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Pharmacological and neuroethological studies of three antiepileptic drugs in the Genetic Audiogenic Seizure Hamster (GASH:Sal)

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ABSTRACT

Epilepsy modeling is essential for understanding the basic mechanisms of the epileptic process. The Genetic Audiogenic Seizure Hamster (GASH:Sal) exhibits generalized tonic–clonic seizures of genetic origin in response to sound stimulation and is currently being validated as a reliable model of epilepsy. Here, we performed a pharmacological and neuroethological study using well-known and widely used antiepileptic drugs (AEDs), including phenobarbital (PB), valproic acid (VPA), and levetiracetam (LEV). The intraperitoneal administration of PB (5–20 mg/kg) and VPA (100–300 mg/kg) produced a dose-dependent decrease in GASH:Sal audiogenic seizure severity scores. The administration of LEV (30–100 mg/kg) did not produce a clear effect. Phenobarbital showed a short plasmatic life and had a high antiepileptic effect starting at 10 mg/kg that was accompanied by ataxia. Valproic acid acted only at high concentrations and was the AED with the most ataxic effects. Levetiracetam at all doses also produced sedation and ataxia side effects. We conclude that the GASH:Sal is a reliable genetic model of epilepsy suitable to evaluate AEDs.

1. Introduction

According to the International League Against Epilepsy (ILAE), epilepsy is defined as a disorder of the brain that is characterized by an enduring predisposition to epileptic seizures, which are defined as a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain [1,2]. Epilepsy is a disorder that has semiologic and genetic heterogeneity because it consists of a variety of syndromes and manifestations. Genetic factors are largely considered to determine the susceptibility to seizure induction and the neuropathological consequences of epilepsy [3,4]. To our knowledge, the occurrence of ictogenesis and epileptogenesis is due to a molecular imbalance generated in the brain between GABA and glutamate, in addition to other neurotransmitters [5,6], that induces modifications in gene transcription, protein expression, and/or the structure of receptors [4,7–10].

Epilepsy modeling is essential for understanding the basic mechanisms of the disease [3,4] and for testing new antiepileptic drugs (AEDs). Currently, there are a variety of animal models of epilepsy, primarily rodent, and fly, fish, and worm models [11–13].

Genetic Audiogenic Seizure Hamsters (GASH:Sal) are a line of Syrian golden hamsters (*Mesocricetus auratus*) that are being validated as a model of epilepsy. They exhibit acute audiogenic seizures (AS) of genetic origin in response to sound stimulation. These AS require the activation of brainstem auditory pathways and originate largely at the level of the inferior colliculus [14,15]. These hamsters exhibit morphological and neurochemical abnormalities in the auditory pathway [13,16].

Neuroethology is a useful tool for studying epilepsy, as it analyzes seizures in an integrated manner using the principle that evaluation of behavioral sequences is more consistent than studies of isolated behaviors [17,18]. Neuroethological studies have been performed in epilepsy models, such as AS in rats [19], in models that use pilocarpine [20,21], and in synapsin knockout mice [22]. Further studies have been performed in patients with temporal lobe epilepsy [23,24]. Associations between pairs of behaviors in a time frame are used to detect frequency, duration, and correlation parameters by statistical association. Groups of behaviors with significant associations are configured as behavioral clusters [17,18].

As a part of the validation process of the GASH:Sal strain, we performed the current study using well-known and widely prescribed AEDs, including phenobarbital (PB), valproic acid (VPA), and levetiracetam (LEV). The goal of this study was to characterize the pharmacology of these AEDs using neuroethological tools in GASH:Sal hamsters.

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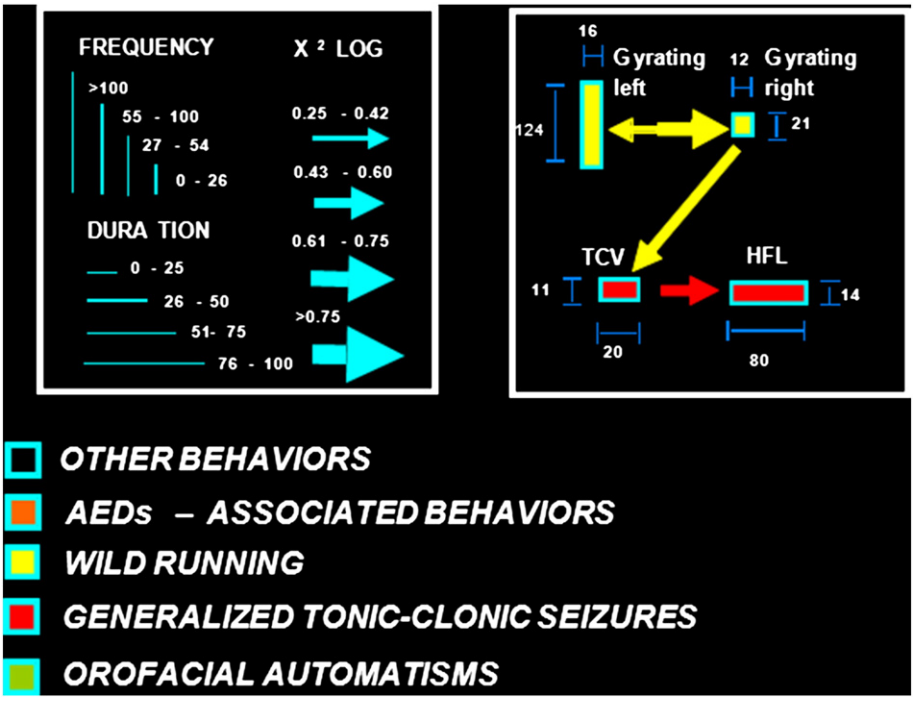

Fig. 1. Flowcharts illustrating the graphical and statistical aspects of the observed behaviors. The frequency and time spent performing each behavior are proportional to the height and width of the rectangle, respectively. The arrow width and direction indicate the statistical intensity and preference association between two items.

2. Materials and methods

2.1. Animals

Two-month-old male GASH:Sal hamsters were used. They were obtained from the inbred strain maintained at the animal facility of the University of Salamanca (USAL, Spain) and were housed during the experiments in the animal house at the Institute of Neuroscience of Castilla y León (INCyL) of the USAL. The experimental animals were

Table 1
Seizure index (SI), according to Garcia-Cairasco et al. [18] using the behavioral descriptions and categorized severity index (cSI) transformed into discreet variables for statistical purposes.
Taken from Rossetti et al. [33].

SI	Seizure behaviors	cSI
0.00	No seizures	0
0.11	One wild running	1
0.23	One wild running (running plus jumping plus atonic fall)	2
0.38	Two wild runnings	3
0.61	Tonic convulsion (*opisthotonus*)	4
0.85	Tonic seizures plus generalized clonic convulsions	5
0.90	Head ventral flexion plus cSI5	6
0.95	Forelimb extension plus cSI6[a]	7
1.00	Hind limb extension plus cSI6[a]	8

[a] Categories, which are generally followed by hind limb clonic convulsions (CCV2).

Table 2
Ataxia index (AI) scores according to Lösher and Hönack [34].
Taken from Rosetti et al. [33].

Score	Behaviors
1	Slight ataxia in hind leg (tottering of hind quarters), no decrease in abdominal muscle tone
2	More pronounced ataxia with dragging of hind legs and slight decrease of muscle tone
3	Further increase in ataxia and more pronounced dragging of hind legs and decrease in muscle tone
4	Marked ataxia, animals lose balance during forward locomotion, total loss of abdominal muscle tone
5	Very marked ataxia with frequent loss of balance during locomotion, loss of abdominal muscle tone

Table 3
Sedation index (SdI) scores according to Lösher and Hönack [34].
Taken from Rosetti et al. [33].

Score	Behaviors
1	Slightly reduced forward locomotion
2	Reduced locomotion with rest periods in between (partly with closed eyes)
3	Reduced locomotion with more frequent rest periods
4	No forward locomotion; animal sits quietly with closed eyes

Table 4
Behavioral dictionary.

Acronym	Behavior	Acronym	Behavior
AF	Atonic fall	GRH	Grooming of head
ATF[a]	Ataxic fall	GRHL	Grooming of head, left
BE	Blinking eyes	GRL	Grooming of body, left
BRL	Barrel rolling, left	GRN	Grooming of neck
BRR	Barrel rolling, right	GRR	Grooming of body, right
CCV1	Clonic convulsions (forelegs)	HFL	Head ventral flexion
CCV2	Clonic convulsions (hind legs)	HP_1	Forelimb extensions
CCVg	Clonic convulsions (generalized)	HP_2	Hind limb extensions
CVL1	Clonic convulsions (forelegs – left)	IM	Immobility
CVL2	Clonic convulsions (hind legs – left)	JP	Jumping
DYS	Dyspnea	LI	Licking
ER	Erect posture	LIC	Licking of claws
EXC	Excretion of feces and urine	MT	Masticatory movements
EXT	Extended posture	PIM	Postictal immobility
FR	Freezing posture	RU	Running
GL	Gyrating, left	SC	Scanning
GN	Gnawing	SN	Sniffing
GR	Gyrating, right	TCP	Tachypnea
GRB	Grooming	WA	Walking
GRF	Grooming of face	WDS	Wet dog shaking
GRG	Grooming of genitals		

[a] Observation: The behavioral item ataxic fall (ATF) was created to show the presence of the toxic effect of drugs on motor behavior.

handled and cared for according to the guidelines of the Spanish (RD 1201/05) and European (2010/63/EU) Directives under the supervision of the corresponding Institutional Animal Care and Use Committee. All efforts were made to avoid unnecessary animal suffering and to reduce the number of animals used in the study.

2.2. Acoustic stimuli

The stimuli were applied in a cylindrical acrylic arena (height: 50 cm; diameter: 37 cm), recorded using a high-pass filter (>500 Hz; microphone Bruel & Kjaer #4134 and preamplifier Bruel & Kjaer #2619), digitized above 4 kHz, and reproduced by a computer coupled to an amplifier (FONESTAR MA-25T, Revilla de Camargo, Spain) and a tweeter (Beyma T2010, Valencia, Spain) in the upper portion of the arena. The final sound was a semirandom sound of 0–18 kHz with an intensity of 115 to 120 dB.

The sound of shaking keys can be downloaded from: <http://www-incyl.usal.es//components/com_gestionusuarios/proyectos/audigenic-sound.mp3>.

2.3. AEDs

Three AEDs were used for these experiments: PB (Luminal®, Kern Pharma), VPA (Depakine®, Sanofi Aventis), and LEV (Kepra®, UCB Pharma).

2.4. Pharmacology of the AEDs

2.4.1. Blood extraction and sample preparation

Blood was extracted at various times postinjection of the AEDs: 30, 60, 120, 240, and 360 min in the case of VPA and LEV and 5, 15, 20, 25, and 30 min in the case of PB. The doses of the drugs were as follows: 20 mg/kg of PB, 500 mg/kg of VPA, and 100 mg/kg of LEV. The blood was extracted from the cranial vena cava according to the procedure described by Picazo et al. [25] under inhalation anesthesia (induction: 4% isoflurane and 1 l/min O_2; maintenance: 3% isoflurane and 0.4 l/min O_2). At each time point, 100 μl of blood was extracted, and the animals were rehydrated with 150 μl of 0.9% saline. The samples were kept at 4 °C until a clot formed and were then centrifuged for 5 min at 10,000 *g* at 4 °C. The supernatant (serum) was collected in 20 μl aliquots and frozen until used. Then, 20 μl of acetonitrile (Panreac, Barcelona, Spain) and an internal standard (IS) were vortexed with the serum aliquot, agitated for 5 min, then centrifuged for 5 min at 11,000 *g*. The supernatant was placed in another tube and left to dry at room temperature. The sample was suspended in 20 μl of 20% methanol in dH_2O. The ISs used were as follows: 25 μg/ml acetanilide (Merck, Darmstadt, Germany) for PB, 20 μg/ml octanoic acid (Panreac) for VPA, and 20 μg/ml caffeine (LiChro test standard, Merck, Darmstadt, Germany) for LEV.

2.4.2. Blood drug concentration

Serum level determinations were carried out to adjust the timing interval for each AED. The blood drug concentrations were determined

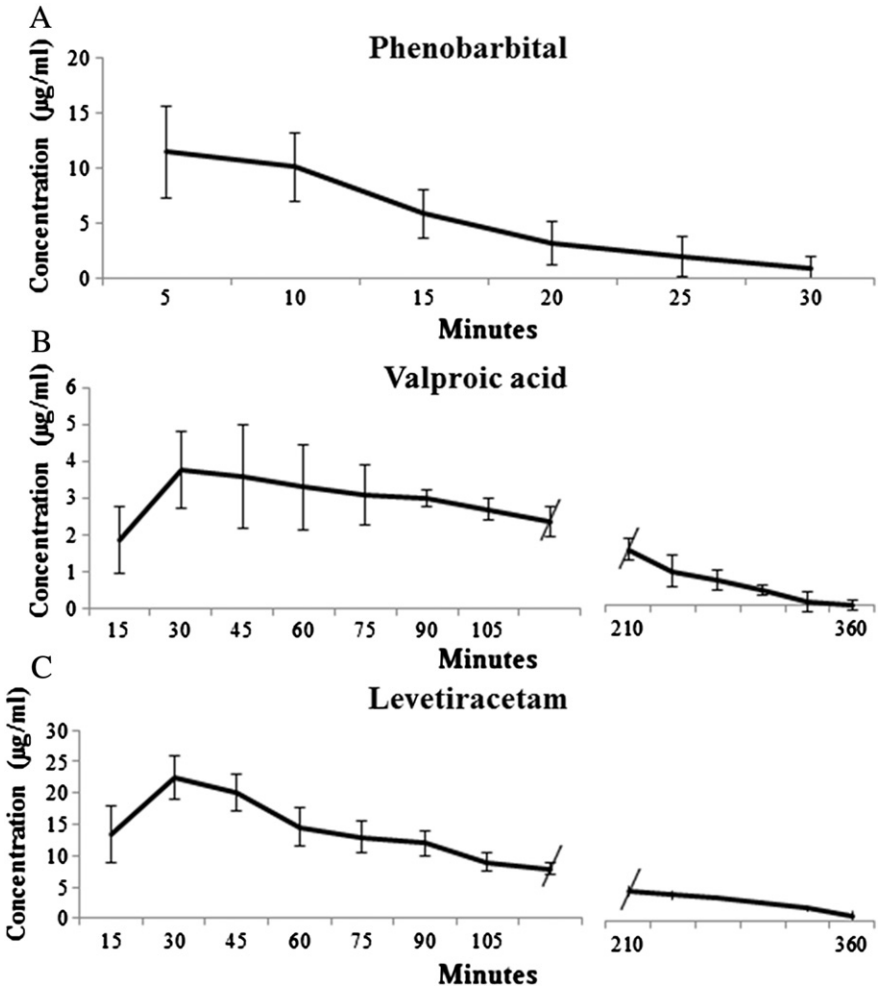

Fig. 2. Serum concentration levels at various extraction times following the intraperitoneal injection of the AEDs: (A) PB (10 mg/kg), (B) VPA (500 mg/kg), and (C) LEV (100 mg/kg). Each point is the mean (±S.E.M.) of 5 animals.

using HPLC/MS at the Mass Spectrometry Service of the USAL. The equipment used for analysis of the samples included an Agilent 1100 (Agilent Technologies, Santa Clara CA, USA) for the HPLC assay and an Agilent trap XCT (Agilent Technologies) for the mass spectrophotometry assay. The mobile phase consisted of A (water containing 0.1% formic acid) and B (acetonitrile). The mobile phase was delivered at a flow rate of 0.2 ml/min to a X-Bridge C-18 column (particle size: 3.5 μm; diameter: 2.1 mm; length: 10 cm) (Waters Corporation, Milford MA, USA). Different methods of elution were used for each drug [26]. The PB method consisted of a 20% constant rate of the B phase, and the total run time was 15 min [27,28]. The VPA method was initiated with 6 min of 40% B phase, which was linearly increased to 100% B phase over 1 min and was maintained at this concentration for 4 min [29]. The LEV method was initiated with 5% B phase, which was linearly increased to 20% B phase from 3 to 8 min and was then maintained at this concentration for 2 min [30].

2.5. Anticonvulsant drug potency: AED doses and acute treatment

The dose of each AED was chosen based on its efficacy at suppressing seizures in other experimental models of epilepsy [27,28,30,31]. The administered doses of each drug were as follows: PB — 5, 10, 15, and 20 mg/kg; VPA — 100, 150, 300, and 500 mg/kg; and LEV — 30, 50, 80, and 100 mg/kg. Each animal received different doses of the same AED 15 to 30 min before the stimuli. In the first week, the animals received the vehicle (0.9% saline; 0.001 ml/g of animal

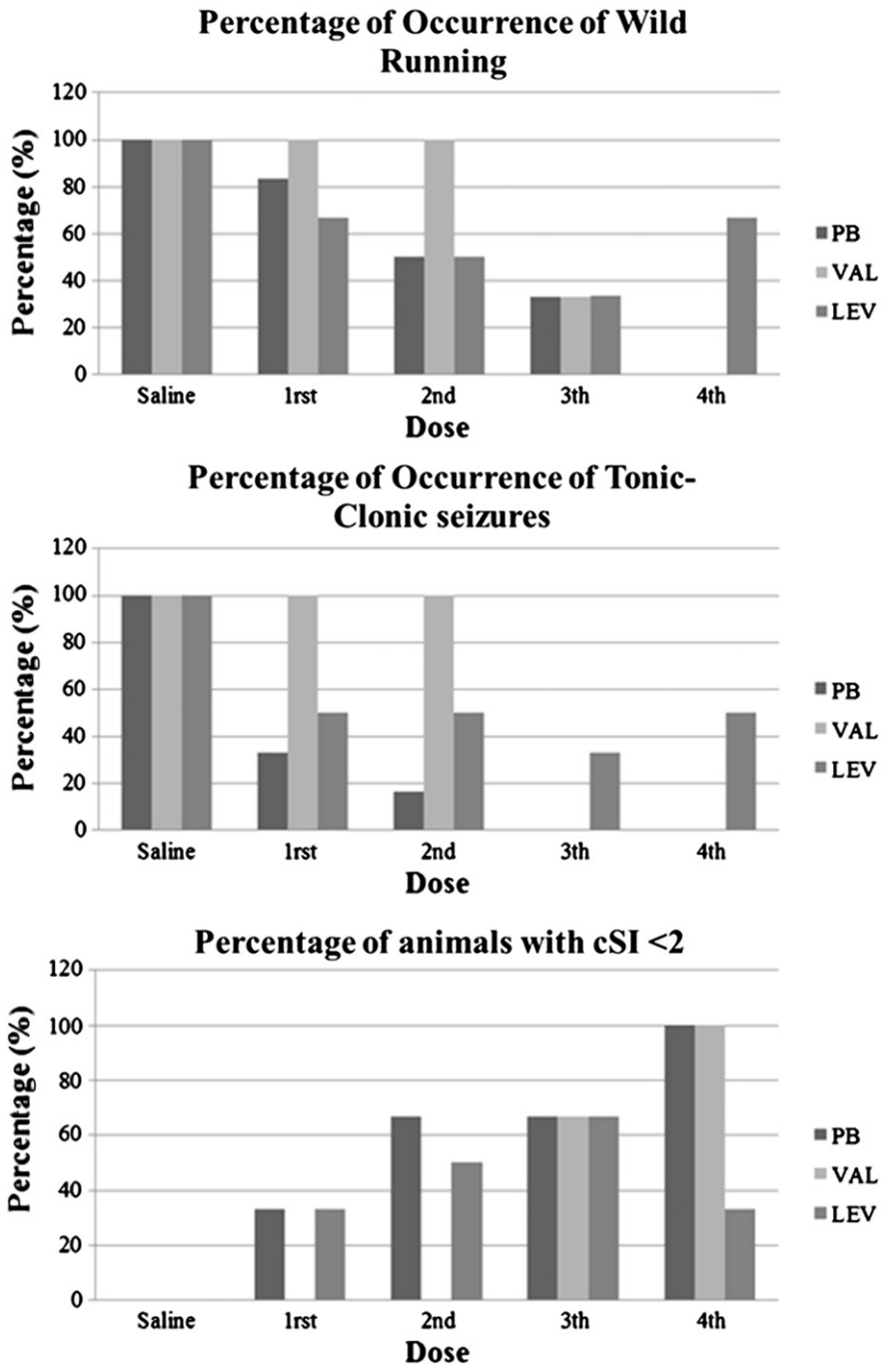

Fig. 3. Percentage of animals that exhibited wild running (top) and tonic–clonic convulsions (middle) under the effects of the AEDs. Observe the percentage of animals (bottom) lacking seizures at the different doses (n = 6). PB (5, 10, 15, and 20 mg/kg), VPA (100, 150, 300, and 500 mg/kg), and LEV (30, 50, 80, and 100 mg/kg).

weight), and during the following testing weeks, the animals received one dose per week for a total of five weeks. A fourth group of animals was added as a control and received saline instead of drug for all five stimuli. Drug potency comparisons were based on administered dosages and were determined at the individual time of the peak anticonvulsant effect. The median effective dose (ED_{50}) values for the various treatment outcomes were calculated following the method of Litchfield and Wilcoxon [32] in PSS PASW Statistical 18 (IBM).

2.6. Neuroethological study

2.6.1. Behavioral observation

The protocols consisted of three phases of behavioral observation: 1 min of presound, 30 s of sound, and 3 min of postsound. All observation windows were recorded for further analysis (Fig. 1).

2.6.2. Seizure severity indexes and neuroethological analysis

Three behavioral evaluations were used. The severity index (Table 1) was used to determine the intensity of the seizures [17,18,33]. The ataxia (Table 2) and sedation (Table 3) indexes were used to measure the toxic effects of the drugs on normal behavior [33,34].

After each animal was scored using the cSI, we divided the animals into two groups for further analysis. Those with a cSI greater than or equal to 2 (SI = 0.23) were considered as animals that maintained seizures, and those with a cSI lower than 2 were considered as animals in which the seizures were blocked. The behavioral sequences observed during the different stimuli were assessed using neuroethological methods. Every behavior presented in a given time window was recorded, second by second, according to a dictionary of behavioral items (Table 4) described by Garcia-Cairasco et al. [18]. Once the data were obtained, the ETHOMATIC [17,18] program was used for statistical analysis of the data. This program displays the mean frequency and the mean duration of each behavioral item in the given observation window. The program also performs statistical analysis, verifying significant associations between pairs of behavioral items and calculating X^2 values. Flowcharts representing all of the statistically significant data were constructed using Microsoft Power Point 2011 (see Fig. 1 for flowchart calibration).

2.6.3. Statistical analysis

All the statistical analyses were performed using SPSS PASW Statistical 18 (IBM) software. For comparisons between the different AED doses, we used the nonparametric Friedman test with Conover post hoc comparisons [35].

3. Results

3.1. Pharmacology of the AEDs in GASH:Sal hamsters

3.1.1. Serum levels

To determine the timing of the maximal levels of the drugs, we measured blood levels after the intraperitoneal administration of an effective anticonvulsant dose. The results obtained from the serum AED concentration analysis (Fig. 2) showed that the blood absorption and elimination of free PB in GASH:Sal hamsters are excessively fast, as nondetectable levels of the drug were recorded 30 min postinjection. Valproic acid and levetiracetam showed a similar kinetic profile, reaching the highest serum levels at 30 min postinjection but had a low but still detectable drug blood concentration after 6 h.

3.1.2. Anticonvulsant effects

The three drugs had anticonvulsant effects at their peak plasma concentrations, i.e., 10 min (PB) or 30 min (VPA and LEV) after the i.p. injections (Fig. 2). At low doses, AEDs modified the seizures (Fig. 3) by prolonging the time of wild running, by suppressing the tonic–clonic phase, and by elongating the time of the postictal period. In the case of PB and VPA, the seizures completely vanished at higher doses.

The anticonvulsant effect of PB was present at the smallest dose (5 mg/kg), at which approximately 33% of the animals did not present any of the components of an epileptic seizure. Valproic acid had a 75% protective effect against seizures at 300-mg/kg dose. Phenobarbital and valproic acid generated anticonvulsant effects in accordance with increasing doses. Levetiracetam did not have a dose-dependent anticonvulsant effect nor did it reach a maximum effect at any of the doses used. In fact, LEV achieved stronger protective effects (50–66%) at lower doses (30–50 mg/kg). Levetiracetam did not alter, or only very slightly altered, wild running at any of the doses used.

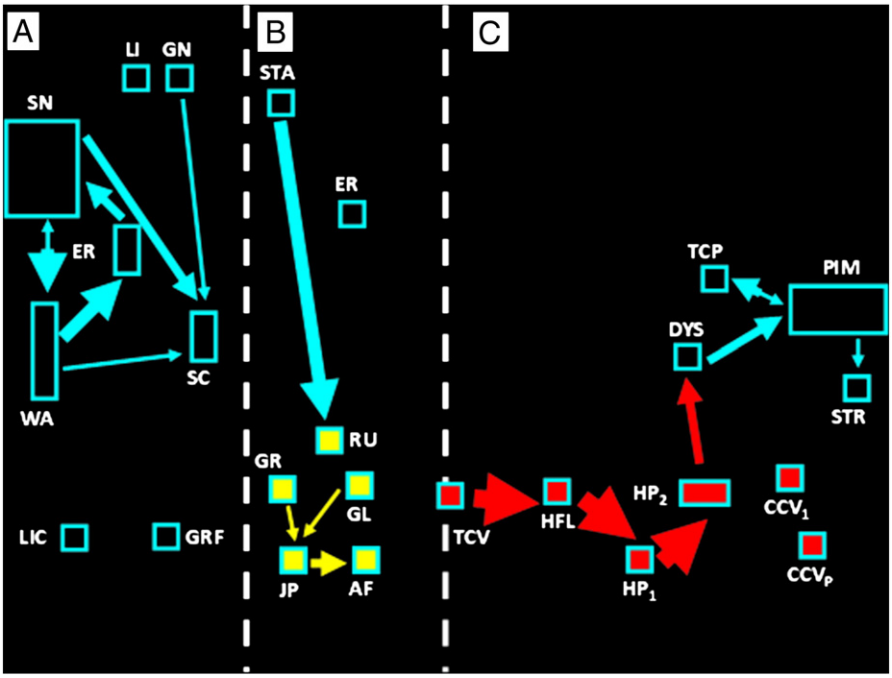

Fig. 4. Characteristic flowchart of the behavioral sequences of the first seizure with no AEDs in GASH:Sal hamsters (n = 24). (A) Presound window, (B) sound window, and (C) postsound window. See behavioral descriptions in Table 4.

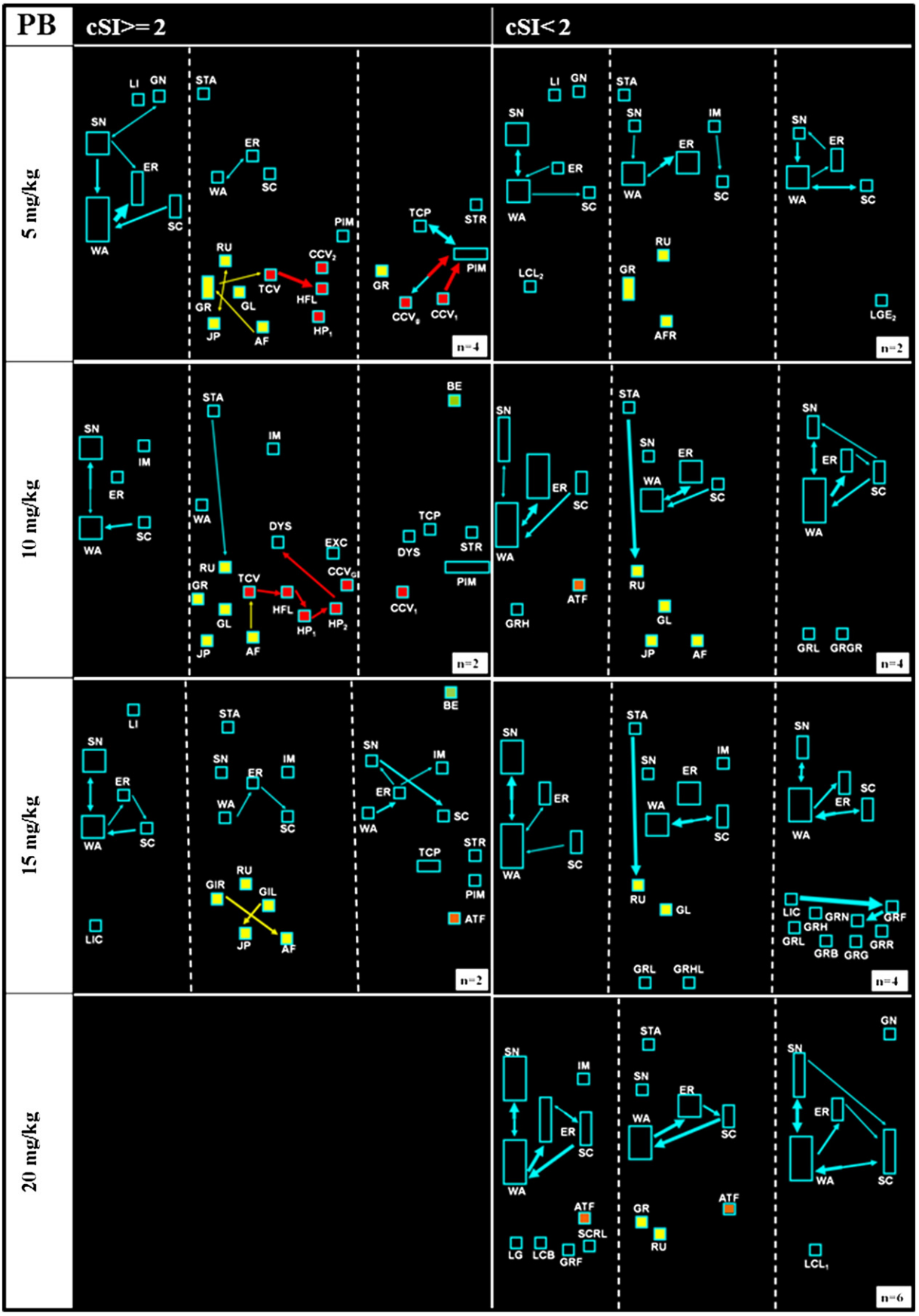

Fig. 5. Flowchart of the sequences of behaviors of the GASH:Sal hamsters injected with different doses of PB.

All three drugs were evaluated for anticonvulsant potency as measured by the ED_{50}, the dose that completely blocks seizures in 50% of animals after stimulation. The dose of PB that produced 50% seizure blockade was 10 mg/kg. In the case of VPA, the calculated ED_{50} was 225 mg/kg. Levetiracetam did not present a linear correlation between the decrease in seizures related to dose, but the ED_{50} range was between 30 and 50 mg/kg.

3.2. Neuroethological study

3.2.1. Characterization of seizures in the GASH:Sal hamster

The seizures of the GASH:Sal hamster, as described by Muñoz and López [36], consist of generalized tonic–clonic seizures that can be divided into different phases, starting with (a) a behavioral arrest, followed by (b) a period of wild running, then (c) tonic–clonic convulsions, with (d) head ventral flexion, forelimb extension, and hind limb extension, and ending with (e) postictal immobility. The duration of the convulsive phases is 25.82 ± 2.44 s.

The representative flowcharts of an acute (first) seizure in the GASH:Sal hamster are shown below in more detail (Fig. 4). During the presound window, the behavioral exploration cluster represents the main activity of the hamsters. Although the animals did not have immobility periods, some isolated grooming behaviors were observed. During the sound window, wild running began approximately 7 s poststimulus. The third window began with a tonic seizure (TCV), followed by a behavioral cluster of generalized clonic seizures with hyperextensions (HP1 and HP2) and ended with postictal immobility (PIM).

3.2.2. Characterization of the anticonvulsant effects of AEDs in the GASH:Sal hamster

At a dose of 5-mg/kg PB, 33.3% of the animals did not have seizures when stimulated. Animals treated with PB had reduced frequencies of the exploratory items and had a reduced number of interactions between the different behaviors involved in this cluster (Fig. 5). During the stimulus window, the animals maintained some of the wild running behavioral components, but there were no interactions between the components, and the animals presented exploratory behavior for the remainder of the duration of the stimulus. During the last period, the animals maintained this exploratory behavior at a level lower than that of control animals. The animals that suffered a seizure at this dose differ from the control animals in the short presence of exploratory behaviors and in the convulsive cluster without hind limb extension. At 10 mg/kg of PB, the animals with no seizure (66.6%) had an increased frequency of exploratory behavior in all three windows, with the presence of drug-related ataxic falling (ATF; orange symbol) in the prestimulus window. These animals maintained some wild running but had no interactions between the components. Some grooming behaviors appeared during the poststimulus observation window. The seizures of the drug-treated animals differed from those of the control animals by a reduction in the interactions of all of the associated behaviors.

Orofacial automatisms (e.g.: blinking eye) were present during the postictal period. At 15 mg/kg of PB, the animals that were seizure-free displayed similar behavior to that of the animals at 10 mg/kg of PB, except for the strong presence of grooming behavior during the poststimulus period. Differences were noted in the animals that had seizures; they only presented wild running behavior items with high interactions, and there was an exploratory cluster of low frequency and duration during the stimulus window. At 20 mg/kg doses, all the animals were seizure-free in all of the observation windows, with the principal behavioral cluster being exploratory with a high frequency of all the items. There was a high number of grooming behaviors at this dose. The cIS and the AI showed significant differences at all the doses in contrast to the vehicle (Fig. 6).

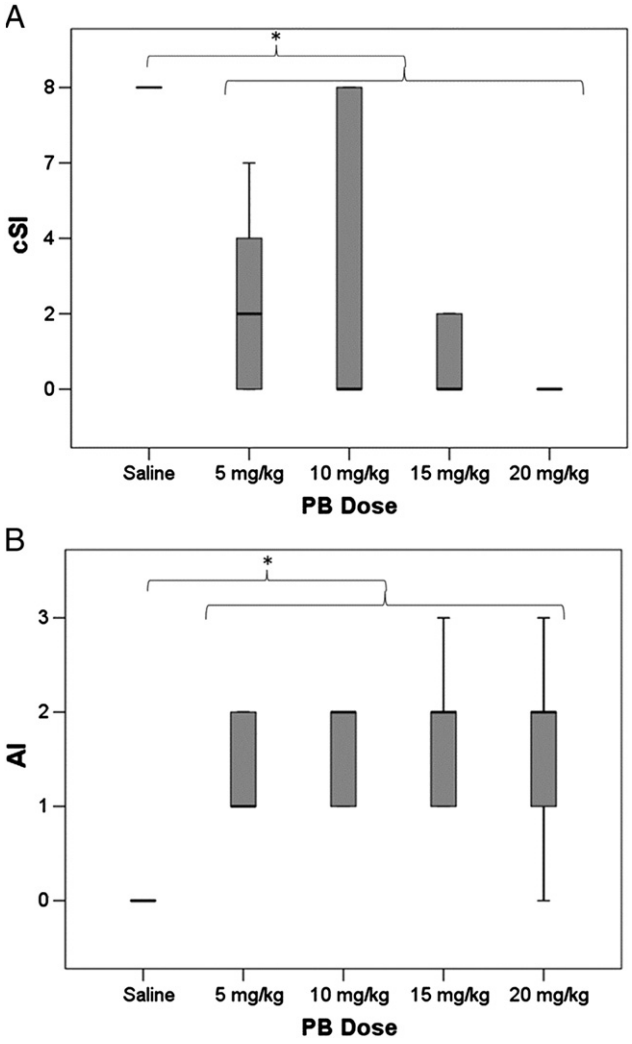

Fig. 6. Box plots with A) the categorized seizure severity index (cSI) and B) the ataxic index (AI) at the different doses of phenobarbital (PB).

The neuroethological sequence (Fig. 7) of the hamsters injected with VPA showed a clear-cut effect of the doses that had an anticonvulsant effect and those that did not. At a lower dose, 100 mg/kg, all the animals had seizures, and no differences were seen in the prestimulus window. When the stimulus began, some exploratory items accompanied the wild running behavior, which decreased the interactions between the different behaviors in the cluster. During the postictal immobility window, orofacial automatisms, such as eye-blinking and mastication, were observed. At 150 mg/kg dose of VPA, some ataxic walking was observed, and there was an increase in the interactions between the exploratory items in the stimulus window. After the exploration cluster, wild running was initiated and was followed by the regular phases of the seizure pattern. Additionally, automatisms were still present in the postictal phase, and some ataxia was noted at this point. At 300 mg/kg VPA, 66.6% of the animals lacked seizures, the exploratory cluster decreased, and the interactions were lower in all three windows. Once the sound stopped, freezing behavior was observed, followed by exploration. The level of ataxia at this point was highly pronounced (Fig. 8). At a higher dose, 500 mg/kg VPA, no animal had seizures, and the main behavioral cluster was exploratory. Some wild running behaviors were present but were not maintained as in the previous dose.

The frequency and duration of the exploratory behavior were lower than normal, except for during the poststimulus window, when they were accompanied by a marked ataxic walk (Fig. 8).

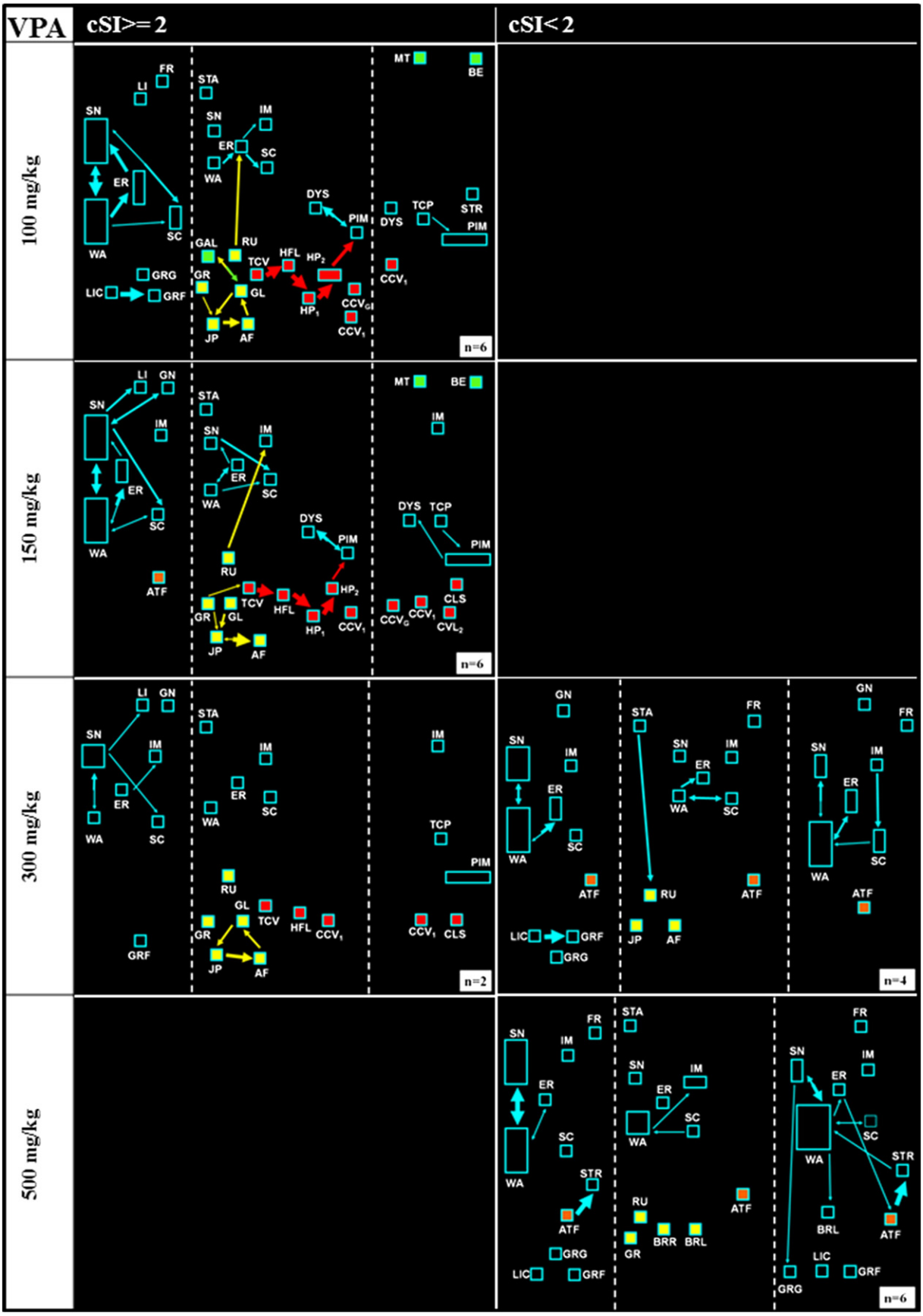

Fig. 7. Flowchart of the behavior of the GASH:Sal hamsters injected with different doses of VPA.

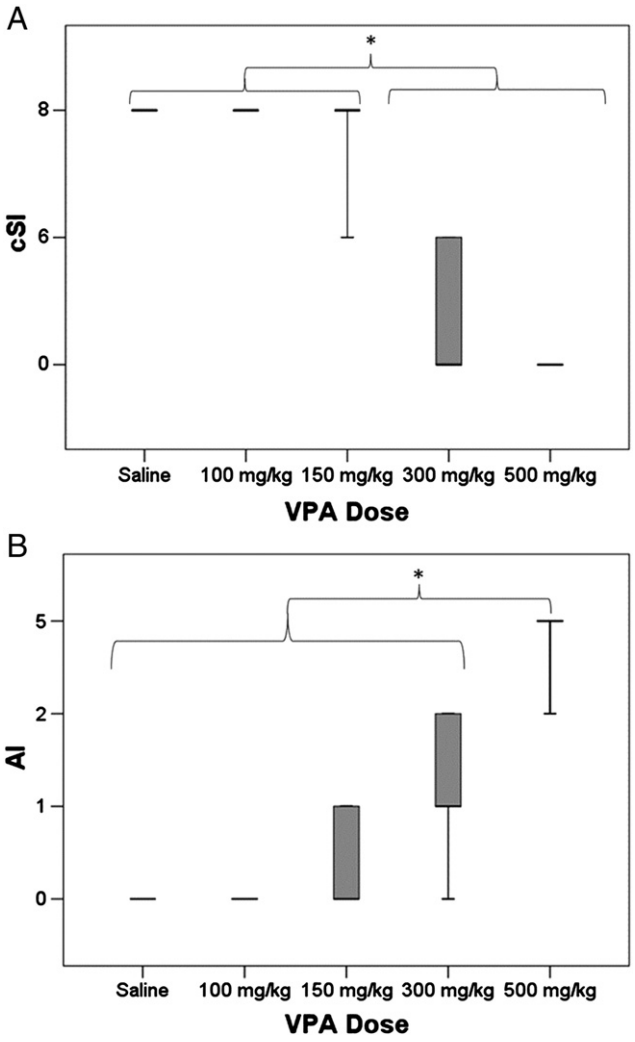

Fig. 8. Box plots showing: A) the categorized seizure severity (cSI) and B) the ataxic index (AI) at the different doses of VPA.

At a dose of 30-mg/kg LEV, 33.3% of the animals did not have seizures. As shown in Fig. 9, the exploration clusters were affected in all three windows (lower frequency and duration), and interactions between the items were highly decreased when compared with the control group. As seen with the other AEDs, in the animals that had seizures, there was an increase in the latency to wild running. At a LEV dose of 50 mg/kg, 50% of the animals lacked seizures, and there was an increase in the interactions between the exploratory behaviors. Some grooming items were seen during the poststimulus window. At 80-mg/kg dose, 66% of the animals had complete seizures, but there was an increase in the latency period to wild running, and orofacial automatisms were present. At the final dose (100 mg/kg), 66% of the animals did not have seizures, and the main component was the exploratory cluster in all three windows. No other cluster had a significant presence in the flowchart. In addition to the lack of an anticonvulsant effect, LEV also produced high toxicity effects, such as ataxia and sedation (Fig. 10).

4. Discussion

Validation of the GASH:Sal hamster as a chronic model of generalized epilepsy may reveal the physiological and neurochemical changes that manifest in the epileptic brain. The present study shows that the anticonvulsant effects of PB and VPA, both with a similar profile, are dose-dependent and that PB and VPA have higher anticonvulsant potency than LEV in the GASH:Sal hamster seizure model. This result contributes to our understanding of the effects of AEDs.

An animal model of epilepsy is validated when, in addition to being able to reliably reproduce the objective symptoms and EEG features of the epilepsy syndrome, it is also able to respond to targeted pharmacological treatment. As an additional strategy to increase our understanding of the pathophysiology of epilepsy, the GASH:Sal hamster has been observed to mimic generalized seizures [37] and to respond to treatment. As such, the GASH:Sal hamster serves as a useful tool for the design and evaluation of new AEDs.

Animal models of disease serve an indispensable function in identifying the biochemical basis of disease and aiding in the development of biological screening methods for molecules with the potential to suppress or stop disease progression. Organisms such as worms, flies, yeast, or fish serve as a complementary approach to in vitro assays and are employed in a wide variety of studies, ranging from developmental and cellular processes to gene mapping and screening. One advantage of a rodent model is its repertoire of complex behaviors [38]; therefore, it has long been the standard choice for pharmaceutical drug target validation. However, when choosing an appropriate model for AED screening, special attention must be paid to the processes underlying the epileptogenesis of a particular epilepsy syndrome. There is an extensive discussion about which types of models are the best for studying AEDs. The use of epilepsy models (e.g., Genetic Absence Epilepsy Rat from Strasbourg) or seizure models (e.g., maximal electroshock seizure) depends on the focus of the study [12]. Lösher [39] suggests that models resulting from spontaneous mutations, such as AS models, are preferred for drug development. The complexity of the epilepsies and the need for a better understanding of the mechanisms underlying them are reflected in the ongoing characterization and enhancement of animal models [11,12,39,40].

When looking to the neuroethological evaluation of GASH:Sal of the current study, we can see that in the prestimulus observation window, the hamsters were hyperactive with high levels of exploratory behavior, which is congruent with the normal anxious behavior of the species [41]. When the behavioral sequence of the drug-free hamster group was observed, it was noticed that, with slight variations, the seizure pattern was quite standard. This is compatible with an already demonstrated stable inherited mutation associated with the epileptogenic circuits in the GASH:Sal inbred strain [36]. Nevertheless, the minimal differences in latency and wild running were considered and explored, although they did not show statistical significance (data not shown), as if there was a kindling component consequence of repeated stimulation. Although this did not seem to be the case, the presence of kindling would naturally affect our interpretation of the effects of the AEDs on the behavioral sequences of the studied hamsters because all doses of a given drug were applied to the same animal but with sufficient time intervals between doses.

Phenobarbital is one of the oldest AEDs used in the clinical treatment of epilepsy and has been validated in different animal epilepsy models [40]. Phenobarbital is rapidly and completely absorbed following i.p. administration. Phenobarbital was the AED that had the highest range of anticonvulsant effect, and it can block seizures in a dose-dependent manner from 5 to 20 mg/kg. With a dose of 10 mg/kg, only 33.33% of the animals seized, and they also showed a longer latency period, most likely because of the protective action of the drug. When the animals finished wild running, they continued to show exploratory behavior. These data indicate that PB at lower doses protects by avoiding the propagation of electric onset generated by the stimulus, possibly due to blockade of the Na^{2+} and Ca^{2+} channels [34,42,43]. At higher doses, PB fully blocks the seizure by enhancing the inhibition that is mediated by longer opening of the GABA-A channels, thereby decreasing the hyperactivity of glutamate and dopamine [44]. By increasing the PB dose, the animals' normal behavior (prestimulus window) was affected by

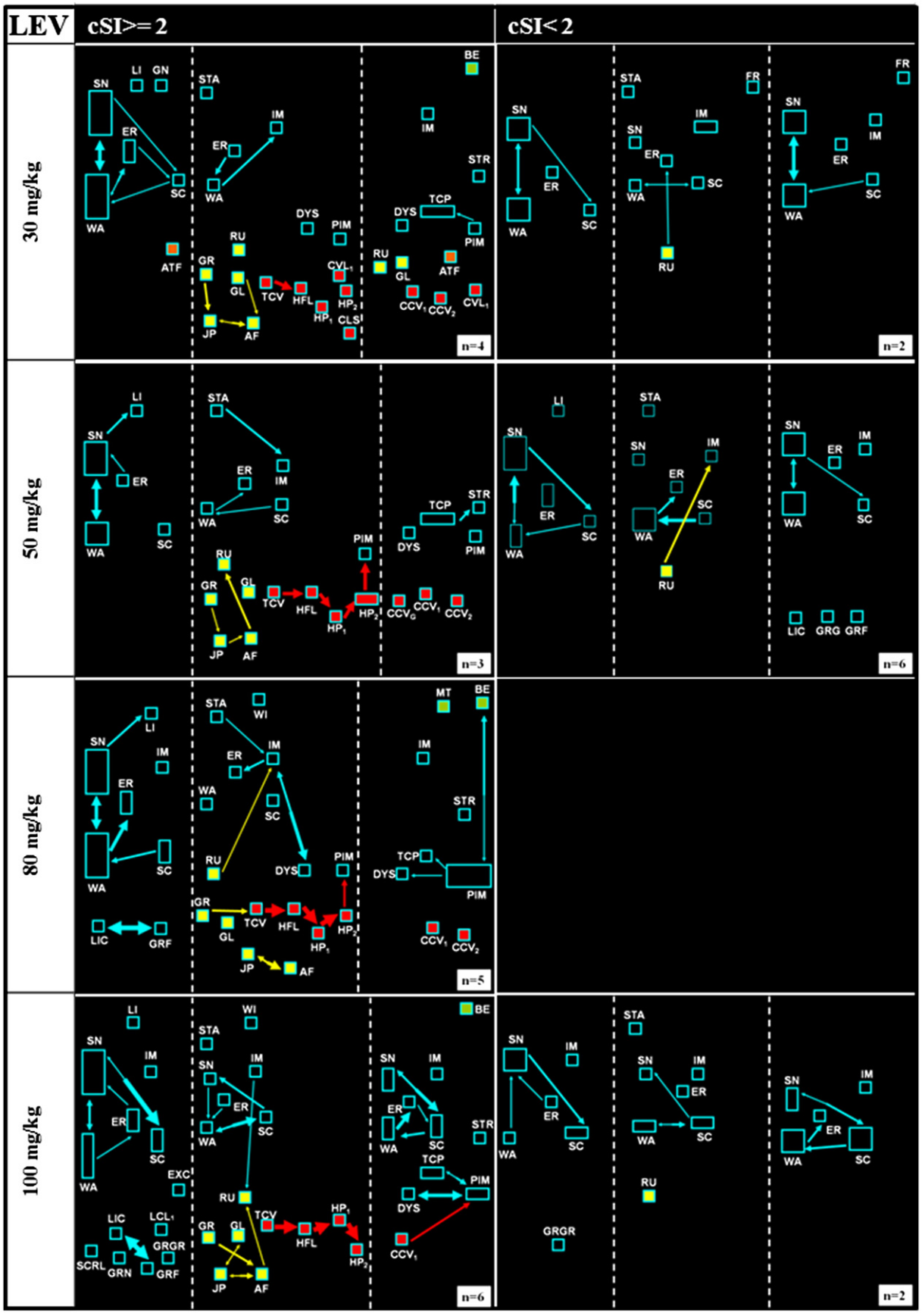

Fig. 9. Flowchart of the behaviors of the GASH:Sal hamsters injected with different doses of LEV.

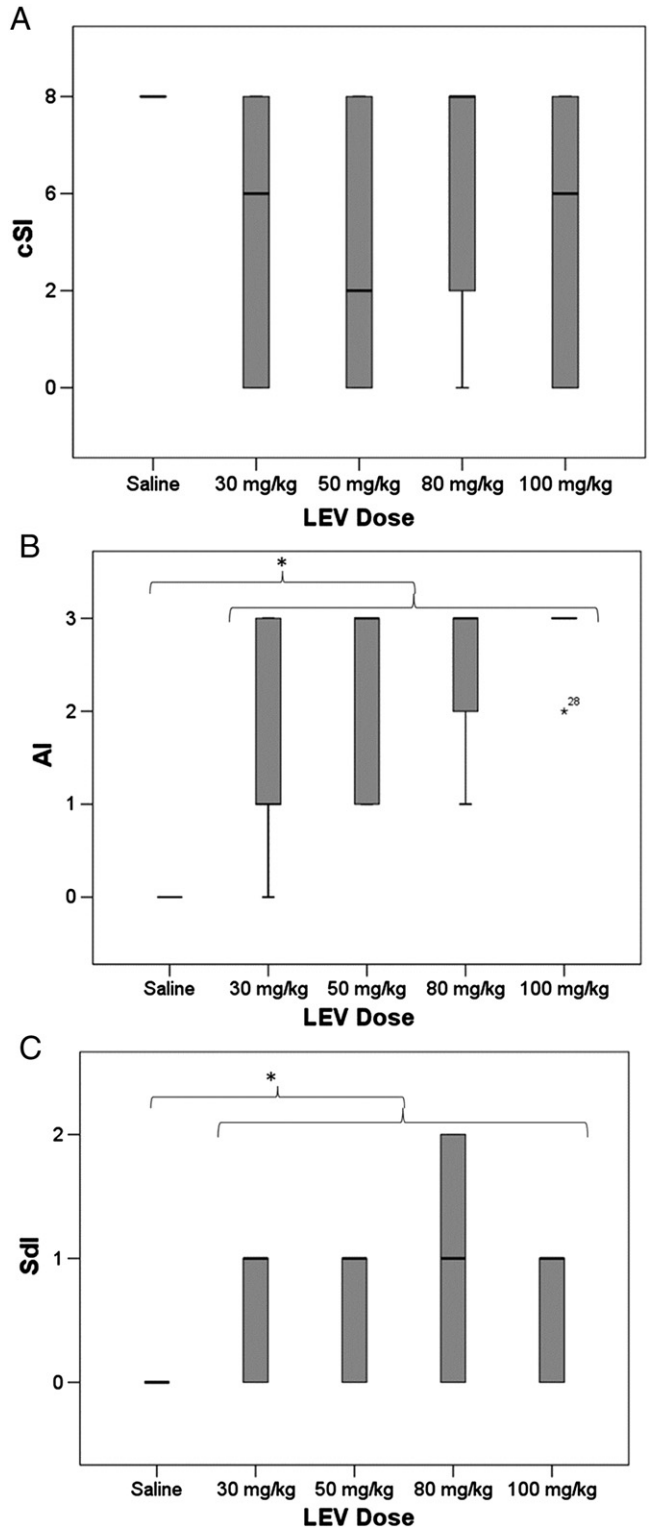

Fig. 10. Box plots of A) the categorized seizure severity (cSI), B) ataxic (AI), and C) sedation (SdI) indexes of the different doses of LEV.

the drug. Ataxic walking and falls, in conjunction with an increase in grooming behavior, accompanied the exploration, except at the highest dose (in which the hamsters had the highest level of ataxia).

In our study, which used a nonselective depressor of the nervous central system, we observed high levels of ataxia, related to high doses of PB, mainly 15 and 20 mg/kg, but similar to Silva Brum and Elisabetsky [40], we did not observe any sedation, which is in contrast to other rodent models [34,40,45–47]. The presence of grooming behavior associated with toxic doses of PB needs further study because this behavior is extremely sensitive to pharmacological treatment and is recognized as a reliable marker of stress-related disturbances and, in some cases, is a useful model for studying the organization and neural mechanisms of movement sequences [48,49].

Valproic acid, one of the oldest classical anticonvulsant drugs, acts similarly to PB to enhance the inhibitory actions of GABA [29,43,44]. The administration of VPA causes an elevation of cerebral GABA levels in rodents [50], which coincides with the period of protection against ASs [51]. In contrast, other authors have demonstrated that VPA induces anticonvulsant activity against AS and electroshock-induced convulsions at doses that are insufficient to raise brain GABA levels [52–54]. Valproic acid blocks seizures induced by pentylenetetrazole and maximal electroshock and shows antikindling properties [55] in a variety of animal models. In our experimental conditions, the maximum plasmatic level was observed 15 min after intraperitoneal administration of the drug, which is in agreement with the results obtained in other animals [56,57]. In our model, VPA only showed anticonvulsant activity at the highest dose, 300 mg/kg, and was accompanied by high levels of ataxia. We observed that animals with VPA at doses below 300 mg/kg maintained some wild running elements despite not seizing. Furthermore, the ataxic effect starts at 150 mg/kg, showing high toxicity of this drug. At the same time, it produces not only anticonvulsant effects but also ataxic walking. According to some authors, VPA has relatively low neurotoxicity in gerbils and rats, and, in mice, some sedation is seen at doses necessary to block convulsions [31,58,59]. The neurotoxic manifestation at anticonvulsant doses could be related to specific characteristics of the hamster species. There is extended information regarding the toxic effects of VPA in embryos, but there is a lack of information on the behavioral effects caused by VPA, mainly because of the low neurotoxicity seen in human adults [58].

Levetiracetam belongs to a group of nootropic drugs called racetams [60] that have anticonvulsant and neuroprotective properties [61] but have failed to reveal a generally accepted mechanism of action and usually show no affinity for the major receptors. Levetiracetam binds to the SV2A vesicle receptor in the cortex and hippocampus, increasing GABA [62] in addition to reducing the Ca^{2+} N type current [44,62,63]. In our study, the effect of LEV was not clear at any of the doses; indeed, at all doses, there were animals that presented seizures. Levetiracetam is devoid of protective effects in acute seizure models [64] but exhibits anticonvulsant activity in a dose-dependent manner in chronic treatment AS animal models [65] and in kindling models [66]. Therefore, it would be useful to observe the effects of LEV in a chronic condition model to determine if LEV has anticonvulsant effects on the GASH:Sal strain. Moreover, LEV was the only AED that induced sedation with ataxia, which is not a surprising result as sedation and somnolence are commonly reported side effects for this AED [67,68].

To completely determine the antiepileptic effect of the AEDs studied in these experiments, all the neuroethological data must be associated to electroencephalographic analysis. In the case of LEV, an EEG study could help us understand the fluctuating effect of the drug at different doses. This would help to determine if the GASH:Sal hamster would be a good model for developing drugs with similar mechanisms of action.

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References

[1] Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia 2005;46(4):470–2.

[2] Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. Epilepsia 2010;51(4):676–85.

[3] Pitkanen A, Lukasiuk K. Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. Epilepsy Behav 2009;14(Suppl. 1):16–25.

[4] Kaneko S, et al. Genetics of epilepsy: current status and perspectives. Neurosci Res 2002;44(1):11–30.

[5] Sgado P, Viaggi C, Pinna A, Marrone C, Vaglini F, Pontis S, et al. Behavioral, neurochemical, and electrophysiological changes in an early spontaneous mouse model of nigrostriatal degeneration. Neurotox Res 2011;20(2):170–81.

[6] Fuentes-Santamaria V, Alvarado JC, Herranz AS, García-Atarés N, López DE. Decreased levels of GABA in the inferior colliculus of the epilepsy-prone hamster (GPG/Vall). Epilepsy Res 2008;79(2–3):224–7.

[7] Gjerstad L, Tauboll E. Epilepsy and genetics. Tidsskr Nor Laegeforen 2003;123(19):2731–4.

[8] Steinlein OK. Channelopathies can cause epilepsy in man. Eur J Pain 2002;6(Suppl. A):27–34.

[9] Guerrini R, Casari G, Marini C. The genetic and molecular basis of epilepsy. Trends Mol Med 2003;9(7):300–6.

[10] Gurnett CA, Hedera P. New ideas in epilepsy genetics: novel epilepsy genes, copy number alterations, and gene regulation. Arch Neurol 2007;64(3):324–8.

[11] Baraban SC. Emerging epilepsy models: insights from mice, flies, worms and fish. Curr Opin Neurol 2007;20(2):164–8.

[12] Schauwecker PE. The relevance of individual genetic background and its role in animal models of epilepsy. Epilepsy Res 2011;97(1–2):1–11.

[13] Fuentes-Santamaria V, et al. Morphologic and neurochemical abnormalities in the auditory brainstem of the genetically epilepsy-prone hamster (GPG/Vall). Epilepsia 2005;46(7):1027–45.

[14] Ross KC, Coleman JR. Developmental and genetic audiogenic seizure models: behavior and biological substrates. Neurosci Biobehav Rev 2000;24(6):639–53.

[15] Faingold CL. Neuronal networks in the genetically epilepsy-prone rat. Adv Neurol 1999;79:311–21.

[16] Fuentes-Santamaria V, Alvarado JC, Herranz AS, García-Atarés N, López DE. Morphologic and neurochemical alterations in the superior colliculus of the genetically epilepsy-prone hamster (GPG/Vall). Epilepsy Res 2007;75(2–3):206–19.

[17] Garcia-Cairasco N, Rossetti F, Oliveira JA, Furtado Mde A. Neuroethological study of status epilepticus induced by systemic pilocarpine in Wistar audiogenic rats (WAR strain). Epilepsy Behav 2004;5(4):455–63.

[18] Garcia-Cairasco N, Wakamatsu H, Oliveira JA, Gomes EL, Del Bel EA, Mello LE. Neuroethological and morphological (Neo-Timm staining) correlates of limbic recruitment during the development of audiogenic kindling in seizure susceptible Wistar rats. Epilepsy Res 1996;26(1):177–92.

[19] Garcia-Cairasco N, Doretto MC, Ramalho MJ, Antunes-Rodrigues J, Nonaka KO. Audiogenic and audiogenic-like seizures: locus of induction and seizure severity determine postictal prolactin patterns. Pharmacol Biochem Behav 1996;53(3):503–10.

[20] Furtado MA, Braga GK, Oliveira JA, Del Vecchio F, Garcia-Cairasco N. Behavioral, morphologic, and electroencephalographic evaluation of seizures induced by intrahippocampal microinjection of pilocarpine. Epilepsia 2002;43(Suppl. 5):37–9.

[21] Castro OW, Furtado MA, Tilelli CQ, Fernandes A, Pajolla GP, Garcia-Cairasco N. Comparative neuroanatomical and temporal characterization of FluoroJade-positive neurodegeneration after status epilepticus induced by systemic and intrahippocampal pilocarpine in Wistar rats. Brain Res 2011;1374:43–55.

[22] Etholm L, Arabadzisz D, Lipp HP, Heggelund P. Seizure logging: a new approach to synchronized cable-free EEG and video recordings of seizure activity in mice. J Neurosci Methods 2010;192(2):254–60.

[23] Dal-Cól MLC, Terra-Bustamante VC, Velasco TR, Oliveira JA, Sakamoto AC, Garcia-Cairasco N. Neuroethology application for the study of human temporal lobe epilepsy: from basic to applied sciences. Epilepsy Behav 2006;8(1):149–60.

[24] Bertti P, Dal-Cól ML, Wichert-Ana L, Kato M, Terra VC, de Oliveira JA, et al. The neurobiological substrates of behavioral manifestations during temporal lobe seizures: a neuroethological and ictal SPECT correlation study. Epilepsy Behav 2010;17(3):344–53.

[25] Picazo MG, Benito PJ, García-Olmo DC. Efficiency and safety of a technique for drawing blood from the hamster cranial vena cava. Lab Anim 2009;38(6):211–6.

[26] Contin M, Mohamed S, Candela C, Albani F, Riva R, Baruzzi A. Simultaneous HPLC-UV analysis of rufinamide, zonisamide, lamotrigine, oxcarbazepine monohydroxy derivative and felbamate in deproteinized plasma of patients with epilepsy. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878(3–4):461–5.

[27] Moriyama M, Yamashita S, Domoto H, Furuno K, Araki H, Gomita Y. Determination of plasma phenobarbital concentration by high-performance liquid chromatography in rat offspring. J Chromatogr B Biomed Sci Appl 1999;723(1–2):301–5.

[28] Tang M, Lau CE, Falk JL. Serum phenobarbital and barbital concentrations in rats on a limited food regimen. Pharmacol Biochem Behav 1979;11(3):359–61.

[29] Cheng H, Liu Z, Blum W, Byrd JC, Klisovic R, Grever MR, et al. Quantification of valproic acid and its metabolite 2-propyl-4-pentenoic acid in human plasma using HPLC–MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 2007;850(1–2):206–12.

[30] Doheny HC, Ratnaraj N, Whittington MA, Jefferys JG, Patsalos PN. Blood and cerebrospinal fluid pharmacokinetics of the novel anticonvulsant levetiracetam (ucb L059) in the rat. Epilepsy Res 1999;34(2–3):161–8.

[31] Chapman A, Keane PE, Meldrum BS, Simiand J, Vernieres JC. Mechanism of anticonvulsant action of valproate. Prog Neurobiol 1982;19(4):315–59.

[32] Litchfield Jr JT, Wilcoxon F. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 1949;96(2):99–113.

[33] Rossetti F, Rodrigues MC, de Oliveira JA, Garcia-Cairasco N. EEG wavelet analyses of the striatum-substantia nigra pars reticulata-superior colliculus circuitry: audiogenic seizures and anticonvulsant drug administration in Wistar audiogenic rats (War strain). Epilepsy Res 2006;72(2–3):192–208.

[34] Löscher W, Hönack D. Comparison of the anticonvulsant efficacy of primidone and phenobarbital during chronic treatment of amygdala-kindled rats. Eur J Pharmacol 1989;162(2):309–22.

[35] Conover WJ. Practical nonparametric statistics. New York: Wiley; 1971, 1980.

[36] Muñoz de la Pascua L, López DE. In: Muñoz de la Pascua Luis, editor. Establecimiento y caracterización de una línea de hámsters sirios propensos a padecer convulsiones audiógenas. ISBN 978-84-609-5027-1; 2005 [Salamanca].

[37] Carballosa-González MM. Hacia un nuevo modelo de epilepsia: el hámster GASH: Sal. Instituto de Neurociencias Castilla y León (INCyL). 2008, Vol. Doctoral thesis, eds. Universidad de Salamanca: Salamanca.

[38] Nef P. Key animal models for the identification and validation of drug targets. Drug Discov Ther 2001;6(15 (Suppl.)):91–6.

[39] Löscher W. Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. Epilepsy Res 2002;50(1–2):105–23.

[40] Silva Brum LF, Elisabetsky E. Antiepileptogenic properties of phenobarbital: behavior and neurochemical analysis. Pharmacol Biochem Behav 2000;67(3):411–6.

[41] Salvador N, Guillén J, Peralta JM. Biología general y mantenimiento de las especies más utilizadas. In: Zuñiga JM, Orellana JM, Tur JA, editors. Ciencia y Tecnología del Animal de Laboratorio. Alcalá: Universidad de Alcalá, Sociedad Española para las Ciencias del Animal de Laboratorio (SECAL); 2008. p. 95–150.

[42] Czuczwar SJ, Turski L, Kleinrok Z. Anticonvulsant action of phenobarbital, diazepam, carbamazepine, and diphenylhydantoin in the electroshock test in mice after lesion of hippocampal pyramidal cells with intracerebroventricular kainic acid. Epilepsia 1982;23(4):377–82.

[43] Gilbert TH, Corley SM, Teskey GC. Conventional anticonvulsant drugs in the guinea pig kindling model of partial seizures: effects of acute phenobarbital, valproate, and ethosuximide. Exp Brain Res 2002;146(3):336–44.

[44] Werner FM, Covenas R. Classical neurotransmitters and neuropeptides involved in generalized epilepsy: a focus on antiepileptic drugs. Curr Med Chem 2011;18(32):4933–48.

[45] Gay MH, Ryan GP, Boisse NR, Guarino JJ. Phenobarbital tolerance and physical dependence: chronically equivalent dosing model. Eur J Pharmacol 1983;95(1–2):21–9.

[46] Munoz-Garcia D, Del Ser T, Bermejo F, Portera A. Truncal ataxia in chronic anticonvulsant treatment. Association with drug-induced folate deficiency. J Neurol Sci 1982;55(3):305–11.

[47] Barcia JA, Rubio P, Alós M, Serralta A, Belda V. Anticonvulsant and neurotoxic effects of intracerebroventricular injection of phenytoin, phenobarbital and carbamazepine in an amygdala-kindling model of epilepsy in the rat. Epilepsy Res 1999;33(2–3):159–67.

[48] Whishaw IQ, Kolb B, editors. The behavior of the laboratory rat. New York: Oxford University Press; 2005.

[49] Kalueff AV, LaPorte JL, Bergner CL, editors. Neurobiology of grooming behavior. New York: Cambridge University Press; 2010.

[50] Godin Y, Heiner L, Mark J, Mandel P. Effects of DI-n-propylacetate, and anticonvulsive compound, on GABA metabolism. J Neurochem 1969;16(3):869–73.

[51] Simler S, Ciesielski L, Maitre M, Randrianarisoa H, Mandel P. Effect of sodium n-dipropylacetate on audiogenic seizures and brain-aminobutyric acid level. Biochem Pharmacol 1973;22(14):1701–8.

[52] Kerwin RW, Taberner PV. The mechanism of action of sodium valproate. Gen Pharmacol 1981;12(2):71–5.

[53] Anlezark G, Horton RW, Meldrum BS, Sawaya CB. Anticonvulsant action of ethanolamine-O-sulphate and di-n-propylacetate and the metabolism of gamma-aminobutyric acid (GABA) in mice with audiogenic seizures. Biochem Pharmacol 1976;25(4):413–7.

[54] Kerwin RW, Olpe HR, Schmutz M. The effect of sodium-n-dipropyl acetate on gamma–aminobutyric acid-dependent inhibition in the rat cortex and substantia nigra in relation to its anticonvulsant activity. Br J Pharmacol 1980;71(2):545–51.

[55] Leviel V, Naquet R. A study of the action of valproic acid on the kindling effect. Epilepsia 1977;18(2):229–34.

[56] Meinardi H, Van Der Kleijn E, Meijer JW, Van Rees H. Absorption and distribution of antiepileptic drugs. Epilepsia 1975;16(2):353–65.

[57] Schobben AFAM. Pharmacokinetics and therapeutics in epilepsy. (Dissertation) Nijmegen the Netherlands: University of Nijmegen; 1979.

[58] Abbott FS, Acheampong AA. Quantitative structure-anticonvulsant activity relationships of valproic acid, related carboxylic acids and tetrazoles. Neuropharmacology 1988;27(3):287–94.

[59] Löscher W, Nau H, Marescaux C, Vergnes M. Comparative evaluation of anticonvulsant and toxic potencies of valproic acid and 2-en-valproic acid in different animal models of epilepsy. Eur J Pharmacol 1984;99(2–3):211–8.

[60] Lim LL, Ahmed A. Limited efficacy of levetiracetam on myoclonus of different etiologies. Parkinsonism Relat Disord 2005;11(2):135–7.

[61] Gualtieri F, Manetti D, Romanelli MN, Ghelardini C. Design and study of piracetam-like nootropics, controversial members of the problematic class of cognition-enhancing drugs. Curr Pharm Des 2002;8(2):125–38.
[62] Boido D, Farisello P, Cesca F, Ferrea E, Valtorta F, Benfenati F, et al. Cortico-hippocampal hyperexcitability in synapsin I/II/III knockout mice: age-dependency and response to the antiepileptic drug levetiracetam. Neuroscience 2010;171(1):268–83.
[63] Luszczki JJ. Third-generation antiepileptic drugs: mechanisms of action, pharmacokinetics and interactions; 2009.
[64] Löscher W, Schmidt D. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Res 1988;2(3):145–81.
[65] Löscher W, Hönack D, Rundfeldt C. Antiepileptogenic effects of the novel anticonvulsant levetiracetam (ucb L059) in the kindling model of temporal lobe epilepsy. J Pharmacol Exp Ther 1998;284(2):474–9.
[66] Silver JM, Shin C, McNamara JO. Antiepileptogenic effects of conventional anticonvulsants in the kindling model of epilespy. Ann Neurol 1991;29(4):356–63.
[67] Gower AJ, Noyer M, Verloes R, Gobert J, Wülfert E. ucb L059, a novel anticonvulsant drug: pharmacological profile in animals. Eur J Pharmacol 1992;222(2–3):193–203.
[68] Gower AJ, Hirsch E, Boehrer A, Noyer M, Marescaux C. Effects of levetiracetam, a novel antiepileptic drug, on convulsant activity in two genetic rat models of epilepsy. Epilepsy Res 1995;22(3):207–13.