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Preincubation with antipsychotic drugs protects against *in vitro* phencyclidine-mediated spontaneous neuronal network suppression

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ABSTRACT

Phencyclidine (PCP), a non-competitive N-methyl-D-aspartate receptor antagonist, is known to produce schizophrenia-like psychosis in humans, including positive and negative symptoms as well as cognitive dysfunction. Moreover, acute administration of PCP can emulate corresponding behavioral symptoms in rodents.

We investigated the effect of PCP and the possible protective potential of typical and atypical antipsychotic drugs (APDs) *in vitro* on spontaneously active neuronal networks. To this end, murine primary cortical cells were cultured on microelectrode arrays (MEAs). Concentration-response curves of PCP ranging from 0.01 to 200 μM were generated and network spike and burst rate as well as burst peak firing rate (PFR) and burst duration was measured in stable 2-min recordings. Measurements were done with and without pre-incubation with the APDs aripiprazole, clozapine, and haloperidol.

We found a concentration-dependent network activity suppression reflected by a decrease in captured spike rate and network PFR upon PCP application relative to baseline. Preexposure with any of the three APDs mediated a right-shift of the PCP concentration-response curve (spike rate, PFR). However, as assessed by their IC_{50} values and Hill coefficients, the atypical APDs aripiprazole and clozapine exhibited a 20- to 30-fold protective potency—higher than the typical APD haloperidol (6-fold).

In summary, a disruptive network effect of PCP as well as a protection by APDs could be demonstrated in the order of potency: clozapine > aripiprazole ≫ haloperidol. We propose this simple, noninvasive setup as a plausible electrophysiological model for testing current and future pharmaceuticals against schizophrenia-spectrum disorders.

1. Introduction

Schizophrenia is a neurodevelopmental psychiatric disorder with a prevalence of 0.5–1 % according to mixed population data. It usually takes a chronic course and can include positive or negative symptoms (Marder and Cannon, 2019; Saha et al., 2005). Antipsychotic drug (APD) treatment is the main therapeutic strategy. Typical APDs can lead to extrapyramidal symptoms due to strong dopamine D_2 receptor (D_2R) affinity (Meltzer, 2013; Meltzer and Huang, 2008). This effect is lesser with atypical APDs due to a different receptor affinity profile involving (among others) serotonin receptor 2A ($5\text{-}HT_{2A}R$) antagonism such as in clozapine and olanzapine or partial D_2R agonism such as in aripiprazole (Meltzer and Huang, 2008; Marder and Cannon, 2019). However, for atypical APDs, a multireceptor action is likely (see below).

Due to the major role of a hypofunction of glutamatergic *N*-methyl-*D*-aspartate receptor (NMDAR) neurotransmission in the pathophysiology of schizophrenia, the non-competitive NMDAR antagonist phencyclidine (PCP) has been established as a pre-clinical model of this disease (for a detailed review see (Li et al., 2024)). Acute administration of a single PCP concentration prior to behavioral experiments particularly yields and reliably models negative and dyscognitive symptoms in rodents, e. g. social withdrawal (Savolainen et al., 2019; Corbett et al.,

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1995) and anhedonia (Baird et al., 2008), as well as deficits in working memory (Moghaddam and Adams, 1998) and inhibitory control (Thomson et al., 2011) as examples of executive dysfunction. The main pathway by which PCP induces schizophrenia-like symptoms is proclaimed as hippocampal to prefrontal (Jodo, 2013) or thalamocortical (Celada et al., 2013) via NMDAR blocking (Marino and Conn, 2002; Goff and Coyle, 2001). Furthermore, influence on the expression of dopamine and 5-HT receptors (Choi et al., 2009), induction of dopamine and glutamate efflux in the prefrontal cortex (Adams and Moghaddam, 1998), increased noradrenaline in the prefrontal cortex (Kehr et al., 2018) and serotonergic effects of PCP are discussed (Maurel-Remy et al., 1995; Meltzer et al., 2011; Oyamada et al., 2015; Huang et al., 2018; Kehr et al., 2018). Recently, PCP promoted a reduced expression of NMDAR subunits in a mouse model (Dutra-Tavares et al., 2023).

Several studies have tried to elucidate the mechanism of action of APD-induced rescue of such PCP effects in electrophysiological and behavioral readouts. Evidence suggests antagonism at the D_2R and even more important at the D_1R (Riga et al., 2023; Llado-Pelfort et al., 2016; Jardemark et al., 2010) and furthermore at the 5-$HT_{2A}R$ (Rajagopal et al., 2022) and noradrenergic $\alpha_{2C}R$ (Savolainen et al., 2019), as well as 5-$HT_{1A}R$ agonism (Llado-Pelfort et al., 2016). Oyamada et al. (2015) report a combined 5-$HT_{1A}R$ agonism and 5-$HT_{2A}R$ and D_2R antagonism to reproduce the rescuing effect of atypical APD in a PCP-impaired novel object recognition mouse model (Oyamada et al., 2015).

NMDARs are tetramers and different splicing variants are known, which can lead to heterogenic ion channels. The receptor gets activated by binding of glutamate and glycine and is not selectively permeable for cations with a focus on calcium. Further, it is not permeable to magnesium which can block the channel and lead to a voltage dependence. PCP is an open channel blocker (non-competitive antagonist) and its binding site overlaps the magnesium site thereby de-blocking the receptor (Bresink et al., 1995; Marino and Conn, 2002).

Cortical neurons grown on microelectrode arrays (MEAs) are a stable neuronal network model and permit extracellular recording of synchronized spontaneous neuronal network activity (SNNA) depending on receptor presence and synaptic function (Chiappalone et al., 2006; Johnstone et al., 2010). MEAs can be used as biosensors to simulate pathological neuronal activity or to analyze drug effects (Görtz et al., 2019; Hondebrink et al., 2016; Dzyubenko et al., 2017).

In the present study, we aimed to investigate (1) electrophysiological effects of PCP on primary cortical networks grown on MEAs as a possible *in vitro* model of schizophrenia focusing on spike and burst activity and (2) if/how typical and atypical APDs may differentially influence these effects.

2. Materials and methods

2.1. Cortical cell culture, drugs and experimental design

Primary cortices were taken from newborn C57BL/6 mice (breeding stock of the Central Institution for Animal Research and Scientific Animal Welfare (ZETT) of the Heinrich Heine University of Düsseldorf, Germany, number O 50/05; procedures approved by Heinrich Heine University of Düsseldorf/State of North Rhine Westphalia animal care committees). We collected cortical tissue (including neurons and astrocytes) for our experiments from four different mouse cortex preparations (i.e. tissue from cortices of at least four different P0 animals). From each preparation, we obtained several networks of 1×10^5 neurons that we cultured separately on different MEAs, with neurons plated on the MEA electrode field in droplets at a density of ~1500 cells/mm^2. Cells were cultured and suspended in nutrient medium consisting of fetal calf serum (10 %), MEM (Eagle, 89 %), glucose (0.8 %), glutamine (2 mM), insulin (0.1 U/ml), and HEPES (10 mM). After seeding, medium was filled up to 1.5 mL Neurobasal medium containing antibiotics, non-essential amino-acids, L-glutamine, and B27 (Life. Technologies, Darmstadt, Germany) as described previously (Görtz et al., 2019).

Stable synchronized burst activity on MEAs was reached around 10–12 days *in vitro* (DIV), and experiments were performed at 14–21 DIV. Networks exhibited comparable and stable synchronous firing patterns at baseline regardless of culture age. We maintained a pH of 7.4 by a constant stream of humidified 95 % air/5 % CO_2 at a flow of 5–10 mL/min. Procedures were standardized by recently defined quality criteria (Vassallo et al., 2017).

For resource-efficient testing of psychotropic substances on MEAs, we employed a cumulative design: we applied increasing concentrations of the same drug to each individual network minimizing within-drug variability (Colombi et al., 2013; Gramowski et al., 2006; Johnstone et al., 2010; Wu et al., 2014).

After 2-min baseline recordings, we preincubated cultured networks for 10 min with either 1 μM aripiprazole (Bristol-Myers-Squibb, Munich, Germany), 1 μM clozapine (Novartis Pharma, Nürnberg, Germany) or 0.75 μM haloperidol (Janssen-Cilag, Neuss, Germany) (stock solutions were made by dissolving APDs in 25 % ethanol and further diluted to final concentrations with PBS), followed by stepwise incubation with 0.01–200 μM of PCP. To exclude any possible sustained alteration of network function following drug exposure, we took care not to reuse neuronal networks after exposure to PCP alone or APD + PCP. Thus, each neuronal network cultured on a MEA was only used for one single experiment and exposed to either PCP alone or PCP after pre-incubation with one specific APD. Ten networks from four preparations were exposed to PCP alone. For each of the three APDs, five networks were exposed to PCP after pre-incubation. In total, our experiments were performed on 25 ($=3 \times 5 + 10$) different networks on the same number of MEAs—10 MEAs for PCP and 5 for each antipsychotic. During pre-incubation, we administered APDs at intermediate concentrations (in the therapeutic range) well below individual IC_{50} identified in extensive experiments presented in our previous work (Görtz et al., 2019), where we found IC_{50} values of 3.3 μM for haloperidol, 4.2 μM for aripiprazole and 9.6 μM for clozapine, and where the spike rate at 1 μM of APD varied between 75 % and 100 % of baseline. As control, we stepwise incubated PCP alone at the same concentrations. Drugs were applied immediately after each 2 min recording, and networks were then allowed to equilibrate for 10 min before the next recording (Fig. 1A). Spontaneous neuronal network activity (SNNA) was recorded on all 60 MEA electrodes, and responses to different drug concentrations were normalized to baseline recordings of the same network.

2.2. Data recording and analysis

Extracellular potentials (spikes) were captured at 37 °C from MEAs (60 planar Ti/TiN microelectrodes, diameter 30 μm, spacing 200 μm, impedance <50 kΩ, MultiChannelSystems, Reutlingen, Germany). Signals were detected simultaneously from all 60 electrodes at 25 kHz, A/D-converted with 12 bits, and visualized using MC_Rack (Multi Channel Systems, Reutlingen, Germany). Spikes and bursts were analyzed using a custom-built software (Result Medical GmbH, Düsseldorf, Germany). Parameters analyzed included spike and network burst rate, network burst duration and -peak firing rate as described previously (Görtz et al., 2019).

2.3. Cell survival

Regarding antipsychotics, we had already performed live cell counting after Trypan blue staining in our previous publication showing that cell viability was not compromised (Görtz et al., 2019).

Regarding PCP we performed washout experiments 24h after incubation indicating preserved synchronous neuronal network activity (SNNA) (Supplementary Fig. 1).

2.4. Statistical analysis

GraphPad Prism 6 (La Jolla, USA) was used for statistics and

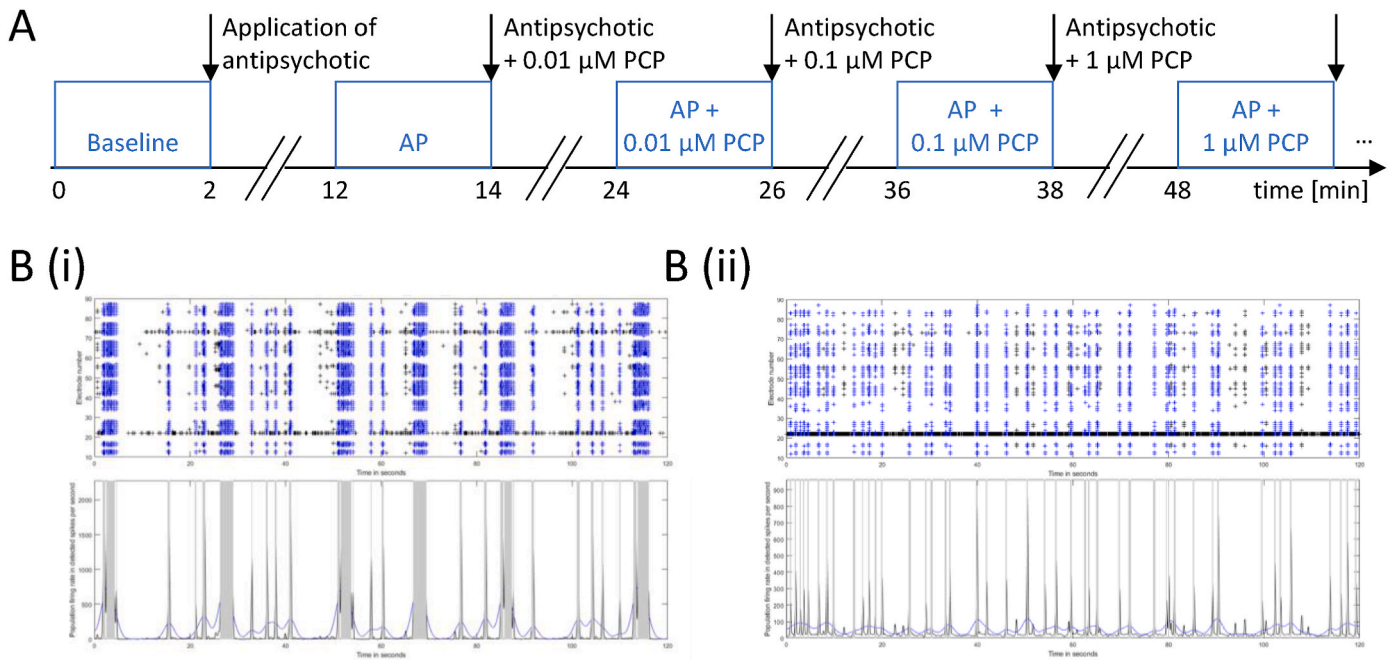

Fig. 1. Experimental Design and Baseline vs. PCP Spike Raster Plots: (A) Schematic of experimental design and workflow: 2-min MEA recordings (blue rectangles) were obtained from neuronal networks at 12-min intervals, allowing for 10-min equilibration (broken axis) after each drug application (APD at respective concentrations of 0.75 or 1 µM plus increasing PCP concentration). (B) Spike raster plots (SRP, top panels) represent network firing patterns of an entire 2-min recording. Spikes fired are depicted by "+"-symbols, with time in seconds on the x-axis and electrode number (12–88) on the y-axis. Bottom panels show total network firing rate in a 100 ms Gaussian smoothed window (gray line) and 1 s average (blue line). Synchronous bursting activity of the entire network appears as vertical spike pattern, indicated by gray shading in the bottom panels. Left SRP B (i) reveals baseline activity composed of a mixture of ~4–5 s long superbursts and 100–200 ms short single bursts. Right SRP B (ii) shows sustained short single burst activity, but at strongly reduced burst strength (PFR, bottom panel) after 10-min incubation with 1 µM PCP. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

graphics. Non-parametric Kruskal-Wallis-test with Dunn's multiple comparisons was used. Results are presented as mean ± standard deviation. Significance was set at $p < 0.05$.

3. Results

3.1. Spike raster plots

At baseline, network firing was dominated by synchronous network bursts across nearly all electrodes—either short single network bursts (~100–200 ms) or long superbursts (4–5 s), separated by quiescent periods. Such network bursts show up as distinct vertical bands in spike raster plots (SRP), where spikes are represented by "+"-symbols with time on the x-axis and electrode numbers on the y-axis (Fig. 1B (i), (ii)). These SRPs permit identifying network firing patterns of an entire recording, like e.g. a mixture of superburst and single burst activity in the exemplary baseline recording shown in Fig. 1B (i). From the SRPs, we derived the quantitative parameters spike rate, network burst rate, network burst duration and network burst peak firing rate (PFR, Fig. 1B, lower panels), which we normalized to the respective baseline recording.

3.2. Drug treatment does not compromise neuronal survival

Seeded cells matured to dense and viable networks on MEAs. After 10–12 DIV, stable synchronous SNNA was established. Incubation with PCP over 24 h followed by a washout revealed functional network activity and ensured cell survival (Supplementary Fig. 1). Further, incubation with any of the applied APDs even at the highest concentrations did not influence the proportion of vital cells between controls and treated cells, as measured by Trypan blue (Görtz et al., 2019).

3.3. Weakly biphasic effect of PCP on network activity

Application of PCP to cultured networks first led to a significant decrease of spike activity beginning at a concentration of 0.25 µM PCP, followed by a transient partial activity recovery between 10 and 50 µM, and eventually activity suppression above 100 µM (Fig. 2A (i), N = 10 MEAs). However, even with strongly reduced activity, synchronous network bursting was generally sustained with short single bursts (~200 ms), while each individual burst contained fewer spikes, so that burst PFR monotonously decreased (Fig. 2A (ii) and (Supplementary Fig. 2A)). In networks with baseline firing patterns dominated by superbursts (5/10 MEAs), PCP had a notable effect of splitting superbursts into a series of short, clearly separated short single bursts already at 0.1 µM. During the partial recovery phase, more but weaker short single bursts dominated network activity patterns.

3.4. Protective effect of typical and atypical APDs prior to PCP exposure

We next examined the impact of pre-incubating cultures with typical (haloperidol) or atypical (aripiprazole, clozapine) APDs at intermediate concentrations (in the therapeutic range well below their IC_{50}, (cf. Methods), prior to applying increasing PCP concentrations across four orders of magnitude (0.01–200 µM PCP). Overall, pre-incubated networks also exhibited a weakly biphasic response to PCP, albeit at higher PCP concentrations (Fig. 2B, C, D (i)). We generally observed a higher tolerance to PCP for all pre-incubated networks as evidenced by activity dropping not until about 10- to 20-fold higher PCP concentrations, and a less steep activity decline (Fig. 2B, C, D (i)). In particular, all 15 networks exposed to one of the three drugs (5 MEAs each) exhibited short single bursts at baseline and sustained these under combined application of drug and PCP. For pre-incubation with any of the three APDs, burst peak firing rates exhibited monotonous decline, but at higher PCP concentrations (Fig. 2B, C, D (ii)). Burst durations consistently remained

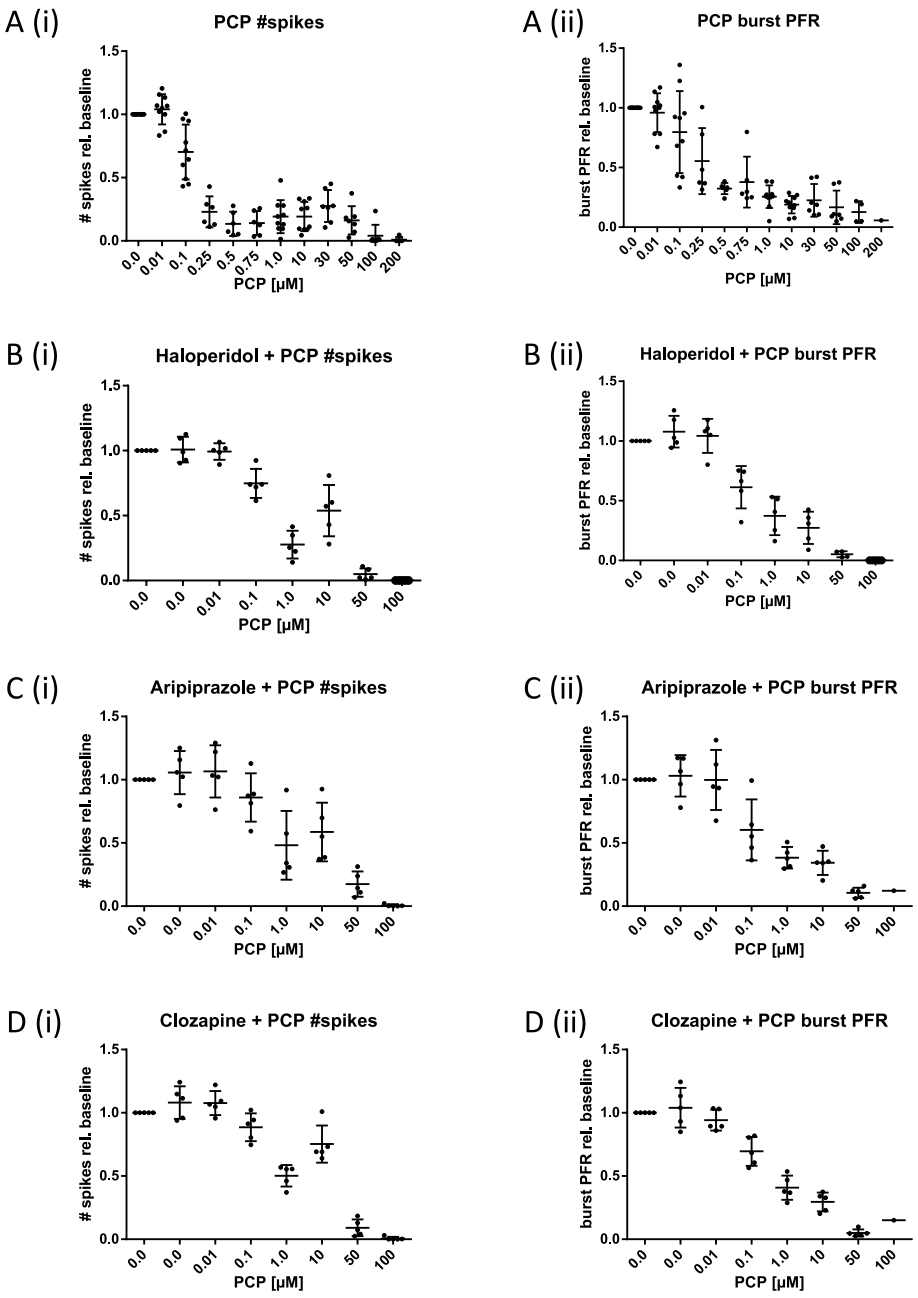

Fig. 2. Spike Rate and Burst Peak Firing Rate For PCP and APDs + PCP: Network response to (A) PCP alone (N = 10 MEAs), and (B–D) after preincubation with APDs: (B) haloperidol, (C) aripiprazole, (D) clozapine (N = 5 MEAs each). Left panels (i) show spike rate normalized by baseline recording, while right panels (ii) show baseline-normalized burst peak firing rate (mean ± standard deviation). Right shift of graphs indicates a clear order of protective effects of clozapine > aripiprazole > haloperidol (> PCP alone).

constant in the narrow range of 100–300 ms (Supplementary Figs. 2B, C, D (ii)), while the number of bursts increased at about 10 µM PCP (Supplementary Figs. 2B, C, D (i)), overcompensating PFR decline and leading to the biphasic response. In short, bursts were sustained while occurring more frequently but being considerably weaker. Changes in burst rate and duration did not reach significance.

3.5. Potency of protective effect of APDs

In order to quantitatively compare the different APDs, we also fitted concentration-response graphs to the spike rate. Fixing the response curve values for no PCP to one and in the high PCP-limit to zero, we fitted different IC_{50} values and Hill coefficients (Fig. 3A—D; Table 1). Concentration-response graphs were consistently shifted to higher PCP concentrations and considerably flattened after pre-incubation with any of the APDs. Respective IC_{50} values and Hill coefficients for PCP and all APDs are given in Table 1. Measured by their IC_{50}, both atypical APDs exhibited a 20- to 30-fold protective potency—higher than the typical APD haloperidol (6-fold). The right-shifts of the concentration-response graphs as well as the IC_{50} values indicate a clear order of protective effects of clozapine > aripiprazole ≫ haloperidol (≫ PCP alone).

At 1 µM of PCP, spike rate was significantly reduced to 19 % of baseline. This reduction could be significantly rescued by preincubation with atypical APDs: to 48 % by aripiprazole (1 µM, *) and to 50 % by clozapine (1 µM, **), but only to 27 % by haloperidol (0.75 µM, n.s.) (Fig. 3E). Neither burst rate nor duration was significantly different in this comparison, and burst PFR barely missed significance for clozapine (Fig. 3F).

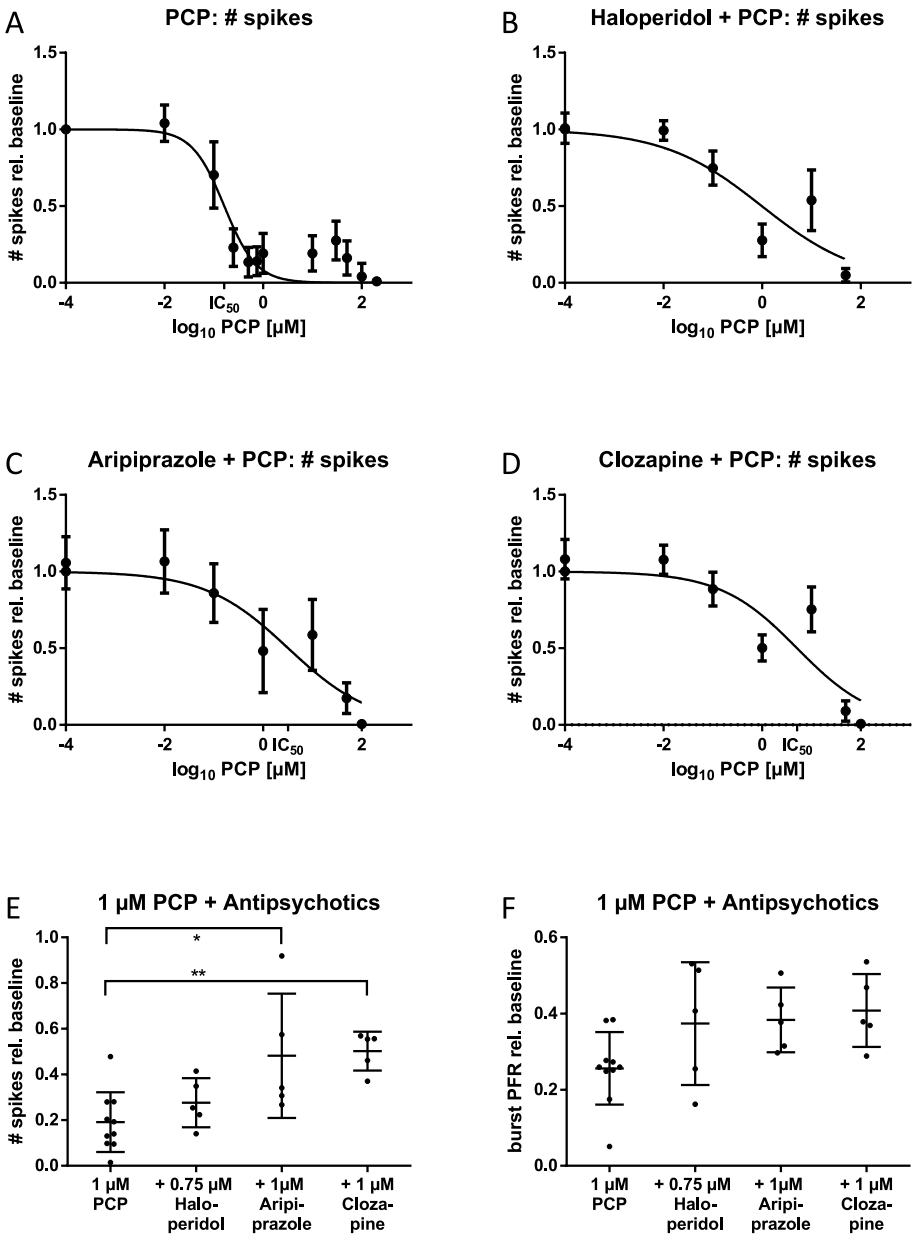

Fig. 3. Concentration-Response Curves For PCP and APDs + PCP; 1 µM PCP + APDs: Concentration-response curves fitted to the number of spikes relative to baseline as function of increasing PCP concentration for (A) PCP alone, (B) 0.75 µM haloperidol + PCP, (C) 1 µM aripiprazole + PCP and (D) 1 µM clozapine + PCP. Concentration-response curves were shifted to higher PCP concentrations and considerably flattened after pre-incubation with any of the APDs. Respective IC_{50} values and Hill coefficients for PCP and all APDs are given in Table 1. Both atypical APDs exhibited a higher protective potency than the typical APD haloperidol. Spike rate under either 1 µM aripiprazole or clozapine +1 µM PCP was significantly higher than under 1 µM PCP alone ($p < 0.01$, E). Burst PFR barely missed significance level for clozapine ($p = 0.07$, F).

Table 1
Presentation of IC_{50} values and Hill Coefficient for PCP and co-incubation of PCP with antipsychotics. PCP: Phencyclidine.

Drug	IC_{50}	Hill coefficient
PCP	0.16 µM	−1.33
Haloperidol + PCP	0.98 µM	−0.43
Aripiprazole + PCP	3.21 µM	−0.52
Clozapine + PCP	5.08 µM	−0.56

4. Discussion

In this work, we studied acute effects of PCP alone and after pre-incubation with the APDs haloperidol, aripiprazole, and clozapine, using electrophysiological recordings of long-term cultured mouse cortical networks on MEAs. While PCP alone concentration-dependently altered network firing patterns, reducing activity at and above 0.25 µM (Fig. 2A (i), (ii)), this effect could reproducibly be shifted to 6- to 30-fold higher concentrations by pre-incubation with ~1 µM of each APD (Fig. 2B, C, D; Fig. 3). Thus, pre-incubation with all three APDs partially counteracted the PCP-induced reduction of SNNA and thus protected synaptic function. In particular, the two atypical APDs exhibited three-to-fivefold higher protective potency than the typical APD haloperidol (Table 1).

Using SNNA in *in vitro* neuronal networks as a read-out, we could reproduce earlier findings of acute PCP-induced suppression of neuronal activity on a single-cell level as assessed by whole-cell patch-clamp recordings. It has long been known, for example, that PCP reduces inward

current flow in (1) cultured rat hippocampal neurons (by specifically blocking NMDAR and—to a much lesser extent—voltage-dependent potassium channels) (ffrench-Mullen and Rogawski, 1989) as well as in (2) pyramidal cells in mPFC cortex slice preparations (Jardemark et al., 2010; Shi and Zhang, 2003). Interestingly, the latter authors also observed an unexpected partial and transient reversal of activity suppression at 10 μM PCP: an increase in spikes per burst in 40 % of cells, which corresponds to our finding of a weakly biphasic response. There is no safe explanation for this finding, however, PCP may promote hyperpolarization by additionally acting on non-NMDA sites at higher concentrations (Shi and Zhang, 2003).

Other authors found that, depending on different populations of cortical neurons, PCP either increased or decreased activity of neurons, using single pyramidal neurons (extracellular recordings) or mPFC local field potentials (LFPs; population activity) *in vivo* in anaesthetized rats (Kargieman et al., 2007, 2008). In both cases, LFP delta oscillation power was profoundly reduced—corresponding to the observed network burst rate in our experiments. Similar to our findings, these effects could be counteracted with the APDs clozapine and haloperidol (but not differentially).

Taken together, findings reported here in our non-invasive *in vitro* MEA model of SNNA correspond to previous results using different and more complex approaches as cited above which altogether demonstrate profound alterations of activity patterns, which *in vivo* are assumed to dramatically reduce the efficiency of cortical information processing, a pathophysiological hallmark of schizophrenia. It is noteworthy that acute SNNA suppression in our model as well as in the above cited single cell studies occurred at PCP concentrations in a range capable of producing psychotomimetic effects (Proksch et al., 2000) justifying its use as a—doubtlessly simplified—*in vitro* model of psychosis. On these grounds, we further tested APD effects in this system.

Reversal of PCP-induced dysfunction of cortical networks by APDs as in the present study has been observed in various *in vitro* and *in vivo* experimental settings. In the above cited patch-clamp study of Jardemark et al., the reduction of current flow promoted by PCP was counteracted by acute administration of the atypical APD asenapine (Jardemark et al., 2010). Corresponding effects mediated by clozapine and haloperidol alike in the *in vivo* studies of Kargieman and colleagues have already been mentioned (see above). Disruption of delta oscillations, corresponding to the burst frequency in our setting, and its reversal by various APDs, including clozapine, olanzapine and aripiprazole, among others, has also been reported in the anaesthetized rat (Llado-Pelfort et al., 2016). Delgado-Sallent and coworkers found that the atypical APDs clozapine and risperidone, but not the typical APD haloperidol rescued PCP-induced disruption of prefrontal and cortico-hippocampal circuitry (Delgado-Sallent et al., 2022). Similar findings were discussed for clozapine in a thalamo-cortical network disrupted by PCP (Riga et al., 2023). Wittmann et al. conducted patch-clamp recordings of *Nucleus accumbens* medium spiny neurons in rat brain slices. Measuring NMDA receptor currents in response to NMDA application, they detected a 42 % increase of current amplitude after bath application of 0.1 μM clozapine, but no change under haloperidol. Their results pointed towards a D_1R, but, interestingly, not 5-HT_{2A} mediated effect thereby supporting a dopaminergic rather than serotonergic pathway (Wittmann et al., 2005).

The role of NMDAR deficiency in schizophrenia has also been extensively examined in transgenic mice. In their classical paper (Mohn et al., 1999), Mohn et al. established a homozygous NMDAR-subunit-1 deficient mouse model ($NR1^{neo-/-}$) with only ~10 % NMDAR expression, and these mice exhibited behavioral symptoms of schizophrenia like increased hyperlocomotion and motor stereotypy—similar to wild type mice treated with PCP or MK-801. Furthermore, administering PCP or MK-801 to $NR1^{neo-/-}$ mice lacked any effect in these typical motor symptoms of schizophrenia, while treatment with the APDs haloperidol or clozapine attenuated these symptoms. Thus, efficacy of APD treatment in $NR1^{neo-/-}$ mice with reduced NMDAR expression has been confirmed *in vivo*. Although the degree of NMDAR expression cannot be easily varied in the $NR1^{neo-/-}$ mice to mimic increasing PCP doses after APD preincubation, future electrophysiological recordings could be performed in $NR1^{neo-/-}$ cell cultures plated on MEAs.

In sum, APDs effectively influence the PCP-induced dysfunction of *in vivo* and *in vitro* cortical networks, and there seems to be a differential effect between typical and atypical APDs. Protection of network function was clearly more pronounced with the latter in our present study (order of declining potency: clozapine > aripiprazole ≫ haloperidol). However, the receptor binding profiles of these drugs differ considerably, with clozapine binding at multiple receptors, e. g., as an antagonist mainly at D_4R, 5-$HT_{2A/C}$R, α_{2C}R and various muscarinergic receptor sites (among others), aripiprazole mainly as a partial D_2R/D_3R agonist, but also with antagonistic properties at various serotonergic receptor sites, and the typical APD haloperidol predominantly as a D_2R antagonist. All of these and other APDs used at present as well as novel compounds that are currently under clinical trial unfold complex molecular interactions at their target sites. Whatever the exact pattern of receptor targeting may be, evidence suggests restoration of a disturbed excitatory-inhibitory balance in schizophrenia as one major downstream effect of APD treatment (Uliana et al., 2024). Our findings provide evidence for a protective effect of APD treatment against network disruptions by PCP, suggesting that this balancing mechanism can be modeled in our *in vitro* MEA system. Future studies using human induced pluripotent stem-cell (hiPSC) both from control subjects and schizophrenia patients on MEAs could help to transfer the model described into humans for developing new therapeutic strategies in schizophrenia and antipsychotic treatment (Izsak et al., 2019, 2021). Such approaches could be supplemented by rodent models, e. g. NMDAR hypofunction mouse models, using behavioral/cognitive alterations and a potential rescue by APDs under study as a readout (Delgado-Sallent et al., 2023).

5. Conclusion

Using electrophysiological recordings of long-term cultured mouse cortical networks on MEAs, we could clearly demonstrate a disruptive network effect of PCP as well as protection by APDs, with a higher effect of the two atypical APDs clozapine and aripiprazole, which is in line with various other studies using different, more complex settings. We propose this straightforward, noninvasive system as a plausible electrophysiological model for testing pharmaceuticals in schizophrenia. MEAs directly assess neuronal network function that can be analyzed in a standardized fashion potentially in a medium-throughput manner. Going forward, using human induced pluripotent stem-cell (hiPSC) derived neuronal networks in our system could permit evaluating candidate compounds on human cells and help reducing *in vivo* assays (Izsak et al., 2019, 2021).

6. Limitations

In the present study we exclusively focused on functional electrophysiological network read-out, leaving single-cell- or even single-channel-effects unobserved. Patch clamp recordings, immunocytochemistry as well as genomic and proteomic analyses would complement this study.

CRediT authorship contribution statement

Timo Jendrik Faustmann: Writing – original draft, Validation, Project administration, Formal analysis, Data curation. **Stephan Theiss:** Writing – review & editing, Visualization, Software, Formal analysis, Data curation. **Philipp Görtz:** Writing – review & editing. **Christian Lange-Asschenfeldt:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejphar.2025.177810.

Data availability

Data will be made available on request.

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