

# Low Doses of Ethanol Enhance LTD-like Plasticity in Human Motor Cortex

Anna Fuhl<sup>1</sup>, Florian Müller-Dahlhaus<sup>1,2</sup>, Caroline Lücke<sup>1,3</sup>, Stefan W Toennes<sup>4</sup> and Ulf Ziemann<sup>\*,1,2</sup>

<sup>1</sup>Department of Neurology, Goethe-University, Frankfurt am Main, Germany; <sup>2</sup>Department of Neurology & Stroke, and Hertie Institute for Clinical Brain Research, Eberhard Karls University, Tübingen, Germany; <sup>3</sup>Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Goethe-University, Frankfurt am Main, Germany; <sup>4</sup>Department of Forensic Toxicology, Goethe-University, Frankfurt am Main, Germany

Humans liberally use ethanol for its facilitating effects on social interactions but its effects on central nervous system function remain underexplored. We have recently described that very low doses of ethanol abolish long-term potentiation (LTP)-like plasticity in human cortex, most likely through enhancement of tonic inhibition [Lücke *et al*, 2014, *Neuropsychopharmacology* 39:1508–18]. Here, we studied the effects of low-dose ethanol on long-term depression (LTD)-like plasticity. LTD-like plasticity was induced in human motor cortex by paired associative transcranial magnetic stimulation (PAS<sub>LTD</sub>), and measured as decreases of motor evoked potential input-output curve (IO-curve). In addition, sedation was measured by decreases in saccade peak velocity (SPV). Ethanol in two low doses (EtOH<sub><10mM</sub>, EtOH<sub><20mM</sub>) was compared to single oral doses of alprazolam (APZ, 1mg) a classical benzodiazepine, and zolpidem (ZLP, 10 mg), a non-benzodiazepine hypnotic, in a double-blinded randomized placebo-controlled crossover design in ten healthy human subjects. EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub> but not APZ or ZLP enhanced the PAS<sub>LTD</sub>-induced LTD-like plasticity, while APZ and ZLP but not EtOH<sub><10mM</sub> or EtOH<sub><20mM</sub> decreased SPV. Non-sedating low doses of ethanol, easily reached during social drinking, enhance LTD-like plasticity in human cortex. This effect is most likely explained by the activation of extrasynaptic  $\alpha 4$ -subunit containing gamma-aminobutyric type A receptors by low-dose EtOH, resulting in increased tonic inhibition. Findings may stimulate cellular research on the role of tonic inhibition in regulating excitability and plasticity of cortical neuronal networks.

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## INTRODUCTION

Humans liberally ingest ethanol (EtOH) at low doses during social drinking for its facilitating effects on personal interactions, but the effects of low doses of EtOH on central nervous system function remain underexplored. Cellular studies demonstrated that low-dose EtOH ( $\leq 30$  mM) enhances tonic inhibition mediated by the extrasynaptic  $\alpha 4$ -subunit containing gamma-aminobutyric acid type A receptor ( $\alpha 4$ -GABAAR) (Hanchar *et al*, 2006; Sundstrom-Poromaa *et al*, 2002; Wallner *et al*, 2003; Wei *et al*, 2004) although not all studies were able to replicate this finding (Borghese *et al*, 2006; Yamashita *et al*, 2006). In addition, low doses of EtOH inhibit N-methyl-D-aspartate receptor (NMDAR)-mediated currents (He *et al*, 2013; Lovinger *et al*, 1989, 1990; Weitlauf and Woodward, 2008).

We have recently reported that non-sedating very low doses of EtOH (<10mM) but not the classical

benzodiazepine alprazolam (APZ), an allosteric positive modulator at synaptic  $\alpha 1$ -,  $\alpha 2$ -,  $\alpha 3$ - and  $\alpha 5$ -GABAARs, and zolpidem (ZLP), a non-benzodiazepine hypnotic with high affinity at the  $\alpha 1$ -GABAAR disrupt long-term potentiation (LTP)-like plasticity induced by paired associative transcranial magnetic stimulation (PAS<sub>LTP</sub>) in human motor cortex (Lücke *et al*, 2014). Similar to LTP, long-term depression (LTD) is thought to play important roles in learning and memory formation (Collingridge *et al*, 2010), but LTD has been much less investigated compared to LTP. EtOH effects on LTD in slice preparations have been very variable, depending on the experimental model. EtOH increased (Hendricson *et al*, 2002) or decreased (Izumi *et al*, 2005) LTD in the hippocampus, led to a switch from LTP to LTD in the dorsal striatum (Yin *et al*, 2007), and decreased LTD in the cerebellum (Belmeguenai *et al*, 2008; Carta *et al*, 2006; He *et al*, 2013; Su *et al*, 2010). In addition, relatively scant evidence showed that enhancement of synaptic GABAergic neurotransmission increased LTD (Choi *et al*, 2002), while its blockade by GABAAR antagonists disrupted LTD (Nishiyama *et al*, 2010; Watanabe *et al*, 2007; Zhu *et al*, 2013), or even resulted in an LTD to LTP shift (Hess and Donoghue, 1996).

At the systems level of human motor cortex, paired associative stimulation (PAS<sub>LTD</sub>) can induce LTD-like plasticity,

\*Correspondence: Professor U Ziemann, Department of Neurology & Stroke, and Hertie Institute for Clinical Brain Research, Eberhard Karls University Tübingen, Hoppe-Seyley-Str. 3, Tübingen, D-72076, Germany, Tel: +49 7071 2982049, Fax: +49 7071 295260,

E-mail: ulf.ziemann@uni-tuebingen.de

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as measured by a decrease in motor evoked potential (MEP) amplitude that outlasts the PAS<sub>LTD</sub> intervention (Huber *et al*, 2008; Müller *et al*, 2007; Rosenkranz *et al*, 2014; Wolters *et al*, 2003). Here we thought to investigate the effects of enhancing extrasynaptic GABAergic inhibition (two low doses of EtOH: <10 mM, <20 mM) vs enhancing synaptic GABAergic inhibition (single oral doses of 1mg of APZ, and 10 mg of ZLP) on PAS<sub>LTD</sub>-induced LTD-like plasticity. We quantified  $\alpha$ 1-GABAAR-mediated sedation by measuring peak velocity of visually guided saccades (SPV) (de Visser *et al*, 2003; Lücke *et al*, 2014).

Given our previous findings on PAS<sub>LTP</sub>-induced LTP-like plasticity (Lücke *et al*, 2014), we hypothesized drug-induced enhancement of PAS<sub>LTD</sub>-induced LTD-like plasticity, predominantly by low-dose EtOH but not or to a lesser extent by APZ and ZLP.

In accordance with the hypothesis, we show that low-dose EtOH but not APZ or ZLP enhanced the PAS<sub>LTD</sub>-induced LTD-like plasticity, while APZ and ZLP but not low-dose EtOH decreased SPV. We discuss carefully that this double dissociation implicates increased tonic inhibition through activation of extrasynaptic  $\alpha$ 4-GABAARs by EtOH as the most likely mechanism for the enhancement of LTD-like plasticity. Findings may stimulate further cellular research on the role of tonic inhibition in regulating excitability and plasticity of cortical neuronal networks.

## MATERIALS AND METHODS

### Subjects

Twelve healthy subjects (mean ( $\pm$ SD) age 24.6  $\pm$  3.95 years, range 19–31 years; body length, 181.5  $\pm$  7.89 cm, range 170–195 cm; body weight, 78.5  $\pm$  10.84 kg, range 60–99 kg; 3 female) participated in the study. Two subjects did not complete the study due to adverse effects under ZLP (nausea, vomiting). The presented data are therefore based on ten subjects. All subjects fulfilled the criteria according to a TMS safety questionnaire (Keel *et al*, 2001), had no history of neurological or psychiatric disease and were non-smokers as nicotine use alters PAS-induced LTD-like plasticity (Thirugnanasambandam *et al*, 2011). Subjects did not take any CNS-active drugs. This was verified by comprehensive urine analysis prior to each experimental session. An additional inclusion criterion for females was the use of a hormonal method of contraception to prevent hormonal fluctuations during menstrual cycle, which might affect motor cortical excitability (Smith *et al*, 2002; Smith *et al*, 1999). All subjects were right-handed as tested by the Edinburgh handedness questionnaire (Oldfield, 1971).

PAS-induced LTD-like plasticity is not expressed in all subjects. Therefore, to identify subjects suitable for this study, volunteers were screened in a session at least 1 week prior to the main study for a resting motor threshold (RMT)  $\leq$  50% of maximum stimulator output (Müller-Dahlhaus *et al*, 2008) and a significant PAS-induced decrease in MEP amplitude <1.0 (ratio of MEP amplitude post-PAS/pre-PAS) (Delvendahl *et al*, 2013; Voytovich *et al*, 2012).

All subjects gave their written informed consent prior to participation. The study was approved by the ethics committee of the Medical Faculty of the Goethe-University

of Frankfurt and conformed to the latest version of the Declaration of Helsinki.

### EMG Recordings

All motor evoked potential (MEP) measurements were conducted by surface electromyography (EMG) from the resting abductor pollicis brevis (APB) muscle of the dominant right hand using Ag-AgCl cup electrodes in a belly-tendon montage, while subjects were seated in a comfortable chair with their arms and hands lying relaxed on the armrests. EMG raw signals were amplified (Digitimer D360, Digitimer Ltd., Welwyn Garden City, UK), bandpass filtered (20 Hz - 2 kHz), digitized at a rate of 5kHz (CED Micro 1401, Cambridge Electronic design, Cambridge, UK) and stored on a laboratory computer. Spike2 v.3.05 software (Cambridge Electronic design, Cambridge, UK) was used for control of the experiment, data recording, and online and offline data analysis. All recordings were performed at rest. Target muscle relaxation was controlled by audio-visual feedback of the raw EMG at high gain (50  $\mu$ V/Div).

### Stimulation Procedures

Focal transcranial magnetic stimulation (TMS) was delivered to the hand area of the left primary motor cortex (M1) using a figure-of-eight stimulating coil (diameter of each wing, 70 mm) and a monophasic current waveform provided by a Magstim, 2002 magnetic stimulator (Magstim Company, Whitland, Wales, UK). The junction of the stimulating coil was positioned tangentially to the skull, with the handle pointing backwards approximately 45° away from the sagittal plane, to induce a current flow in the brain directed from lateral-posterior to medial-anterior. This way, the cortico-spinal system is excited predominantly trans-synaptically via horizontal cortico-cortical connections (Di Lazzaro *et al*, 2008). The coil was placed over the hot spot, defined as the optimal site over the left M1 to elicit consistently largest MEPs in the right APB at a slightly suprathreshold stimulation intensity. This coil position was marked on the scalp with a pen for correct placement of the coil throughout the experiment.

The individual resting motor threshold (RMT) was determined using a relative frequency method (Groppa *et al*, 2012), defined as the lowest stimulus intensity that elicited MEPs of  $\geq$  50  $\mu$ V in at least five out of ten consecutive trials in the relaxed APB. In addition, for each subject the individual minimum stimulation intensity to elicit a peak-to-peak MEP amplitude of 1mV ( $SI_{1mV}$ ) was determined. Stimulation intensity is reported as a percentage of the maximum stimulator output (%MSO).

Motor cortical excitability was assessed measuring input-output curves of MEPs (IO-curves). Eleven stimulation intensities were applied at stimulus intensities of 0.5 to 1.5  $\times$   $SI_{1mV}$  in steps of 0.1  $\times$   $SI_{1mV}$  (Lücke *et al*, 2014; Rosenkranz *et al*, 2007a). Eight trials per stimulation intensity were recorded in randomized order to avoid hysteresis effects (Möller *et al*, 2009). The single-trial peak-to-peak MEP amplitudes were analyzed and then averaged according to stimulation intensity. MEP IO-curves rather than MEP amplitude at one single stimulus intensity were

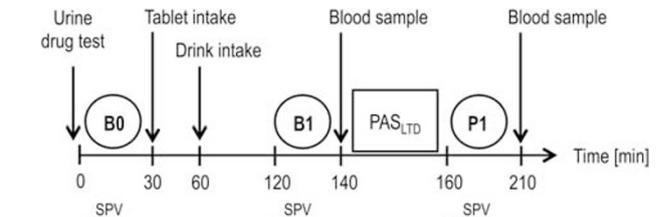
chosen as primary readout for drug-induced and PAS<sub>LTD</sub>-induced changes in corticospinal excitability for the following reasons: (1) Previous studies demonstrated that PAS-induced LTD-like plasticity was expressed predominantly in the high-intensity range of the IO-curve (Ni *et al*, 2014; Rosenkranz *et al*, 2007a; Rosenkranz *et al*, 2007b). (2) Different parts of the IO-curve represent different physiological mechanisms. While the low-intensity part represents relatively direct excitation of the corticospinal neurons (I1-wave) the high-intensity part reflects more indirect excitation through interneurons (late I-waves) (Di Lazzaro *et al*, 2008). Therefore, differential drug effects on selected parts of the IO-curve after PAS<sub>LTD</sub> would allow assignment of more specific mechanisms to the observed effects. (3) Finally, we wanted to be compatible with our previous research, where we also investigated IO-curves to study the effects of the same drugs as in the present study on PAS-induced LTP-like plasticity (Lücke *et al*, 2014).

### Paired Associative Stimulation (PAS<sub>LTD</sub>)

PAS<sub>LTD</sub> was applied according to an established protocol (Jung and Ziemann, 2009; Müller *et al*, 2007; Voytovich *et al*, 2012) to induce LTD-like plasticity in M1. In brief, electrical stimulation of the right median nerve followed by focal single-pulse TMS of the contralateral M1 was applied 225 times at a rate of 0.25 Hz with an interstimulus interval (ISI) of  $15.0 \pm 1.1$  ms (mean ( $\pm$  SD)). ISI was calculated based on the individual N20 latency, measured by median nerve somatosensory-evoked potentials, minus 5 ms (N20-5 ms). Electrical stimulation was delivered through a bipolar electrode (constant current, pulse duration 1.0 ms) with an intensity of three times the perceptual sensory threshold. To control the attention directed to the stimulated hand during PAS (Stefan *et al*, 2004), subjects were instructed to count the number of flashes emitted from an LED fixed at the right wrist.

### Saccadic Peak Velocity (SPV) Measurements

We measured visually guided saccadic peak velocity (SPV) (Velazquez-Perez *et al*, 2004) to assess drug-induced sedation. This is important, as we were concerned that sedation and a possible concomitant decrease in attention could have contributed to drug-related changes in PAS-induced LTD-like plasticity (Kamke *et al*, 2014). In addition, SPV is a behavioral marker of inhibition mediated through the  $\alpha$ 1-GABAAR (de Visser *et al*, 2003). Horizontal saccades were recorded by electronystagmography using Ag-AgCl surface electrodes over the outer canthus of each eye. Subjects were positioned in front of a screen (viewing distance, 0.9 m) and were instructed to make visually guided saccades in response to a white dot subtending an angle of view of  $1^\circ$  on a black background while the head was kept in straight position. The dot jumped at random intertrial intervals of 2–3 s (to limit anticipation of the next event) horizontally from one lateral edge to the opposite edge of the screen, subtending an angle of view of  $40^\circ$ . In each block 50 trials were recorded. Offline analysis was performed with MATLAB (version 6.1; Natick, MA) and customized software. Saccade onset and offset were identified manually,



**Figure 1** Timeline of experiments. Motor evoked potential input-output curves (IO-curve) and saccadic peak velocity (SPV) were measured at baseline (time point B0), post drug (time point B1) and post paired associative stimulation for induction of LTD-like plasticity (PAS<sub>LTD</sub>, time point P1). The study medication was administered immediately (tablet) and 30 min (drink) after B0. Post drug measurements at B1 were performed 60 min after drink intake. PAS<sub>LTD</sub> followed immediately after B1. Blood samples for measuring serum drug levels were taken at B1 and P1.

**Table 1** Scheme of Study Drugs in the Five Visits that were Randomized in Order Across Participants

Condition	Tablet	Drink
1	APZ (1 mg)	PBO
2	ZLP (10 mg)	PBO
3	PBO	EtOH (female, 0.25 g/kg, male 0.29 g/kg)
4	PBO	EtOH (female 0.47 g/kg, male 0.55 g/kg)
5	PBO	PBO

Abbreviations: APZ, alprazolam; EtOH, ethanol; PBO, placebo; ZLP, zolpidem. In each visit a tablet followed by a drink were administered, separated by 30 min (cf. Figure 1).

before applying a third-order polynomial fit to obtain SPV in  $^\circ$ /s.

### Pharmacokinetic Measurements

Pharmacokinetic analyses in blood and urine samples were performed at the Department of Forensic Toxicology, Goethe-University Frankfurt using chromatographic-mass spectrometry. Before each experiment, a urine drug screening was performed to test for CNS-active medical drugs and drugs of abuse. Blood plasma concentrations of the study medication were assessed immediately before (time point B1) and after (time point P1) PAS<sub>LTD</sub> (Figure 1).

### Experimental Design

We performed a randomized, double blind, double-dummy, placebo-controlled crossover study with five sessions (Figure 1). Study visits of a given participant were separated by at least 1 week. Experiments started with a urine drug test, followed by baseline (time point B0) measurements of SPV, RMT, SI<sub>1mV</sub> and MEP IO-curve. Study medication was administered as a tablet (immediately after the B0 measurements) and a drink (30 min later) (Table 1). Post drug measurements (SPV and IO-curve) were done 90 min after tablet intake and 60 min after drink intake (time point B1) to accommodate for the expected times of peak plasma

**Table 2** Pharmacokinetic Data of Tested Drugs

Drug	GABAAR BS	$t_{max}$ (h)	$t_{1/2}$ (h)	Single oral dose	Blood serum concentration	
					B1	P1
APZ	$\alpha 1, \alpha 2, \alpha 3, \alpha 5$	0.7–1.8	9–16	1 mg	$12.6 \pm 1.5$ ng/ml	$12.8 \pm 0.8$ ng/ml
ZLP	$\alpha 1 > \alpha 2 = \alpha 3$	0.75–2.6	0.7–3.5	10 mg	$110.0 \pm 16.8$ ng/ml	$100.8 \pm 18.6$ ng/ml
EtOH <sub>&lt;10mM</sub>	$\alpha 4, \alpha 6$			females: 0.25 g/kg; males: 0.29 g/kg	$0.19 \pm 0.02\%$ $\approx 4.16 \pm 0.5$ mM Range: 2.39–5.64 mM	$0.06 \pm 0.01\%$ $\approx 1.23 \pm 0.26$ mM Range: 0.22–1.95 mM
EtOH <sub>&lt;20mM</sub>				females: 0.47 g/kg; males: 0.55 g/kg	$0.61 \pm 0.04\%$ $\approx 13.17 \pm 0.89$ mM Range: 10.20–15.8 mM	$0.44 \pm 0.03\%$ $\approx 9.52 \pm 0.72$ mM Range: 7.16–11.5 mM

Abbreviations: APZ, alprazolam; BS, specific allosteric binding site at GABAAR;  $t_{1/2}$ , plasma half-life;  $t_{max}$ , plasma peak time; ZLP, zolpidem.

Drug serum concentrations ( $n = 10$ , mean  $\pm$  SEM) were assessed pre (time point B1) and post PAS<sub>LTD</sub> (time point P1). Note the rapid decrease of blood alcohol level in EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub>.

concentrations of APZ (Greenblatt and Wright, 1993), ZLP (de Haas *et al*, 2010) and EtOH (Welling *et al*, 1977) in healthy young adults. Immediately before PAS<sub>LTD</sub> a blood sample was taken. Five minutes after PAS<sub>LTD</sub> (time point P1, Figure 1) SPV and IO-curve were measured and a second blood sample was taken. The stimulation intensities for measurement of the IO-curve were kept constant at all time points.

In each session a drink and a pill were administered in a double-dummy design according to the scheme in Table 1 to ensure blinding of the subjects. Tablets of APZ (1 mg) or ZLP (10 mg) were combined with a placebo drink (conditions 1 and 2 in Table 1). Ethanol was mixed with orange juice and bitter syrup to obfuscate the characteristic taste and administered in two dosages (see below) in combination with a placebo tablet (conditions 3 and 4 in Table 1). In the PBO session, the placebo tablet was given combined with the placebo drink (condition 5 in Table 1).

Alcohol dosages were calculated individually considering gender, body weight and body height (Ulrich *et al*, 1987; Widmark, 1932). Male subjects received ethanol doses of 0.29 g/kg and 0.55 g/kg, and female subjects 0.25 g/kg and 0.47 g/kg in the EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub> conditions, respectively. These dosages were expected to result at time point B1 (i.e., 80 min after ingestion, and immediately prior to PAS<sub>LTD</sub>, Figure 1) in plasma levels of approximately 0.2% ( $\approx 4.6$  mM) for EtOH<sub><10mM</sub> and 0.5% ( $\approx 11.6$  mM) for EtOH<sub><20mM</sub> (Table 2). Plasma levels at time point B1 were slightly but not significantly higher than those measured approximately 20 min later, at time point P1, in the previous study (Lücke *et al*, 2014). The naming of the lower-dose EtOH condition was accommodated in the present study from EtOH<sub><5mM</sub> to EtOH<sub><10mM</sub>, as the dose range exceeded 5 mM (Table 2).

To put the applied alcohol doses into the context of social drinking, a 70 kg male subject, for example, received 1.45 and 2.75 standard drink equivalents for the EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub> conditions, respectively.

## Statistics

All statistics were performed with SPSS Statistics software (IBM, v.22.0.0).

Drug effects on SPV were analyzed by a repeated measures analysis of variance (rmANOVA) with DRUG (5 levels: APZ, ZLP, EtOH<sub><10mM</sub>, EtOH<sub><20mM</sub>, PBO) and TIME (3 levels: B0, B1, P1) as within-subject factors.

Differences in RMT, SI<sub>1mV</sub>, and SPV at baseline (time point B0) between conditions were tested using rmANOVAs with DRUG (5 levels) as within-subject effect. Differences in IO-curve between conditions were tested at baseline (time point B0) and post-drug intake immediately before PAS<sub>LTD</sub> (time point B1) using rmANOVAs with DRUG (5 levels) and SI (11 levels: 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4 and  $1.5 \times$  SI<sub>1mV</sub>) as within-subject effects. Drug effects on IO-curve (i.e., comparison of time point B1 vs B0) were analyzed by rmANOVA with the within-subject effects DRUG (5 levels), TIME (2 levels: B0, B1) and SI (11 levels). This analysis showed a significant DRUG\*TIME\*SI interaction that justified pairwise comparisons of PBO with all drugs in rmANOVAs with the effects of DRUG (2 levels: PBO vs drug of interest), TIME (2 levels) and SI (11 levels). In case of a significant triple interaction, *post hoc* comparisons tested the drug effect at each of the SIs using two-way rmANOVAs with the effects of DRUG and TIME, using Fisher's Least Significant Difference test. The effects of drug on PAS<sub>LTD</sub>-induced changes of the IO-curve (i.e., comparison of time point P1 vs B1) were similarly analyzed by performing an rmANOVA with the within-subject effects of DRUG (5 levels), TIME (2 levels: B1, P1) and SI (11 levels). *Post hoc* tests were analogously performed as above. MEP amplitudes recorded during PAS<sub>LTD</sub> were averaged in bins of 25 consecutive trials (i.e., 9 bins in total for 225 trials). Drug effects on MEP amplitudes during the PAS<sub>LTD</sub> intervention were then assessed by a two-way rmANOVA with the within-subject effects of DRUG (5 levels) and BIN (9 levels).

For all rmANOVAs, violation of sphericity was tested by Mauchly's test, and the Greenhouse-Geisser correction was applied when appropriate.

Two metrics were calculated that summarize PAS-induced changes of IO-curve in one single value: slope (Rosenkranz *et al*, 2007a; Rosenkranz *et al*, 2007b) and area under the IO-curve (AUIOC) (Lücke *et al*, 2014). Both metrics were determined for each subject, time point, and drug condition. Slope was defined as the linear regression of MEP amplitudes

over the stimulus intensity range of the steepest part of the IO-curve ( $0.8\text{--}1.2 \times SI_{1mV}$ ), while AUIOC was the sum of MEP amplitudes over the full IO-curve. These metrics did not reveal a significant LTD-like decrease in the PBO condition (slope at time point B1:  $0.051 \pm 0.008$  mV/%MSO, slope at P1:  $0.046 \pm 0.011$  mV/%MSO,  $P=0.44$ ; AUIOC at B1:  $15.02 \pm 2.19$  mV, AUIOC at P1:  $13.39 \pm 2.60$  mV,  $P=0.15$ ). These nil results were explained by the fact that the LTD-like decrease in MEP amplitude in the PBO condition was expressed only in the high-intensity range of the IO-curve (see Results, and previous studies (Ni et al, 2014)). As we wanted to analyze drug-induced changes (increases and decreases) of LTD-like plasticity, its expression in the PBO condition was a prerequisite. Therefore, slope and AUIOC were not used for any further statistical analyses.

Linear regression analyses were performed to test for correlations between the drug-induced changes in LTD-like plasticity and drug serum concentrations. For those SIs that showed a significant difference of LTD-like plasticity between DRUG and PBO,  $(MEP_{P1} - MEP_{B1})_{DRUG} - (MEP_{P1} - MEP_{B1})_{PBO}$  was calculated and related to drug serum concentration by calculating the Pearson coefficient.

In all tests,  $P \leq 0.05$  was considered to be statistically significant. All values are given as means  $\pm$  SEM, if not stated otherwise.

## RESULTS

The ten subjects who completed the study tolerated the study medications well without relevant adverse effects. None of the subjects had a positive urine drug screen prior to any of the sessions.

### Drug Plasma Concentrations at Time Points B1 and P1

The blood concentrations of drugs measured before (time point B1) and after  $PAS_{LTD}$  (time point P1) are reported in Table 2. The blood concentrations of  $EtOH_{<10mM}$  and  $EtOH_{<20mM}$  were significantly lower at P1 compared to B1 (all  $P < 0.0001$ ), due to fast metabolism of ethanol.

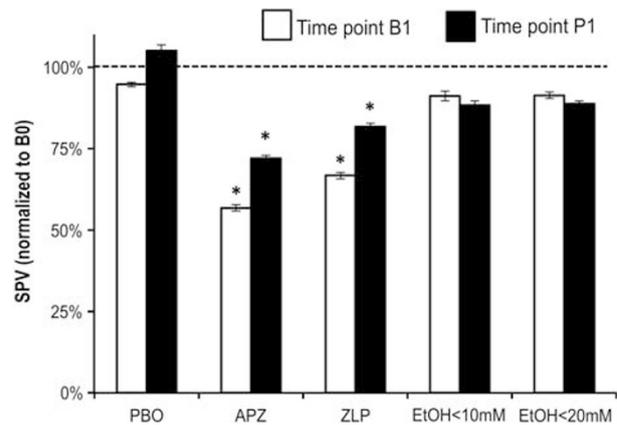
### Drug Effects on Saccade Peak Velocity (SPV)

The baseline SPV was not different between drug conditions (rmANOVA, all  $P > 0.05$ , Table 3). RmANOVA of the PBO condition showed no effect of TIME (3 levels) ( $F_{1,22,18} = 0.728$ ,  $P = 0.441$ ), demonstrating that subjects performed a stable level for the period of the experiment (Figure 2). RmANOVA of all drug conditions and time points revealed significant effects of DRUG ( $F_{4,36} = 11.309$ ,  $P < 0.0001$ ), TIME ( $F_{2,18} = 8.588$ ,  $P = 0.004$ ) and DRUG\*TIME interaction ( $F_{8,72} = 2.293$ ,  $P = 0.034$ ). Post hoc testing revealed that APZ caused a significant reduction of SPV at time points B1 ( $P = 0.004$ ) and P1 ( $P = 0.012$ ) compared to baseline (Figure 2). ZLP also caused a significant reduction at B1 ( $P < 0.0001$ ) and P1 ( $P = 0.05$ ) (Figure 2). In summary, APZ and ZLP caused similar sedative effects, due to their enhancement of neurotransmission through the  $\alpha 1$ -GABAAR.  $EtOH_{<10mM}$  and  $EtOH_{<20mM}$  did not change SPV (all  $P > 0.1$ ). There was no correlation between SPV and drug concentration in any of the drug conditions (all  $P > 0.05$ ).

**Table 3** Baseline Data (time point B0) for SPV, RMT, and SI1 mV

Drug condition	SPV (°/s)	RMT (%MSO)	SI <sub>1mV</sub> (%MSO)
APZ	524 $\pm$ 41	41.8 $\pm$ 1.0	52.4 $\pm$ 1.1
ZLP	535 $\pm$ 35	40.7 $\pm$ 1.0	51.3 $\pm$ 1.1
$EtOH_{<10mM}$	564 $\pm$ 34	40.4 $\pm$ 1.1	53.5 $\pm$ 0.7
$EtOH_{<20mM}$	535 $\pm$ 37	41.6 $\pm$ 1.2	52.9 $\pm$ 0.9
PBO	570 $\pm$ 39	40.0 $\pm$ 1.0	51.4 $\pm$ 0.9

Abbreviations: APZ, alprazolam; EtOH, ethanol; %MSO, percentage of maximum stimulator output; RMT, resting motor threshold; SI1 mV, minimum intensity needed to elicit motor evoked potentials of 1 mV in peak-to-peak amplitude; SPV, Saccadic peak velocity; ZLP, zolpidem.



**Figure 2** Saccadic peak velocity (SPV) at time points B1 (post drug, white bars) and P1 (post  $PAS_{LTD}$ , black bars) normalized to SPV at baseline (B0) in the placebo (PBO), alprazolam (APZ), zolpidem (ZLP), and ethanol ( $EtOH_{<10mM}$ ,  $EtOH_{<20mM}$ ) conditions. All data are means  $\pm$  SEM ( $n = 10$ ). Asterisks indicate significant difference to baseline ( $P < 0.05$ ). Note that APZ and ZLP but not EtOH decrease SPV.

### RMT and SI<sub>1mV</sub> at Baseline (Time Point B0)

RmANOVA did not show any differences of RMT ( $F_{4,36} = 1.698$ ,  $P = 0.17$ ) or SI<sub>1mV</sub> ( $F_{4,36} = 1.816$ ,  $P = 0.15$ ) at baseline between drug conditions (Table 3).

### Drug Effects on IO-Curve (Comparison of Time Point B1 vs B0)

There was no difference in baseline IO-curves between drug conditions ( $F_{4,36} = 0.618$ ,  $P = 0.65$ , Figure 4a and d). In the PBO condition, there was no change in IO-curve pre (B0) versus post (B1) drug intake (TIME:  $F_{1,9} = 1.153$ ,  $P = 0.311$ ; TIME\*SI:  $F_{10,90} = 1.085$ ,  $P = 0.382$ ), indicating stability of IO-curve recordings (Figure 3a).

The rmANOVA with all drugs revealed a significant DRUG\*SI ( $F_{40,360} = 1.772$ ,  $P = 0.004$ ) and DRUG\*TIME\*SI ( $F_{40,360} = 1.708$ ,  $P = 0.006$ ) interaction, suggesting a drug effect on IO-curve depending on stimulus intensity (Figure 4a–d). The significant triple-interaction justified pairwise comparisons of the  $PAS_{LTD}$ -induced changes in IO-curve between any of the drugs with PBO. The comparison

of PBO vs APZ revealed significant effects of DRUG ( $F_{1,9} = 5.761$ ,  $P = 0.040$ ), DRUG\*SI ( $F_{10,90} = 4.392$ ,  $P < 0.0001$ ) and DRUG\*TIME\*SI ( $F_{10,90} = 3.186$ ,  $P = 0.002$ ). *Post hoc* two-way rmANOVAs with the effects of DRUG and TIME at the single SIs showed that APZ caused a significant MEP amplitude decrease at  $1.4 \times SI_{1mV}$  ( $P = 0.048$ , Figure 4a), but not at any of the other SIs.

The comparison of PBO vs ZLP revealed significant effects of DRUG ( $F_{1,9} = 5.207$ ,  $P = 0.048$ ), DRUG\*SI ( $F_{10,90} = 2.239$ ,  $P = 0.022$ ) and DRUG\*TIME\*SI ( $F_{10,90} = 1.949$ ,  $P = 0.048$ ). *Post hoc* two-way rmANOVAs with the effects of DRUG and TIME at the single SIs showed that ZLP caused a significant MEP amplitude decrease at  $1.1 \times SI_{1mV}$  ( $P = 0.016$ , Figure 4b), but not at any of the other SIs.

The comparison of PBO vs EtOH<sub><10mM</sub> showed a significant DRUG\*TIME\*SI interaction ( $F_{10,90} = 2.127$ ,  $P = 0.030$ ). However, *post hoc* two-way rmANOVAs with the effects of DRUG and TIME did not reveal a significant effect of EtOH<sub><10mM</sub> at any of the single SIs (Figure 4c).

The comparison of PBO vs EtOH<sub><20mM</sub> did not show a significant effect of DRUG or any of its interactions (Figure 4d).

In summary, APZ and ZLP resulted in marginal MEP amplitude decreases at single SIs while EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub> did not change MEP amplitude. This is an important nil finding given the effects of EtOH on PAS<sub>LTD</sub>-induced LTD-like plasticity (see below).

### Drug Effects on MEP Amplitude During the PAS<sub>LTD</sub> Intervention

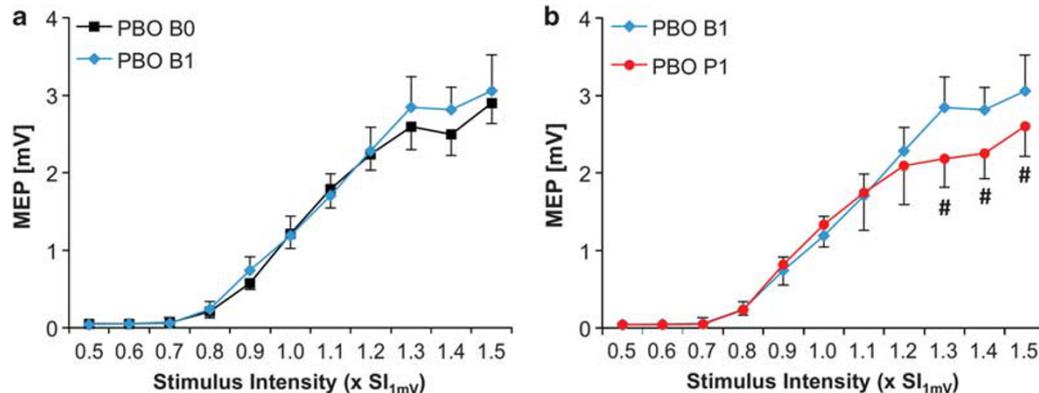
MEP amplitudes recorded during the PAS<sub>LTD</sub>-intervention did not differ between drug conditions, as demonstrated by a

non-significant effect of DRUG ( $F_{4,32} = 0.347$ ,  $P = 0.84$ ) and DRUG\*BIN ( $F_{32,256} = 1.179$ ,  $P = 0.24$ ) interaction. This is an important nil finding, indicating that drug condition did not alter the PAS<sub>LTD</sub> intervention *per se*, while it did change the induction of plasticity by PAS<sub>LTD</sub> (see below). Irrespective of drug condition, MEP amplitude increased over the course of PAS<sub>LTD</sub> (BIN:  $F_{2,286,18,285} = 7.735$ ,  $P = 0.003$ ) (Figure 5), similar to previous observations (Ilic et al, 2011).

### Drug Effects on PAS<sub>LTD</sub>-Induced Changes of IO-Curve (Comparison of Time Point P1 vs B1)

At time point B1, there was no significant effect of DRUG ( $F_{4,36} = 5.886$ ,  $P = 0.06$ ) but a significant DRUG\*SI interaction ( $F_{40,360} = 2.250$ ,  $P < 0.001$ ). *Post hoc* pair-wise comparisons of IO-curves at time point B1 between all study drugs and PBO revealed significant differences between APZ and PBO for DRUG ( $F_{1,9} = 7.420$ ,  $P = 0.023$ ) and the DRUG\*SI interaction ( $F_{10,90} = 7.119$ ,  $P < 0.001$ ), and between ZLP and PBO for DRUG ( $F_{1,9} = 6.583$ ,  $P = 0.030$ ) and the DRUG\*SI interaction ( $F_{10,90} = 2.893$ ,  $P = 0.004$ ). In contrast, there were no significant effects of DRUG or DRUG\*SI interaction on IO-curves at time point B1 between EtOH<sub><10mM</sub> or EtOH<sub><20mM</sub> and PBO (all  $P > 0.15$ ). The effects of APZ and ZLP were explained by a depression of the IO-curve (Figure 4). It is important to note that the lack of effects of ethanol on the IO-curve excludes a significant contribution of changes in corticospinal excitability on the enhancing effects of ethanol on PAS-induced LTD-like plasticity (see below).

In the PBO condition, there was no significant effect for TIME ( $F_{1,9} = 2.39$ ,  $P = 0.157$ ), but the TIME\*SI interaction on PAS<sub>LTD</sub>-induced changes of IO-curve was significant ( $F_{10,90} = 3.849$ ,  $P < 0.0001$ ). *Post hoc* testing revealed that

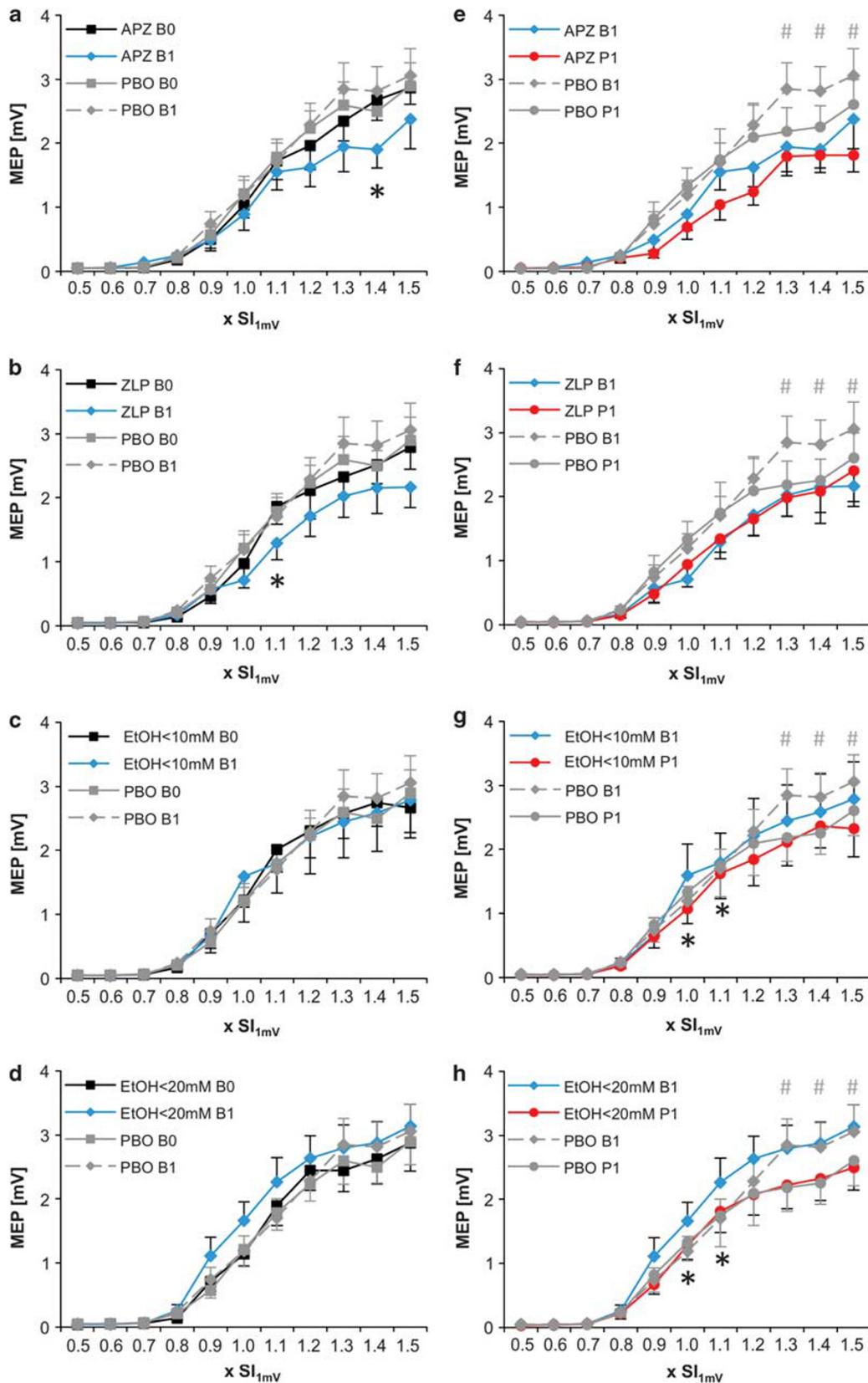


**Figure 3** (a) IO-curve (y-axes: MEP amplitude in mV, x-axes: stimulus intensity in multiples of  $SI_{1mV}$ ) at baseline (B0, black squared symbols) vs post drug (B1, blue diamonds), and (b) post drug (B1, blue diamonds) vs post PAS<sub>LTD</sub> (P1, red circles) in the placebo (PBO) condition. Note that PBO had no effect on IO-curve when comparing time points B1 with B0, indicating reliability of IO-curve measurements. Also note that PAS<sub>LTD</sub> resulted in a significant IO-curve depression at SIs of  $1.3-1.5 \times SI_{1mV}$  ( $^{##}P < 0.05$ ).

**Figure 4** (a-d) Drug effects (A: alprazolam (APZ), (b): zolpidem (ZLP), (c): ethanol < 10 mM (EtOH<sub><10mM</sub>), (d): ethanol < 20 mM (EtOH<sub><20mM</sub>)) on IO-curve (y-axes, MEP amplitude in mV) at time point B1 (blue diamonds) vs B0 (black squares) compared to PBO (grey symbols). (e-h) Drug effects (e: APZ, f: ZLP, G: EtOH<sub><10mM</sub>, h: EtOH<sub><20mM</sub>) on PAS<sub>LTD</sub>-induced change of IO-curve at time point P1 (red circles) vs B1 (blue diamonds) compared to PBO (grey symbols). X-axes: stimulus intensity (in multiples of  $SI_{1mV}$ ). Number signs indicate LTD-like plasticity in the PBO condition ( $^{##}P < 0.05$ ), asterisks indicate significant difference of drug effects on IO-curve (B1 vs B0) or LTD-like plasticity (P1 vs B1) when compared to PBO ( $^{*}P < 0.05$ ). Note that APZ and ZLP but not EtOH depressed IO-curve, while both doses of EtOH but not APZ or ZLP increased PAS<sub>LTD</sub>-induced LTD-like IO-curve depression.

PAS<sub>LTD</sub> resulted in a decrease of IO-curve at P1 exclusively at high stimulation intensities of 130–150%SI<sub>1mV</sub> (all  $P < 0.05$ , Figure 3b). This result is in line with previous studies, which

showed that changes after PAS<sub>LTD</sub> occurred predominantly in the high-intensity range of the IO-curve (Rosenkranz *et al*, 2007a; Rosenkranz *et al*, 2007b).



The rmANOVA including all drug conditions showed a significant effect of TIME ( $F_{1,9} = 11.562$ ,  $P = 0.008$ ), TIME\*SI ( $F_{10,90} = 5.228$ ,  $P < 0.0001$ ) and DRUG\*TIME\*SI ( $F_{40,360} = 1.489$ ,  $P = 0.033$ ), indicating that drugs modified the PAS<sub>LTD</sub>-induced changes in IO-curve dependent on stimulation intensity (Figures 4e and h). The significant triple-interaction justified pairwise comparisons of the PAS<sub>LTD</sub>-induced changes in IO curve between any of the drugs with PBO. The comparison of PBO vs APZ revealed significant effects of DRUG ( $F_{1,9} = 8.604$ ,  $P = 0.017$ ), TIME ( $F_{1,9} = 13.159$ ,  $P = 0.006$ ), DRUG\*SI ( $F_{10,90} = 6.997$ ,  $P < 0.0001$ ) and DRUG\*TIME\*SI ( $F_{10,90} = 2.890$ ,  $P = 0.004$ ). However, *post hoc* two-way rmANOVAs with the effects of DRUG and TIME showed that APZ did not significantly alter the PAS<sub>LTD</sub> effect in the PBO condition at any of the single SIs (Figure 4e).

The comparison of PBO vs ZLP showed a trend for DRUG ( $F_{1,9} = 5.038$ ,  $P = 0.051$ ) and a significant DRUG\*SI interaction ( $F_{10,90} = 2.101$ ,  $P = 0.032$ ) but there were no effects of DRUG\*TIME or DRUG\*TIME\*SI (Figure 4f). Therefore, ZLP did not significantly alter the PAS<sub>LTD</sub>-induced IO-curve depression observed in the PBO condition.

The comparison of PBO vs EtOH<sub><10mM</sub> revealed a significant interaction DRUG\*TIME\*SI ( $F_{10,90} = 2.371$ ,  $P = 0.015$ ). *Post hoc* comparisons of the PAS<sub>LTD</sub> effect at the single SIs showed that EtOH<sub><10mM</sub> enhanced the MEP depression at  $1.0 \times SI_{1mV}$  ( $P = 0.032$ ) and at  $1.1 \times SI_{1mV}$  ( $P = 0.033$ , Figure 4g).

The comparison of PBO vs EtOH<sub><20mM</sub> revealed a significant interaction DRUG\*TIME\*SI ( $F_{10,90} = 1.90$ ,  $P = 0.05$ ). *Post hoc* comparisons of the PAS<sub>LTD</sub> effect at the single SIs showed that EtOH<sub><20mM</sub> enhanced the MEP depression at  $1.0 \times SI_{1mV}$  ( $P = 0.033$ ) and at  $1.1 \times SI_{1mV}$  ( $P = 0.032$ , Figure 4h).

In summary, EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub> but not APZ or ZLP enhanced PAS<sub>LTD</sub>-induced LTD-like plasticity in human MI. None of these effects correlated with serum ethanol levels at B1 or P1.

## DISCUSSION

The principal novel findings of this study were that EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub> but not APZ or ZLP enhanced

the PAS<sub>LTD</sub>-induced LTD-like plasticity, while APZ and ZLP but not EtOH<sub><10mM</sub> or EtOH<sub><20mM</sub> decreased SPV, indexing  $\alpha 1$ -GABAAR-mediated sedation. The following paragraphs will discuss these findings in detail.

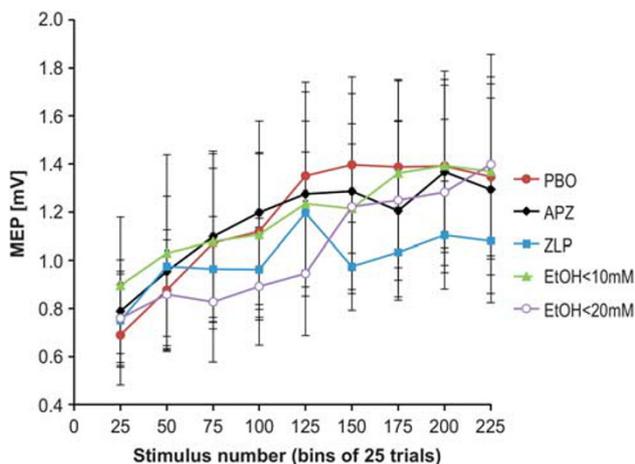
### Drug Effects on Saccadic Peak Velocity (SPV)

We replicated our earlier findings that APZ and ZLP reduce SPV (Lücke et al, 2014; Premoli et al, 2014) while EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub> had no significant effect (Lücke et al, 2014). SPV is an established sensitive electrophysiological surrogate marker of  $\alpha 1$ -GABAAR-mediated sedation (Blom et al, 1990; de Visser et al, 2003). In contrast to the classical benzodiazepine lorazepam, a selective agonist at  $\alpha 2$ -/ $\alpha 3$ -GABAARs did not decrease SPV unless high doses were given (de Haas et al, 2009). In addition, the largely selective  $\alpha 1$ -GABAAR agonist ZLP reduced SPV (de Haas et al, 2010), in accordance with our results. The nil results of EtOH on SPV are important because they demonstrate that EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub> had no impact on  $\alpha 1$ -GABAAR-mediated neurotransmission and, therefore, sedation cannot be attributed to account for the enhancing effects of EtOH on LTD-like plasticity (see below). Our findings on a behavioral surrogate marker of  $\alpha 1$ -GABAAR-mediated inhibition are concordant with the notion that even high doses of EtOH (25–300 mM) had no effect on  $\alpha 1$ -GABAAR-mediated currents of acutely dissociated neurons from various brain regions (Criswell et al, 2003).

### Drug Effects on IO-Curve

We demonstrated that APZ and ZLP but not EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub> resulted in marginal IO-curve decreases at single stimulus intensities (Figure 4a and d). These data are largely in agreement with our previous study (Lücke et al, 2014). In particular, the present data replicate the nil effect of ethanol on IO-curve. This is also compatible with one earlier study where EtOH at a higher blood concentration (16.5 ± 2.3 mM) than in the present study had no effect on IO-curve (Ziemann et al, 1995). This nil finding is of particular importance because it excluded changes in corticospinal excitability at the time of PAS<sub>LTD</sub> that could have been responsible for the significant effects of EtOH on PAS<sub>LTD</sub>-induced LTD-like plasticity (see below). Furthermore, EtOH has been implicated to inhibit NMDAR-mediated currents at doses of <25 mM (He et al, 2013; Lovinger et al, 1989, 1990; Weitlauf and Woodward, 2008). Since one previous study demonstrated a significant and dose-dependent IO-curve increase by ketamine, another NMDAR antagonist (Di Lazzaro et al, 2003), the present and previous (Lücke et al, 2014) nil findings of EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub> on IO-curve make it unlikely that these low dosages of EtOH inhibited NMDARs to a significant extent.

In the previous study, we did not find significant IO-curve depression by APZ or ZLP (Lücke et al, 2014), but the effects in the present study were also only marginal, and significant only at single stimulation intensities. Different populations of subjects may have caused this slight discrepancy in otherwise identical experimental conditions. Also, the literature reported somewhat diverse results, with benzodiazepines such as lorazepam or diazepam having either no effect (Ilic et al,



**Figure 5** MEP amplitudes measured during PAS<sub>LTD</sub> intervention in the placebo (PBO), alprazolam (APZ), zolpidem (ZLP), EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub> conditions. Each data point represents the mean ± SEM of 25 consecutive trials of 10 subjects (i.e., 9 data points for 225 trials in total).

2002; Ziemann *et al*, 1996) or producing a moderate IO-curve depression (Borojerdi *et al*, 2001; Kimiskidis *et al*, 2006).

### Drug Effects on PAS<sub>LTD</sub>-Induced Changes of the IO-Curve

PAS<sub>LTD</sub>-induced IO-curve depression is an established model of LTD-like plasticity at the systems level of human motor cortex (for review, (Müller-Dahlhaus *et al*, 2010)). Although this is not equivalent to LTD as studied at the cellular level, several properties suggest that PAS<sub>LTD</sub>-induced corticospinal plasticity has overlapping mechanisms with LTD, and therefore has been termed LTD-like plasticity: it outlasts the PAS<sub>LTD</sub> intervention by > 30 min (Müller *et al*, 2007; Wolters *et al*, 2003), is associative as intervals between the electrical stimulus to median nerve and TMS of the contralateral M1 significantly different from N20-5 ms or 10 ms do not result in MEP depression (Wolters *et al*, 2003), is input specific, i.e. MEP depression occurs only in hand muscles supplied by the stimulated median nerve but not in those supplied by the ulnar nerve (Weise *et al*, 2006), and can be abolished by the NMDAR antagonist dextromethorphan and the voltage gated calcium channel blocker nimodipine (Wolters *et al*, 2003). In addition, epidural recordings from the cervical spinal cord and brain stem stimulation experiments verified that the PAS<sub>LTD</sub>-induced MEP depression originates at the level of M1 rather than in the spinal cord (Di Lazzaro *et al*, 2009; Wolters *et al*, 2003). Finally, the neurobiological relevance of this form of LTD-like plasticity has been demonstrated by its homeostatic interactions with motor learning (Jung and Ziemann, 2009; Rosenkranz *et al*, 2007a; Ziemann *et al*, 2004), and its exaggerated expression in musicians (Rosenkranz *et al*, 2007b), focal hand dystonia (Weise *et al*, 2006), or after upper limb immobilization (Rosenkranz *et al*, 2014).

In agreement with previous studies (Rosenkranz *et al*, 2007a; Rosenkranz *et al*, 2007b), we found that the PAS<sub>LTD</sub>-induced depression of the IO-curve in the PBO condition occurred specifically in the high-intensity range ( $1.3\text{--}1.5 \times SI_{1mV}$ , Figure 3b). This may reflect that the so called I1-wave caused by presumably direct transsynaptic activation of corticospinal neurons by TMS remained unchanged by PAS<sub>LTD</sub>, while late I-waves (I2-I4-waves) caused by presumably indirect transsynaptic activation of corticospinal neurons through higher-order interneurons (for review, (Di Lazzaro *et al*, 2008)) were depressed by PAS<sub>LTD</sub> (Di Lazzaro *et al*, 2009). This notion is supported by the finding that the low-intensity part of the IO-curve, not changed by PAS<sub>LTD</sub>, is largely based on recruitment of the I1-wave if the induced current in M1 runs in posterior-to-anterior direction, while the high-intensity part of the IO-curve depressed by PAS<sub>LTD</sub> is based on additional recruitment of late I-waves (Di Lazzaro *et al*, 1998a; Di Lazzaro *et al*, 1998b). The implication is that late I-waves but not the I1-wave are controlled by GABAergic inhibition (Di Lazzaro *et al*, 2000; Di Lazzaro *et al*, 1998c). Therefore, we had speculated that increased GABAergic neurotransmission by the drugs tested here would result in enhanced PAS<sub>LTD</sub>-induced LTD-like IO-curve depression.

We found that EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub> but not APZ or ZLP enhanced significantly PAS<sub>LTD</sub>-induced LTD-like plasticity in human cortex at stimulus intensities of  $1.0\text{--}1.1 \times SI_{1mV}$  (Figure 4e and h). The enhancing effect of EtOH

on PAS<sub>LTD</sub>-induced LTD-like IO-curve depression specifically at these mid-range intensities may be explained by the fact that late I-waves are just coming into play here (Di Lazzaro *et al*, 1998a; Di Lazzaro *et al*, 1998b), so that it may require the synergistic action of PAS<sub>LTD</sub> and drug to result in MEP depression.

An important implication, also given the discussion above, is that the enhancement of the PAS<sub>LTD</sub>-induced LTD-like plasticity by low-dose EtOH but not APZ or ZLP strongly suggests that this effect was mediated by increased tonic inhibition mediated by the extrasynaptic  $\alpha 4\beta 3\delta$ -GABAAR. The double dissociation of the effects of EtOH vs APZ and ZLP on SPV (see above) vs PAS<sub>LTD</sub>-induced LTD-like plasticity clearly speaks against a mechanism mediated by the  $\alpha 1$ -GABAAR. Modification of synaptic inhibition mediated through the  $\alpha 2$ -,  $\alpha 3$ - or  $\alpha 5$ -GABAAR is similarly unlikely because alprazolam exerts full positive allosteric modulation at the benzodiazepine-binding site of all of these receptors. Short-interval intracortical inhibition, a putative paired-pulse TMS marker of neurotransmission through the  $\alpha 2$ - and  $\alpha 3$ -GABAAR (Di Lazzaro *et al*, 2007; Di Lazzaro *et al*, 2006; Ziemann *et al*, 2014) has not been measured in the present study. This is a limitation, but previous studies demonstrated that classical benzodiazepines and ethanol increased short-interval intracortical inhibition, while zolpidem did not (Di Lazzaro *et al*, 2007; Di Lazzaro *et al*, 2006; Ziemann *et al*, 1995; Ziemann *et al*, 1996). Therefore, the uniform effects of benzodiazepines and ethanol on short-interval intracortical inhibition, i.e.,  $\alpha 2$ - and  $\alpha 3$ -GABAAR mediated inhibition, would not explain their different effects on PAS-induced LTD-like plasticity observed in the present study. Finally a mechanism related to blockade of the NMDAR by EtOH was also unlikely to be involved because effective blockade of the NMDAR by dextromethorphan resulted in abolition rather than enhancement of PAS<sub>LTD</sub>-induced LTD-like plasticity (Wolters *et al*, 2003).

These conclusions are compatible with the reported high sensitivity of the  $\alpha 4\beta 3\delta$ -GABAAR to low-dose EtOH (Hanchar *et al*, 2006; Sundstrom-Poromaa *et al*, 2002; Wallner *et al*, 2003; Wei *et al*, 2004) even if this was not a unanimously supported finding in all studies (Borghese *et al*, 2006; Yamashita *et al*, 2006). The extrasynaptic  $\alpha 6\beta 3\delta$ -GABAAR also exhibits high sensitivity to EtOH (Hanchar *et al*, 2006; Wallner *et al*, 2003; Wei *et al*, 2004) but is expressed exclusively in cerebellar granule cells, in contrast to the  $\alpha 4\beta 3\delta$ -GABAAR that is widely distributed in the brain including neocortex (Pirker *et al*, 2000). As PAS<sub>LTD</sub>-induced LTD-like plasticity occurs in M1 (Di Lazzaro *et al*, 2009; Wolters *et al*, 2003) we believe that the most likely candidate mechanism for the enhancement of PAS<sub>LTD</sub>-induced LTD-like plasticity by low-dose EtOH is enhancement of extrasynaptic tonic inhibition through the  $\alpha 4\beta 3\delta$ -GABAAR.

In our previous study we have reported a similarly selective suppression of LTP-like plasticity by EtOH<sub><10mM</sub> and EtOH<sub><20mM</sub> in the absence of significant effects by APZ or ZLP (Lücke *et al*, 2014). Together, these findings support the novel view that extrasynaptic tonic inhibition may constitute a key mechanism in regulating synaptic plasticity in human neocortex: enhancement of tonic inhibition leads to a rightward shift of a cell's input-output relationship through a more hyperpolarized compared to normal state (Mitchell and Silver, 2003), a condition that favors LTD over

LTP in neocortical circuits (Artola *et al*, 1990). Findings may stimulate cellular research on the role of tonic inhibition in regulating excitability and plasticity of cortical neuronal networks.

### Behavioral Significance of the Present Findings

LTD of excitatory synaptic neurotransmission, besides LTP, is a leading candidate mechanism for memory and learning processes (Collingridge *et al*, 2010; Malenka and Bear, 2004). In addition, several studies have provided evidence that LTD in the nucleus accumbens and prefrontal cortex of rats plays a pivotal supportive role in drug addiction (Brebner *et al*, 2005; Van den Oever *et al*, 2008). It may be speculated that the enhancing effect of low-dose ethanol on LTD-like plasticity in the present study relates to detrimental effects of acute ethanol ingestion on memory formation and learning (Lister *et al*, 1991; Mattila *et al*, 1998; Zorumski *et al*, 2014). However, this remains to be tested in further experiments as memory or learning processes have not been studied here. Furthermore, the existence of any relation between enhancement of LTD by ethanol in primary motor cortex in the present study and LTD-dependent addictive behavior mediated by non-overlapping neurocircuits such as nucleus accumbens and prefrontal cortex (Collingridge *et al*, 2010; Noori *et al*, 2012) is entirely unclear. Therefore, whether the increase of PAS-induced LTD-like plasticity in motor cortex by low-dose ethanol provides a surrogate marker for the addictive potential of ethanol in given individuals would require further deliberate testing. On the other hand, it is of interest to note that several recent therapeutic trials applied high-frequency repetitive TMS to the dorsolateral prefrontal cortex of alcohol addicts and observed a decrease in spontaneous alcohol craving (Gorelick *et al*, 2014; Höppner *et al*, 2011; Mishra *et al*, 2010). High-frequency repetitive TMS typically leads to LTP-like plasticity (for review, (Ziemann *et al*, 2008)) so that these therapeutic effects may be explained by counteracting LTD-dependent mechanisms of addiction, in addition to other potential mechanisms, such as modulation of dopaminergic neurotransmission in addiction-related subcortical target areas of the dorsolateral prefrontal cortex (Feil and Zangen, 2010; Naim-Feil and Zangen, 2013).

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The authors declare no conflict of interest.

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