The effects of MDMA on *in vitro* hippocampal network dynamics

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Introduction

Neural synchronization involves bursts of rapid membrane potential activity in specific frequencies [1]. Previous research has shown that 3,4-Methylenedioxymethamphetamine (MDMA) has a range of effects on the brain, including changes in neuronal activity and neurotransmitter release correlating with changes in memory consolidation [2]. The microelectrode array (MEA) is a novel device that can be used to measure neuronal synchronization [3]. The effect of MDMA on neural synchronization and electrophysiology remains largely unexplored.

MDMA has been shown to impact neuronal activity. The addition of MDMA facilitates the release of both dopamine and serotonin throughout various regions in the brain. Preliminary evidence shows that MDMA facilitates hyperactivity in hippocampal cells, strengthening the claim that cognitive impairment of MDMA is attributed to its effects in other brain regions [4].

Due to the need for both temporal and spatial resolution for study of network dynamics, an array of microelectrodes (MEA) can record extracellular signals from plated cultured neurons or brain slices [5, 6]. MEAs allow high temporal resolution recordings and the multiple electrodes of the array provide information on the spatial flow of information that cannot by measured with a traditional single electrode system. Therefore, using an MEA can facilitate understanding the effects of MDMA on network bursting activity.

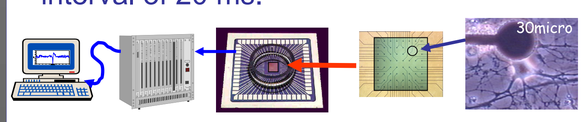
Methods

All methods were approved by the Georgetown University Animal Care and Use Committee.

Generation of the neuronal network: Hippocampal cultures were harvested from E17-18 mice, with a plating density of 700 cells/mm^2. Initial plating medium included Neurobasal, B27, 200 mM pen/strep, 0.5 mM glutamine and 25 mM glutamate. At DIV4 half of the volume of media was replaced with a glutamate-free media. Cultures were maintained in a 37^{o}C incubator (10% CO_2).

Recording: Extracellular electrical activity from the MEAs were recorded between DIV15-21. Recordings were performed on a heated stage at 37^{o}C and the signal from the electrodes was passed through a 64-channel preamplifier (standard gain 1100; preamp filter 300 Hz to 3 kHz) and transmitted onto a computer (Dell Computing) for visualization and recording for offline analysis.

Data Analysis: 60 seconds of continuous recording were subjected to a high-pass filter (25Hz), and spike waveforms were detected using a Spike Detector tool (Multichannel Systems). The threshold for a spike was defined to be 5σ of average noise amplitude. Timestamps for spikes were processed using a Spike Analyzer tool (Multichannel Systems) to generate raster plots. A burst was defined as a series of 10 spikes (minimum) with a maximum inter-spike interval of 20 ms.



Acknowledgements

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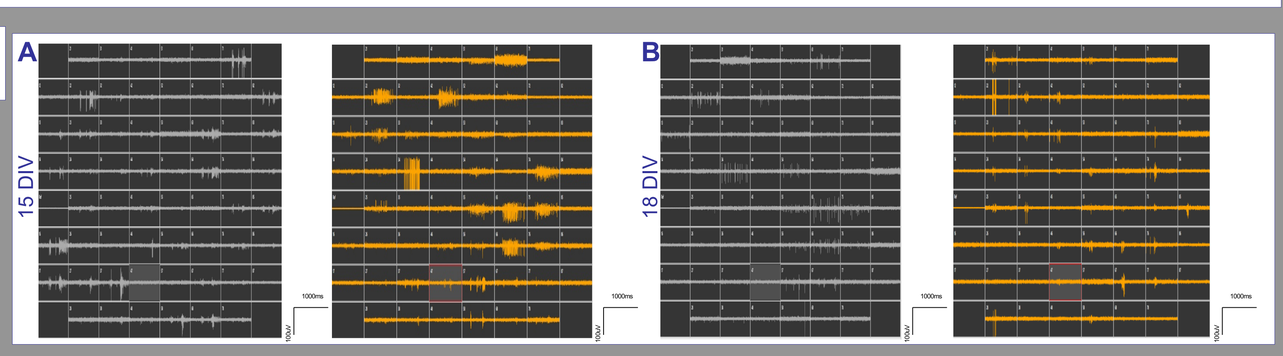
Results



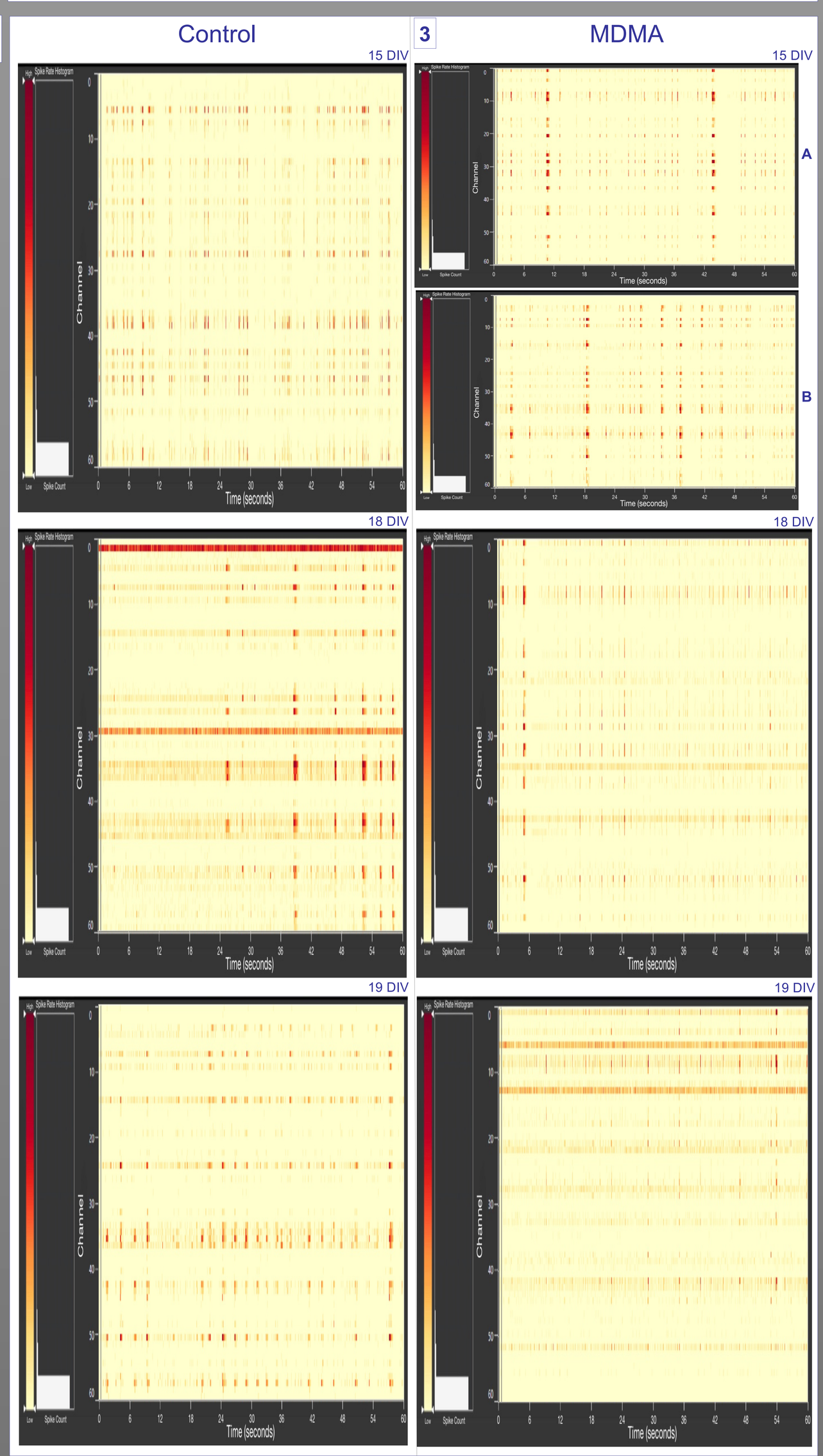


Figure Legends:

1.Recordings from hippocampal cultures grown on an MEA.
Action potentials are shown as thin downward-deflecting lines. Several units with bursts of activity appear as a cluster vertical of lines. Recordings are from a control culture (white traces) and from a culture treated with MDMA (orange traces) and are dated 15 days in vitro (DIV) **(A)** and 18 DIV **(B)**.

2.Spike frequency plot of the control network.
One-minute recordings from 15, 18, and 19 DIV for the control culture. Red indicates a high density of spiking at a given time (burst) and white indicates a low density of spiking.

3. Spike frequency plot of the MDMA network.
One-minute recordings from 15, 18, and 19 DIV for the MDMA culture. Baseline activity of 15 DIV network prior to modulation **(A)** and post-MDMA modulation **(B)** are shown. Red indicates a high density of spiking at a given time (burst) and white indicates a low density of spiking.

Conclusions

- MDMA resulted in increased neuronal spike bursts directly after modulation.
- MDMA resulted in decreased time intervals between high frequency neuronal spike bursts directly after neuromodulation.
- Preliminary evidence suggests up to three days after treatment with MDMA, the hyperactivity is no longer present and there is no visual difference between MDMA-modulated networks and control networks suggesting the effects of MDMA are transient.

Future Studies

- *Ongoing:* Perform more pharmacological treatments using MDMA at various concentrations.
  - Is the magnitude of MDMA's effect on hippocampal neuronal networks concentration dependent?
- Determine the minimum concentration necessary to produce significant changes in network dynamics.
- Study the effect of MDMA in inducing potentiation in hippocampal neuronal networks.
- Perform MDMA modulations on hippocampal slices.

References

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