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Do *in vitro* assays in rat primary neurons predict drug-induced seizure liability in humans?

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ABSTRACT

Drug-induced seizures contribute to the high attrition rate of pharmaceutical compounds in development. The assessment of drug-induced seizure liability generally occurs in later phases of development using low throughput and intensive *in vivo* assays. In the present study, we evaluated the potential of an *in vitro* assay for detecting drug-induced seizure risk compared to evaluation in rats *in vivo*. We investigated the effects of 8 reference drugs with a known seizurogenic risk using micro-electrode array (MEA) recordings from freshly-dissociated rat primary neurons cultured on 48-well dishes for 28 days, compared to their effects on the EEG in anesthetized rats. In addition, we evaluated functional responses and mRNA expression levels of different receptors *in vitro* to understand the potential mechanisms of drug-induced seizure risk. Combining the functional MEA *in vitro* data with concomitant gene expression allowed us to identify several potential molecular targets that might explain the drug-induced seizures occurring in both rats and humans. Our data 1) demonstrate the utility of a group of MEA parameters for detecting potential drug-induced seizure risk *in vitro*; 2) suggest that an *in vitro* MEA assay with rat primary neurons may have advantages over an *in vivo* rat model; and 3) identify potential mechanisms for the discordance between rat assays and human seizure risk for certain seizurogenic drugs.

1. Introduction

Within drug development, preclinical investigation includes focus on the potential of new medical entities (NME) that cause hazardous or adverse effects on different physiological systems within and above the expected therapeutic range of exposures. Drug-induced seizures (both convulsive and non-convulsive) are life-threatening adverse reactions, and have resulted in the withdrawal of several drugs from the market (Authier et al., 2016; Onakpoya et al., 2016) or termination of further drug candidate development throughout the various R&D stages. *In vivo* models have been developed and are widely used to study seizure liability. However, the general CNS safety study using modified Irwin test (Irwin, 1968) is generally conducted in the later phases of the drug development process (Kelly, 2004).

Seizure, or ictus, is defined as an uncontrolled electrical activity in the brain, which may produce a clinical convulsion, minor physical signs, thought disturbances, or a combination of these symptoms. Convulsions, tonic or clonic, are typically characterized by persistent or alternate contraction and spasm or relaxation of a set of voluntary muscles. Seizures with no external behavioral changes may also occur (Pitkänen et al., 2006). Such seizures can only be detected using typical EEG recordings, making detection of drug-induced abnormal neuronal activity in preclinical studies based solely on behavioral observations very difficult. Therefore, the facilitation of seizure risk identification of compounds early in the drug candidate selection process through assessment based on disturbances of neuronal activity *in vitro* that are indicative of seizure risk would represent an important and strategic step forward.

Defining seizures *in vitro* is very challenging and complex due to multiple pharmacological targets (Easter et al., 2009) and cellular mechanisms that need to be studied in a neuronal network. *In vitro* electrophysiology using hippocampal slice preparations from neonatal rodents has been a technique of choice to reproduce abnormal *seizure-like* activity and can detect the seizurogenic effects of a wide range of compounds. Single electrode and micro-electrode arrays (MEAs) for recording neuronal activity in hippocampal slices have also been

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successfully used in early drug development and safety pharmacology (Easter et al., 2007). However, the low throughput, the use of only a small portion of brain tissue, such as the hippocampus, and the use of large numbers of animals per study limit its utility and efficiency. Primary neuronal cultures from neurons dissociated from different regions of the central nervous system (CNS) have also long been used to study neurological mechanisms using the patch-clamp technique (Sakmann and Neher, 1984).

The combination of dissociated primary neurons with MEAs has more recently been introduced for neurotoxicity assessment (Gramowski et al., 2000). Recent studies have shown that re-aggregated cortex cell cultures allow exploration of network properties, while preserving the morphological, molecular and functional properties of the individual neurons (Chiappalone et al., 2006; Frega et al., 2012; Hondebrink et al., 2016). Moreover, another study demonstrated reproducibility and reliability of the MEA measurements across different laboratories using re-aggregated cells (Vassallo et al., 2016). However, some published studies investigated drug-induced neuronal toxicities by either analyzing the changes in the mean firing rate (Novellino et al., 2011; Vassallo et al., 2016), or by using a comprehensive set of parameters based on spike train characteristics (Hammer et al., 2015). Information resulting from MEA recordings can be utilized to classify compounds acting on different targets as reported previously by Gramowski et al., 2004 (Gramowski et al., 2004).

In the present study, to challenge these limitations of both *in vitro* and *in vivo* approaches used for safety pharmacology assessments of drug-induced seizure liability, we investigated the translatability between an *in vivo* assay in anesthetized rats and an *in vitro* assay using MEA recordings from rat primary neuronal cultures. To capture information on the electrical behavior of seizure-like patterns *in vitro*, we quantified different MEA parameters (or metrics) from the key combined bursting, network, and synchrony of the neuronal activity to establish potential characteristics for drug-induced seizure tendency *in vitro*. Because of the wide variety of molecular targets and off-targets of reference drugs with seizurogenic risk (Supplementary data Table S2), we investigated both functional responses of different receptors and their gene expression data to identify potential mechanisms and/or pathways that could be responsible for inducing seizures.

2. Materials and methods

2.1. Drugs

Chlorpromazine hydrochloride (CAS 69-09-0, purity ≥ 98%), amoxapine (CAS 14028-44-5, purity 99.5%, lot number BCBB6438V), pentylenetetrazol (CAS 54-95-5, purity ≥ 99%, lot number SLBF5034V), picrotoxin (CAS 124-87-8, purity ≥ 98%, lot number SLBN2682V), strychnine (CAS 57-24-9, purity ≥ 98%, lot number SLBP8489V), acetylcholine chloride (CAS 60-31-1, purity ≥ 99%, lot number BCBH3758V), epinephrine (CAS 52-43-4, purity ≥ 99%, lot number 044K1252), γ-Aminobutyric acid (GABA, CAS 56-12-2, purity ≥ 99%, lot number 38H4702), dopamine hydrochloride (CAS 62-31-7, purity ≥ 98%), pilocarpine hydrochloride (CAS 54-71-7, purity ≥ 98%, lot number MKBV5022V) and glutamate (CAS 6106-04-3, purity ≥98%, lot number BCBK6359V) were obtained from Sigma (Sigma-Aldrich, Diegem, Belgium). Serotonin hydrochloride (CAS 153-98-0, purity 98%, lot number 10158506) was obtained from Alfa Aesar (Alfa Aesar, Lancashire, UK). Histamine dihydrochloride (CAS 56-92-8, purity 99%, lot number A0249142) was obtained from Acros Organics (Thermo Fisher Scientific, Geel, Belgium). Isoniazid (CAS 553-53-7, purity 97%, lot number FI246851501) was obtained from Carbosynth (Carbosynth Ltd., Berkshire, UK). Amitriptyline (CAS 50-48-6, purity > 95%) was obtained from Sequoia research products (Sequoia research products Ltd., UK).

2.2. Culture of rat primary neurons in vitro

Experiments were conducted using rat cortical cells that contain glutamatergic and GABAergic neurons and glia (Mundy and Freudenrich, 2000; Hogberg and Bal-Price, 2011). Primary neurons were freshly dissociated from embryonic E18-19 rat cortices as described previously in literature (Banker and Goslin, 1998) and 80,000 cells/well were plated onto 48-well MEA plates (Maestro system, Axion Biosystems). One day before plating the cells, each 48-well MEA plate was pre-coated with a polyethyleneimine (PEI) (0.1%) solution (Sigma), washed for four times with sterile distilled water and then allowed to dry overnight. On the day of plating, Laminin (20 μg/ml) (Sigma) was added to each plate which was then incubated for 1 h at 37 °C. Thereafter the neurons were cultured at 37 °C, 5% CO_2, 95% air atmosphere, in Neurobasal medium (Thermofisher cat. No. 21103-049) supplemented with 0.5 mM L-Glutamine (Thermofisher cat No 25030149) and 5% FBS (fetal bovine serum, from Thermofisher (cat No A3160802). The media was fully exchanged on the first day *in vitro* (DIV1) with Neurobasal media supplemented with glutamine and 2% B27 (Thermofisher cat. No 17504044) up to DIV5. From DIV5 onwards and every other day thereafter 50% of the media was changed with Neurobasal media containing 2% B27.

At DIV28, spontaneous neuronal activity obtained for 40 min in culture solution (Neurobasal + 2% B27) was defined as baseline. All the reference drug compounds were added at a single concentration per well (n = 8 per concentration within the same plate to avoid plate to plate variations) and plates were kept in the incubator (37 °C, 5% CO_2 and 95% O_2 atmosphere) for 60 min before being recorded for 40 min. Experiments with the neurotransmitters: GABA, epinephrine, serotonin, glutamate, dopamine and acetylcholine were analyzed after 3 min of exposure allowing us to capture transient effects of the neurotransmitters.

2.3. Anesthetized rats

All reported studies described here have been conducted in accordance with “The provision of the European Convention” on the protection of vertebrate animals which are used for experimental and other scientific purposes, and with “the Appendices A and B”, made at Strasbourg on March 18, 1986 (Belgian Act of October 18, 1991). Female Sprague-Dawley rats with a body weight range of 200 to 250 g were used in all experiments.

2.4. Anesthetic regime

Anesthesia was induced with a mixture of 5 mg/kg etomidate (Janssen Pharmaceutica NV, Beerse, Belgium) and 0.025 mg/kg fentanyl (Fentadon®, Eurovet Animal Health B.V., Bladel, the Netherlands) (van der Linde et al., 2011a). This mixture was injected *via* the tail vein. During the experiment, anesthesia was maintained with a continuous infusion of 10 mg/kg/h etomidate and 0.015 mg/kg/h fentanyl by i.v. infusion in the femoral vein. Just before the start of the infusion of drug compound, the rats received a subcutaneous injection of 0.5 mg/kg of the muscle relaxant succinylcholine (Janssen Pharmaceutica NV, Beerse, Belgium).

2.5. Experimental procedure

Rats were intubated with an endotracheal tube (Intramedic Polyethylene PE-200 tubing) for mechanical ventilation (Harvard Apparatus, Inspira asv) (tidal volume 3 ml, 60 resp/min). Three needle electrodes were placed on the cranium under the skin against the skull: two electrodes on the left and right and one reference electrode on the nose. Electrodes were connected to the Narcotrend (TM Monitor Technik, Germany) and a one lead EEG signal was monitored. Both left and right femoral arteries and veins were cannulated with Intramedic

Polyethylene PE-50 tubing. The right vein was used for drug compound infusion, whereas, the anesthetic maintenance was continued *via* the left vein. The left femoral artery was used to monitor blood pressure during the experiment and the right femoral artery was used for blood sampling (0.2 ml/sample every 5 min). Blood samples were centrifuged immediately (8000 rpm for 2 min) and plasma was collected. At the end of each experiment (60 min) cerebrospinal fluid (CSF) was sampled *via* a cisterna magna puncture. Rats were decapitated, the skull was removed and brains collected and dissected into the neocortex and hippocampus. All fluid (plasma and CSF) and tissue (neocortex and hippocampus) samples were stored in a freezer at −20 °C and analyzed.

2.6. Data analysis

2.6.1. In vitro using micro-electric array (MEA)

Data analysis was performed using AxIs software (Axion Biosystems Inc.) and GraphPad Prism (version 7.00; GraphPad Software Inc., San Diego, CA). Active electrodes, AEs, (16 electrodes per well) were defined as an electrode having an average of > 10 spikes/min (Wallace et al., 2015) and an active well as a well containing > 50% AEs. All wells below this threshold were discarded based on these quality criteria. The threshold for the spike detection was ≥6× the standard deviation of the rms (root mean square) noise. Statistical analysis consisted of expressing the treatment ratio of exposed wells (percentage change between the baseline and the treatment) normalized to the treatment ratio of 100% in control experiments. Normalized treatment ratios of n = 8 wells were averaged per condition. Each well of the MEA served as its own control, and the changes in electrical activity elicited by the treatments were expressed as percent of control activity and normalized to the wells treated with the vehicle control DMSO. The final concentration of DMSO added to each well was 0.1% (1 μl/ml), which did not alter the pH or the ionic concentration of the medium. The heat map was based on the tolerance interval calculated from DMSO treatment (n = 160 wells) to define baseline activity ± SD at −24 to +13%. Any decrease below the DMSO standard deviation (−24%) and increase above the DMSO standard deviation (+13 to +2000%) were considered as a significant effect of the compound on the neuronal activity where we defined low increase (+13 to +25%), significant increase (+26 to 200%) and high increase (+201 to 2000%) based on picrotoxin (n = 114 wells) and acetylcholine (n = 96 wells) effects on the rat cortical neurons. Differences were determined using one-way ANOVA with Dunnett's correction; p values below 0.05 were considered significant. Data are expressed as means ± SEM.

2.7. In vivo EEG

To quantify seizure activity, we calculated EEG spiking by visually counting the spikes for over 1 min of EEG every 5 min over 60 min of recording. The seizure threshold in mg/kg and the seizure duration in seconds were calculated and reported (van der Linde et al., 2011b). Statistical significance was determined using Fisher's test and p values below 0.05 were considered significant. Values are expressed as means ± SEM.

2.8. Microarray assay and analysis

For microarray analysis, rat cortical neurons at DIV28 plated on the MEA and cells dissociated from rat cortical brain tissues were lysed using RLT buffer (Qiagen) and RNA was extracted with the RNeasy 96 kit (p/n 74,181 Qiagen). All microarray-related steps for target preparation, including the amplification of total RNA and labeling, were carried out as described in the GeneChip®3' IVT Express Kit User Manual (Affymetrix 2004). Biotin-labeled target samples were hybridized to the GeneChip® Clariom_S_Rat_HT Array containing probes for almost 20 k genes. Target hybridization was processed on the GeneTitan® Instrument according to the instructions provided in the User Guide for Expression Array Plates (P/N 702933). Images were analyzed using the GeneChip® Command Console Software (AGCC) (Affymetrix).

All microarray data were processed using the statistical computing R-program (R version 3.2.2; R Core Team, 2015) and Bioconductor tools (Gentleman et al., 2004). The gene expression values were normalized using RMA (Irizarry et al., 2003). Grouping of the individual probes into gene-specific probe sets was performed based on Entrez Gene using the metadata package (clariomdratrnentrezg, version 21.0.0) (Dai et al., 2005).

3. Results

3.1. Response of in vitro rat cortical neuron cultures using defined MEA parameters for drug-induced seizures

To study the acute effect of increasing concentrations of compounds, we analyzed the changes exhibited by the electrophysiological network activity of freshly dissociated primary cortical neurons plated onto 48-well MEA plates. All assays were performed on DIV28, after which stable electrophysiological activity and synchronization of bursts were observed in culture, indicative of a good level of connectivity amongst cells (Frega et al., 2012). Spontaneous electrical activity in neuronal networks consists of action potentials (spikes) and patterns of action potential bursts organized across time and space within the network. The MEA recordings yield meaningful information on the neuronal network activity resulting in data that can be considered high content. Many studies have often used the changes in mean firing rate as a metric for observing chemical effects, as this measure is sensitive and can be quickly extracted from data files (Shafer et al., 2008; Novellino et al., 2011; Hondebrink et al., 2016). However, multiple parameters are necessary to determine and distinguish seizurogenic potential (characterized by a partial or complete change in firing activity pattern and in network activity) from excitatory potential (*e.g.* cognitive enhancer) *in vitro*.

We used different parameters from the bursting analysis including the mean firing rate, the number of bursts, burst duration, number of spikes in bursts, the inter-burst interval (IBI) and its coefficient of variation. Other parameters were left out due to their redundancy with the parameters we selected or were used internally for quality control of the data (such as the number of active electrodes, number of bursting electrodes, etc). The network burst metric in each well was determined across time and electrodes within the MEA. Bursts in one electrode containing at least 10 spikes with a maximum inter spike interval of 100 ms were considered part of the network burst activity. The network is then defined as bursts spread over a minimum of 50% of active electrodes. This allows extraction of information on the neuronal network activity including the number of network bursts, network burst duration, number of spikes per network burst and the network IBI coefficient of variation. For synchronicity, we used the half width at half height of normalized cross-correlation. All of the parameters and their definition are listed in Table 1.

We derived a set of concentration-response curves and a heat map of activity based on different spike and burst parameters from the neuronal activity of the rat cortical cells observed after the addition of 8 seizurogenic compounds. As an example, Fig. 1A shows the heat map of activity of the seizurogenic compound **picrotoxin** on rat cortical neurons. Fig. 1 (B-G) shows the main effects on the most representative electrical activity parameters following exposure to increasing concentrations of picrotoxin, a $GABA_A$ receptor antagonist. For each concentration of picrotoxin we observed a change of pattern of activity characterized by an increase in network burst duration with an increase in spikes within the bursts and within the network bursts. Picrotoxin produced a 42% increase from baseline in mean firing rate of cortical neurons at 10 μM (Fig. 1B) and an increase in burst duration of 77% from the baseline (Fig. 1C). Both the number of bursts and the number

Table 1
MEA parameters used to identify drug-induced seizure potential *in vitro*.

Parameters	Definition	Literature
Mean firing rate	Mean firing rate weighted by the corresponding total number of active electrodes	(Frega et al., 2012; Vassallo et al., 2016)
ISI coefficient of variation	The coefficient of variation of the inter-spike intervals for all spikes in the recording. This is a measure of spike regularity.	(Nawrot et al., 2008)
Number of bursts	Total number of single-electrode bursts over the duration of the analysis	(Frega et al., 2012; Hammer et al., 2015; Vassallo et al., 2016)
Burst duration	Length of time for the average burst	(Frega et al., 2012; Hammer et al., 2015; Vassallo et al., 2016)
Number of spikes in bursts	Average number of spikes in a single-electrode burst	(Hammer et al., 2015; Avoli and Jefferys, 2016)
Inter burst interval (IBI)	Time between bursts	(Pasquale et al., 2010; Hammer et al., 2015)
IBI coefficient of variation	Variability of the inter-burst interval. This metric provides a measure of burst rhythmicity - bursts occurring at regular intervals have a small coefficient of variation, whereas sporadic bursting has a larger coefficient of variation	
Number of network bursts	Total number of network bursts over the duration of the analysis	(Pasquale et al., 2010; Frega et al., 2012; Hammer et al., 2015)
Network burst duration	Average time from the first spike to last spike in a network burst	(Pasquale et al., 2010; Hammer et al., 2015)
Number of spikes per network burst	Average number of spikes in a network burst	
Network IBI coefficient of variation	The coefficient of variation (standard deviation/average) for the inter-network burst interval, the time between network bursts. This is a measure of network burst regularity	(Pasquale et al., 2010; Hammer et al., 2015)
Half width at half height of normalized cross correlation	Synchronicity of the network	

of network bursts decreased by 34% and 46.6%, respectively, at 3 μM (Fig. 1D and E). The effect of picrotoxin seemed to be more pronounced, with the number of spikes within network bursts increasing by 154% (Fig. 1F) and thus, the network burst duration increasing by 123% from baseline (Fig. 1G). Finally, the synchronicity of the neural network was determined with the half width at half height of normalized cross-correlation, which showed a decrease of 26%, corresponding to a higher synchrony of firing neurons (Fig. 1H).

Pentylenetetrazol (PTZ), another $GABA_A$ receptor antagonist, produced a pronounced effect on the mean firing rate, which reached a maximum (129%) at 1000 μM. Similarly, the burst duration and number of network bursts of the cortical neurons showed an important increase in activity at 1000 μM (87 and 169% respectively) (Fig. 2). **Pilocarpine** (PLC), a non-selective muscarinic receptor agonist (muscarinic acetylcholine M3) showed an overall increase of general activity. The mean firing rate reached a peak at 1 μM (81%) and decreased at higher concentrations (29% at 10 μM). Similarly, the number of network bursts reached a peak at 1 μM (87%) and decreased at higher concentration (22% at 10 μM), while the burst duration at 1 μM was 14% and increased to 78% at 10 μM. On the other hand, **strychnine** (STRY), an antagonist of glycine and acetylcholine receptors, showed no changes in mean firing rate and the number of network bursts at low concentrations (0.1 to 3 μM) and showed a decrease in activity from 3 to 30 μM (Fig. 2).

Subsequently, we investigated the CNS-active compounds, amoxapine, a tetracyclic antidepressant, the tricyclic antidepressant amitriptyline, and chlorpromazine, a tricyclic antipsychotic and antagonist of dopamine receptors. Amoxapine and amitriptyline possess a wide array of pharmacological effects and are mainly reuptake inhibitors of serotonin and norepinephrine. In the measurements on the MEA, amoxapine showed no effect on the main parameters: mean firing rate, burst duration and number of network bursts (Fig. 3). On the other hand, both amitriptyline and chlorpromazine displayed a strong concentration-dependent inhibition of overall neural activity, with complete cessation of activity at 3 μM (Fig. 3).

Isoniazid, an antibiotic that may block GABA synthesis chronically (Iadarola and Gale, 1981), was also used as a reference drug. Isoniazid showed no *in vitro* changes in overall electrical activity of the primary neurons (Fig. 4).

Overall, the effect of the compounds and their potential risk for seizures is based on the number of parameters that either increase or decrease (Fig. 4). PTZ, picrotoxin, and pilocarpine would be considered as obvious seizure risk compounds. Amitriptyline and chlorpromazine with important decreases in mean firing rate and number of bursts as well as increased inter-burst interval (IBI) show a strong inhibitory effect. Strychnine has a similar but milder effect than amitriptyline and chlorpromazine. An inhibitory effect is considered a potential risk by reducing the threshold for seizures. Therefore, antiepileptics and sedatives are classified in the same category as having potential risk for seizures. Amoxapine and isoniazid didn't show any inhibitory or excitatory effect and their main parameters, MFR, number of bursts or number of network bursts remained unchanged. A few changes were observed such as a slight increase of the IBI for both compounds and a decrease in burst duration for amoxapine. But these few changes are not sufficient to determine the potential risk of amoxapine and strychnine and are thus, classified as no effect. Additional compounds were tested *in vitro* and results presented and compared to their clinical adverse effects in Supplementary data Table S1.

3.2. Spiking and seizure activity in EEG in anesthetized rat

We also conducted *in vivo* studies in anesthetized rats employing an anesthetic regime suitable for seizure research (van der Linde et al., 2011a; van der Linde et al., 2011b). Spiking in the EEG recordings was counted every 5 min for 1 min over a period of 1 h for each reference compound (Table 2). The samples for plasma analysis were taken every 5 min from all animals. Picrotoxin (0.05 mg/kg/min i.v.) and pentylenetetrazol (6 mg/kg/min i.v.) induced dose-dependent spiking in the EEG signal. Seizures were observed in all picrotoxin treated rats (n = 6) with a threshold dose of 1.57 ± 0.15 mg/kg and a seizure duration of 23.3 ± 4.5 s. Pentylenetetrazol induced seizures in five out of six rats, with a threshold dose of 174 ± 23 mg/kg and a seizure duration of 20 ± 14 s. The drug plasma level (C_{max}) at the moment of seizures (30 min) was 1.67 mM with brain levels of 2 mM at 30 min. Overall, pentylenetetrazol was well distributed in fluids and tissues collected after 60 min, with plasma and CSF levels of 2.8 mM and 2.9 mM, respectively, and levels in the hippocampus and neocortex tissue of 1.9 and 2 mM, respectively. *In vitro*, we observed the first increase in neuronal activity at a 10 μM concentration of pentylenetetrazol, a 167-fold lower concentration compared to the first seizures *in vivo*.

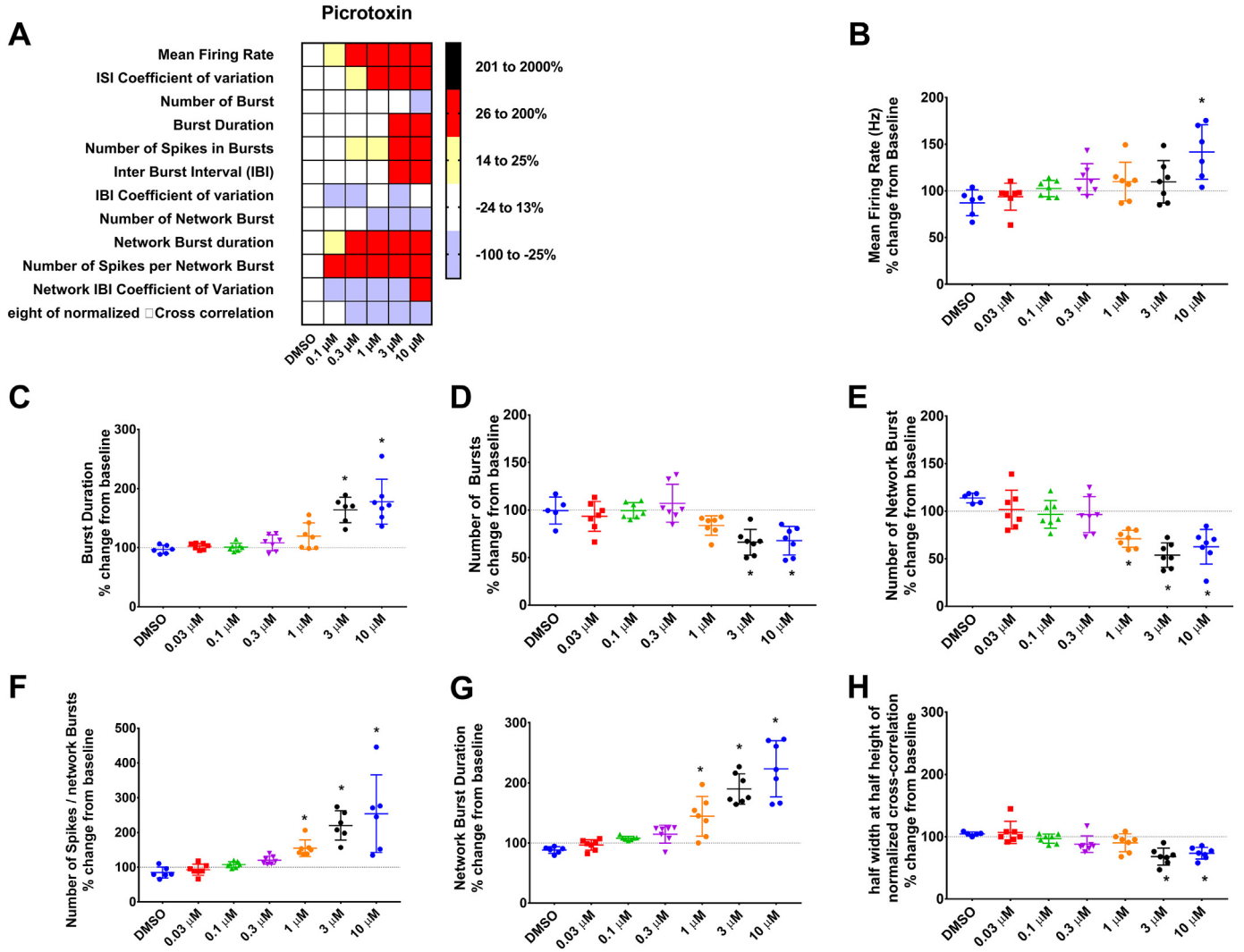

Fig. 1. Heat map, or fingerprint, activity of picrotoxin (PTX) on extracellular recordings of action potentials of a network of primary rat neurons *in vitro* by analyzing MEA parameters (Fig. 1A) Significant decrease: −100 to −24% (blue), baseline activity ± SD: −24 to 13% (white), Low increase: 13 to 25% (yellow), significant increase: 26 to 200% (red), high increase: 201 to 2000% (black). Examples of main changes in MEA parameters induced by PTX on extracellular recordings of action potentials of primary rat neurons: spike pattern (mean firing rate, burst duration and number of bursts), network (number of network bursts, number of spikes/network, and network burst duration) and synchronicity (half width at half height of normalized cross-correlation) (Fig. 1B-G). Data are expressed as mean ± SEM (n = 8). Statistical analysis performed using one-way ANOVA (Dunnett's test), *p < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In comparison, levels of picrotoxin in the plasma and brain tissues could not be determined.

Strychnine (0.04 mg/kg/min i.v.) and isoniazid (1 mg/kg/min i.v.) did not affect spiking and did not induce seizures in rats. For these 2 compounds, EEG activity was stable over the 60 min of infusion. Strychnine concentrations in plasma and CSF were 0.4 µM and 0.2 µM, respectively, but there was significant accumulation in brain tissues (2.63 µM and 2 µM in hippocampus (HPC) and neocortex (NC), respectively). The inhibitory effect of strychnine *in vitro* was observed at concentrations above 3 µM. Isoniazid was well distributed and showed high levels in plasma and CSF (414 µM and 298 µM respectively), and was also well distributed in the brain tissues (186 µM and 183 µM in HPC and NC, respectively). *In vitro*, isoniazid was tested up to 300 µM with no apparent effect.

Pilocarpine was tested at 2 different doses, 10 mg/kg/min and 0.5 mg/kg/min i.v. Pilocarpine effects on EEG were not evaluated at the high dose due to serious systemic effects consisting of blood pressure decrease, pulmonary oedema, respiratory problems and acute ischemia leading to death. No seizures were observed in the EEG prior to death. At the lower dose (0.5 mg/kg/min i.v.), no seizures were observed. Interestingly, pilocarpine showed a difference in distribution at the two doses. At the low dose pilocarpine levels in brain tissues were higher than in the CSF and plasma. At the higher dose, pilocarpine levels were elevated in CSF (3.7 mM), ~2 fold the level found in brain tissues. At both doses of pilocarpine, peak plasma levels were very low. The *in vitro* data for pilocarpine indicated a seizure risk with an overall increase in neuronal activity at 1 µM. Plasma levels in the *in vivo* assay 60 min after the 30 mg/kg dose of pilocarpine were 5.6 µM and brain levels were 107 µM, while plasma levels 60 min after the 600 mg/kg dose were 13 µM and brain concentrations were 1.4 mM.

Seizurogenic effects of amoxapine (0.02 mg/kg/min), amitriptyline (0.5 mg/kg/min) and chlorpromazine (1 mg/kg/min) were not detected *in vivo*. *In vitro* we observed inhibitory activity with amitriptyline and chlorpromazine but observed no changes with amoxapine up to 0.1 µM. The plasma, CSF and brain levels of amoxapine 60 min after a dose of 1.2 mg/kg were 0.89 µM, 0.08 µM and 4.6 µM, respectively. Plasma levels of amitriptyline 60 min following dosing were 8.4 µM, which is 280-fold greater than the threshold concentration for inhibitory effects

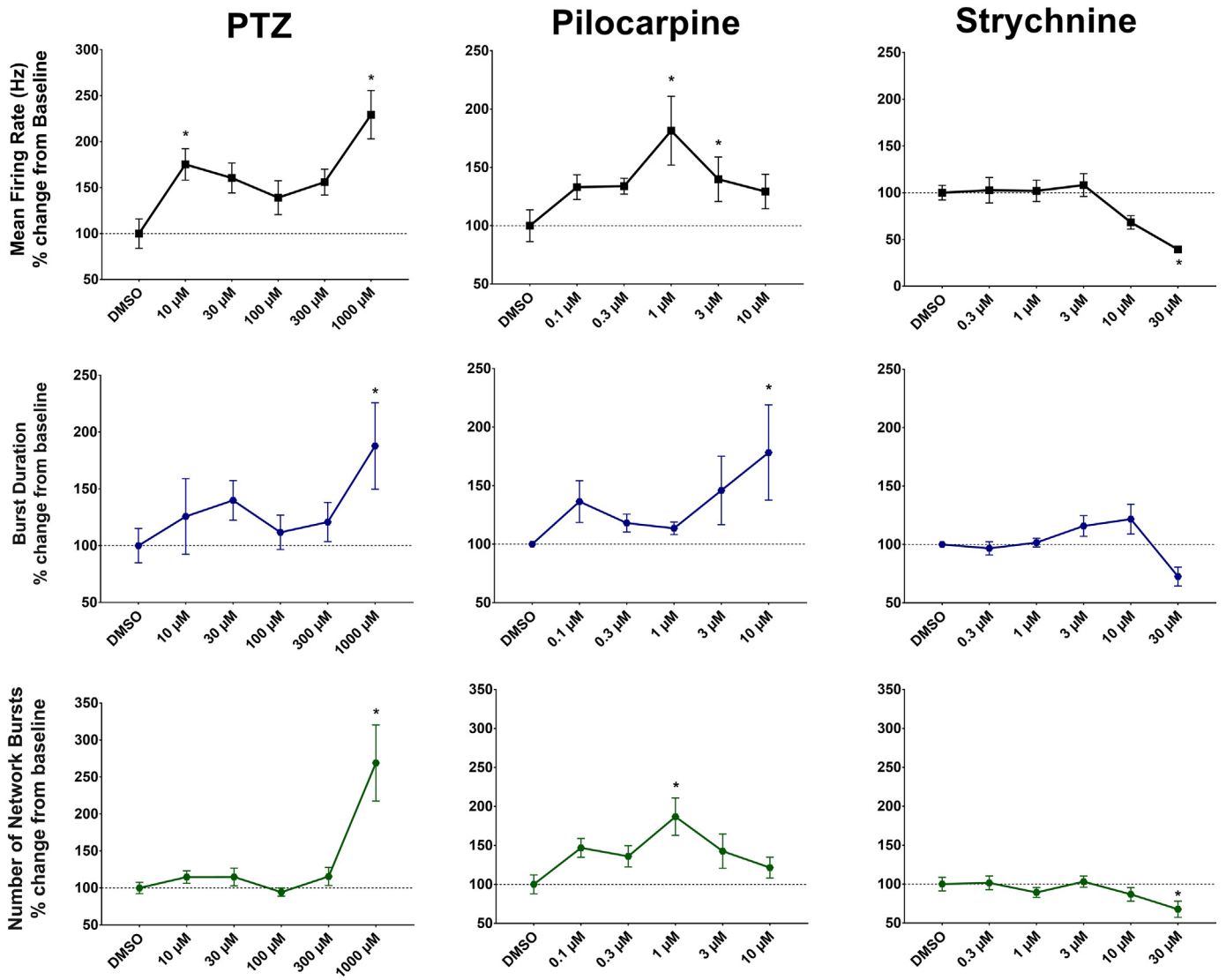

Fig. 2. Concentration-dependent effects of pentylenetetrazol (PTZ), pilocarpine and strychnine on extracellular recordings of action potentials of a network while strychnine showed no effects up to 10 µM in rat primary neurons *in vitro*. Data are expressed as mean ± SEM (n = 8). Statistical significance was determined using one-way ANOVA and Dunnett's test; *p < 0.05.

in vitro (0.03 µM); brain levels were 175 µM, which is 58-fold higher than the concentration that fully inhibits neuronal activity *in vitro* (3 µM). Chlorpromazine decreased neuronal activity *in vitro* at concentrations above 0.3 µM. Plasma levels of chlorpromazine 60 min after dosing *in vivo* were 0.2 µM, and CSF and brain levels were 0 µM and 743 µM, respectively. While the plasma levels *in vivo* are comparable to active concentrations *in vitro*, brain levels were > 248-fold higher than the chlorpromazine concentration that fully inhibited neuronal activity *in vitro* (3 µM).

3.3. Responses to different neurotransmitters in rat primary neurons

To understand and characterize the *in vitro* rat cortical neurons used, we determined the functional response to neurotransmitter receptor activation in cortical neurons using MEAs and a neurotransmitter concentration range close to rodent brain levels (Kim et al., 2014; Wojnicz et al., 2016). Concentration-dependent effects of GABA, epinephrine, serotonin, glutamate, dopamine and acetylcholine are presented in Fig. 5. GABA evoked a concentration-dependent inhibition of the mean firing rate (70% decrease in neuronal activity). Epinephrine produced an increase in neuronal activity up to 0.1 µM, followed by a lesser increase at higher concentrations (0.1 to 1 µM). Interestingly, exposure to serotonin up to 100 µM did not change neuronal activity, whereas a strong decrease (67% decrease from baseline) was observed at 300 µM, similar to the observations made in an earlier study by Hondebrink et al. (Hondebrink et al., 2016). Acetylcholine and glutamate increased neuronal activity up to the maximal concentrations tested. On the other hand, dopamine had a dual effect, with a strong increase in neuronal activity at 0.1 µM (205% increase), a slight increase at 3 µM (61% increase) and a greater increase at 10 µM (150% increase).

3.4. Gene expression levels of neurotransmitter receptors in the rat neuronal cultures and in brains of adult rats

To understand the characteristics of the neurons in the *in vitro* assay, we identified differences in neurotransmitter receptor expression between rat cortical neuronal cells after 28 days in culture and the cortical tissues taken from rats (Sprague-Dawley rats, 2 weeks old) without treatment.

The Volcano plot (Fig. 6) illustrating the significance and magnitude of differential expression for all measured genes, shows relative

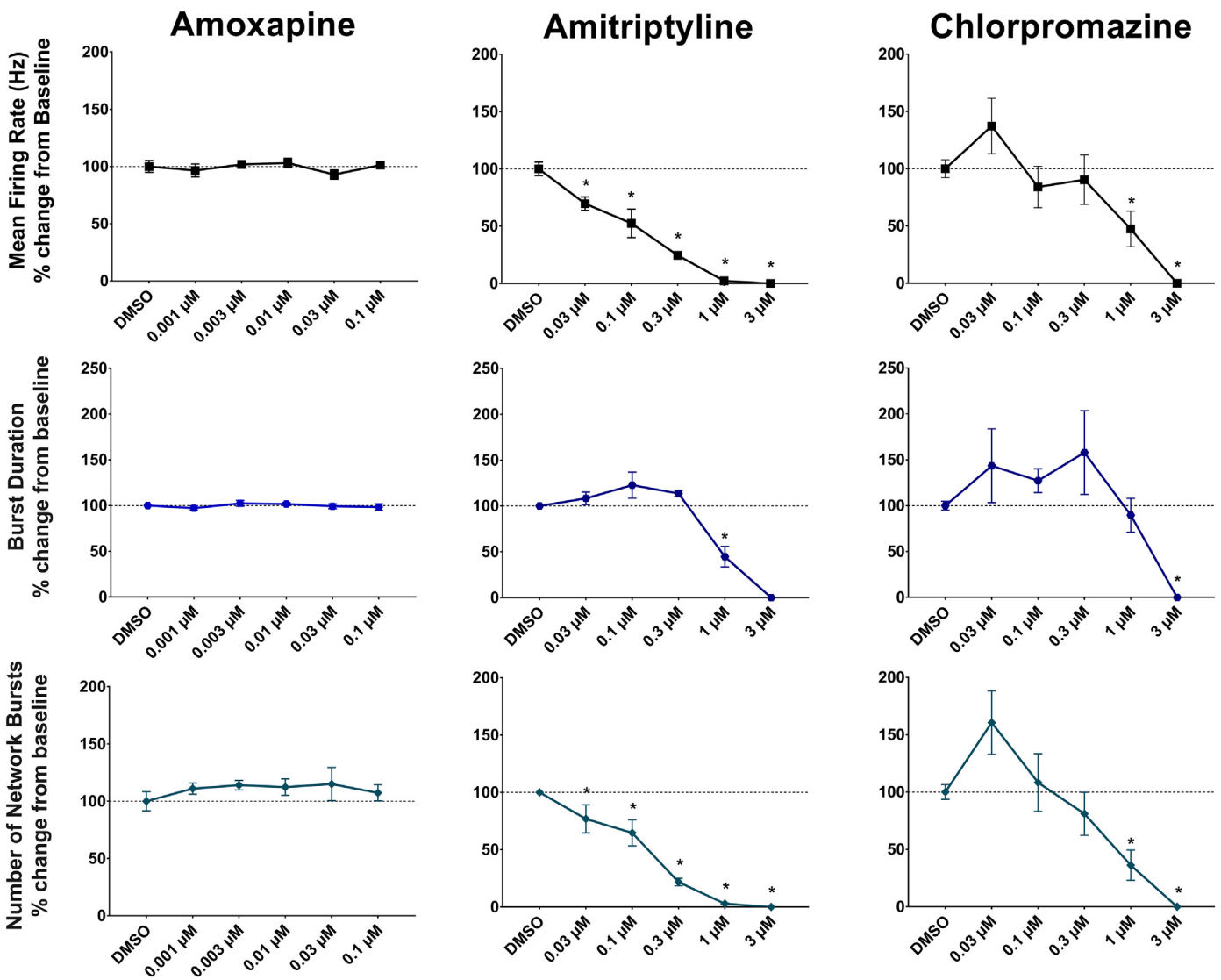

Fig. 3. Concentration-dependent effects of amoxapine, amitriptyline and chlorpromazine on the MEA electrical activity in rat cortical neurons *in vitro*. Data are expressed as mean ± SEM (n = 8). Statistical significance was determined using one-way ANOVA and Dunnett's test; *p < 0.05.

differences (increases or decreases) between cell or tissue expression of significantly regulated genes. Top genes based on significance, differential expression and their relation to seizures are highlighted in red. The rat cortical neurons from the *in vitro* system show a lower expression of mRNA for serotonin receptor proteins, *e.g.* Htr1a, Htr2a and Htr2c, compared to brain tissue samples. The functional assay with serotonin neurotransmitter showed no effect on the neuronal activity measured with MEA up to 100 µM. NMDA type subunits 1 and 2A, kainate type subunit 1, muscarinic acetylcholine receptors M1 and M3 and $GABA_A$ and $GABA_B$ receptors, appear to be less expressed as well. Nonetheless, in the functional assay the rat cortical neurons did respond to most neurotransmitters tested.

4. Discussion

In the present study, we demonstrated the utility MEA recordings in rat primary neurons for detecting potential drug-induced seizure risk *in vitro*. Moreover, we showed that this *in vitro* MEA assay with rat primary neurons may have advantages over an *in vivo* rat model both in terms of better throughput and sensitivity. In addition, we were able to identify potential mechanisms for the discordances between rat assays and human seizure risk for certain seizurogenic drugs.

4.1. In vivo/in vitro correlation for drug-induced seizures

Anesthetized rats were used for detection of drug-induced seizure risk to compare with the *in vitro* model findings (same animal species). Previous studies have also demonstrated the utility of an *in vitro* assay using rat hippocampal slice preparations to detect drug-induced seizures. Both models focus on the induction of seizure activity and burst suppression rather than on convulsions or tremors. The correlation between the *in vivo* anesthetized rat results and the *in vitro* MEA assay with rat cortical neurons using different reference compounds known to induce seizures in man and animals is summarized in Table 3 together with data collected from different studies on the *in vitro* assay with the rat hippocampal slice preparation. The $GABA_A$ receptor antagonists, picrotoxin and pentylenetetrazol, produced an increase in overall neuronal activity in cultured rat cortical neurons and seizures in anesthetized rats. While the picrotoxin plasma and brain levels could not be measured, the *in vitro* MEA assay showed a statistically significant effect at 3 µM. In comparison, effects of picrotoxin in hippocampal slices occurred at a concentration range of 5–300 µM (Corrigall and Linseman, 1980; Ameri et al., 1996; Easter et al., 2007), which is higher than that producing effects in rat cortical neurons. Plasma and brain pentylenetetrazol levels at the time of seizure observation *in vivo* were

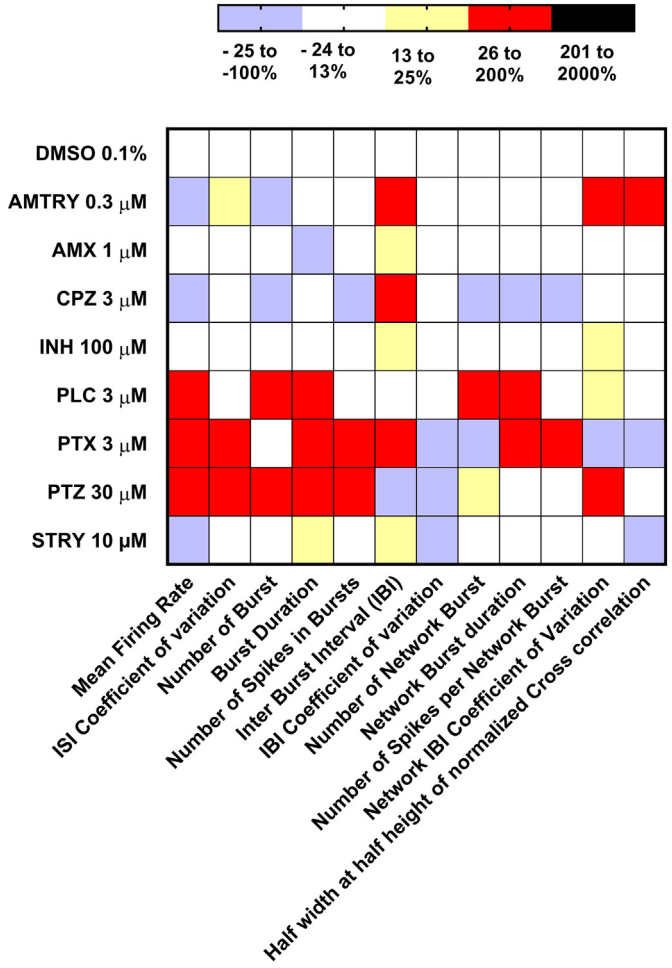

Fig. 4. Summary of the Heat map activity of 8 seizurogenic drugs (around their free Cmax or E/IC_{50} concentration) on extracellular recordings of action potentials of a network of primary rat neurons by analyzing a small and defined set of 12 MEA parameters. Significant decrease: −100 to −24% (blue), baseline activity ± SD: −24 to 13% (white), Low increase: 13 to 25% (yellow), significant increase: 26 to 200% (red), high increase: 201 to 2000% (black). AMTRY: amitriptyline; AMX: amoxapine; CPZ: chlorpromazine; INH: isoniazid; PLC: pilocarpine; PTX: picrotoxin; PTZ: pentylenetetrazol; STRY: strychnine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

relatively high (1.67 mM and 2 mM, respectively). The *in vitro* assay was more sensitive, with an increase in mean firing rate at 10 μM up to 1 mM, and a clear increase of the number of network bursts starting at 1 mM. Pentylenetetrazol induced a clear effect in hippocampal slices at 2–10 mM, concentrations that are closer to those observed *in vivo* (Rostampour et al., 2002; Easter et al., 2007; Accardi et al., 2017). In our *in vitro* assay, higher concentrations of pentylenetetrazol were not tested.

Pilocarpine did not induce seizures in the anesthetized rats at low doses (30 mg/kg/h) but at the high dose it induced death in all animals (600 mg/kg/h). Our *in vivo* findings with pilocarpine are similar to previously published data (Curia et al., 2008) reporting significant changes in respiration, heart rate and blood pressure leading to death within the first minutes of infusion. As such, the seizure risk of pilocarpine is difficult to detect in this *in vivo* model due to a narrow safety window and pronounced M1 muscarinic receptor activation when administered intravenously. Brain levels of pilocarpine were 107 μM and 1.4 mM following 30 mg/kg/h and 600 mg/kg/h, respectively. *In vitro*, statistically significant effects were observed at 1 μM in the MEA assay and a study in hippocampal slices reported seizure induction at 1–10 μM (Nagao et al., 1996; Priel and Albuquerque, 2002). These pilocarpine concentrations in hippocampal slices are in good agreement with those obtained in our *in vitro* assay.

The CNS-active compounds, amoxapine, amitriptyline and chlorpromazine, did not produce seizures *in vivo*, while only chlorpromazine caused a slight increase of the number spikes in the EEG. Although amoxapine had no effect *in vitro*, amitriptyline and chlorpromazine produced 100% inhibition of neuronal firing *in vitro* at 3 μM in the MEA assay. Brain concentrations of amitriptyline and chlorpromazine associated with lack of seizure activity *in vivo* were 58- and 248-fold, respectively, the concentrations that were active in the MEA assay *in vitro*. Chlorpromazine has been shown to induce seizures in rat hippocampal slices at concentrations between 10 and 100 μM (Easter et al., 2007). Thus these findings represent apparent discordance between *in vitro* and *in vivo* activities for amitriptyline and chlorpromazine.

Strychnine also showed no changes in activity *in vivo* while *in vitro* showed an inhibition at high concentrations (> 3 μM). Strychnine is known to induce violent generalized muscle spasm and convulsions in humans and rodents by blocking the physiological inhibitory action of glycine by a non-competitive action (Curtis and Johnston, 1974; Marvizon et al., 1986). Because the convulsions induced by strychnine do not originate from the brain but rather from the spinal cord, strychnine seizures are not considered epileptiform (Towe et al., 1981) and are not always observed in the EEG (Somjen, 2004).

Finally, isoniazid is an example of a drug acting mainly *via* its metabolites and as such demonstrates delayed effects *in vivo*. In rodents, Preziosi showed that isoniazid formed mainly acetylated active metabolites, leading to a chronic effect of isoniazid (Preziosi, 2007). This mechanism that is dependent on drug metabolism is consistent with lack of detection of isoniazid effect in both acute studies *in vivo* in rats, where there would not be sufficient time to generate active metabolites, and in the *in vitro* MEA model, where metabolism is absent. However, some studies (Carta et al., 2008; Easter et al., 2009) using a rat cerebellar slice preparation have shown the potential of isoniazid to cause a significant decrease of GABA concentration leading to the potential for seizure activity.

4.2. Potential mechanisms for drug-induced seizures

Drugs can directly or indirectly affect neuronal activities (decreases or increases) in the central nervous system, which may cause seizures by disturbing the delicate balance between neuronal excitation and inhibition. Many different cellular or biochemical changes induced by altering ion channel function and/or neurotransmitter level/receptor function can mediate drug-induced seizures, as discussed in the current study (Fig. 7).

4.3. GABA receptor antagonists

Drug-induced inhibition of GABA receptors are one of the most common mechanisms producing acute seizures. Many antagonists of GABA receptors have been shown to cause epileptiform events in neocortical slices (Gutnick et al., 1982), in hippocampal slices (Miles and Wong, 1983), as well as in re-aggregated hippocampal neurons on the MEA (Colombi et al., 2013). In our *in vitro* assay with rat cortical neurons, picrotoxin and pentylenetetrazol (both known to inhibit $GABA_{A/B}$ receptors) had a distinct fingerprint of activity corresponding to an increase in the firing rate and in the synchronicity of network bursting (Fig. 2 and Fig. 3). There was abundant expression of $GABA_{A/B}$ receptors in rat cortical neurons, which correlates with the inhibition of the neuronal activity observed when γ-aminobutyric acid (GABA) was added to the cell culture (Figs. 5 and 7).

4.4. Glycine receptor antagonist

Strychnine is a toxin known to cause seizures in man (Edmunds

Table 2
EEG spiking at baseline and just before seizure induction (Max), and drug concentration in plasma and brain in anesthetized rats.

In vivo anesthetized rat											In vitro rat cortical neurons
Compounds	Dose mg/kg/min	n	Spikes on EEG		Seizures (n)		Plasma Cmax 60 min µM	CSF 60 min µM	HPC 60 min µM	NC 60 min µM	(Drug concentration at effects)
			Baseline n/min	Max n/min	Threshold mg/kg	Durations sec					
Vehicle	0.1	19	1 ± 1	0 ± 1	–	–	–	–	–	–	–
Picrotoxin	0.05	6	1 ± 1	31 ± 5*	1.57 ± 0.2 (6/6)	23.3 ± 4.5 (6/6)	*n.d.*	*n.d.*	*n.d.*	*n.d.*	Excitatory (> 1 µM)
Strychnine	0.04	6	1 ± 1	1 ± 1	–	–	0.4	0.2	2.63	2	(−) (< 3 µM)/inhibitory (> 3 µM)
Pilocarpine	0.5	6	1 ± 1	3 ± 2	–	–	5.6	59	121	107	Excitatory (1 µM)
Pilocarpine	10	6	*n.d.*	*n.d.*	*n.d.*	*n.d.*	13	3700	1910	1400	Excitatory (1 µM)
Chlorpromazine	1	6	1 ± 1	10 ± 2*	–	–	0.2	0	760	743	Inhibitory (0.3 µM)
Pentylenetetrazol	6	6	1 ± 1	52 ± 4*	174 ± 23 (5/6)	20 ± 14 (5/6)	2837	2890	1961	2055	Excitatory (10 µM)
Isoniazid	1	6	2 ± 1	1 ± 1	–	–	414	298	186	183	(−) (up to 300 µM)
Amitriptyline	0.5	6	1 ± 1	2 ± 2	–	–	8.4	0.25	189	175	Inhibitory (0.03 µM)
Amoxapine	0.02	6	2 ± 1	1 ± 1	–	–	0.89	0.08	4.4	4.6	(−) (up to 3 µM)

Threshold dose and duration of seizures are calculated for all drugs. Drug concentrations in brain tissues: neocortex (NC) and Hippocampus (HPC) and in CSF and plasma are also presented. Values are mean ± sem. Statistical significance was determined using Fisher's test; n.d., not determined.
* $p < 0.05$.

et al., 1986; Hernandez et al., 1998) *via* glycine receptor (GlyR) inhibition. It did not produce an increase in activity *in vitro* at low concentrations but inhibited neuronal activity at concentrations above 10 µM, likely due to another mechanism, *i.e.* the inhibition of cholinergic receptors. To understand the lack of excitatory effect of strychnine, we performed a gene expression analysis. The data from the rat primary neurons clearly demonstrated expression of the glycine receptor subunits GLRA2 and GLRB. However, a single amino acid difference in GLRA2 subunit, from glycine in the rat and in human, to glutamate in the neonatal rat cortical neurons (Becker et al., 1988) leads to lower affinity of strychnine for glycine receptors in neonatal rat cortical neurons (Becker et al., 1988; Kuhse et al., 1990; Schmieden et al., 1992), which might explain the lack of *in vitro* effects of strychnine in the current study.

4.5. Multiple receptors involved in seizures

Antipsychotic or neuroleptic drugs such as chlorpromazine are known to lower the seizure threshold and induce spike-wave discharge pattern in the human EEG (Torta and Monaco, 2002). Chlorpromazine is an antagonist of postsynaptic and presynaptic receptors for dopamine (Kapur and Seeman, 2002; Seeman, 2002), but is also an antagonist of 5-HT receptors, histamine H1 receptors, α1/α2 adrenergic receptors and M1/M2 muscarinic acetylcholine receptors (Cosi and Koek, 2001; Cahir and King, 2005). Chlorpromazine induced a transient increase of neuronal activity (Supplementary data S1) that drops significantly after 60 min incubation. Van Zessen et al. suggested that chlorpromazine creates an imbalance in dopaminergic-cholinergic activity leading to a lower seizurogenic threshold in the brain due to suppression of dopaminergic neuronal activity by GABA activation (van Zessen et al., 2012). Chlorpromazine showed a strong inhibition of the electrical activity of rat cortical neurons recorded on the MEA. Overall, in anesthetized rats, chlorpromazine slowly increased the number of spikes after 1 h (10 ± 2 spikes per minute) but did not induce seizures, even though the plasma exposure level was 6.3 µg/ml (12- to 210-fold the therapeutic blood plasma level) after 1-hour infusion at 60 mg/kg/h. Clinical data show that chlorpromazine is associated with a high incidence of seizures in humans (4.35%).

We also tested two tricyclic antidepressants, amoxapine, a derivative of an antipsychotic agent (loxapine) with high incidence of seizures in humans (8.44%) and amitriptyline, known to have a lower incidence of seizure in humans (2.82%). Both molecules share similar targets, including the sodium-dependent serotonin and noradrenaline transporters (Tatsumi et al., 1997) as well as histamine H1 receptor, dopaminergic receptors, adrenergic receptors and serotonergic receptors (Richelson and Nelson, 1984; Cusack et al., 1994). *In vivo*, no effect was observed at therapeutic doses of amoxapine and amitriptyline. At a higher dose level (30 mg/kg/h), seizures could be induced in 50% of the rats with amoxapine (Table 3). *In vitro*, amoxapine had no effect, while amitriptyline showed a very pronounced decrease in neuronal activity. This difference between the two antidepressants results from the low expression level of serotonin receptors and the presence of muscarinic acetylcholine receptors, confirmed by the effect of agonists of both serotonin and acetylcholine receptors (Fig. 7). Amitriptyline inhibits muscarinic receptors (McKinney et al., 1988), inducing a decrease of neuronal activity. Moreover, amoxapine has a high affinity for D2 receptors but only *via* its metabolite, 7-OH-amoxapine (Calvo et al., 1985), which is not produced *in vitro*. Lower gene expression of serotonin receptor subtypes, 5HT1, 5HT2 and 5HT3 (as well as 5HT6) were observed in our gene expression data. Moreover, the response to the neurotransmitter serotonin showed no effect up to 100 µM and at higher concentrations (300 µM) showed a strong decrease of neuronal activity that may relate to non-specific effects of the neurotransmitter. The low gene expression of 5HT receptors and the lack of serotonin receptor function in the *in vitro* model would explain the inability to detect the direct or indirect effect of certain compounds like amoxapine that induce seizure-like activities *in vitro*.

4.6. Other possible mechanisms

Finally, we used isoniazid, an antibiotic that decreases GABA synthesis and is known to induce peripheral neuropathy in humans after chronic administration and seizures after overdose (Romero and Kuczler Jr., 1998). In our assay, isoniazid showed no adverse effect *in vitro* or *in vivo*. Isoniazid acts mainly *via* its metabolites, including the very toxic metabolites hydrazine, acetyl-diazine and NH_3 (Ramappa and Aithal, 2013). The mechanisms by which isoniazid induces seizures are not fully understood. The ammonia released by isoniazid can be directly linked to the seizurogenic propensity of isoniazid (Preziosi, 2007). Indeed, the NH_3, originating from the hydrazine that is released by CYP-dependent oxidative scission of isoniazid, can be responsible for seizure induction. Other studies have shown that the effect of isoniazid

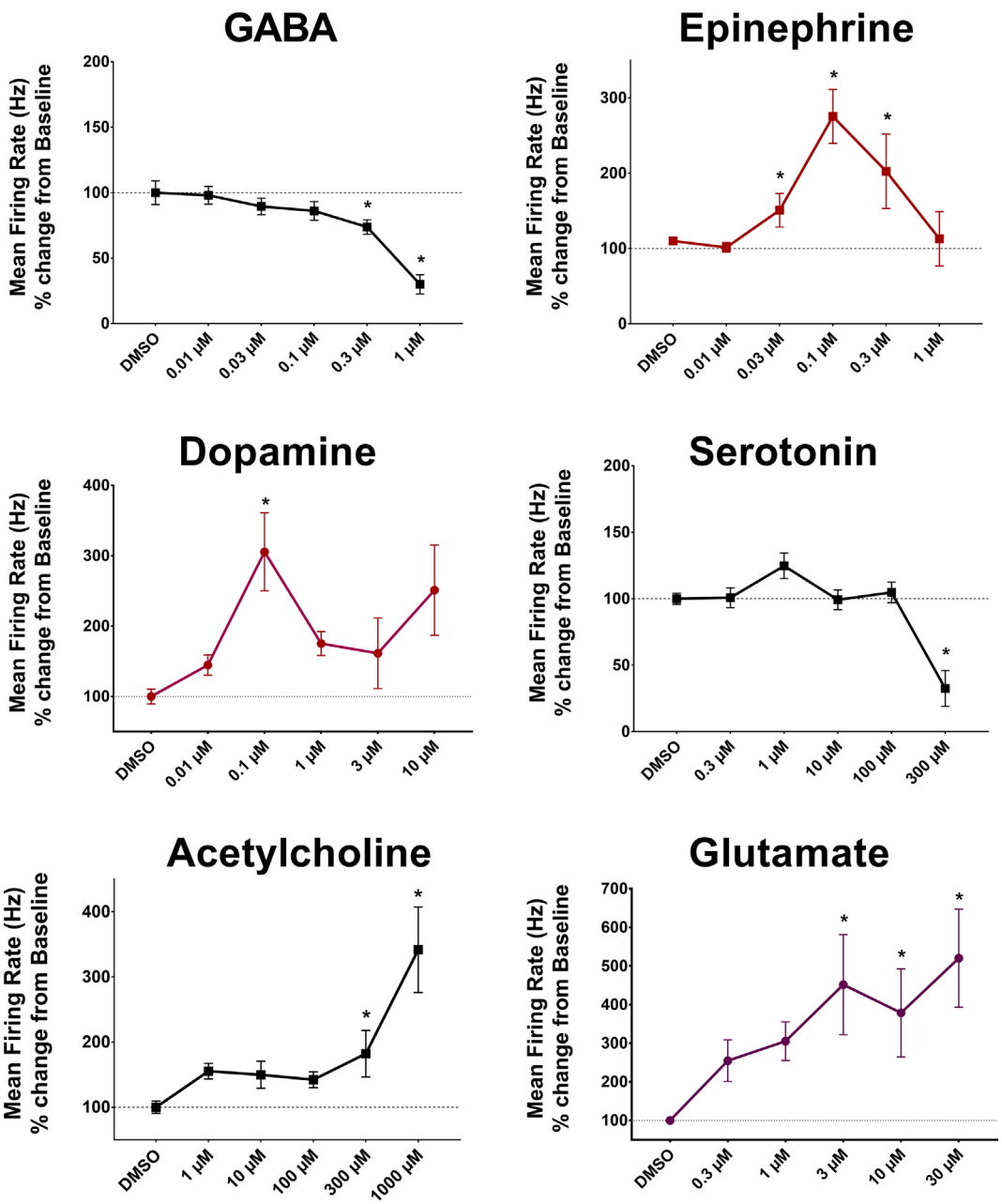

Fig. 5. Effects of neurotransmitters GABA, epinephrine, serotonin, acetylcholine, dopamine and glutamate on neuronal activity of rat cortical neurons measured with MEA. Data are expressed as mean ± SEM (n = 8). Statistical significance was determined using one-way ANOVA and Dunnett's test. *p < 0.05. Data in which the maximum effect (transient effect) was induced were used for analysis.

on the central nervous system is associated with its effect on pyridoxine (vitamin B6) homeostasis since isoniazid condenses with pyridoxine to form hydrazine, thereby depleting pyridoxine. The seizures observed after administration of isoniazid or its main metabolite hydrazine are similar to those caused by pyridoxine deficiency (Horton et al., 1979; Tong, 2014). Isoniazid pathology is very complex with possibly more reactions, either acute or chronic, which can lead to seizures and other neurotoxicological damage (Preziosi, 2007).

5. Limitations

Although the MEA assay appears to be reliable and sensitive for detection of compound effects, we identified some limitations of this *in vitro* assay. For instance, drug-induced seizures *via* metabolite formation or caused by chronic treatment (delayed onset) (*e.g.* isoniazid) are not detectable. Our assay correctly detected the effect of different compounds known to induce seizures in the clinic with some exceptions, *e.g.* amoxapine, due to lack of gene expression and functional effects of serotonin receptors in rat primary neurons. The latter highlights a species difference between animal and human and as such, a human-based assay such as human induced pluripotent stem cell derived neurons (hiPSC-neurons) may be better for prediction and screening of drug-induced seizure liability in humans in the future. Furthermore, the dissociated rat cortical neurons present a neonatal phenotype which differs from adult phenotype (Becker et al., 1988; Schmieden et al., 1992). For example, the glycine receptor shows a clear difference between neonatal and adult rat neurons, leading to a change in pharmacology of the glycine receptor.

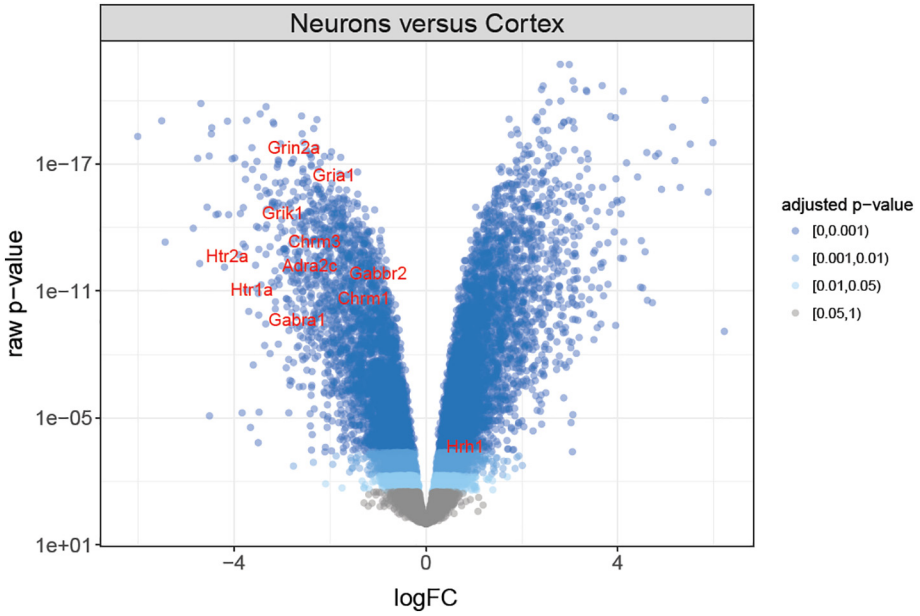

Fig. 6. Volcano plot of the differential expression of genes between the rat cortical neurons after 28 days in *in vitro* culture ("Neurons") *versus* the rat brain tissue ("Cortex") with indication of specific seizure genes with raw p–value < 0.001. The significance and magnitude are calculated as described in Materials and methods.

Table 3
Comparison between the rat models *in vivo*, the rat cortical neurons cultured on MEAs with published *in vitro* brain slice data and clinical seizure/convulsion risk in man.

	Clinical seizure risk (VigiAccess, 2017) *Free plasma level (μM)*	Published In vitro Brain slices *Concentration range (μM)*	In vivo anesthetized rat	In vitro rat cortical neurons
Vehicle/DMSO	–	–	(−)	(−)
Picrotoxin	NA –	Excitatory[a] *5–300*	Seizures	Excitatory
Strychnine	NA –	Excitatory[b] –	(−)	(−)
Pilocarpine	0.4% *0.03–0.55*	Excitatory[c] *1–10*	(−)/death (high dose)	Excitatory
Chlorpromazine	4.35% *0.05–0.15*	Excitatory[d] *0.3–100*	(−)	Inhibitory
Pentylenetetrazol	NA –	Excitatory[e] *2000–10,000*	Seizures	Excitatory
Isoniazid	1.05% *3.5–35*	Excitatory[f] *10,000*	(−)	(−)
Amitriptyline	2.82% *0.2–0.7*	–	(−)	Inhibitory
Amoxapine	8.44% *0.04–0.6*	–	(−)	(−)

(−): no incidence *in vivo* or no potential seizure risks *in vitro*; NA: not available; Incidence of seizure in man is extracted from the WHO database for adverse drug reactions, VigiBase®, from 1968 to 2017. a. (Corrigall and Linseman, 1980; Ameri et al., 1996; Easter et al., 2007); b. (Easter et al., 2009); c. (Nagao et al., 1996; Priel and Albuquerque, 2002); d. (Easter et al., 2007); e. (Rostampour et al., 2002; Easter et al., 2007; Accardi et al., 2017); f. (Carta et al., 2008; Easter et al., 2009).
The number in italics correspond to the free plasma level in clinical seizure risk column and the concentration range in Published in vitro brain slices.

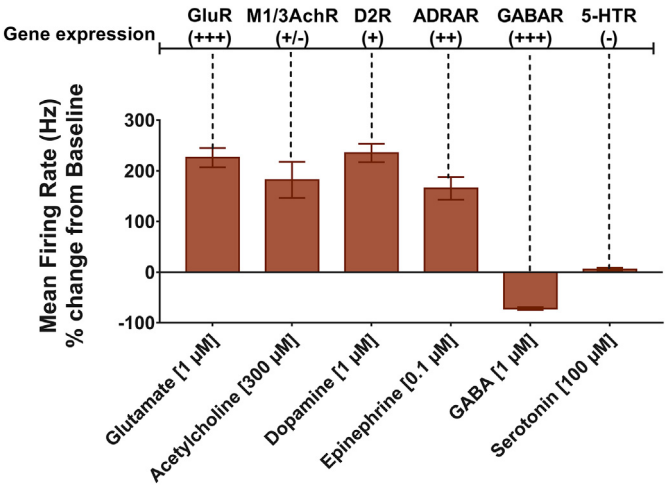

Fig. 7. Correlation between functional effects of key neurotransmitters and their respective receptor gene expression levels, that are most likely associated with mechanisms for different seizurogenic drugs according to literature. (+ + +) genes that are well expressed in the rat cortical neurons in culture (> 8 $\log^2$ intensity); (+) genes that are downregulated but well expressed; (+/−) genes that are downregulated with a lower expression level (> 5 $\log^2$ intensity); (−) genes that are downregulated and not expressed or have very low expression level (< 5 $\log^2$ intensity).

6. Conclusions

In summary, our data show that rat cortical neuronal cells coupled to MEA platforms are a suitable tool for identifying seizure liability. By combining functional assays with gene expression data, we identified the possible mechanisms of investigated drugs that provide a basis for differences *in vivo* and *in vitro* results and clinical data. Overall, we have demonstrated that this *in vitro* assay approach has good potential translational value in detecting seizurogenic drugs early in drug development.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.taap.2018.03.028.

Conflict of interest

All authors are employees of Janssen Pharmaceutica N.V. The authors declare having no conflict of interest.

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