Chapter 11

Human Brain Tissue as a Model for the Study of Epilepsy

Leonardo Lara-Valderrábano, Ivette Bañuelos-Cabrera, Víctor Navarrete-Modesto, and Luisa Rocha

Abstract

Epilepsy surgery is widely used to treat pharmacoresistant epilepsy. This therapeutic strategy offers the opportunity to study human brain tissue in vitro. We describe three approaches that represent an excellent opportunity for the study of the underlying mechanisms of pharmacoresistant epilepsy: in vitro electrophysiological recordings, in vitro procedures for receptor evaluation, and genomic analyses. These procedures could allow individual diagnosis and personalized treatment for patients with pharmacoresistant epilepsy.

Key words Temporal lobe epilepsy, Human brain tissue, Electrophysiology, Receptor analysis, Genomic analysis

1 Introduction

The human epileptic brain exhibits unique network, cellular, and molecular properties. Therefore, using brain tissue from patients with mesial temporal lobe epilepsy (MTLE) is a first-rate opportunity to characterize the electrophysiological, histological, molecular, and genetic properties in this pathological condition. There is an increase of interest in the study of epilepsy directly in human tissue, estimated by the number of publications on this topic between 1976 and 2005 [1] (Fig. 1).

Epilepsy is one of the most common neurologic disorders [2]. It is estimated that approximately 50 million people is affected with epilepsy worldwide [3]. Despite the severity and high prevalence of this disorder [4], the underlying neurobiology mechanisms associated with epilepsy are poorly understood. Indeed, most of what is known about epilepsy has been obtained from the research done in animal models. Studying human brain tissue in vitro offers a crucial opportunity for the understanding of the disease. The increment of epilepsy surgery [5] and its effectiveness to reduce pharmacoresistant epilepsy [6, 7] has increased the availability of brain tissue from

Alan Talevi and Luisa Rocha (eds.), *Antiepileptic Drug Discovery: Novel Approaches*, Methods in Pharmacology and Toxicology, DOI 10.1007/978-1-4939-6355-3_11,

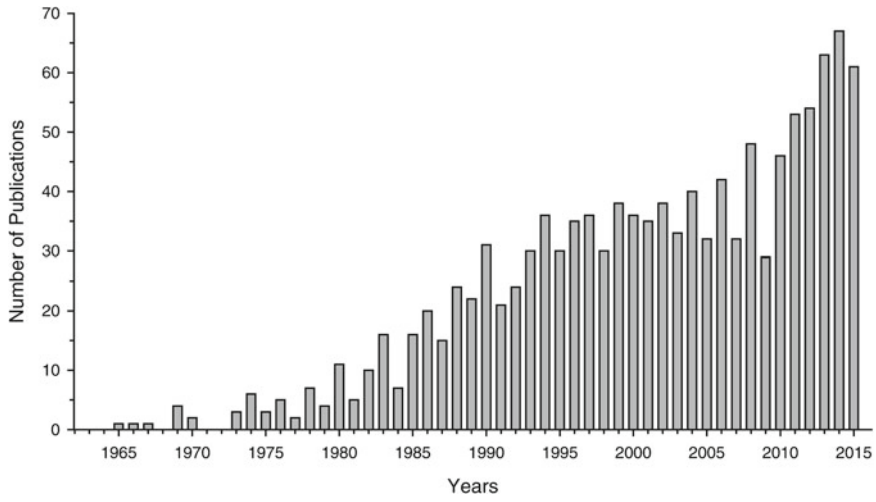

Fig. 1 Histogram showing the number of publications containing "human brain tissue" in PubMed search

patients with this disorder. In this review, we focus in the relevance of evaluating the human epileptic tissue using the following approaches: electrophysiology, receptor analysis, and genetic evaluation.

2 Epilepsy and In Vitro Electrophysiology

The first topic to be addressed is the electrophysiological approach to study the brain tissue obtained from patients with pharmacoresistant epilepsy. This situation allows us to explore directly the activity of human single neurons and networks of brain structures of patients with drug-resistant epilepsy (Fig. 2).

Once the focus has been identified, surgery often involves the resection of specific brain structures involved in the generation and propagation of the seizure activity [6]. It is essential to proceed fast in order to preserve the tissue in optimal conditions. After brain tissue samples have been excised, it is optimal to place them immediately in ice-cold (4 °C) artificial cerebrospinal fluid (ACSF) gassed with a 95% O_2–5% CO_2 mixture. The ACSF composition used for slice perfusion during electrophysiological experiments varies, but it generally includes (in mM): NaCl 124–129, KCl 2–4, $CaCl_2$ 1.6–2.4, $MgSO_4$ 1.3–2, NaH_2PO_4 1.24–1.25, $NaHCO_3$ 21–26, and glucose 10 [1, 8]. When tissue transportation takes long periods (30 min), it is advised to use ACSF reduced in Na^+ (to prevent hypoxia-induced Na^+ influx) and α-tocopherol in addition (as a free radical scavenger) [9].

The thickness of brain slices varies depending if the procedure involves the evaluation of individual neurons or networks. Commonly it goes from 300 to 750 μm [8–14], although it is recommended a maximum of 600 μm for a proper slice oxygenation [1].

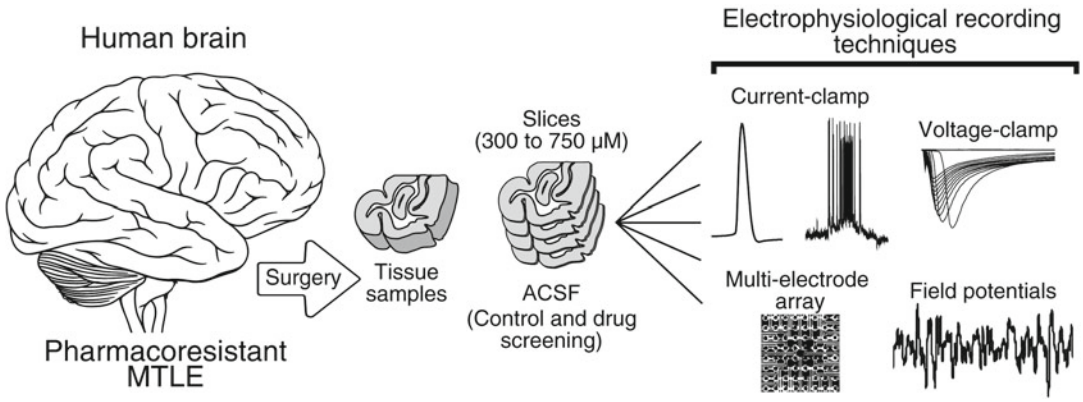

Fig. 2 Schematic representation of the standard protocol used to evaluate electrophysiological activity in brain tissue obtained from patients with pharmacoresistant epilepsy. After slicing, the tissue is available for different techniques of electrophysiological recording (*right panels*); each one provides distinct kind of information depending on the aim of the study. For example, current-clamp allows the study of epileptiform activity and action potential dynamics of individual neurons; voltage-clamp could be used to analyze currents and channel properties; multielectrode arrays provide the opportunity of simultaneously stimulating and recording field potentials, and/or action potentials in different areas of the tissue; field potentials are used to evaluate multiple neuron activity of during epileptiform activity

It is important to consider that some types of epilepsy, such as mesial temporal lobe epilepsy (MTLE), are associated with neuronal damage and hippocampal sclerosis [15–17]. This condition makes the tissue difficult to be penetrated by the pipette or electrode. Also, the usual method to visualize neurons in patch-clamp mode (infrared differential interference contrast optics) could be difficult to implement.

Reports about the evaluation of human cortex unitary activity in vivo exist since 1956 [18]. Extracellular field potentials or unitary neuron recordings in vitro are valuable alternatives for the study of the underlying cellular mechanisms of epilepsy depending on the main goal of the study. Field potential recordings are used to study extracellular activity of network synchronization activity in hippocampal preparations [11] and synaptic plasticity properties [19]. This approach allows the description of spontaneous and rhythmic activity initiated in the subiculum, a condition that resembles the epileptiform discharges recorded by intracranial electrodes [20]. Concurrent to field potential recordings, other measurements as extracellular ionic concentrations can be assessed [21].

The evaluation of unitary extracellular neuronal activity requires the incubation of the human brain tissue immediately after its resection [12]. The study of Schwartzkroin and Prince in 1976 represents the first evaluation of intracellular recording of neurons from the human cortex neurons [22]. These authors employed the same criteria used for assessing neurons of animal

cortex and used sharp electrodes (20–50 MΩ). The results obtained demonstrated that human cortical neurons present stable resting membrane potential (RMP), overshooting action potentials, and absence of rhythmic high-frequency spike firing. Although the number of neurons recorded was small, the authors stood out that the in vitro electrophysiology techniques can be used to obtain valuable data from human brain tissue [20, 22–24].

The firing properties of human neurons evaluated by in vitro electrophysiology methods have shown to be similar to those identified in rodents and other species [1, 24, 25]. Other important approach represents the evaluation of neurotransmitter effects. For example, when GABA is applied to the brain slice, it may exert inhibitory effects crucial for controlling cerebral excitability [10]. This situation is perturbed in the cerebral tissue of patients with pharmacoresistant epilepsy [26, 27]. The in vitro evaluation of the effects of other neurotransmitters such as acetylcholine, adenosine, histamine, norepinephrine, and serotonin reveals their effects and their contribution in the neuronal activity modulation [28].

The electrophysiological activity should be correlated with the cell morphology. Cellular labeling with fluorescent dyes as Lucifer yellow [23] or biocytin [24, 27] are techniques available for morphological reconstructions after the electrophysiological evaluation of the tissue. Morphological data of neurons in epileptic tissue should be carefully compared with data obtained from autopsies [29, 30]. Biocytin-filled neurons in combination with immunochemistry [26] provide more information about the expression of particular proteins.

There are some approaches that reduce the clamp problems such as the dissociation of the cells or neuronal cultures [31]. The isolation of neurons allows the characterization of essential events for the neuronal excitability, such as the transient currents in neocortical neurons [32]. The evaluation of astrocytes in cultures from medically intractable TLE allowed to establish that cells from the seizure focus exhibited action potential-like events in response to electrical stimulation [33].

Another new approach is the evaluation of electrical activity by multielectrode arrays (MEAs). This procedure allows the analysis of spatiotemporal dynamics of the epileptiform activity in specific networks, including the areas of seizure initiation and propagation. It also facilitates the analysis of effects induced by antiepileptic drugs at cellular and network levels, field potentials, and action potentials of multiple neurons from diverse regions of the tissue [34].

One of the most interesting perspectives of the evaluation of the cerebral tissue obtained from patients with pharmacoresistant epilepsy using in vitro electrophysiology is the investigation on the mechanisms underlying the pharmacoresistance to antiepileptic drugs. For example, previous studies demonstrated that the blockage of sodium channels induced by carbamazepine (i.e., use-dependent

blocker of voltage-dependent sodium channels) is not detected in tissue obtained from some patients with pharmacoresistant epilepsy [35]. In another study, the modulation of Na^+ currents induced by valproic acid was tested in isolated neurons from patients with and without hippocampal sclerosis. The cellular response to valproic acid was consistent with previous reports in animal models. In spite of the important damage found in patients with mesial sclerosis, they did not demonstrate differences in the biophysical properties of the voltage-gated sodium currents, when compared with the non-sclerotic tissue [36].

It is evident that the in vitro electrophysiology approaches can contribute to trace the underlying cellular mechanisms of epilepsy and the screening of new antiepileptic drugs and their effects. This approach can be used to determine the final diagnosis and subsequent individual therapy. However, it presents relevant limitations such as the scarce spontaneous epileptiform activity found in human brain slices possibly due to the isolation of complex networks from other brain areas.

3 Evaluation of Receptor Changes in Human Brain Tissue

A receptor is a cellular macromolecule, or an assembly of macromolecules, that is involved in chemical signaling between and within cells [37]. Receptors should present at least the following characteristics to identify them: (1) Binding, specific, and saturable ligand binding are hallmarks of receptors. (2) Affinity, the ability of a drug to bind to the specific receptor to form the drug-receptor complex. The dissociation constant (Kd) or affinity of most receptors for their ligands is in the nanomolar or subnanomolar range. (3) Ligand binding to the receptors should be able to activate signaling pathways to trigger physiological responses [38].

The evaluation of the tissue of patients with epilepsy represents a great opportunity to investigate receptor changes associated with this disorder such as number, affinity, binding, and coupling to transduction mechanisms. The knowledge obtained allows the design of new therapeutic strategies to strengthen or inhibit transductional mechanisms [39]. Receptors in the cerebral tissue of patients with epilepsy have been evaluated through different in vitro and in vivo techniques. The method of choice depends upon the aim of the study.

Western blot is a useful procedure to determine changes in the protein expression of the receptors [40]. In general, the methodology for Western blot is as follows: samples of human brain tissue are suspended in lysate buffer containing protease inhibitor cocktail including 50 mM HEPES buffer, 0.05% Triton X-100, 0.62 mM phenylmethylsulfonyl fluoride, and 20 mM sodium molybdate. Proteins from tissue are isolated by

electrophoresis using 8 % SDS-polyacrylamide Tris-glycine gels. Once separated, the proteins are transferred to a membrane of nitrocellulose in which each protein is located in specific bands according to the molecular weight. The membrane is then incubated with specific antibodies to label the protein of interest. The thickness and density of the band corresponds to the amount of protein present [40, 41]. Although the results obtained from Western blot experiments give information about the protein expression of specific receptors, they do not give data about the capacity of these proteins to bind specific agonists and induce transductional mechanisms.

Some in vivo and in vitro techniques are useful to determine receptor binding changes in the brain of patients with epilepsy. Positron emission tomography (PET) is an in vivo procedure that let us identify the distribution and density of specific receptors in specific brain areas of patients with epilepsy (Fig. 3a). This procedure involves the systemic administration of ligands that may label particular receptors. However, be cautious regarding the interpretation of results due to the presence of endogenous ligands in the brain that may compete with the exogenous ligands.

In vitro autoradiography is a useful procedure to label specific receptors and proteins involved in the transductional mechanisms in brain sections obtained from patients with pharmacoresistant epilepsy and submitted to epilepsy surgery. In vitro autoradiography procedures avoid endogenous ligands and give information about the anatomical localization of the receptors (Fig. 3b) [42].

For in vitro autoradiography, brain sections are exposed to ligands labeled with a radioactive isotope and subsequently exposed to a film [43, 44]. The more commonly used isotopes for in vitro autoradiography are the following: ^{3}H gives the highest resolution and is used to label proteins, nucleic acids, and other molecules frequently involved in binding assays; ^{14}C replaces the nonradioactive carbon in order to trace chemical and biochemical reactions involving pharmacological substances; and ^{35}S is used to label proteins, nucleic acids, and amino acids containing a thiol group [45].

The in vitro autoradiography experiments can be carried out using brain sections of patients with pharmacoresistant epilepsy. Initially, the brain sections are washed to remove endogenous ligands and then incubated in a solution containing the specific radioligand at a concentration necessary to occupy 50 % of receptors (Kd) [39]. Nonspecific binding has to be detected in parallel sections from the same tissue and incubated under similar experimental conditions but including a high concentration of a nonlabeled ligand (a concentration 500–1000 higher than that used for the ^{3}H-ligand). Specific binding is obtained from the difference between nonspecific and total binding. After incubation, the sections are rinsed in buffer, dipped into distilled water, and dried under cold air stream. The dried sections are exposed to a sensitive

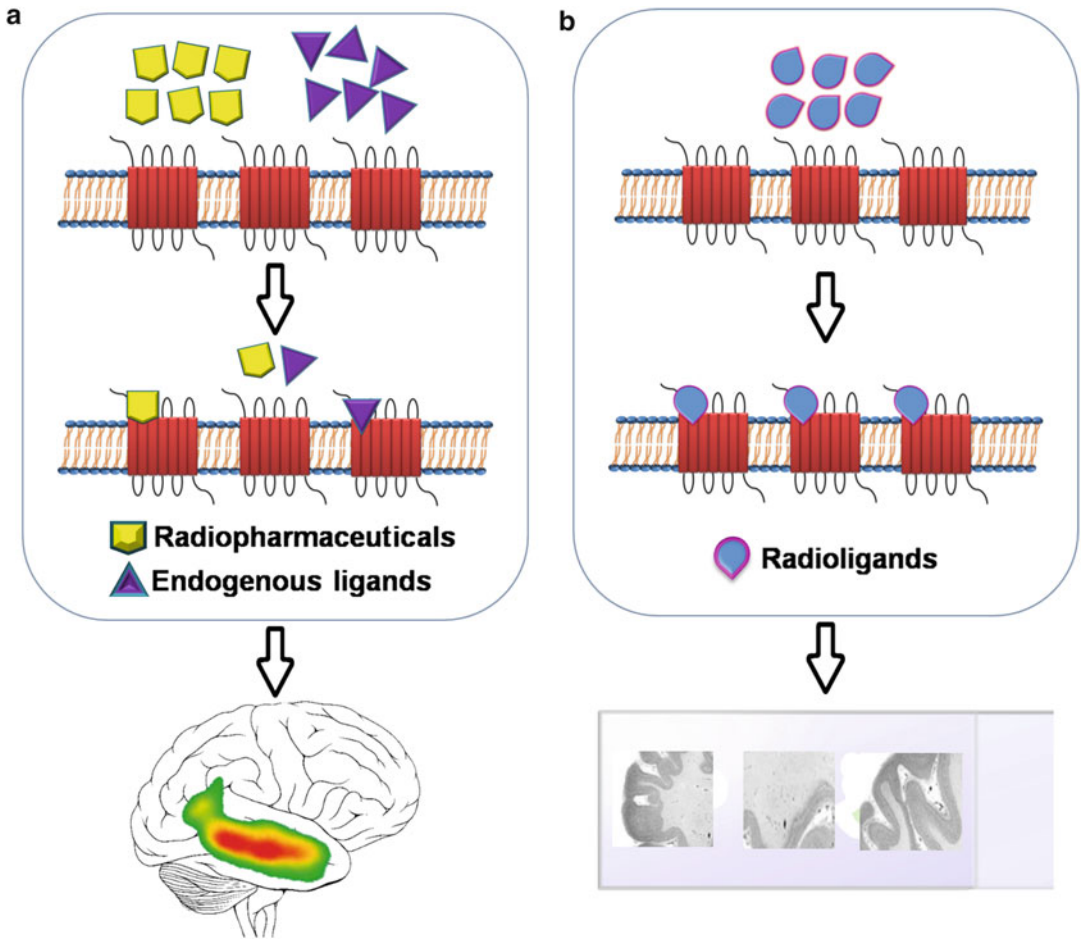

Fig. 3 Schematic representation of in vivo positron emission tomography (PET) (**a**) and in vitro autoradiography (**b**) procedures. Notice that in PET radiolabeled ligands compete with the endogenous ligands for the binding site in the receptors. In contrast, for autoradiography, the radioligand binds directly to the receptors because the endogenous ligands were previously washed

film together with standards of the isotope used. After the exposure period, the film is developed and used to determine optical densities of specific brain areas. The optical density is converted to radioactivity values (dpm/mm^2) and then to amount of receptors (fmol/mg protein) [46–48].

The in vitro autoradiography allows to determine the distribution of receptors labeled with a specific ligand in selected brain areas of patients with pharmacoresistant epilepsy [43, 49]. The results obtained will depend on the affinity and number of receptors labeled. However, in vitro autoradiography is not an appropriate procedure to determine specific characteristics of the receptor binding like maximal binding (Bmax) and affinity (Kd).

Binding of an agonist to its receptor induces conformational changes in the receptor molecule. This situation leads to activation

of the G protein, a process during which the α subunit replaces the GDP with GTP. Functional autoradiography is a technique used to know if the receptor is capable to activate this reaction and subsequent transductional signaling pathways. During the functional assay, the tissue is exposed to [^{35}S]-GTPγS that is resistant to hydrolysis. This situation provokes that [^{35}S]-GTPγS replaces the GDP upon receptor activation and remains irreversibly bound to the G proteins [39, 42].

To perform functional autoradiography, the sections are initially dipped in assay buffer (50 mM Tris, 3 mM $MgCl_2$, 0.2 mM EGTA, 100 Mm NaCl, pH 7.4) at 25 °C for 10 min and then incubated with 2 mM GDP in assay buffer for 30 min at 25 °C. Thereafter, the process involves different assays in parallel sections under different conditions (Table 1). The specific binding results from the difference between the values obtained from the basal and nonspecific binding assays. At the end of each assay, slides are rinsed twice, 2 min each in 50 mM Tris–HCl buffer and once in deionized H_2O at 4 °C. Slices are dried overnight and exposed to film for 5 days in film cassettes containing [^{14}C] microscales. A standard curve is generated from [^{14}C] microscales values. Optical density readings are converted into nanocuries of [^{35}S] per milligram of tissue. Net agonist-stimulated [^{35}S]-GTPγS binding is calculated by subtracting basal binding (obtained in absence of agonist) from agonist-stimulated binding [46, 50].

A main limitation of the [^{35}S]-GTPγS autoradiography is that not all the G proteins can be detected with this method due to their low number or efficiency. In addition, it results difficult to identify the types of G proteins labeled with [^{35}S]-GTPγS [42, 51].

Binding assays in membranes are valuable procedures to determine important receptor characteristics such as Bmax (propor-

Table 1
Experimental conditions to implement functional autoradiography

Basal activity	G proteins are evaluated in presence of 2 mM GDP and 40 pM [^{35}S]-GTPγS. No agonists are included in the assay
Agonist-stimulated [^{35}S]-GTPγS	This step is carry out in presence of 2 mM GDP, 40 pM [^{35}S]-GTPγS, and a receptor agonist at a concentration necessary to produce maximal stimulation (μM) of the G proteins. The [^{35}S]-GTPγS labels the G proteins activated as consequence of the agonist
Antagonist blockade of agonist-stimulated [^{35}S]-GTPγS	A parallel assay has to be focused to evaluate the specificity of the receptor that activates the labeled G protein. The assay is carry out according to the conditions described for the agonist-stimulated [^{35}S]-GTPγS but containing a specific antagonist at a concentration that blocks >90% of the receptor-induced G protein activation
Nonspecific binding	The assay is similar to the agonist-stimulated [^{35}S]-GTPγS but with an excess of unlabelled GTPγS (10 μM).

tional to the number of receptors present in the brain tissue), Kd (equilibrium dissociation constant), Emax (concentration of an agonist to produce maximal stimulation), and potency of stimulation (EC_{50}).

Binding assays can be used to determine changes in Bmax and Kd, and [^{3}H] is the most frequently used isotope for this purpose. For this procedure, brain tissue (~50 mg) is thawed and homogenized into a mixture of ice-cold 50 mM Tris–HCl and 1 mM EGTA, pH 7.4. After centrifugation (13,000 rpm, 20 min, 4 °C), the pellet is resuspended in 10 ml of 50 mM Tris–HCl buffer solution (pH 7.4) and centrifuged again. The resulting pellet (crude membranes) is suspended in incubation buffer (50 mM Tris–HCl, 5 mM $MgCl_2$, pH 7.4). Receptor binding assays are performed in triplicate in 0.5 ml of incubation buffer containing membranes (30–70 μg protein) and in presence of different concentrations of the radioligand with and without a non-labeled ligand. Specific binding is determined by subtracting the binding in the presence of non-labeled ligand from total binding. Incubations are terminated by rapid filtration through Whatman GF/C filters presoaked in 0.3% polyethylenimine. Filters are washed three times with ice-cold 50 mM Tris–HCl buffer (pH 7.4), dried, and then immersed in Sigma-Fluor™ scintillation cocktail (Sigma). Radioactivity is determined using a Beckman LS6000SC liquid scintillation counter. Data are expressed in fmol/mg of tissue.

Binding assays in membranes can be also used to evaluate the ligand-induced activation of G proteins subsequent to the receptor stimulation (Emax and EC_{50}). The binding assays are as follows: brain tissue (~50 mg) is thawed and individually homogenized in 10 mM Tris–HCl solution containing 1 mM EGTA (pH 7.4) and centrifuged (10 min, 1800 rpm, 4 °C). The supernatants are collected and centrifuged at 13,000 rpm (20 min, 4 °C) and the pellet obtained is incubated (30 min, 30 °C) in 5 ml of assay buffer (50 mM Tris–HCl, 100 mM NaCl, 0.2 mM EDTA, pH 7.4). The incubation is stopped with the addition of 10 ml of ice-cold buffer. The obtained membranes are incubated (60 min, 60 °C) in 0.5 ml of assay buffer containing 0.02% bovine serum albumin, [^{35}S]-GTPγS (50 pM), and increasing concentrations (10^{-10}–10^{-6} M) of a selective receptor agonist in the presence of excess GDP (10 μM). Basal binding is measured in the absence of the tested compound. Nonspecific binding is determined in the presence of 20 μM unlabeled GTPγS and subtracted from total binding to calculate the specific binding. The reaction is initiated by adding [^{35}S]-GTPγS and terminated by filtration of the samples through Whatman GF/B glass fiber filters. Filters are washed three times with ice-cold 50 mM Tris–HCl buffer (pH 7.4), dried, and dipped in Sigma-Fluor™. Finally, bound radioactivity is determined using a liquid scintillation counter. Data are subjected to nonlinear regression analysis of concentration effect curves performed by Prism (GraphPad Software) to determine Emax

and EC_{50} values. Data are expressed in percentage of stimulation or in fmol/mg of protein [52].

One important limitation of the binding assays is that the changes evaluated correspond to big areas of the cerebral tissue. This procedure does not allow the evaluation of small brain areas.

4 Genomic Analysis of Brain Tissue from Patients with Pharmacoresistant Epilepsy

According to the dogma of molecular biology (Fig. 4), nucleic acids (DNA and RNA) and proteins, through the processes of replication, transcription, and translation, are key to perform genomic analyses. These represent the main tools in research, diagnosis, evaluation, treatment, and prognosis of many diseases, including various types of epilepsies. At present, genetic alterations are considered relevant in the development of epilepsy [53] and various types of seizures, such as juvenile myoclonic epilepsy [54]. It is important to consider that some of the studies that demonstrate the role of genes in the development of epilepsy and the associated pathological processes (e.g., drug resistance and hippocampal sclerosis) have been carried out using tissue obtained from patients subjected to epilepsy surgery [55].

When the cerebral tissue is removed from the human body, the neuronal cells begin to die. During this process, there is an activation of specific caspases that in turn activate endo- and exonucleases responsible for the degradation of RNA [56]. In contrast with DNA, which has a double-stranded arrangement and a chemical structure that favors its preservation, RNA is more labile and sensitive to degradation due to its single-stranded conformation while the presence of ribose facilitates the reaction with oxygen [57]. Therefore, the experimental conditions to manipulate postsurgical

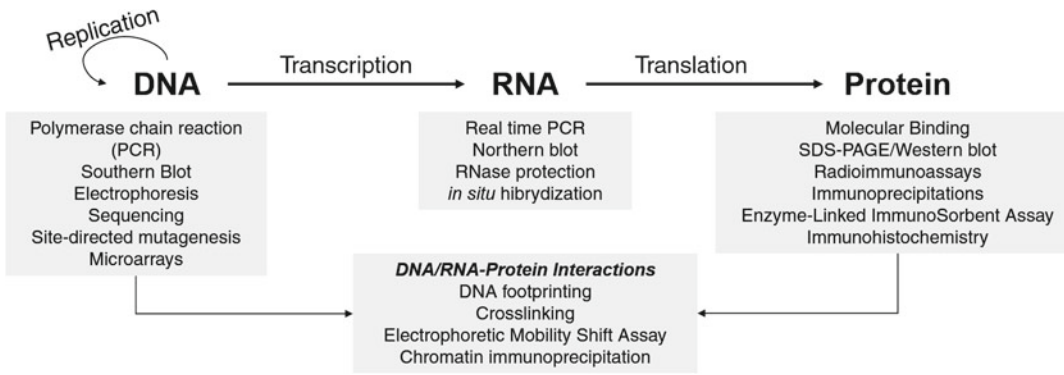

Fig. 4 Central dogma of molecular biology. Notice the different methodological approaches that can be carried out to evaluate DNA, RNA, and protein expression

tissue are essential to maintain the quality of proteins and nucleic acids and give validity and reliability to the results obtained [58].

In practice, after surgical resection, the epileptic tissue should be immediately frozen and stored at −80 °C for the subsequent mRNA evaluation. Low temperatures (−60 °C or lower) prevent the activation of caspases and hence the degradation of nucleic acids and proteins [59]. However, RNA seems to maintain some reactivity at low temperatures. RNases and some ribozymes are still active at −20 °C and −70 °C, respectively [60, 61]. At present, there are commercial compounds such as RNAlater® (Qiagen) which are useful to stabilize and protect the structure of cellular RNA and prevent its oxidation. Formalin fixation with paraffin embedding represents other procedure for mRNA preservation and maintaining histology [62]. With this procedure, the proteins are kept whole and the DNA can be isolated with very good quality for several years after [63–65]. Other important factor for consideration is the period of storage. The integrity of nucleic acids and proteins are compromised when the tissue is stored for long periods after the surgical resection.

Some considerations are relevant to keep in mind when isolating nucleic acids or proteins obtained from cerebral tissue: (1) one must work on ice and under complete sterile conditions; (2) the use of inhibitors of endo- and exonucleases in the case of nucleic acids is essential, and protease inhibitors in the case of proteins, (3) the material must be treated with diethylpyrocarbonate (DEPC) to 0.1 %, which is a potent inhibitor of RNase [66].

The nucleic acid isolation of cerebral tissue from patients with epilepsy is often achieved by common protocols. However, isolation of DNA, RNA, and proteins has to be done separately if we want to preserve the quality of the molecules. For DNA isolation, it is necessary to use extraction with organic solvents such as ethanol or acetone. This procedure preserves the DNA of high molecular weight. The RNA is isolated by phenol–chloroform extraction in addition to commercial solutions of guanidine isothiocyanate and ammonium thiocyanate (e.g., TrizolThermoFisher Sci.). Proteins are isolated through different buffers containing detergent and ionic salts. Once the RNA is obtained, it is necessary to confirm its purity and integrity. The purity is determined by commonly used spectrophotometric assays, according to the ratio 260/280 nm. Using this procedure, an index of 1.8 for DNA and RNA 2 indicates high purity, whereas lower values indicate contamination with proteins or organic solvents. The integrity is determined by electrophoretic assays using 1 % agarose gel. Using this procedure, a single band must be observed for DNA (no sweep), while for the RNA, it is common to detect electrophoretic migration patterns of the 28S, 18S, 5.8S, and 5S ribosomal subunit. These values represent the RNA integrity number (RIN) [67]. Pure samples of DNA should

be stored in solution because their dehydration leads to denaturation. In contrast, the RNA is best preserved dehydrated [59].

Concerning epilepsy and regardless of the level and the techniques used, the study of patients with this disorder is focused on the search for the "epilepsy gene." Unfortunately this approach is utopian because it is impossible to attribute the cause of epilepsy to a single gene since it represents a multifactorial disorder from a physiological point of view [68]. Also, the molecular analysis of genes has to be associated with different factors, such as type of epilepsy, the epileptic focus, the treatments administered prior to surgical resection, whether or not it is drug resistant, and other clinical variables.

Despite the plurality in studies, when a search in PubMed (NCBI) is performed with the words "epilepsy and genes in humans," they found only 6285 items, in which research ranges from the characterization and analysis of gene expression (the gene encoding the $GABA_A$ receptor subunit δ [69]) to variants associations (atypical variants, p.G257R, p.R323Q, p.I389V in $GABA_A$ receptor associated with Rolandic epilepsy [70]), polymorphisms and mutations (the SNP c.3435C > T in the MDR1 gene encoding the P-glycoprotein transporter [71]), identification and global study of microRNAs (miRNAs such as miR-204 and miR-218 that are downregulated in patients with TLE [72]), identification of biomarkers (TGFβ1 that is increased in CSF of patients with drug-resistant epilepsy [73]), epigenomic analysis (hypermethylation-associated genes observed in neurotransmission and synaptic transmission in patients with TLE [74]), and inheritance (the deletion of 18q21.32 relatives inherited from a family with cases of idiopathic epilepsies [75]). Thanks to all the studies using tissue of patients with epilepsy, many genetic aspects of the disease have been identified. However, there is still a long way to go in understanding the mechanisms. An undeniable fact is that studies of gene expression have opened the door to a new era where the final goal is the optimization and customization of treatments, thus improving the quality of life of patients.

5 Autopsy and Control Human Tissue

A good research design should always consider a control condition to eliminate variables that could lead to the wrong conclusion. The evaluation of human brain tissue of patients with epilepsy implies the analysis of control tissue obtained from neurologically healthy subjects. However, this situation is difficult to obtain.

Appropriate controls represent a crucial situation that must be addressed when human tissue is used for electrophysiology recordings [76]. It is clear that normal tissue samples cannot be used for comparison for obvious ethical causes. Resected samples of non-epileptic patients due to removal of deeply laying tumors have been

Table 2
Factors that affect the quality of the brain samples for molecular studies in epilepsy

Antemortem	Postmortem
Diseases	Postmortem interval (PMI)
Premedication	Structural alterations of the pathophysiology of epilepsy (hippocampal sclerosis and cortical dysplasia)
Fever	pH of the brain or cerebrospinal fluid
Hypoxia-ischemia	Ambient temperature in the postmortem period
Acidosis	Harvesting procedures
Agonal conditions	Storage temperature
	Accidental or systematic thawing and refreezing

used as "control tissue" [65]. Nevertheless, this tissue is not adequate for comparison because it comes from patients with a different pathology.

Many studies include the evaluation of autopsy samples obtained from subjects who died of different causes and without evidence of neurological illness. However, it is important to consider various antemortem and postmortem factors that can directly impact on the quality and therefore the validity of the results obtained from autopsy samples. For example, the evaluation of nucleic acids from autopsies is particularly difficult (Table 2) [76, 77]. Indeed, there are controversial studies indicating that the RNA of the brain tissue is stable and preserves integrity even several hours after death [78, 79], while other studies suggest a rapid and definitive RNA degradation as the postmortem interval progresses [76, 80].

6 Concluding Remarks

Certain epilepsy animal models have received great acceptance as they exhibit similar development of spontaneous seizures and a spectrum of histological changes like those of showed by patients with epilepsy [81]. However, the study of brain tissue obtained from patients with pharmacoresistant epilepsy provides valuable information about human epilepsy pathophysiology [22] and represents an excellent tool for individual diagnosis and specialized treatment. Indeed, correlations between the results obtained and different clinic data from the patients with pharmacoresistant epilepsy represent a valuable procedure to identify the possible influence of relevant conditions that can be manipulated to modify the expression of the disease.

Acknowledgments

We thank Francia Carmona for her technical assistance and the National Council for Sciences and Technology of Mexico for the support provided for developing this work (grant 220365 (LR) and scholarships 232762 (LL), 243422 (IB), and 377494 (VN)) and by the Fogarty International Center of the National Institutes of Health (Award Number R21TW009384).

References

1. Köhling R, Avoli M (2006) Methodological approaches to exploring epileptic disorders in the human brain in vitro. J Neurosci Methods 155:1–19. doi:10.1016/j.jneumeth.2006.04.009
2. Ngugi AK, Bottomley C, Kleinschmidt I et al (2010) Estimation of the burden of active and life-time epilepsy: a meta-analytic approach. Epilepsia 51:883–890. doi:10.1111/j.1528-1167.2009.02481.x
3. PAHO/WHO (2013) Report on Epilepsy in Latin America and the Caribbean. http://www.who.int/mental_health/neurology/epilepsy/paho_report_2013.pdf. Accessed on 27 Mar 2016
4. Magiorkinis E, Sidiropoulou K, Diamantis A (2010) Hallmarks in the history of epilepsy: epilepsy in antiquity. Epilepsy Behav 17:103–108. doi:10.1016/j.yebeh.2009.10.023
5. Engel J, Wiebe S, French J et al (2003) Practice parameter: temporal lobe and localized neocortical resections for epilepsy: report of the Quality Standards Subcommittee of the American Academy of Neurology, in association with the American Epilepsy Society and the American Association of Neuro. Neurology 60:538–547. doi:10.1212/01.WNL.0000055086.35806.2D
6. Jobst BC, Cascino GD (2015) Resective epilepsy surgery for drug-resistant focal epilepsy. JAMA 313:285–293. doi:10.1001/jama.2014.17426
7. Tonini C, Beghi E, Berg AT et al (2004) Predictors of epilepsy surgery outcome: a meta-analysis. Epilepsy Res 62:75–87. doi:10.1016/j.eplepsyres.2004.08.006
8. Ledri M, Sørensen AT, Madsen MG (2015) Differential effect of neuropeptides on excitatory synaptic transmission in human epileptic hippocampus. J Neurosci 35:9622–9631. doi:10.1523/JNEUROSCI.3973-14.2015
9. Kivi A, Lehmann TN, Kovács R et al (2000) Effects of barium on stimulus-induced rises of [K+]o in human epileptic non-sclerotic and sclerotic hippocampal area CA1. Eur J Neurosci 12:2039–2048.doi:10.1046/j.1460-9568.2000.00103.x
10. Mccormick DA (1989) GABA as an inhibitory neurotransmitter in human cerebral cortex. J Neurophysiol 62:1018–1027
11. Cohen I, Navarro V, Clemenceau S et al (2002) On the origin of interictal activity in human temporal lobe epilepsy in vitro. Science 298:1418–1421. doi:10.1126/science.1076510
12. Kato H, Ito Z, Matsuoka S et al (1973) Electrical activities of neurons in the sliced human cortex *in vitro*. Electroencephalogr Clin Neurophysiol 35:457–462
13. Roseti C, Fucile S, Lauro C et al (2013) Fractalkine/CX3CL1 modulates GABAA currents in human temporal lobe epilepsy. Epilepsia 54:1834–1844. doi:10.1111/epi.12354
14. Foehring RC, Waters RS (1991) Contributions of low-threshold calcium current and anomalous rectifier (Ih) to slow depolarizations underlying burst firing in human neocortical neurons in vitro. Neurosci Lett 124:17–21. doi:10.1016/0304-3940(91)90812-8
15. Thom M, Mathern GW, Cross JH et al (2010) Mesial temporal lobe epilepsy: how do we improve surgical outcome? Ann Neurol 68:424–434. doi:10.1002/ana.22142
16. Margerison JH, Corsellis JA (1966) Epilepsy and the temporal lobes. Brain 89:499–530
17. Blümcke I, Coras R, Miyata H et al (2012) Defining clinico-neuropathological subtypes of mesial temporal lobe epilepsy with hippocampal sclerosis. Brain Pathol 22:402–411. doi:10.1111/j.1750-3639.2012.00583.x
18. Ward AA, Thomas LB (1955) The electrical activity of single units in the cerebral cortex of man. Electroencephalogr Clin Neurophysiol 7:135–136. doi:10.1016/0013-4694(55)90067-5
19. Dietrich D, Kral T, Clusmann H et al (2002) Presynaptic group II metabotropic glutamate receptors reduce stimulated and spontaneous transmitter release in human dentate gyrus. Neuropharmacology 42:297–305. doi:10.1016/S0028-3908(01)00193-9
20. Köhling R, Schwartzkroin PA, Avoli M (2006) Studying epilepsy in the human brain in vitro.

In: Pitkänen A, Schwartzkroin PA, Moshé SL (eds) Models of seizures and epilepsy, 1st edn. Elsevier, Burlington, VT

21. Avoli M, Drapeau C, Louvel J et al (1991) Epileptiform activity induced by low extracellular magnesium in the human cortex maintained in vitro. Ann Neurol 30:589–596. doi:10.1002/ana.410300412
22. Schwartzkroin PA, Prince DA (1976) Microphysiology of human cerebral cortex studied in vitro. Brain Res 115:497–500. doi:10.1016/0006-8993(76)90366-8
23. Avoli M, Olivier A (1989) Electrophysiological properties and synaptic responses in the deep layers of the human epileptogenic neocortex in vitro. J Neurophysiol 61:589–606
24. Koch UR, Musshoff U, Pannek HW et al (2005) Intrinsic excitability, synaptic potentials, and short-term plasticity in human epileptic neocortex. J Neurosci Res 80:715–726
25. Schwartzkroin PA (1994) Cellular electrophysiology of human epilepsy. Epilepsy Res 17:185–192. doi:10.1016/0920-1211(94)90049-3
26. Huberfeld G, Wittner L, Clémenceau S et al (2007) Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. J Neurosci 27:9866–9873. doi:10.1523/jneurosci.2761-07.2007
27. Wittner L, Huberfeld G, Clémenceau S et al (2009) The epileptic human hippocampal cornu ammonis 2 region generates spontaneous interictal-like activity in vitro. Brain 132:3032–3046. doi:10.1093/brain/awp238
28. McCormick DA, Williamson A (1989) Convergence and divergence of neurotransmitter action in human cerebral cortex. Proc Natl Acad Sci U S A 86:8098–8102
29. Milatovic D, Montine TJ, Zaja-Milatovic S et al (2010) Morphometric analysis in neurodegenerative disorders. Curr Protoc Toxicol 12:1–14. doi:10.1002/0471140856.tx1216s43
30. Rosoklija GB, Petrushevski VM, Stankov A et al (2014) Reliable and durable Golgi staining of brain tissue from human autopsies and experimental animals. J Neurosci Methods 15:20–29. doi:10.1016/j.jneumeth.2014.04.006
31. Vreugdenhil M, Hoogland G, van Veelen CWM et al (2004) Persistent sodium current in subicular neurons isolated from patients with temporal lobe epilepsy. Eur J Neurosci 19:2769–2778. doi:10.1111/j.1460-9568.2004.03400.x
32. Rüschenschmidt C, Köhling R, Schwarz M et al (2004) Characterization of a fast transient outward current in neocortical neurons from epilepsy patients. J Neurosci Res 75:807–816. doi:10.1002/jnr.20055
33. O'Connor ER, Sontheimer H, Spencer DD et al (1998) Astrocytes from human hippocampal epileptogenic foci exhibit action potential-like responses. Epilepsia 39:347–354. doi:10.1111/j.1528-1157.1998.tb01386.x
34. Dossi E, Blauwblomme T, Nabbout R et al (2014) Multi-electrode array recordings of human epileptic postoperative cortical tissue. J Vis Exp 26:1–9. doi:10.3791/51870
35. Remy S, Gabriel S, Urban BW et al (2003) A novel mechanism underlying drug resistance in chronic epilepsy. Ann Neurol 53:469–479. doi:10.1002/ana.10473
36. Vreugdenhil M, van Veelen CWM, van Rijen PC (1998) Effect of valproic acid on sodium currents in cortical neurons from patients with pharmaco-resistant temporal lobe epilepsy. Epilepsy Res 32:309–320. doi:10.1016/S0920-1211(98)00061-8
37. Neubig RR, Spedding M, Kenakin T et al (2003) International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXXVIII. Update on terms and symbols in quantitative pharmacology. Pharmacol Rev 55:597–606. doi:10.1124/pr.55.4.4
38. Feener EP, King GL (1998) The biochemical and physiological characteristics of receptors. Adv Drug Deliv Rev 29:197–213
39. Rocha L, Neri-Bazán L, Orozco-Suárez SA et al (2009) Studying receptors in the human epileptic brain using in vitro autoradiography. In: Rocha L (ed) Models of neuropharmacology, 1st ed. Research Signpost, Kerala
40. Mahmood T, Yang PC (2012) Western blot: technique, theory, and trouble shooting. N Am J Med Sci 4:429–434. doi:10.4103/1947-2714.100998
41. Pujols L, Mullol J, Roca-Ferrer J et al (2002) Expression of glucocorticoid receptor alpha- and beta-isoforms in human cells and tissues. Am J Physiol Cell Physiol 283:C1324–C1331. doi:10.1152/ajpcell.00363.2001
42. Sóvágó J, Dupuis DS, Gulyás B et al (2001) An overview on functional receptor autoradiography using [35S]GTPgammaS. Brain Res Brain Res Rev 38:149–164. doi:10.1016/S0165-0173(01)00106-0
43. Solon EG (2015) Autoradiography techniques and quantification of drug distribution. Cell Tissue Res 360:87–107. doi:10.1007/s00441-014-2093-4
44. Sharif N, Eglen R (1993) Quantitative autoradiography: a tool to visualize and quantify receptors, enzymes, transporters, and second messenger systems. In: Sharif N (ed) Molecular imaging in neuroscience: a practical approach, 1st edn. Oxford University Press, Oxford

45. Barthe N, Maîtrejean S, Cardona A (2012) High-resolution beta imaging. In: L'Annunziata MF (ed) Handbook of radioactivity analysis, 3rd edn. Academic, Oxford
46. Rocha L, Orozco-Suarez S, Alonso-Vanegas M et al (2009) Temporal lobe epilepsy causes selective changes in mu opioid and nociceptin receptor binding and functional coupling to G-proteins in human temporal neocortex. Neurobiol Dis 35:466–473. doi:10.1016/j.nbd.2009.06.008
47. Jin CY, Anichtchik O, Panula P (2009) Altered histamine H3 receptor radioligand binding in post-mortem brain samples from subjects with psychiatric diseases. Br J Pharmacol 157:118–129. doi:10.1111/j.1476-5381.2009.00149.x
48. Pollard H, Moreau J, Arrang JM et al (1993) A detailed autoradiographic mapping of histamine H3 receptors in rat brain areas. Neuroscience 52:169–189
49. Waeber C, Chiu ML (1999) In vitro autoradiographic visualization of guanosine-5′-O-(3-[35S]thio) triphosphate binding stimulated by sphingosine 1-phosphate and lysophosphatidic acid. J Neurochem 73:1212–1221. doi:10.1046/j.1471-4159.1999.0731212.x
50. Potier S, Sénécal J, Chabot JG (2005) A pentylenetetrazole-induced generalized seizure in early life enhances the efficacy of muscarinic receptor coupling to G-protein in hippocampus and neocortex of adult rat. Eur J Neurosci 21:1828–1836. doi:10.1111/j.1460-9568.2005.04026.x
51. Sim L, Selley D, Childers S (1997) Autoradiographic visualization in brain of receptor-G protein coupling using [35S] GTPγS binding. In: Challiss RAJ (ed) Receptor signal transduction protocols, 1st edn. Humana Press, Totowa, NJ
52. Cuellar-Herrera M, Velasco AL, Velasco F et al (2012) Mu opioid receptor mRNA expression, binding, and functional coupling to G-proteins in human epileptic hippocampus. Hippocampus 22:122–127. doi:10.1002/hipo.20891
53. Panayiotopoulos CP (2012) The new ILAE report on terminology and concepts for the organization of epilepsies: critical review and contribution. Epilepsia 53:399–404. doi:10.1111/j.1528-1167.2011.03381.x
54. Helbig I, Hartmann C, Mefford HC (2013) The unexpected role of copy number variations in juvenile myoclonic epilepsy. Epilepsy Behav 28:S66–S68. doi:10.1016/j.yebeh.2012.07.005
55. Mirza N, Vasieva O, Marson AG et al (2011) Exploring the genomic basis of pharmacoresistance in epilepsy: an integrative analysis of large-scale gene expression profiling studies on brain tissue from epilepsy surgery. Hum Mol Genet 20:4381–4394. doi:10.1093/hmg/ddr365
56. Wagner E, Lykke-Andersen J (2002) mRNA surveillance: the perfect persist. J Cell Sci 115:3033–3038
57. Elliott D, Ladomery M (2011) Molecular biology of RNA. Oxford University Press, Oxford
58. Neumeister VM (2014) Tools to assess tissue quality. Clin Biochem 47:280–287. doi:10.1016/j.clinbiochem.2014.02.016
59. Fabre AL, Colotte M, Luis A et al (2014) An efficient method for long-term room temperature storage of RNA. Eur J Hum Genet 22:379–385. doi:10.1038/ejhg.2013.145
60. Ma S, Huang Y, van Huystee RB (2004) Improved plant RNA stability in storage. Anal Biochem 326:122–124. doi:10.1016/j.ab.2003.10.026
61. Seyhan AA, Burke JM (2000) Mg2+-independent hairpin ribozyme catalysis in hydrated RNA films. RNA 6:189–198
62. Su JM, Perlaky L, Li XN et al (2004) Comparison of ethanol versus formalin fixation on preservation of histology and RNA in laser capture microdissected brain tissues. Brain Pathol 14:175–182. doi:10.1111/j.1750-3639.2004.tb00050.x
63. Shibata D, Martin WJ, Arnheim N (1988) Analysis of DNA sequences in forty-year-old paraffin-embedded thin-tissue sections: a bridge between molecular biology and classical histology. Cancer Res 48:4564–4566
64. Foss RD, Guha-Thakurta N, Conran RM et al (1994) Effects of fixative and fixation time on the extraction and polymerase chain reaction amplification of RNA from paraffin-embedded tissue: comparison of two housekeeping gene mRNA controls. Diagn Mol Pathol 3:148–155
65. Kashofer K, Viertler C, Pichler M et al (2013) Quality control of RNA preservation and extraction from paraffin-embedded tissue: implications for RT-PCR and microarray analysis. PLoS One 8:e70714. doi:10.1371/journal.pone.0070714
66. Williams MA (2010) Stabilizing the code-methods to preserve RNA prove their worth. Biomark Insights 5:139–143. doi:10.4137/BMI.S6094
67. Schroeder A, Mueller O, Stocker S et al (2006) The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol 7:3. doi:10.1186/1471-2199-7-3
68. Stafstrom CE, Tempel BL (2000) Epilepsy genes: the link between molecular dysfunction and pathophysiology. Ment Retard Dev Disabil Res Rev 6:281–292. doi:10.1002/1098-2779(2000)6:4<281::AID-MRDD7>3.0.CO;2-9

69. Windpassinger C, Kroisel PM, Wagner K et al (2002) The human γ-aminobutyric acid A receptor delta (GABRD) gene: molecular characterisation and tissue-specific expression. Gene 292:25–31. doi:10.1016/S0378-1119(02)00649-2
70. Reinthaler EM, Dejanovic B, Lal D et al (2015) Rare variants in γ-aminobutyric acid type A receptor genes in rolandic epilepsy and related syndromes. Ann Neurol 77:972–986. doi:10.1002/ana.24395
71. Manna I, Gambardella A, Labate A et al (2015) Polymorphism of the multidrug resistance 1 gene MDR1/ABCB1 C3435T and response to antiepileptic drug treatment in temporal lobe epilepsy. Seizure 24:124–126. doi:10.1016/j.seizure.2014.09.010
72. Kaalund SS, Venø MT, Bak M et al (2014) Aberrant expression of miR-218 and miR-204 in human mesial temporal lobe epilepsy and hippocampal sclerosis-convergence on axonal guidance. Epilepsia 55:2017–2027. doi:10.1111/epi.12839
73. Yu W, Zou Y, Du Y et al (2014) Altered cerebrospinal fluid concentrations of TGFβ1 in patients with drug-resistant epilepsy. Neurochem Res 39:2211–2217. doi:10.1007/s11064-014-1422-z
74. Miller-Delaney SF, Bryan K, Das S et al (2015) Differential DNA methylation profiles of coding and non-coding genes define hippocampal sclerosis in human temporal lobe epilepsy. Brain 138:616–631. doi:10.1093/brain/awu373
75. Tiron C, Campuzano O, Pérez-Serra A et al (2015) Further evidence of the association between LQT syndrome and epilepsy in a family with KCNQ1 pathogenic variant. Seizure 25:65–67. doi:10.1016/j.seizure.2015.01.003
76. Birdsill AC, Walker DG, Lue L et al (2011) Postmortem interval effect on RNA and gene expression in human brain tissue. Cell Tissue Bank 12:311–318. doi:10.1007/s10561-010-9210-8
77. Trabzuni D, Ryten M, Walker R et al (2011) Quality control parameters on a large dataset of regionally dissected human control brains for whole genome expression studies. JNeurochem119:275–282.doi:10.1111/j.1471-4159.2011.07432.x
78. Ervin JF, Heinzen EL, Cronin KD et al (2007) Postmortem delay has minimal effect on brain RNA integrity. J Neuropathol Exp Neurol 66:1093–1099. doi:10.1097/nen.0b013e31815c196a
79. Durrenberger PF, Fernando S, Kashefi SN et al (2010) Effects of antemortem and postmortem variables on human brain mRNA quality: a BrainNet Europe study. J Neuropathol Exp Neurol 69:70–81. doi:10.1097/NEN.0b013e3181c7e32f
80. Beach T, Sue L, Walker DG et al (2008) The sun health research institute brain donation program: description and experience, 1987–2007. Cell Tissue Bank 9:229–245. doi:10.1007/s10561-008-9067-2
81. Sharma AK, Reams RY, Jordan WH et al (2007) Mesial temporal lobe epilepsy: pathogenesis, induced rodent models and lesions. Toxicol Pathol 35:984–999. doi:10.1080/01926230701748305