Differential Role of NR2A and NR2B Subunits in *N*-methyl-D-aspartate Receptor Antagonist-induced Aberrant Cortical Gamma Oscillations

Supplemental Information

Supplemental Methods and Materials

Experimental Procedures

All experiments were performed in accordance with National Institute of Health guidelines and were approved by the Institutional Animal Care and Use Committee of Beth Israel Deaconess Medical Center. The rats were housed in a temperature and humidity-controlled room with 12 hr/12 hr light/dark cycle; food and water was available ad libitum both in the home cage and during recordings. A total of 33 rats were implanted with chronic electroencephalographic (EEG) and electromyographic (EMG) electrodes. Stainless steel screws were used to record cortical EEG in the frontal cortex on both sides (1 mm anterior and 2 mm lateral to bregma), and over the occipital cortex (6.5 mm posterior and 3 mm lateral to the bregma) and pairs of twisted wires were implanted in the hippocampus to record field potentials. Two additional screw electrodes were inserted, one ~5 mm anterior to bregma and the other over the cerebellum, for ground and reference. Muscle tone was recorded using multithreaded wires in the neck muscles, in both sides. All electrodes were connected to a miniature connector and the wires and the connector were fixed to the skull with dental acrylic. In two rats, in addition to frontal and occipital cortex EEG and neck muscle EMG, the animals' movements were also monitored using accelerometers (RodentPack, EMKA Technologies, Falls Church, VA) continuously recording locomotion along two horizontal coordinates and rearing. Electrophysiological recordings started after a 7-10 day recovery period. Experiments with drug injections started after several daily control recordings. For recording sessions, the rats were placed in a recording box and connected to a slip-ring commutator (PlasticOne, Inc., Roanoke, VA) or had the telemetric transmitter mounted on the head connector. The recordings started early morning and lasted 10-24 hours; the drugs were administered after 4 hr control recording. Other than the drug injection, the rats were left undisturbed. Each rat received 1-5 injections (in 1 ml/kg volume, intraperitoneal (i.p.) or subcutaneous (s.c.) injections), separated by at least 4 days to allow time for washout.

Prepulse Inhibition (PPI) of Startle

Prepulse inhibition of startle was tested in a subset of animals (*n* = 11) on three occasions separated by 4-5 days. Six rats were taken to the PPI apparatus once before an injection, once 60-90 min after MK801 injection on the height of the induced gamma oscillation, and once 5-6 hours after the injection, i.e. after the large on-going gamma subsided and the normal sleep-wake cycles returned, verified by electrophysiological monitoring. In another 5 rats, PPI was tested in control, after injection of Ro-25-6891 and after injection of NVP-AAM077. Cortical EEG was monitored before and after the PPI test to ensure timing at the height of the changes in EEG gamma power. The order of the three measurements was randomized between animals and was performed on different days. PPI measures the gating of the startle response induced by a 120 dB auditory stimulus (40 ms white noise click) by a preceding subthreshold (80 dB, 20 ms, 100 ms delay) stimulus (1, 2) and was performed using standard equipment and procedures (Columbus Instruments, Columbus, OH).

Drugs

The list of test compounds and their origin and principal features of NMDA-R subunit selectivity is as follows. non-selective NMDA-R antagonists ketamine (10 mg/kg, *n* = 10, Fort Dodge Animal Health, Overland Park, KS) and MK801 (0.2 mg/kg, *n* = 12, Tocris, Ellisville, MO). NR2A-preferring antagonists NVP-AAM077 (10 and 20 mg/kg s.c., *n* = 12 or i.p. *n* = 8, Novartis, Basel, Switzerland, 12-120 fold IC_{50} differences for NR2A vs. NR2B) and PEAQX (10 mg/kg, *n* = 5, Sigma, St. Louis, MO, it is the racemic version of NVP-AAM077, i.e. the PEAQX dose used in this study was equivalent to 5 mg/kg of this NVP-AAM077). NR2B-selective antagonists, ifenprodil (5 and 10 mg/kg, *n* = 6, Tocris; NMDA antagonist acting on at the polyamine site), threo-ifenprodil (10 mg/kg, *n* = 4, Tocris; IC_{50} values are 0.22 and 324 μM at NR2B and NR2A, respectively), and Ro25-6985 (5, 10, 20, and 30 mg s.c., *n* = 13, Tocris; IC_{50} values are 0.009 and 52 μM for cloned receptor subunit combinations NR1C/NR2B and NR1C/NR2A, respectively). Selective antagonist of NR2C/D receptors (PPDA 10 and 20 mg/kg, *n* = 8, Tocris; Ki values are 0.096, 0.125, 0.31 and 0.55 μM for NR2C, NR2D, NR2B and NR2A subunits, respectively). Vehicle: saline. All drugs were injected s.c., except NVP-AAM077 which was also tested in one series of 20 mg/kg i.p. injections.

Electrophysiology and Data Analysis

Cortical field potentials were filtered below 100 Hz, using also a 60 Hz notch filter, and sampled at 250 Hz. In 14 rats, the EEG signals were also recorded with lowpass filter setting of

300 Hz and with a 1 kHz sampling rate. In these experiments, the notch filter was off and the 60 Hz noise was eliminated during the processing. EEG signals were subjected to Fast Fourier transform to generate power spectra for consecutive 16 s windows. Gamma oscillations were assessed in frontal and occipital cortex EEGs using the average spectral power in the gamma band surrounding 40 Hz (+/-10 Hz, i.e. 30-50 Hz) and in the higher gamma band, above the notch filter (65-90 Hz). In the recordings with wider filter settings and the notch filter off (n = 14), the low and high gamma bands were widened to 30-58 Hz and 62-90 Hz. For detailed examination of the features of spectral components altered by the injected compounds in these rats (saline, MK801, NVP-AAM077 and Ro-25 6891 at one dose, each), average power spectra of two hours of control recording and two hours at the height of the drug effect were generated and the difference between the two was used. For comparison between rats and different compounds, including all drugs at all doses, the time course of the changes in gamma band power was built for each recording session and the values for gamma power were normalized using the average of gamma power during the first hr of control recording, i.e. the changes in this case were expressed as after vs. before injection ratio. The present study focused on cortical gamma oscillations, and thus only the analysis of cortical EEGs at the frontal and occipital leads are reported here, whereas thorough analysis and assessment of the effects on hippocampal EEG requires further experiments. Statistical analysis was based on pair-wise comparison between each individual drug with saline using Student's t-test and on ANOVA and post-hoc Bonferroni for testing dose-dependence.

Intrastructural theta-gamma cross-frequency coupling was calculated using the algorithm of Canolty *et al.* (3, 4). Briefly, the EEG signals were filtered using butterworth bandpass filter to isolate theta (5-10 Hz) and gamma (30-55 Hz) oscillations and the instantaneous theta phase and gamma amplitude were determined using the Hilbert transform. These two measures were then used to construct a continuous, complex-valued signal and the modulus length of this signal (Mraw) was compared with the distribution of surrogate lengths to obtain a statistical measure of coupling strength. The modulation index, M=(Mraw-m)/SD, where m and SD are the mean and standard deviation of surrogate lengths, is a normalized z-score value which can directly be used to determine the probability that the results would be due to chance. When it is calculated using recording boxes of the same length, the comparison of this metric between different signals and between signals recorded under different conditions are valid (see (3, 4) for more details and discussions). The intrastructural M index was calculated on consecutive 5 min segments and averaged over 60 min periods for the frontal and occipital cortex EEGs,

separately, in 8 rats before and after MK801 (0.2 mg/kg) injection and in 6 of these rats also before and after administration of NVP-AAM077 (20 mg/kg) and Ro-25 6891 (10 or 20 mg/kg).

Neck muscle EMG and the accelerometer signals were processed by calculating the root-mean square (RMS) values in the same time windows as used for the EEG signals. EMG/movement signals were correlated with gamma band power using average gamma power vs. RMS over 5 min segments. Thus, these correlations detect co-occurrence of fluctuations rather than correlation on the short time scale (or interference). Besides a certain steady tone present when the animal is awake, neck muscle activity fluctuates with motor activity as most movements of the rat, confined to a limited space (e.g. digging, turning, rearing, grooming, wiskering, sniffing, etc.), involve head movement.

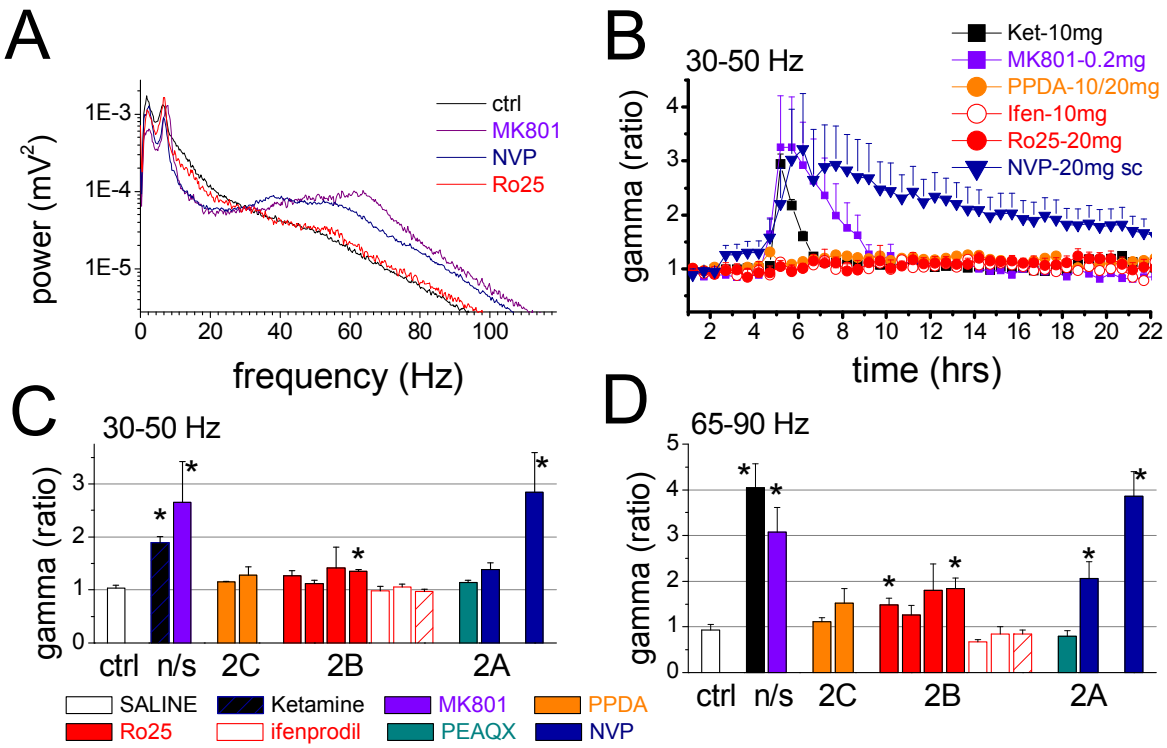

Figure S1. Changes in gamma oscillations over the occipital cortex after injection of NMDA antagonists acting on different subunit-containing receptors. **(A)** Comparison of power spectra (shown on semi-logarithmic scale) of occipital cortex EEG after different NMDA antagonists with control recording in a representative experiment (the spectra were made using 2 hr segments on the top of the reactions). **(B)** Time course of integrated gamma power (30-50 Hz, group averages) over the frontal cortex in consecutive 30 min segments for 3 hrs before and 18 hrs after injections. **(C and D)** Group averages and SEM of relative gamma power (post/pre-injection ratio) in the 30-50 Hz **(C)** and 65-90 Hz range **(D)** calculated in a 30 min (ketamine) or a 2 hr (all other compounds) segment at the peak of the reaction after injection of saline, non-selective NMDA antagonists ketamine (10 mg/kg) and MK801 (0.2 mg/kg), the NR2C/D-selective PPDA (10 mg/kg and 20 mg/kg), NR2B-selective Ro25-6985 (5, 10, 20, and 30 mg/kg), ifenprodil (5 and 10 mg/kg), and threo-ifenprodil (10 mg/kg), and NR2A-selective PEAQX (10 mg/kg), and NVP-AAM077 (10, 20 mg/kg i.p., and 20 mg/kg s.c.). These signals were simultaneously recorded with frontal cortex EEG and were processed the same way as those shown in Figure 1. ctrl, control; EEG, electroencephalogram; Ifen, ifenprofil; Ket, ketamine; NMDA, *N*-methyl-D-aspartate.

Supplemental References

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