SUPPORTING INFORMATION

Effects of Pregnanolone Glutamate and its Metabolites on $GABA_A$ and NMDA Receptors and Zebrafish Behavior

Vera Abramova[1,8], Vanessa Leal Alvarado[1], Martin Hill[2], Tereza Smejkalova[1], Michal Maly[1], Karel Vales[1,3], Ivan Dittert[1], Paulina Bozikova[4], Bohdan Kysilov[1], Barbora Hrcka Krausova[1], Vojtech Vyklicky[1], Ales Balik[1], Klevinda Fili[1,8], Miloslav Korinek[1], Hana Chodounska[5], Eva Kudova[5], David Ciz[6], Jan Martinovic[6], Jiri Cerny[1], Petr Bartunek[7,9], and Ladislav Vyklicky*[1]

[1] Institute of Physiology CAS, Videnska 1083, 142 20 Prague 4, Czech Republic
[2] Institute of Endocrinology, Narodni 8, 116 94 Prague 1, Czech Republic
[3] National Institute of Mental Health, Topolova 748, 250 67 Klecany, Czech Republic
[4] Institute of Biotechnology CAS, Prumyslova 595, 252 50 Vestec, Czech Republic
[5] Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nam. 2, 166 10 Prague 2, Czech Republic
[6] IT4Innovations National Supercomputing Center, Studentska 6231/1B, 708 00 Ostrava - Poruba, Czech Republic
[7] Institute of Molecular Genetics CAS, Videnska 1083,142 20 Prague, Czech Republic
[8] Charles University, Third Faculty of Medicine, Ruska 87, 100 00 Prague 10, Czech Republic
[9] CZ-OPENSCREEN, Institute of Molecular Genetics CAS, Videnska 1083, 142 20 Prague 4, Czech Republic

Corresponding author: *Prof. Ladislav Vyklicky, MD, Ph.D., DSc Email: Ladislav.Vyklicky@fgu.cas.cz

Table of Contents

Supplementary Figures:

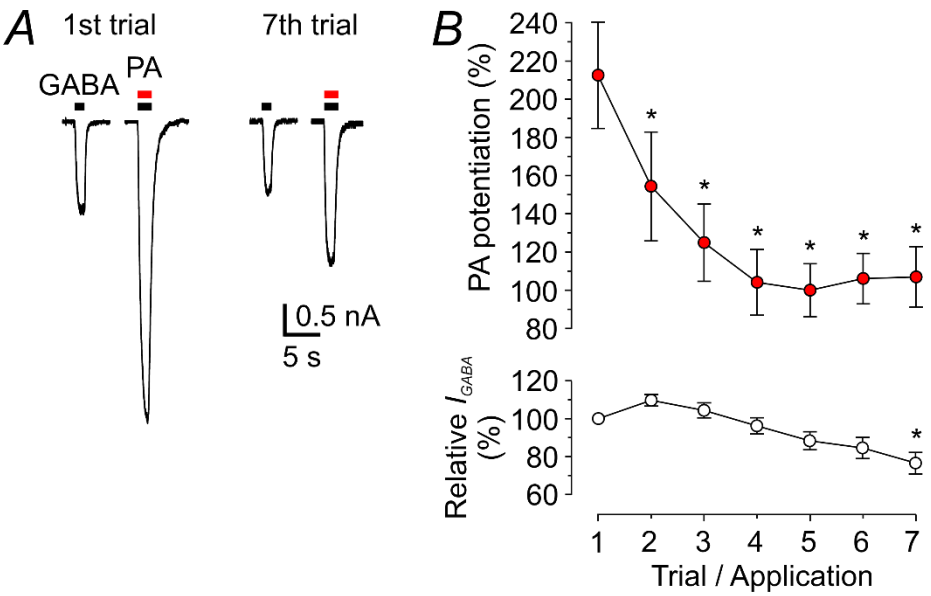

Figure S1. Potentiation of GABAR responses decreases in the course of repeated steroid and GABA co-applications**.** (**A**) Representative responses are shown for the first and seventh trial. The first trial was recorded ~2 min after whole-cell formation, and each trial consisted of the response to 1 μM GABA and the response to co-application of 1 μM GABA and 300 nM PA made 40 s later. Trials were repeated at 100 s intervals (0.01 Hz). (**B**) Graph of relative PA-induced potentiation of GABAR responses determined for each trial (above, red symbols) and relative responses to GABA (1 μM) recorded during the trial (below, open symbols). *, indicates statistically significant differences (One Way Analysis of Variance followed by Multiple Comparisons *versus* Control (PA potentiation determined during the first trial or GABAR response determined during the first trial); Holm-Sidak method).

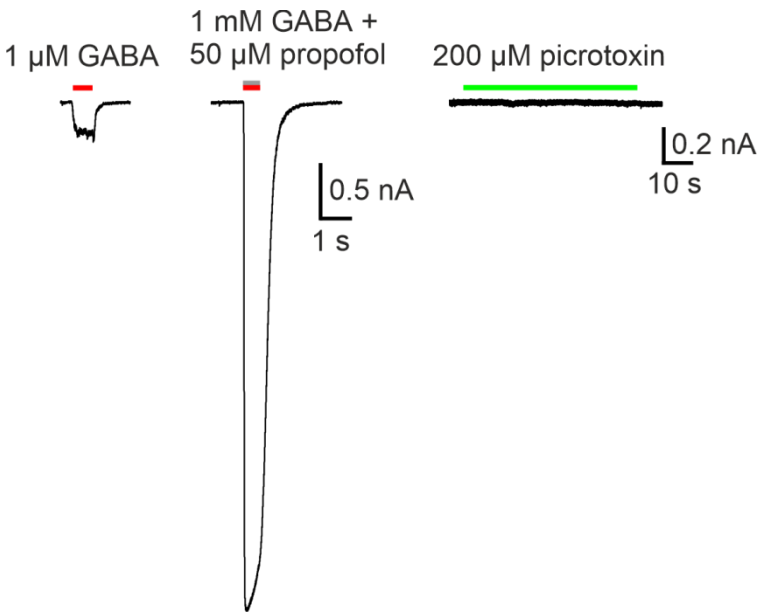

Figure S2. Effects of propofol and picrotoxin on GABAR responses in hippocampal neurons. Representative responses are shown for the response to 1 μM GABA, to co-application of 1 mM GABA and 50 μM propofol, and to application of 200 μM picrotoxin.

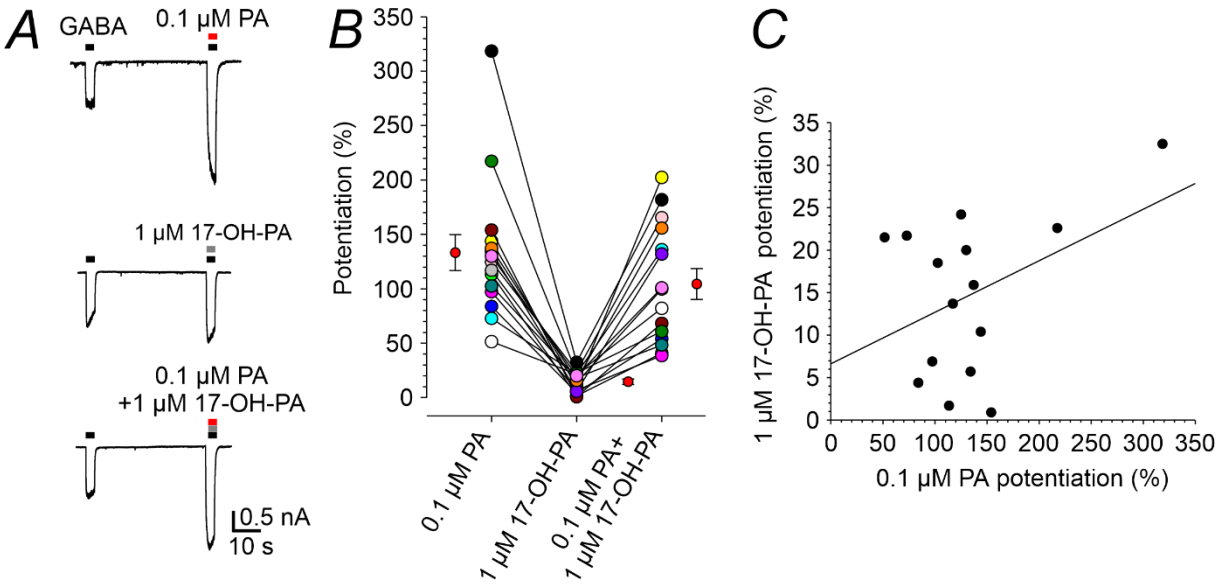

Figure S3. Combined effect of PA and 17-OH-PA at GABARs. (**A**) Representative responses are shown for the three steroid application trials. These were recorded ~2 min after the formation of the whole-cell configuration and each trial consisted of the response to 1 µM GABA and to GABA co-application with either PA (0.1 µM), 17-OH-PA (1 µM), or both steroids. The order of trials with individual steroid applications was randomized, and each neuron was exposed to PA, 17-OH-PA, and PA+17-OH-PA only once. (**B**) Plots of the relative steroid effects on responses to 1 µM GABA. Data from individual cells (color symbols) are plotted normalized with respect to the control response induced by 1 µM GABA. The red-filled symbols give mean ± SEM. The degree of potentiation induced by PA alone and by co-application of PA with 17-OH-PA were not significantly different. (**C**) Graph represents the relative potentiating effect of 0.1 µM PA on responses to 1 µM GABA plotted *versus* the relative potentiating effect of 1 µM 17-OH-PA in the same neuron. Data were fit by a linear regression (Pearson product moment correlation coefficient r = 0.414; $p = 0.125$).

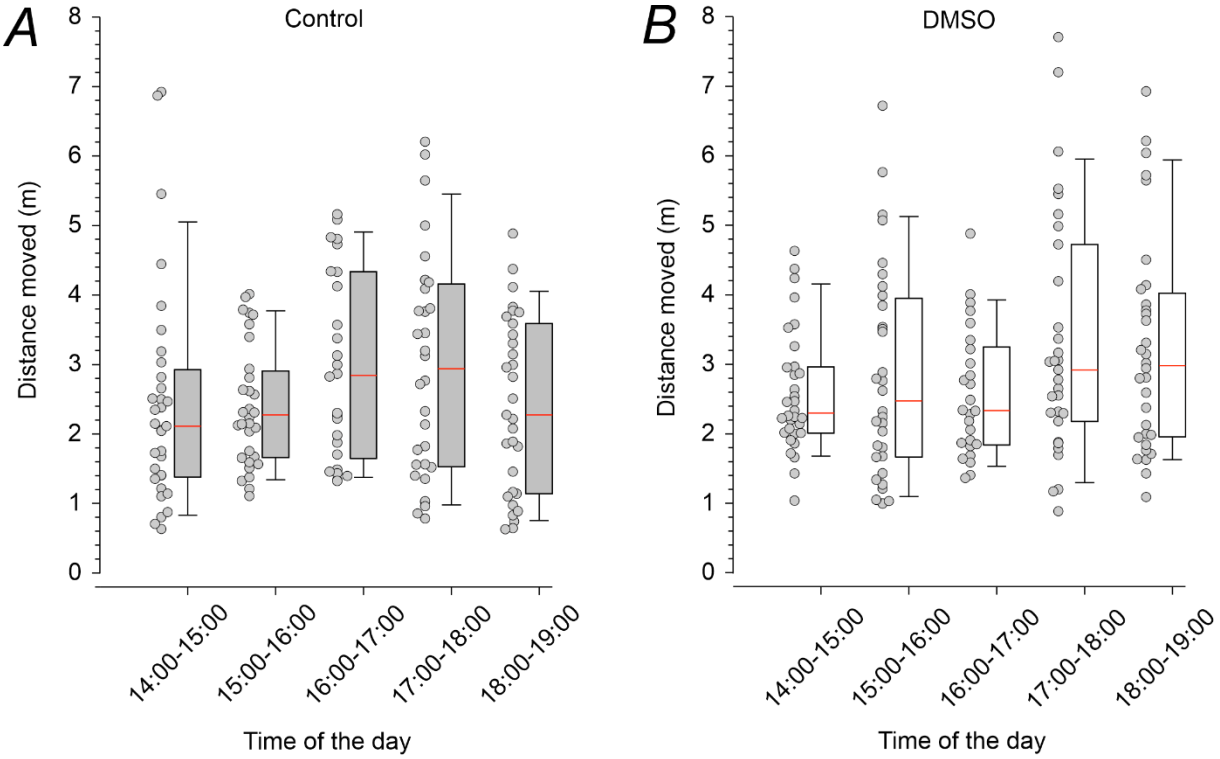

Figure S4. Effect of time of day on locomotor activity of zebrafish larvae. Larvae were first incubated in a dish containing either control medium (Control) or DMSO (1%) at 28.5°C. Following 1-hour incubation period, larvae were placed individually in wells in a new dish containing the same medium as that used for the initial incubation period (see Methods for details). Locomotor activity was tracked continuously for 1 hour (**A**-**B**). Abscissa indicates the time of day during the monitoring period. In the box plots, the boundary of the box closest to zero indicates the 25th percentile, the red line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Dot plots show the distance moved by each individual. No differences in the median values of the locomotor activity were observed either for Control or DMSO (1%)-containing media in epochs from 14:00-15:00 to 18:00-19:00 (Kruskal-Wallis One-Way Analysis of Variance on ranks). No differences in the median values of the locomotor activity were observed for the same epochs recorded in DMSO (1%) and Control media (Mann-Whitney rank sum test DMSO *versus* Control 14:00-15:00 to 18:00-19:00.