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# Levodropropizine suppresses seizure activity in rats with pentylenetetrazol-induced epilepsy

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ABSTRACT

*Background:* Millions of individuals worldwide suffer from epilepsy, and up to 25% of patients have seizures that are resistant to currently available antiepileptic drugs. Hence, there continues to be a need for more seizure medications that are effective yet tolerable. Levodropropizine (LVDP) is an established antitussive drug that, based on preclinical data, may also have antiepileptic activity.

*Methods:* We treated rats with either intraperitoneal (IP) LVDP at two different doses or placebo in randomized fashion and then exposed them to IP pentylenetetrazol (PTZ), a potent seizure-inducing compound. We measured the rats' subsequent seizure activity with electroencephalography (EEG), Racine's convulsion scale (RCS) and time to first myoclonic jerk (TFMJ) to determine whether LVDP has antiepileptic properties in our murine model for epilepsy.

*Results:* When compared to placebo, LVDP at both doses significantly suppressed seizure activity. Mean EEG spike wave percentage score decreased from 76.8% (placebo) to 13.1% (lower dose) and 7.6% (higher dose, both $p < 0.0001$). RCS decreased from a mean of 5.8 (placebo) to 1.83 (lower dose) and 1.16 (higher dose, both $p < 0.05$). TFMJ had increased from a mean of 65.1 s (placebo), to 247.3 s (lower dose) and 295.5 s (higher dose, both $p < 0.0001$).

*Conclusions:* Levodropropizine, a common antitussive drug, suppresses seizure activity in rats with PTZ-induced status epilepticus. Given the ongoing need to find effective therapies for refractory epilepsy, the possibility of using levodropropizine as an antiepilepticshould be further explored.

## 1. Introduction

Epilepsy is a common and often debilitating neurological disease that is characterized by recurrent spontaneous seizures arising from abnormal electrical activity in the brain. Worldwide, up to 3% of the general population have epilepsy at some point in their lives (Wyllie, 2015), and in the United States, it is estimated that nearly two million people have epilepsy. Epileptic seizures have various aetiologies that range from genetic and congenital to neuronal abnormalities that arise from prior trauma such as hypoxia, infection, and inflammation. It may also be due to an underlying disorder or associated with psychiatric symptoms (Motamedi and Meador, 2003; Schmidt, 2009; Eddy et al., 2011; Kanner et al., 2012). While we've made tremendous advances in our understanding of epilepsy over the past 50 years, many instances of epilepsy remain idiopathic with unclear pathophysiology (Chang and Lowenstein, 2003). It may be surprising to some that cases of epilepsy with an identifiable underlying cause—often classified as structural abnormalities, metabolic disturbances or genetic factors—only account for only 25–45% of all cases (Cowan, 2002).

Likewise, there continue to be great challenges in the treatment of epilepsy. In spite of the growing diversity of antiepileptic drugs, approximately 30% of epilepsy patients continue to experience seizures despite optimal antiepileptic therapy (Reddy and Kuruba, 2013; Shin et al., 2011), and thus they are deemed to have resistant or refractory

*Abbreviations:* AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ANOVA, one-way analysis of variance; EEG, electroencephalogram; EU, European Union; GABA, γ-aminobutyric acid; Hz, hertz; IP, intraperitoneal; KA, kainate receptor; LVDP, levodropropizine; NMDA, *N*-methyl-D-aspartate; NTS, nucleus tractus solitarius; PTZ, pentylenetetrazol; RCS, Racine's convulsion scale; SP, substance P; SSSE, self-sustaining status epilepticus; TFMJ, time to first myoclonic jerk

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disease. Furthermore, many antiepileptic drugs—including ones that are most commonly used—have a narrow therapeutic window and (or) low tolerability and therefore clinically significant adverse effects are not uncommon (Hancock et al., 2001). Because of this, even patients who are able to attain seizure–free status with therapy may still live with adverse effects that persist for the duration of treatment (Elger et al., 2004). For these reasons, finding novel therapies for epilepsy that possess excellent antiepileptic properties while at the same time a wider therapeutic window and fewer toxicities should continue to be an area of interest for epileptologists and the biomedical research community.

Epileptogenesis is a process that takes place at the cellular and molecular level by which previously normal brain synaptic function is altered and biased towards the formation of abnormal electrical activity that subserves seizure activity. There are several theories that address the precise mechanisms of epileptogenesis at the molecular, anatomical and synaptic level which include dysregulation of ionic channels or neurotransmitter receptors, abberations in prominent neuronal cells, and scarring or inflammation in the microenvironment (Curia et al., 2014).

One prominent theory for epileptogenesis involves the altered metabolism of γ-aminobutyric acid (GABA), a dominant and well-known inhibitory neurotransmitter in the human and mammalian brain (Kang and Macdonald, 2009). In the course of epileptogenesis, decreased GABA production, availability, or activity is known to cause neuronal hyperexcitability, and conversely, ligands that increase GABA activity have been shown to possess antiepileptic properties (Olsen et al., 1999). There are also other molecules that likely play important roles in epileptogenesis. For instance, overabundance of glutamate—one of the most common excitatory neurotransmitters in the brain—as well as Substance P (SP) increase the risk of seizure (McNamara et al., 2006; Nalivaiko et al., 1997). In one study, SP was observed to trigger a cascade of events that lead to self-sustaining status epilepticus (SSSE), one mediated through glutamate release (Liu et al., 1999). Conversely, antagonists of N-methyl-D-aspartate (NMDA) receptors have a neuroprotective effect (Penix et al., 1996). Interestingly, cellular processes and molecules that are associated with heightened seizure activity often also have a significant role in the central and peripheral mechanisms of coughing.

In this study, we investigate the potential antiepileptic effects of levodropropizine, a non-opioid compound that is commonly used as an efficient and well-tolerated antitussive agent in clinical practice (Braga, 1989), at least in some parts of the world. Levodropropizine, also less commonly known as S(-)-3-(4-phenyl-piperazin-1-yl)-propane-1,2-diol or simply LVDP, is believed to exert its antitussive effect through both peripheral and central mechanisms. While LVDP possess central sedative effects similar to other antitussive drugs, these fortunately appear to be minimal (Malandrino et al., 1988; Melillo et al., 1988). Since overlapping mechanisms and targets appear to exist between coughing and seizure activity, we were motivated to assess whether levodropropizine can antagonize seizure activity.

## 2. Materials and methods

### 2.1. Ethics approval

The experimental procedures used in this study were approved by the governing animal ethics comittee of the institution in which the experiments were carried out. All experiments were performed in accordance with the ARRIVE guidelines, the U.K. Animals (Scientific Procedures) Act of 1986 and associated guidelines, the European Union (EU) Directive 2010/63/EU for animal experiments, and the Guide for the Care and Use of Laboratory Animals as per the US National Institutes of Health (NIH Publications No. 8023, revised 1978).

### 2.2. Care of study animals

We used 48 male Sprague–Dawley rats, weighing 200 to 250 g each, for this study, with 24 rats randomized to the electroencephalogram (EEG) based experiment and 24 rats randomized to the behaviour-based experiment. The rats were maintained in a 12 h – 12 h light–dark cycle, with light being provided from 0700 to 1900 h, in quiet rooms, with the ambient temperature set to 22 to 24 °C. The rats were fed by standard laboratory food and tap water *ad libitum*.

### 2.3. Experimental procedures

We performed two experiments in which we induced seizures in the rats with the chemoconvulsant PTZ, and measured the effects of LVDP on seizure activity either through the analysis of electroencephalogram (EEG) recordings or a behavioural scoring system. Out of a total of 48 rats, 24 were randomized to Group A for the EEG-based experiment, and 24 were randomized to Group B for the behavioral experiment. All EEG recordings and behavioral assessments were performed in accordance to protocols that were previously described (Erbaş et al., 2015).

### 2.4. EEG experiment (Group A)

Prior to the experiment, rats in group A underwent electrode implantation to facilitate EEG recording. The rats were deeply anesthetized with ketamine at 80 mg/kg and xylazine at 4 mg/kg intraperitoneally (IP). Then, we drilled—using precise stereotactic methods—small burr holes through the cranium for electrode placement. The EEG electrodes we used were polyamide-coated stainless steel wires, 0.1 mm diameter with an electrical resistance of less than 1Ω per 10 mm. We implanted electrodes on the dura over left frontal cortex, 2.0 mm lateral to the midline, and 1.5 mm anterior to the bregma. We implanted a reference electrode over the cerebellum, 1.5 mm posterior to the lambda on the midline as per previously published protocols (Kubin et al. (2006)). Following successful placement, the electrodes were fixed with dental acrylic, a mixture of alloys and hydrocarbons that are typically used in dental restoration.

After a recovery period of 12 days, the 24 rats in group A were further equally randomized into 4 subgroups. Group A1 was a control group that received no seizure-inducing chemicals or other interventions. Group A2 was the placebo group that received IP saline. Group A3 was treated with a lower dose of IP LVDP at 20 mg/kg and a concentration of 6 mg/ml. Lastly, group A4 was treated with a higher dose IP LVDP at 40 mg/kg with identical concentration.

To induce seizures in groups A2, A3, and A4, pentylenetetrazol (PTZ) was administered at a dose of 35 mg/kg intraperitoneally (IP) 30 min after the administration of either LVDP or saline placebo. PTZ at 35 mg/kg IP results in epileptiform activity on EEG without observable behavioral changes, while at a higher dose of 70 mg/kg, observable behavioral changes can be consistently seen alongside EEG changes consistent with seizure. However, at this higher dose, the EEG signal-to-noise ratio may be impaired.

EEG recordings were started 5 min after PTZ administration and continued for 60 min. The rats were left awake without sedation and placed in special containers for the entire duration of their EEG recording, which were done with a BIOPAC MP150 Data Acquisition System that is available through Biopac System Incorporated at Santa Barbara, California, United States. We recorded each rat's EEG tracing for 60 min, at a sampling rate of 240 Hz (Hz). The signal was amplified 10,000 times and filtered within a range of 1–60 Hz. After the EEG recording was done, we euthanized the test subject.

The presence and severity of seizures in our animal model was quantified using the spike wave percentage method, which is a well-accepted and reproducible way of evaluating epileptiform activity in this type of research, and has recently been used in similar experiments

(Erbaş et al., 2015). The validity of spike wave percentages in evaluating seizures has been investigated and discussed in prior studies (Aeby et al., 2005; Fernández et al., 2012). To further minimize errors in quantitative evaluation, we employed two blinded clinical neurophysiologists to score the EEG data.

To generate the spike wave percentage score, we separate the EEG tracing into one-second bins and our neurophysiologists evaluate each bin for the presence of spike waves. The spike wave is defined by an elevation in the amplitude of the EEG tracing that is at least two-fold higher than baseline activity. If at least one spike wave exists in a one-second bin, that bin is considered to be positive for the presence of spike waves. At 2-minute intervals (120 bins), the number of positive bins is divided by the total number of bins (120) to get the spike-wave percentage. The overall spike-wave percentage is obtained by averaging the percentages found in each 2-minute run.

### 2.5. Behavioral experiment (Group B)

The 24 rats in group B were evaluated for visually observable seizure activity (the "behavioral experiment"). No brain electrodes were installed in this group. Like in the EEG experiment, the rats in group B were randomized to 4 subgroups with 6 rats in each subgroup. Group B1 was a control group that received no intervention. Groups B2, B3, and B4 received PTZ for seizure induction at a higher dose of 70 mg/kg IP to induce clinically observable seizures. Like in the EEG experiment, about 30 min prior to the administration of PTZ, group B2 received IP saline placebo, while group B3 received LVDP at 20 mg/kg IP, and group B4 received LVDP at 40 mg/kg IP.

We used two metrics to evaluate the presence and severity of seizures: (1) Racine's Convulsion Scale (RCS) (Lüttjohann et al., 2009), and (2) time to first myoclonic jerk (TFMJ). RCS, as previously described, is a simple and reproducible 6-point scoring system for evaluating murine epilepsy. A score of 0 indicates no visible convulsion. A score of 1 indicates twitching of vibrassae and pinnae. A score of 2 indicates motor arrest with more pronounced twitching. A score of 3 indicates motor arrest with generalized myoclonic jerks. In this experiment, the elapsed time (in seconds) upon which a score of at least 3 is obtained represents the rat's TFMJ (Erbaş et al., 2015). A score of 4 indicates tonic-clonic seizure activity while the animal still able to stay on its feet. A score of 5 indicates tonic-clonic seizure with loss of the righting reflex, and finally a score of 6 indicates a lethal seizure.

The TFMJ is recorded in seconds following administration of PTZ. In our experiment, almost all animals that demonstrated tonic generalized extension died from seizure activity. The observation period for PTZ-induced seizures were limited to 30 min, similar to previous experiments of this nature in the literature (Erbaş et al., 2015). After this 30-minute evaluation, surviving animals were euthanized.

### 2.6. Statistical analysis

We analyzed our data with SPSS version 15.0 for Windows. We applied the Shapiro-Wilk test to determine the normality of our population, evaluated our RCS data with the Kruskal Wallis test, and our TFMJ data with one-way analysis of variance (ANOVA). We looked for differences between our experimental groups using the post hoc Bonferonni and Mann Whithney U tests. The limit of statistical significance was set to a $p$-value of $< 0.05$.

## 3. Results

### 3.1. Results of EEG experiment

We found that administration of LVDP at 20 mg/kg significantly decreased seizure activity as measured via spike wave percentage when compared to saline placebo (13.1% versus 76.8%, $p < 0.0001$). The higher dose of LVDP also suppressed seizure activity with a trend towards greater effectiveness (7.6% versus 76.8%, $p < 0.0001$). However, the difference in seizure suppression between the lower and higher dose of LVDP was not statistically significant (see Table 1). Representative tracings from the EEG experiment for each subgroup is provided in Fig. 1, and in Fig. 2 at a higher resolution for better characterization of epileptiform activity.

**Table 1**
Results from the EEG experiment.

| Group | Intervention | Spike wave (%) | $p$-value vs A2 |
|---|---|---|---|
| A1 | None | 0.0% ± 0.0% | – |
| A2 | Saline IP (placebo) | 76.8% ± 4.2% | – |
| A3 | Saline IP + LVDP 20 mg/kg IP | 13.1% ± 2.6% | $p < 0.0001$ |
| A4 | Saline IP + LVDP 40 mg/kg IP | 7.6% ± 1.9% | $p < 0.0001$ |

**Description**: We randomly allocated 6 rats to each treatment groups A1, A2, A3, and A4. After receiving their respective interventions, the rats in groups A2, A3, and A4 were given pentylenetetrazol (PTZ) at a dose of 35 mg/kg intraperitoneally (IP) to induce status epilepticus. Electroencephalogram (EEG) recording was started 5 min after PTZ administration and continued for 60 min. Surviving animals were euthanized thereafter. The EEG recordings were evaluated by two trained neurophysiologists to look for spike waves in 1-second bins. The spike wave percentage reflects how many bins contained at least one spike wave out of the total number of bins available in the EEG records. The higher the spike wave percentage, the higher the seizure activity. In this experiment, we found that levodropropizine (LVDP) at both 20 and 40 mg/kg IP significantly reduced seizure activity on EEG as measured by spike wave percentage ($p < 0.0001$). The further reduction in spike wave percentage with LVDP dose escalation from 20 mg/kg to 40 mg/kg was not statistically significant ($p > 0.05$).

### 3.2. Results of behavioral experiment

The results from our behavioral experiment also suggest that LVDP exert antiepileptic effects in our murine model for epilepsy (Tables 2 and 3). When compared to the placebo-treated group, LVDP significantly reduced RCS scores (via the Kruskal–Wallis test) and delayed TFMJ (via the one-way ANOVA and *post hoc* Bonferroni tests).

The mean RCS score decreased from 5.8 (which is quite severe, since a score of 6 indicates fatal seizure activity) to 1.83 ($p < 0.05$) with the lower dose of LVDP. The mean RCS score decreased to 1.16 ($p < 0.05$) with the higher dose of LVDP. There was a trend towards lower RCS scores with the higher dose of LVDP as opposed to the lower dose of LVDP, however this trend was not statistically significant (Mann–Whitney U test).

Likewise, LVDP significantly increased the TFMJ at both the lower and higher doses ($p < 0.05$). As compared to the untreated group B2 with a mean TFMJ of 65.1 s, in group B3 with the lower dose of LVDP, the TFMJ had increased to a mean of 247.3 s ($p < 0.0001$). In group B4 with the higher dose of LVDP, the TFMJ had increased to a mean of 295.5 s ($p < 0.0001$). The difference in mean TFMJ between the lower and higher dose of LVDP was not statistically significant.

## 4. Discussion

In this experiment, we explored the antiepileptic potential of LVDP, which has been classically utilized as a cough suppressing medication for both children and adults, in a murine model for epilepsy. We were motivated to conduct this study since there is emerging evidence that the pathophysiologies of seizure and cough have overlapping cellular and neurochemical pathways (Canning, 2007; Walker et al., 1999).

Epilepsy is one of the oldest medical conditions that has been characterized in the history of medicine (Epilepsy, 2018) and continues to be the most common neurological disease that affects individuals of all ages. Worldwide, it is estimated that 50 million people live with epilepsy (Epilepsy, 2018). At the cellular level, the pathogenesis of epilepsy is complex and implicate many mechanisms (Avoli et al.,

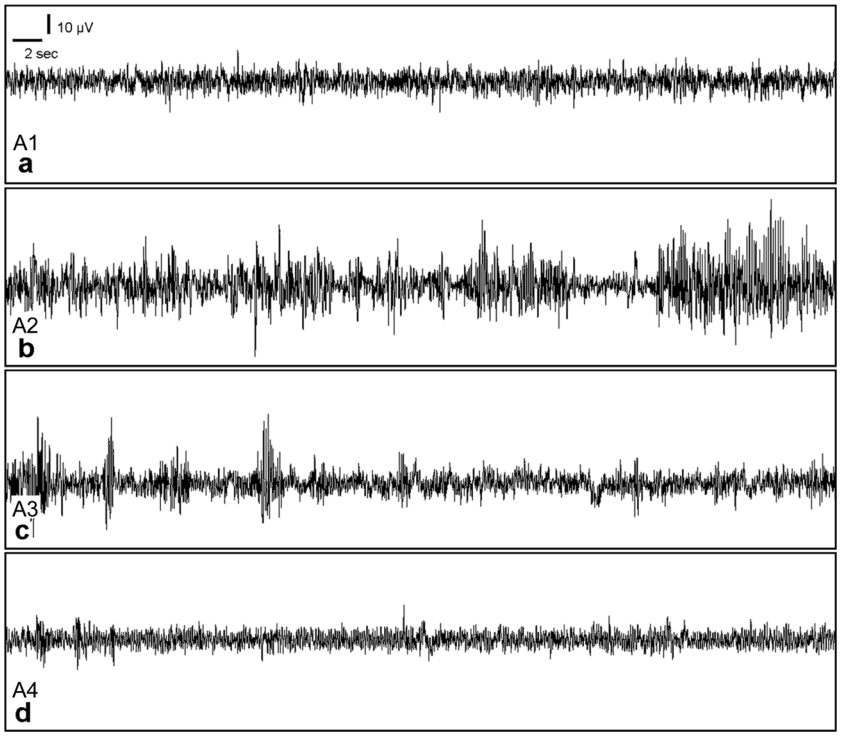


**Fig. 1.** Representative tracings from the EEG experiment.
**Caption**: Representative EEG recordings from groups A1 (control), A2 (PTZ and saline), A3 (PTZ and LVDP at 20 mg/kg) and A4 (PTZ and LVDP at 40 mg/kg). As expected, there were virtually no spike waves seen in group A1. Dense spike wave activity was seen in group A2 due to the unopposed induction of seizures with 35 mg/kg of IP PTZ, with a mean spike wave percentage score of 76.8%. There was significant abatement ($p < 0.0001$) of seizure activity—as quantified by spike wave percentages—in groups A3 and A4 with the addition of LVDP at 20 mg/kg (A3, 13.1%) and 40 mg/kg (A4, 7.6%) as compared to A2. However, the marginal improvement seen between groups A3 and A4 were not statistically different from one another.

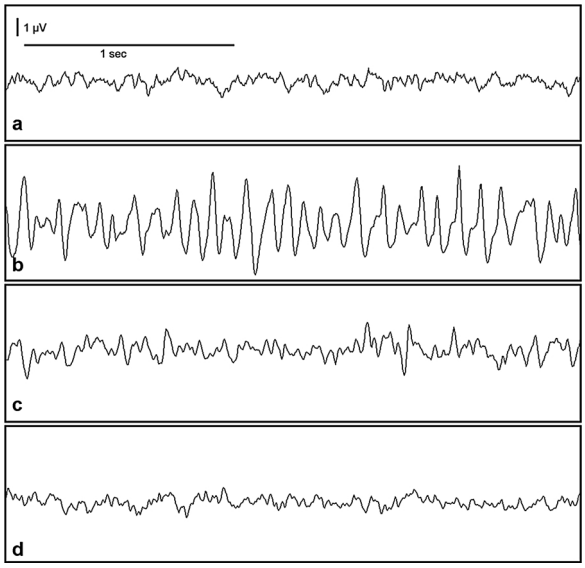


**Fig. 2.** Representative tracings from the EEG experiment in higher resolution.
Caption: Representative EEG recordings from groups a—control), b—PTZ and saline, c—PTZ and LVDP at 20 mg/kg and D-PTZ and LVDP at 40 mg/kg displayed at a higher resolution (about 2,500 ms in duration) to allow for better characterization of epileptiform waves especially in group b, which received PTZ to induce status epilepticus but not LVDP.

2002). Mutated ion channels can also result in seizures as they can augment the delicate balance that exists between excitatory and inhibitory neurotransmission, and thus alter the overall excitability of neurons (Mantegazza et al., 2010). Mutations that cause a "gain of function" or "upregulation" of excitatory neurotransmission, or a "loss of function" or "downregulation" of inhibitory neurotransmission, results in disinhibition and hyperexcitability in the brain, and subsequently a lowering of the seizure threshold (Kang and Macdonald, 2009). In epileptogenesis, there is commonly the presence of both increased neuronal excitability and synchronization (Curia et al., 2014).

**Table 2**
Improvement in RCS with LVDP.

| Group | Intervention | RCS | *p*-value vs B2 |
|---|---|---|---|
| B1 | None | 0.0 ± 0.0 | – |
| B2 | Saline IP (placebo) | 5.8 ± 0.1 | – |
| B3 | Saline IP + LVDP 20 mg/kg IP | 1.83 ± 0.2 | $p < 0.05$ |
| B4 | Saline IP + LVDP 40 mg/kg IP | 1.16 ± 0.2 | $p < 0.05$ |

**Description**: similar to the EEG experiment, we randomly allocated 6 rats to each treatment groups B1, B2, B3, and B4. After receiving their respective interventions, the rats in groups A2, A3, and A4 were given pentylenetetrazol (PTZ) at a dose of 70 mg/kg intraperitoneally (IP) to induce behaviorally apparent seizures. In our experiment, LVDP at 20 mg/kg and 40 mg/kg IP significantly reduced the Racine's convulsion score (RCS) when compared to placebo. We also found that the marginal improvement in RCS at the higher dose of LVDP (40 mg/kg) was statistically significant when compared to the lower dose of LVDP (20 mg/kg) with a *p*-value of < 0.05.

**Table 3**
Improvement in TFMJ with LVDP.

| Group | Intervention | TFMJ | *p*-value vs B2 |
|---|---|---|---|
| B1 | None | – | – |
| B2 | Saline IP (placebo) | 65.1 ± 1.5 | – |
| B3 | Saline IP + LVDP 20 mg/kg IP | 247.3 ± 18.2 | $p < 0.05$ |
| B4 | Saline IP + LVDP 40 mg/kg IP | 295.5 ± 3.3 | $p < 0.05$ |

**Description**: In our experiment, LVDP at 20 mg/kg and 40 mg/kg IP significantly reduced the time to first myoclonic jerk (TFMJ) when compared to placebo. We also found that the marginal improvement in TFMJ at the higher dose of LVDP (40 mg/kg) was statistically significant when compared to the lower dose of LVDP (20 mg/kg) with a *p*-value of < 0.05.

In addition to channelopathies, alterations in the availability and (or) activity of various neurotransmitters, in particular GABA and glutamate, can alter the seizure threshold. A reduction in GABA, or a decrease in GABAergic presynaptic inhibition alongside altered calcium, sodium, chloride and potassium currents, is known to be a common cause of seizure onset (Badawy et al., 2009). Pre-synaptic and post-

synaptic glutamate activity, via N-methyl-D-aspartate (NMDA) and other receptors, also play an important role in epileptogenesis (Werner and Coveñas, 2011). It is for these reasons that pathways related to GABA, the main inhibitory neurotransmitter of the brain, and glutamate, have become common targets of antiepileptics. For instance, drugs that enhance GABA-mediated inhibition can act as antiepileptics that is used clinically to treat various syndromes of focal and generalized epilepsy, with the notable exception of absence seizures which are aggravated by such treatments. Glutamate receptor antagonists—both NMDA and non-NMDA—are also potent antiepileptics used in many animal models of epilepsy (Meldrum, 1995).

As previously mentioned, there appears to be overlapping cellular and neurochemical pathways between coughing and seizures. Coughing is unique among the bronchopulmonary reflexes in that it is highly dependent on threshold regulation and requires an epileptiform-like dischange in respiratory motor neurons to produce the characteristic enhanced inspiratory and expiratory efforts, separated by closure of the glottis during the compressive phase of the reflex (Canning, 2007; Bolser et al., 2006). This highly synchronized process is controlled by interactions that ocur centrally and peripherally.

Physiologically, the cough reflex arc starts with the afferent pathway, which consists of sensory nerve fibers in the vagus nerve that often start in the ciliated epithelium of the upper airways. A noxious sensation which originates there travel up the vagus nerve to the central pathway (cough center), which is a central coordinating region for in the upper brain stem and pons. If the threshold is met, the efferent pathway is activated. Coordinated impulses from the cough center travels down multiple nerves including the vagus, phrenic, and spinal motor nerves to innervate the glottis, diaphragm, and muscles of the thoracic and abdominal wall to generate the cough (Polverino et al., 2012).

On a cellular level, bronchopulmonary vagal afferents comprised of mostly unmyelinated C fibers act synergistically with cough receptors in the lungs and airway to initiate the cough reflex. The cell bodies of these C fibers are believed to reside in the jugular ganglion (Canning and Mori, 2011; Coleridge and Coleridge, 1984). These vagal afferent fibers enter the brainstem and interface in the nucleus tractus solitarius (NTS) (Canning, 2007), which is located in the ventrolateral region of the medulla oblongata and can be considered as the main control center for coughing (Shannon et al., 1998, 2000). This special region in the brainstem processes the afferent information and coordinates the cough reflex. To facilitate conscious (non involuntary) coughing, there are cortical and subcortical pathways that also interface with this region (Canning et al., 2014). Neurons in the NTS are known to express receptors for y-aminobutyric acid (GABA), *N*-methyl-D-aspartate (NMDA) and AMPA/KA. Here, increased availability and binding of GABA to its receptor is known to result in inhibition of signal transmission, whereas L-glutamate plays a stimulatory role by binding to NMDA and AMPA/KA receptors (Walker et al., 1999).

More recent studies have uncovered a potential relationship between the NTS and propagation of seizure activity. It has been found that epileptic activity can spread throughout the brain through the NTS through glutamatergic excitatory pathways embedded in the NTS, and the physiologic connections between the NTS and various forebrain structures (Jhamandas and Harris, 1992; Rutecki, 1990). Subsequently, by enhancing GABA activity or antagonizing glutamate binding in the NTS has been shown to suppress limbic motor seizures evoked by either focal or systemic stimulation (Walker et al., 1999). Another study has demonstrated that afferent fibers entering the NTS can be suppressed with antiepileptic medications (Jhamandas and Harris, 1992). The behaviour or these neurons is more complex than an on-off switch, however. High-frequency afferent vagal stimulation may paradoxically suppress activity in the NTS. Results from metabolic mapping studies suggest that this phenomenon may be mediated via 2-deoxyglucose, with induced reductions of glucose metabolism in the NTS (Walker et al., 1999).

Racemic dropropizine or its formal name (+)-3-(4-phenyl-1-piperazinyl)-1,2-propanediol, has long been used as an antitussive drug. In the late 1980s, separation of the two isomers, levo (S) (-) and dextro (R) (+), was achieved and the activity of each stereoisomer was investigated and compared against each other and the racemic mixture (De Blasio et al., 2012). These studies revealed that the (S) (-) enantiomer possessed similar antitussive activity when compared to the racemic mixture across a panel of different animal models of cough, while showing improved tolerability especially with regards to unwanted sedation. Thus, the (S) (-) enantiomer, *a.k.a.* LVDP or S(-)-3-(4-phenyl-piperazin-1-yl)-propane-1,2-diol emerged as the preferred stereoisomer, and since then it has been widely used as an effective and well-tolerated non-opioid antitussive drug in clinical practice (Braga, 1989).

It has been suggested that LVDP exerts its antitussive effect mainly on peripheral nerve structures, with mild central sedative effects (Malandrino et al., 1988; Melillo et al., 1988). It is believed that LVDP prevents cough by interfering with the activation of peripheral sensory C nerve fibres by modulating the activity of substance P (SP) and other neuropeptides that are involved in the cough reflex (Kohrogi et al., 1988; Lavezzo et al., 1992; Daffonchio et al., 1993; Yamawaki et al., 1993). The ability of LVDP to prevent cough was markedly reduced when these neuropeptides were in a depleted state (Gamse et al., 1981). This observation supports the theory that the peripheral activity of LVDP is mediated by—and dependent on—adjunct neuropeptides. These peptides, in particular neuropeptide Y, galanin, cholecystokinin, leptin, adiponectin, and growth hormone-releasing peptides (GHRPs), are potent modulators of synaptic activity (Giordano et al., 2014). Further studies demonstrate that LVDP prevents noxious stimuli from activating sensory nerve endings by interfering with SP and other neuropeptides in these nerve endings (Shams et al., 1996).

Outside the cough reflex literature, SP appears to be also involved in mediating epileptiform responses in neurons (Nalivaiko et al., 1997), and that heightened SP activity can evoke seizure activity. In one study, SP and glutamate mediated excitation of hippocampal neurons results in a cascade of events that led to self-sustaining status epilepticus (SSSE) and neuronal death. Subsequently, antagonism of the SP receptor was successful in distrupting the maintenance phase of SSSE that was resistant to diazepam, a widely used and highly effective emergency antiepileptic. These results raise the possibility that refractory status epilepticus—one that is impervious to standard therapies—may respond to antagonists of the SP receptor and other neurokinins (Liu et al., 1999).

The effect of LVDP on seizures has not been previously investigated in detail in the literature, although few limited studies do exist. In one study by Melillo et al, a racemic mixture of dropropizine as well as LVDP were able to delay the onset of convulsions in a dose-dependent manner (Melillo et al., 1988). We report similar findings in our study, in which TFMJ was significantly lengthened with the administration of LVDP. To the best of our knowledge, our study represents the most detailed investigation into the potential antiepileptic activity of LVDP in a murine model of epilepsy, which includes information on its efficacy at different doses as well as a quantitative analysis based on EEG data.

In the EEG data, we observed strong evidence of seizure suppresion with LVDP. Rats that were not treated with LVDP demonstrated florid EEG abnormalities that include delta, theta and spike waves. Treatment with LVDP at 20 mg/kg and 40 mg/kg IP resulted in abatement of epileptiform activity, and thus we conclude that LVDP is effective in mitigating PTZ-induced seizures in mice. This is a novel finding as previous investigations into this matter only documented that LVDP increased the time to first convulsion in a dose-dependent manner (Melillo et al., 1988). The results of our experiments not only provide confirmation for this earlier finding, but also expand our collective knowledge regarding dosing, impact on EEG tracing, and the effect of LVDP on murine seizures that are induced specifically by PTZ. In sum,

to the best of our knowledge, our study represents the first detailed clinical and electrophysiological investigation into the antiepileptic effects of LVDP in a pre-human model for epilepsy. We believe that the antiepileptic property of LVDP is mediated through its ability to alter the activity of SP, GABA and glutamate neurotransmission in the NTS and perhaps other regions of the central nervous system.

Despite our positive *in vivo* data, many uncertainties exist and further work needs to be done to determine the clinical meaningfulness of our findings. Although the PTZ-induced murine model for epilepsy is one that is widely used in pre-clinical epilepsy research, this model is not etiologically similar to the typical epilepsy syndromes found in humans. Furthermore, the dosing of LVDP in rats is quite different than the doses found in typical clinical use. It remains to be seen whether LVDP can be administered to humans at doses that would result in meaningful antiepileptic activity, with an acceptable toxicity profile.

## 5. Conclusions

We demonstrate that LVDP at a dose of 20 mg/kg IP and 40 mg/kg IP significantly mitigates PTZ-induced seizures in rats, with quantifiable suppression of seizure activity on EEG telemetry, a reduction in mean RCS, and prolongation of TFMJ. There was a trend towards better seizure suppresion with the higher dose of LVDP as compared to the lower dose of LVDP, but these differences were not statistically significant in any of the experiments we did. Our findings replicate earlier published findings which demonstrated that LVDP can delay the onset of seizure activity in another murine model for epilepsy, and add to the body of evidence that LVDP may serve as an antiepileptic drug.

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## Declaration of competing interests

None: the authors have no competing interests, commercial, pecuinary, patent-related or otherwise, with regards to this study, its contents, and findings.

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