Chapter 1
Introduction

Men think epilepsy divine, merely because they do not understand it. But if they called everything divine which they do not understand, why, there would be no end to divine things.”

(Hippocrates)
Chapter 1.1

General introduction and outline
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Case Description

A 52-year-old woman presented with a seizure. On examination her temperature and blood pressure were normal. She was orientated and obeyed commands (score on Glasgow Coma Scale 15). Neurological examination showed normal strength in all extremities, but loss of skilled movements of the left arm. CT and MRI scans of the brain showed a right frontoparietal lesion surrounded by finger-shaped edema (figure 1).

Anti-epileptic drug therapy was started (valproic acid 1000 mg/day). Epilepsy surgery could not be performed due to the location of the tumor and a biopsy of the lesion was planned. Three days after the start of the anti-epileptic drugs she was seizure free and in expectation of the biopsy she was discharged. Ten days later (the biopsy had not taken place yet) she presented with a partial status epilepticus. In the next 48 hours diazepam, midazolam, phenytoin and clonazepam were given subsequently without response, and eventually she was transferred to the intensive care for a continuous infusion of clonazepam. At last, she improved, but seizures persisted and occurred twice daily despite the use of a combination of levetiracetam, valproic acid, phenytoin and clobazepam. The seizures consisted of clonic movements of her left arm during one minute. Biopsy of the lesion was further delayed by a low trombocytic count, probably caused by valproic acid, but eventually the biopsy was performed. Histological examination demonstrated an anaplastic astrocytoma WHO grade III. A few days later she displayed an intense red, confluent exanthema on the trunk of her body. The exanthema was attributed to a drug reaction to phenytoin and disappeared after cessation of phenytoin. A couple of weeks later she was again admitted to the hospital with a partial status epilepticus. This time she had a paresis of her left arm. Meanwhile radiotherapy (30 x 2 Gy) had started. MRI follow-up did not show any signs of local recurrence or increase of edema explaining the persistence of the seizures. Since epilepsy surgery could not be performed, anti-epileptic drugs remained the only option. Once again she was given a continuous infusion of clonazepam and pregabalin was started. Despite the high seizure frequency, the infusion was stopped, because she experienced severe somnolence. Pregabalin and levetiracetam were replaced by topiramate. At first the seizure frequency decreased, allowing her to rehabilitate in a nursing home. However, within a few weeks in the nursing home, she deteriorated quickly and became confused and disorientated, not recognizing her husband and daughter anymore, not eating or drinking, and dependent on constant care. The seizure frequency increased to several times per hour, with a left hemiparesis in between. Since cognitive impairment due to topiramate had been reported, the topiramate was stopped. Within a few weeks the confusion cleared up. After a week, she communicated adequately and was able to take care of herself. Remarkably, the seizure frequency also decreased and she was almost seizure free on valproic acid, clobazepam.
Brain tumors

The case above is an extreme example, illustrating the impact that seizures may have on a patient’s daily functioning and further, it illustrates the difficulties in treating epilepsy in brain tumor patients. The case provides a clinical rational for this thesis. The presented patient was diagnosed with a primary brain tumor: an anaplastic oligodendroglioma WHO grade III. Brain tumors are classified in primary brain tumors, arising from intracranial tissue e.g. meningioma, ganglioglioma, dysembryoplastic neuroepithelial tumors and glioma, and secondary brain tumors, originating from malignancies elsewhere in the body. This thesis is focused mainly on primary brain tumors, namely glioneuronal tumors and glioma. The incidence of primary brain tumors is 5-7 per 100,000. This means that approximately 800 new patients per year are diagnosed with a primary brain tumor in The Netherlands. In children, 40% of primary brain tumors are localized in the cerebellum or brain stem. In adults most tumors are located supratentorially. Primary brain tumors are more frequent in males (male/female ratio 1.5). Eighty percent of the tumors in adults are from glial origin and the frequency is highest between 20-69 years of age.

Tumor type and grading

Primary brain tumors are categorized according to cell type and grade: Grading of tumor types is a way to predict the behavior of a neoplasm. Grade I include tumors with low proliferative potential and possibility of cure after surgical resection. Grade II tumors are infiltrative with low proliferative activity (slow-growing). These tumors often recur after treatment. Grade III tumors display histological evidence of malignancy (e.g. nuclear atypia and high mitotic activity). Grade IV tumors are mitotically active, show necrosis and often infiltrate surrounding tissue (fast-growing). In the long run most low-grade tumors tend to progress to higher grades of malignancy. The neuropathological assessment of brain tumor typing and grading is further complemented by molecular tests for clinically relevant tissue-based biomarkers.

Glial tumors arise from the supporting tissue (glial tissue) of the brain. Glial tumors are roughly divided in low-grade glioma (astrocytomas, mixed oligo-astrocytomas as well as oligodendrogliomas) and high-grade glioma (anaplastic astrocytoma, anaplastic oligodendroglioma, anaplastic oligo-astrocytoma and glioblastoma multiforme). The most common tumor type in adults is the glioblastoma multiforme. Figure 2 shows a grade II astrocytoma. Glioneuronal tumors consist of a mixture of dysplastic neurons and neoplastic glial cells. The most common glioneuronal tumors are gangliogliomas and dysembryoplastic neuroepithelial tumors (DNTs).

Figure 2: Grade II astrocytoma. A (Toluidine blue): intraoperative smear preparations of diffuse astrocytoma, showing glial cells with some variation in nuclear shape and size and fibrillary processes. B (HE): fibrillary astrocytoma with microcyst formation. C: GFAP immunoreactivity in a fibrillary astrocytoma. D (HE), E (NeuN): entrapped neurons within a diffuse astrocytoma.
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Prognosis

The prognosis depends on patient factors (e.g. age, Karnofsky performance status), tumor factors (e.g. grade, type and genetics) and in lesser extent treatment factors.\(^5,6\) Despite a variety of treatment strategies (e.g. surgery, chemotherapy, radiotherapy and experimental therapies) the prognosis of patients with a glioma remains poor. It is not a question of ‘if’ the tumor recurs, but ‘when’ the tumor recurs. The median survival of patients with a low-grade glioma is 5 – 15 years.\(^7\) Patients suffering from glioblastoma have a median survival of 1 year and patients with anaplastic glioma have a median survival of 2-3 years.\(^6,8\) Oligodendroglioma and oligo-astrocytoma give a better prognosis than astrocytoma.

Given this limited prognosis, treatment of glial tumors is not only aimed at prolonging survival, but also at decreasing symptoms (e.g. with dexamethasone and anti-epileptic drugs). Glioneuronal tumors are largely WHO grade I tumors with a more favorable prognosis than glioma. Of patients with a ganglioglioma following surgery, 90% have a progression free long survival. Only a small percentage of gangliogliomas display a more aggressive behavior with regard to progression. The same holds true for DNTs, which usually are slow growing WHO I tumors. Less than one percent of DNTs show more accelerated growth and anaplastic behaviour.\(^9\)

Symptoms in brain tumor patients

The patient above presented with epilepsy and loss of skilled movements of her left arm. The neurological symptoms in brain tumor patients do not differ from symptoms in patients with other space occupying lesions in the brain such as abscesses. Patients may present with seizures, mental changes, focal neurological deficits related to the location of the tumor (e.g. paresis, aphasia, sensory deficits, loss of coordination and vision problems) or signs of increased intracranial pressure (e.g. headaches, vomiting, nausea, vision problems and disturbed vigilance).\(^10\) Signs of increased intracranial pressure and rapidly progressive focal deficits are more often associated with fast growing tumors (e.g. glioblastoma). In approximately 20-45% of tumor patients, seizures lead to the diagnosis of the underlying brain tumor, and in another 15-30% of patients seizures occur later in the course of the disease.\(^11,12\) The incidence of epilepsy differs among tumor types and ranges from 30-100%.\(^13\) Although any brain tumor, regardless of tumor location, may cause epilepsy, patients with temporal or frontal low-grade tumors are more likely to develop seizures, either as a presenting sign or during the course of the disease.\(^9,13\) Tumor-related epilepsy is often refractory to treatment. Here will be further elaborated upon later in this thesis.

The majority of glioma patients is confronted with cognitive deficits at some point in the course of their disease.\(^14-17\) Both low-grade and high-grade gliomas may display cognitive deficits.\(^16,18-20\) Factors that may influence cognitive functioning are the tumor itself, tumor growth,\(^21\) and side-effects of both anti-epileptic and anti-tumor treatment,\(^22\) but also epilepsy itself.\(^23,24\) Cognitive deficits that occur most frequently in brain tumor patients concern mainly attentional functioning, working memory, psychomotor speed and executive functioning.\(^25\)
Aims and outline of the thesis

The general aim of this thesis is to improve anti-epileptic drug therapy in brain tumor patients. Treatment of epilepsy in brain tumor patients is far from easy. Chapter 1.2 outlines the challenges that are met in tumor-related epilepsy and provides an overall picture of the hypothesis of how brain tumors eventually lead to epilepsy. Understanding the mechanisms that lead to epilepsy might provide new tools for the development of an effective treatment of epilepsy.

What makes lesions epileptogenic?
Silencing the expression of components of a pathway that ultimately leads to epilepsy, might prevent epileptic seizures. The first aim of the research that has led to this thesis, therefore, is to investigate pathways that could be related to epilepsy. Most research directed at epileptogenesis in lesional epilepsy aims at cortical malformations. Starting from here, we can extrapolate this knowledge to brain tumors later. The synaptic vesicle protein (SV2A) is the binding site for levetiracetam, an effective and well-tolerated anti-epileptic drug. Dysfunction of SV2A is also believed to trigger epilepsy. We investigated whether SV2A expression is altered in epilepsy patients with focal cortical dysplasia (FCD) and tuberous sclerosis cortical tubers (TSC) (Chapter 2.1).

In this section we also investigate another pathway that possibly plays a role in epileptogenesis. Adenosine kinase (ADK) represents the key metabolic enzyme for the regulation of extracellular adenosine levels in the brain. Several lines of experimental evidence support a critical role of ADK in different types of brain injury associated with astrogliosis, which is also a prominent morphological feature of temporal lobe epilepsy (TLE). We hypothesize that deregulation of ADK is a ubiquitous pathological hallmark of TLE (Chapter 2.2).

What makes brain tumors epileptogenic?
The knowledge we gained on SV2A and ADK in non-tumorous lesions in the previous chapter, can be extrapolated to brain tumor patients. Dysfunction of components that lead to epilepsy in several non-tumor lesions possibly also leads to epilepsy in brain tumors. Levetiracetam is effective and well-tolerated in glioma patients, and SV2A is its ligand. We hypothesize that SV2A is involved in epileptogenesis in glioma and, therefore, we determine if SV2A expression is altered in tumor and peritumoral tissue (Chapter 3.1). As we show that adenosine kinase (ADK) is involved in epileptogenesis in other lesions than brain tumors, we also investigate if the adenosine metabolism is modified in epilepsy-associated tumors (Chapter 3.2).

How do molecular changes due to brain tumors lead to alterations in the whole brain?
This section focuses on the role of brain networks in epilepsy. Synchronization of neurons may be pivotal for optimal brain functioning and it reflects dynamics related to epilepsy. In Chapter 4.1 we investigate whether functional connectivity can be used as a predictor of epilepsy. Brain tumors may also influence functional interactions in brain networks, possibly leading to epilepsy. In chapter 4.2 functional connectivity and network topology are investigated in brain tumor patients with epilepsy over time.

It has been proposed that molecular changes lead to alterations in functional network properties. We hypothesize in chapter 4.3 that an imbalance between inhibition and excitation at the receptor, neuronal and astrocytic level due to brain tumors can cause networks to be disorganized. We correlate the expression of specific proteins that are known to be altered due to brain tumors and play an role in epilepsy (SV2A, PgP, GAD 65/67), to local neural networks.
Treatment of epilepsy in brain tumor patients

Despite the availability of a wide variety of anti-epileptic drugs pharmacotherapy of epilepsy remains an enormous challenge. Levetiracetam has many favorable characteristics for brain tumor patients. It has few side-effects, no enzyme-inducing or -inhibiting properties and is only a weak substrate to multi-drug transporters. Though these properties seem favorable, no reliable data on the effects of levetiracetam on cognitive functioning in glioma patients exist. Other AEDs, such as phenytoin, have shown to cause cognitive impairment. Also without AED treatment glioma patients have impaired cognition in comparison to age- and education-matched healthy individuals and to other cancer patients. Additional deteriorating effects of AEDs should be avoided whenever possible. We investigate the effect of levetiracetam monotherapy on cognitive functioning in a group of glioma patients (Chapter 5.1). Older anti-epileptic drugs are believed to negatively affect cognitive functioning to a greater extent than newer anti-epileptic drugs. Subsequently, the effects of several anti-epileptic drugs, including levetiracetam, on cognitive functioning are compared (Chapter 5.2).

Now we have established that levetiracetam is well-tolerated with respect to side-effects as well as cognitive functioning, it seems obvious to prescribe levetiracetam to all brain tumor patients. However, not all patients are seizure free on levetiracetam and no clinical parameters exist that predict the efficacy of an anti-epileptic drug. In view of the fact that SV2A is the binding site of levetiracetam and SV2A seems to be involved in epileptogenesis, we investigate if SV2A expression predicts the efficacy of levetiracetam (Chapter 5.3).

In the final section the results of the studies are summarized and discussed and suggestions for future research are proposed (Chapter 6).