# Translational Utility of Mismatch Negativity:
## Implementing MMN in rats for Drug Discovery

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## BACKGROUND

Mismatch negativity (MMN) is a validated, objective measure of central auditory processing and has become a key clinical biomarker in schizophrenia. MMN is an auditory evoked potential (AEP) elicited clinically by an auditory oddball paradigm in which a different, deviant ('oddball', DEV) auditory (tonal) stimuli occur infrequently and unexpectedly within a sequence of repetitive identical tonal stimuli ('standards', STD) and reflects pre-attentive processing dependent upon NMDA receptor function.

Here we demonstrate back-translatability of this AEP in Sprague-Dawley (SD) rats and show that NMDA receptor antagonism can impair rat MMN. First, we developed a system whereby we utilized the Data Sciences International (DSI) telemetry system to provide the flexibility and high-throughput nature of wireless recording up to 16 SD rats simultaneously in sound-attenuated chambers with the robust data handling and timestamping capabilities of the Cambridge Electronic Design (CED) micro1401 processor. A custom sequencer file enabled Spike2 software to generate tonal stimuli delivered to all rats simultaneously and timestamp the EEG data with digital precision. Rats were subjected to a standard flip-flop oddball paradigm using 6 kHz and 8 kHz tones

## METHODS

### Auditory Evoked Potential Platform

Adult male Sprague-Dawley rats (>300 grams) were implanted with wireless DSI transmitters (F40 or F50) and supradural screw electrodes placed above frontal cortex with reference placed above cerebellum. 16 rats were kept in their home cages and placed into sound-attenuated chambers and positioned below a speaker atop the DSI receiver. EEG was recorded continuously during each flip-flop sessions of audio stimuli (MMN protocol).

Flip-flop MMN protocol: Approximately 1,000 tones of 6.0kHz were delivered at 90% probability (i.e. the low standard) and 100 tones of 8.0kHz were delivered at 10% probability (i.e. the high deviant) in pseudo-random order (random except that two deviants were not presented back-to-back). In the second protocol, following the first protocol by 60-90 seconds, the frequencies were flipped to deliver 1000 tones of 8 kHz as the high standard (STD) stimulus (90% probability) and 100 tones of 6 kHz as the low deviant (DEV) stimulus (10% probability). Each tone was 50 ms in duration. The interstimulus interval (ISI) between tones was 350 ms. The combination of these two protocols constituted the 'flip-flop' session.

Difference Waves (DIFF) were calculated for each 1 ms bin (-200 to 400 ms) by subtracting the µV values of STD from DEV. The N1 or P2 components were extracted from 20-60 ms or 50-150 ms time windows, respectively.

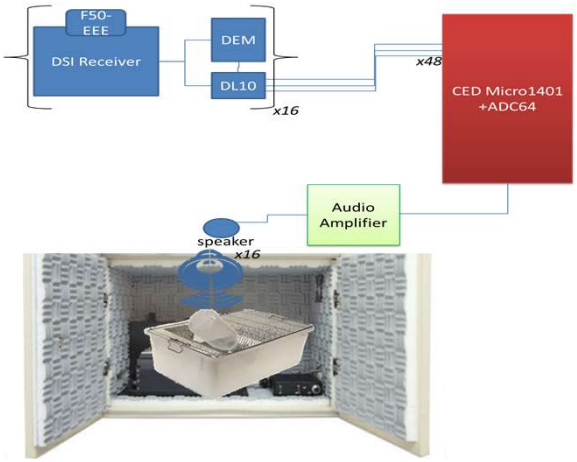
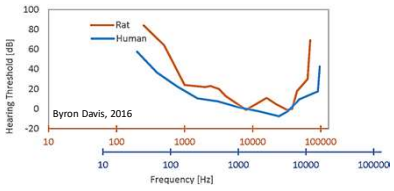


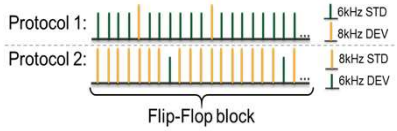

***Figure 1: Leveraging the high-throughput capability of the DSI system's wireless EEG with the temporal precision and data processing of the CED system provides a reliable turn-key solution to running up to 16 animals in the MMN protocol simultaneously. The CED system through custom sequencer files delivers tonal stimuli that were digitally timestamped and logged with the continuously-recorded EEG for AEP generation.***

***Figure 2: The auditory stimulation protocol to elicit MMN in rats was directly back-translated from clinical protocols with 2 exceptions:***

***(1) Higher frequency tones (6-8kHz) were used in the rat to compensate for different hearing ranges.***




***(2) Since different tonal frequencies elicit different AEP amplitudes, a flip-flop paradigm was used and Difference Waves were calculated using tones of the same frequency for when that tone was presented as Standard (STD) or when its presented as Deviant (DEV)***




## RESULTS

Vehicle-treated rats had a significantly larger response to DEV than STD as measured by peak and area under the curve components of the AEP waveform, while MK-801 (0.1-0.3 mg/kg) showed dose-dependent effects on impairing MMN. Specifically, Vehicle-treated rats had a significantly larger response to DEV than to STD as measured by the N1 peak (between 20-60 ms) and the area under the curve (AUC) for N1 (30-60 ms) while the N1 and N1-AUC for STD and DEV did not differ after MK-801 (0.3 mg/kg), revealing clearly inhibited AEPs after NMDA antagonism. Further, the Difference Waves (DIFF), generated by subtracting STD from DEV, revealed MK-801 significantly suppressed both N1 and P2 (between 50-150 ms) peak amplitudes and AUCs. Showing clear dose-dependency, MK-801 (0.1 mg/kg) had intermediate effects but did not demonstrate as robust an impairment of MMN as 0.3 mg/kg. This work further validates that rats have a MMN correlate to that of the human and that this biomarker is dependent upon NMDA receptor function. Further, we demonstrate the ability to perform this in a higher-throughput manner necessary for drug discovery, which will lead to the further work needed to validate the translatability and predictive validity of the rat MMN.

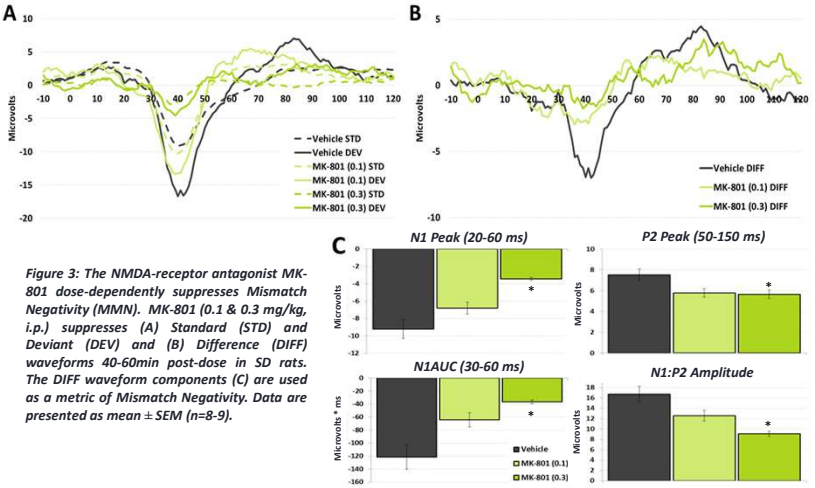



***Figure 3: The NMDA-receptor antagonist MK-801 dose-dependently suppresses Mismatch Negativity (MMN). MK-801 (0.1 & 0.3 mg/kg, i.p.) suppresses (A) Standard (STD) and Deviant (DEV) and (B) Difference (DIFF) waveforms 40-60min post-dose in SD rats. The DIFF waveform components (C) are used as a metric of Mismatch Negativity. Data are presented as mean ± SEM (n=8-9).***

To further probe the NMDA receptor's role in regulation of MMN, we tested Glycine, which is known to modulate NMDA channel function. We show that the MK-801 (0.2 mg/kg)-impaired MMN can be restored by Glycine (1.6 g/kg) as seen in the Difference waveforms (A) and its waveform components (B). This result is similar to clinical observations, where acute high-dose Glycine attenuated MMN in healthy human controls (Leung et al., 2007; Greenwood, et al., 2018).

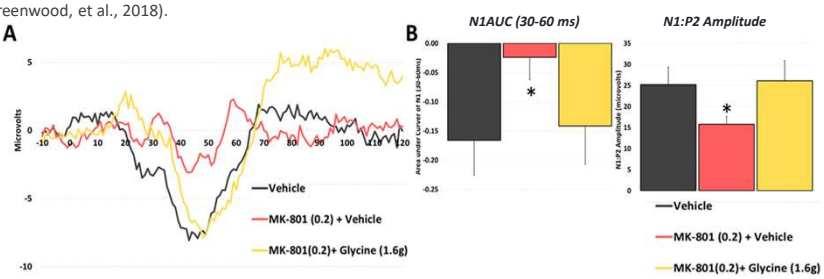



***Figure 4: MK-801 (0.2 mg/kg)-impaired MMN can be restored by Glycine (1.6 g/kg) as seen in the Difference waveforms (A) and its waveform components (B). Data are presented as mean ± SEM (n=13-16), *p<0.05, t-test vs. vehicle. MK-801 (0.2) and Glycine were given 60 and 30 minutes prior to MMN respectively.***

Similar to Oranje et al., 2008, and Wienberg et al., 2009, who showed that Escitalopram increased MMN in healthy volunteers (HVs), we demonstrated in healthy SD rats that Escitalopram (10 mg/kg, s.c.) increased components of the MMN waveforms (both STD and DEV). In the rat, the N1 was only moderately increased, yet the P2 was more widely effected and showed a shorter latency, likely reflecting an increased processing speed also reported clinically.

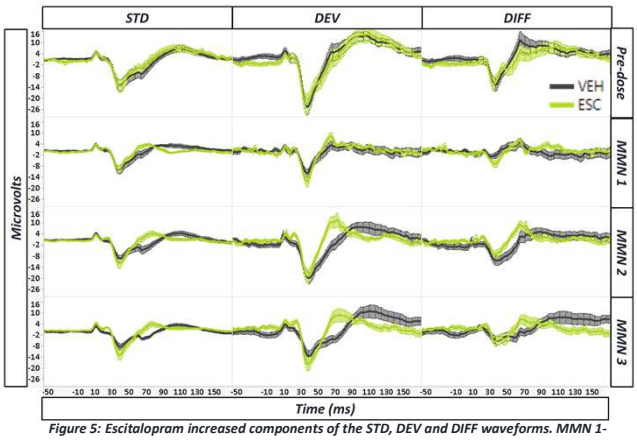



***Figure 5: Escitalopram increased components of the STD, DEV and DIFF waveforms. MMN 1-3 are 0-20, 20-40, and 40-60 minutes post-dose, respectively. Mean ±SEM (n=15 each).***

## DISCUSSION

MMN is an index of cognitive decline and disturbed central auditory processing in ageing and many neurological and neuropsychiatric disorders and thus presents as a highly attractive biomarker in drug discovery. Our work further validates that rats have a MMN correlate to that of the human MMN and that this biomarker is dependent upon similar mechanisms and receptor function and pharmacologically pliable. Further, we demonstrate the ability to perform this in a high-throughput manner necessary for drug discovery, which will lead to the further work needed to validate the translatability and predictive validity of the rat MMN.

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*Schizophrenia International Research Society, April 2019*