Differential electrographic signatures generated by mechanistically-diverse seizurogenic compounds in the larval zebrafish brain

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1. Differential electrographic signatures generated by mechanistically-diverse seizurogenic compounds in the larval zebrafish brain

2. LFP's of seizurogenic compounds in the zebrafish brain

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Abstract

We assessed similarities and differences in the electrographic signatures of local field potentials evoked by different pharmacological agents in zebrafish larvae. We then compared and contrasted these characteristics with what is known from electrophysiological studies of seizures and epilepsy in mammals, including humans. Ultimately, our aim was to phenotype neurophysiological features of drug-induced seizures in larval zebrafish for expanding knowledge on the translational potential of this valuable alternative to mammalian models. Local field potentials were recorded from the midbrain of 4-day old zebrafish larvae exposed to a pharmacologically diverse panel of seizurogenic compounds, and the outputs of these recordings were assessed using frequency domain analysis. This included analysis of changes occurring within various spectral frequency bands of relevance to mammalian CNS circuit pathophysiology. From these analyses, there were clear differences in the frequency spectra of drug-exposed local field potentials, relative to controls, many of which shared notable similarities with the signatures exhibited by mammalian CNS circuits. These similarities included the presence of specific frequency components comparable to those observed in mammalian studies of seizures and epilepsy. Collectively, the data presented provide important information to support the value of larval zebrafish as an alternative model for the study of seizures and epilepsy. These data also provide further insight into the electrophysiological characteristics of seizures generated in non-mammalian species by the action of neuroactive drugs.

Significance Statement

In this study we offer novel insight into the frequency domain of the local field potentials (LFPs) for a range of seizurogenic compounds in zebrafish larvae. We make a direct comparison of seizurogenic compounds with varying mechanisms of action and, where possible, compare the effects of these compounds in zebrafish larvae with those recorded in mammals in terms of the frequency components of their LFPs. This study adds to the mounting body of evidence supporting the use of the larval zebrafish as a powerful alternative model organism for seizure and epilepsy research.

1 1. Introduction

2 Seizures are defined as periods of excessive or hyper-synchronous brain activity (Fisher et al.,
3 2014a) which, when recurrent and unprovoked, define the chronic disease epilepsy (Falco-Walter et
4 al., 2018). In addition, seizure occurrences and myoclonus appear to be a feature of the
5 pathophysiology of a number of other CNS diseases, including Alzheimer's disease and other forms of
6 dementia (Beagle et al., 2017). Seizures themselves have a wide-ranging aetiology that includes a
7 substantial proportion attributable to the adverse action of drugs. It has been estimated, for example,
8 that approximately 6% of new-onset seizures and 9% of status epilepticus cases are as a result of drug
9 toxicity (Chen et al., 2016), which includes the inadvertent action of multiple marketed drugs (Easter
10 et al., 2009; Ruffmann et al., 2006). Seizures themselves can be definitively identified in human
11 patients and non-clinical animal models using neurophysiological assessment techniques such as local
12 field potential (LFP) recordings and electroencephalography (EEG) (Lévesque and Avoli, 2019; Usman
13 et al., 2019). Using these techniques, seizures can be observed to present diverse electrographic
14 dynamics, with some common components including low voltage fast activity, e.g. high frequency
15 oscillations (HFOs) (Jiménez-Jiménez et al., 2015; Wang et al., 2020) in the fast ripple frequency bands
16 (250-500 Hz), or high amplitude periodic spikes (Jiménez-Jiménez et al., 2015; Perucca et al., 2014;
17 Wang et al., 2020). However, precisely how, and where, they are manifest can vary between different
18 forms of epilepsy and different causes of seizure (Jiménez-Jiménez et al., 2015; Perucca et al., 2014;
19 Wang et al., 2020).

20 In order to better understand why seizures occur and how to treat epilepsy the use of
21 experimental models is important. Clearly such models should include an appropriate, often complex
22 neural architecture, and the best models are those performed *in vivo*, preferably in the absence of
23 confounds generated by anaesthesia. With this in mind, a drive towards more ethical and cost-
24 effective approaches for studying complex neurological disorders has increased interest in alternative,
25 non-mammalian, vertebrate models for such studies. Of these models, the larval zebrafish offers
26 considerable potential as a highly genetically tractable alternative for screening epilepsy related genes

27 and for the study of genetic mutation-induced spontaneous seizures (Baraban et al., 2013a; Griffin et
28 al., 2021). To date, zebrafish have been relatively widely used for assessing drug-induced neural
29 activity, and various studies have described the effects of seizurogenic chemicals on their behaviour
30 (Afrikanova et al., 2013; Alfaro et al., 2011; Baraban et al., 2013b; Berghmans et al., 2007; Winter et
31 al., 2021), electrophysiology (Afrikanova et al., 2013; Baraban et al., 2013b, 2007; Cho et al., 2017;
32 Copmans et al., 2019; Griffin et al., 2017; Hortopan et al., 2010; Hunyadi et al., 2017; Kim et al., 2010b;
33 Liu and Baraban, 2019), and functional imaging phenotypes (Burgstaller et al., 2019; Burrows et al.,
34 2020; Ghannad-Rezaie et al., 2019; Winter et al., 2021, 2017). Electrophysiological assessments, in
35 particular, offer extremely high temporal resolution and allow direct comparison of zebrafish-derived
36 data with that obtained from neurophysiological assessments undertaken in mammals. Consequently,
37 the recording of larval zebrafish electrophysiological data offers the opportunity to directly compare
38 abnormal electrographic dynamics between these two taxonomically diverse sets of model organisms.
39 In larval zebrafish, typically, LFP recordings from small clusters of neurons in easily identified
40 anatomical targets (such as the optic tectum) are used to measure the response of the brain to drug
41 treatment. Indeed, LFP recordings have been used to assess the electrographic response of the larval
42 zebrafish to a few seizurogenic compounds including picrotoxin, pilocarpine (Baraban, 2013;
43 Hortopan et al., 2010), and, most often, pentylenetetrazole (PTZ) (Baraban et al., 2007; Copmans et
44 al., 2019; Kim et al., 2010b; Liu and Baraban, 2019). Despite the relatively widespread use of
45 electrophysiology in larval zebrafish, little is actually known about the specific electrographic
46 characteristics of seizures induced by diverse chemicals in this model organism, other than the
47 aforementioned exceptions. There are few published studies which characterise LFP profiles in larval
48 zebrafish across a range of excitatory mechanisms, for example, or that have compared their
49 characteristics with those used to define seizures in more traditional models of seizures and epilepsy
50 using mammalian electrophysiology and human EEG data. Here we sought to address this knowledge
51 gap by assessing the LFP-based response of 4 days post-fertilisation (dpf) larval zebrafish, to treatment
52 with a variety of seizurogenic compounds which act through a range of pharmacological mechanisms

of action. Using frequency domain analysis on the resulting data we aimed to assess the similarities and differences in the electrographic signatures of LFP's between different pharmacologies, and to compare and contrast these characteristics with what is known from electrophysiological studies of seizures and epilepsy in mammals including humans, thereby adding considerably to our knowledge of the translational value of this model.

2. Methods

The experimental approach used is detailed in the following sections and the process is summarised in **Fig. 1A**. Throughout, the data shown in graphs and tables are the means, $\pm$ SEM of data points from individual animals (n). All analyses were performed using MATLAB (MATLAB version 9.8.0, 2020), including the signal processing toolbox 8.4 (MATLAB signal processing toolbox 8.4, 2020) and wavelet toolbox 5.4 (MATLAB wavelet toolbox 5.4, 2020).

2.1 Experimental animals

For this work, 4dpf transgenic zebrafish, of unknown sex, with a pan-neuronal Ca^{2+} sensor (*elavl3:GCaMP6s*) were used. This transgenic line allowed us to compare the electrographic recording data generated here, with functional imaging data obtained from a separate study (Winter et al., 2021). 4dpf larval zebrafish were selected as these are not considered protected vertebrates under European animal legislation and as such are ethically preferable to the use of older, protected, animals. The use of 4dpf larval zebrafish also enabled the use of neuromuscular blocking agents and for electrophysiological recordings to be performed in the absence of a potentially confounding general anesthetic.

Adult *elavl3:GCaMP6s* broodstock (Ahrens et al., 2014) (kindly supplied by Dr Misha Ahrens, Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, Virginia, USA) were held in aquaria at $28 \pm 1^\circ\text{C}$ under optimal conditions for spawning. Briefly, animals were held in dechlorinated mains tap water (referred to here as 'culture water'), which was routinely monitored for water quality

79 parameters. Fertilized eggs were collected shortly after spawning and transferred to Petri dishes which
 80 were filled with culture water and held at $28 \pm 1^\circ\text{C}$ until use in experiments at 4dpf (see (Winter et al.,
 81 2017) for full details). All work was undertaken under project and personal licences granted by the UK
 82 Home Office under the UK Animals (Scientific Procedures) Act.

83

84 **2.2 Test compounds and concentration range selection**

85 Test compounds were selected based upon their known seizurogenic potential in mammals,
 86 including humans, as defined by case studies of seizure incidence and assays in model organisms as
 87 outlined in **Table 1**. Appropriate exposure concentration ranges ensuring CNS responsiveness without
 88 generalised toxicity were also selected based upon previously published data (Winter et al., 2021).
 89 The exposure conditions adopted are summarised in **Table 1**. All compounds were dissolved in
 90 extracellular solution (ECS) and pH adjusted to ~ 7.5 prior to use. The composition of ECS was: 1 mM
 91 NaCl, 2.9 mM KCl, 10 mM HEPES, 1.2 mM MgCl_2 , 10 mM Dextrose, and 2.1 mM CaCl_2 (Baraban, 2013).
 92 For each compound, there were 3 exposure concentrations, and for each experimental group, 7-8
 93 larvae were used per treatment group. All test chemicals and other reagents were obtained from
 94 Sigma-Aldrich (Gillingham, UK) or Tocris (Bristol, UK).

95

96 **2.3 In vivo local field potential recordings from zebrafish brains**

97 Individual 4dpf larvae were paralysed using 4 mM tubocurarine and then positioned dorsal side
 98 up in the recording chamber in 1.5% low melting point agarose containing ECS. Throughout recording
 99 the mounted fish were kept in a static bath containing ECS. Under 4x magnification, a glass 2M NaCl
 100 filled microelectrode (resistance of 3–5 $\text{M}\Omega$) was inserted into the midbrain to record extracellular
 101 LFPs from a small network of neurons (**Figure 1B**). The area chosen for electrode placement was based
 102 on clear anatomical landmarks, and the electrode was placed a small distance (circa $\sim 30\mu\text{m}$) lateral
 103 from the midline of the brain. This was decided upon to ensure consistency of electrode placement in
 104 a specific brain area. Recordings were captured using an Axon CNS Multiclamp 700B amplifier and

105 digitised using an Axon Digidata 1440A (Amplifier settings were Mode: I=0, Gain: 20, Bessel: 2 kHz),
106 and data were recorded with the Clampex 10.4 software. Following introduction of the recording
107 electrode, larvae were equilibrated for 300 seconds prior to beginning data acquisition. The LFP
108 recording procedure consisted of acquiring 7.5 minutes of baseline data following which the test
109 compound was added to the ECS by pipette, followed by recording in the presence of compound for
110 17.5 minutes. The exposure epoch was defined as the period between 17.5 and 25 minutes, thus
111 allowing 10 minutes for the test compound to penetrate the larva and reach equilibrium. All raw
112 electrophysiological signals were recorded at 10 kHz, downsampled to 2 kHz and digitally filtered,
113 using a Butterworth filter, with high pass at 1 Hz and low pass at 500 Hz, to filter out spiking activity
114 from individual neurons while preserving LFP. Throughout recording and pre-equilibration larva were
115 flashed with two flashes of blue (488 nm) light, each lasting 100 ms and separated by a 500 ms gap,
116 repeated every 4.5 seconds. This was undertaken for two purposes: to provide visual stimulation that
117 could be detected using electrophysiology so as to ensure healthy nervous system functioning and to
118 provide regular periodic CNS stimulation by a standard sensory pathway. Moreover, previous studies
119 have successfully used light stimulation protocols to encourage spontaneous seizures in zebrafish
120 expressing genes related to Dravet syndrome, thus we hypothesised it may also help to additionally
121 sensitise animals to drug treatment (Eimon et al., 2018). Following completion of the compound
122 exposure recording period, larval heart rate was visually assessed to confirm fish health. Additional
123 vehicle-only experiments (identical except for no test compound exposure) were performed at regular
124 intervals during the data acquisition phase to ensure that any changes in LFPs were not associated
125 with the time/date of assessment.

126

127 ***2.4 Data analysis: comparisons of area under the curve of the LFP baseline and exposure period***

128 As a first step in our analyses we aimed to assess if the test compounds had an effect on the
129 overall brain activity levels, over time. Seizures are associated with excessive hypersynchronous
130 activity, therefore fish exposed to seizurogenic drugs might be expected to display a change in overall

signal amplitude. The area under the curve (AUC) was calculated, in 30 second time bins, for the absolute value of the Hilbert transform of the filtered LFP signal to allow an assessment of the 'volume' of activity over time. This allowed us to visualise the time-course of oscillatory activity, whether it was increasing or decreasing throughout the exposure or baseline periods **see Figure 5A**. Moreover, the total AUC over the baseline and exposure period was also calculated and a Wilcoxon signed rank test (corrected for multiple comparisons using Benjamini and Hochberg method (Benjamini and Hochberg, 1995)) was used to identify differences between baseline and drug-exposure recording epochs, **see Figure 5B**.

2.5 Data analysis: spectral analysis

Among the most commonly used approaches for assessing changes in neural activity patterns is frequency domain analysis, which allows investigation into the contribution of oscillations at different frequencies to the overall signal. Commonly in human EEG or mammalian animal model LFP datasets, oscillations are binned into bands of behavioural relevance, and changes in the power of these bands can correlate with transitions in behavioural or cognitive state (Başar et al., 2001). While the same frequency bands may not be as behaviourally-relevant in zebrafish, we hypothesised that due to the similar underlying neurophysiology of mammals and zebrafish, interpreting spectral changes in the context of the same bands may provide useful insights into underlying mechanisms of seizurogenesis. Furthermore, performing such analysis in the 4dpf larval zebrafish served to provide a shared context which, it was hypothesised, would be important for making interspecies comparisons of electrophysiological responses to seizurogenic chemical treatment. Fourier transforms were performed on the signal from baseline and exposure periods of each fish, and the resulting spectra during each exposure period were normalised to the corresponding spectra of the baseline recordings. Next, the mean normalised spectra for each fish were averaged within treatment groups (**see Figure 6B**). Finally, normalised spectra were binned into frequency bands used for studying mammalian and human EEG data (Moffett et al., 2017; Wang et al., 2020). These bands were: δ (1–4 Hz), θ (4–7 Hz),

157 α/μ (8–13 Hz), β (beta, 15–30 Hz), γ (gamma, 30–80 Hz) and high γ (80–150 Hz) and HFO (150–500
 158 Hz). The activity in each band was then compared between test compound-treated and control fish
 159 using Mann-Whitney U-tests corrected for multiple comparisons using the Benjamini and Hochberg
 160 method (Benjamini and Hochberg, 1995) **see Figure 6A and Table 2.**

161

162 **2.6 Data analysis: classical multidimensional scaling (MDS) of baseline normalised spectral data**

163 In order to visualise the similarity or differences between representative spectra for each
 164 treatment group, classical multi-dimensional scaling was performed across the average normalised
 165 power spectra for each fish. Subsequently, the mean of the first two coordinates was calculated across
 166 treatments groups, and were plotted. The eigenvalues for these coordinates accounted for >74% of
 167 the sum of all the eigenvalues.

168

169 **2.7 Data analysis: Event detection**

170 The analysis described above was derived from averaging the activity over long periods.
 171 However we also wanted to examine the presence of differences in the LFP on shorter time scales,
 172 akin to examining the morphology of epileptiform spikes or rhythm. To achieve this, we algorithmically
 173 selected shorter time periods based on differences from baseline in their frequency components,
 174 which we defined as ‘events’. These events were defined as periods of time 1 second long whose
 175 frequency spectra deviated significantly from the baseline. Each larva has a ‘drug free’ baseline period
 176 and ‘events’ that were considered significantly different from baseline were selected. In the case of
 177 control larva this ‘exposure period’ was also a drug free baseline period. However, due to the
 178 sensitivity of the equipment used there were still some ‘events’ that differed enough from the baseline
 179 in the control fish to be included, although compared to the drug treated fish, these were very few in
 180 number (**see figure 8**). These ‘erroneous’ events are likely to be the result of electrical noise common
 181 in these kinds of recordings. For event detection, initially wavelet transformations were performed on
 182 the recorded voltage timeseries for both the exposure and the baseline periods in order to produce

highly temporally resolved frequency spectra. For this a continuous 1-D wavelet transform was used, which was obtained using the analytic Morse wavelet with the symmetry parameter (γ) equal to 3, and the time-bandwidth product equal to 60, using 10 voices per octave. The frequencies of the resultant wavelets were binned into the following bands: 1–4 Hz, 4–7 Hz, 8–13 Hz, 15–30 Hz, 30–80 Hz, 80–150 Hz, and 150–500 Hz (see Figure 2A). These bands broadly correlate with neural frequency bands used in mammalian models (Moffett et al., 2017; Wang et al., 2020). Next, the average over time in each of these frequency bands was calculated across the entire baseline period. Subsequently the Euclidean distance between the frequency spectra of every time-point in the wavelets and the average baseline frequency spectra was calculated. This allowed identification of where in the wavelet transformations deviations from the mean of the baseline occurred. After plotting the resultant Euclidean distances as shown in Figure 2B, we used MATLAB’s ‘findpeaks’ function to identify where the Euclidean distances were greater than two standard deviations from the mean of the baseline period, selecting 1 second long events. Next, taking all of the events selected using this first pass approach, the event wavelets for each fish were plotted separately in Euclidean space. From this, only the exposure events that were further from the centroid of the baseline events than the most distant baseline event, were selected. This process ensured we selected only the events whose spectra were distinct from those that occurred during the drug-free baseline period. The numbers of these events were calculated for each larva and compared between control and treatment groups using a Wilcoxon rank sum test, corrected for multiple comparisons using the Benjamini and Hochberg method (Benjamini and Hochberg, 1995).

203

204 **2.8 Data analysis: Wavelet of selected events**

In order to better understand how the temporal profile of events differed between compounds, event epochs were selected from each treatment group of each compound. The events with the smallest Euclidean distance from the mean spectra of all the events selected for that treatment group (using the method outlined above), were selected as best representing events produced by that

209 treatment. In addition to the presentation of the filtered timeseries, a heatmap of the wavelet
 210 transforms was also generated, as shown in **Figure 3**.

211 **Code Accessibility**

212 The code/software described in the paper is freely available online at:
 213 <https://github.com/jp908/Ephys-Seizure-ZF>. The code is available as Extended Data.

214

215 **3. Results**

216 **3.1 Changes in AUC between baseline and exposure**

217 The results of the changes in AUC from baseline to exposure are summarised in **Figure 5**. Only
 218 picrotoxin ($p=0.0081$, corrected- $p=0.0104$, $W=51$, $z\text{-value}=-2.6461$) and SB205607 ($p=0.0006$,
 219 corrected- $p=0.0015$, $W=270$, $z\text{-value}=3.4286$) exposure resulted in significant changes in AUC between
 220 the baseline and exposure periods, exhibiting increased and decreased AUC respectively. The AUC of
 221 picrotoxin appeared to oscillate slightly across 30 second time bins with a slight upward gradient.

222

223 **3.2 Spectral Frequency Band Analysis**

224 The results of the spectral analysis of the *in vivo* LFP data are summarised in **Figure 6 and table**
 225 **2**. From these data, it can be seen that exposure to the highest concentrations of chlorpromazine and
 226 picrotoxin resulted in a significant increase in activity in the slower delta-frequency band compared
 227 with the control animals. In the higher frequency γ , high γ , and HFO bands, significant increases in
 228 activity were observed after exposure to RST, picrotoxin and aminophylline. Interestingly, exposure to
 229 chlorpromazine resulted in significantly reduced, β , γ , high γ , and HFO band activity at all of the
 230 concentrations tested, and this was not observed for any other compounds assessed here. Exposure
 231 to SB205607 and donepezil resulted in no significant changes in any of the frequency bands measured.
 232 In addition, exposure to picrotoxin resulted in increases in α band oscillatory activity, while
 233 aminophylline and picrotoxin exposure resulted in increases in β power. Notably, picrotoxin resulted
 234 in an increase in power in every single frequency band, but only at the highest concentration.

235

236 **3.3 Classical multi-dimensional scaling**

237 Classical multi-dimensional scaling of the spectral data revealed the distribution of each
 238 treatment group in 2-dimensions. The outer regions of the resultant scatter plot (**Figure 7**) are
 239 occupied by the highest concentration of chlorpromazine, donepezil, RST and picrotoxin. Notably
 240 there was overlap of the middle concentration of aminophylline and picrotoxin, likely due to similar
 241 increases in β , γ and high frequency oscillations. SB205607 and the lowest concentration of
 242 aminophylline lie closest to the control reflecting the relative absence of induced effects on the
 243 parameters measured. The highest concentration of chlorpromazine is located furthest from RST and
 244 picrotoxin likely reflecting its reduction in oscillations at >15 Hz. Furthermore, RST and picrotoxin are
 245 located on the right hand side of the scatterplot likely due to their notable induced increases in the
 246 magnitude of oscillations at >15 Hz.

247

248 **3.4 Number of Detected Events**

249 Exposure to aminophylline, chlorpromazine, donepezil and RST appeared to increase the number of
 250 events detected in a dose-dependent manner (**see figure 8**). The highest concentrations of, donepezil
 251 ($p=0.0354$ $z\text{-value}=-2.764$, test statistic=239.5), picrotoxin ($p=0.0273$, $z\text{-value}=-2.715$, test
 252 statistic=234), and RST ($p=0.0159$, $z\text{-value}=-3.220$, test statistic=229.5) resulted in a significant
 253 increase in the number of detected events as compared to the control.

254

255 **3.5 Mean Event and Wavelet Analysis**

256 The results of the mean event and wavelet analysis are summarised in **Figure 3**. Exposure to the
 257 compounds tested resulted in bursts of rhythmic activity of specific frequencies consistent with
 258 changes in the spectra observed in the previous analyses (**Figure 6**). Chlorpromazine's wavelet
 259 spectrograms, for example, showed a distinct amelioration of >80 Hz activity particularly in the higher
 260 concentrations, in addition to bursts of theta, delta and alpha range activity. The events identified in

261 animals exposed to donepezil were categorised by a gradual polarisation followed by a large low
262 frequency event in the <15 Hz range with an additional high frequency component at the peak of, and
263 subsequent to, the main depolarisation. However, there was also evidence of bursting type high
264 frequency activity. Picrotoxin exposure resulted in wavelet spectrograms characterised by a high
265 amplitude activity in the 80 Hz or less frequency range, and appeared to show a distinct dose-
266 dependent increase in activity. RST exposed animals were observed to contain a bursting type of high
267 frequency activity in addition to sharp spike-type discharges, as illustrated most clearly at the middle
268 concentration. Notably RST appeared to lack much of the lower frequency component. Both
269 aminophylline and SB205607 appeared to induce medium sized events, although notably the highest
270 concentration of aminophylline appeared to have some similarities with donepezil's high
271 concentration event profile.

272

273

274

275 **4. Discussion**

276 Using *in vivo* mid-brain LFP recordings in 4dpf larval zebrafish, we demonstrate responsiveness
277 to a range of pharmacological agents implicated in the induction of seizures in mammals. The data
278 we present show that exposure to chlorpromazine (a phenothiazine antipsychotic), donepezil (an
279 acetylcholinesterase inhibitor), picrotoxin (a GABA_AR antagonist) and RST (an NMDAR agonist) results
280 in quantifiable, concentration-dependent, altered neuronal electrophysiology. Furthermore, the
281 electrographic responses exhibited differed between each drug, and in some cases exhibited
282 characteristics commensurate with the induction of interictal and ictal electrophysiology phenotypes
283 similar to those classified in humans (Fisher et al., 2014b).

284 ***4.1 Electrographic Phenotypes of Representative Traces***

285 Although seizure phenotypes from electrographic recordings are extremely diverse, the main
 286 aim of our study was to explore what ‘abnormal’ LFP recordings might emerge following exposure of
 287 zebrafish larvae to drugs acting via known seizurogenic mechanisms. Furthermore, from these
 288 phenotypes, we aimed to classify such patterns in the context of their frequency components, and to
 289 compare these components to human EEG data and models of seizure in mammals. To this end we
 290 compared the representative traces that were selected using our event detection algorithm with
 291 specific examples of pre-ictal, inter-ictal and ictal type waveforms, as reported by Fisher et al (Fisher
 292 et al., 2014b). The representative trace for the highest concentration of chlorpromazine, for example,
 293 appeared to comprise of rhythmical evolving theta, delta and alpha frequencies, which is an
 294 electrophysiological phenotype commonly seen preceding seizure in EEG traces recorded clinically
 295 (Fisher et al., 2014b). Similarly, the high amplitude, high frequency, discharges observed in the
 296 representative traces for the intermediate exposure concentrations for both donepezil and RST share
 297 similarities with intracerebral stereo-EEG recordings of seizures reported in mammals (Fisher et al.,
 298 2014b). These discharges also contained very high frequency (80-500 Hz) components similar to
 299 examples of seizures reported previously in mammals (Gliske et al., 2017; Jiruska et al., 2017, 2013;
 300 Mackenzie et al., 2002; Shiri et al., 2015; Wang et al., 2020). The representative traces for the highest
 301 concentration of aminophylline, in addition to that seen for the lowest and highest concentrations of
 302 donepezil, appear consistent with electrodecremental EEG patterns, a electrophysiological phenotype
 303 commonly seen preceding seizure in EEG traces recorded clinically (Fisher et al., 2014b).

304 Qualitative comparisons of representative traces for the highest concentration of
 305 aminophylline, donepezil and picrotoxin in our study, with traces of spontaneous ictal and inter-ictal
 306 activity identified by (Griffin et al., 2021) in their recording of LFPs in zebrafish (where they were
 307 screening for potentially seizurogenic genetic modifications), showed strong similarities. Moreover,
 308 the traces for the medium and high concentrations of RST appeared similar to their definition of an
 309 ictal-type waveform. These similarities support a good degree of consistency between drug induced
 310 seizures and spontaneous seizures, although a more extensive comparison would be necessary to

confirm this. Compared with behavioural and imaging-based assessments of seizures in zebrafish (13,22,26,34–39), *in vivo* LFP recordings provide a direct measure of the electrographic response of model organisms to neuroactive drug treatment. This is important as the data generated more readily allows comparisons between model species (see Table 3), a feature which is crucial for evaluating the translational power of the zebrafish as a surrogate for understanding the effects of neuroactive drugs in higher vertebrates, including humans.

4.2 Trace Amplitude and Seizure

Given that seizures are considered to be high amplitude events, measuring the change in the average AUC induced by the different compounds was initially undertaken. Notably the largest increase in AUC was observed after exposure to picrotoxin which is commonly used in animal models as a seizure precipitant (Baraban, 2013; Fan et al., 2019; Ridler et al., 2018; Winter et al., 2008). This is consistent with previous studies in zebrafish exposed to picrotoxin that reported the presence of high amplitude events (Baraban, 2013). Interestingly, however, the NMDA-receptor agonist RST, which is also a well-known model convulsant, induced no change in AUC despite inducing changes in other measured parameters. It is worth noting that seizure like states are often characterised by periods of bursting activity with intermittent quiescence (Barnett et al., 2013). In this context it is possible that periods of seizure like activity induced via RST are bursting in nature, with long intervals in-between and thus not best detected using our protocol. In support of this, upon a more detailed analysis of the spectral components of its electrographic signature, the resultant data suggested that RST exposure was associated with elevated neural activity in specific frequency-bands, rather than showing a more generalised elevated activity (see below). In a previous study using Ca^{2+} imaging (Winter et al., 2021), it was also reported that there was a relative absence of oscillatory activity after exposure to RST (and the pharmacologically similar NMDA). Given that here we saw elevated activity only in the >15 Hz frequency range (see below), and notwithstanding the sensitivity of Ca^{2+} dye based imaging, it is possible that in the previous study, the temporal resolution of the imaging-based approach used was insufficient to capture such rapid oscillations thus highlighting the relative

337 advantages and disadvantages of each type of approach when studying drug-induced neuronal events.

338 ***4.3 Neural Oscillations Zebrafish versus Mammals***

339 The binning of generated frequency spectra into specific frequency bands is a commonly used
 340 approach in the analysis of mammalian electrophysiological data (Başar et al., 2001). We adopted this
 341 approach primarily to directly compare neural activity signatures between 4dpf larval zebrafish and
 342 mammalian models of drug-induced seizures. It should be noted that we consider the changes seen
 343 in the different frequency bands for all drugs to be as a result of bursts of rhythmic activity. This means
 344 there are both periodic and aperiodic contributions to the frequency spectra. We believe this is the
 345 case, because the event detection algorithm utilised here identified bursts of rhythmic activity whose
 346 spectra were consistent with the frequency spectra performed on the full recordings. Moreover, the
 347 number of these events was not adequate to constitute the full traces. As such our analyses suggested
 348 that drug exposure resulted in transient bursts of rhythmic activity that altered the overall spectral
 349 profile of the recordings, as opposed to sustained oscillatory activity.

350 In multiple EEG studies undertaken in mice, the antipsychotic medications haloperidol and
 351 clozapine have been shown to significantly reduce gamma power (Olszewski et al., 2013; Sun et al.,
 352 2020). Here, we also observed a significant reduction in all of the frequency bands of >15Hz upon
 353 exposure to chlorpromazine. Both chlorpromazine and haloperidol broadly reduce monoamine
 354 signalling, a neurotransmitter sub-group that is highly conserved between larval zebrafish and
 355 mammals in both their molecular underpinnings and behavioural functionality (Maximino et al., 2016).
 356 Indeed, haloperidol has been shown to induce locomotor impairments in zebrafish, an effect mirrored
 357 in the common side effects in humans (e.g. drowsiness, dizziness and neuromuscular dysfunction
 358 (Giacomini et al., 2006)). Chlorpromazine has also been shown to induce significant alterations in 4dpf
 359 larval brain functional connectivity (Winter et al., 2021) and our data here also show comparable
 360 oscillatory activity induced by this antipsychotic compound between zebrafish and mammals. The
 361 confirmation here of shared brain oscillatory changes, in addition to the aforementioned similar
 362 behavioural manifestations, suggests that the established links between neural frequency bands and

363 mammalian behaviour may have some translatability to zebrafish.

364 Exposure to the acetylcholinesterase inhibitor donepezil increased power in the theta
365 frequency bands in larval zebrafish. In mammals theta and gamma oscillations are associated with
366 successful memory recall (Barnett et al., 2013). Notably, a mechanistically similar compound and
367 cholinesterase inhibitor, physostigmine, has been shown to reverse scopolamine-induced learning
368 impairment in zebrafish, suggesting effectiveness in reversing impaired cognition (Kim et al., 2010a).
369 In this context it would be interesting to assess the behavioural response of zebrafish to donepezil
370 specifically given the apparent enhancement of theta oscillations in the electrophysiology data we
371 present here. Indeed, observation of donepezil-induced cognitive enhancement in larval zebrafish
372 would provide further evidence of a similar relationship between brain oscillatory patterns and
373 behavioural manifestations in larval zebrafish as those observed in mammals.

374 Perturbations in oscillations in the gamma and high gamma ranges are common across a variety
375 of psychiatric disorders, including attention deficit hyperactivity disorder, schizophrenia and, of
376 particular relevance here, epilepsy (Herrmann and Demiralp, 2005). Epilepsy specifically is associated
377 with an increase in gamma oscillations likely due to cortical excitation (Herrmann and Demiralp, 2005).
378 Indeed, in human EEGs, oscillations in the gamma and fast gamma range precede interictal
379 epileptiform spikes in the seizure onset zone (Jiruska et al., 2013). Similarly, in the nucleus accumbens
380 of rats, there are increases in gamma power when seizure kindling is induced using kainic acid (Ma
381 and Leung, 2010). Here we observed significant increases in gamma and fast gamma activity after
382 exposure to the two seizure precipitants picrotoxin and RST, in addition to aminophylline. Previously
383 published work in mice has shown that treatment with picrotoxin has been shown to increase gamma
384 oscillations in the olfactory bulb, an effect that could be suppressed via inhibition of ionotropic
385 glutamate receptors (Lepousez and Lledo, 2013). In addition, in rats, NMDA agonists induce
386 acetylcholine release and affect an increase in both beta and gamma oscillations in the basal forebrain
387 (Fournier et al., 2004). In our study, both picrotoxin and RST appeared to induce abnormal
388 electrographic events, similar to those recorded in previous studies in zebrafish (Afrikanova et al.,

2013; Baraban et al., 2005; Copmans et al., 2019; Hortopan et al., 2010; Löscher, 2017) and consistent with seizures observed in human EEG (Fisher et al., 2014b). Notably our data suggest that these events, much like in humans and rats, were characterised by an increase in gamma and high gamma frequency oscillations. The fact that chlorpromazine appeared to uniquely reduce gamma and high gamma oscillations is interesting and raises the issue of primary versus secondary pharmacological activity and dosing levels. Certainly, chlorpromazine is known to have potent sedative effects linked to its well-known H_1 activity (von Coburg et al., 2009), and is typically associated with a seizure-threshold lowering effect rather than a direct seizurogenic effect as measured using our exposure method (Chi et al., 2017).

The highest frequency band we measured, in the range between 150 and 500 Hz, contained high frequency oscillations (HFOs) (Wang et al., 2020). HFOs are strongly correlated with the epileptogenic zone and have been implicated as a useful biomarker of epilepsy (Lévesque and Avoli, 2019), although their use to prospectively define the epileptogenic zone is not clear (Jacobs et al., 2018). Indeed, fast ripples (250-500 Hz) appear to be highly indicative of epileptic tissue in both human conditions and animal models (Jiruska et al., 2017). HFOs are believed to occur as a result of the synchronisation of fast firing within populations of interconnected neurons generating high-frequency population spikes which, when recorded extracellularly, present as an HFO event (Jiruska et al., 2017). Individual Pyramidal neurons cannot fire fast enough to account for oscillations higher than 300Hz, thus, fast ripple oscillations have been proposed to be generated via the action of synchronised, but out-of-phase, neurons (Jiruska et al., 2013). Here, a significant increase in the magnitude of HFOs was observed after exposure to aminophylline, picrotoxin and RST, while chlorpromazine appeared to reduce the magnitude of HFOs. This is consistent with studies in mouse brain slices showing induction of HFOs via picrotoxin exposure (Shiri et al., 2015). Moreover, automated event detection designed to categorize events unique from baseline identified several events across these compounds that contained HFOs, including after exposure to donepezil, RST and picrotoxin across all concentrations.

4.4 Study Limitations

415 Exposure to the δ -opioid agonist, SB205607, showed little evidence of altered brain
416 electrophysiology despite showing significantly elevated activity in a number of brain regions at 500
417 μ M (the top concentration used here) in an imaging based assessment undertaken in 4dpf zebrafish
418 (Winter et al., 2021). Other studies have reported insensitivity of the larval zebrafish to specific δ OR
419 agonists such as SNC80 (Rodriguez and Gonzalez-Nunez, 2006) although this does not explain why
420 previous work with SB205607 suggested some degree of neuroactivity (Winter et al., 2021). One
421 possible reason for this could be explained by the relative strengths and weaknesses of
422 electrophysiological versus imaging-based neural functional assessments. Electrophysiological
423 assessment offers unparalleled levels of temporal resolution, whereas imaging based approach offer
424 much greater spatial coverage. Given the LFP approach used here measures electrical activity in a
425 small population of neurons in one part of the brain it is possible that this region was not activated by
426 SB205607 exposure. Certainly this would be supported by the data from our Ca^{2+} imaging work (Winter
427 et al., 2021) which suggested comparatively low levels of activity in mid brain regions compared with
428 the hind brain. This could be investigated further by the use of multi-electrode arrays to allow for
429 greater spatial coverage during LFP recordings (e.g. (Hong et al., 2016)).

430 Despite the existence of notable similarities between the LFPs recorded in zebrafish and
431 mammalian electrophysiology data, there are limitations to the larval zebrafish as a model.
432 Specifically, *in vitro* LFP recordings taken in mammals are often performed on specific anatomical
433 structures which in the case of seizure and epilepsy research are recognised as hyper-excitatory in
434 nature, such as the hippocampus. In zebrafish larvae, recording from specific brain structures is
435 extremely challenging due the small size of the brain, thus recordings are generally taken from the
436 midbrain or the forebrain with little ability to differentiate between specific sub regions of higher or
437 lower relevance. In this respect, the use of genetically modified models in which specific neural circuits
438 or populations are labelled may prove extremely useful and allow more precise placement of
439 electrodes in structures that have been identified as especially appropriate for measuring the type of
440 brain activity being investigated.

441

442 **5. Conclusions**

443 In summary, *in vivo* LFP-mediated assessment of neural activity in 4dpf zebrafish larvae revealed
 444 responsiveness to seizurogenic compounds that act via a range of pharmacological mechanisms of
 445 action. Furthermore, the resultant electrographic profiles exhibited by 4dpf zebrafish larvae exposed
 446 to a number of these compounds show clear differences in their characteristics and in some cases
 447 share notable similarities with the signatures exhibited by mammalian, including human,
 448 electrophysiological profiles. The data generated here add to the body of data supporting the use of
 449 the larval zebrafish as a complimentary and potentially alternative model for the study of seizures and
 450 epilepsy and also provide further insight into the electrophysiological characteristics of seizures
 451 generated in non-mammalian species.

452

453

454 **Declaration of competing interests**

455 All authors declare no potentially competing interests.

456

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660 **Legends for Tables and figures**

661 **Table 1) Test compounds and exposure concentration ranges used for *in vivo* electrophysiological**
 662 **recording in 4dpf *elavl3:GCaMP6s* zebrafish larvae.** Also shown are published evidence to support
 663 seizurogenicity. All compounds were sourced from Sigma-Aldrich (Gillingham, UK) or Tocris (Bristol,
 664 UK).
 665

666 **Figure 1) A schematic showing the experimental process and example data from *in vivo***
 667 **electrophysiological recording in 4dpf *elavl3:GCaMP6s* zebrafish larvae. Panel A)** The experimental
 668 process used. **Panel B) Left:** A paralysed, mounted zebrafish larva with glass electrode inserted into
 669 midbrain. The Red circle indicates the placement of the tip of the electrode, while the blue dashed
 670 line indicates landmarks used to consistently place the electrode. **Right:** Representative LFP recording
 671 from midbrain of zebrafish larva at baseline and after administration of 30 mM picrotoxin.

672 **Figure 2) Example data obtained from *in vivo* electrophysiological recording in a 4dpf**
 673 ***elavl3:GCaMP6s* zebrafish larva exposed to donepezil A)** A representative frequency binned wavelet
 674 transformation from a single larva, the top graph shows the baseline and the bottom wavelet
 675 transform after drug administration. These transformations were used to identify events from the full
 676 timeseries. **B)** A plot of the Euclidean distance of each time point from the average of the baseline.
 677 The black dotted line represents the threshold for selecting events. All peaks above this line were
 678 selected and used to find events which were subsequently clustered.
 679

680 **Figure 3) Example of an event plus 2 seconds either side for each treatment group.** For each
 681 treatment group, the timeseries' are displayed for each event with a wavelet transform below each
 682 one displaying the frequency domain over the same time period. The events selected were the events
 683 whose spectra were closest in Euclidean distance to the mean event spectra for that treatment group,
 684 meaning that these are representative of the events shown for each compound. The bottom bar

685 shows the colour scaling for the magnitude of the wavelet transformation. Each column represents a
686 different concentration set and each row represents a different compound.

687 **Figure 4) Mean number of events detected per treatment group.** Bar graph showing the mean
688 number of events per treatment group. Error bars represent the SEM (n =7-8). *adjacent to the bars
689 indicate a statistically significant difference from control (p<0.05) using a Wilcoxon rank sum test
690 corrected for multiple comparisons using the Benjamini and Hochberg method.

691
692 **Figure 5) Analysis of the area under the curve (AUC) of the Hilbert transform of the LFP recordings.**
693 **Panel A)** Shows the mean baseline normalised AUC of the absolute value of the Hilbert transform of
694 the LFP averaged into 30 second time bins. The shadows represent the SEM (n=20-24). The left hand
695 side of the graph represents the baseline period, while the right-hand side represents the exposure
696 period. The gap in time from 7-17 minutes is time allotted for compounds to take effect. **Panel B)**
697 Shows the baseline normalised AUC for each of the compounds tested. The bars show the Normalised
698 AUC averaged across all treatment groups for each compound, while the normalised AUC of individual
699 larvae are represented as transparent grey circles. Data are shown as the mean with error bars
700 depicting the SEM (n=20-24).* indicates a statistically significant difference between baseline and
701 exposure periods (p<0.05) using Wilcoxon signed rank test and corrected for multiple comparisons
702 using the Benjamini and Hochberg method (Benjamini and Hochberg, 1995).

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705 **Figure 6) Data generated for larvae exposed to each of the test compounds after spectral analysis**
706 **and categorisation into specific frequency bands. Panel A)** The bars show the baseline normalised
707 mean amplitude for each of the neural frequency bands frequently used for categorising mammalian
708 electrophysiological data (Moffett et al., 2017; Wang et al., 2020). Data are shown as the mean with
709 error bars showing the SEM (n=7-20). The baseline normalised power for individual larvae are
710 represented by the transparent grey circles.*indicates a statistically significant difference from control
711 (p<0.05) using Mann Whitney U-tests and corrected for multiple comparisons using the Benjamini and
712 Hochberg method (Benjamini and Hochberg, 1995). **Panel B)** shows the mean baseline normalised
713 power spectra for each compound treatment group. In this case the x-axis is scaled to the common
714 logarithm. Shading represents the SEM for each data point (n=7-20) across the power spectra. Black
715 lines here indicate the mean of the control animal power spectra, and therefore are the same for each
716 graph.

717
718 **Table 2) All statistical test outputs from frequency band analyses:** The activity in each frequency band
719 compared between test compound-treated and control fish using unpaired Mann-Whitney U-test and
720 corrected for multiple comparisons using the Benjamini and Hochberg method (Benjamini and
721 Hochberg, 1995). The columns from left to right contain the relevant frequency band, compound,
722 concentration of exposure, p-value, Benjamini and Hochberg corrected p-value, Rank Sum test
723 statistic and corresponding z-statistic computed when the method is 'approximate'. All statistics were
724 undertaken using the MatLab statistics and machine learning toolbox ("Wilcoxon rank sum test -
725 MATLAB ranksum - MathWorks United Kingdom," n.d.).

726
727 **Figure 7) Multidimensional Scaling of the normalised power spectra: Right-hand image:** A scatter
728 graph of the first two coordinates produced after classical multi-dimensional scaling of the mean
729 normalised power spectra of each treatment group (see Figure 6B): **Left-hand image:** A scatter graph
730 of the first two coordinates produced after classical multidimensional scaling of each individual larva.
731 In both plots the circles represent the lowest concentration, the triangles the middle concentration
732 and the diamonds the highest concentration used for each treatment group. The black square
733 represents the control group.

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737 **Table 3) Summary of information comparing the effect of selected drugs on EEG in mammals from**
738 **published data with the data obtained for zebrafish in this study.**

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740
Table 1
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Compound Name	Pharmacodynamics	Concentrations Used	Seizure liability
Aminophylline	Adenosine Receptor Antagonist and phosphodiesterase inhibitor	1 mM, 2 mM, 4 mM	Various cases of seizure in humans, and evidence of kindling in rats (Albertson et al., 1983; Schwartz and Scott, 1974).
Chlorpromazine	Dopamine, Serotonin, Histamine, Muscarinic and α 1- and α 2-adrenergic receptor antagonist	31.25 μ M, 62.5 μ M, 125 μ M	Increased risk of seizure in patients receiving anti-psychotic drug (APD) treatment, particularly polytherapy. Seizure risk 5-fold higher in individuals receiving low/medium potency APDS such as chlorpromazine (Bloechliger et al., 2015; Druschky et al., 2019).
Donepezil	Acetylcholinesterase inhibitor	125 μ M, 62 μ M, 31 μ M	Among the top 10 drugs most commonly associated with seizures in world health organisation adverse drug reaction database (Kumlien and Lundberg, 2010).
Picrotoxin	GABA _A receptor Antagonist	30 μ M, 60 μ M, 120 μ M	Commonly used convulsant compound used for modelling seizures (Mackenzie et al., 2002).
(RS)-(Tetrazol-5-yl)glycine	NMDA receptor Agonist	62.5 μ M, 125 μ M, 250 μ M	Convulsant compound used for modelling seizures (Schoepp et al., 1991).
SB205607 (TAN-67)	δ -opioid receptor agonist	125 μ M, 250 μ M, 500 μ M	Increases incidence of convulsions in bicuculline kindled rats (Yajima et al., 2000).

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Table 2

Frequency Band	Compound	Concentration	P-Value	Corrected P-Value	Test Statistic	Z-Value
δ	Chlorpromazine	62.5 μ M	0.013812	0.03942	235	-2.46211
δ	Chlorpromazine	125 μ M	0.010224	0.035258	239	-2.56817
δ	Picrotoxin	120 μ M	0.013812	0.038106	235	-2.46211
θ	Picrotoxin	120 μ M	0.002566	0.013276	225	-3.01539
α	Picrotoxin	120 μ M	0.000544	0.003464	217	-3.45802
β	Aminophylline	2 mM	0.010089	0.036306	233	-2.57277
β	Chlorpromazine	31.25 μ M	0.011825	0.037643	340	2.517311
β	Chlorpromazine	62.5 μ M	0.00438	0.018126	332	2.849409
β	Chlorpromazine	125 μ M	0.011825	0.036249	340	2.517311
β	Picrotoxin	120 μ M	0.000442	0.003329	216	-3.51335
γ	Aminophylline	2 mM	0.002566	0.012495	225	-3.01539
γ	Chlorpromazine	31.25 μ M	0.0001	0.002071	367	3.890389
γ	Chlorpromazine	62.5 μ M	0.000188	0.002223	348	3.734663
γ	Chlorpromazine	125 μ M	0.000053	0.004369	370	4.042954
γ	Picrotoxin	120 μ M	0.00029	0.002668	214	-3.62401
γ	RST	250 μ M	0.011825	0.034954	240	-2.51731
High γ	Aminophylline	2 mM	0.002566	0.011801	225	-3.01539
High γ	Aminophylline	4 mM	0.011821	0.039135	234	-2.51744
High γ	Chlorpromazine	31.25 μ M	0.000081	0.002236	368	3.941244
High γ	Chlorpromazine	62.5 μ M	0.000188	0.001945	348	3.734663
High γ	Chlorpromazine	125 μ M	0.000053	0.002184	370	4.042954
High γ	Picrotoxin	120 μ M	0.000442	0.003052	216	-3.51335
High γ	RST	250 μ M	0.00293	0.012763	231	-2.975
HFO	Aminophylline	2 mM	0.001466	0.008088	222	-3.18138
HFO	Aminophylline	4 mM	0.008586	0.032303	232	-2.6281
HFO	Chlorpromazine	31.25 μ M	0.0001	0.001657	367	3.890389
HFO	Chlorpromazine	62.5 μ M	0.000995	0.00588	340	3.292036
HFO	Chlorpromazine	125 μ M	0.000123	0.0017	366	3.839535
HFO	Picrotoxin	120 μ M	0.00029	0.002401	214	-3.62401
HFO	RST	250 μ M	0.004766	0.018784	234	-2.82244

Table 3

Compound	Effects observed in 4dpf zebrafish LFP recordings undertaken in this study	Effects reported from EEG recordings undertaken in mammals
Aminophylline	Increase in the amount of beta and gamma oscillations (figure 6) and the presence of electrodecremental type pre-ictal discharges (figure 3).	Little frequency domain analysis of EEG exists for aminophylline however visual assessment of EEG traces from rats exposed to high doses of aminophylline show epileptiform discharges of high amplitude and frequency (Chu, 1981)
Chlorpromazine (Antipsychotic)	We observed a significant reduction in all of the frequency bands >15Hz upon exposure to chlorpromazine (figure 6).	Antipsychotic medications haloperidol and clozapine significantly reduce gamma power (Olszewski et al., 2013; Sun et al., 2020).
Donepezil	Apparent increase in theta oscillations (the largest mean difference of all the compounds) although it did not reach statistical significance (figure 6). Events with a large theta component (figure 3).	Rats exposed to Donepezil show increases in cortical EEG oscillations in the theta frequency band (Ahnaou et al., 2014).
Picrotoxin	Significant increases in gamma, fast gamma activity, and HFO amplitude (figure 6). This is consistent with studies showing high frequency bursting type LFPs (Baraban, 2013)	Picrotoxin increases gamma oscillations in the olfactory bulb, an effect that could be suppressed via inhibition of ionotropic glutamate receptors (Lepousez and Lledo, 2013). Mouse brain slices show induction of HFOs via picrotoxin exposure (Shiri et al., 2015)
RST	Significant increases in gamma and fast gamma activity (figure 6).	In rats, NMDA agonists induce acetylcholine release and affect an increase in both beta and gamma oscillations in the basal forebrain (Fournier et al., 2004).
SB205607	Few effects of SB205607 on LFP in zebrafish.	No studies found for the electrophysiology of SB205607 in mammals.

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