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Impairment of neural coordination in hippocampal neuronal ensembles after a psychotomimetic dose of dizocilpine

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ABSTRACT

The discoordination hypothesis of schizophrenia posits discoordination of neural activity as the central mechanism that underlies some psychotic symptoms (including 'hallmark' cognitive symptoms) of schizophrenia. To test this proposition, we studied the activity of hippocampal neurons in urethane anesthetized Long Evans rats after 0.15 mg/kg dizocilpine (MK-801), an *N*-Methyl-D-aspartate (NMDA) glutamate receptor antagonist, which can cause psychotic symptoms in humans and cognitive control impairments in animals. We observed that MK-801 altered the temporal coordination, but not rate, of neuronal firing. Coactivation between neurons increased, driven primarily by increased coincident firing of cell pairs that did not originally fire together before MK-801 injection. Increased pairwise coactivation manifested as disorganized discharge on the level of neuronal ensembles, which in turn could lead to disorganization in information processing. Disorganization of neuronal activity after a psychotomimetic dose of MK-801 supports the discoordination hypothesis of psychosis.

1. Introduction

According to the discoordination hypothesis of schizophrenia, discoordination of activity within neuronal networks is the neural mechanism that underlies symptoms of cognitive disorganization (Phillips and Silverstein, 2003). Central to this hypothesis is the concept of cell assemblies: coalitions of coactive cells that constitute the fundamental functional units of information encoding and processing in the brain (Hebb, 1949; Harris et al., 2003). The importance of coordination in neuronal firing for information processing is supported by a substantial body of evidence; for example, synchronization of neuronal activity in the visual cortex has been implicated in visual scene segmentation (Engel et al., 1991), and coordinated activation of cell assemblies in the hippocampus was shown to organize distinct, competing spatial representations (Kelemen and Fenton, 2010, 2016; Jezek et al., 2011). Organization of neuronal activity has been characterized on timescales ranging from tens of milliseconds to seconds (Olypher et al., 2002; Harris et al., 2003; Jezek et al., 2011; Kelemen and Fenton, 2010, 2013). The timing of synchronized neuronal firing within cell assemblies is organized by rhythmical gamma (Engel et al., 1991; Harris et al., 2003) and theta activity (O'Keefe and Recce, 1993; Dragoi and Buzsáki, 2006; Jezek et al., 2011).

Disturbances in the temporal organization of neuronal firing are the hypothesized cause of impaired information processing in mental disorders, including schizophrenia (Uhlhaas and Singer, 2006; Olypher et al., 2006; Jones, 2010; Moghaddam & Wood, 2014; Fenton, 2015). According to this hypothesis, psychosis is related to impairments in coordination of activity between neurons – a phenomenon that we call discoordination. Discoordination would occur for example, if neurons that normally discharge together stopped firing together, or if neurons that normally discharge separately started firing together, or if combination of the two effects occurred. In this theoretical framework, discoordination manifests on the cognitive level by incomplete, fuzzy or otherwise damaged mental representations. Discoordination in firing between different cell assemblies leads to errors in separating different representations, and to the formation of improper associations between representations, which can be manifested as cognitive disorganization characteristic of psychosis.

NMDA receptor antagonists induce psychotic symptoms in humans (Krystal et al., 1994; Adler et al., 1998, 1999; Lahti et al., 1995, 2001) and are used as a model of psychosis in experimental animals (Stuchlik et al., 2004; Lobellova et al., 2013; Zemanova et al., 2013, Svoboda et al., 2015). Studies in rat models have also confirmed the detrimental effect of NMDA receptor antagonists on cognitive organization; for

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example, in hippocampus-dependent spatial tasks that required animals to segregate and coordinate two types of information (e.g., information about their position in the room and information about their position on the arena: Cimadevilla et al., 2000) NMDA receptor antagonists such as MK-801 impaired acquisition (Stuchlík and Vales, 2005; Kubík et al., 2014) as well as reversal learning (Lobellova et al., 2013; Svoboda et al., 2015).

Within the framework of the discoordination hypothesis we set out to test the effects of MK-801 on the activity of hippocampal neurons. To study effects of MK-801 on hippocampal neuronal dynamics, whilst eliminating confounding effects on hippocampal neuronal firing associated with animal's position (O'Keefe and Dostrovsky, 1971; Muller et al., 1987), motor activity (Stuchlík and Vales, 2005), or attention (Fenton et al., 2010), we used urethane anesthesia in our experimental preparation. Although urethane affects multiple neurotransmitter receptors, including glutamate receptors, the effect is only mild at anesthetic doses (Hara and Harris, 2002), and unlike other anesthetics, it preserves an activity pattern resembling that of an awake animal, including hippocampal theta rhythm (Fox et al., 1986). We predicted that MK-801 would alter the coordination of the timing of neuronal firing (without affecting the firing rates of the neurons), and that this discoordination would be manifested as changes in the timing of action potentials between pairs of cells, changes in coordinated firing within cell assemblies, or changes of phase-locked firing of neurons relative to ongoing theta field oscillations.

2. Materials and methods

2.1. Animals

Six adult male Long–Evans rats (300–400 g) from the breeding colony of the Institute of Physiology of the Czech Academy of Sciences were used. Rats were housed in an air-conditioned room with stable temperature (22 ± 2 °C), humidity (60 ± 10%) and controlled 12 h/12 h light/dark cycle; food and water were available ad libitum. Experiments were performed during the light phase. All experimental procedures were conducted in accordance with the Animal Protection Code of the Czech Republic and the corresponding directive of the European Community Council on the use of laboratory animals (2010/63/EC).

2.2. Drug treatment

MK-801 hydrogen maleate (dizocilpine; supplied by Sigma-Aldrich, Czech Republic), a non-competitive antagonist of the NMDA receptor, was dissolved in sterile physiological saline at a concentration of 0.15 mg/ml. It was injected intraperitoneally at a dose of 0.15 mg/kg.

2.3. Surgery and acute single cell recordings

Rats were anesthetized with urethane (1.2 g/kg, intraperitoneal) and their head was fixed horizontally in a stereotaxic frame. The scalp was removed and burr holes were drilled bilaterally in the skull overlying the left and right hippocampi (AP = −4, L ± 2.5 relative to Bregma) for tetrodes and reference electrodes. Another hole was drilled over the cerebellum for a ground electrode.

Electrodes prepared from an insulated nichrome wire (25 μm in diameter, California Fine Wire, Grover Beach, CA, USA) were used in tetrode configuration. The tips of the electrode wires were cleaned by current application and then gold-plated so that their impedance was 50–200 kΩ.

Two tetrodes were used during each recording session. The tips of the tetrodes were gradually advanced into the pyramidal CA1 layer of the hippocampus, until characteristic complex spike discharges were detected. Before the start of recording, the signals from neurons in the CA1 field had to remain stable for at least 30 min. After the stability of the detected signals was confirmed, signals were recorded for 60 min before, and at least 120 min after MK-801 injection. Heart rate (ECG) and temperature were monitored throughout the experiment. The body temperature of the animals was maintained at 36–37 °C during all of the procedures.

The electrophysiological signal was amplified 5000–10,000 times. The signal from single units was filtered between 300 and 9000 Hz, and digitized at ~32 kHz (Neuralynx, Bozeman, MT, USA and Power1401, Cambridge Electronic Design, Cambridge, UK). The local field potential (LFP) signal was filtered between 0.1 and 500 Hz and digitized at ~2 kHz. Action potential waveforms (2 ms duration) and continuous LFP data were stored and analyzed offline. Discrimination of units was performed based on spike amplitude and wave shape using clustering algorithms in the Spike2 software (Cambridge Electronic Design, Cambridge, UK). Data from 1 h before MK-801 administration and 1 h after the administration were analyzed.

2.4. Correlation analysis

Coactivity between pairs of neurons on a timescale of tens of milliseconds was assessed by cross-correlation. Cross-correlation histograms were computed for all simultaneously recorded cell pairs at 5 ms and 10 ms resolution. Cell pairs were further studied if cross-correlation histograms contained at least 1000 events within a 500 ms time window.

Correlation in firing between pairs of neurons on slower timescale was assessed using non-parametric Kendall's correlations. The cell's firing rate was calculated for 400 ms intervals and Kendall's correlation (tau) was computed for 15 min segments of recording. By comparing tau values across subsequent 15 min intervals we could assess whether positively correlated cell pairs remained positively correlated and vice versa. This way we could detect and compare spontaneous changes in pairwise coordination of neuronal discharge and changes induced by MK-801.

2.5. Ensemble vectors and their correlations

The ensemble activity during each 2 min time interval was characterized by the ensemble spike count vector of the number of action potentials that each cell emitted during the time interval (Fig. 4A). The similarity of ensemble discharge during two time intervals was quantified by computing the Pearson product-moment correlation of the two corresponding spike count vectors. To visualize the change in ensemble discharge patterns before and after MK-801 injection, a correlation matrix was constructed (Kelemen and Fenton, 2013). In the correlation matrix, each interval is compared with every other interval and the correlation coefficient is color coded (Fig. 4B).

2.6. Theta phase modulation

The presence of theta oscillations in local field potentials (LFP) was assessed using the fast Fourier transformation. To study the modulation of neuronal activity by theta rhythm, the LFP signal was filtered between 3 and 10 Hz and the Hilbert transformation was used to determine the theta phase and amplitude at times of neuronal spikes. The distribution of firing in different phases of the theta rhythm was calculated for each cell (Fig. 5E). The significance of theta modulation of a cell firing was determined using runs test. For an additional analysis, each action potential of a neuron was represented by a vector with direction determined by the theta phase (Fig. 5F). The average vector was then calculated as a representation of the theta modulation of the cell's firing. The length of the average vector was used to assess the strength of theta modulation, and the direction of the vector represented the preferred theta phase of the neuron's activity.