Electrophysiological properties and carbamazepine sensitivity of epileptic human cortical neurons

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There are between 20 and 30 drugs available for the treatment of seizures but despite this ~30% of the epilepsy population remains unresponsive to treatment. The underlying mechanisms behind drug resistance in temporal lobe epilepsy (TLE) have not been completely identified. The purpose of this study was to determine if distinct types of neuronal firing patterns occurring in human epileptic cortex are altered by carbamazepine (CBZ). We used whole-cell patchclamp techniques combined with intracellular labeling to electrophysiologically and morphologically characterize neuronal populations in resected cortical tissue from patients with drug resistant epilepsy. We then determined if cells were uniformly resistant to carbamazepine or whether only a subset did not respond. Cortical spiking patterns were segregated in six main clusters: adapting high frequency cluster 1 and 2 (AHF1 and AHF2), adapting low frequency cluster 1 and 2 (ALF1 and ALF2), strongly adapting low frequency group (sALF) and one spike cluster (OS). A morphological analysis showed that some spiking patterns tend to be associated with specific neuronal morphology. OS group included only pyramidal cells while adapting high frequency groups (AHF1 & AHF2) displayed typical interneuron phenotype. Finally, we found that CBZ does not uniformly suppress neuronal activity as only ~27% of interneurons and ~40% of pyramidal cells were carbamazepine insensitive. These data indicate that in humans with DRE there is a heterogeneous CBZ insensitivity in all subpopulations of neurons.

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Introduction

A significant number of TLE patients are resistant to anti-epileptic drug (AED) therapy [1-4]. Although different hypotheses like Multi Drug Transporter (increased expression/function of multidrug transporters) or Target sensitivity (changes in molecular drug targets for AEDs) have been suggested to account for drug resistant epilepsy (DRE) (see review [5]), to date neither of these theories have been conclusively proved or refuted.

Carbamazepine (CBZ) is an example of a highly used antiepileptic drug (AED) that can become non-effective in many patients with chronic epilepsy [6, 7]. The primary mechanism of CBZ is thought to be related to its action on voltage-gated Na⁺ channels which are integral to the generation of seizure discharges [8]. Carbamazepine is known to inhibit voltage-dependent Na⁺ currents by two mechanisms; one is activity- or use-dependent [5, 8] while the other is voltage-dependent [9, 10]. Use-dependent block preferentially reduces Na⁺ channel availability during high-frequency firing. This mechanism is thought to be a key factor that predicts and accounts for the efficacy of this AED. This inability to follow a high frequency excitatory input is primarily due to the pronounced slowing of Na⁺ channel recovery from inactivation [8]. This mechanism of action is not unique to carbama-
epileptic human cortical neurons

Cezar Gavrilovici, et al.

Epileptic human cortical neurons

Carbamazepine as other AEDs such as phenytoin, lamotrigine and oxcarbazepine all reduce the excitability of Na\(^+\) channels during high-frequency excitatory drive \(^{[19]}\), and this activity is thought to explain their clinical efficacy. However less is known about how CBZ becomes non-effective in many patients with chronic epilepsy and whether there is a uniform loss of sensitivity for carbamazepine or only specific neuronal populations are affected. Thus, one of the goals of our study was to determine if distinct types of neuronal firing patterns occurring in human epileptic cortex are altered by carbamazepine (CBZ).

In spite of some efforts made to characterize the functional properties of neurons in human epileptic cortex \(^{[11-16]}\) there is still a need for a more detailed functional classification and analysis of electrophysiological properties of neurons. Thus, the purpose of this study is twofold: first we wished to examine the functional diversity of human cortical firing and second to determine whether carbamazepine insensitivity is uniform (affecting all cell types), restricted to one cell type or affecting only a sub set of many types. Understanding what particular subpopulation(s) of neurons are resistant to drugs like CBZ is important as it may represent novel pharmacological targets for new AEDs.

**Experimental Procedures**

**Patients**

All procedures on human tissue were approved by the ethics committee of the University of Western Ontario. Appropriate patients with intractable epilepsy who underwent surgery for resection of their epileptic focus were identified before surgery and informed consent was obtained to allow this tissue to be used for these studies. The identity of patient was not used. Patient age, gender and area from which the tissue was collected are summarized in Table 1.

**Tissue collection, preservation, slicing procedures and maintenance**

No tissue was removed for this research that would not normally be excised during routine surgery. Gathering and processing of the human samples are as follows and are based on established procedures \(^{[17]}\). On the day of surgery a Choline solution was prepared [containing (in mM): Choline Cl 110, KCl 2.5, NaH\(_2\)PO\(_4\) 1.2, NaHCO\(_3\) 25, CaCl\(_2\) 0.5, MgCl\(_2\) 7, Na pyruvate 2.4, ascorbate 1.3, dextrose 20] as previously described \(^{[18, 19]}\). A 1 cm\(^3\) region was removed from an electrographically active area of cortex (identified by the presence of interictal spikes or seizures) as demonstrated by intraoperative ECoG recording or prior recording using subdural electrodes. Immediately after resection, the excised cortex was dropped into an ice-cold Choline solution (see above) and taken to electrophysiology lab where the tissue was immediately processed for patch clamp recordings. Coronal brain slices (350 \(\mu\)m) from single gyri were performed according to published methodology \(^{[18, 19]}\). All solutions were maintained at pH 7.4 and bubbled with 5%CO\(_2\)/95%O\(_2\) (carbogen).

**Electrophysiology**

Patch electrodes were pulled from borosilicate glass capillaries and filled with K\(^+\)-gluconate solution having a composition (in mM) of: potassium gluconate 147, KCl 1, CaCl\(_2\) 2, HEPES 10, EGTA 10, Glucose 10, MgATP 2, GTP 0.3 (300 mOsm, pH 7.3-7.4) as described in \(^{[20]}\). Whole-cell patch clamp recordings from cortical neurons were made with an EPC 9/2 amplifier (HEKA, Lambrecht, Germany). Series resistance compensations were performed in all recordings. The initial access was < 20 MΩ and compensated by 50-70%. All experiments were performed at 32°C. The excitability of the cells was monitored by means of current clamp protocols (PulseFit v8.0; Heka, Germany). Cell responses were obtained in less than 5 minutes after forming whole-cell configuration by injecting hyperpolarizing and depolarizing current steps (500 ms pulse; 50 pA increments). Input resistance (R\(_i\)) was calculated by linear regression of the current-voltage relationship in response to hyperpolarizing steps (as described in \(^{[21]}\)) using Origin software (Microcal, Northampton, MA). Firing pattern analysis was performed at the current level that produced reliable repetitive firing (twice the firing threshold), in presence of NMDA, AMPA and kainite channel blockers (20 mM 2-amino phosphonovaleric acid, APV & 10 mM dinitroquinoxaline-2,3-dione, DNQX; Research Biochemicals, Natick, MA, USA) as described in \(^{[20]}\). Interspike interval ratio (I\(_{IR}\)) was obtained by dividing the last interspike interval (measured in milliseconds) by the duration of the first interval (as previously described in \(^{[20]}\)). Carbamazepine effect was tested after 10 min of drug perfusion. Final carbamazepine concentration was 50 \(\mu\)M which corresponds to upper therapeutically relevant serum levels \(^{[22-24]}\).

**Cluster Analysis**

Unsupervised cluster analysis was performed on cortical neuronal populations using Ward’s method with z-score normalization and intervals calculated by Euclidian squared distances (SPSS 13, Chicago, Illinois, USA) according to described methodology \(^{[25]}\). The analysis was based on their main electrophysiological
In order to reconstruct the morphology and understand where the recordings were made, patch electrodes included 0.2% Biocytin. After the completion of a recording, the slice was removed from the microscope chamber and fixed in paraformaldehyde solution (4% paraformaldehyde in 0.1 phosphate buffer) for 5–7 days. The sections were washed for 15 min in PBS followed by a 30 min rinse in PBS-TX and 90 min incubation in streptavidin-conjugated Alexa Fluor-594 (5μg/ml; Molecular Probes) at room temperature. After rinsing in PB-TX for 30 min, the slices were mounted on slides for viewing on a confocal microscope. See Supplemental Figure 1 for examples of reconstructed cells.

Image acquisition and morphological reconstruction of patched neurons

Confocal images were taken on an Olympus IX 60 inverted microscope outfitted with a Perkin Elmer Spinning Disk Confocal attachment with a 20X (N.A. = 0.50) objective. The microscope was equipped with a Hamatsu Orca ER CCD camera (1300 X 1030 pixels), and images were acquired in Volocity software (Improvision, Lexington, MA). Each image represents a stack of 40–50 images 0.2 μm apart in the z-plane. For morphological reconstruction of the dendritic arborization of patched neurons, the stacks of confocal images were deconvolved with Auto-Quant software (Auto quant X2, Media Cybernetics, Bethesda, MD) and then processed with Imaris Filament Tracer module in “Surpass mode” (Bitplane, Zurich, Switzerland). Filament tracer creates dendritic arborization patterns based on an algorithm that predicts arborization pathways. These pathways are set up by the user-set criteria of a start point, namely, the size of cell somata, and an end point representing minimum thickness of processes.

Start points were set to 10 μm, and endpoints were set to 1 μm. The resultant filament lines were converted from lines to two-pixel-thick cones. To mark the cell bodies, an “Isosurface” was then created. This process creates a cell body from the stack of images that is then merged with the dendritic morphology.[20, 28] We used criteria for classification of pyramidal versus non-pyramidal cells as previously described[18, 19] and were based on: 1) soma morphology; 2) presence of dendritic spines for pyramidal cells; and 3) spiking properties. Most of our interneuron recordings were in superficial layers (1 and 2) while the recordings in pyramidal cells were done in layer 3.

Results
The purpose of this study was to determine if distinct types of neuronal firing patterns occurring in human epileptic cortex are altered by carbamazepine. The resected mesial temporal lobe epilepsy (MTLE) neocortical tissue did not show any apparent structural damage, but only a mild to moderate degree of gliosis. A total of 107 recordings from human cortical neurons, according to the criteria indicated in Methods, are included in this study.

All recordings could be broadly classified into two types of firings patterns: most cells (93 cells; ~87%) responded by firing tonically throughout the current injection, while the rest (14 cells; ~13%) fired phasically (i.e. they stopped firing before the end of the depolarizing step).

Similar to the regular spiking neurons from other human MTLE cortical recordings \cite{11, 13} we also found that continuously firing neurons (tonic firing) had various degrees of adaptation. The occurrence of adaptation was defined by determining whether the last interspike firing interval was 25% longer than the first interval ($\Delta I_{R} > 1.25$) as previously described \cite{20}.

In our study we did not find any cells displaying bursting firing as this type of firing is not common \cite{12} or was seen only very rarely in slices from human neocortical slices during injection of depolarizing current \cite{11}.

We also found that adapting firing cells fired at both high (> 50 Hz) and low (< 50 Hz) frequencies. An exact (unbiased) functional grouping was provided by cluster analysis. Cluster analysis using neuronal electrophysiological characteristics (see Methods), provided six distinct groups: adapting high frequency cluster 1 (AHF1) and moderate frequency cluster 2 (AHF2), strongly adapting low frequency cluster 3 and 4 (ALF1 and ALF2), strongly adapting low frequency (sALF), and one spike (OS) cluster (OS).

The resting membrane potential (RMP) of neurons ranged from -55 mV to -90 mV, the only significant difference among groups was found in OS neuronal cluster which had the lowest RMP values among other clusters (~ -68 mV, p < 0.05; Fig. 1B).
High firing frequency neurons

High frequency spiking cells (firing frequency; FF > 50 Hz) accounted for about 32% of all recordings (34 of 107 cells; Fig. 1, Fig. 2). They had higher firing frequency and an increased number of spikes per pulse and the smallest firing adaptation (II_R) among all other neuronal groups (Fig. 1B). High frequency spiking neurons segregated in two different clusters termed AHF1 and AHF2. AHF1 neurons were more excitable than those in AHF2 group (Fig. 1B, Fig. 2) as they had an increased input resistance (338.7 ± 19.7 MΩ vs 275.1 ± 21.4 MΩ; p < 0.05; Fig. 1B), firing frequency (102.9 ± 4.8 Hz vs 74.2 ± 4.5 Hz; p < 0.005) and number of spikes evoked by current injection (50 ± 2 vs 27 ± 2; p < 0.005).

Low firing frequency neurons

Low frequency firing patterns (firing frequency; FF < 50 Hz) segregated into four different clusters named adapting low frequency 1 and 2 (ALF1 & ALF2), strongly adapting low frequency (sALF) and one spike cluster (OS). The most common low frequency pattern was the adapting low frequency 2 type (ALF2; FF = 102.9 ± 4.8 Hz; Fig. 1B; Fig. 3). They accounted for about 51% (37 of 73) of the low frequency group and they were 34.5% (37 of 107) of all recordings. Although ALF2 cluster had a lower input resistance and a higher current threshold than ALF1 neurons (I_R = 134.8 ± 6.4 MΩ vs 290.2 ± 12.5 MΩ, p < 0.005; and 136.4 ± 9.8 pA vs 75.0 ± 10.1 pA, p < 0.005; see Fig.1B) they displayed a similar firing frequency. Second most common low firing pattern was represented by both ALF1 and OS neurons (~19% each cluster). Although ALF1 had the highest input resistance among the rest of the low frequency group and they showed a trend to a higher firing frequency and number of spikes (Fig. 1B), the difference did not reached significance value when compared to other low frequency groups (ALF1, sALF and OS clusters had small n values). The main feature of sALF cluster was represented by their adaptation ratio.
which was the highest among all other low- and high frequency clusters (II_R = 3.93 ± 0.21, p < 0.005; Fig. 1B, Fig 3). Finally OS neurons had a lower RMP than ALF1, AHF1 and AHF2) and the characteristic one spike evoked by depolarizing current steps, which placed them at the bottom of firing frequencies found in human cortical slices.

Correlation of electrophysiological properties with morphological attributes

Our study showed that at least in some cases there was a correlation between electrophysiological properties and a specific neuronal type. For example, we found that AHF1, AHF2 clusters were represented only by interneurons while OS group had only pyramidal cells (Fig. 4). However the other clusters (sALF, ALF1 and ALF2) did not show a preference towards the interneuron or pyramidal phenotype (see Fig.4).

Carbamazepine insensitivity in human cortical neuronal populations

We monitored the effect of carbamazepine (50 μM) on 65 cells. Overall we found that ~32% of cells were CBZ insensitive, as this drug did not reduce the number of spikes or firing frequency (11 ± 3 vs 12 ± 3 spikes and 23.6 ± 6.0 vs 24.3 ± 6.3 Hz). In the rest of neuronal population (~68% of cells) CBZ was effective in reducing the number of spikes (from 19 ± 2 to 9 ± 2 spikes after carbamazepine perfusion; p < 0.005).

Next we determined the effect of CBZ on the 6 firing clusters. We found that carbamazepine insensitivity was different among the six clusters (see Fig. 5). For example, while most of neurons in OS cluster were CBZ insensitive (~90%; Fig. 5), in AHF1 and ALF2 clusters only a reduced proportion of cells failed to reduce their spiking in presence of CBZ (11.2% in AHF1 and 4.2% in ALF2 group; Fig. 5).

Finally by monitoring the CBZ effect on interneuron and pyramidal cell populations we found that a similar proportion in these two groups of cells were insensitive to CBZ (27% of interneuron population and 40% of pyramidal cells were CBZ resistant; χ^2 = 0.463; Fig. 6).

Discussion

Our study identifies multiple subpopulations of neurons with different firing patterns and membrane properties in epileptic human cortex that are not uniformly sensitive to CBZ. Based on their electrophysiological properties, cortical neurons were classified in six clusters in concordance with previously developed criteria [27]. Some of the firing clusters were correlated with a specific neuronal type as AHF1 and
Thus as expected there were frequency clusters we found that in two cases (OS and ALF) most of the cells were CBZ sensitive (88% in AHF1 and 66% in AHF2 groups).

Beside these different effects on the six functional clusters, we have also determined that CBZ did not reduce spiking in a subpopulation of pyramidal cells and interneurons (27% of interneuron population and 40% of pyramidal cells were CBZ insensitive). The lack of CBZ effect on ~40% of pyramidal cell might have an negative impact in reducing network activity as their firing remain unaltered and cannot be pharmacologically modulated/ decreased. On the other hand, as ~73% of interneurons are still sensitive to CBZ, their decreased inhibitory output might in fact counteract CBZ’s intended role in damping network activity. Finally, the interneuron population insensitive to CBZ (~ 27%) might also have deleterious effects on network function. One possible explanation here is that CBZ may fail to control the activity of interneurons that target interneurons that are in turn responsible for inhibiting excitatory cells. The result would be an increase in disinhibition and increased excitation and/or ineffective seizure control.

One of the shortcomings of a study like this which relies on obtaining human tissue is that normal controls (age and sex matched, drug free) are rarely obtained. During the course of this study (> 3yrs) we were able to obtain only one control which was tissue isolated from a tumour resection. From this sample we obtained 7 recordings of which only one neuron was insensitive to CBZ (14%). As this sample is not sufficiently powered to do any kind of statistical evaluation we have not included these data in the results. Nevertheless, this rate is about half of what we found in epileptic tissue across all cell types. Thus it seems that epileptic tissue is less sensitive to CBZ than control. This conclusion is supported by results obtained in some animal models of epilepsy where sham rats are uniformly sensitive to CBZ but those treated with pilocarpine were less sensitive [31]. An important difference in these data from ours is that the CA1 neurons were all less sensitive while we found that only a minority of interneurons and pyramidal cells were CBZ insensitive. As well this study showed that dentate gyrus was less sensitive to CBZ than CA1 cells so differing cell types were not equally affected. In another study that isolated dentate gyrus cells from resected human hippocampus CBZ activity was reduced uniformly in all recordings [32]. Our data was obtained from cortical tissue is much more heterogenous that either of these two studies and so it is difficult to determine whether the varying sensitivity is due to recording from differing cells types or that same cell types are differentially sensitive. According to our functional classifications it seems that the latter possibility is more likely. That said, and although we

AHF2 contained only interneurons while OS cluster was represented by pyramidal cells. The remaining clusters (sALF, ALF1 and ALF2) were morphologically heterogenous containing both spiny and pyramidal as well as aspiny nonpyramidal cells. Firing patterns observed in our study correlated with previous observations. For example, high frequency interneuron firing (as seen in our AHF1 and AHF2 clusters) were previously described in human [14] or primate cortex [28]. Likewise, lower firing frequency interneuron types (FF <50 Hz) were previously shown in human temporal neocortex [16]. Pyramidal cells were only found in low frequency clusters, in agreement with data from other studies in human cortex [16, 29]. Thus as expected there was a high degree of functional heterogeneity with regard to excitability and morphology.

The second goal of our study was to identify what kind of neurons fail to have their electrical activity attenuated by carbamazepine. We have found that CBZ does not uniformly suppress neuronal activity. For example, in the low frequency clusters we found that in two cases (OS and ALF) most of the cells (70% and 62.5% respectively) did not change their firing when CBZ was added to the bath. This might correlate with the CBZ mechanism of action which is known to mostly inhibit high frequency but not low frequency firing [10]. However the opposite situation was seen in other low frequency clusters: in ALF2 cluster (but in a lesser manner in sALF group) most of the cells were CBZ sensitive (95% in ALF2 and 60% in sALF). When we analyzed the higher frequency clusters, we found that most of the cells were CBZ sensitive (88% in AHF1 and 66% in AHF2 groups).

Figure 6 Correlation between neuronal type and carbamazepine sensitivity. Vertical bars represent the percent of carbamazepine sensitive (grey) and insensitive cells (black) in each morphological group. Int, interneurons; Pyr, pyramidal cells.
have classified our recordings as best as we can, interneurons in particular are very heterogeneous, varying not only by spiking properties and morphology but also neuropeptide content which could not been done here. Another important distinction from these two past studies is that neither looked at how the spiking properties of these cells were affected. Both were voltage clamp studies that examined the effect CBZ on the inactivation kinetics of sodium current. Here we used current clamp recordings as we were interested in understanding how CBZ may affect the firing properties of the differing kinds of neurons in the brain slices. Overall, in spite of the lack of control recordings, our data is in agreement with these studies showing that in epileptic tissue CBZ is unable to affect sodium channel function in some or all cells.

There are two main hypotheses that attempt to explain DRE. The first is multidrug transporter hypothesis and the second is the altered target hypothesis [33]. The first suggests that DRE occurs because AEDs cannot achieve sufficient concentrations in the brain to be effective. This occurs because of the increased expression of multi drug resistance pumps expressed on the blood brain barrier that actively “empty” the brain of small organic molecules. The second states that DRE occurs due to the alteration of drug target by some chemical modification (phosphorylation, glycosylation etc.). The data reported here support the latter hypothesis as in absence of blood brain barrier CBZ was still ineffective in some cells. This suggests therefore that sodium channel sensitivity is changed by some alteration in structure. This could occur not only as mentioned above but also through the expression of accessory β subunits of the sodium channels [34]. We tested this possibility by QPCR analysis of these tissues but found no changes in the expression levels (unpublished observations). Thus at this point we are left with the conclusion that some other modification is occurring that reduces the activity of CBZ in select populations of neurons.

Summary

Our data indicates that in humans with DRE there is a loss of drug sensitivity in a subpopulation of neurons, both pyramidal and non-pyramidal. Unraveling the mechanism(s) by which this occurs will be a difficult task as it will require identifying structural changes in the sodium channel that occur from a mixed population of cells that are and are not sensitive. Given that this is unlikely, it seems that new drugs developed against sodium channels are required.

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We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Disclosure

None of the authors has any conflict of interest to disclose.

References

1. Spencer DD, Spencer SS. Hippocampal resections and the use of human tissue in defining temporal lobe epilepsy syndromes. Hippocampus 1994; 4: 243-249. http://dx.doi.org/10.1002/hipo.450040305 PMid:7842044
2. Zentner J, Hufnagel A, Wolf HK, Ostertun B, Behrens E, Campos MG, et al. Surgical treatment of temporal lobe epilepsy: clinical, radiological, and histopathological findings in 178 patients. J Neurol Neurosurg Psychiatry 1995; 58: 666-673. http://dx.doi.org/10.1136/jnnp.58.6.666 PMid:7608662 PMCID:PMC1073541
3. Wiebe S, Blume WT, Girvin JP, Eliaziw M. A randomized, controlled trial of surgery for temporal-lobe epilepsy. N Engl J Med 2001; 345: 311-318. http://dx.doi.org/10.1056/NEJM200108023450501 PMid:11484687
4. Clusmann H, Kral T, Fackeldey E, Blumcke I, Helmstaedter C, von Oertzen J, et al. Lesional mesial temporal lobe epilepsy and limited resections: prognostic factors and outcome. J Neurol Neurosurg Psychiatry 2004; 75: 1589-1596. http://dx.doi.org/10.1136/jnnp.2003.024208 PMid:15489392 PMCID:PMC1738802
5. Remy S, Beck H. Molecular and cellular mechanisms of pharmacoresistance in epilepsy. Brain 2006; 129: 18-35. http://dx.doi.org/10.1093/brain/awh682 PMid:1637026
6. Hermanns G, Noachtar S, Tuxhorn I, Holthausen H, Ebner A, Wolf P. Systematic testing of medical intractability for carbamazepine, phenytoin, and phenobarbital or primidone in monotherapy for patients considered for epilepsy surgery. Epilepsia 1996; 37: 675-679. http://dx.doi.org/10.1111/j.1528-1157.1996.tb00632.x PMid:8681900
7. Wieser HG, Hane A. Antiepileptic drug treatment before and after selective amygdalohippocampectomy. Epilepsy Res 2003; 55: 211-223. http://dx.doi.org/10.1016/S0920-1211(03)00116-5
8. Rogawski MA, Loscher W. The neurobiology of antiepileptic drugs. Nat Rev Neurosci 2004; 5: 553-564. PMid:15208697

9. Kuo CC, Chen RS, Lu L, Chen RC. Carbamazepine inhibition of neuronal Na+ currents: quantitative distinction from phenytoin and possible therapeutic implications. Mol Pharmacol 1997; 51: 1077-1083. PMid:9187275

10. Rush AM, Elliott JR. Phenytoin and carbamazepine: differential inhibition of sodium currents in small cells from adult rat dorsal root ganglia. Neurosci Lett 1997; 226: 95-98. http://dx.doi.org/10.1016/S0304-3940(97)00258-9

11. Avoli M, Olivier A. Electrophysiological properties and synaptic responses in the deep layers of the human epileptic neocortex in vitro. J Neurophysiol 1989; 61: 589-606. PMid:2709102

12. Foehring RC, Lorenzon NM, Herron P, Wilson CI. Correlation of physiologically and morphologically identified neuronal types in human association cortex in vitro. J Neurophysiol 1991; 66: 1825-1837. PMid:1812219

13. Avoli M, Mattia D, Siniscalchi A, Perreault P, Tomaiuolo F. Pharmacology and electrophysiology of a synchronous GABA-mediated potential in the human neocortex. Neuroscience 1994; 62: 655-666. http://dx.doi.org/10.1016/0306-4522(94)00467-7

14. Tasker JG, Hoffman NW, Fisher RS, Peacock WJ, Dudek FE. Electrical properties of neocortical neurons in slices from children with intractable epilepsy. J Neurophysiol 1996; 75: 931-939. PMid:8714665

15. Avoli M, Hwa G, Louvel J, Kurcewicz I, Pumain R, Lacaille JC. Functional and pharmacological properties of GABA-mediated inhibition in the human neocortex. Can J Physiol Pharmacol 1997; 75: 526-534. http://dx.doi.org/10.1139/y97-037

16. Menendez dLP, Benavides-Piccione R, Sola R, Pozo MA. Electrophysiological properties of interneurons from intraoperative spiking areas of epileptic human temporal neocortex. Neuroreport 2002; 13: 1421-1425. http://dx.doi.org/10.1097/00001225-200208070-00015

17. Kohling R, Avoli M. Methodological approaches to exploring epileptic disorders in the human brain in vitro. J Neurosci Methods 2006; 155: 1-19. http://dx.doi.org/10.1016/j.jneumeth.2006.04.009 PMid:16753220

18. Gavrilovic C, D’Alfonso S, Dann M, Poulter MO. Kindling-induced alterations in GABA receptor mediated inhibition and neurosteroid activity in the piriform cortex of rat. Eur J Neurosci 2006; 24: 1373-1384. http://dx.doi.org/10.1111/j.1460-9568.2006.05012.x PMid:16987222

19. McIntyre DC, Hutcheon B, Schwabe K, Poulter MO. Divergent GABA(A) receptor-mediated synaptic transmission in genetically seizure-prone and seizure-resistant rats. J Neurosci 2002; 22: 9922-9931. PMid:12427849

20. Gavrilovic C, Pollock E, Everest M, Poulter MO. The loss of interneuron functional diversity in the piriform cortex after induction of experimental epilepsy. Neurobiol Dis 2012; 48: 317-328. http://dx doi.org/10.1016/j.nbd.2012.07.002 PMid:22801084

21. Dietrich D, Podlogar M, Ortmanns G, Clusmann H, Kral T. Calbindin-D28K content and firing pattern of hippocampal granule cells in amygdala-kindled rats: a perforated patch-clamp study. Brain Res 2005; 1032: 123-130. http://dx.doi.org/10.1016/j.brainres.2004.10.060 PMid:15680950

22. Strandjord RE, Johannessen SI. Single-drug therapy with carbamazepine in patients with epilepsy: serum levels and clinical effect. Epilepsia 1980; 21: 655-52. http://dx.doi.org/10.1111/j.1528-1157.1980.tb04319.x

23. Semah F, Gimenez F, Longer E, Laplane D, Thuiller A, Baulac M. Carbamazepine and its epoxide: an open study of efficacy and side effects after carbamazepine dose increment in refractory partial epilepsy. Ther Drug Monit 1994; 16: 537-540. http://dx.doi.org/10.1097/00007691-199412000-00001 PMid:7878690

24. Neels HM, Sierens AC, Naelaerts K, Scharpe SL, Hatfield GM, Lambert WE. Therapeutic drug monitoring of old and newer anti-epileptic drugs. Clin Chem Lab Med 2004; 42: 1228-1255. http://dx.doi.org/10.1515/CCLM.2004.245 PMid:15576287

25. Cauli B, Porter JT, Tsuzuki K, Lambolez B, Rossier J, Quenel B, et al. Classification of fusiform neocortical interneurons based on unsupervised clustering. Proc Natl Acad Sci U S A 2000; 97: 6144-6149. http://dx.doi.org/10.1073/pnas.97.11.6144 PMid:10823957 PMcid:PMC18572

26. Kroner S, Krierner LS, Lewis DA, Barriouneau G. Dopamine increases inhibition in the monkey dorsolateral prefrontal cortex through cell type-specific modulation of interneurons. Cereb Cortex 2007; 17: 1020-1032. http://dx.doi.org/10.1093/cercor/bhl012

27. Ascoli GA, Alonso-Nanclares L, Anderson SA, Barriouneau G, Benavides-Piccione R, Burkhalter A, et al. Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. Nat Rev Neurosci 2008; 9: 557-568. http://dx.doi.org/10.1038/nrn2402 PMid:18568015 PMcid:PMC2868386

28. Gavrilovic C, D’Alfonso S, Poulter MO. Diverse interneuron populations have highly specific interconnectivity in the rat piriform cortex. J Comp Neurol 2010; 518: 1425. http://dx.doi.org/10.1002/cne.22291 PMid:20187146

29. McCormick DA. GABA as an inhibitory neurotransmitter in human cerebral cortex. J Neurophysiol 1989; 62: 1018-1027. PMid:2573696

30. Elliott P. Action of antiepileptic and anaesthetic drugs on Na- and Ca-spikes in mammalian non-myelinated axons. Eur J Pharmacol 1990; 175: 155-163. http://dx.doi.org/10.1016/0014-2999(90)90226-V

31. Schaub C, Uebachs M, Beck H. Diminished response of CA1 neurons to antiepileptic drugs in chronic epilepsy. Epilepsia 2007; 48: 1339-1350. http://dx.doi.org/10.1111/j.1528-1167.2007.01103.x PMid:17441992

32. Reckziegel G, Beck H, Schramm J, Urban BW, Elger CE. Carbamazepine effects on Na+ currents in human dentate granule cells from epileptogenic tissue. Epilepsia 1999; 40: 401-407.
33. Loscher W, Schmidt D. Experimental and clinical evidence for loss of effect (tolerance) during prolonged treatment with antiepileptic drugs. Epilepsia 2006; 47: 1253-1284.
http://dx.doi.org/10.1111/j.1528-1167.2006.00607.x
PMid:16922870

34. Ellerkmann RK, Remy S, Chen J, Sochivko D, Elger CE, Urban BW, et al. Molecular and functional changes in voltage-dependent Na(+) channels following pilocarpine-induced status epilepticus in rat dentate granule cells. Neurosci 2003; 119: 323-333.
http://dx.doi.org/10.1016/S0306-4522(03)00168-4

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