

Effects of the Glycine Transporter-1 Inhibitor Iclepertin (BI 425809) on Sensory Processing, Neural Network Function, and Cognition in Animal Models Related to Schizophrenia[§]

Holger Rosenbrock, Cornelia Dörner-Ciossek, Riccardo Giovannini, Bernhard Schmid, and Niklas Schuelert

Department of CNS Discovery Research (H.R., C.D.-C., N.S.), Department of Medicinal Chemistry (R.G.), and Department of Drug Discovery Sciences (B.S.), Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany

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ABSTRACT

N-methyl-D-aspartate (NMDA) receptor hypofunction leading to neural network dysfunction is thought to play an important role in the pathophysiology of cognitive impairment associated with schizophrenia (CIAS). Increasing extracellular concentrations of the NMDA receptor co-agonist glycine through inhibition of glycine transporter-1 (GlyT1) has the potential to treat CIAS by improving cortical network function through enhanced glutamatergic signaling. Indeed, the novel GlyT1 inhibitor iclepertin (BI 425809) improved cognition in a recent clinical study in patients with schizophrenia. The present study tested the ability of iclepertin to reverse deficits in auditory sensory processing and cortical network function induced by the uncompetitive NMDA receptor antagonist, MK-801, using electroencephalography (EEG) to measure auditory event-related potentials (AERPs) and 40 Hz auditory steady-state response (ASSR). In addition, improvements in memory performance with iclepertin were evaluated using the T-maze spontaneous alternation test in MK-801-treated mice and the social recognition test in naïve rats. Iclepertin reversed MK-801-induced deficits in the AERP readouts N1 amplitude and N1 gating, as well as reversing deficits in

40 Hz ASSR power and intertrial coherence. Additionally, iclepertin significantly attenuated an MK-801-induced increase in basal gamma power. Furthermore, iclepertin reversed MK-801-induced working memory deficits in mice and improved social recognition memory performance in rats. Overall, this study demonstrates that inhibition of GlyT1 is sufficient to attenuate MK-801-induced deficits in translatable EEG parameters relevant to schizophrenia. Moreover, iclepertin showed memory-enhancing effects in rodent cognition tasks, further demonstrating the potential for GlyT1 inhibition to treat CIAS.

SIGNIFICANCE STATEMENT

Despite the significant patient burden caused by cognitive impairment associated with schizophrenia, there are currently no approved pharmacotherapies. In this preclinical study, the novel glycine transporter inhibitor iclepertin (BI 425809) reversed sensory processing deficits and neural network dysfunction evoked by inhibition of *N*-methyl-D-aspartate receptors and enhanced working memory performance and social recognition in rodents. These findings support previous clinical evidence for the procognitive effects of iclepertin.

Introduction

Patients with schizophrenia experience debilitating cognitive symptoms related to deficits in sensory processing and neural network function (O'Donnell et al., 2013; Javitt and Freedman, 2015; Schuelert et al., 2018; Shen et al., 2020); however, there are currently no approved pharmacotherapies targeting cognitive impairment associated with schizophrenia (CIAS) (Sinkeviciute et al., 2018). Thus, there is a need for better understanding of the mechanisms underlying sensory processing deficits and neural network dysfunction in CIAS to support the development of effective treatments.

Sensory processing deficits and neural network dysfunction in patients with schizophrenia can be assessed using neurophysiological biomarkers detectable with electroencephalography (EEG)

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ABBREVIATIONS: AC, auditory cortex; AERP, auditory event-related potential; AP, anterior posterior; ASSR, auditory steady-state response; CIAS, cognitive impairment associated with schizophrenia; CSF, cerebrospinal fluid; EEG, electroencephalography; E/I, excitatory/inhibitory; GlyT1, glycine transporter-1; ITC, intertrial coherence; NMDA, *N*-methyl-D-aspartate; PFC, prefrontal cortex; PK, pharmacokinetic; T1, trial 1; T2, trial 2.

(O'Donnell et al., 2013; Javitt and Freedman, 2015; Schuelert et al., 2018; Shen et al., 2020). Since the relevant sensory processes are phylogenetically conserved, these deficits have the potential to be modeled in preclinical studies (Bickel and Javitt, 2009; Rosen et al., 2015; Liodice et al., 2021) for use as translatable biomarkers in the development of novel pharmacotherapies for CIAS (Javitt et al., 2008; Light and Swerdlow, 2015; Kim et al., 2020; Light et al., 2020). Patients with schizophrenia exhibit deficits in sensory gating, the process by which redundant or unnecessary stimuli are filtered out to prevent overload of higher-order processes (Shen et al., 2020). Deficits in auditory event-related potentials (AERPs) in paradigms such as the 40 Hz auditory steady-state response (ASSR), which demonstrates the ability of auditory circuits to support entrainment to temporally or continuous amplitude modulated tones, have been described in patients with schizophrenia (O'Donnell et al., 2013; Javitt and Freedman, 2015). Patients also exhibit disturbances in local field potential oscillations in the gamma frequency range (~25–100 Hz), which are critical for coordinating network activity during typical brain functioning (Schuelert et al., 2018) and integral to several aspects of learning and memory (Uhlhaas and Singer, 2010; Cohen et al., 2015; Stefanescu and Shore, 2015).

N-methyl-D-aspartate (NMDA) receptors play a key role in cognition and sensory processing by providing excitatory input to gamma aminobutyric-acid (GABA) inhibitory interneurons, supporting the generation of gamma oscillations in cortical regions (Collingridge et al., 2013; Cohen et al., 2015). Thus, NMDA receptor hypofunction and GABAergic interneuron dysfunction are implicated in the pathophysiology of CIAS (Dauvermann et al., 2017; Uno and Coyle, 2019). NMDA receptor hypofunction is thought to disturb the excitatory/inhibitory (E/I) balance in prefrontal cortex (PFC) through impaired interneuron function resulting in disinhibition of pyramidal cells, leading to the disruption of gamma oscillations and impaired network connectivity in PFC (Gonzalez-Burgos and Lewis, 2012; Lisman, 2012; Cohen et al., 2015; Schoonover et al., 2020).

Postmortem studies of patients with schizophrenia reveal altered expression of NMDA receptor, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptor subunits, and proteins associated with clustering of both receptors (Beneyto and Meador-Woodruff, 2008; Vrajová et al., 2010; Forsyth and Lewis, 2017; Uno and Coyle, 2019). Large-scale genomic studies have demonstrated a strong link between schizophrenia and gene variants related to glutamatergic signaling, including the NMDA receptor complex (Kirov et al., 2012; Fromer et al., 2014; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Forsyth and Lewis, 2017). Moreover, postmortem studies of patients with schizophrenia have found a reduced density of small interneurons and a 73% reduction in GABAergic interneurons expressing the NR2A NMDA receptor subunit in the cingulate cortex (Cohen et al., 2015). These data support a role for inhibitory interneuron dysfunction mediated by NMDA receptor hypofunction in the pathophysiology of CIAS (Dauvermann et al., 2017; Uno and Coyle, 2019).

The link between NMDA hypofunction, E/I imbalance, and CIAS is supported by the effects of noncompetitive NMDA receptor antagonists such as ketamine and phencyclidine, which induce psychosis and schizophrenia-like cognitive symptoms in healthy volunteers and exacerbate symptoms in patients with schizophrenia (Krystal et al., 1994; Moghaddam

and Krystal, 2012). NMDA receptor antagonists also induce sensory deficits and neural network dysfunction in animal models and humans similar to those observed in patients. Exposure to NMDA receptor antagonists causes heterogeneous disturbances in gamma oscillations in rodents (Pinault, 2008; Hakami et al., 2009) and healthy volunteers (Hong et al., 2010; Ahnaou et al., 2017), which may impair synchrony and reduce signal-to-noise ratio in neural networks (Hakami et al., 2009). These findings are in line with reports describing atypical gamma oscillations in patients with schizophrenia compared with healthy controls (Cho et al., 2006; Hirano et al., 2015; Tanaka-Koshiyama et al., 2020), although the precise nature of these abnormalities varies across experimental paradigms (Molina et al., 2020).

Therefore, novel pharmacotherapies to ameliorate NMDA receptor hypofunction have the potential to reverse deficits in sensory processing, restore neural network function, and improve cognition (Collingridge et al., 2013). Examples of novel pharmacotherapies include NMDA receptor positive allosteric modulators (PAMs) that directly activate the NMDA receptor, D-amino acid oxidase (DAAO) inhibitors that increase the synaptic concentration of the NMDA receptor co-agonist D-serine, and kynurenine aminotransferase-2 (KAT-2) inhibitors that decrease the concentration of the endogenous competitive NMDA receptor antagonist kynurenic acid (Yoshida et al., 2019; Pei et al., 2021; Wu et al., 2021; Geoffroy et al., 2022). NMDA receptor PAMs and DAAO inhibitors have reached phase I and II clinical trials, respectively, whereas the KAT-2 inhibitors are still in preclinical development. Another novel strategy to ameliorate NMDA receptor hypofunction, which has progressed into phase III clinical trials, involves increasing the synaptic concentration of glycine, an obligatory co-agonist at NMDA receptors, by inhibition of glycine transporter-1 (GlyT1) (Harvey and Yee, 2013; Pinaud et al., 2018; Pei et al., 2021; Wu et al., 2021). Synaptic glycine concentration is regulated by GlyT1, which is expressed both pre- and postsynaptically at glutamatergic synapses and on astrocytes (Zafra and Giménez, 2008; Harvey and Yee, 2013; Marques et al., 2020). Thus, increasing synaptic glycine concentration by inhibiting GlyT1 is thought to enhance NMDA receptor function (Depoortère et al., 2005; Alberati et al., 2012; Harvey and Yee, 2013; Ahnaou et al., 2020), which would be expected to normalize network function and therefore ameliorate cognitive impairment. Several GlyT1 inhibitors have been developed previously and evaluated in clinical trials for indications such as schizophrenia (positive, negative, or cognitive symptoms), obsessive-compulsive disorder, and addiction (Cioffi and Guzzo, 2016; Pei et al., 2021). One of the first GlyT1 inhibitors, the prototype inhibitor sarcosine, showed efficacy for the treatment of positive, negative, and cognitive symptoms of schizophrenia in small clinical trials (Tsai et al., 2004; Lane et al., 2010). However, development of sarcosine and other GlyT1 inhibitors has been later discontinued due to adverse effects, negative results in phase II, or undisclosed reasons (Cioffi and Guzzo, 2016; Pei et al., 2021). The most advanced GlyT1 inhibitor, bitopertin, showed promising phase II results for treatment of negative symptoms in schizophrenia, but efficacy on negative symptoms was not confirmed in larger phase III trials (Pinaud et al., 2018).

Iclepertin is a novel GlyT1 inhibitor under development for treatment of CIAS. Previous studies have demonstrated that iclepertin is a potent and selective inhibitor of GlyT1 that shows functional target engagement by increased glycine levels in the cerebrospinal fluid (CSF) of rodents and healthy

volunteers (Moschetti et al., 2018a,b; Rosenbrock et al., 2018). Furthermore, iclepertin has demonstrated procognitive effects in a phase II trial in patients with schizophrenia (Fleischhacker et al., 2021). Phase III trials investigating the efficacy and safety of iclepertin in patients with schizophrenia are currently underway (NCT04846868, NCT04846881, and NCT04860830). We report preclinical studies characterizing the effects of iclepertin on EEG biomarkers related to sensory processing and neural network function and on rodent cognition tasks related to schizophrenia.

Materials and Methods

Drugs

Iclepertin (BI 425809) was synthesized at Boehringer Ingelheim Research Italia (Milan, Italy). The molecular structure of iclepertin is shown in Fig. 1. The uncompetitive NMDA receptor antagonist, MK-801, was obtained from Sigma (Saint-Quentin-Fallavier, France).

Ethical Considerations

Procedures involving animals and their care were conducted in conformity with institutional and European Union guidelines (EEC Council Directive 86/609 and Directive 2010/63/EU) and were approved by the ethical committees of the respective regional councils.

In Vivo Pharmacokinetics of Iclepertin in Rats

To assess plasma exposure of iclepertin, male Han Wistar rats (Charles River, Sulzfeld; 8 weeks old, 250–270g, $n = 3$) were treated orally with iclepertin 2.6 mg/kg in a Natrosol (Ashland, Krefeld)/Tween-80 suspension (Dr. W. Kolb AG, Stuttgart) used as vehicle. Iclepertin was suspended under stirring in 0.5% Natrosol/0.01% Tween-80 in water and then sonicated for 10 minutes, resulting in a fine, slightly cloudy suspension. After retrobulbar puncture, blood was collected in ethylenediaminetetraacetic acid-containing tubes at 0, 0.25, 0.5, 1, 2, 4, 8, and 24 hours after administration of iclepertin. Samples were centrifuged at 2000 g for 10 minutes at 4°C, and the upper phase (plasma) was stored at –20°C. Iclepertin plasma concentrations were determined using liquid chromatography coupled to mass spectrometry. Iclepertin plasma concentrations were determined by liquid chromatography tandem-mass spectrometry (LC-MS/MS) using an API 4000 system (SCIEX). Plasma was precipitated using acetonitrile/water (80/20 v/v). After centrifugation at 2000 g for 10 minutes at 4°C, an aliquot of the upper phase was directly injected into the LC-MS/MS system. A linear calibration standard curve (13 concentrations) with 1/X weighting was used to determine iclepertin concentrations over 1–10,000 nmol/l.

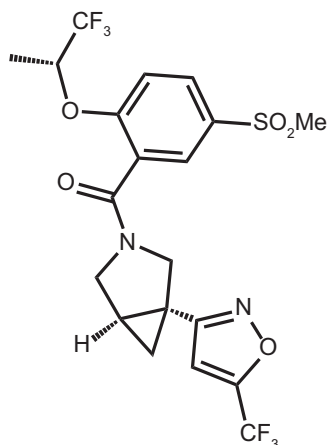


Fig. 1. Structure of iclepertin.

EEG Biomarkers in the MK-801 Challenge Model in Mice

Animals. Male C57BL/6J mice (12–14 weeks old, 28–30 g, $n = 21$) (Janvier Laboratories, Le Genest-Saint-Isle, France) were group housed prior to surgery (four animals per cage) and single housed after surgery under standard light-dark conditions (12 hour/12 hour, lights on 06:00–18:00) with food and tap water available ad libitum. C57BL/6J mice were used as they are well suited for neurophysiological cortical network investigations using EEG techniques (Schuelert et al., 2018). Five epidural EEG electrodes were placed 10 days prior to recording (PFC: anterior posterior (AP) = +1.7 mm, medial lateral (ML): ± 1.0 mm; auditory cortex (AC): AP = –2.7 mm, ML: ± 4.0 mm; reference electrode placement: AP = –5.7 mm, ML: ± 0 mm). Blood samples were collected from satellite mice ($n = 3$ per dose group) to measure plasma drug exposure 60 minutes after subcutaneous administration.

Procedure. Mice were placed in a plexiglass cage in an attenuated sound box and allowed to adapt to their environment for 20 minutes before subcutaneous administration of iclepertin 0.3, 1, or 4 mg/kg or vehicle (20% HP-β-CD in water). Iclepertin was dissolved in 20% HP-β-CD in water and then sonicated for 10 minutes, resulting in a clear solution. After 15 minutes, all animals then received a subcutaneous dose of the MK-801 0.15 mg/kg or vehicle (saline). All injections had a total volume of 10 ml/kg. A crossover treatment schedule was used such that each mouse received all treatment conditions separated by a 3-day washout period. EEG, AERP, and auditory evoked oscillation recordings in PFC and AC began 15 minutes after administration of either MK-801 or vehicle, and were conducted as described previously (Schuelert et al., 2018).

EEG Analysis. EEG parameters were analyzed as previously described (Schuelert et al., 2018). Briefly, four recording channels (one AC channel and one PFC channel for each hemisphere) and one trigger channel (to identify the timing of tone presentations) were analyzed per animal. The sampling rate of recording channels and trigger channels was set to 1000 Hz. Any EEG recording segments with artifacts were removed. Analyzer 2 software (Brain Products GmbH, Munich, Germany) was used for data analysis.

EEG parameters of interest that were tested using a double-click protocol (Schuelert et al., 2018) included N1 amplitude, N1 gating, and basal and evoked gamma power. N1 (also known as N100) is a large negative potential elicited by sensory stimuli, which peaks approximately 100 milliseconds after stimulus onset in humans (Rosburg et al., 2008; Joos et al., 2014; Du et al., 2017) and 40 milliseconds after stimulus onset in rodents (Aguilar et al., 2021). In this study, N1 was defined as the maximum negative deflection between 30 and 50 milliseconds after the click. A representative trace of the N1 response is shown in Supplemental Fig. 1A. Sensory gating of N1 amplitude between paired click stimuli was measured using the ratio between the N1 amplitude of the response evoked by click 1 and the N1 amplitude of the response evoked by click 2 to evaluate the capacity of the sensory system to filter repeated stimuli. Basal gamma power (50–100 Hz) was defined as power outside of the auditory evoked response (3 seconds before click presentations), whereas evoked gamma power (35–80 Hz) was defined as the total event-related power (evoked plus basal power) minus the basal power. These frequency ranges were selected to demonstrate the largest effect of MK-801 compared with vehicle, thus providing greater sensitivity to detect any effects of iclepertin on MK-801-induced changes in basal and evoked gamma power (Supplemental Fig. 2). ASSR intertrial coherence (ITC) and ASSR evoked gamma power were assessed by presentation of a click train 2 seconds in length with a frequency of 40 Hz. The ASSR paradigm explores cortical capacity to support an entrained 40 Hz oscillation. ASSR ITC is a measure of the phase synchronization of brain oscillations in response to auditory stimuli across trials, indicating the ability of the sensory network to stereotypically process repeated input (Schuelert et al., 2018). A representative trace of the ASSR response is shown in Supplemental Fig. 1B. An ITC of 0 represents an absence of synchrony (no phase locking), whereas a score of 1 equates to perfect synchronization (fully phase-locked).

T-Maze Spontaneous Alternation Task in Mice

Animals. Male CD-1 mice (4–5 weeks old, 25–30g; Janvier Laboratories) were group housed (10 mice per cage) in a temperature-controlled room (21–22°C) on a reversed light-dark cycle (12 hour/12 hour, lights on: 17:30–05:30) with food and water available ad libitum. Satellite mice ($n = 3$ per dose group) were used for blood sample collection to measure plasma drug exposure 60 minutes after oral administration. CD1 mice were used as they are well suited for the T-maze due to their strong exploratory behavior, especially if tested when in their active phase under a reversed light cycle.

Procedure. Mice ($n = 10$ in each treatment group) were randomly assigned to receive iclepertin 0.005, 0.05, 0.15, 0.5, 1.5, or 4.5 mg/kg or vehicle (0.5% Natrosol/0.01% Tween-80) orally 60 minutes prior to the T-maze test, in addition to MK-801 0.1 mg/kg or vehicle (saline) administered subcutaneously 30 minutes after administration of iclepertin. Iclepertin was suspended under stirring in 0.5% Natrosol/0.01% Tween-80 in water and then sonicated for 10 minutes, resulting in a fine, slightly cloudy suspension. The T-maze spontaneous alternation task was performed at Neurofit SAS. (Illkirch, France) using a non-transparent T-maze as previously described (Rosenbrock et al., 2019). The experimental protocol comprised an individual session with one forced-choice trial preceded by 14 free-choice trials. During the forced-choice trial, either the left or right arm of the T-maze was blocked to allow mice to explore the open arm. Thereafter, mice were allowed to enter either arm in the 14 free-choice trials. An error response was recorded if a mouse entered the same arm on two subsequent trials. A session ended when 14 free-choice trials had been completed or 10 minutes had elapsed, whichever occurred first. All mice completed the 14 free-choice trials within the 10-minute time frame. Working memory performance was assessed by the percentage of successful alternation over the 14 free-choice trials.

Social Recognition Task in Rats

Animals. The social recognition task was performed in rats rather than mice due to species differences in social cognition; rats are more likely to seek the company of other rats and are less territorial and less aggressive in social contexts than mice. This makes the rat a more appropriate species to address social cognition preclinically (Kent Scientific Corporation, 2019; Kondrakiewicz et al., 2019). Adult male Wistar rats (12–15 weeks old, 300–330 g, $n = 72$) and juvenile males (23–25 days old, 50–70 g, $n = 72$) (Janvier, Le Genest-Saint-Isle, France) were group housed (three animals per cage) under standard light-dark conditions (12 hour/12 hour, lights on: 07:00–19:00) with food and water available ad libitum.

Procedure. Animals were randomly assigned to receive iclepertin 0.2 ($n = 17$), 0.6 ($n = 18$), or 1.8 mg/kg ($n = 18$) or vehicle (0.5% Natrosol/0.01% Tween-80; $n = 18$) administered orally 60 minutes prior to trial 1 (T1) and trial 2 (T2) with a volume of 5 ml/kg. Iclepertin was suspended under stirring in 0.5% Natrosol/0.01% Tween-80 in water and then sonicated for 10 minutes, resulting in a fine, slightly cloudy suspension. Only the adult rats were administered with iclepertin; the juvenile rats received no treatment. The social recognition task was performed as previously described (van der Staay et al., 2008). Briefly, rats were tested in a black square box with the floor covered with bedding material and under dimmed light conditions. A camera above the box recorded video footage for analysis using a DVD recorder. The social recognition test was performed across two consecutive days. On day 1 (T1), a conspecific cogenitor juvenile rat was placed into the testing arena with the adult rat, and they were allowed to interact for 3 minutes. At the end of T1, both rats were returned to their home cage. After 24 hours, the adult rat was reintroduced to the testing arena for another 30 minutes of habituation, whereas juveniles were kept singly housed. After habituation, the familiar juvenile (used in T1) was introduced to the testing arena with the adult rat and allowed to interact for 3 minutes (T2). All digitalized trials were analyzed by a trained observer blinded to the experimental conditions and treatment group. Sniffing and grooming of body parts, anogenital sniffing, and close following were scored. The difference in

social investigation time between T1 and T2 was used as an index of social memory performance. Overall investigation time in T1 and T2 was also noted to detect nonspecific treatment effects on arousal or attention.

Statistical Analysis

EEG data were analyzed using a generalized linear model with factors of ‘animal,’ ‘day,’ and ‘treatment.’ Mean and standard error of the mean (S.E.M.) were calculated over all treatment days. To focus on the procognitive effects of iclepertin, only findings from PFC are reported in the main manuscript; EEG data from AC are shown in the supplement. One-way analysis of variance (ANOVA) was performed on the T-maze spontaneous alternation task data with Fisher’s Protected Least Significant Difference for pairwise comparisons. For the rat social recognition task, quantitative data are presented as mean and S.E.M. and were analyzed using two-way ANOVA with post hoc Bonferroni tests. The significance threshold was set at $P < 0.05$ for all analyses.

Results

In Vivo Pharmacokinetics of Iclepertin

Mean plasma concentration of iclepertin over 24 hours in rats after an oral dose of 2.6 mg/kg is shown in Fig. 2. The mean maximum concentration in plasma of 1010 nM was reached at 1.67 hours, and the resulting area under the concentration time-curves from time zero to infinity was 21 400 nM·h. In a previous study, the concentration of iclepertin in CSF, which was used as a surrogate for free brain concentration, was determined on average as 2% of the plasma concentration (Rosenbrock et al., 2018).

Effects of Iclepertin on Sensory Processing and Cortical Network Function in Mice

In PFC, treatment with iclepertin 0.3 ($P < 0.05$), 1 ($P < 0.01$), or 4 mg/kg ($P < 0.05$) significantly reversed MK-801-induced deficits in N1 amplitude and N1 gating (Fig. 3, A and B), whereas iclepertin 0.3 ($P < 0.01$) or 1 mg/kg ($P < 0.05$) significantly reversed deficits in 40 Hz ASSR evoked gamma power (Fig. 3C). Iclepertin 1 mg/kg reversed deficits in 40 Hz ASSR ITC ($P < 0.05$; Fig. 3D) and reversed MK-801-induced increases in basal gamma power ($P < 0.05$; Fig. 3E). Similarly, iclepertin 0.3, 1, or 4 mg/kg significantly reversed the effects of MK-801 in AC for N1 amplitude, N1 gating, and basal gamma

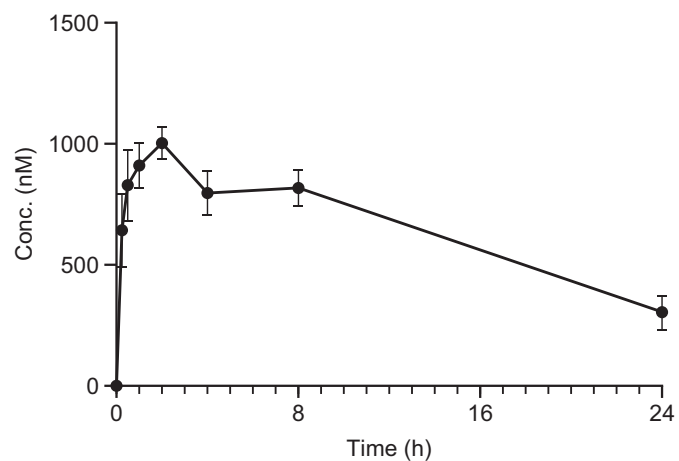


Fig. 2. In vivo pharmacokinetics of iclepertin in rats. Mean \pm S.E.M. plasma concentration of iclepertin over 24 hours in rats ($n = 3$) after an oral dose of 2.5 mg/kg.

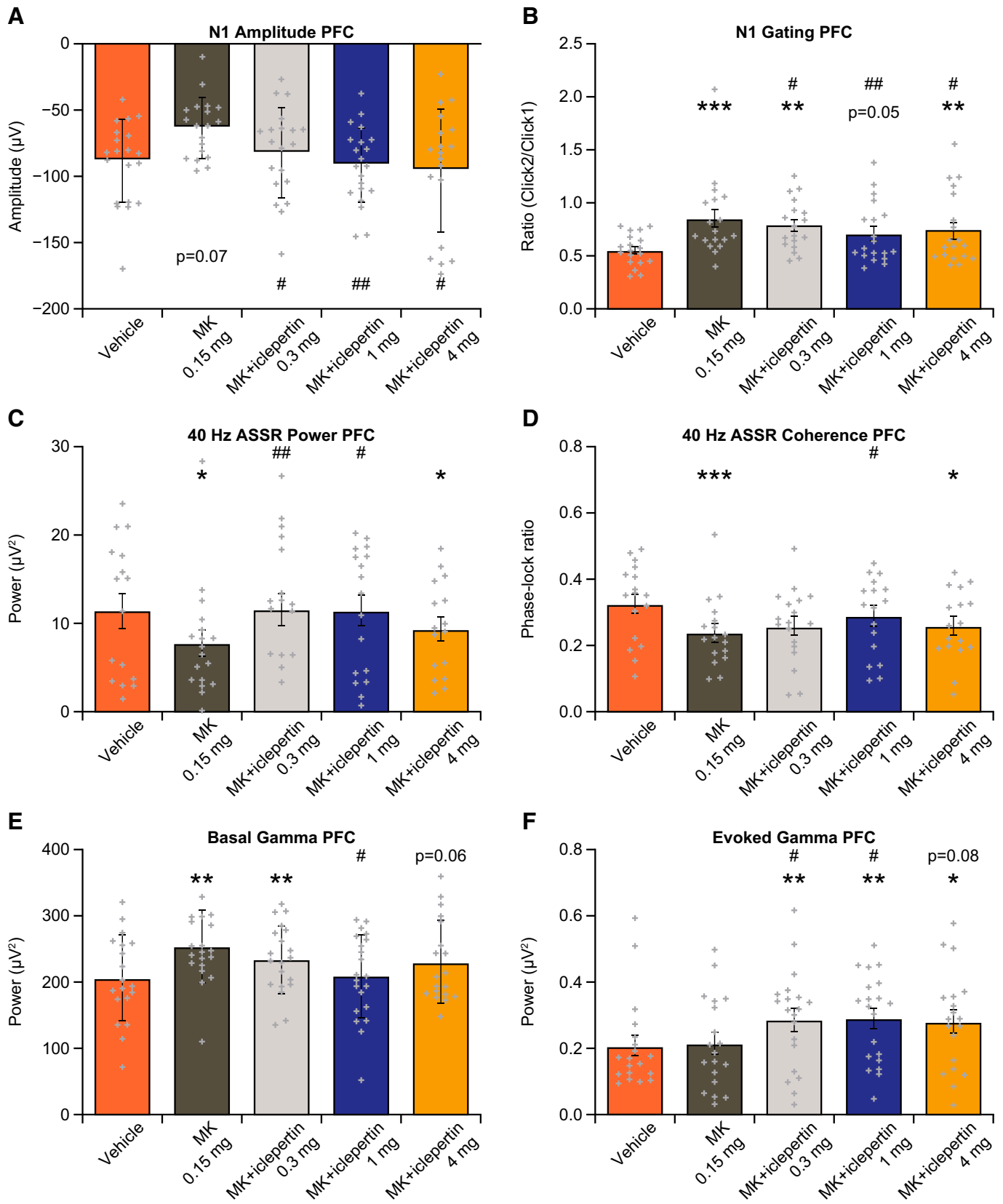


Fig. 3. Effects of iclepertin on EEG measurements in PFC. The effects of orally administered iclepertin 0.3, 1, or 4 mg/kg on EEG measurements in PFC in mice after administration of MK-801 0.15 mg/kg or vehicle. Data are shown as mean \pm S.E.M. ($n = 21$ for each treatment group). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ compared with vehicle; # $P < 0.05$, ## $P < 0.01$ compared with MK-801.

power ($P < 0.01$; Supplemental Fig. 3), but no MK-801-induced deficit compared with vehicle was observed in AC for ASSR evoked gamma power or ASSR ITC (Supplemental Fig. 3, C and D). No MK-801-induced deficits were observed in either brain region for evoked gamma power in the click paradigm; treatment with icleperlin 0.3, 1, or 4 mg/kg significantly increased evoked gamma power compared with vehicle in both PFC and AC ($P < 0.05$; Fig. 3F; Supplemental Fig. 3F), whereas icleperlin 0.3 or 1 mg/kg significantly increased evoked gamma power in PFC compared with MK-801 ($P < 0.05$; Fig. 3F).

Mean plasma exposures in satellite mice after subcutaneous administration of icleperlin 0.3, 1, and 4 mg/kg were 251, 842, and 2650 nM, respectively. Based on a rat CSF/plasma ratio of 0.02, these values correspond to estimated icleperlin CSF levels in the range of 1- to 10-fold of the GlyT1 half-maximal inhibitory concentration (IC_{50}) (IC_{50} on GlyT1 = 5.2 nM; Rosenbrock et al., 2018).

Effects of Icleperlin on Working Memory in Mice

Administration of MK-801 0.1 mg/kg led to a significant reduction in spontaneous alternation (i.e., the percentage of trials in which correct alternation was observed) from a mean of 67.9% with vehicle to 40.7% with MK-801 ($P < 0.05$; Fig. 4), corresponding approximately to chance level (50% correct alternation). Oral treatment of mice with icleperlin at doses of 0.5–4.5 mg/kg led to a significant attenuation or reversal of the MK-801-induced reduction in spontaneous alternation compared with the MK-801-treated group ($n = 10$ animals per group; $P < 0.05$; Fig. 4). For the assessment of a potential procognitive compound in the T-maze spontaneous alternation test, a pharmacologically induced impairment of performance by an NMDA receptor antagonist such as MK-801 is needed to evaluate the drug effect. This is because naïve mice perform well in the test, with an average alternation of approximately 70% that is unlikely to be substantially further improved by the study drug.

Mean plasma exposures in satellite mice after oral administration of 0.5, 1.5, and 4.5 mg/kg were 51, 509, and 1523 nM, respectively, corresponding to estimated CSF levels of icleperlin in the range of 0.2- to 6-fold of the GlyT1 IC_{50} .

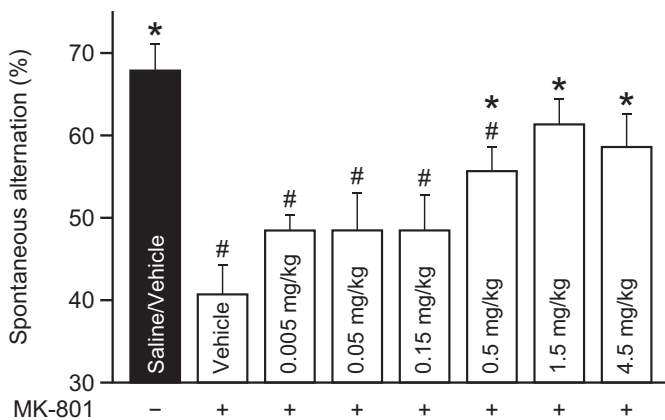


Fig. 4. Effects of icleperlin on working memory in mice. The effects of orally administered icleperlin 0.005, 0.05, 0.15, 0.5, 1.5, or 4.5 mg/kg on spontaneous alternation in the T-maze task in mice, after administration of subcutaneous MK-801 0.1 mg/kg or vehicle. Data are shown as mean \pm S.E.M. ($n = 10$ in each treatment group). * $P < 0.05$ vs. MK-801 0.1 mg/kg/vehicle; # $P < 0.05$ vs. saline/vehicle.

Effects of Icleperlin on Social Recognition Memory in Rats

Treatment with icleperlin 0.2, 0.6, and 1.8 mg/kg, but not vehicle, improved social recognition memory as shown by the significant reduction in social investigation times between T1 and T2 for all dose groups (icleperlin 0.2, 0.6 mg/kg: $P < 0.01$; icleperlin 1.8 mg/kg: $P < 0.001$) (Fig. 5). As this is a test for assessment of long-term memory (episodic/recognition memory), a potential procognitive compound like icleperlin is tested without an additional pharmacological challenge since the rats are already compromised in their memory performance due to the 24-hour delay between T1 and T2. In addition, there were no significant differences in overall investigation time observed in T1 ($P > 0.2$), suggesting that there were no detectable nonspecific effects of icleperlin on behavior. Further, there were no overt effects on locomotion or other non-specific effects of icleperlin observed during T1 and T2.

Based on these findings as well as the previously reported rat CSF/plasma ratio of 0.02 and IC_{50} for inhibition of GlyT1 by icleperlin of 5.2 nM (Rosenbrock et al., 2018), the doses used in the social recognition test would be expected to result in plasma levels of icleperlin in the range of 180–1800 nM at T1 and in the range of 250–2500 nM at T2. These estimated values correspond to CSF levels of icleperlin in the range of 0.7- to 7-fold of the GlyT1 IC_{50} at T1 and in the range of 1- to 10-fold of the GlyT1 IC_{50} at T2. The increase in exposure at T2 of approximately 40% is due to residual exposure after administration at T1 (Fig. 2).

Discussion

Previous studies have demonstrated that the GlyT1 inhibitor icleperlin has shown high potency against GlyT1 ($IC_{50} = 5.0$ nM and 5.2 nM on human and rat GlyT1, respectively) and is highly selective, as demonstrated by a lack of activity toward GlyT2 ($IC_{50} > 10 \mu\text{M}$), or 102 other off-targets ($IC_{50} > 10 \mu\text{M}$) (Rosenbrock et al., 2018). Central target engagement of icleperlin was demonstrated by a dose-dependent increase

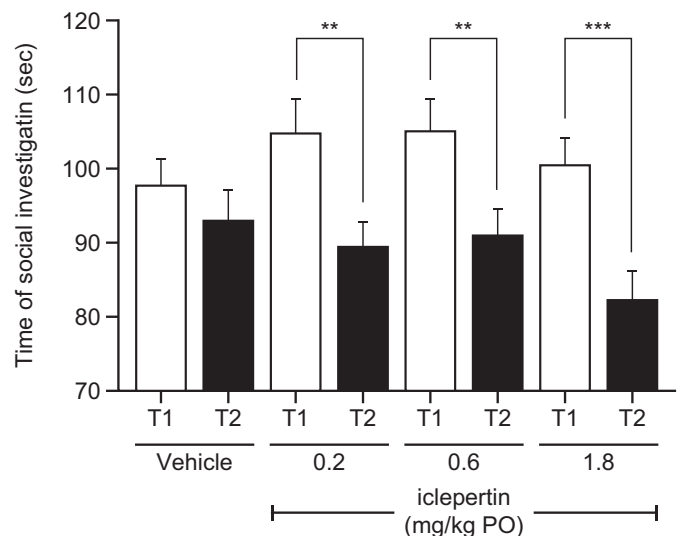


Fig. 5. Effects of icleperlin on social recognition in rats. The effects of orally administered icleperlin 0.2, 0.6, or 1.8 mg/kg on social investigation times in rats, compared with vehicle. Data are shown as mean \pm S.E.M. (vehicle: $n = 18$; icleperlin 0.2 mg/kg: $n = 17$; icleperlin 0.6 mg/kg: $n = 18$; icleperlin 1.8 mg/kg: $n = 18$). ** $P < 0.01$, *** $P < 0.001$. PO, orally administered.

of glycine in rat and human CSF (Rosenbrock et al., 2018), and the compound was generally well tolerated in single and multiple rising dose studies in healthy volunteers at doses and exposure levels expected to be clinically relevant (Moschetti et al., 2018a,b).

In these preclinical studies, iclepertin attenuated deficits in EEG parameters induced by the NMDA receptor antagonist MK-801 and improved working and episodic memory performance in rodent cognition tasks. With regard to quantitative EEG parameters, treatment with iclepertin reversed MK-801-induced deficits in N1 amplitude and N1 gating and significantly reversed an MK-801-induced increase in basal gamma power. Furthermore, iclepertin attenuated MK-801-induced deficits in ASSR ITC and ASSR evoked gamma power and also significantly attenuated an MK-801-induced increase in basal gamma power. Overall, these data suggest a normalization of E/I balance and improved signal-to-noise ratio, based principally on the normalization of N1 gating and basal gamma power alongside an increase in evoked gamma power after treatment with iclepertin.

With regard to cognition, iclepertin reversed MK-801-induced deficits in working memory in the spontaneous alternation task in mice and led to significant improvement of episodic memory function in social recognition in rats using a 24-hour forgetting paradigm (time interval between T1 and T2). Thus, this study demonstrates the ability of iclepertin to reverse deficits in clinically relevant EEG biomarkers and working memory in a rodent model of NMDA receptor hypofunction related to schizophrenia (Deserno et al., 2012; Gao et al., 2022). Iclepertin also improved recognition/episodic memory in naïve rats, a memory domain that is also impaired in patients with schizophrenia (Bonner-Jackson et al., 2005; Guo et al., 2019).

The main objective of the EEG part of the study was to test the ability of iclepertin to attenuate cortical network disturbances in a rodent model of NMDA receptor hypofunction related to schizophrenia. Thus, potential effects of iclepertin alone on EEG parameters were not explored. Nonetheless, a previous rodent EEG study using the GlyT1 inhibitor bitopertin showed similar attenuating effects on MK-801-induced EEG disturbances (Voehringer et al., 2019). In that study, effects of the GlyT1 inhibitor were also investigated without MK-801 challenge; no relevant effects on EEG were observed compared with vehicle, suggesting that iclepertin alone is unlikely to have relevant effects on EEG parameters. Disturbances in sensory processing and neural network function, thought to underlie CIAS, are commonly observed in patients with schizophrenia (O'Donnell et al., 2013; Javitt and Freedman, 2015; Shen et al., 2020). N1 (or N100) is an AERP associated with neurons in primary and association AC, and therefore pathology affecting the connectivity and projections of these areas may reduce N1 responses (Gonzalez-Heydrich et al., 2015). Deficits in auditory N1 amplitude are commonly found in patients with schizophrenia (Rosburg et al., 2008; Shen et al., 2020) and have also been observed in unaffected first-degree relatives of patients, suggesting that these deficits may reflect genetic predisposition for schizophrenia (Ahveninen et al., 2006; Force et al., 2008; Foxe et al., 2011). Patients with schizophrenia also exhibit an impaired ability to filter sensory stimuli, as evidenced by deficits in sensory gating such as decreased N1 gating (Rosburg et al., 2008; Shen et al., 2020). Our findings demonstrate similar MK-801-induced deficits in N1 amplitude and N1 gating in mice, supporting the translatability of our findings. These data are

also consistent with a previous EEG study in rodents, in which low doses of MK-801 (0.03 mg/kg) increased N1 amplitude whereas higher doses of 0.1 and 0.3 mg/kg reduced N1 amplitude (Schuelert et al., 2018); this is also in line with reported findings in patients with schizophrenia (Rosburg et al., 2008; Mota et al., 2020; Shen et al., 2020).

Patients with schizophrenia also exhibit robust deficits in 40 Hz ASSR (Sivarao, 2015), including decreased ITC (Spencer et al., 2009; Mulert et al., 2011; O'Donnell et al., 2013; Hirano et al., 2015; Thuné et al., 2016) and reduced power (Spencer et al., 2009; O'Donnell et al., 2013; Thuné et al., 2016). These deficits are present from the first psychotic episode (Spencer et al., 2008; Wilson et al., 2008), and ITC deficits have been observed before onset in ultrahigh-risk individuals (Tada et al., 2016). In line with these observations, the current study demonstrated MK-801-induced deficits in ASSR evoked gamma power and ITC in PFC in mice. Further, comparable deficits in auditory evoked gamma power and ITC have been shown in a clinical trial in healthy volunteers treated with subanesthetic doses of the NMDA receptor antagonist ketamine (Curic et al., 2019).

The MK-801-induced increase in basal gamma power reported here has also been previously described in mice (Saunders et al., 2012; Hiyoshi et al., 2014) and is thought to represent electrophysiological “noise” that impairs detection and discrimination of transient stimuli or event-related changes in gamma oscillations (Sivarao, 2015; Schuelert et al., 2018). Patients with schizophrenia display similar abnormal increases in basal gamma power (Grent-’t-Jong et al., 2018) as well as deficits in evoked gamma power (Ferrarelli et al., 2008). It should be noted that in the present study, treatment with MK-801 only had an effect on ASSR evoked gamma power in PFC but not in the double-click paradigm. This is in contrast to previous preclinical studies, which have demonstrated an MK-801-induced reduction in evoked gamma power in addition to increased basal gamma power (Saunders et al., 2012; Sokolenko et al., 2019). This disparity may again be attributable to differences in the doses of MK-801 administered; in the present study mice received a 0.15-mg/kg dose of MK-801, whereas in previous studies the reduction in evoked gamma was observed at higher doses of MK-801 (0.3–1 mg/kg) (Saunders et al., 2012; Sokolenko et al., 2019).

Iclepertin showed a relatively consistent pharmacokinetic (PK) exposure-effect relationship in the current study. In all of the models used, efficacy could be achieved at CSF concentrations in the range of approximately 1- to 6-fold of the previously reported IC_{50} for inhibition of GlyT1 by iclepertin (Rosenbrock et al., 2018), and efficacy on working memory in the T-maze task was achieved even at lower iclepertin exposure levels. These findings are consistent with the results of phase I and II clinical trials (Moschetti et al., 2018a,b; Rosenbrock et al., 2018). In fact, data from a phase I study on functional target engagement in healthy volunteers demonstrated that after a single oral dose of iclepertin, CSF glycine was increased by 50%–60% at iclepertin CSF concentrations approximately equal to the GlyT1 IC_{50} (Rosenbrock et al., 2018). Furthermore, in the recent phase II trial in patients with schizophrenia, iclepertin improved cognition after 12 weeks of treatment at 10 mg and 25 mg doses (Fleischhacker et al., 2021), corresponding to mean steady-state postdose plasma concentrations of 246 and 529 nM (Fleischhacker et al., 2021). Based on the previously reported human CSF/plasma ratio of approximately 0.08 (Rosenbrock et al., 2018), patients exhibited improvements in cognitive

function at estimated icleptin CSF concentrations in the range of 3.5- to 8-fold of the GlyT1 IC₅₀. Thus, the PK exposure-effect relationship in the rodent cognition tasks described here and the previously reported evidence of functional target engagement (Rosenbrock et al., 2018) (i.e., glycine increase in CSF) are in agreement with the PK exposure-effect relationship underlying cognitive improvement in patients with schizophrenia.

Overall, this study demonstrates EEG disturbances in animal models of NMDA receptor hypofunction, indicative of neural network dysfunction similar to those observed among patients with schizophrenia. Our data in this study demonstrate that icleptin can reverse sensory and neural network deficits related to NMDA receptor hypofunction, indicating its potential for further development as a viable treatment option for CIAS. GlyT1 inhibitors have previously been shown to reverse MK-801-induced deficits in EEG parameters related to lower-frequency oscillations (Depoortère et al., 2005). To our knowledge the present study is the first to report positive effects of a GlyT1 inhibitor on AERPs in an animal model related to schizophrenia, evaluating basal and evoked gamma oscillations and ASSR.

An advantage of exploring the effects of treatment on EEG biomarkers in animal models is that for many relevant biomarkers, homologous or analogous processes can be studied in humans, meaning that EEG data are readily translatable (Javitt et al., 2008). Indeed, our findings are largely consistent with known deficits in patients with schizophrenia, supporting the clinical translatability of these EEG parameters as neurophysiological biomarkers of CIAS (Javitt et al., 2008; Light and Swerdlow, 2015; Kim et al., 2020; Light et al., 2020).

One potential limitation of this study is that the animal models used in this study do not capture all aspects of schizophrenia but instead reflect certain key features of the behavioral phenotype and pathophysiology associated with schizophrenia. Nonetheless, previous studies have shown that MK-801 administration in rodents induces a range of schizophrenia-like behavioral phenotypes, including hyper-locomotor activity, reduced sensorimotor gating, deficits in gamma oscillations and ASSR, decreased social interaction, and cognitive impairments including learning and memory deficits, supporting the validity of this model for drug development in schizophrenia (Bubeníková-Valesová et al., 2008; Svoboda et al., 2015; Schuelert et al., 2018; Lee and Zhou, 2019; Mabunga et al., 2019). It should be noted, however, that the current study assessed gamma oscillations at a higher frequency (basal: 50–100 Hz; evoked: 35–80 Hz) since the effects of MK-801 in rodents are most prominent in this frequency range (Ahnaou et al., 2017; Schuelert et al., 2018) compared with the lower frequencies (< 50 Hz) that are typically analyzed to explore deficits in patients with schizophrenia (Ferrarelli et al., 2008; Grent-*t*-Jong et al., 2018).

In conclusion, this study provides additional evidence for the role of NMDA receptor hypofunction in neural network dysfunction and sensory processing deficits relevant to CIAS. Our data also demonstrate that the GlyT1 inhibitor icleptin can reverse deficits in clinically relevant EEG parameters relating to sensory processing and neural network activity. In addition, the reported data demonstrate that icleptin has pro-cognitive effects in learning and memory tasks in rodents. The preclinical efficacy demonstrated in this study provides solid support for the development of icleptin, which is currently being tested in phase III trials [Connex-1 (NCT04846868), Connex-2

(NCT04846881), and Connex-3 (NCT04860830)] as a potential treatment of CIAS.

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Authorship Contributions

Participated in research design: Rosenbrock, Dorner-Ciossek, Giovannini, Schmid, Schuelert.

Conducted experiments: Schuelert.

Contributed new reagents or analytic tools: Rosenbrock, Giovannini, Schmid, Schuelert.

Performed data analysis: Rosenbrock, Giovannini, Schmid, Schuelert.

Wrote or contributed to the writing of the manuscript: Rosenbrock, Dorner-Ciossek, Giovannini, Schmid, Schuelert.

References

- Aguilar DD, Radzik LK, Schifffo FL, Folorunso OO, Zielinski MR, Coyle JT, Balu DT, and McNally JM (2021) Altered neural oscillations and behavior in a genetic mouse model of NMDA receptor hypofunction. *Sci Rep* 11:9031.
- Ahnaou A, Broadbelt T, Biermans R, Huysmans H, Manyakov NV, and Drinkenburg WHIM (2020) The phosphodiesterase-4 and glycine transporter-1 inhibitors enhance in vivo hippocampal theta network connectivity and synaptic plasticity, whereas D-serine does not. *Transl Psychiatry* 10:197.
- Ahnaou A, Huysmans H, Van de Castelee T, and Drinkenburg WHIM (2017) Cortical high gamma network oscillations and connectivity: a translational index for antipsychotics to normalize aberrant neurophysiological activity. *Transl Psychiatry* 7:1285.
- Ahveninen J, Jääskeläinen IP, Osipova D, Huttunen MO, Ilmoniemi RJ, Kaprio J, Lönnqvist J, Manninen M, Pakarinen S, Therman S, et al. (2006) Inherited auditory-cortical dysfunction in twin pairs discordant for schizophrenia. *Biol Psychiatry* 60:612–620.
- Alberati D, Moreau JL, Lengyel J, Hauser N, Mory R, Borroni E, Pinard E, Knoflach F, Schlotterbeck G, Hainzl D, et al. (2012) Glycine reuptake inhibitor RG1678: a pharmacologic characterization of an investigational agent for the treatment of schizophrenia. *Neuropharmacology* 62:1152–1161.
- Beneyto M and Meador-Woodruff JH (2008) Lamina-specific abnormalities of NMDA receptor-associated postsynaptic protein transcripts in the prefrontal cortex in schizophrenia and bipolar disorder. *Neuropsychopharmacology* 33:2175–2186.
- Bickel S and Javitt DC (2009) Neurophysiological and neurochemical animal models of schizophrenia: focus on glutamate. *Behav Brain Res* 204:352–362.
- Bonner-Jackson A, Haut K, Csernansky JG, and Barch DM (2005) The influence of encoding strategy on episodic memory and cortical activity in schizophrenia. *Biol Psychiatry* 58:47–55.
- Bubeníková-Valesová V, Horáček J, Vrajová M, and Höschl C (2008) Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. *Neurosci Biobehav Rev* 32:1014–1023.
- Cho RY, Konecky RO, and Carter CS (2006) Impairments in frontal cortical gamma synchrony and cognitive control in schizophrenia. *Proc Natl Acad Sci USA* 103:19878–19883.
- Cioffi CL and Guzzo PR (2016) Inhibitors of glycine transporter-1: potential therapeutics for the treatment of CNS disorders. *Curr Top Med Chem* 16:3404–3437.
- Cohen SM, Tsien RW, Goff DC, and Halassa MM (2015) The impact of NMDA receptor hypofunction on GABAergic neurons in the pathophysiology of schizophrenia. *Schizophr Res* 167:98–107.
- Collingridge GL, Volianskis A, Bannister N, France G, Hanna L, Mercier M, Tidball P, Fang G, Irvine MW, Costa BM, et al. (2013) The NMDA receptor as a target for cognitive enhancement. *Neuropharmacology* 64:13–26.
- Curic S, Leicht G, Thiebes S, Andreou C, Polomac N, Eichler IC, Eichler L, Zöllner C, Gallinat J, Steinmann S, et al. (2019) Reduced auditory evoked gamma-band response and schizophrenia-like clinical symptoms under subanesthetic ketamine. *Neuropsychopharmacology* 44:1239–1246.
- Dauvermann MR, Lee G, and Dawson N (2017) Glutamatergic regulation of cognition and functional brain connectivity: insights from pharmacological, genetic and translational schizophrenia research. *Br J Pharmacol* 174:3136–3160.
- Depoortère R, Dargazanli G, Estenne-Bouhtou G, Coste A, Lanneau C, Desvignes C, Poncelet M, Heaulme M, Santucci V, Decobert M, et al. (2005) Neurochemical,

- electrophysiological and pharmacological profiles of the selective inhibitor of the glycine transporter-1 SSR504734, a potential new type of antipsychotic. *Neuropsychopharmacology* **30**:1963–1985.
- Deserno L, Sterzer P, Wüstenberg T, Heinz A, and Schlagenhaut F (2012) Reduced prefrontal-parietal effective connectivity and working memory deficits in schizophrenia. *J Neurosci* **32**:12–20.
- Du X, Choa FS, Summerfelt A, Rowland LM, Chiappelli J, Kochunov P, and Hong LE (2017) N100 as a generic cortical electrophysiological marker based on decomposition of TMS-evoked potentials across five anatomic locations. *Exp Brain Res* **235**:69–81.
- Ferrarelli F, Massimini M, Peterson MJ, Riedner BA, Lazar M, Murphy MJ, Huber R, Rosanova M, Alexander AL, Kalin N, et al. (2008) Reduced evoked gamma oscillations in the frontal cortex in schizophrenia patients: a TMS/EEG study. *Am J Psychiatry* **165**:996–1005.
- Fleischhacker WW, Podhorna J, Gröschl M, Hake S, Zhao Y, Huang S, Keefe RSE, Desch M, Brenner R, Walling DP, et al. (2021) Efficacy and safety of the novel glycine transporter inhibitor BI 425809 once daily in patients with schizophrenia: a double-blind, randomised, placebo-controlled phase 2 study. *Lancet Psychiatry* **8**:191–201.
- Force RB, Venables NC, and Sponheim SR (2008) An auditory processing abnormality specific to liability for schizophrenia. *Schizophr Res* **103**:298–310.
- Forsyth JK and Lewis DA (2017) Mapping the consequences of impaired synaptic plasticity in schizophrenia through development: an integrative model for diverse clinical features. *Trends Cogn Sci* **21**:760–778.
- Foxe JJ, Yeap S, Snyder AC, Kelly SP, Thakore JH, and Molholm S (2011) The N1 auditory evoked potential component as an endophenotype for schizophrenia: high-density electrical mapping in clinically unaffected first-degree relatives, first-episode, and chronic schizophrenia patients. *Eur Arch Psychiatry Clin Neurosci* **261**:331–339.
- Fromer M, Pocklington AJ, Kavanagh DH, Williams HJ, Dwyer S, Gormley P, Georgieva L, Rees E, Palta P, Ruderfer DM, et al. (2014) De novo mutations in schizophrenia implicate synaptic networks. *Nature* **506**:179–184.
- Gao WJ, Yang SS, Mack NR, and Chamberlin LA (2022) Aberrant maturation and connectivity of prefrontal cortex in schizophrenia—contribution of NMDA receptor development and hypofunction. *Mol Psychiatry* **27**:731–743.
- Gonzalez-Burgos G and Lewis DA (2012) NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. *Schizophr Bull* **38**:950–957.
- Gonzalez-Heydrich J, Bosquet Enlow M, D'Angelo E, Seidman LJ, Gumlak S, Kim A, Woodberry KA, Rober A, Tembulkar S, Graber K, et al. (2015) Early auditory processing evoked potentials (N100) show a continuum of blunting from clinical high risk to psychosis in a pediatric sample. *Schizophr Res* **169**:340–345.
- Grent-t-Jong T, Gross J, Goense J, Wibral M, Gajwani R, Gumley AI, Lawrie SM, Schwannauer M, Schultze-Lutter F, Navarro Schröder T, et al. (2018) Resting-state gamma-band power alterations in schizophrenia reveal E/I-balance abnormalities across illness-stages. *eLife* **7**:e37799.
- Guo JY, Ragland JD, and Carter CS (2019) Memory and cognition in schizophrenia. *Mol Psychiatry* **24**:633–642.
- Hakami T, Jones NC, Tolmacheva EA, Gaudias J, Chaumont J, Salzberg M, O'Brien TJ, and Pinault D (2009) NMDA receptor hypofunction leads to generalized and persistent aberrant gamma oscillations independent of hyperlocomotion and the state of consciousness. *PLoS One* **4**:e6755.
- Harvey RJ and Yee BK (2013) Glycine transporters as novel therapeutic targets in schizophrenia, alcohol dependence and pain. *Nat Rev Drug Discov* **12**:866–885.
- Hirano Y, Oribe N, Kamba S, Onitsuka T, Nestor PG, and Spencer KM (2015) Spontaneous gamma activity in schizophrenia. *JAMA Psychiatry* **72**:813–821.
- Hiyoshi T, Kambe D, Karasawa J, and Chaki S (2014) Involvement of glutamatergic and GABAergic transmission in MK-801-increased gamma band oscillation power in rat cortical electroencephalograms. *Neuroscience* **280**:262–274.
- Hong LE, Summerfelt A, Buchanan RW, O'Donnell P, Thaker GK, Weiler MA, and Lahti AC (2010) Gamma and delta neural oscillations and association with clinical symptoms under subanesthetic ketamine. *Neuropsychopharmacology* **35**:632–640.
- Javitt DC and Freedman R (2015) Sensory processing dysfunction in the personal experience and neuronal machinery of schizophrenia. *Am J Psychiatry* **172**:17–31.
- Javitt DC, Spencer KM, Thaker GK, Winterer G, and Hajós M (2008) Neurophysiological biomarkers for drug development in schizophrenia. *Nat Rev Drug Discov* **7**:68–83.
- Joos K, Gilles A, Van de Heyning P, De Ridder D, and Vanneste S (2014) From sensation to percept: the neural signature of auditory event-related potentials. *Neurosci Biobehav Rev* **42**:148–156.
- Kim HK, Blumberger DM, and Daskalakis ZJ (2020) Neurophysiological biomarkers in schizophrenia-P50, mismatch negativity, and TMS-EMG and TMS-EEG. *Front Psychiatry* **11**:795.
- Kirov G, Pocklington AJ, Holmans P, Ivanov D, Ikeda M, Ruderfer D, Moran J, Chambert K, Toncheva D, Georgieva L, et al. (2012) De novo CNV analysis implicates specific abnormalities of postsynaptic signalling complexes in the pathogenesis of schizophrenia. *Mol Psychiatry* **17**:142–153.
- Kondrakiewicz K, Kostecki M, Szadzińska W, and Knapska E (2019) Ecological validity of social interaction tests in rats and mice. *Genes Brain Behav* **18**:e12525.
- Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers Jr MB, and Charney DS (1994) Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* **51**:199–214.
- Lane HY, Lin CH, Huang YJ, Liao CH, Chang YC, and Tsai GE (2010) A randomized, double-blind, placebo-controlled comparison study of sarcosine (N-methylglycine) and D-serine add-on treatment for schizophrenia. *Int J Neuropsychopharmacol* **13**:451–460.
- Lee G and Zhou Y (2019) NMDAR hypofunction animal models of schizophrenia. *Front Mol Neurosci* **12**:185.
- Light GA, Joshi YB, Molina JL, Bhakta SG, Nungaray JA, Cardoso L, Kotz JE, Thomas ML, and Swerdlow NR (2020) Neurophysiological biomarkers for schizophrenia therapeutics. *Biomarkers in Neuropsychiatry* **2**:100012 DOI: 10.1016/j.bionps.2020.100012.
- Light GA and Swerdlow NR (2015) Future clinical uses of neurophysiological biomarkers to predict and monitor treatment response for schizophrenia. *Ann N Y Acad Sci* **1344**:105–119.
- Lisman J (2012) Excitation, inhibition, local oscillations, or large-scale loops: what causes the symptoms of schizophrenia? *Curr Opin Neurobiol* **22**:537–544.
- Loidice S, Drinkenburg WH, Ahnaou A, McCarthy A, Viardot G, Cayre E, Rion B, Bertaina-Anglade V, Mano M, L'Hostis P, et al. (2021) Mismatch negativity as EEG biomarker supporting CNS drug development: a transnomic and translational study. *Transl Psychiatry* **11**:253.
- Mabunga DFN, Park D, Ryu O, Valencia ST, Adil KJL, Kim S, Kwon KJ, Shin CY, and Jeon SJ (2019) Recapitulation of neuropsychiatric behavioral features in mice using acute low-dose MK-801 administration. *Exp Neurol* **28**:697–708.
- Marques BL, Oliveira-Lima OC, Carvalho GA, de Almeida Chiarelli R, Ribeiro RI, Parreira RC, da Madeira Freitas EM, Resende RR, Klempin F, Ulrich H, et al. (2020) Neurobiology of glycine transporters: from molecules to behavior. *Neurosci Biobehav Rev* **118**:97–110.
- Moghaddam B and Krystal JH (2012) Capturing the angel in “angel dust”: twenty years of translational neuroscience studies of NMDA receptor antagonists in animals and humans. *Schizophr Bull* **38**:942–949.
- Molina JL, Thomas ML, Joshi YB, Hochberger WC, Koshiyama D, Nungaray JA, Cardoso L, Sprock J, Braff DL, Swerdlow NR, et al. (2020) Gamma oscillations predict pro-cognitive and clinical response to auditory-based cognitive training in schizophrenia. *Transl Psychiatry* **10**:405.
- Moschetti V, Desch M, Goetz S, Liesenfeld KH, Rosenbrock H, Kammerer KP, Wunderlich G, and Wind S (2018a) Safety, tolerability and pharmacokinetics of oral BI 425809, a glycine transporter 1 inhibitor, in healthy male volunteers: a partially randomised, single-blind, placebo-controlled, first-in-human study. *Eur J Drug Metab Pharmacokinet* **43**:239–249.
- Moschetti V, Schlecker C, Wind S, Goetz S, Schmitt H, Schultz A, Liesenfeld KH, Wunderlich G, and Desch M (2018b) Multiple rising doses of oral BI 425809, a GlyT1 inhibitor, in young and elderly healthy volunteers: a randomised, double-blind, phase I study investigating safety and pharmacokinetics. *Clin Drug Invest* **38**:737–750.
- Mota A, Tomé D, Campos C, and Rocha N (2020) The auditory N1 in schizophrenia: comparative analysis with a monoaural stimuli paradigm. *Psychiatr Danub* **32**:210–213.
- Mulert C, Kirsch V, Pascual-Marqui R, McCarley RW, and Spencer KM (2011) Long-range synchrony of γ oscillations and auditory hallucination symptoms in schizophrenia. *Int J Psychophysiol* **79**:55–63.
- O'Donnell BF, Vohs JL, Krishnan GP, Rass O, Hetrick WP, and Morzorti SL (2013) The auditory steady-state response (ASSR): a translational biomarker for schizophrenia. *Suppl Clin Neurophysiol* **62**:101–112.
- Pei JC, Luo DZ, Gau SS, Chang CY, and Lai WS (2021) Directly and indirectly targeting the glycine modulatory site to modulate NMDA receptor function to address unmet medical needs of patients with schizophrenia. *Front Psychiatry* **12**:742058.
- Pinard E, Borroni E, Koerner A, Umbricht D, and Alberati D (2018) Glycine transporter type I (GlyT1) inhibitor, bitopertin: a journey from lab to patient. *Chimia (Aarau)* **72**:477–484.
- Pinault D (2008) N-methyl d-aspartate receptor antagonists ketamine and MK-801 induce wake-related aberrant gamma oscillations in the rat neocortex. *Biol Psychiatry* **63**:730–735.
- Rosburg T, Boutros NN, and Ford JM (2008) Reduced auditory evoked potential component N100 in schizophrenia—a critical review. *Psychiatry Res* **161**:259–274.
- Rosen AM, Spellman T, and Gordon JA (2015) Electrophysiological endophenotypes in rodent models of schizophrenia and psychosis. *Biol Psychiatry* **77**:1041–1049.
- Rosenbrock H, Desch M, Kleiner O, Dorner-Ciossek C, Schmid B, Keller S, Schlecker C, Moschetti V, Goetz S, Liesenfeld KH, et al. (2018) Evaluation of pharmacokinetics and pharmacodynamics of BI 425809, a novel GlyT1 inhibitor: translational studies. *Clin Transl Sci* **11**:616–623.
- Rosenbrock H, Giovannini R, Schänzle G, Koros E, Runge F, Fuchs H, Marti A, Reymann KG, Schröder UH, Fedele E, et al. (2019) The novel phosphodiesterase 9A inhibitor BI 409306 increases cyclic guanosine monophosphate levels in the brain, promotes synaptic plasticity, and enhances memory function in rodents. *J Pharmacol Exp Ther* **371**:633–641.
- Saunders JA, Gandai MJ, and Siegel SJ (2012) NMDA antagonists recreate signal-to-noise ratio and timing perturbations present in schizophrenia. *Neurobiol Dis* **46**:93–100.
- Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014) Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**:421–427.
- Schoonover KE, Diemel SJ, and Lewis DA (2020) Prefrontal cortical alterations of glutamate and GABA neurotransmission in schizophrenia: insights for rational biomarker development. *Biomark Neuropsychiatry* **3**:100015.
- Schuelert N, Dorner-Ciossek C, Brendel M, and Rosenbrock H (2018) A comprehensive analysis of auditory event-related potentials and network oscillations in an NMDA receptor antagonist mouse model using a novel wireless recording technology. *Physiol Rep* **6**:e13782.
- Shen CL, Chou TL, Lai WS, Hsieh MH, Liu CM, Liu CM, and Hwu HG (2020) P50, N100, and P200 auditory sensory gating deficits in schizophrenia patients. *Front Psychiatry* **11**:868.
- Sinkevičiute I, Begemann M, Prikken M, Oranje B, Johnsen E, Lei WU, Hugdahl K, Kroken RA, Rau C, Jacobs JD, et al. (2018) Efficacy of different types of cognitive enhancers for patients with schizophrenia: a meta-analysis. *NPJ Schizophr* **4**:22.
- Sivarao DV (2015) The 40-Hz auditory steady-state response: a selective biomarker for cortical NMDA function. *Ann N Y Acad Sci* **1344**:27–36.
- Sokolenko E, Hudson MR, Nithianantharajah J, and Jones NC (2019) The mGluR_{2/3} agonist LY379268 reverses NMDA receptor antagonist effects on cortical gamma oscillations and phase coherence, but not working memory impairments, in mice. *J Psychopharmacol* **33**:1588–1599.

- Spencer KM, Niznikiewicz MA, Nestor PG, Shenton ME, and McCarley RW (2009) Left auditory cortex gamma synchronization and auditory hallucination symptoms in schizophrenia. *BMC Neurosci* **10**:85.
- Spencer KM, Salisbury DF, Shenton ME, and McCarley RW (2008) Gamma-band auditory steady-state responses are impaired in first episode psychosis. *Biol Psychiatry* **64**:369–375.
- Stefanescu RA and Shore SE (2015) NMDA receptors mediate stimulus-timing-dependent plasticity and neural synchrony in the dorsal cochlear nucleus. *Front Neural Circuits* **9**:75.
- Svoboda J, Stankova A, Entlerova M, and Stuchlik A (2015) Acute administration of MK-801 in an animal model of psychosis in rats interferes with cognitively demanding forms of behavioral flexibility on a rotating arena. *Front Behav Neurosci* **9**:75.
- Tada M, Nagai T, Kirihaara K, Koike S, Suga M, Araki T, Kobayashi T, and Kasai K (2016) Differential alterations of auditory gamma oscillatory responses between pre-onset high-risk individuals and first-episode schizophrenia. *Cereb Cortex* **26**:1027–1035.
- Tanaka-Koshiyama K, Koshiyama D, Miyakoshi M, Joshi YB, Molina JL, Sprock J, Braff DL, and Light GA (2020) Abnormal spontaneous gamma power is associated with verbal learning and memory dysfunction in schizophrenia. *Front Psychiatry* **11**:832.
- Thuné H, Recasens M, and Uhlhaas PJ (2016) The 40-Hz auditory steady-state response in patients with schizophrenia: a meta-analysis. *JAMA Psychiatry* **73**:1145–1153.
- Tsai G, Lane HY, Yang P, Chong MY, and Lange N (2004) Glycine transporter 1 inhibitor, N-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry* **55**:452–456.
- Uhlhaas PJ and Singer W (2010) Abnormal neural oscillations and synchrony in schizophrenia. *Nat Rev Neurosci* **11**:100–113.
- Uno Y and Coyle JT (2019) Glutamate hypothesis in schizophrenia. *Psychiatry Clin Neurosci* **73**:204–215.
- van der Staay FJ, Rutten K, Bärfacker L, Devry J, Erb C, Heckroth H, Karthaus D, Tersteegen A, van Kampen M, Blokland A, et al. (2008) The novel selective PDE9 inhibitor BAY 73-6691 improves learning and memory in rodents. *Neuropharmacology* **55**:908–918.
- Voehringer P, Schuelert N, Plano A, Dorner-Ciossek C, and Rosenbrock H (2019) Effects of the glycine transporter-1 inhibitor bitopertin on rodent electroencephalography and auditory event-related potential biomarkers related to schizophrenia. *Eur Neuropsychopharmacol* **29** (Suppl 6):S390 DOI: 10.1016/j.euroneuro.2019.09.558.
- Vrajobá M, Stastný F, Horáček J, Lochman J, Serý O, Peková S, Klaschka J, and Höschl C (2010) Expression of the hippocampal NMDA receptor GluN1 subunit and its splicing isoforms in schizophrenia: postmortem study. *Neurochem Res* **35**:994–1002.
- Wilson TW, Hernandez OO, Asherin RM, Teale PD, Reite ML, and Rojas DC (2008) Cortical gamma generators suggest abnormal auditory circuitry in early-onset psychosis. *Cereb Cortex* **18**:371–378.
- Wu K, Castellano D, Tian Q, and Lu W (2021) Distinct regulation of tonic GABAergic inhibition by NMDA receptor subtypes. *Cell Rep* **37**:109960.
- Zafra F and Giménez C (2008) Glycine transporters and synaptic function. *IUBMB Life* **60**:810–817.

Address correspondence to: Dr. Holger Rosenbrock, Boehringer Ingelheim Pharma GmbH & Co. KG, Birkendorfer Str 65, 88400, Biberach an der Riss, Germany. E-mail: holger.rosenbrock@boehringer-ingenlheim.com

Supplement Figure 1

