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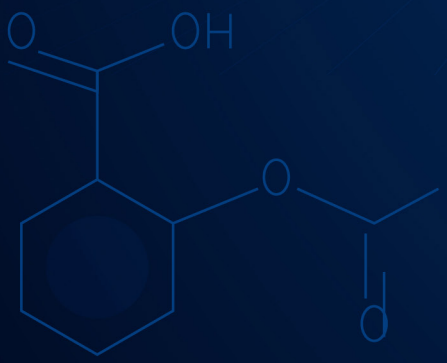

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Investigating how Noscapine Influences Activity and Seizures Caused by the Intake of Alkaloids in Mice

Noorullah Neekbin[1*], Mohammad Yonus Sahil[2], Mahdi Mohammadi[2], Abdul Qayom Rajabi[3]

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ABSTRACT

This study investigates the effects of noscapine on seizure-like activity and vital functions in mice exposed to alkaloid dependence, with the aim of elucidating its potential therapeutic properties. Specifically, the research explores whether noscapine can mitigate or modulate seizure events triggered by alkaloid exposure, thereby contributing to a broader understanding of its pharmacological profile. Findings from this study may provide valuable insights for the development of novel therapeutic strategies for the management of seizures and other neurological disorders associated with alkaloid consumption. A total of forty newborn healthy mice were randomly selected for the experiment. To induce alkaloid dependence, hydromorphine was administered subcutaneously at doses of 2, 4, 8, 16, and 32 mg/kg (0.1 cc) once daily from postnatal day 14 to day 17. On postnatal days 18 and 19, brain sections were prepared, and epileptiform activities were induced through cerebrospinal fluid perfusion with reduced magnesium levels. The experimental design evaluated the effects of hydromorphine at concentrations of 10, 100, and 1000 µM, as well as noscapine at 10 µM. Quantitative parameters including the number, onset latency, and amplitude of seizure-like activities were assessed as indicators of drug effects. The results demonstrated that hydromorphine exhibits a concentration-dependent, triphasic effect on seizure-like activity during the lactation period: low and high concentrations attenuated seizure activity, whereas moderate concentrations enhanced it. By contrast, noscapine exerted a significant anticonvulsant effect, supporting its potential as a modulator of alkaloid-induced seizures.

INTRODUCTION

Seizure disorders in humans are complex neurobehavioral conditions arising from abnormal neuronal excitability in distinct regions of the brain. Among the neural circuits implicated, the limbic system plays a central role in the initiation, propagation, and termination of seizure activity. In the hippocampus, the onset of pseudo-ictal activity appears to be in the CA1 region, while interracial activity is found in the CA3-CA2 region (Salmani *et al.*, 2007). Seizure disorder is a chronic neurological condition characterized by recurrent episodes of epileptic activity. It is commonly associated with underlying factors such as brain injury and genetic abnormalities. Within the seizure onset zone, neuronal function and network dynamics undergo profound alterations, which in certain cases may lead to structural damage and cellular loss. During the transition to epilepsy, nerve cells in the epileptic area begin to discharge electrical signals intensely, simultaneously, excessively, or with an abnormal pattern (Ghadimkhani, 2016). The transformation of seizures into epilepsy can start only in specific brain structures (such as the cerebral cortex and amygdala, for example) and then spread to other structures (Castillo *et al.*, 2011). Multiple mechanisms have been proposed to explain the pathogenesis of various forms of seizures and epilepsy.

LITERATURE REVIEW

Homayoun (2002) mentioned that, early hypothesis posits that epileptic seizures arise from a reduction in synaptic inhibition. Another hypothesis is that the enhancement of the glutamate system leads to epilepsy Experimental studies have demonstrated that seizures can be induced in the absence of chemical synaptic activation. This observation suggests that future research should focus on delineating the distinct mechanisms underlying various types of seizures and epilepsy. Extensive efforts and funding have been invested in elucidating the molecular and cellular pathophysiological mechanisms and fundamental pharmacodynamics processes of epileptic syndromes, but the relevant connection remains questionable (Brodie, 2002). Although animal models in vivo have been standard experimental methods for decades, experimental seizure models have been used for screening the effects of potential drugs (Arias & Bowlby, 2005). The hippocampus represents a highly organized neural structure that plays critical roles in learning, memory processing, and the pathophysiology of neurological disorders such as Alzheimer's disease and epilepsy, where it contributes to synchronized neuronal discharges. Notably, among brain regions, the hippocampus exhibits the lowest threshold for seizure generation. Preparation of hippocampal slices, if synaptic circuits and cellular structures are preserved, is therefore essential for electrophysiological studies in laboratory conditions (Gáll *et al.*, 2015).

Seizure activity in experimental conditions in the

[1] Department of Chemistry, Faculty of Education, Saripul University, Saripul, Afghanistan
[2] Department of Pharmacognosy-Microbiology, Faculty of Pharmacy & Department of Chemistry, Faculty of Science, Balkh University, Afghanistan
[3] Department of Chemistry, Faculty of Chemical industrial Engineering, Jawzjan University, Afghanistan
[*] Corresponding author's e-mail: neekbin.neekbin786@gmail.com

interneuron brain slice is induced by removing magnesium from the perfusion solution, causing the opening of NMDA glutamate receptor channels (Velíšek, 1998). The N-methyl-D-aspartate (NMDA) receptor ion channel exhibits high permeability to calcium ions, with approximately 10% of the total ionic current being carried by Ca^{2+}. Calcium influx can initiate several calcium-dependent intracellular processes, including seizure activity (Motin & Yasnetsov, 2015). Pharmacological agents play a pivotal role in both the initiation and termination of seizures.

Acute systemic administration of μ-, δ-, and ϰ-opioid receptor agonists, including hydromorphine, fentanyl, pentazocine, and meperidine, has been shown to exert protective effects against seizure activity. Notably, hydromorphine and fentanyl demonstrate biphasic, dose-dependent actions, with low concentrations producing anticonvulsant effects, whereas higher doses elicit proconvulsant activity. Furthermore, administration of the opioid antagonist hydromorphine has been reported to attenuate postictal depression, a transient state following seizure termination characterized by reduced neuronal excitability, thereby decreasing seizure recurrence. Intracerebral administration of endorphins, met-encephalin, and hydromorphine derivatives has anticonvulsant effects in adult rodents (Gáll *et al*., 2015). Early-life exposure to drugs may occur either directly, through medical administration, or indirectly, through environmental factors such as parental substance use. Such exposure has the potential to influence neurological development and may predispose individuals to seizures later in life. Accordingly, the present study aims to compare the acute and chronic effects of hydromorphine on seizure activity induced by low-magnesium cerebrospinal fluid in ex vivo brain slices from mice exposed to hydromorphine during early lactation. In addition, this study investigates the concentration-dependent effects of hydromorphine on seizure activity in perfused brain slices prepared from both hydromorphine-dependent and control mice.

MATERIALS AND METHODS

Medicines and chemicals

The reagents required for the preparation of artificial cerebrospinal fluid (aCSF), including NaCl, $NaHCO_3$, KCl, $MgSO_4$, $CaCl_2$, and glucose, were procured from Merck (Germany). Hydromorphine and noscapine were obtained from the Department of Medicines, People's Friendship University of Russia. Both compounds were dissolved in low-magnesium aCSF to achieve the desired concentrations. All pharmacological agents were administered via perfusion during electrophysiological recordings.

Animals

A total of 40 healthy male and female mice were obtained and maintained at the Animal Facility of the People's Friendship University of Russia under controlled environmental conditions (22 ± 2 °C) with ad libitum access to food and water. The animals were randomly assigned to two groups: control (n = 20) and hydromorphine-dependent (n = 20). Each group was further subdivided into four experimental subgroups, corresponding to treatment with hydromorphine at 10 μM, 100 μM, or 1000 μM, or noscapine at 10 μM, yielding a total of eight subgroups. All procedures related to animal care, handling, anesthesia, and euthanasia were carried out in compliance with the ethical standards of the Helsinki Declaration.

The control group received daily subcutaneous injections of sterile normal saline (1.0 mL) from postnatal day (PND) 14 to PND 17. The hydromorphine-dependent group was administered escalating doses of hydromorphine (2, 4, 8, 16, and 32 mg/kg, s.c.) during the same period. On PND 18 and 19, animals from both groups were utilized for electrophysiological recordings.

Preparation of live brain slices

For this study, live brain slices were obtained from both hemispheres of 80 healthy mice. On postnatal days 18 and 19, animals were anesthetized with ether in a desiccator. Decapitation was performed rapidly using a smooth-bladed instrument, and the brain was immediately extracted and immersed in ice-cold artificial cerebrospinal fluid (aCSF; 0–4 °C) continuously equilibrated with carbogen gas (95% O_2, 5% CO_2). Following removal of the cerebellum and frontal brain regions, the remaining tissue was mounted on a cutting platform using cyanoacrylate or beeswax adhesive. Horizontal slices, 400 μm in thickness, were prepared using a vibratome (Campden, UK). Only slices of sufficient quality, in which the dentate gyrus and CA subfields of the hippocampus were clearly visible under a stereomicroscope at 4× magnification, were selected for subsequent experiments. Prepared slices were transferred into aCSF using a specialized pipette and maintained at physiological temperature for at least 90 minutes prior to electrophysiological recordings.

Following incubation, brain slices of adequate quality were transferred to a specialized surface-contact recording chamber and positioned on a nylon mesh. Slices were continuously perfused with normal artificial cerebrospinal fluid (aCSF) at 1 mL/min for 20 minutes to establish stable baseline conditions.

Two types of aCSF solutions were employed for perfusion. The first was standard aCSF, while the second was a low-magnesium, high-potassium aCSF used to induce seizure-like activity. The ionic composition of the seizure-inducing solution (in mM) was as follows: $NaHCO_3$ (26), KCl (5), $CaCl_2$ (2), $MgSO_4$ (0.2), NaH_2PO_4 (1.25), NaCl (125), and glucose (10). The osmolality of all aCSF solutions ranged from 300 to 307 mOsm/L.

Electrophysiology recording

Glass microelectrodes (TW150F-4; World Precision Instruments, USA) with electrical resistances of 3–5 MΩ were prepared and filled with normal artificial cerebrospinal fluid (aCSF). Extracellular recordings were

obtained from the CA1 region of the hippocampus, with the electrode tip positioned in the pyramidal cell layer to monitor seizure-like activity. Perfusion of the aCSF was maintained at a rate of 1 mL/min, and the temperature was controlled at 32 °C using a temperature regulation device (Campden, UK).

Seizure-like activities, previously described as "stable seizure activities" in the literature, were continuously recorded during perfusion with low-magnesium aCSF until drug administration. Data acquisition and analysis were performed using a research workstation (Rays of Knowledge Company) and E-Trace software. Quantitative measures, including the number of spikes and amplitude of recorded waves, were analyzed before and after drug application. In this study, hydromorphine was tested at concentrations of 10, 100, and 1000 µM, and noscapine at 10 µM, with each drug dissolved directly in low-magnesium aCSF for perfusion. The number of spikes generated during perfusion with low-magnesium aCSF and subsequent drug exposure was recorded and analyzed to evaluate drug effects on seizure activity.

Data analysis

The analysis of the data was conducted using SPSS software (version 22), which is a robust tool for statistical analysis. The first step involved assessing the normality of the data distribution through the Kolmogorov-Smirnov (K-S) test. This test is essential for determining whether the data meet the assumptions required for parametric tests. In this case, the results indicated that the data did not follow a normal distribution, which is a crucial finding as it influences the choice of statistical methods used for analysis.

Given the non-normality of the data, non-parametric statistical tests were deemed appropriate. The Wilcoxon signed-rank test was utilized to compare two related groups, providing a method to analyze differences without relying on the normality assumption. This test is particularly useful in studies where the same subjects are measured under different conditions.

For comparisons among independent groups, the Friedman test was employed. This test is an extension of the Wilcoxon signed-rank test for more than two groups and is effective in analyzing repeated measures or related samples while adhering to the non-parametric framework. The results are presented as mean ± standard error of the mean (SEM), which offers a clear indication of the central tendency and variability of the data. Finally, a P-value threshold of < 0.05 was set to determine statistical significance. This indicates that any observed differences are unlikely to be due to random chance, thus providing confidence in the findings.

In summary, the choice of non-parametric methods reflects a careful consideration of the data's characteristics, ensuring that the analysis is both appropriate and robust.

RESULTS AND DISCUSSION

In the majority of cortical brain slices used in this study, perfusion with low-magnesium artificial cerebrospinal fluid (aCSF) induced pseudo-seizure-like activities, referred to as "stable" events. These activities were characterized by repetitive discharges with durations ranging from less than one second to 10 seconds, typically lasting 2–5 seconds per event, and repeated continuously until the magnesium concentration in the perfusing solution reached zero, while the brain slice remained of sufficient quality. Because most recorded events lasted less than one second, the analysis primarily focused on the number of spikes and their amplitudes.

This study investigated the effects of acute and chronic hydromorphine administration (10, 100, and 1000 µM) and 10 µM noscapine on hippocampal seizure-like activity in both normal and hydromorphine-dependent Syrian mice at postnatal days 19 and 20. The results are summarized in Figures 1–4. All pseudo-seizure activities observed across the 80 brain slices were of the pseudo-stable type; however, detailed features such as spike morphology, frequency, and amplitude varied among slices.

In slices obtained from normal mice, seizure-like activities emerged 5–10 minutes after perfusion with low-magnesium aCSF, whereas in slices from hydromorphine-dependent mice, these activities appeared after 10–15 minutes. In both groups, seizure-like activity persisted throughout the perfusion period. The number of spikes

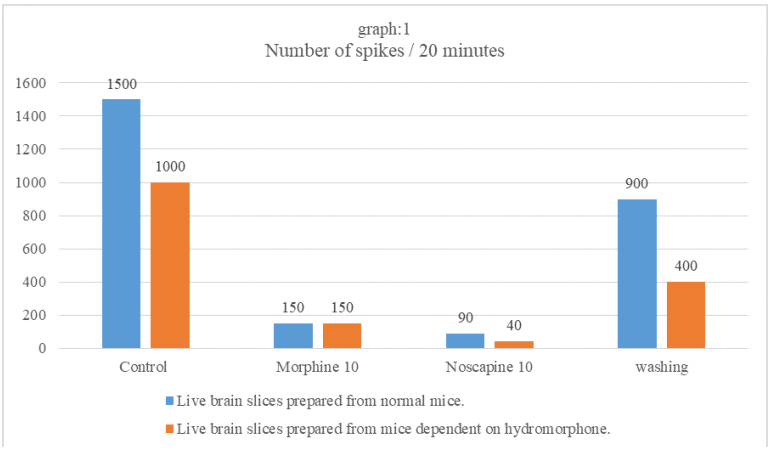

Figure 1:

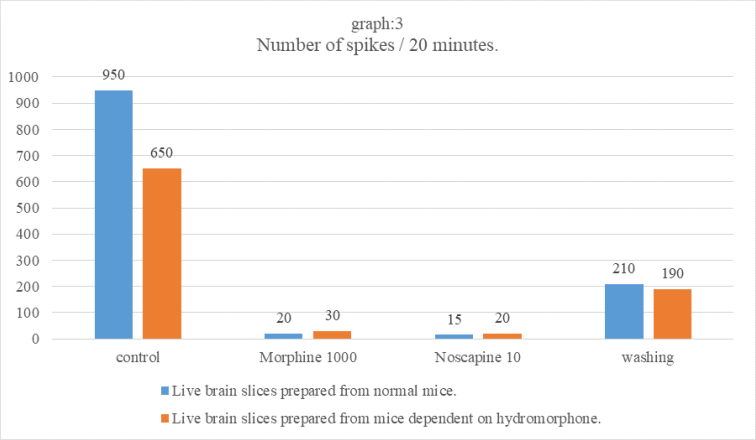

Figure 2:

per unit time under baseline conditions and during perfusion with different concentrations of hydromorphine and noscapine was quantified. The results indicated that hydromorphine at 10 and 1000 μM significantly reduced spike counts relative to baseline in slices from both normal and hydromorphine-dependent mice (Figures 1 and 3).

Perfusion of the targeted slices with 10 μM noscapine alone did not significantly alter seizure-like activity. In contrast, perfusion of slices from both normal and hydromorphine-dependent Syrian mice with 100 μM hydromorphine resulted in an increased number of spikes relative to baseline conditions. Notably, subsequent perfusion of these same slices with 10 μM noscapine significantly reduced the number of spikes induced by 100 μM hydromorphine (Figure 2).

Perfusion of live brain slices from both normal and

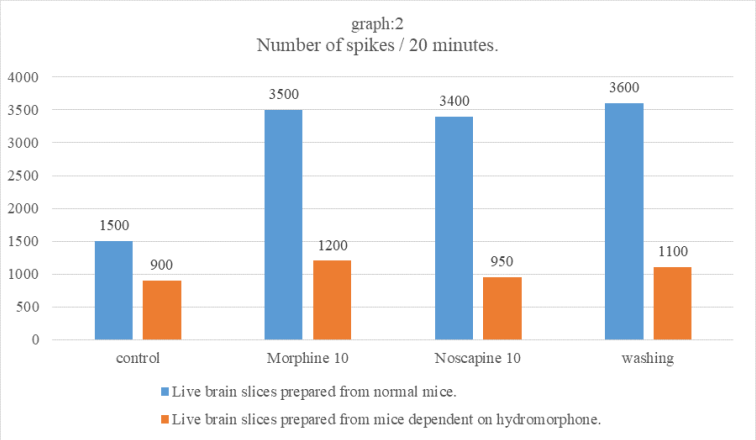

Figure 3:

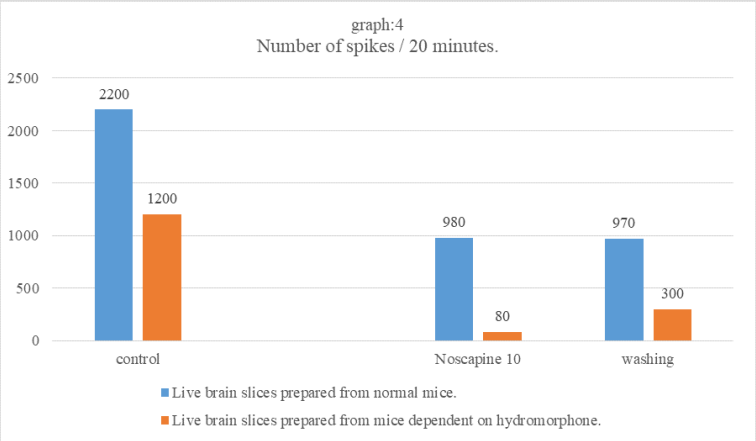

Figure 4:

hydromorphine-dependent mice with 10 μM noscapine resulted in attenuation of baseline seizure-like activity in both groups. Notably, the reduction in activity was more pronounced in slices from hydromorphine-dependent mice compared to controls, and this difference was statistically significant (Figure 4).

CONCLUSION

This study examined the effects of acute and chronic administration of hydromorphine and noscapine on seizure-like activities induced by low-magnesium cerebrospinal fluid (CSF) in CA1 hippocampal slices from neonatal and hydromorphine-dependent mice. Neonatal mice were chosen as this developmental stage parallels the third trimester of human gestation, a critical period for cognitive neural development and synaptic maturation. The physiological immaturity of neonatal mice makes them particularly relevant for assessing the impact of drug exposure on neuronal excitability and seizure susceptibility.

Chronic exposure to hydromorphine is anticipated to alter seizure activity due to significant changes in the nervous system (Saboory *et al*., 2007). While the low-magnesium CSF model may not fully replicate human seizure mechanisms, it is a widely accepted method for inducing epileptiform activity. The primary aim was to quantify seizure activity by measuring spike frequency. Drug concentrations were critical, with hydromorphine administered at 10, 100, and 1000 μM, and noscapine at 10 μM. The effects of these agents were evaluated for both proconvulsant and anticonvulsant properties, which varied according to concentration and context.

The findings indicate that hydromorphine at 10 μM and 1000 μM increases seizure activity in hippocampal slices from both healthy neonatal and hydromorphine-dependent mice. This aligns with previous animal studies demonstrating similar effects at high doses of hydromorphine (Saboory *et al*., 2007). Chronic agonist exposure leads to receptor downregulation, a key mechanism for drug tolerance. Additionally, hydromorphine-pretreated animals exhibited rapid seizure onset and reduced sensitivity to kindling stimuli (Hofmann *et al*., 2006).

Prior research has shown that chronic hydromorphine exposure in neonatal mice alters sensitivity to pentylentetrazole-induced seizures in an age-dependent manner. Consistent with this, our study confirms a biphasic effect of hydromorphine on seizure activity, where high concentrations exert anticonvulsant effects, while moderate concentrations demonstrate proconvulsant actions (Gholami *et al*., 2012).

Using the low-magnesium CSF model, which enhances neuronal excitability by reducing membrane charge screening and NMDA receptor inhibition (Frenk *et al*., 1983), we found that noscapine, typically effective as an antagonist to hydromorphine's anticonvulsant effects, did not adequately reverse hydromorphine's impact at a concentration of 10 μM. Notably, noscapine's inhibitory effects were stronger in hydromorphine-dependent mice than in controls.

In summary, hydromorphine exhibits a biphasic influence on seizure activity induced by low-magnesium CSF, with similar patterns of acute and chronic exposure but significantly greater effects in normal mice. This discrepancy may be attributed to opioid receptor desensitization resulting from chronic hydromorphine administration.

REFERENCES

Arias, R. L., & Bowlby, M. R. (2005). Pharmacological characterization of antiepileptic drugs and experimental analgesics on low magnesium-induced hyperexcitability in rat hippocampal slices. *Brain research, 1047*(2), 233-244. https://doi.org/10.1016/j.brainres.2005.04.052.

Binder, D. K., & Steinhäuser, C. (2006). Functional changes in astroglial cells in epilepsy. *Glia, 54*(5), 358-368. https://doi.org/10.1002/glia.20394.

Brodie, M. J. (2010). Antiepileptic drug therapy the story so far. *Seizure, 19*(10), 650-655. https://doi.org/10.1016/j.seizure.2010.10.027.

Brodie, M. J. (2011). Antiepileptic drug therapy: does mechanism of action matter? *Epilepsy & Behavior, 21*(4), 331-341. https://doi.org/10.1016/j.yebeh.2011.05.025.

Castillo, P. E., Chiu, C. Q., & Carroll, R. C. (2011). Long-term plasticity at inhibitory synapses. *Current opinion in neurobiology, 21*(2), 328-338. https://doi.org/10.1016/j.conb.2011.01.006.

D'Antuono, M., (2010). Antiepileptic drugs abolish ictal but not interictal epileptiform discharges in vitro. *Epilepsia, 51*(3), 423-431. https://doi.org/10.1111/j.1528-1167.2009.02273.

Frenk, H., Urca, G., & Liebeskind J. C. (1987). Properties of leucine-and methionine-enkephalin: comparison with hydromorphine and reversibility by hydromorphine. *Brain Research, 147*(2), 327-337.

Frenk, H. (1983). Pro-and anticonvulsant actions of hydromorphine and the endogenous opioids: involvement and interactions of multiple opiate and non-opiate systems. *Brain Res, 6*(2), 197-210. https://DOI.org/10.1210/er.2009-0009.

Gáll, Z., Orbán-Kis, K., & Szilágyi, T. (2015). Differential effects of sodium channel blockers on in vitro induced epileptiform activities. *Archives of Pharmacia Research*, 1-10. https://doi.org/10.1007/s12272-015-0676-6

Ghadimkhani, M. (2016). Effect of magnesium sulfate on hyperthermia and pentylen-tetrazol-induced seizure in developing rats. *Iranian Journal of Basic Medical Sciences, 19*(6), 608.

Gholami, M., Saboory, E., Zare, S., Roshan-Milani, S., & Hajizadeh-Moghaddam, A. (2012). The effect of dorsal hippocampal administration of nicotinic and muscarinic cholinergic ligands on pentylenetetrazol-induced generalized seizures in rats. *Epilepsy &*

Behavior, 25(2), 244-249. https://doi.org/10.1016/j.yebeh.2012.07.004.

Hofmann, A. (2006). Myoclonus as an acute complication low-dose hydromorphine in multiple system atrophy. *Journal of Neurology, Neurosurgery & Psychiatry, 77*(8), 994-995.

Motin, V., & Yasnetsov, V. (2015). Effect of NMDA, a Specific Agonist to NMDA Receptor Complex, on Rat Hippocampus. *Bulletin of experimental biology and medicine, 159*(6), 704-707. https://doi.org/10.1007/s10517-015-3053-z

Homayoun, H. (2002). The role of nitric oxide in anticonvulsant and proconvulsant effects of hydromorphine in mice. *Epilepsy Research, 48*(1-2), 33-41. https://doi.org/10.1016/s0920-1211(01)00316-3.

Potschka, H., Friderichs, E., & Löscher, W. (2000). Anticonvulsant and proconvulsant effects of tramadol, its enantiomers and its M1 metabolite in the rat kindling model of epilepsy. *British journal of pharmacology Research, 131*(2), 203-212. https://doi.org/10.1038/sj.bjp.0703562

Saboory, E. (2007). Mechanisms of hydromorphine enhancement of spontaneous seizure activity. *Anesth Analg Research, 105*(6), 1729-1735. https://doi.org/10.1213/01.ane.0000287675.15225.0b.

Salmani, M., Mirnajafizadeh, J., & Fathollahi, Y. (2007). Offsetting of aberrations associated with seizure proneness in rat hippocampus area CA1 by theta pulse stimulation-induced activity pattern. *Neuroscience, 149*(3), 518-526. https://doi.org/10.1016/j.neuroscience.2007.08.019.

Velíšek, L. (1998). Prenatal hydromorphine exposure alters ovarian steroid hormonal regulation of seizure susceptibility. *Brain research, 796*(1), 247-256. https://doi.org/10.1016/S0006-8993(98)00367-9.

Zieglgansberger, W. (1979) Opioid peptides may excite hippocampal pyramidal neurons by inhibiting adjacent inhibitory interneurons. *Science, 205*(4), 415-417. https://doi.org/10.1126/science.451610