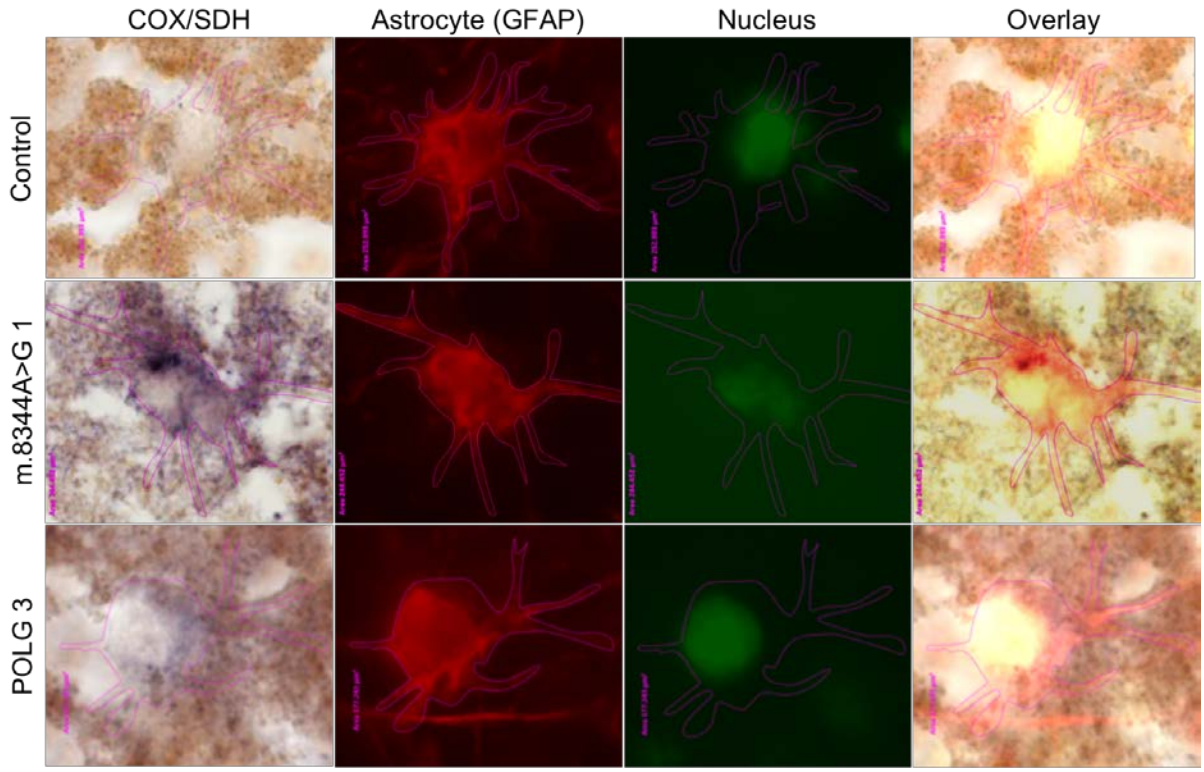
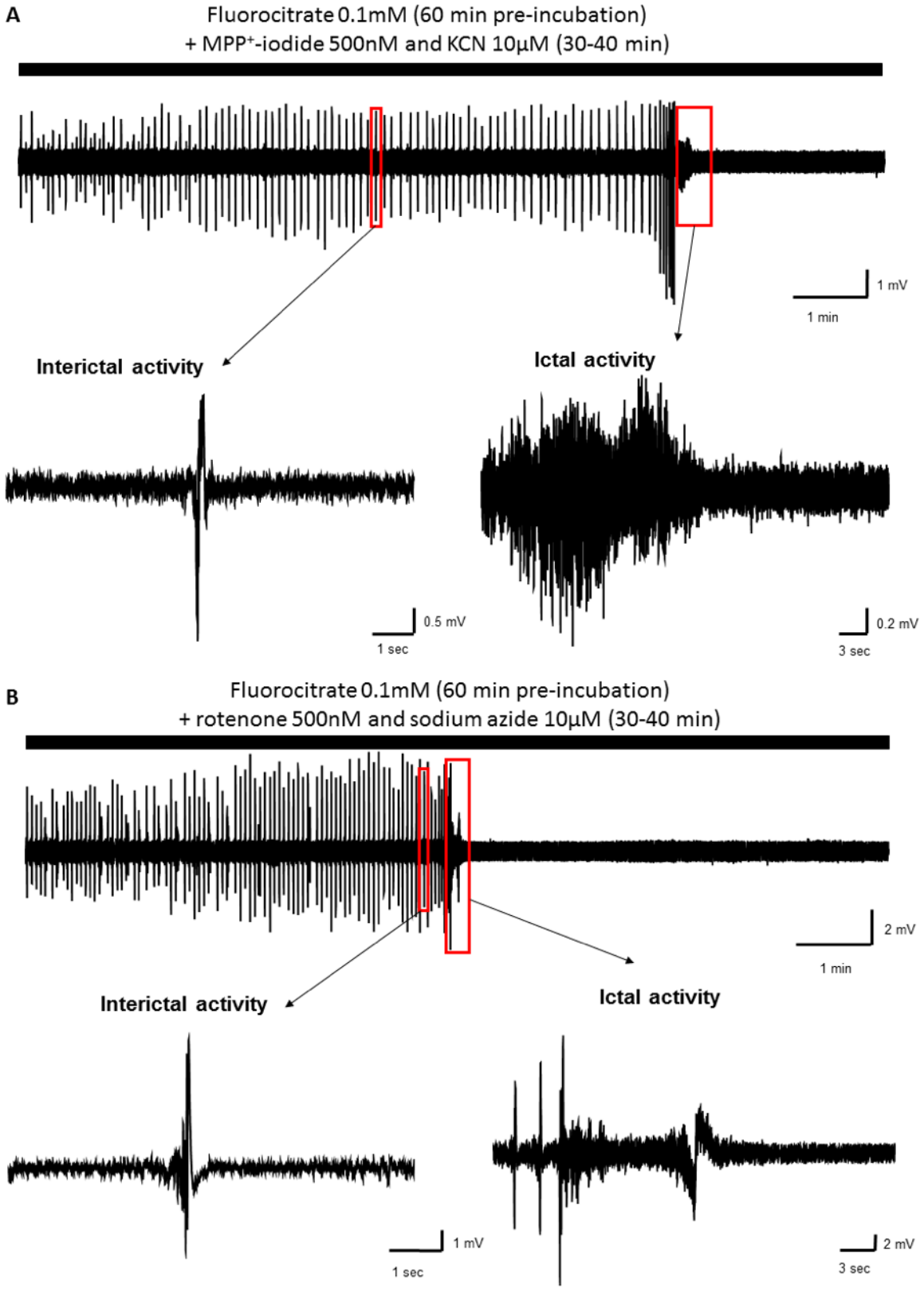
Supplementary material



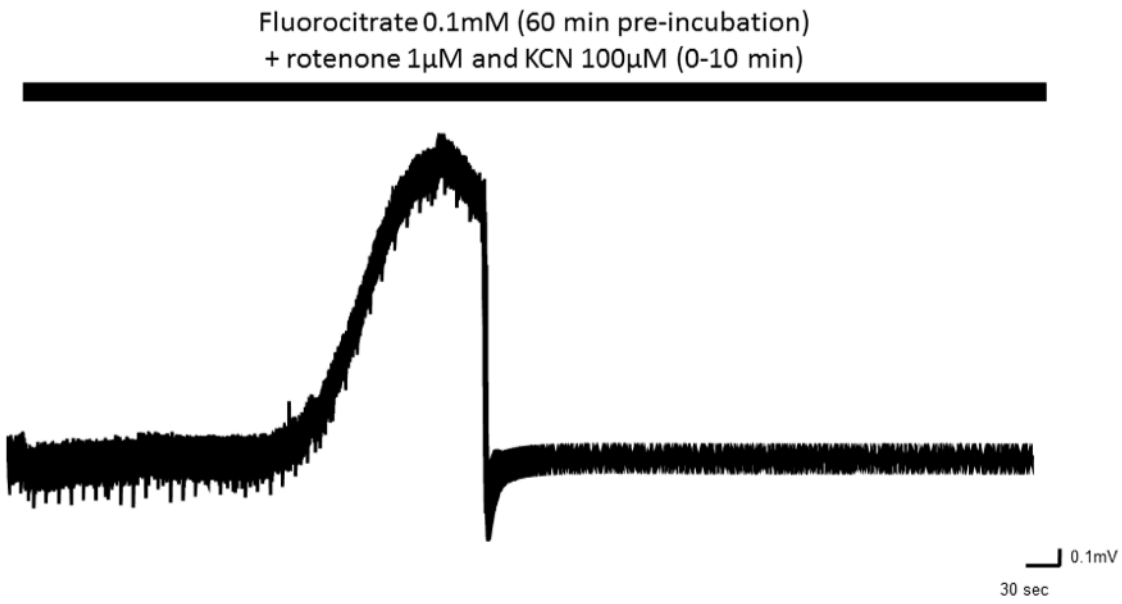
Supplementary Figure 1: Identification of cytochrome c oxidase (COX)-deficient and succinate dehydrogenase (SDH)-positive GFAP-immunoreactive astrocytes in patient tissues indicate the presence of respiratory chain deficiency affecting astrocytes.

COX-SDH histochemistry followed by sequential immunofluorescent labelling of astrocytes (GFAP; red) reveals COX-positive (brown staining) astrocytes in control tissues while patients show COX-deficiency (blue staining) affecting astrocytes, indicating mitochondrial respiratory chain deficiency.



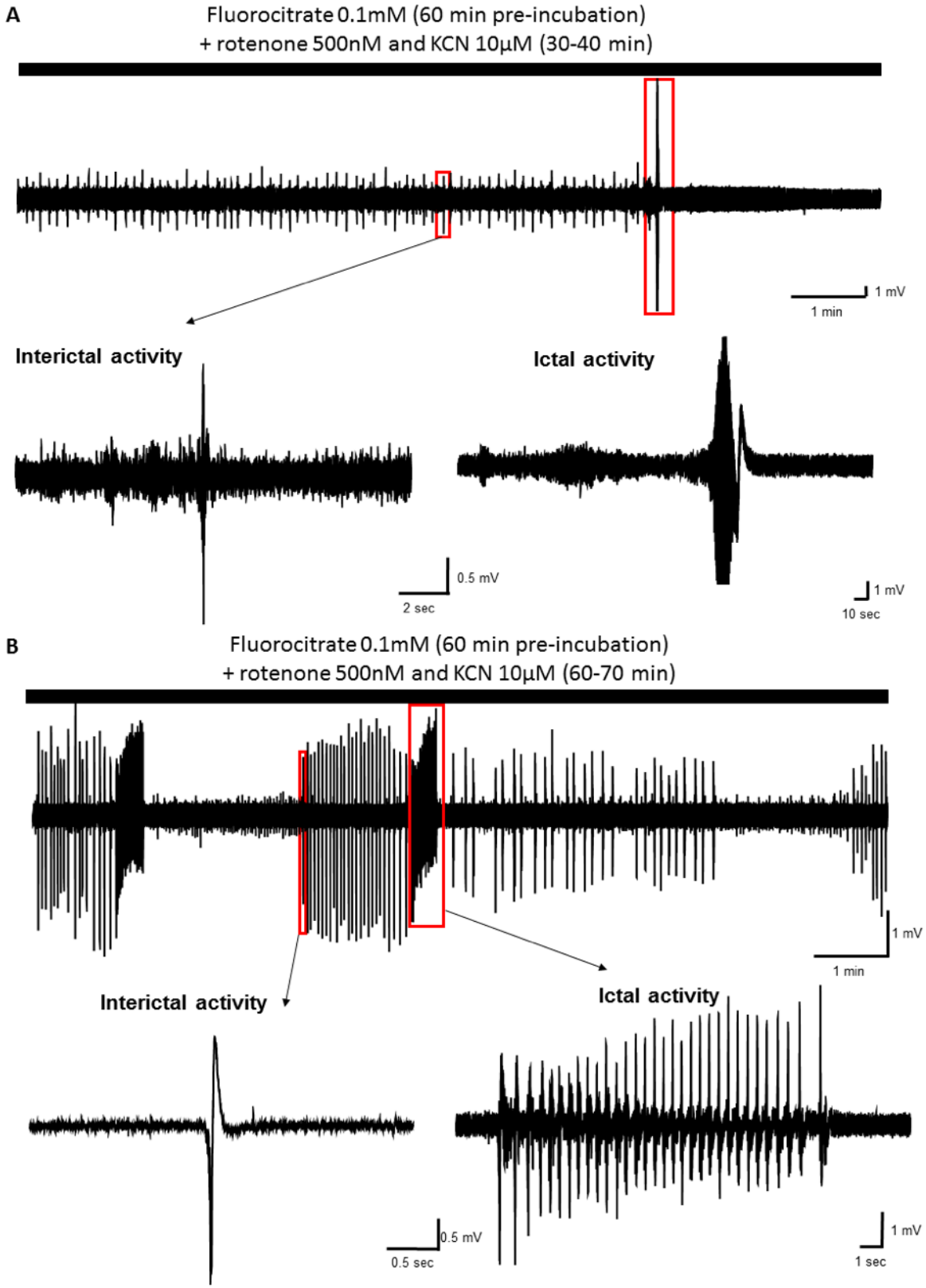
Supplementary Figure 2: Mitochondrial epileptic activity induction is not dependent on the specific use of rotenone or potassium cyanide.

Rather, it appears that mitochondrial epileptic activity induction in this model is dependent on the concomitant inhibition of mitochondrial complex I and IV. Substitution of rotenone with equimolar MPP^+-iodide (A) was adequate for induction of mitochondrial epileptiform discharges in the same pattern as it would in the presence of rotenone; the interictal – ictal cycle for the first 120 minutes (n=8). Similarly, sodium azide (B) was able to substitute for potassium cyanide as a complex IV inhibitor for the induction of mitochondrial epileptiform discharges (n=6). Again, the same pattern of interictal – ictal cycle was observed in the first 120 minutes. In both cases, the activity progressed in the similar manner to the late recurrent interictal discharges.



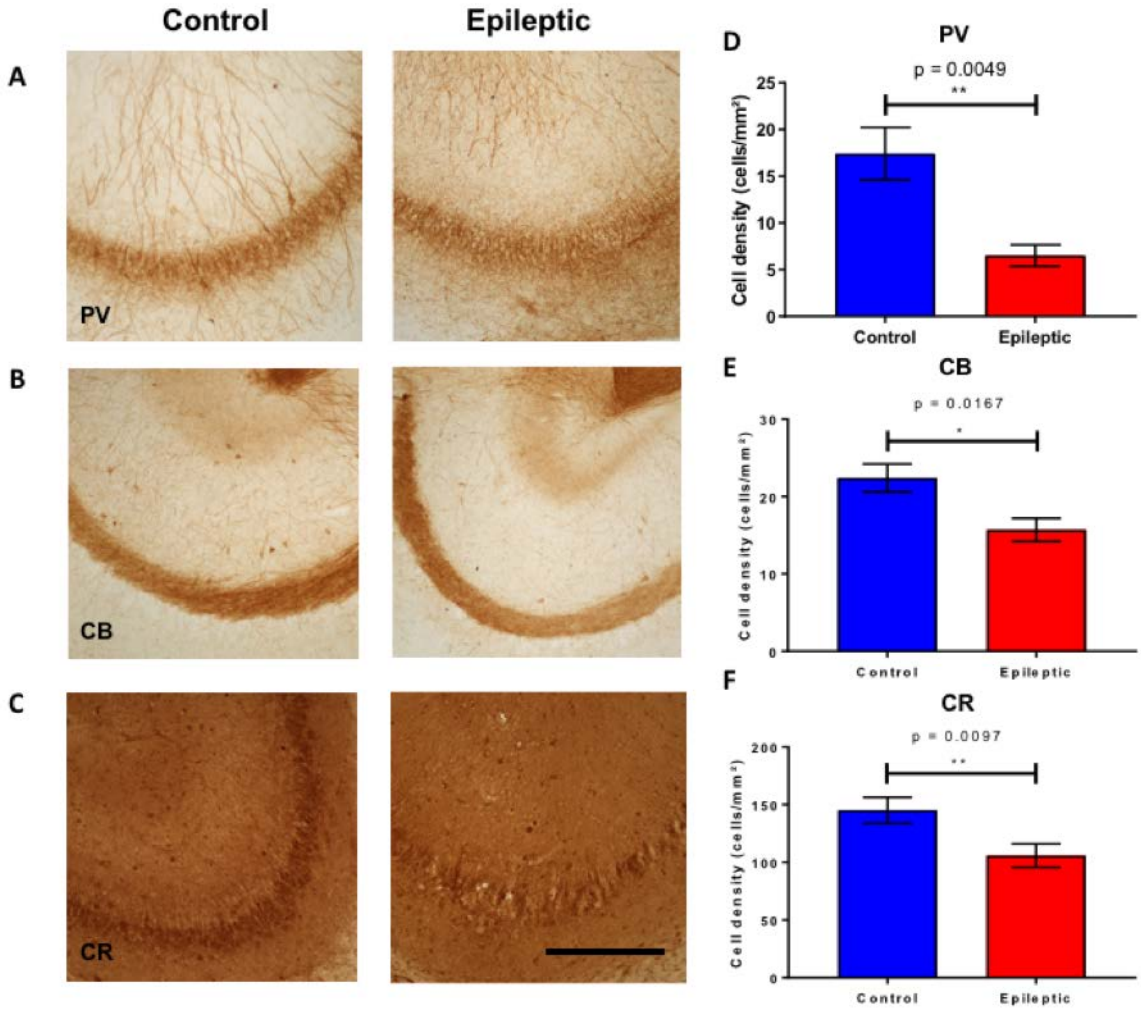
Supplementary Figure 3: Higher concentrations of mitochondrial respiratory chain inhibitors led to the induction of spreading depression instead of epileptiform discharges.

Washing in a higher concentration of the mitochondrial respiratory chain inhibitors; rotenone at 1µM and potassium cyanide at 100µM, induced generalized spreading depression rather than the classic cyclical interictal – ictal discharges (n=8). This generalized spreading depression is a giant wave of neuronal and glial depolarization that is followed by a long-lasting period of electrical silence in the brain slice. To us, the spreading depression represents a progression of electrophysiological manifestation of energy failure where in low to moderate energy failure, epileptiform discharges are induced and in a more severe form of energy failure, generalized spreading depression is induced instead.



Supplementary Figure 4: Mitochondrial epileptic activity induction is conserved across species.

The figure demonstrates the typical interictal – ictal activity that was induced with the protocol of pre-incubation with fluorocitrate followed by concomitant application of rotenone and cyanide. These activities were recorded from acutely prepared mice brain slice (A) (n=10) and acutely resected human brain tissue (B) (n=6). In both species, the same progression to late recurrent interictal discharges was also noted.



Supplementary Figure 5: There is a generalized loss of interneuron marker expression in the epileptic brain slices.

Following the observed loss of GABAergic cells, we examined interneuron marker expression in the epileptic brain slices. There appeared to be a significant loss of parvalbumin expression in the epileptic slices as compared against control (A) and the same was observed with calbindin (B) and calretinin (C) expression. These are quantified in the graphs (D-F) showing the cell density of the expression of parvalbumin (n=6 control, 6 epileptic), calbindin (n=5 control, 7 epileptic), and calretinin (n=9 control, 11 epileptic); all of which

showed significant reduction in epileptic slices. * $p \leq 0.05$ ** $p \leq 0.01$. Data are presented as mean ± s.e.m. in (D-F).

Supplementary Table 1

No	Age at surgery (years)	Gender	Seizure semiology	Pathology	Type of surgery*	Brain region	Surgical outcome	Antiepileptic medication**
1	25	F	Complex partial seizures	Mesial temporal sclerosis	SAH	Right temporal lobe	Seizure-free with post-op nystagmus	CBZ
2	38	M	Complex partial seizures	Hippocampal sclerosis	SAH	Left temporal lobe	Seizure-free	VPA/ECBZ/LEV
3	23	F	Complex partial seizures	Mesial temporal sclerosis	SAH	Left temporal lobe	Seizure-free	LEV
4	29	F	Complex partial seizures	Mesial temporal sclerosis	SAH	Right temporal lobe	Seizure-free	LEV/LTG
5	36	M	Focal onset seizures	Grade III anaplastic astrocytoma	AL	Left temporal lobe	No reduction in seizure	CBZ
6	53	F	Generalised seizures	Glioblastoma multiforme	FC	Left frontal lobe	Seizure-free	PHT/CBZ

* SAH – selective amygdalohippocampectomy, AL – anterior lobectomy, FC – frontal craniotomy.

** CBZ – carbamazepine, VPA – valproate, ECBZ – eslicarbazepine, LEV – levetiracetam, LTG – lamotrigine, PHT – phenytoin

Supplementary Table 2

Drug name	Supplier	Product code
Fluorocitric acid barium salt	Sigma Aldrich	F9634
Rotenone	Sigma Aldrich	R8875
Potassium cyanide	Sigma Aldrich	60178
MPP^+-iodide	Sigma Aldrich	D048
Sodium azide	Sigma Aldrich	52002
Carbamazepine	Tocris	4098
Lamotrigine	Sigma Aldrich	L3791
Levetiracetam	Sigma Aldrich	L8668
Valproate acid	Sigma Aldrich	P4543
Diphenylhydantoin (phenytoin)	Sigma Aldrich	D4505
Midazolam	Tocris	2832
Lorazepam	Sigma Aldrich	L1764
Sodium pentobarbital	Sigma Aldrich	P3761
GABA	Tocris	0344
L-glutamine	Sigma Aldrich	G3126
L-glutamic acid	Sigma Aldrich	G1251

Supplementary Table 3

Target protein	**Supplier**	**Product code**	**Host species**	**Dilution**
NeuN	Merck Millipore	ABN90P	Guinea pig	1:1000
GABA	Sigma Aldrich	A0310	Mouse	1:1000
CaMKII	Abcam	AB52476	Rabbit	1:1000
GFAP	Merck Millipore	AB5804	Rabbit	1:1000
PV	Sigma Aldrich	P3088	Mouse	1:1000
CB	Swant	CB38	Rabbit	1:10,000
CR	Swant	CG1	Goat	1:1000
Secondary Antibody			**Supplier**	**Product code**
Biotinylated goat-anti rabbit IgG antibody			Vector Laboratories	BA-1000
Biotinylated horse-anti mouse IgG antibody			Vector Laboratories	BA-2000
Biotinylated goat-anti guinea pig IgG antibody			Vector Laboratories	BA-4000