NMDA Induced Rhythmic Motor Patterns in Wild Type Zebrafish Larvae

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Introduction

Neural networks for locomotion are localized in the spinal cord. Rhythmic patterns of behavior, such as walking and swimming, are induced by central pattern generators (CPG), which are neural networks that can produce endogenous rhythmic patterned motor outputs in the absence of inputs from higher centers in the brain and other sensory afferents. Studies have shown that rhythmic motor patterns can be induced by the application of exogenous sources of excitation to *in vitro* spinal cords. The presence of coordinated locomotor activity patterns without higher-order processing reveals that the CPGs responsible for generating locomotion are intrinsic to the spinal cord.

Recently, the zebrafish larvae has arisen as a good model for studying neuronal circuits responsible for generating behaviors. Electrophysiology, genetics, and optical imaging techniques can be applied in studies using this model. Spinalized zebrafish larvae at 3 days post-fertilization were shown to produce rhythmic motor patterns with the application of the excitatory amino acid agonist N-methyl-D-aspartate (NMDA). Here we monitored motor activity induced by NMDA in motor neuron peripheral nerves using extracellular recording techniques in an intact (non-spinal) preparation. The optimal concentration of NMDA required to induce rhythmic swimming behavior is determined by generating a dose-response-curve. Our analysis of the curve provides a foundation for future research that examine CPG induced motor patterns that utilize NMDA as an exogenous source of excitation.

Methods

Animals: Experiments were done on wild type zebrafish larvae [4-6 days post-fertilization (dpf)].

Experimental preparation: Zebrafish larvae were anesthetized in 0.02% Tricaine (MS222) in fish saline and then pinned on their side to a Sylgard-lined glass bottom petri dish with short pieces (~1 mm) of fine tungsten wire (0.001 in dia.). The pins were pushed through the notocord rostrally and caudally. Fine forceps were used to remove the skin between pins. The preparation was then paralyzed with α-bungarotoxin **(Fig. 1)**.

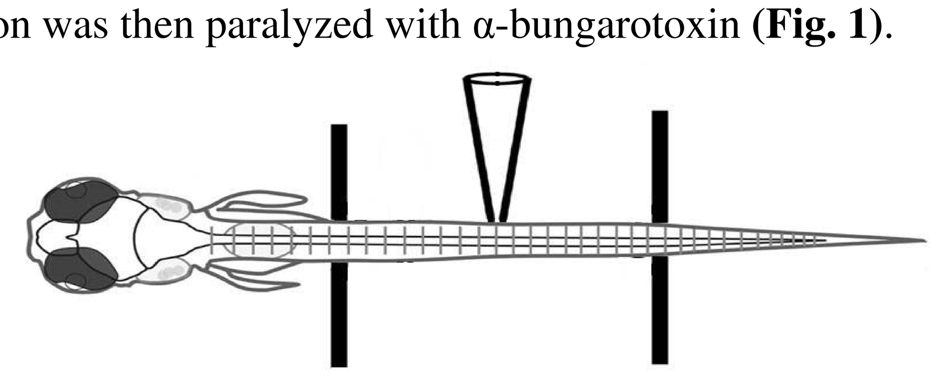


Figure 1. A schematic diagram of a larval zebrafish at 4–6 dpf. Fine tungsten wires (thick vertical bars) were pushed through the notochord to secure the fish in a lateral position. Extracellular electrode shown at midbody (cone).

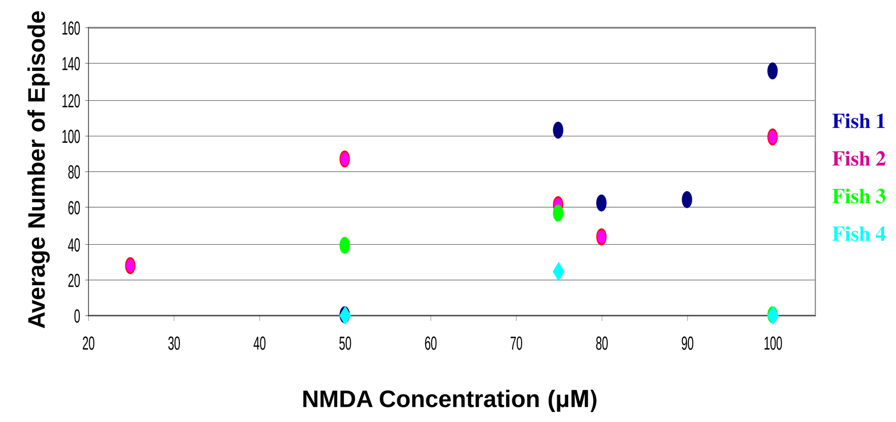
Electrophysiology: Fictive swimming behavior was monitored using extracellular recordings. Extracellular suction electrodes placed at the myotomal clefts along the midbody axis, body segments 7 to 20, monitored activity in peripheral nerves.

Pharmacology: Drugs used in fish preparation are as follows: 0.02% MS222 and 0.05% α-bungarotoxin. Fish remained in extracellular recording solution [fish saline ("fish-sauce")]. NMDA concentrations used ranged from 25-100 µM.

Data acquisition and analysis: Extracellular recordings were digitized and acquired using a digitizing board and pClamp 8.2 software, respectively. A custom software program written in MATLAB code was used to find a number of behavioral-related components of the digitized signal, including episode duration, burst frequency, burst duration, cycle period, and duty cycle. Episode number was counted by hand.

1. Episode Number

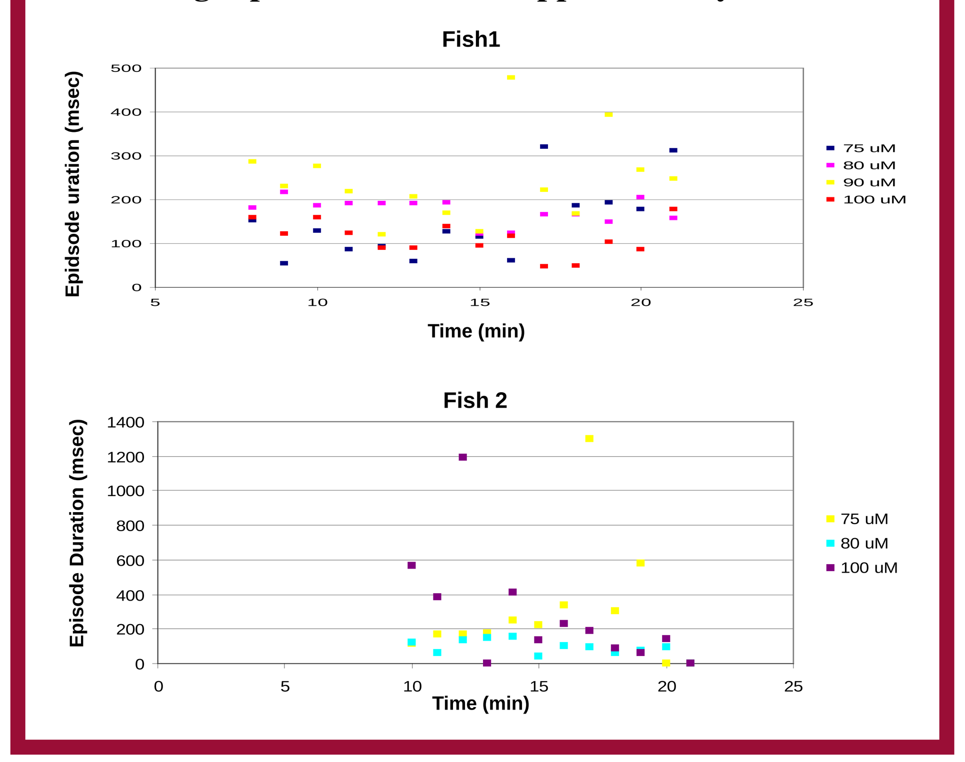
- **NMDA concentration dependent**
- **Episode number increases with increasing NMDA concentration**



Application of 100 µM NMDA to Fish 3 and 4 elicited excessive activity and could not be analyzed

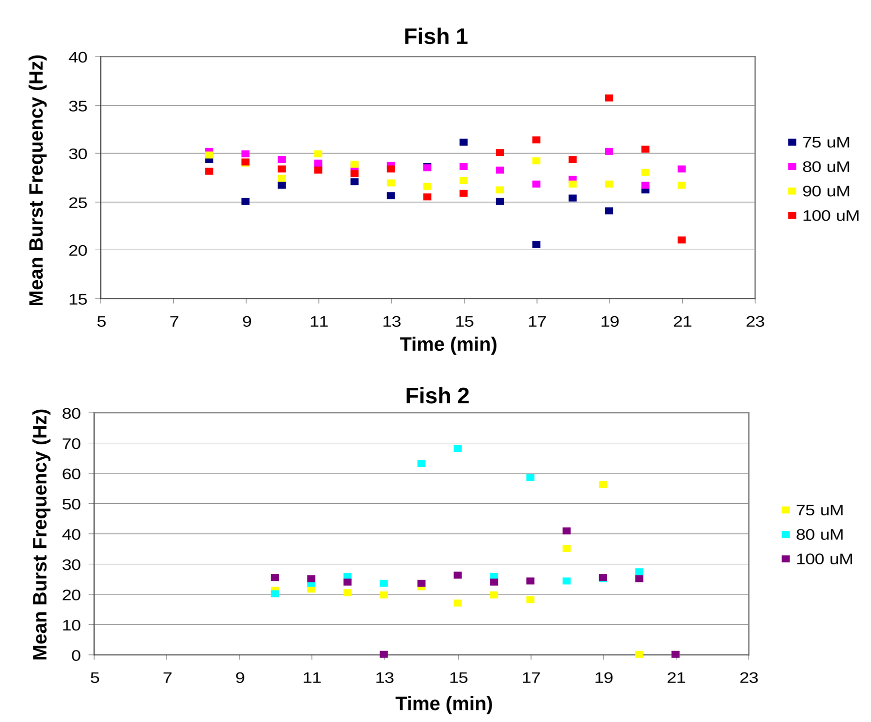
2. Episode Duration

- **NMDA concentration independent**
- **Average episode duration is approximately 150 msec**



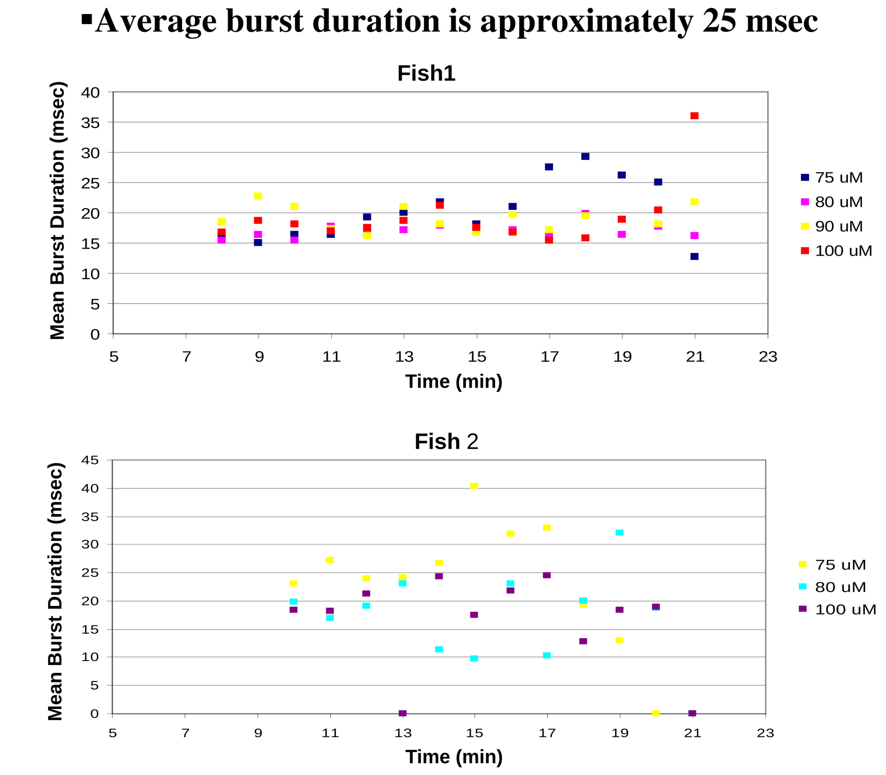
3. Burst Frequency

- **NMDA concentration independent**
- **Average burst frequency is approximately 25 Hz**



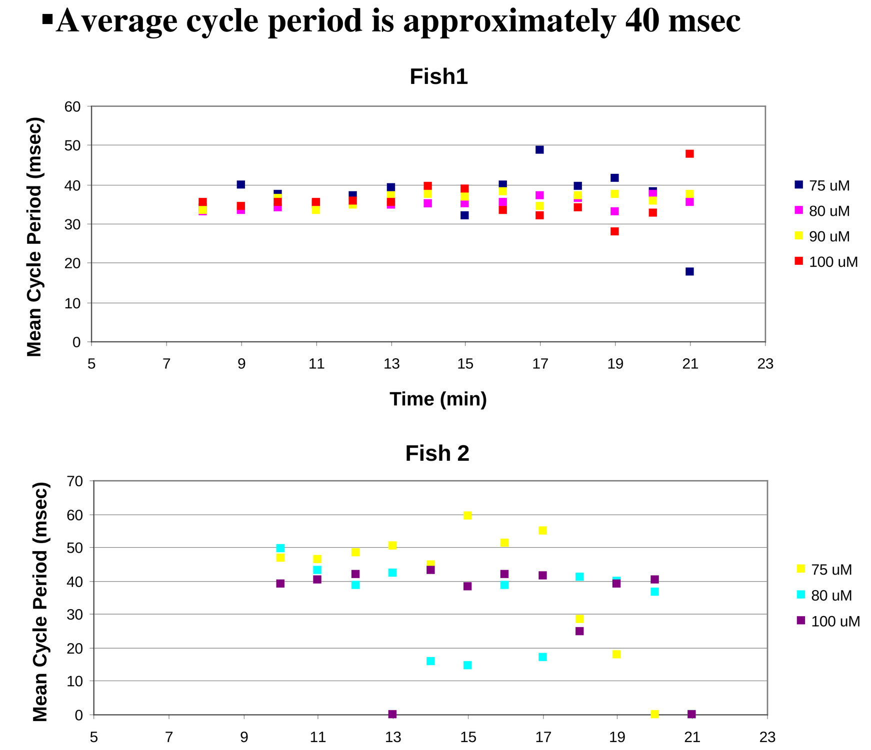
4. Burst Duration

- **NMDA concentration independent**
- **Average burst duration is approximately 25 msec**



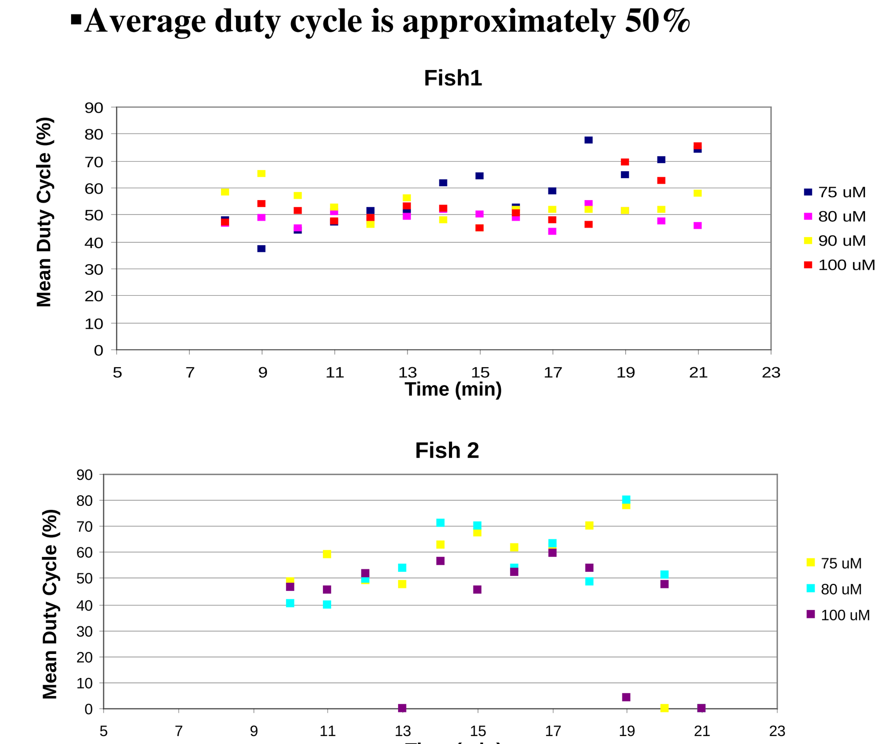
5. Cycle Period

- **NMDA concentration independent**
- **Average cycle period is approximately 40 msec**



6. Duty Cycle

- **NMDA concentration independent**
- **Average duty cycle is approximately 50%**



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Behavioral Features

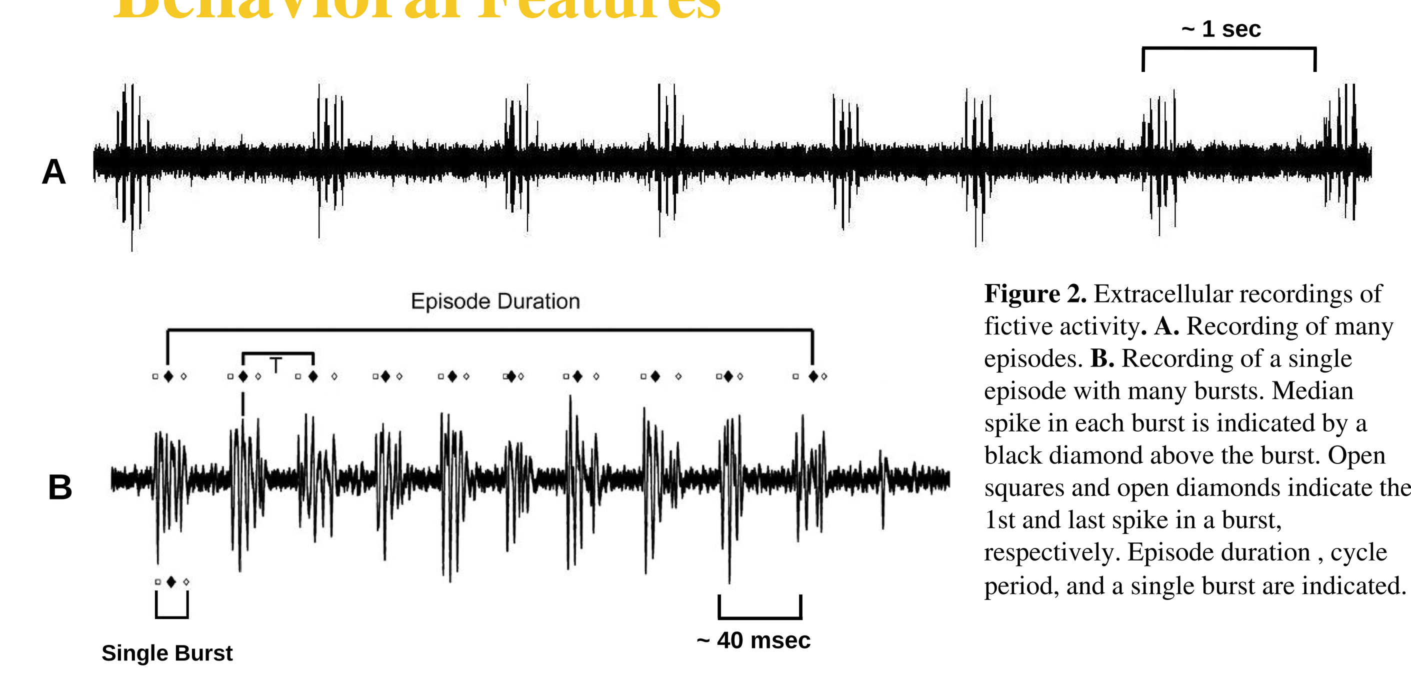


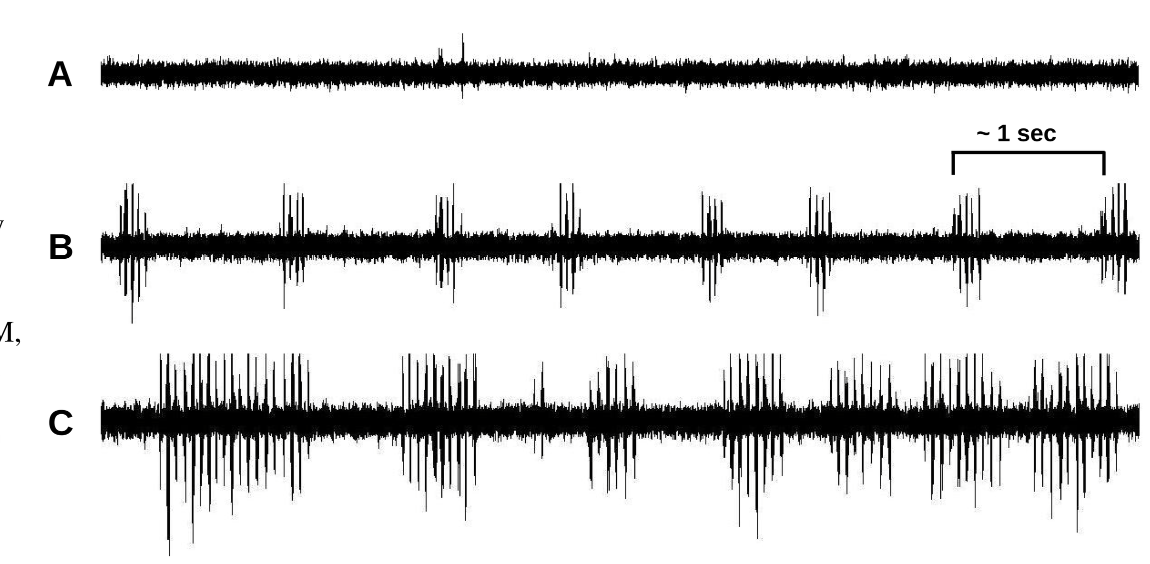
Figure 2. Extracellular recordings of fictive activity. **A.** Recording of many episodes. **B.** Recording of a single episode with many bursts. Median spike in each burst is indicated by a black diamond above the burst. Open squares and open diamonds indicate the 1st and last spike in a burst, respectively. Episode duration , cycle period, and a single burst are indicated.

Episode number:	number of swimming episodes in a one minute window
Episode duration:	interval from the first spike of the first burst to the last spike of the last burst, msec
Cycle period (T):	interval from the median spike to median spike of consecutive bursts, msec
Burst frequency:	inverse of the cycle period (1/T), Hz
Burst duration (BD):	portion of period (T) occupied by spike activity, msec
Duty cycle (D):	percentage of T occupied by BD, [(D=BD/T) X 100], %

Optimal NMDA Concentration

75 – 80 µM NMDA elicits regular rhythmic swimming pattern

Figure 3: Extracellular recordings displaying swimming motor pattern elicited by different concentrations of NMDA: **A.** 50 µM, **B.** 80 µM, and **C.** 100 µM. Application of 80 µM elicited the most regular pattern.



Conclusion

- **Episode number is NMDA concentration dependent**
- **Episode duration, bout frequency, bout duration, cycle period, and duty cycle are NMDA concentration independent**
- **NMDA concentrations of 75 – 80 µM were most effective at evoking regular patterns of swimming behavior**
- **It takes ~ 10 minutes of an NMDA wash to observe regular motor pattern (data not shown)**

References

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