute to slower Glu uptake and enhanced Glu transporter reversal.

Of neurotransmitters, γ aminobutyric acid (GABA) plays a prominent role in epilepsy by eliciting the long-standing hyperpolarization required to stimulate low threshold calcium channels to initiate sustained burst firing when targeting $GABA_B$ receptors. GABA is the main inhibitory neurotransmitter in the brain and is present in about 30 % of the human brain neurons. GABA is known to activate two types of receptors; the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptors (Chebib and Johnston 1999). $GABA_B$ receptors are located in the interneurons of the cortex, the thalamus, the nRt of the thalamus, the stellate, Golgi, basket and Purkinje cells of the cerebellum, and the basket cells of the hippocampus. $GABA_B$ agonists, such as baclofen, aggravate, and $GABA_B$ antagonists suppress, typical absences. GABA plays a very important role in the inhibitory/excitatory imbalance found in epilepsy. GABA receptors are clearly essential in this imbalance. Several antiepileptic drugs (benzodiazepine derivatives and barbiturates) enhance GABA inhibition. The loss of GABAergic interneurons and impaired inhibitory function have frequently been considered characteristics of the epileptic brain (Magloczky and Freund 2005). The GABA neurotransmitters have not been investigated throughout the studies included in this thesis.

It is important to highlight the dopaminergic influence on the striatum. Dopaminergic neurons in the mammalian brain have received considerable attention in the past given their essential role in several body functions and behaviors. The major dopaminergic population is found in two nuclei of the ventral midbrain. Cells of the substantia nigra pars compacta are involved in the control of voluntary movements and postural reflexes. Cells of the ventral tegmental area (VTA) modulate reward and cognitive behaviors. This innervation results in four major dopamine (DA) circuits in the brain: the nigrostriatal, mesocortical, mesolimbic, and tuberoinfundibular circuits. DA is synthesized in the presynaptic terminal of several metabolic pathways. First, tyrosine in the cell is converted to L-DOPA by the enzyme tyrosine hydroxilase. L-DOPA is then converted into DA by the enzyme aromatic amino acid decarboxylase (Vermeulen, Drukarch et al. 1994; Vermeulen, Drukarch et al. 1994). The synthesised DA molecules in the presynaptic terminal are reuptaken by synaptic vesicles. After the DA is released from the vesicles into the synaptic cleft, the remaining molecules are taken back into the synaptic terminal by DA transporters (DAT) in the membrane. DA also has the property to modulate the effect of other neurotransmitters impinging on striatal neurons (Jaber, Robinson et al. 1996).

Dysfunctions in DA networks result in disturbances of several functions. Experimental depletion of the DA or blockade of DA receptors results in cognitive deficits such as impaired working memory (WM) (Brozoski, Brown et al. 1979), decision making (Rogers, Everitt et al. 1999), attention (Brown and Marsden 1988), and mental flexibility (Cools, van den Bercken et al. 1984). Motor changes after modulation of DA receptors or DA depletion are a change in neuronal activity of the striatum, the globus pallidus, and the cortex (Sharott, Magill et al. 2005).

Basal ganglia function is modulated by the dopaminergic influence originating mainly from the substantia nigra. This region displays the densest staining in the central nervous system for the dopaminergic markers (Prensa, Cossette et al. 2000). Since the striatum expresses a high density of D1 and D2 receptors (Jaber, Robinson et al. 1996), the effects of DA depend on the relative proportion of these two receptor types, the concentration of DA, and the ongoing excitatory and inhibitory synaptic activity (Johnson, Jaju et al. 1981; Hall, Sedvall et al. 1994). Drug-induced seizures in D2 knock-out mice show increased levels of Glu acid decarboxilase in the cortex and striatum, and deficient GABA neurotransmission in the neostriatum (An, Bae et al. 2004). Glu may play a key role in this process. DA in low concentrations can depress the glutamatergic synaptic transmission in the neostriatum by decreasing the neurotransmitter release from corticostriatal presynaptic terminals (Choi and Lovinger 1997). The dopamine D1 and D2 receptors control neuronal signaling and modulate many important behaviors antagonistically. Thus, D2 postsynaptic receptor stimulation is associated with inhibitory, anticonvulsive effects, whereas stimulation of D1 receptors is associated with excitatory, proconvulsive effects. Injections of DA D2 agonists and D1 antagonists in the dorsal parts of the striatum are reported to suppress absence seizures (Deransart, Riban et al. 2000). Experimentally induced kindling leads to temporary decreases of D1 and D2 receptors in the striatum (Tchekalarova, Sotiriou et al. 2004). At the cortical level, the primary role of the D1 receptors is the modulation of glutamatergic input to cortical pyramidal cells (Smiley, Levey et al. 1994). It also plays a selective role in the mnemonic, predictive functions of the prefrontal cortex (Sawaguchi and Goldman-Rakic 1991). D2 receptors are present in all regions of the cortex, although with densities less than that measured in the neostriatum (Lidow, Goldman-Rakic et al. 1989).

Blockade of GABA innervation in the substantia nigra leads to EEG spiking in the striatum (Stevens, Wilson et al. 1974). This suggests a role of the DA system in spike generation. The mechanism by which DA has been proposed to play a role in the occurrence of SWDs is described by Buzsáki et al. (Buzsaki, Smith et al. 1990): a constant level of firing of DA neurons in the substantia nigra pars compacta (SNc) provides a stable inhibitory innervation to the dorsal striatum, thus inhibiting the GABAergic projections from the substantia nigra pars reticulata (SNr) and globus pallidus (GP) to the thalamus. The consequence of this is a disinhibition of the TC neurons. Data confirming this idea come from studies in which an enhancement in SWD incidence was established subsequent to injections of DA blockers into the dorsal striatum (Buzsaki, Smith et al. 1990) and lesioning of the SNc neurons (Danober, Deransart et al. 1998). Indirect evidence that underlines the role of striatal DA in SWD occurrence come from studies in which DA agents were systemically injected: DA agonists decreased and DA antagonists increased SWD incidence (Avakyan and Arushanyan 1976; Warter, Vergnes et al. 1988; Midzianovskaia 1999; Deransart, Riban et al. 2000; Deransart, Le-Pham et al. 2001). The interictal neurotransmitter imbalance that characterizes IGE might therefore be altered in these patients.

There are several ways to investigate the dopaminergic system in humans (Volkow, Fowler et al. 1996). So far, only two studies have examined the DA system in epilepsy patients (Biraben, Semah et al. 2004; Bouilleret, Semah et al. 2005). Bouillert et al report decreased reuptake of DA in the striatum and midbrain (Biraben, Semah et al. 2004; Bouilleret, Semah et al. 2005). However, only quantification of DAT can offer data about the dopaminergic innervation, as DAT is the only marker of DA innervation in the brain (Volkow, Fowler et al. 1996) Quantification of the midbrain DAT in vivo is now possible (Halldin, Erixon-Lindroth et al. 2003; Jucaite, Fernell et al. 2005; Jucaite, Odano et al. 2006). We propose that DAT activity and a decreased level of DA receptors might have a crucial role in idiopathic generalized epilepsy. DA transmission at the level of the striatum could be either increased in IGE as compensation, thereby preventing seizures/hyperexcitability. Or, seizures in IGE may be a consequence of low DA tone. In both events, changes in the DA system are expected.

1.3.4 Interictal neuropsychological performance

Epilepsy is generally associated with cognitive impairment (Dodrill 1978). Idiopathic epilepsies are an interesting model in which the effects of epileptic discharge on development and brain plasticity can be studied. This is due to the absence of evident brain lesions in these epilepsies. Many factors influence the neuropsychological assessment in patients with epilepsy, such as medication, age of onset of epilepsy, duration of epilepsy, control of seizures, as well as the social stigma of having epilepsy. It is also difficult to assess whether the changes in behavior are the results of continuous epileptic discharges or if alteration is due to medication. Several behavioral changes have been noted, particularly in social integration and personality, and this have led to more profound studies of IGE. The intellectual capacity of IGE patients appear unaffected, or have a tendency to be lower (Farwell, Dodrill et al. 1985; Mirsky and Duncan 2001; Chaix, Laguitton et al. 2006). However, executive functions, such as memory performance, psychomotor speed, and predictable learning have been observed to be reduced in children with IGE (Bailet and Turk 2000). Most studies that target the cognitive profile in IGE have focused their attention on JME patients, due to more marked differences of behavior and cognition (Sonmez, Atakli et al. 2004). As opposed to several other IGE syndromes, a larger portion of JME patients have interictal cognitive dysfunctions (Swartz, Halgren et al. 1994; Swartz, Simpkins et al.

1996; Devinsky, Gershengorn et al. 1997; Janz 1997). JME patients have been described as emotionally unstable (Janz 1985), impaired at planning, organization, cognitive speed and abstract reasoning (Devinsky, Gershengorn et al. 1997). Working memory (WM) is also impaired in JME patients. WM is believed to have two major neuronal substrates – the dorsolateral prefrontal cortex and the basal ganglia (D'Esposito, Detre et al. 1995); additionally training of WM was associated with increased activity in the middle frontal gyrus and superior and inferior parietal cortices (Olesen, Westerberg et al. 2004). It is, therefore, of interest that low WM performance in JME patients correlates with frontal lobe reductions of interictal glucose metabolism and concentrations of *N*-acetyl-aspartate (Swartz, Simpkins et al. 1996; Savic, Osterman et al. 2004). Swartz et al. performed an FDG-PET activation study while testing WM. Results show a reduction of glucose uptake in the dorsolateral prefrontal cortex in JME patients during a WM task (Swartz, Halgren et al. 1994). Thus, the cognitive dysfunctions seen in JME (Swartz, Simpkins et al. 1996; Sonmez, Atakli et al. 2004; Pascalicchio, de Araujo Filho et al. 2007) suggest that these differences might be related to an underlying effect of DA signaling.

The majority of studies in JME patients indicate alteration of several cognitive functions together with subtle alterations detected in cortical, predominantly frontal, areas and some subcortical changes such as thalamic volumetric changes, neurochemical changes, and decreased striatal DA uptake. Previous research indicates that cognitive functions are dependent on the DA signaling to the target regions. Thus, to observe the correlation between the neuropsychological profile of JME patients and the DA system function in our group of patients, a battery of tests specific to frontal lobe function has been used in study III.

1.4 Partial epilepsy

Partial epilepsies are characterized by partial epileptic seizures (with or without generalized seizures) (Engel 1989). The partial seizures are sub-classified into simple partial seizures, complex partial seizures, and partial seizures with secondary generalization to clonic and/or tonic seizures. The most common form of partial epilepsy is mesial temporal lobe epilepsy (MTLE).

1.4.1 Mesial temporal lobe epilepsy

Most of the temporal lobe seizures begin in the mesial temporal lobe structures. Mesial temporal lobe epilepsy (MTLE) is associated with a history of febrile seizures, particularly complex, or prolonged febrile seizures. Observations have also associated MTLE with other acute neurological injuries such as episodes of partial-onset status epilepticus, closed head injury, brain tumours, and stroke. Hippocampal sclerosis is, however, the most common pathology and is found in ~65% of patients undergoing resective surgery (Blumcke, Thom et al. 2002).

In patients with severe hippocampal sclerosis, atypical spread of ictal discharges can be detected with scalp EEG recordings on the opposite side from the hippocampal sclerosis,. These can, however, be well controlled after hippocampal resection (Mintzer, Cendes et al. 2004). The existence of hippocampal sclerosis is often associated with the duration of epilepsy and it is currently discussed whether MTLE may be a progressive condition. Clinical evidence for progression of dysfunction in TLE is changes in seizure character, decreasing response to treatment, and a

development of bilateral temporal foci. These signs of progression are detected in one third of patients with refractory TLE observed on EEG (Engel, Rausch et al. 1981; Morrell 1985). Specific dysfunctions, such as memory deficit (Delaney, Rosen et al. 1980), personality changes (Bear and Fedio 1977), and mood disturbances (de Souza and Salgado 2006) are very common in MTLE. These behavioral disturbances imply a greater involvement of the limbic system.

1.4.2 Interictal dysfunction in MTLE

It has been suggested that interictal dysfunction in severe MTLE spreads from the epileptogenic region, possibly as a result of a seizure related excitotoxicity (Heinemann 2004; Avoli, Louvel et al. 2005). Extensive PET research on neuroreceptor binding and glucose consumption has provided important information about the extent of dysfunction in MTLE. FDG-PET is the most established functional imaging modality in the evaluation of patients with epilepsy. Numerous studies using this technique have shown regional hypometabolism not only in the epileptogenic lobe, but also in the ipsilateral thalamus and basal ganglia (Henry, Mazziotta et al. 1990; Henry, Mazziotta et al. 1993). The sensitivity for detecting relative temporal lobe hypometabolism with FDG-PET in MTLE ranges between 80 and 90% (Gaillard, Bhatia et al. 1995; Ryvlin, Bouvard et al. 1998).

Flumazenil has proved to be a very efficient ligand in detecting epileptogenic regions (Savic, Ingvar et al. 1993; Van Paesschen 2004). It is extensively used in the preoperative evaluation of patients with medically refractory epilepsy. Multiple limbic reductions of benzodiazepine receptor binding have been detected in MTLE patients (Savic, Persson et al. 1988; Hammers, Koepp et al. 2001). The 5-HT_{1A} receptor binding potential has been found reduced not only in the seizure generating hippocampus and parahippocampus, but also in several limbic areas such as the ipsilateral anterior cingulate, and the insular cortex (Savic, Lindstrom et al. 2004). Whether, and to what extent, the observed changes imply abnormal response to external stimuli in the limbic circuits is, however, presently unknown. Investigating this issue is important when trying to understand the mechanisms behind the anxiety, depression, attention, and memory difficulties highly prevalent in severe MTLE (Blumer, Montouris et al. 1995; Engel 1997), and could, at least to a certain extent, be attributed to limbic dysfunctions (Blumer, Montouris et al. 1995; Gloor 1997). Investigations complementary of functional response of the limbic system to external stimuli are thus essential. This has, however, not been studied. Therefore, we specifically address this issue in Study IV.

1.4.3 Limbic circuits and olfaction

The limbic lobe refers to the structures that surround the brain stem. This includes the subcallosal gyrus, cingulate gyrus, isthmus, parahippocampal gyrus, and uncus. *The limbic system* includes the limbic lobe and cortical and subcortical structures connected to it. The following structures are known to connect to the limbic lobe: the septal nuclei, amygdala, hypothalamus (mostly the mamillary body), thalamus (anterior and medial thalamic nuclei), reticular formation of the brainstem, neocortical areas in the basal fronto-temporal region, olfactory cortex, and ventral parts of the striatum. These structures play an important role in emotional and sexual behavior, memory, motivation, and integration of various homeostatic responses (Gloor 1997).

After entering the cranial cavity, the olfactory nerve fibers connect to the mitral and tufted cells of the olfactory bulb, which via the olfactory tract and olfactory tubercle

projects to the ipsilateral primary olfactory cortex (consisting of the piriform, periamygdaloid and transenthorhinal cortex). The olfactory tract also connects directly to the ipsilateral periamygdaloid cortex, and the olfactory tubercle to the mediodorsal thalamic nucleus (Paxinos 1990). These principal anatomical connections indicate that the perception of odors has to be mediated mainly by the cerebral hemisphere, ipsilateral to the stimulated nostril. The two olfactory tracts are, however, connected to each other via the anterior olfactory nuclei and the anterior commissure (Paxinos 1990), two secondary connections that could allow a certain extent of contralateral processing.

Imaging of olfaction offers new data for understanding odor processing. Our group's previous studies demonstrate bilateral activation of the amygdala, piriform cortex and parts of the anterior insular cortex in males and females during passive smell of pure olfactory odorants (Bengtsson, Berglund et al. 2001). Thus, when studying the processing of olfactory odorants, the study groups need not be separated with respect to sex. Furthermore, according to the study of Savic and Gulyas, odor perception is processed by both hemispheres, despite the predominantly ipsilateral anatomical projections (Bengtsson, Berglund et al. 2001). Odor stimuli, independently administered to each nostril, show bilateral activation of the amygdala, piriform cortex, anterior cingulate, and the right orbitofrontal cortex. These findings suggest the existence of functional connections between the two hemispheres, probably through the anterior commissure (Kettenmann, Hummel et al. 1997), and the anterior and ventral medial thalamic nuclei (Savic, Gulyas et al. 2000; Savic, Gulyas et al. 2002).

Subjects' handedness slightly influences odor discrimination. When the odor stimulus is presented to the right nostril in right-handed subjects the performance is better compared to results following presentation to the left nostril (Hummel, Mohammadian et al. 1998). The nostril-specific response detected in the primary olfactory cortex is linked to the cross-modal integration of olfactory information in a multi modal superior temporal gyrus (Porter, Anand et al. 2005). There seems to exist a semantic link during encoding of odors, if they are familiar. During smelling of familiar odors, Savic and Berglund showed activation in the Brodmann areas 44,45,47 on the left side, in addition to activation of the limbic structures (Savic and Berglund 2004).

The orbitofrontal cortex (OFC) is one of the secondary olfactory areas. The first study to find right OFC activation as a response to odorants was carried out by Zatorre in 1992 (Zatorre, Jones-Gotman et al. 1992). A later study, specifically investigating the various levels of olfactory processing, showed a specific involvement of the OFC during odor memory and discrimination, although minor portions on the right side were activated during mere perception (Savic et al., 2000). OFC activation was detected also during evaluation of pleasantness and intensity of odors (Zatorre, Jones-Gotman et al. 2000). Different circuits seem to mediate odor intensity and valence; odor valence activates the OFC, while the amygdala is activated by the odor intensity. Furthermore, depending on the pleasantness of the odor, different portions of the OFC are activated. Pleasant odors activate the medial OFC, while unpleasant activate the lateral OFC (Anderson, Christoff et al. 2003). The activation of the left OFC is, on the other hand, reported to be linked to the judgement of familiarity of an odor (Royet, Hudry et al. 2001). The left OFC is also involved in judgment of aversive odors, but not of pleasant odors (Zald and Pardo 1997).

1.4.4 Olfactory changes in MTLE

By investigating the olfaction of patients with brain damage, correlations between impaired structure and function can be discerned. There are several reports about olfactory functions in MTLE. Although the majority of studies show impaired performance, West and Doty reviewed the literature on epilepsy and olfactory functions a decade ago, and concluded that studies were inconsistent with respect to odors used, testing methods, and subject selection, highlighting the remarkably inconsistent findings from one study to the next (West and Doty 1995).

There is now a general agreement that epilepsy is *not* linked to increased olfactory thresholds (decreased sensitivity) (Eskenazi, Cain et al. 1986; West and Doty 1995; Lehrner, Baumgartner et al. 1997; Kohler, Moberg et al. 2001). In retrospect, this observation is hardly surprising in light of the findings in patient H.M, who underwent bilateral temporal lobectomies for treatment of uncontrolled seizures (Scoville and Milner 1957), with normal postoperative odor detection thresholds (Eichenbaum, Morton et al. 1983). This suggests that the mesial temporal structures are not needed for normal *odor detection* in humans. Whether this also applies to *odor perception* is yet to be evaluated, which motivates study IV.

In respect to olfactory quality, MTLE patients showed an impaired capacity to discriminate familiar odors ipsilateral to the epileptogenic side. This was found using the University of Pennsylvania Smell Identification Test (UPSIT performance- a standardized microencapsulated test of olfactory function using 50 % familiar odors, such as lemon or bubble gum etc.), as was the odor recognition memory (Savic, Bookheimer et al. 1997). Therefore, the testing of olfactory memory and discrimination was proposed to be a valuable diagnostic tool to determine lateralization of seizure generation in MTLE patients without lobectomy (Savic, Bookheimer et al. 1997). Again, using familiar odors, impaired bilateral odor identification was demonstrated in patients presurgically (Eskenazi, Cain et al. 1986).

In evaluating the olfaction in patients with TLE, several contradictions occur. The information converging from different studies use patients before or after surgery, and apply different ranges of odors. For instance, the above-mentioned study by Eskenazi et al indicates that odor memory is impaired in patients with resected temporal lobes. There is, however, no difference between the groups when the familiar odors were given ipsilateral to the epileptogenic side prior to surgery (Eskenazi, Cain et al. 1986). Again, UPSIT test showed impairment in odor identification in patients with anterior temporal lobe lesions (piriform cortex) (Jones-Gotman, Zatorre et al. 1997). This allows the conclusion that the piriform cortex is involved in odor identification. In our group's previous study, using the UPSIT odors (Savic, Bookheimer et al. 1997), patients having undergone temporal lobectomy were shown to perform worse in the odor discrimination task if the stimulus (unfamiliar odors) is given to the right nostril in patients with right MTLE and to the left nostril in patients with left MTLE (Zatorre and Jones-Gotman 1991). It seems that familiarity of odors given to the subjects is of importance. In line with our group's findings, passive smelling of familiar odors engage semantic circuits, which is not the case when using unfamiliar odors (Savic and Berglund 2000; Savic and Berglund 2004). This might explain why the use of odors unfamiliar to subjects give contradictory results from different studies; the other reason might be the selection of patient group, since right and left MTLE patients differ in olfactory processing capacities.

In contrast to normal or supranormal odor detection, patients with epilepsy are usually impaired in tests of odor naming, discrimination, and recall. These impairments are

found in patients with left and right sided MTLE. However, these deficits are often (Carroll, Richardson et al. 1993; Kohler, Moberg et al. 2001), but not always (Eskenazi, Cain et al. 1986; Hudry, Perrin et al. 2003) found to be more pronounced in patients with right TLE than in those with left TLE or other epilepsy syndromes. Birhinic odor identification and recall of common nameable odors in 30 patients with epilepsy also indicate that patients with right TLE have abnormally low retention of namable odors in comparison to unnamable odors. This might occur due to a right sided localization of structures implicated in short-term memory odor retention, and a disruption of function of these olfactory memory encoding circuits in right TLE (Carroll, Richardson et al. 1993). Additionally, right MTLE patients seem to show an impaired odor matching (Abraham and Mathai 1983). A recent fMRI study demonstrated greater impairment of odor recognition and discrimination in left than in right TLE patients, with both groups performing worse than controls (Hudry, Perrin et al. 2003).

Interestingly, the performance disparity between left and right TLE patients was tentatively attributed to group differences in psychosocial traits, as opposed to any difference in perceptual or memory processes. These findings highlight the role of mesial temporal structures in olfactory encoding. This impairment was not seen in patients with left TLE (Kohler, Moberg et al. 2001). These results were attributed to abnormal olfactory encoding and short-term memory function (Hudry, Perrin et al. 2003).

It is worth mentioning that most of the studies that use odor stimulation apply additional cognitive tasks, which activate olfactory and extraolfactory areas that are involved in olfactory processing. We have shown that passive smelling of familiar and unfamiliar odors activate primary olfactory areas, with additional activation of semantic areas when smelling familiar odors; mostly areas related to language. Familiar odors involve episodic odor memory and semantic or verbal factors that play a role in more complex odor processing. For example, patients performed better in memory- and discrimination tests when smelling familiar odors compared to unfamiliar odors (Lyman and McDaniel 1986; Schab and Crowder 1995; Larsson and Backman 1998; Savic and Berglund 2000). Thus, when combining this information with additional information from language related studies in MTLE, where impaired language processing is observed in left MTLE (Field, Saling et al. 2000), this motivates the use of both types of odors during passive smelling in search of olfactory areas possibly altered in MTLE.

In conclusion, most studies demonstrate that epilepsy patients have normal olfactory thresholds, and impaired odor discrimination, odor identification, and odor memory. These olfactory deficits are most likely more distinct in right than in left TLE, consistent with the association of olfactory symptoms and disturbances in right TLE patients. Whether these deficits are consequences of a disturbance of perception or memory is not clear, as this distinction is particularly difficult to make in olfaction due to the central role of the limbic system in both processes. However, the extent of these changes seems to depend on the side of epileptogenesis, the spread of dysfunction, and the involvement of limbic regions. The limbic regions are essential considering their great involvement in olfactory processing. Investigating limbic system integrity in patients with MTLE is a complicated task due to the lack of stimuli that trigger the limbic system primarily. Odor stimuli trigger the primary olfactory areas, such as the amygdala and piriform cortex, which are components of the limbic system. The transmission bypasses the thalamus, as is necessary for stimuli used to investigate limbic circuits. Therefore, the use of an olfactory paradigm will ensure the targeting of

primary olfactory areas. Functional connections of regions and presumably impaired circuitry are investigated in patients using functional connectivity analysis.

1.4.5 Functional connectivity

In neuroimaging, *functional connectivity* is defined as the correlation between spatially remote neurophysiological events, which imply a simple characterization of functional interactions. Alternatively, *effective connectivity* is the influence one neuronal system exerts over another. Essentially, functional connectivity is a conclusion made upon observed correlations; a term that provides no information about mediation of these connections. Of importance is the fact that functional connectivity is not necessarily the effect of effective connectivity (Frackowiak 2004).

In absence of pathology it is assumed that the structural interconnections between regions remains relatively stable, a fact that, if studied, provides information about the extent to which different components of a certain network interact. Characterization at the functional level focuses on how neural activity within one component of the network changes in relation to neural activity in other components of the network. When the activity in two regions displays a high degree of covariance, the regions involved are defined as functionally connected (Friston 1994). Thus, the level of covariance or correlation between two regions arising over multiple measurements may be taken as an empirical estimate of their functional connectivity. If two regions demonstrate a high degree of functional connectivity they may be referred to as functionally coupled (Horwitz 1991). By defining regions that are functionally coupled, one could assume that the regions might influence each other, or be commonly influenced by another region, but no source or direction of the connection can be detected. The level of functional connectivity can vary noticeably depending on the task that the network performs at any given instant. Since most of the stimuli necessitate the interaction of different brain regions, two areas may be functionally coupled during one task, but not during another, in spite of having a stable level of anatomic connectivity. In this regard, when the activity in two regions becomes independent from one another, the regions may be described as "functionally uncoupled." The networks might also exhibit disintegration of the circuits in a pathological condition, such as MTLE. PET quantifies activity in multiple brain regions simultaneously, thereby enabling the assessment of the functional couplings and uncoupling within distributed neurocognitive networks. Several related statistical techniques allow the extraction of information on the functional connectivity between brain regions from PET data. All methods rely heavily on the covariance of measured activity in different brain regions. In each method, the covariance between different regions provides estimates of the level of functional connectivity between regions during a given task. Analysis of covariance analysis of PET data is used to explain functionally connected brain regions during a task. Cerebral regions (e.g. the limbic system) can exhibit different patterns of functional connectivity during different tasks.

There are two approaches to measure functional connectivity: over time and over subjects. The over time approach (temporal correlation of activity between brain regions) requires many scans and execution of the same task across the examination. This is difficult to achieve using PET. In study IV we therefore employed functional connectivity across subjects.

We have used resting state and two activation conditions to investigate functionally connected regions in controls and MTLE patients. The piriform cortex was chosen as

the region of interest because of its involvement in odor processing and its anatomical connections with mesial temporal structures involved in the epileptogenic process such as the amygdala, entorhinal cortex, dorsal medial thalamic nucleus, insular, perirhinal, and entorhinal cortices (Engel 1997).

2 AIMS OF THE THESIS AND THE UNDERLYING HYPOTHESES

◊ We hypothesized that our previously found reduction of NAA in the thalamus of patients with IGE, could be the result of neuronal excitotoxicity reflected by increased Glx in the thalamus. Therefore, in a mixed population of IGE we investigated the metabolic and structural integrity of the thalamus with MR imaging (manual volumetry and voxel based morphometry) and single volume magnetic resonance spectroscopy (Study I).

◊ A second hypothesis was that GTCS is associated with regional changes in gray/white matter fractions and structural volumes, and that the location of these changes is relevant for the behavioral expression of seizures. To test our hypothesis, manual volumetry and voxel-based morphometry were employed (Study II).

◊ We also hypothesized that JME is associated with changes in the dopamine system and that these changes are linked to interictal cognitive dysfunctions in these patients. This hypothesis was tested using positron emission tomography, and the state of the art ligand for the dopamine transporter. Additionally, batteries of tests of cognitive and psychomotor function were used (Study III).

◊ Finally, we hypothesized that cerebral activation during simple odor perception could delineate functional disintegration of the limbic circuits in MTLE. The reason was that very little is presently known about how these circuits (frequently showing metabolic and neuroreceptor changes in MTLE) process common external stimuli. We tested our hypothesis using positron emission tomography during passive smelling of familiar and unfamiliar odors, and adding analysis of functional connectivity to the simple evaluation of the activation patterns (Study IV).

3 METHODS

3.1 Patients

All patients were recruited from the Epilepsy Clinic at Karolinska University Hospital, Stockholm, Sweden. All studies were approved by the local Ethical Committee and, for studies using PET, the Radiation Safety Committee. All subjects participating in these studies gave informed written consent.

All patients included in studies I, II, and III were diagnosed with IGE according to the International Classification of Epilepsies from 1989. The details of the patients are:

- Forty-three (twenty females) adult patients (age 32±8 years, age at onset 16±5 years with a seizure history of 16±10 years) were included in the study I (twenty-three had JME and twenty one suffered from GTCS).
- Nineteen (nine females) GTCS patients (age 34±9 years, age at onset 18±6 years and the duration of epilepsy 16±10 years) were included in study II.
- Twelve (nine males) JME patients (age 38 ± 12 years, age at onset age 15 ± 3 years with duration of epilepsy 23 ± 11 years) participated in the study III.

JME was diagnosed based on seizure history, seizure semiology as described by relatives or recorded during hospitalization, and results of scalp electroencephalographic (EEG) recordings. EEG showed bilateral and generalized spike-and-wave or polyspike-and-wave activity. Patients had late childhood or teenage onset of awakening myoclonic jerks. Some reported myoclonias at any time of the day.
With respect to GTCS, the differentiation from secondarily generalized seizures is of particular importance and, therefore, only the patients who had a complete clinical evaluation with a documented seizure phenomenology were included in studies II and I. Evidence against secondarily generalized epilepsy include: an objective description of their seizure semiology by relatives and medical personnel, the age at seizure onset, the absence of lateralized abnormalities on MRI, and the presence of bilaterally synchronous SWD in EEG recordings.

All the patients had a normal routine MRI scan of the brain. JME patients had late childhood or teenage onset of awakening myoclonic jerks, most often in the upper, but sometimes also in the lower extremities. Most of the patients had a history of GTCS in addition to myoclonias.
Patients were treated with sodium valproate, lamotrigine, carbamazepine, leviteracetam, and/or phenytoin.

Patients included in study IV had MTLE. MTLE was diagnosed independently by two senior neurologists (Ivanka Savic, Per Lindström), based on the International Classification of the Epileptic Syndromes (1989). In the thirteen patients with left MTLE (age 23-59, seven women, education 13 ± 2) the age at seizure onset was 13 ± 7 years and the duration of epilepsy was 27 ± 9 years. The age at seizure onset in the ten patients with right MTLE (age 23-56, four women, education 13 ± 2) was 12 ± 4 years and the duration of epilepsy was 22 ± 9 years. The clinical manifestations included stereotyped seizures with epigastric sensation followed by lip smacking, staring, masticatory automatisms, and impaired consciousness. Four patients had febrile convulsions. Apart from epilepsy, the patients were healthy and their treatment was

restricted to antiepileptic drugs (carbamazepine, valproate, topiramate, lamotrigine, levetiracetam, gabapentine).

Sixteen patients underwent [^{18}F] FDG PET, (with thirteen showing a regional hypometabolism in the epileptogenic region). Eight patients were investigated with a ligand-PET ([^{18}F] Flumazenil and/ or [^{11}C] WAY 100635). [^{18}F] Flumazenil is the [^{18}F] analogue to [^{11}C] Flumazenil (Savic, Persson et al. 1988; Ryzhikov, Seneca et al. 2005), whereas [^{11}C] WAY 100635 binds to the 5-HT$_{1A}$ receptor binding (Savic, Lindstrom et al. 2004). The two latter PET methods showed unilateral mesial temporal lobe reductions on the epileptogenic side in all investigated patients. The routine clinical investigation included long-term video EEG recordings of at least four habitual seizures. Ictal EEG telemetry was carried out using the 10-20 systems. In fourteen of the patients this included sphenoidal electrodes. Three patients had subdural recordings. The subdural plates were placed under the mesial, lateral temporal, and inferior frontal lobes. Thirteen patients were diagnosed with left sided MTLE and ten with right-sided MTLE. Ten of the patients had hippocampal sclerosis and/or atrophy on the side of seizure onset. None had bilateral hippocampal changes. One patient had cortical dysplasia, and one showed tissue defect (secondary to cerebral hemorrhage) in the left temporal lobe. In one patient, we detected increased T2 signal in the left insular cortex, and in another patient a temporal lobe ganglioglioma. The remaining nine patients had normal MRIs. Handedness was tested in all subjects (Oldfield 1971). All the subjects were right handed, except for one patient with right MTLE and three with left MTLE who were ambidextrous.

3.2 Controls

The control group (study I) for structural brain imaging consisted of thirty-eight right-handed healthy volunteers (age 29±8 years, twenty females). Ten of the controls were also examined with MRS. All control subjects underwent routine MRI of the brain. None of them were on medication at the time of the study, and none had a family history of epilepsy or other neuropsychiatric disorders.

The control group (study II) consisted of fifty-two right-handed healthy volunteers (30 males, 22 females, age 29±8 years). None of the volunteers were taking medication at the time of the study. There was no family history of epilepsy or other neuropsychiatric disorders. Therefore, these controls were not investigated with EEG.

The control group for the PET study (study III) consisted of ten men (age 30 ± 6 years) who had no medication, denied nicotine consumption, and whose MRI of the brain was normal. Because the controls for the PET study were not age and sex matched, a separate control group was used for the neuropsychological evaluation. The control group for the neuropsychological tests (study III) consisted of 3 women and 9 men (age: 36 ± 11 years, education: 13 ± 2 years) and was age, education, and gender matched to the JME group. All the controls were deemed to be healthy.

The control group (study IV) consisted of twenty-one right-handed, non-smoking healthy subjects (age 20-28 years, eleven women) with no heredity for epilepsy or other neuropsychiatric disorders. They had normal results in routine physical, blood and urine examination, a normal MRI of the brain, and no on-going medical treatment. No EEG recordings were performed on the controls.
All the groups were matched with respect to age and gender.

3.3 Magnetic resonance spectroscopy

Methods used to assess the development of cerebral alterations need to be quantitative, reliable, reproducible, and safe.

Magnetic resonance spectroscopy imaging (MRS), is a non-invasive method to assay various classes of cerebral metabolites from neurons and glial cells. Whereas MRI depicts the spatial distribution of protons of water, MRS detects various molecules present at concentrations on the order of mM (millimolar). The method is based on the detection of signal intensity vs frequency. This signal provides information about the biochemical content of a tissue. The concentration of water macromolecules (phospholipides, proteins, DNA, RNA), metabolites, and neurotransmitters such as acetylcholine, norepinephrine, DA and serotonin can be measured in the brain. The nuclei of many atoms, such as ^{1}H, have magnetic properties. When a radio frequency (RF) pulse of a particular frequency (Larmor frequency) is transmitted into a nuclear spin under a static magnetic field, the nuclear spin resonates and absorbs energy (Kato, Inubushi et al. 1998). When the spin returns to its previous state, it emits electromagnetic waves known as free induction decay (FID). This process is referred to as relaxation. The concentration of nuclei is related to the intensity of FID or echo observed. Generally, many FID or echoes must be averaged to obtain enough signals for a quantitative spectroscopic analysis of the sample. FIDs, or echoes, are averaged with a specific interval of repetition time (TR). Usually, the TR is set comparable to the T_1 relaxation time. Under this condition, the signals are partly saturated and the signal intensity is decreased, which complicates the interpretation of data. The small difference in resonance frequency depends on the position of the nuclei in a molecule. The chemical shift enables us to discriminate different molecules containing the same nuclei.
To obtain MR signals from a certain region of the brain, the signal localization method is used. Obtaining MR spectra from FID, or echoes, requires many kinds of data processing (Kato, Inubushi et al. 1998). It was suggested that MR spectroscopy may be more sensitive than regular MRI for detecting abnormalities, such as tumor tissue, MS plaques, however, the test-retest reliability of MRS is less proficient (Duncan 2002).

A few previous studies have described abnormal levels of NAA in the frontal lobes (Savic, Thomas et al. 2000; Simister, McLean et al. 2003) and thalamus (Bernasconi, Bernasconi et al. 2003; Mory, Li et al. 2003; Savic, Osterman et al. 2004) using MRS. The impaired level of NAA may be an effect of excitotoxicity, possibly induced by Glu. Assuming that Glu regulation may also be impaired in IGE patients, we conducted an MRS study by analyzing the combined levels of Glu and Gln (denoted as Glx = Glu + Gln) in parallel with levels of NAA. The differentiation between Gln and Glu at 1.5 Tesla is quite complicated due to the similarities of these compounds. The Glx value can be detected because the fitting error of the sum is much smaller than those of the single compounds. Due to time limitation and application of single voxel MRS, only the thalamus and the occipital cortex (serving as a reference region) were examined with this method. In parallel, voxel-based morphometry of the thalamus was performed to evaluate whether a possible NAA reduction could be attributed to cell loss.
A number of methods can be used to quantify of the observed metabolite peaks. We conducted an MRS study by choosing volumes-of-interest (VOIs) positioned on the T2-weighted images as previously described (Pouwels and Frahm 1998). The average VOI size was 4.1 ml in the thalamus and 7.2 ml in the occipital cortex. Due to time constraints, only the right thalamus was investigated. A stimulated echo sequence (STEAM) was used for single volume MRS with a short echo-time (TE = 30 ms) and a

long repetition time (TR = 6 s) to reduce relaxation weighting. In accordance with our group's previous work (Savic, Lekvall et al. 2000; Savic, Osterman et al. 2004), sixty-four single acquisitions were corrected for effects of residual eddy currents and patient motion (Helms and Piringer 2001) and analyzed by a linear combination of model spectra (LCModel 5.2-2). Concentration estimates in units of mM (millimole/litre) were corrected for coil loading, B1 inhomogeneity, and for partial volume of cerebro-spinal fluid (Helms 2000).

LCModel is an automated, user independent, curve fitting routine for the quantification of metabolite concentrations from *in vivo* MR spectra (Provencher 1993). The program uses a library of spectra at calibrated concentrations of each individual metabolite. This allows discrimination between metabolites with partly overlapping signals by exploiting spectroscopic information from resonances at different frequencies. LCModel was used to quantify the *in vivo* proton MR spectra. Gunther Helms performed the quantification. The advantages of this method are: that it is fully automated, non-interactive, and reproducible. The method was successfully used in several studies, including investigations of epilepsy (Woermann, McLean et al. 1999; Simister, McLean et al. 2003; Jang, Lee et al. 2005).
The methodological error of the Glx concentration estimate is mainly due to low signal-to-noise ratios (SNR) and strong coupling effects. These errors could have masked putative correlations between NAA and clinical parameters.

3.4 Magnetic resonance imaging

Structural images were acquired on a GE 1.5 T clinical MRI system. The MRI protocol consisted of axial T2 weighted Fast Spin Echo (FSE) images and axial T1 weighted 3D spoiled GRASS images (SPGR). An experienced neuroradiologist concluded that none of the subjects showed morphological abnormalities of the central nervous system.

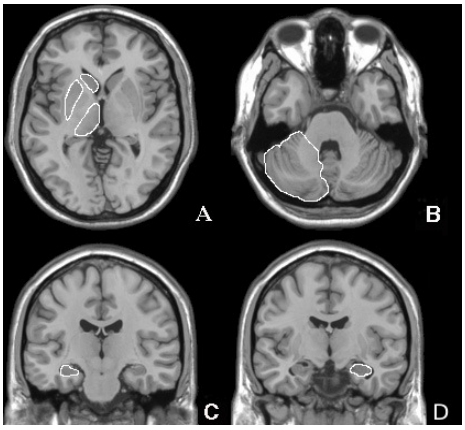
The MRI protocol consisted of axial T2-weighted fast spin echo (FSE) images (effective TE = 56 ms, TR = 2500 ms, FOV = 24 cm, 23 slices of 3-mm thickness), and axial T1 weighted three-dimensional spoiled grass images (SPGR, TE = 7 ms, TR = 23 ms, FOV = 24 cm, flip angle 50°, 156 slice partitions of 1.2 mm thickness, 2 NEX).

To evaluate possible hippocampal pathology (study IV), additional coronal T2 images, coronal fast inversion recovery images (flip angle 3°, 16 sections), and coronal three-dimensional SPGR (2, 5/0, 60 slices, 256 x 256, 1 NEX) were acquired perpendicularly to the long axis of the hippocampus. An experienced neuroradiologist concluded that none of the subjects showed morphological abnormalities of the central nervous system.

3.4.1 Volumetry

MR volumetric measurements of subcortical structures have recently gained importance in the evaluation of healthy subjects (Szabo, Xiong et al. 2001; Szabo, Lancaster et al. 2003) patients with schizophrenia (Gur, Maany et al. 1998), and patients with epilepsy (DeCarli, Hatta et al. 1998; Dreifuss, Vingerhoets et al. 2001). MRI structural analysis of patients with epilepsy has revealed a number of anatomical abnormalities, which are very often subtle, and therefore necessitate a careful evaluation.

The main advantage of manual volumetric analysis compared to automated procedures, is that the latter is more dependent on the quality of the MRI images. Furthermore, the capability to distinguish between gray and white matter is better using the manual technique. For the manual technique, ROIs are drawn and checked in a 3D view.



Two trained investigators, uninformed about the identity of the subjects, manually delineated volumes of interest on T1 images. ROI were drawn for the thalamus (Study I and II), amygdala, hippocampus (Study II and IV), cerebellar hemispheres (Study II), putamen, and the caudate (Study III).

Figure 4. Anatomical landmarks for: (A) caudate, putamen, thalamus, (B) cerebellum, (C) hippocampus (D) amygdala.

We were mostly interested in structures involved in motor control. However, amygdala and hippocampus were of interest since these areas have been previously involved in the generation of generalized seizures if kindled (Study II). Amygdala and hippocampus were also used to assess volumetric changes of these structures (Study IV) in patients with MTLE. The anatomical landmarks and boundaries used for the volumetric method were based on the morphological description and tracing guidelines previously described (Duvernoy Henri 1991; Watson, Andermann et al. 1992; Kettenmann, Hummel et al. 1997; Raz, Rodrigue et al. 2003) (Figure 3). Quantitative volumetry was performed using the MRIcro software package (Rorden and Brett 2000). All the volumetric data have been assessed in relation to the total brain volume. Calculation of the total brain volume was problematic. Most available software uses an automatic stripping algorithm that works on the interim of the image intensities and often misclassifies CSF or gray matter. We quantified the total brain volume by segmenting the images with statistical parametric mapping software (SPM) (Wellcome Department of Cognitive Neurology, London, http://www.fil.ion.ucl.ac.uk/spm). After determining the volumes of gray and white matter, brain volume was calculated by summing up the compartmental volumes.

However, the volumetric approach has its limitations. First, it is a time consuming method when performed manually. This also implies a biased approach. In order to minimize bias, it is of essence that the investigator is unaware of the subject's identity and group. Furthermore, established landmarks must be used in delineation of structures and the same investigator must perform the analysis in all subjects. Depending on the quality of images it is sometimes difficult to determine the boundaries of structures. For instance, delineation of the hippocampal volume is difficult since subjective judgment is inevitable when delineating the anterior and posterior boundaries. Even with 1.2 mm thick slices used in our studies, it is difficult to distinguish the anterior hippocampus from the posterior amygdala.

Therefore, our approach was to employ both a manual and automatic volumetry technique and compare and validate the volumes of the subcortical structures in the same subjects.

3.4.2 Voxel based morphometry

MRI has been useful in revealing subtle structural brain abnormalities in epileptic patients. Studies I and II used the quantification of gray, white matter, and CSF based on image voxels. This method is termed voxel-based morphometry (VBM) and is defined as a voxel-wise comparison of the local concentration of gray matter between two groups of subjects (Ashburner and Friston 2000). The usual procedure involves spatially normalizing all images into the same stereotactic space and segmenting the normalized images into three tissue classes - gray, white, and CSF. Furthermore, all segmented images are smoothed using a convolution with a Gaussian kernel. The method is fully automated, operator-independent, and avoids potential investigator bias (Ashburner and Friston 1999).

We applied an optimized VBM protocol (Good, Johnsrude et al. 2001) in Study II. This method has an improved normalization, improved inter-subject registration, and creation of own templates for gray, white, and CSF. The warping step has its limitations as it uses a standard T1 template. Areas cannot be expected to overlap precisely when comparing two or more subjects. However, by enrolling a reliable number of subjects, inferences about the structures can be made. The normalized images were then segmented into gray matter, white matter, and CSF.

Analysis of subject differences between aligned voxels in the data sets uses a conventional Gaussian statistic and the output creates statistical significance maps representing the detected differences. Searching for the t-statistic in the differences between different tissue classifications results in regional differences in gray matter, white matter, and CSF.

Since we were particularly interested in regional group differences between the gray and white matter fractions and the CSF fractions, comparisons were performed using the ANCOVA model to correct for the differences in the total brain volume. The interpretation of results is based on the differences in the intensities in the images at the regional level. The observed differences may have several interpretations, e.g the volumetric changes are due to structure changes or morphological changes.

The segmented images were carefully evaluated for possible misclassification. No images were excluded. The t-test showed an extensive cluster in the midline. This can be the effect of gray matter fraction misclassification. Although, plotting a regressor did not reveal outliers (none of the values were outside lowest and highest quartiles). The application of a specific mask in this region yielded similar results and is described in Study II.

In addition to the common manual evaluation of volume changes, VBM has provided an additional potential to independently detect significant structural differences in subcortical and cortical gray matter. This strengthens the findings from the manual volumetry work and helps validate our results.

3.5 Positron emission tomography

3.5.1 PET procedure

Positron emission tomography (PET) it is an imaging technique, which produces a three-dimensional image, or map, of the functional processes of the brain. PET involves the acquisition of physiologic images based on the detection of radiation from the emission of positrons. After injection of a tracer compound labeled with a positron emitting radionuclide, the head of the subject is placed within the field of view (FOV) of a number of detectors capable of registering incidents from the positron's elucidation annihilation of gamma rays. Positron-emitting radioisotopes have an excess of protons. This unstable state ends once the excess positron is emitted. It travels a certain distance, 1-3 mm, before it undergoes annihilation with an electron creating a pair of collinear photons that can be detected by the detectors surrounding the subject. The detector electronics are linked so that two detection events unambiguously occurring within a certain time window may be called coincident and thus be determined to have come from the same annihilation. These "coincidence events" can be stored in arrays corresponding to projections through the patient and reconstructed using standard tomographic techniques. The resulting images show the tracer distribution throughout the brain of the subject.
The combined use of PET and MRI allows for image co-registration so that detailed quantitative data on the function of the brain with PET can be associated with specific regions by detailed structural images obtained from MRI (Turner and Leigh 2000). Broadly, there are two types of PET study.
PET ligand/tracer studies use ligands that bind to specific receptors, for example [11C] PE2I for DA receptors, used to evaluate in detail the structural and functional integrity of neural systems. In study III we use a [11C] PE2I ligand which has a high affinity DAT compound N-(3-iodoprop-2 E-enyl)-2beta-carbomethoxy-3beta-(4-methylphenyl) nortropane. [11C] PE2I is has high affinity, specificity, and selectivity to central DAT specifically located in striatum and midbrain.
PET activation studies with tracers such as radiolabelled water [15O] H2O identify changes in cerebral blood flow whilst performing a particular task. Under normal circumstances, regional cerebral blood flow can serve as a reliable indicator of changes in neuronal activity. The short half-life (about 2 minutes) of [15O] H2O allows both successive measurements of cerebral blood flow in a single session and the acquisition of experimental and control images with the same subject. Development of functional imaging led to functional magnetic resonance imaging (fMRI), which has some disadvantages when compared with PET in neuroimaging of the olfactory system.

Advantages of using [11C] PE2I in study III:

◊ Is the only way to investigate *in vivo* the dopaminergic system since DAT is a presynaptic DA marker.

◊ [11C] PE2I is the only ligand today that has the capacity to bind to DAT not only in the striatum, but also in the midbrain.

Disadvantages of using [11C] PE2I:

◊ Limitation of number of subjects included in the study due to expensive costs of the experiments.

◊ Long time acquisition of PET images (90 minutes). The previous two studies using this ligand (Jucaite, Fernell et al. 2005; Jucaite, Odano et al. 2006) did not observe a decay in ligand binding after 60 minutes in the striatum (See example of time

activity curve for 90 minutes). The control group used in study III was previously scanned for 60 minutes and the calculation of binding potential for both groups was based on 60 minutes acquisition time. However, arterial blood was sampled during the 90 minutes acquisition time and will be used in a separate methodological study to investigate whether a 90-minute acquisition is preferable for calculating the DAT binding with this ligand.

We decided to use PET rather than fMRI for study IV for several reasons:

◊ PET allows the assessment of neuronal activation across the entire brain simultaneously.

◊ PET offers a good visualization of activation in the mesial temporal and orbitofrontal cortex where primary and secondary olfactory regions reside. These structures are confounded with artefacts in fMRI.

◊ Odors can be administrated directly to subjects without additional equipment (olfactometer).

◊ Consistency with our previous studies (Savic and Gulyas 2000; Savic, Gulyas et al. 2000; Bengtsson, Berglund et al. 2001; Savic, Berglund et al. 2001; Kovacs, Gulyas et al. 2004; Savic and Berglund 2004; Savic, Berglund et al. 2005; Hillert, Musabasic et al. 2007).

The major disadvantages for activation PET studies are:

◊ Exposure to radioactivity. In study IV, the number of scans for each patient was limited to six because patients have been examined previously with other radioligands.

◊ PET analysis cannot provide information on the individual level, which is important in epilepsy, since even patients with identical syndromes may differ in their functional deficits.

◊ The spatial resolution is worse than in fMRI.

◊ An even greater limitation of PET activation imaging is that tracer kinetics and the relatively poor counting statistics of PET tomographs necessitate that each experimental measurement be integrated over a time period of about 40 seconds. Considering that the cognitive processes we must investigate, such as olfactory activation, it is apparent that the temporal resolution of PET is several orders of magnitude slower than the neuronal events of interest. This means that PET experiments necessitate the repetition of the tasks.

3.5.2 PET data analysis - activation study

PET data was acquired with a Siemens ECAT Exact HR 47 (Siemens/CTI, Knoxville, Tennessee). All PET measurements were interictal, and evaluated by the neurologists present during each experiment.

3.5.2.1 PET data processing

The images were co-registered (Woods, Cherry et al. 1992), smoothed with a 10 mm-Gaussian filter, and spatially normalized to the human brain atlas brain (Roland 1994). The cerebral blood flow was calculated according to Fox et al. (Fox, Mintun et al. 1984) during each scan (80 seconds acquisition). The images were realigned, normalized to the arbitrary global mean of 50 ml/100g/min.

The statistical analyses were performed using Matlab 6.5.1 (Math Works, Natick, MA, USA), SPM2, and the MarsBaR program (Bret 2002) installed on a Unix based system. First-level single subject statistical contrasts were created using the general linear model. Statistical parametric maps were generated using t-statistics and contrasts were

performed, activation conditions vs baseline condition, to identify the activated regions. Second level, random effect analysis (mixed effect analysis) exploratory whole brain contrasts, within the group and between the groups were performed. Statistical images were thresholded at $p<0.001$ and sometimes $p<0.01$ uncorrected (FDR- false discovery rate study II < 0.01).

3.5.2.2 Olfactory stimulation

All patients were scanned interictal. [^{15}O] H2O was used as the PET tracer. Subjects were blindfolded throughout all the scans (with eyes closed, and ears plugged).
Three different conditions were applied: 1. Passive, birhinal, smelling of the four familiar odors (FAM), 2. Passive, birhinal, smelling of the four unfamiliar odors (uFAM), 3. Passive, birhinal, smelling of odorless air (AIR), which also served as the baseline condition.

During odor smelling, each of the four odorants were presented for 15 seconds, from a glass bottle at a distance of about 10 mm from the nostrils with an interval of five seconds of breathing the air in the scanner room in between. Each presentation started five seconds after the bolus tracer injection and was indicated by touching the subject's right index finger. One PET session lasted 80 seconds (Savic and Berglund 2000; Bengtsson, Berglund et al. 2001; Savic, Gulyas et al. 2002). During AIR, the identical on-off mode was applied, but the stimulus was odorless air. The order of conditions was counterbalanced and randomized across subjects and scans.

The general instruction to subjects was that they would smell an odor or odorless air and to not sniff. The subjects were also prompted to concentrate on the perception of the presented item without trying to categorize or label it. Subjects were not aware of the identity, the category of odor, nor the type of condition (odor or base line). Following each scan, the subjects were asked whether they had smelt any odor. After all the scans, each odor was presented again and the subject was asked to score it for familiarity, pleasantness, intensity, and irritability using a visual analogue scale (Savic and Berglund 2000). Odor thresholds were tested prior the scanning.

3.5.2.3 Functional connectivity

Analysis of covariance with PET activation data is used to explain functionally connected brain regions during a task or rest. The region of interest was left and right piriform cortex. Average rCBF of all voxels in the seed region was then used as a covariate of interest in a whole-brain statistical parametric analysis. These activity values were used as condition specific covariates of interest regressed over the whole brain in the general linear model using SPM2, This produced a functional connectivity map. The covariates were interacted with conditions and centered to the condition mean.

3.5.3 PET data analysis - binding study

The binding potential (BP) of [^{11}C] PE2I was calculated using manually delineated ROIs and a simplified reference tissue model (SRTM) (Lammertsma and Hume 1996) using the cerebellum as a reference region, as previously described (Jucaite, Fernell et al. 2005). Several regions of interest have been used: dorsal striatum, substantia nigra, midbrain that contains mostly dopaminergic cells –ventral tegmentum and substantia nigra, and midbrain that included both coliculli (ROI used for adhoc analysis). Distribution of [^{11}C] PE2I is illustrated below in a control and a JME patient (Figure 5). The data are corrected for decay and normalized for injected radioactivity.

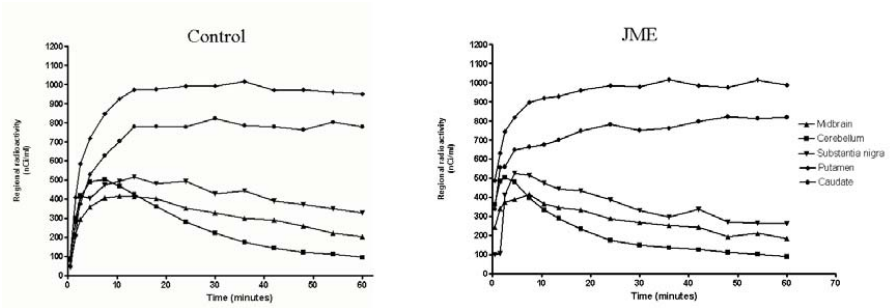


Figure 5. Time activity curves.

The SRTM is an alternative method for the estimation of binding potential (BP) from reversible binding studies. This model does not require, or use plasma samples, but instead uses a reference region with low or non-existent specific binding. The cerebellum was described to lack DAT and therefore no DAT binds in the cerebellum (Hall, Halldin et al. 1999). The parameters R_1 (ratio of the K_1 values of regions of interest and reference tissue), k_2, k_3, and BP (k_3/k_4) can be estimated using nonlinear fitting (Cunningham, Hume et al. 1991).

$$C_t(t)=R_1 \bullet C_r(t)+ k_2 - R_1 \bullet k_2/(1+BP) \bullet C_r(t)\exp -k_2 \bullet t/(1+BP)$$

C_t is the radioligand concentration in the tissue (region of interest). C_r denotes the radioligand concentration in the reference tissue (cerebellum). This model has some advantages over the Logan plot: dynamic study can be used from the beginning with no need to wait for any equilibrium or search for a linear phase. Depending on the input data, it can be used to calculate (with arterial input), or using the slope and intercept, - 1 to give BP (with reference region, for example). The slope assumes a linear regression line and equilibration of the ligand. A previous study (Jucaite, Odano et al. 2006) indicates that the equilibrium was reached in part of the subjects and SRTM was used to calculate the BP. The distribution volume in the cerebellum was calculated based on arterial input function in our groups. Preliminary data are presented in the results section.

SRTM can be used when a two compartmental model could reasonably describe the kinetics of the tracer in the tissue (Lammertsma and Hume 1996). However, if the reference region has specific binding, the BP will be underestimated (Gunn, Lammertsma et al. 1997).

4 RESULTS AND DISCUSSION

4.1 Changes of thalamic NAA and Glx concentrations (Study I)

Voxel based morphometry (VBM) and MR volumetry were employed to investigate possible thalamic structural and neurochemical alterations in patients with IGE. The major finding – a reduction of thalamic NAA accompanied by an elevation of Glx concentrations, and reduced thalamic gray matter fractions – support this assumption.

The thalamic Glx concentrations were found to be significantly higher in IGE patients than in controls (12.2±2.6 mM vs. 8.9±4.1 mM, p=0.0022), while the thalamic concentrations of NAA were lower (9.9±1.0 mM vs.10.7±0.9 mM) in controls, (p=0.017). No significant differences were found in the occipital cortex. This supports our hypothesis.

Patients with more than 10 GTC seizures during their lifetime (22 patients) had lower thalamic NAA concentrations than those that had experienced less than 10 seizures. The results of the VBM analysis, which took into account the number of GTCS seizures per lifetime, did not differ between subjects.

Neither the MRS, nor volumetric or VBM analysis showed any significant difference between patients with JME and GTCS (two-sided unpaired t-test with unequal variances, $p<0.05$).

Several putative mechanisms could explain the chemical shifts found in Study I

MRS is a valuable technique in assessing the metabolic content in several pathological conditions, including IGE. The purpose of the current study was to examine if patients with IGE had signs of thalamic affection; gray/white matter disorganization, tissue atrophy or metabolic changes. Based on previous data (Meencke and Janz 1984; Bernasconi, Bernasconi et al. 2003; Mory, Li et al. 2003; Savic, Osterman et al. 2004) the major hypothesis was to observe whether reduced NAA would be accompanied with an increase in Glx. The thalamus was included because there is data supporting thalamic dysfunction in IGE. This study could not offer separate information about Glu or Gln content in the thalamus. Neither could we determine whether the content of Glx was located in the pre- or postsynaptic part, or in the synaptic cleft.

What could possibly determine the elevation in Glx?

One suggested mechanism for the elevated Glu concentration in epilepsy is the reduction in extracellular Glu clearance from the postsynaptic cleft by the Glu transporters. Glu transporters have an important role in the maintenance of low extracellular Glu concentrations (Jabaudon, Shimamoto et al. 1999). This clearance limits the duration of the synaptic message (Diamond and Jahr 1997), preventing extrasynaptic diffusion (Asztely, Erdemli et al. 1997), and indirectly modulating neurotransmitter release (Maki, Robinson et al. 1994). Decreased Glu transporter levels have been assessed prior to seizure occurrence in the cortex and the thalamus (Dutuit, Touret et al. 2002). The results suggest a possible involvement of Gln and Glu transmission in this region, and could be determined by changes in both neurons and/or glia.

Another possible mechanism could be that high Glx concentrations are brought about by an excessive Glu release resulting from continuous epileptiform discharges. The concentration of Glu is increased significantly after an epileptic seizure due to transcranial magnetic stimulation (Michael, Gosling et al. 2003). Furthermore, studies with microdialysis show increased ictal and postictal extracellular Glu (During and Spencer 1993; Neppl, Nguyen et al. 2001).

Impaired Glu clearance and excessive Glu release are two suggested mechanism explaining the elevated thalamic Glx concentration found in our study. These mechanisms are not mutually exclusive, and can exist in tandem in epilepsy. The present data add new information for understanding the underlying pathophysiology of IGE, and suggests that an elevation in Glu and/or Gln levels is a common characteristic of the epileptogenic tissue.

The other finding, the decrease of NAA in the thalamus, was previously reported in several studies (Savic, Lekvall et al. 2000; Bernasconi, Bernasconi et al. 2003; Mory, Li et al. 2003; Savic, Osterman et al. 2004; Haki, Gumustas et al. 2007). NAA is the most abundant metabolite visible using MRS. NAA is an amino acid that binds poorly to macromolecules and proteins, and is located in neurons (cytoplasm, proximal dendrites, axons) (Birken and Oldendorf 1989). Thus, NAA is a neuronal marker. It is widely accepted that a decrease in NAA reflects neuronal damage, neuronal loss, or altered mitochondrial metabolism (Petroff, Pleban et al. 1995).

To conclude, the reduced gray matter fractions and reduced thalamic volumes, along with the elevated fractions of Glx and decreased NAA levels, suggest thalamic atrophy in IGE patients, possibly as a result of excitotoxicity.

4.2 Volumetric cortical, thalamic and subcortical changes

No asymmetries in volumes were detected between the right and left side in study I-III.

4.2.1 Study I

The volumetric analysis of the thalamus in the mixed IGE population yielded significant reduction ($p<0.001$) comparing thalamic volumes in patients and controls. There was also a reduction seen when comparing total brain volumes.
The significant reduction of gray matter in both thalami, and the increase of white matter in the right thalamus were detected with VBM. The peak was located in the right ventro-medial thalamus.

4.2.2 Study II

Volumetric analysis of the thalamus, caudate, putamen, cerebellum, hippocampus, and amygdala was performed. Structures involved in motor control were of particular interest. We also investigated the hippocampus and the amygdala because these structures are susceptible to hypoxic damage (Gale 1992).

Patients with GTCS had a significantly smaller caudate, putamen, cerebellum, and thalamus than controls. Although they also had a smaller whole brain volume, $p=0.0008$; the VOI/whole brain ratios remained reduced for patients in the caudate,

putamen, and cerebellum. The thalamus/whole brain ratio did not pass the level of significance. No volume differences were observed in the hippocampus and the amygdala.

No correlation was found between the duration of seizures, age at onset, seizure frequency, number of lifetime seizures and the respective VOI/whole brain ratios. There were no clinical parameters that correlated with the estimates of global gray matter, white matter or CSF.

The groups were also compared with respect to total gray matter, white matter and CSF fractions. Patients showed significantly decreased gray matter fractions (p=0.0007), whereas their overall fraction of CSF was significantly increased (p=0.0003).

In addition, VBM analysis revealed regional gray matter reductions bilaterally in the middle and inferior temporal gyri, the fusiform gyri, the insular and cingulate cortex, the superior and medial frontal gyri, the precentral and postcentral gyri, and in the precuneus and inferior parietal lobuli. Patients also showed subcortical reductions of gray matter, which were located bilaterally in the thalamus, and in the quadrangular and semilunar lobuli of the cerebellum. The degree of reduction was uneven, and most pronounced in the medial frontal gyrus, the ventral anterior, and ventral lateral thalamic nuclei.

In parallel we found an elevated fraction of CSF in the frontal lobes with a maximum within the pre- and postcentral gyri.

The VOI and VBM analyses are based on different principles and should be used in tandem. They may generate disparate results in regions in which the structural landmarks are well defined, but the gray-white matter contrast is poor. A typical example is the caudate and putamen where the voxel-averaged, landmark based VOI-analysis is more reliable than the signal based VBM measurements. The VBM did not show any group difference in these particular structures, whereas the respective volumes were reduced in GTCS, which may be explained by limitations of the methods applied. To investigate this apparent inconsistency, we carried out a post hoc random effect analysis of the gray matter images (with total gray matter fraction as a covariate), using a reduced search space (rectangular explicit mask with Talairach's coordinates x = –36 to +36, y = +22 to -40, z = –12 to +26). The VBM analysis then showed an area in the putamen with gray matter reduction, which was significant on the left side (x= -28; y= –13; z= -4; p=0.027, z-score 3.9, cluster size 4.0 cm^3).

4.2.3 Study III

The volume of the putamen was reduced in patients (p = 0.036), as was their total brain volume (p = 0.014), but the putamen/brain ratios were similar. The caudate volume, absolute as well as relative, was similar in patients and controls. Unpublished data in 33 JME patients and 62 controls indicated decreased volume of the putamen (p = 0.001), the caudate (p = 0.0053) and total brain volume (p = 0.0017) in patients. However, no significant difference between patients and controls was detected after correction for the total brain volume [putamen/total brain ratio p = 0.711, caudate/total brain ratio p = 0.6767].

Preliminary VBM data from JME patients indicate increased gray matter fractions located in the frontobasal region, whereas a decrease of gray matter was found in the left thalamus, inferior temporal gyri and inferior semilunar lobuli of the cerebellum.

4.3 General discussion of volumetric and neurochemical changes from Studies I-III

Are the anatomical and metabolic changes of the thalamus specific for JME and GTCS?

We detected elevated Glx and decreased NAA levels in the thalamus of JME and GTCS patients, adding to the theory that the underlying mechanism of the generalized seizures is the abnormal thalamocortical circuitry (described in detail in the Introduction, subchapter 1.3.1). Thalamic structural changes were found in Studies II and I. Furthermore, subcortical gray matter reductions in our patients were confined to the ventral anterior and ventral lateral nuclei of the thalamus (Study I and II). This anatomical framework thus provides a basis for major involvement of the affected regions in the ictal behavioral manifestations of JME and GTCS. This is congruent with recent reports concerning thalamic changes in generalized epilepsy (Savic, Pauli et al. 1994; Bernasconi, Bernasconi et al. 2003; Mory, Li et al. 2003; Salek-Haddadi, Lemieux et al. 2003; Aghakhani, Bagshaw et al. 2004; Savic, Osterman et al. 2004).
In CAE thalamic atrophy has been reported. The thalamic atrophy has been explained with excitotoxcicity, or as a primary defect that causes the pathology (Chan, Briellmann et al. 2006). The thalamic alterations are the common findings throughout the studies, supporting the thalamic implication in IGE.

Are the changes similar in GTCS and JME?

Localized, rather than diffuse changes have been previously described in JME, such as frontal cortical alterations (Woermann, Free et al. 1999; Savic, Lekvall et al. 2000; Savic, Osterman et al. 2004). Our preliminary VBM data from JME patients indicate an increase of gray matter in the frontobasal region, replicating the previous results from Cendes group (Betting, Mory et al. 2006). However, the Duncan group found increased gray matter fractions in mesiofrontal regions in JME patients; regions in which we found a decrease of gray matter in GTCS. In GTCS patients we also found a decrease of gray matter in the superior and medial frontal gyri, parietal and temporal cortices, and the cerebellum. In CAE, the changes also involve the thalamus, superior and mesial frontal cortical regions, but also temporal and parietal regions (Salek-Haddadi, Lemieux et al. 2003; Aghakhani, Bagshaw et al. 2004). This suggests differing involvement of the cortical regions in IGE. The common finding between our study and previous studies is the chemical and volumetric alterations in the thalamus in both JME and GTCS. The cortical and subcortical alterations are somehow different across the studies and suggest that there might be syndrome specific changes in IGEs.

Could the observed changes be the effects of excitotoxic alteration?

Whereas the motor cortex sends excitatory output to the thalamus, the basal ganglia and cerebellum, the feed back from the basal ganglia and cerebellum to the cortex is mainly inhibitory (Purves and Williams 2001). Such an organization of the excitatory and inhibitory neuronal connections between the motor cortex, thalamus, basal ganglia and cerebellum provides a substrate for a *vicious* loop in which seizure activity may lead to

excitotoxic lesions in the striatal and cerebellar targets to the synchronously firing of cortical pyramidal cells, resulting in an impaired inhibitory feedback from these structures, and a facilitation of further seizures (Savic, Persson et al. 1988). Excitotoxic lesions, an expected result of hyperexcitability, would be localized in the specific networks supporting seizures, rather than distributed to the areas particularly susceptible to diffuse hypoxia or diffuse excitotoxicity, for example the hippocampus. It is, therefore, of special interest that we found no hippocampal changes in GTCS, whereas reductions in gray matter and increases in CSF fractions were detected in the premotor cortex. However, whilst supported by certain animal studies (McBean and Roberts 1985; Sloviter and Dempster 1985) the idea of seizure related excitotoxic lesions finds no direct support in the present study, as no quantitative relationship was found between seizure load and the observed regional changes (possibly because of the narrow range in the number of seizures during the lifetime).

Could the presented data be a result of broad changes during the development of cellular and molecular mechanisms?

An alternative explanation for our findings is developmental abnormality. It was proved that changes of the environment at the molecular and cellular level during migration of cells from the ventricular zone result in heterotopic cells (Rakic 2000). This could be due to immature inhibitory/excitatory mechanisms. According to Ben-Ari et al, the migration disorders may be generated by environmental factors that include the effects of GABA-acting agents (Ben-Ari 2006). Although we did not find any macroscopic developmental abnormalities, microscopic abnormalities such as heterotopias may still have been present in our patients but remained undetected by our methods. It has been reported that cortical dysplasia may be detected by VBM as dispersed voxels of gray matter in the white matter regions (Merschhemke, Mitchell et al. 2003). Subtle widespread frontal lobe microdysgenesis has been detected in one study of patients with IGE (Meencke and Janz 1984) but not in another (Opeskin, Kalnins et al. 2000). The two alternatives (seizure related and developmental abnormalities) are not mutually exclusive, and the observed changes could be an effect of subtle developmental malformations increasing the susceptibility to epileptiform and seizure activity, which, in turn, may lead to excitotoxic lesions.

Can the presented changes be a result of drug treatment?

The majority of our patients were treated with valproate; some with carbamazepine, leviteracetam, lamotrigine, and one with phenytoin. Although valproate has been reported to decrease global cerebral blood flow and glucose consumption (Gaillard, Zeffiro et al. 1996), this treatment has, to the best of our knowledge, not been associated with brain atrophy, with the exceptions being a patient with progressive generalized epilepsy and a reversible cortical atrophy (Straussberg, Kivity et al. 1998) and two patients with a reversible diffuse cerebellar and cortical atrophy (Papazian, Canizales et al. 1995). Idiosyncratic pharmacological effects of valproate are extremely unusual, and it seems unlikely that the present observations were a mere effect of drug toxicity. In addition, carbamazepine and lamotrigine in doses that totally suppress convulsions cause no inhibition in the striatum and at best a 50% inhibition in the brain cortex, and are not associated with induced atrophy (Waldmeier, Martin et al. 1996). Leviteracetam, another drug used to treat our patients, is a relatively new drug, whose mechanism of action is yet unknown. No study associates leviteracetam with brain atrophy. It is of importance that drug naïve JME patients show reductions of rCBF predominantly in the cerebellum, midbrain and thalamus, and an increase in the

frontal regions, suggesting that changes are regional and centered to a network that involves mostly the fronto-thalamo-midbrain structures (Tae, Joo et al. 2007). A more detailed implication of the midbrain in IGE will be discussed further. Thus, it appears unlikely that antiepileptic medication is responsible for the detected changes in our patients.

Even with extensive research in the area of the pathophysiology of epilepsy, it is still unknown which structure is responsible for the generation of a seizure. Our findings support the concept that structural cortical changes may be associated with abnormalities within the mesiofrontal and frontobasal neocortex and between cortical and subcortical structures in IGE.

The present data allow no cause – effect conclusions. Nevertheless, using various independent methods, we add information concerning the existence of morphological changes in the brain in human IGE. Furthermore, the findings of regional changes in selective networks in JME and GTCS patients adds new arguments to the discussion of whether generalized seizures are truly generalized, and calls for a reevaluation of the current diagnostic criteria for IGE.

4.4 Dopaminergic innervation in JME (Study III)

4.4.1 [^{11}C] PE2I binding potential

To assess dopaminergic innervation in JME patients, the [^{11}C] PE2I ligand that binds to DAT was chosen for its unique capacity to bind to DAT in the midbrain and striatum. The DAT presence in the midbrain and striatum was previously not possible to evaluate in vivo. Several regions of interest have been chosen to evaluate the binding potential (BP). The substantia nigra is a very small structure, approximately 6 mm thick.
The substantia nigra, as well as the midbrain ROIs, were delineated on three horizontal sections. None of the groups showed asymmetry in the respective binding potentials, and the group comparisons were based on the mean values for each separate region. As previously reported (Jucaite, Odano et al. 2006) the BP for [^{11}C] PE2I was higher in the caudate and putamen than in the substantia nigra and the midbrain. Reduced BP was also found when using the midbrain ROI, irrespective of how it was defined.
The DAT binding in the substantia nigra was lower in JME patients, as was their BP in the midbrain (p = 0.002 and p = 0.004), (Figure 6).

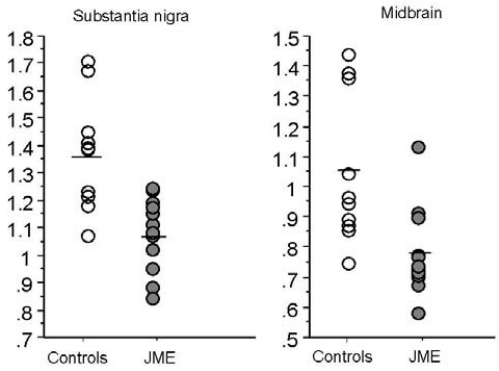


Figure 6. The [^{11}C] PE2I binding potential in the midbrain in controls (empty circles) and JME patients (filled circles). Horizontal lines denote mean values. The binding potential has no units. In two patients the midbrain values were overlapping.

No group differences in volume were detected in the putamen or caudate irrespective of whether the ROI volume and age was employed as a covariate (5.8 ± 0.9 cm^3 patients vs. 6.0 ± 1.1 cm^3 controls in the caudate, and 7.3 ± 1.2 cm^3 patients vs 7.3 ±1.7 cm^3 controls in the putamen). The ROI volumes used for calculations of the BP were similar in patients and controls.

The BP was negatively related to age only in the putamen (patients: R = -0.395, p = 0.024; controls: R = -0.654, n = 10, p = 0.040).
The BP in the putamen was significantly negatively correlated with duration of epilepsy (r = -0.595, n = 12, p = 0.041). No significant correlation was found for the caudate (r = -0.395, n = 12, p = 0.204), substantia nigra (r = -0.355, n = 12, p = 0.257), or midbrain (r = -0.324, n = 12, p = 0.305). Duration of epilepsy was also correlated with age (r = 0.977, n = 12, $p < 0.0001$).

The calculated BP was not correlated with the age of onset, seizure frequency or number of generalized tonic and clonic seizures over lifetime, in any of the ROIs. Correlation was detected only when using the midbrain ROI, which could be attributed to the fact that the midbrain ROI comprises both the ventral tegmental area and substantia nigra.

Our main finding, the decrease of the presynaptic markers of dopamine innervation – DAT (Volkow, Fowler et al. 1996) in the midbrain, suggests that nigrostriatal projections arising from the substantia nigra and mesocorticolimbic projections arising from the VTA (Björklund and Lindvall 1984), send impaired dopaminergic signals to the target regions, mainly the frontal cortex and the striatum. The Semah group (Bouilleret, Semah et al. 2005) has found decreased DA reuptake in the midbrain and in the dorsal striatum in IGE patients. This confirms our results and conclusions concerning impaired signaling to the striatum. It is, however, worth mentioning that all the patients in their study had daily seizures, which was not the case in our patients, who had sporadic seizures occurring 72 hours or more prior to PET investigation. Previous studies in animal models of generalized epilepsy indicated that after seizures the DA (Hiramatsu, Fujimoto et al. 1982), as well as homovanilic acid (the major DA metabolite) (Garelis and Sourkes 1973), are decreased in CBF. DA concentrations seems to be altered in structures involved in the epileptogenesis; DA is clearly reduced in resected tissue of patients with intractable epilepsy (Mori, Hiramatsu et al. 1987). DA seems to have an important role in seizure interruption as the application of DA agonists on D2 and antagonists on D1 receptors have been reported to stop the seizures (Starr 1996). The DA receptors, especially D2, are decreased in the striatum after kindling (Tchekalarova, Sotiriou et al. 2004). The D1 receptors in the substantia nigra are increased post electric-induced seizures (Robertson 1992), which is related to increased motor and seizure behavior.

There is a substantial body of evidence for the antiepileptic effect of DA. It is particularly interesting for the JME patients, who have myoclonias as the predominant type of seizures. Myoclonias are abolished after administration of dopaminergic medication (Greer and Alpern 1977; Obeso, Rothwell et al. 1986). JME patients can also suffer from photosensitivity. In Papio Papio baboons, photosensitivity is accompanied by myoclonias which are suppressed after administration of apomorphine, a dopaminergic agonist (Meldrum 1978). Apomorphine also inhibits the spike and wave discharges induced by photic stimulation in patients with photosensitive generalized epilepsy (Quesney, Andermann et al. 1980). The superior colliculi, the

rostral part of the midbrain, have an important role in visual sensory transmission, besides being connected to the nigra. Therefore, when discussing midbrain involvement in JME, thalamic implication in visual processing together with known thalamic alteration in JME could suggest that the photosensitivity in JME might be related to the dopaminergic innervation. In addition, studies have shown a decrease of rCBF in the midbrain (Tae, Joo et al. 2007). The stimulation of midbrain dopaminergic structures result in suppression of seizures induced by kindling (Veliskova, Garant et al. 1994; Shi, Luo et al. 2006). These data indicate that the DA system is altered in JME. This alteration might be primarily located in the midbrain dopaminergic structure.

What factors could have influenced our findings of low DAT levels in patients?

1. *Age*. As previously shown, DAT levels in the striatum are decreased with age, about 6 to 10 % per decade (Volkow, Fowler et al. 1994; Volkow, Ding et al. 1996). We have used age as a covariate of interest when performing all the calculations for determining the BP in both groups, especially since the control group was younger. It did not highlight any significant difference in the BP of the caudate and the putamen. Before and after controlling for age, the difference between the groups was highly significant in BP. The difference can therefore not be attributed to age effects and in accordance with the report of Leroy et al., (Leroy, Comtat et al. 2007) DAT levels in the midbrain are not dependent of age.

2. *Gender*. Among 12 patients, only three were women. It seems, however, that if anything, inclusion of three women should contribute to increased levels of DAT, since studies indicate either increased (Lavalaye, Booij et al. 2000) or similar levels between sexes (van Dyck, Seibyl et al. 1995). We have repeated the group comparisons after exclusion of the female patients, and the results remained similar.

3. Definition of ROI and selection of the method of analysis. Even though we have used three different ROIs for quantification of DAT in the midbrain, the substantia nigra, midbrain without and with coliculli, all the results were robust and showed significantly lower BP in JME patients compared with controls. We have employed the simple reference tissue model (SRTM), using the cerebellum as the reference tissue. Employment of a reference region can be challenging when comparing controls and patients, and even though no specifically bound [^{11}C] PE2I has been detected in the cerebellum, the concentration of non-specifically bound ligand could, theoretically, be higher in patients, leading to an underestimation of the BP in the target ROIs. The standard method to determine TACs for the reference tissue involves the manual definition of ROIs. We used the cerebellum, since it was previously reported to have a negligible amount of DAT (Hall, Halldin et al. 1999; Halldin, Erixon-Lindroth et al. 2003). DAT can be detected only in the cerebellar vermis (Melchitzky and Lewis 2000), which was excluded from the ROI. However, the BP was reduced in the substantia nigra and midbrain but not in the caudate and putamen, and the nigral and striatal DAT binding were not correlated, which would be expected if the uptake in the reference region had a major impact on the estimated binding.
Preliminary comparisons between nine patients and eight controls whose arterial input was used to calculate the cerebellar volume of distribution of [^{11}C] PE2I, showed similar values in patients (3.8 ± 0.8) and controls (4.0 ± 0.9), p = 0.654 (Ikuo Odano, personal communication).

What is the possible impact of low DAT levels in JME?

DAT is a presynaptic marker of the dopaminergic neurons whose function is to remove extracellular DA by DA reuptake from the synaptic cleft (Jaber, Robinson et al. 1996).

DAT is located on the somatodendritic and axon terminal membrane of the DA neurons. The attribution of DAT function to reuptake DA from the synaptic cleft seems to be relevant only at the target regions, but not at the origin-mostly nigra and VTA, and has been revised in the last few years. It seems that DAT at the dendritic level has the role of a releasing factor, thus regulating the amount of DA released (Falkenburger, Barstow et al. 2001). The dynamic interactions with extracellular DA are regulated by the presynaptic D2 autoreceptors in the substantia nigra (Mortensen and Amara 2003). The finding of a reduced DAT binding in the substantia nigra implies an impaired DA reuptake with increased autoinhibition of DA signalling in the striatum and the frontal lobe.

Since PET binding studies measure the total concentration of membrane protein in a tissue volume, they cannot differentiate among DAT reduction due to cell loss, impaired dendritic arborisation or dysregulation of DAT protein expression. However, low DAT expression in the midbrain and not at the target region - striatum (a negligible amount of DAT is detected in the cortex (Jucaite, Odano et al. 2006)) suggests the involvement in all the functions in which our patients showed poor performance (Nieoullon 2002).

Low DAT levels in the midbrain could imply loss of nigral neurons, selective and seizure related excitotoxic lesions, effects of the antiepileptic medication, and the epileptogenic condition per se. We could only speculate about any potential mechanism underlying the low DAT. *Loss of nigral cells* and low DAT, common findings in Parkinson's disease (Volkow, Fowler et al. 1996) are also associated with striatal DAT decrease. However, DAT binding was normal in the striatum of our JME patients, excluding this possibility. Status epilepticus for more than 24 hours in rats is accompanied by lesions in the substantia nigra pars reticulata (Schmidt-Kastner, Heim et al. 1991), however, seizures lasting less then two hours leave no neuronal damage in the nigra and none of our patients had a history of status epilepticus. Excitotoxic lesions are primarily expected in the striatum [which is a target for epileptic seizures, (Auer, Ingvar et al. 1986)].

The low DAT in the midbrain might be due to *epileptic condition per se*, as a consequence of continuous background epileptogenic discharges or as a primary deficiency, possibly genetically induced.

Antiepileptic medication should also be considered when discussing possible factors affecting DAT levels. The majority of our patients were treated with valproate. A recent study indicated increased DAT gene expression due to treatment with valproate (Wang, Michelhaugh et al. 2007), which, if anything, will lead to increased DAT levels due to medication. No other drugs in our study group have been associated with changed DAT levels.

4.5 Neuropsychological findings in JME (Study III)

Patients with JME showed lower psychomotor speed, motor function and attention (illustrated by their performance in the Finger Tapping, Grooved Pegboard, Dual Task Condition and TMT-A, and tracing in TMT-A). They also had impaired executive functions (shown in TMT-B and digit symbol tests), and poorer WM reaction time. In contrast, the primary and episodic memory was intact. Correlation with performance was assessed only in patients, due to the fact that the controls used

in our PET study were significantly younger, and therefore another control group was used to make neuropsychological comparisons. Performance in the dual condition of tapping with the right hand and placing the pegs with the left hand correlated with the BP of [^{11}C] PE2I in the midbrain (r = 0.601, n = 12, p = 0.039). The midbrain BP of [^{11}C] PE2I was also significantly correlated with WM level (verbal WM: r = 0.621, n = 12, p = 0.031, and visual WM: r = 0.639, n = 12, p = 0.025). In addition, the BP in the putamen was positively correlated with tracing performance in the Trail Making Test A (r = 0.607, n = 12, p = 0.036). No correlations were observed with the BP in the caudate for any of the tests. No correlations were observed between the neuropsychological tests and the duration of epilepsy.

Janz was first to describe JME. He summarized that neurophysiology, neuropsychology, and neuroanatomical results point toward frontal lobe dysfunction in JME (Janz 1985). Indeed, some of JME personality traits, such as limited self-control, immaturity, suggestibility, distractibility, and indifference to physical needs, correlated with cognitive dysfunction and neuroradiological alterations, suggesting an involvement of the frontal lobe (Janz 1985). A role of the frontal lobe is also stressed by the pattern of increased bilateral frontal or frontocentral spike-wave activity seen in JME (Santiago-Rodriguez, Harmony et al. 2002), and the multitude of abnormalities in the frontal lobe found with use of various techniques (Meencke and Janz 1984; Koepp, Richardson et al. 1997; Woermann, Sisodiya et al. 1998; Savic, Osterman et al. 2004). Besides the anatomical changes described above, JME patients also show decreased glucose uptake in the dorsolateral prefrontal cortex and a decreased activation in the dorsolateral prefrontal area during performance of WM tasks (Swartz, Simpkins et al. 1996). The same group (Swartz, Halgren et al. 1994) analysed primary WM, attention, motivation, and motor function in nine JME patient compared with frontal lobe epilepsy patients and healthy controls. The frontal lobe epilepsy patients had deficits in primary memory functions, as did the JME patients.

Motor dysfunction detected in our patients has hitherto been reported primarily in children and adolescent patients with IGE. It has been attributed to antiepileptic drugs (AED) in some studies (Dodrill 1978), and the epileptic condition in others (Vermeulen, Kortstee et al. 1994; Aldenkamp, Overweg-Plandsoen et al. 1999).
The present findings may be independent of the antiepileptic medication, and just a part of the epileptogenic process. The two alternatives are not mutually exclusive, and cannot be distinguished by the present data. Both are, however, compatible with an impaired DA signalling. It seems possible that both ictal and interictal activity in JME may involve DA transmission. Therefore, the therapeutic strategies might be selected with alertness to the patient's neurobehavioral component. The observed changes are attributed to JME, but the DA transmission might accompany other types of IGE, and it encourages research aiming to specifically characterize the cognitive and psychomotor performance in separate IGE syndromes, in relation to specific neurobiological parameters, including DA signalling.

4.6 Odor activation in MTLE (Study IV)

4.6.1 Pattern of activation in patients and controls

In accordance with our group's previous publications (Savic and Gulyas 2000; Bengtsson, Berglund et al. 2001; Savic and Berglund 2004; Savic, Berglund et al. 2005;

Berglund, Lindstrom et al. 2006), smelling of uFAM activated the amygdala, piriform and agranular insular cortex, the anterior and ventromedial thalamic nuclei, and the anterior cingulate cortex in healthy controls. These regions were also recruited with FAM odors, but the cluster covering the left insular cortex extended to, and included the BA 44, 45 and 47. Furthermore, the cluster centered at the right amygdala extended to the right parahippocampal gyrus. No hippocampal or orbitofrontal activation was observed.

Patients failed to activate the amygdala and piriform cortex and the agranular insular cortex ipsilateral to the epileptogenic region. The pattern of activation was slightly different in patients with left compared to right MTLE, in that the left MTLE group failed to recruit the left BA 44 when smelling FAM.

One group random effect analysis of the entire patient population (after flipping the images of patients with right MTLE, and constructing a group of 23 patients with left-sided MTLE) showed activation in the amygdala, piriform+insular cortex ipsilateral to the epileptogenic region, and the anterior cingulate cortex with FAM, as well as uFAM. Smelling of FAM yielded no significant cluster in the left BA 44, 45, and 47, as the corresponding activation in patients with right MTLE was now on the right side. Neither was there a significant cluster in the right parahippocampus (probably due to a loss of resolution with the flip, and a loss of significance by mixing two populations, one of which did not activate this region). As expected, the activations in combined flipped left MTLE with right MTLE, treated as 23 patients with right-sided MTLE, showed the corresponding clusters, but on the opposite side.

We performed post hoc analysis, selecting 9 MR negative subjects and 13 subjects whose MR indicated pathology. The pattern of activation was similar in both groups.

Group comparisons confirmed the observations from the within-group analyses: thus, when smelling uFAM, controls activated the right amygdala, the right piriform and agranular insular cortex (+12, -22 -6; z = 4.2; size 2.4 cm^3), and the right middle temporal gyrus (+48, -22, -10; z = 4.2; size 10.4 cm^3), significantly more than patients with right MTLE. When using familiar *odors* the difference included the right fusiform gyrus (+30, -54, -18; z = 3.7; size 2.4 cm^3), in addition to the right amygdala and parahippocampal gyri (+34, -22, -16; z = 4.7; size 1.4 cm^3).

Patients showed no additional areas of activation compared to those detected in controls.

Direct comparison between the two MTLE groups did not show any significant difference, possibly reflecting the combined effect of certain contralateral affection and minor ipsilateral increase in rCBF.

What could be the substrate for impaired activation during olfactory stimulation in MTLE?

The *odor* stimulation is transmitted from the amygdala and piriform cortex via cortico-cortical projections to the ipsilateral insular, the orbitofrontal and the anterior cingulate cortices, and via the dorsal medial and anterior thalamic nuclei, the corpus callosum and the anterior commissure to the contralateral homologous cortical areas (a minor portion) (Powell 1965; Prince 1990). The poor activation in the amygdala and piriform cortex in our patients with MTLE cannot, therefore, simply be attributed to de-

afferentation from the epileptogenic hippocampus. Furthermore, hippocampus is bypassed during odor perception. Rather, it seems to reflect a dysfunction in these regions.

4.6.2 Psychophysical data

Congruent with our previous reports (Savic and Berglund, 2000), the controls rated FAM and uFAM differently only with respect to odor familiarity. All the subjects perceived the odors during scanning, and none reported odor perception during presentations of odorless air. The main effect of odor categorization was significant for odor familiarity (P = 0.007), and odor intensity (P = 0.009). A significant between subjects main effect of familiar odors was found for the rating of familiarity [F = 5.49, P = 0.008)] and intensity [F = 7.29, P = 0.002)] respectively. Employing the Scheffe's post-hoc test, significant differences were found between controls and patients with left MTLE in familiarity ratings of the familiar odors (P =0.011). The intensity ratings on the other hand, were significantly different for familiar odors between controls and patients with right MTLE (P = 0.002). There was a similar tendency for unfamiliar odors for RTLE, which did not pass the level of significance (P = 0.053). Familiarity ratings were then separately compared for FAM and uFAM in the three groups using pared t-tests (P < 0.016 after Bonferroni correction). Congruent with our previous reports (Savic and Berglund, 2000), the controls rated FAM and uFAM differently only with respect to odor familiarity, as did patients with right MTLE (P = 0.004, df 20, and P = 0.011, df = 9, respectively). No such difference was detected in patients with left MTLE (P = 0.018, df = 12).

It could be that the observed difference in the functional brain activity could be driven by the asymmetry in detection thresholds for odors. However, in accordance with previous studies of MTLE (Jones-Gotman and Zatorre 1988; Savic, Bookheimer et al. 1997), the odor thresholds were similar in patients and controls (patients: 0.0004 ± 0.0003 M ipsilateral to epileptogenic region, and 0.0003 ± 0.0005 M contralateral to the epileptogenic region; controls: 0.0002 ± 0.00003 M, bilaterally), *ns?*, and no side differences were detected.

No group differences were observed in respiratory parameters, independent of the type of stimulus (AIR, uFAM, FAM).

4.6.3 Connectivity analysis

To investigate the pattern of functionally connected regions a piriform cortex ROI was selected, as the piriform cortex is not directly connected to the epileptogenic hippocampus. Even though excitation of a small region in the piriform cortex is capable of provoking seizures, with a secondary generalization (McIntyre and Plant 1989), later studies contradict these findings and suggest that the piriform cortex has no implication in secondary generalization of hippocampal triggered seizures and various manipulations of the level of piriform cortex just delay the seizure onset, but do not stop it (Kelly, Staines et al. 2002). From an olfactory point of view, odor stimulation was used and was proven to be effective in stopping seizures, especially if these seizures were preceded by an olfactory aura (Efron 1956; Efron 1957). This might be due to a task that is attributed to the piriform cortex; to perceive and discriminate the odor, and therefore the propagation of the seizure is impaired. Therefore, it is possible to consider this structure not only the olfactory primary cortex, but also as part of a potential chain in the pathophysiological mechanism of MTLE.

Covariation analyses revealed a more extensive network of communicating brain areas than activation analyses. The piriform and temporal neocortex showed bilateral covariations (the latter region was not activated). Furthermore, the right piriform ROI covaried with the right orbitofrontal cortex during smelling of uFAM, although no orbitofrontal activation was detected. The remaining clusters corresponded to those found during activations (the anterior cingulate, piriform, amygdala and insular cortex); they were bilateral, independent of condition, but more pronounced during odor smelling than during the base line.

In patients the seed ROIs showed, independently of condition, only ipsilateral piriform, amygdala and insular clusters, whereas no connection was detected with the corresponding regions on the contralateral side. Both seed ROIs covaried with the anterior cingulate, which was not significantly activated in patients. In addition, there was a covariation between the piriform and temporal neocortex in the epileptogenic hemisphere. Finally, during smelling familiar odors covariations were detected between the left piriform ROI and the left BA44 in patients with right MTLE (and in controls), but not in those with left MTLE.

The altered pattern in patients was confirmed when directly comparing patients and controls with respect to between-task covariations (covariations in odor-related increase of rCBF). Thus, covariations with the contralateral amygdala+piriform+agranular insular cortex were reduced in both patient groups. Furthermore, the covariation between the right seed ROI and the right orbitofrontal cortex was reduced in patients with right MTLE when smelling uFAM, whereas the covariation between the left piriform and left frontal cortex (BA 47) was reduced in patients with left MTLE when smelling FAM. Finally, both patient groups had reduced covariation between the seed ROI and the temporal neocortex contralateral to the epileptogenic region.

4.6.4 Discussion of results from Study IV

In study IV we investigated the functional integrity of the limbic circuits in patients with mesial temporal lobe epilepsy by using the unique capacity of odors to selectively activate these areas of the brain (Savic and Berglund 2004). We opted for passive smelling of odors as the activation paradigm, rather than higher olfactory functions, which in earlier psychophysical studies have shown specific impairments. Our study material is inhomogenous especially with respect to the degree of hippocampal pathology. Furthermore, patients with MTLE can be ambidextrous due to reorganization of language networks. However, the results of the post hoc analyses showed similar patterns of activation in the MR negative patients and MR positive patients, indicating that the alteration of the olfactory processing is a more extensive process, possibly due to circuitry dysfunction.

The pattern of activation was slightly different in patients with left compared to right MTLE, which failed to recruit Broca's area when smelling the familiar (FAM) odors. *Left MTLE* patients also rated familiar odors as less familiar, possibly because of an impaired odor signal-related neuronal input from the left piriform and insular cortices. The question is, however, to what extent cerebral processing during judgement of odor familiarity, which was carried out after the PET scans, can be related to the pattern of activation during passive perception of odors in the scanner? Odor judgment is reported to activate the right orbitofrontal cortex, and the left BA 47 (Royet, Koenig et al. 1999). None of these regions were involved when patients with left MTLE perceived FAM

odors (functional connectivity with right orbitofrontal cortex was detected with uFAM odors). It is, therefore, possible that both the rating and the perception of odor familiarity in left MTLE patients reflected the same functional disconnections. Whether patients with left MTLE should also therefore be less prone to memorize and discriminate FAM odors compared to uFAM is presently unknown since such specific comparative studies have, to the best of our knowledge, not been done in epilepsy patients. Carroll and col. (Carroll, Richardson et al. 1993), however, showed that odor identification, which involved semantic processing (Royet, Koenig et al. 1999), was more impaired in patients with left compared to right sided epilepsy when the FAM odors were employed. Odor recognition and discrimination is prone to be more impaired in left MTLE (Hudry, Perrin et al. 2003) than in right MTLE.

Right MTLE patients rated familiar odors as less intense. Odor intensity is believed to be coded at the levels of the olfactory epithelium and bulb (Duchamp-Viret, Duchamp et al. 2000) and the primary olfactory cortex (Wilson 1997). Interestingly, it has been shown that odor detection improves by bilateral summation and recruitment of both hemispheres' olfactory circuits (Risse, LeDoux et al. 1978). The lower intensity ratings in patients can, therefore, be attributed to the functional disintegration of the amygdala and piriform cortex in the epileptogenic hemisphere. This assumption is indeed supported by the recent publication by Sobel's group, showing that odor intensity is mediated by the amygdala and piriform cortex whereas odor valence is processed by the orbitofrontal cortex (Anderson, Christoff et al. 2003).

The *explorative connectivity* analysis reveals the entire neuronal network that participates synchronously in an activation related function. In describing two areas as functionally coupled, we imply that the two regions participate in processing of the given task, influence each other, or are normally influenced by another source, with no suggestion about the source or causality of their functional connection. Thus, in regions with non-significant, but synchronous activation related changes in cerebral blood flow, the connectivity analysis may reveal significant co-variations. For example, as in our previous studies with an identical design, OFC activation was not detected. The orbitofrontal cortex is believed to process conscious perception of odors (Zatorre, Jones-Gotman et al. 1992; Yousem, Williams et al. 1997; Zald and Pardo 1997; Sobel, Prabhakaran et al. 1998; Savic, Gulyas et al. 2000; Savic 2002), and is activated during sniffing of odorless air or odors (Sobel, Prabhakaran et al. 1998). Therefore, lack of activation could be due to the training, carried out on several occasions prior to scanning, to perceive the odors passively and to avoid analyzing their characteristics. However, the right orbitofrontal CBF covaried positively with the right seed ROI in controls and patients with left MTLE, but not in patients with right MTLE, suggesting a synchronous recruitment of this area in the first two populations, and its absence in the third, congruent with their functional disintegration in the right amygdala and the piriform cortex and the insular cortex, and, thus, do not transmit odor signal to the orbitofrontal cortex. This is of interest when considering that the majority of studies of higher olfactory function (such as odor discrimination and memory) show the greatest impairments in patients with right MTLE (Rausch and Serafetinides 1975; Henkin, Comiter et al. 1977; Rausch, Serafetinides et al. 1977; Abraham and Mathai 1983; Eichenbaum, Morton et al. 1983; Eskenazi, Cain et al. 1983; Jones-Gotman and Zatorre 1988; Zatorre and Jones-Gotman 1991; Jones-Gotman and Zatorre 1993; Jones-Gotman, Zatorre et al. 1997).

When interpreting the results from the analysis of regional correlations during baseline it should be taken into account that the smelling of odorless air is processed by portions

of the piriform cortex (Sobel, Prabhakaran et al. 1998). When mapping the functional connectivity during odor activation we therefore chose not to use the contrasts uFAM-AIR and FAM-AIR. Instead, the connectivity was based on rCBF during smelling of the respective odor category, thereby avoiding failure to detect areas in which the rCBF correlated both during baseline and activation. The contrasts were, on the other hand, employed when trying to detect how changes in odor related rCBF covaried in patients compared to controls.

There is an on-going discussion as to whether the task related decreases in rCBF reflect active inhibition or a resting state network whose activity is suspended during task performance (Greicius, Krasnow et al. 2003; Fox, Snyder et al. 2005). Considering that negative co-variations appeared in similar areas as the AIR-ODOR clusters, one may assume that these regions really were deactivated. This particular issue requires additional analyses.

As in controls, patients showed odor-induced extralimbic co-variations in the ipsi- and contralateral multimodal neocortex (temporal neocortex sometimes extending to the lingular gyrus), less pronounced in MTLE than in controls. The temporal neocortex is a multimodal area (Mesulam and Mufson 1985), which in some studies of olfactory processing shows significant activations (Porter, Anand et al. 2005), and may also be recruited during odor smelling.

One of the questions in this study is whether connectivity analysis can provide additional information about the interictal dysfunction in MTLE. Evaluation of connectivity revealed a more extensive functional network, which included the temporal neocortex and the right orbitofrontal cortex. Both regions are involved in olfaction (Savic 2002), and whilst temporal lobe activation has been reported only in some studies (Savic, Gulyas et al. 2000; Savic, Gulyas et al. 2002; Zelano, Bensafi et al. 2005), activation of the right orbitofrontal represents a frequent finding (Zatorre, Jones-Gotman et al. 1992; Zald and Pardo 1997; Royet, Koenig et al. 1999). That it was not detected in the present setting can be attributed to the orbitofrontal processing of odor judgment, which was employed in several previous studies (Royet, Plailly et al. 2003), but presently avoided in order to minimize the cognitive load.

In addition to the previously commented detection of functional disintegration of the right orbitofrontal cortex in right MTLE, the findings with respect to the temporal neocortex deserve a comment. In contrast to controls patients showed covariations only between the piriform and temporal neocortex ipsilateral to the epileptogenic region. This suggests that both areas are dysfunctional, a finding that is in accordance with the well known interictal hypometabolism in the temporal neocortex on the focus side in MTLE (Henry, Mazziotta et al. 1993; Savic, Ingvar et al. 1993). That we fail to detect a corresponding connection on the contralateral side is more difficult to explain, but it could be attributed to a minor affection of the mesial but not lateral temporal structures on the contralateral side (Engel 1997).

Finally, the connectivity analysis confirmed that the left piriform and left frontal cortex are functionally connected and that this connection is impaired in subjects with left MTLE.

In conclusion, this approach offers additional information in defining the extent of functional disintegration in MTLE, and may, potentially, help understand the biological substrates for the interictal behavioral and cognitive impairments in MTLE.

There is an on-going discussion as to whether the task related decreases in rCBF reflect active inhibition, or a resting state network, whose activity is suspended during task performance (Greicius, Krasnow et al. 2003; Fransson 2005). The present study was not aimed at addressing this issue, nor was the base-line state entirely comparable with the 'default resting state' defined in previous publications, as our subjects were concentrated on smelling.

Are there other factors that could influence the odor processing in MTLE?

1. *Different odor threshold.* The olfactory thresholds were not significantly different between the two nostrils, or between the groups, which is in accordance with previous publications (Eskenazi, Cain et al. 1986; West and Doty 1995; Kohler, Moberg et al. 2001). Considering that all the odors were presented in an identical manner, and suprathresholded concentrations were presented to both controls and patients, this cannot be attributed to a methodological bias.
2. *Gender.* The set of odors used in this study was successfully used in several pheromone activation studies by our group (Savic, Berglund et al. 2001; Savic 2002; Savic 2002; Savic, Berglund et al. 2005; Berglund, Lindstrom et al. 2006). Independent of the sexual preferences and gender of the subjects included, the pattern of activation during smelling of familiar and unfamiliar odors was similar irrespective of odor used.
3. *Change in physical properties of experimentally used odors.* Right MTLE patients rated familiar odors as less intense, whereas left MTLE patients rated these odors as less familiar. These changes could not be attributed to change in odor quality. We have investigated olfactory processing in an identical design in several other study populations and during the same time period. The ratings of odor intensity in these studies did not differ between subjects and controls (Savic, Berglund et al. 2005; Berglund, Lindstrom et al. 2006).
4. *AED.* An effect of antiepileptic drugs on perceptual function was not found in most studies that specifically addressed this issue (Campanella, Filla et al. 1978; Knecht, Henningsen et al. 1996). The criticism is questionable since our findings suggest a strict lateralization in MTLE.

The major limitation of the present data is that is not informative at the individual level. This is an inherent problem of PET activation studies, due to restrictions of radioactivity exposure. PET was, however, preferred as the imaging tool in this initial study of odor processing in MTLE because of the well-known problems with signal loss in the orbitofrontal regions when using fMRI. We wanted to investigate the general pattern of activation, before proceeding with the fMRI studies of individual patients.

The altered patterns of activation in our patients were reflected by altered psychophysical responses. Thus, a disconnection, or impaired responsiveness of a portion of the network mediating a certain function, eg odor perception, may lead to changes of this function. This brings attention to the possible behavioral impact of the observed functional disintegrations of the limbic circuits in MTLE. Both lateral and medial nuclei of the amygdala have prominent connections with the frontal lobe, with particularly dense reciprocal projections to orbitofrontal, medio-frontal and anterior cingulate cortices (Stefanacci, Farb et al. 1992)**.** These cortical regions are critical nodes in the distributed network, which regulates affective processing (Davidson and Irwin 1999; Davidson 2002), suggesting that anxiety, phobia, compulsion, and depression, are all co-morbid with MTLE and may be a direct neurobiological

consequence of the epileptogenic dysfunction. A further implication of the present results is that a number of important components of social functioning, mediated by the limbic networks, such as recognition of emotional codes, may also be disturbed in MTLE and contribute to the social maladaptation of many persons with severe MTLE. These subtle and frequently undiscovered dysfunctions need further attention.

Most of the studies highlight impaired odor discrimination, odor recall and odor identification in epilepsy. The olfactory processing seems to be more affected in patients with right MTLE than left MTLE. This might be due to a slight right lateralization of the olfactory system, and the impaired processing of the right side in right MTLE indirectly reflects an extension of lesions beyond the temporal mesial structures. The same conclusion is valid for left MTLE, where the olfactory processing is, to some extent, less severely affected, but where other circuits are more impaired e.g. learning and language. Future studies of olfactory function in epilepsy patients, with special attention to the odor characteristics in relation to the side of epileptogenesis, are, therefore, highly warranted.

5 CONCLUSIVE REMARKS

Three of the studies presented in this thesis had the purpose of adding new information about the pathophysiological mechanism behind generalized and partial epilepsy.

Study I - The finding of altered gray and white matter fractions, reduced volume and changed NAA and Glx concentration in the thalamus of IGE patients, questions the current definition of IGE as a condition without regional tissue changes.

Study II - The regional, rather than diffuse alteration of gray matter in GTCS, particularly in the fronto-parieto-temporal regions, cerebellum and thalamus, together with reduced volumes of the subcortical structures known to be involved in the motor pathway found with two independent MR methods in study II, further underlines this statement.

Additional prospective studies of larger numbers of patients (including comparisons with patients having secondarily generalized seizures) are, however, necessary to further elucidate the cause-effect relationship between epileptic seizures and their subsequent effect on the thalamus in IGE.

Study III - The reduction in midbrain DAT sugests in low DA signalling to the target regions, and a poor performance of the executive functions, WM, and motor tasks. The findings support the note that IGE is associated with multiple cerebral changes, and add a new aspect to the pathophysiology of JME, and its future treatment strategies.

The current ILAE Classification of Epileptic Seizures and Epileptic Syndromes suggests that the seizures in IGE are generalized, implying a widespread subcortical and cortical neuronal involvement from onset, with impairment of consciousness as the clinical hallmark. Our findings from Studies I-III add new information about the pathophysiology of IGE and emphasize the possibility of syndrome specific changes.

Study IV - During limbic activation patients with documented MTLE fail to recruit several extrahippocampal limbic regions on the side of seizure onset. The presence of specific perceptual disturbances in partial epilepsy syndromes is consistent with the view that epilepsy is a network disease, with the potential to affect neural circuits distant from the seizure focus. Imaging of olfaction in MTLE may be used to study functional activation of the limbic system. The observation of functionally uncoupled regions may be an indirect indication of interictal and ictal discharges having an impact on structures beyond the hippocampus and amygdala.

6 FUTURE WORK

1. To increase study material for the JME group and investigate the pattern of structural alterations (preliminary data indicates increase of gray matter in frontobasal region, and decrease in the thalamus, cerebellum and temporal regions).

2. Use a D2 ligand, such as [^{11}C] FLB457, in the same population of patients that we have used previously to assess the receptor density primarily in cortical areas. D2 receptor agonists have been suggested to improve WM performance at visuo-spatial tasks (Luciana, Collins et al. 1998). Since many reports indicate impaired WM in JME patients, and since epilepsy is associated with reduced D2 receptor density, changes in receptor density might be expected.

3. Examine GTCS and CAE (childhood absence epilepsy) patients in similar settings as in Study III. It might be of interest to evaluate the dopamine system in association with neuropsychological functions in these groups of patients.

4. To set up the logistics to study olfactory processing using olfactometer and fMRI that will allow asswesment of possible abnormalities in individual patients.

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