

# Inducing Neuronal Bursting Activity in the CNS of *L. stagnalis* Using Dimethylformamide

John DiCecco<sup>1</sup>, Michael Segala<sup>2</sup>, and Ying Sun<sup>1</sup>, PhD

<sup>1</sup>Department of Electrical, Computer, and Biomedical Engineering, <sup>2</sup>Department of Physics  
University of Rhode Island, Kingston, RI, USA

**Abstract**—Dimethylformamide (DMF) is a hydrophilic aprotic solvent often used in peptide coupling, an essential process in the pharmaceutical industry. Its use in biological study is typically in the role of a peptide solvent when evaluating the efficacy of peptides and their effect on the cellular membrane. In evaluating the effect of the peptide *gramicidin* on neurons of the central nervous system (CNS) of the pond snail *Lymnaea stagnalis*, an interesting cellular response was noted when applying DMF without gramicidin. In the presence of DMF alone, the regular beating pattern of neuron VD1, an identified neuron in the dorsal presentation of the visceral ganglion, changed to bursting pattern. A slight lowering of the membrane potential was also noted.

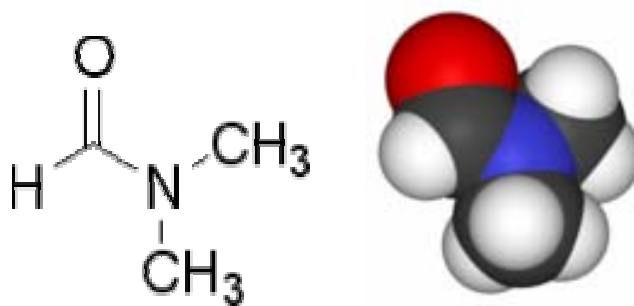


Figure 1. Molecular model of Dimethylformamide (DMF). DMF is a hydrophilic aprotic solvent that has been shown to increase cellular membrane permeability [4].

## I. INTRODUCTION

In evaluating the efficacy of peptide-peptide or peptide-cell interactions, a control substance is used to determine whether the effects noticed are due to the peptide or the solvent in which the peptide is dissolved. A common solvent choice for evaluating peptide-cell interactions is Dimethylformamide (DMF). In cellular permeability evaluations of a number of substances, DMF was shown to nominally affect cellular permeability [1].

An interesting cellular response was noticed when performing control experiments for evaluating the effect of gramicidin on the cellular bilayer lipid membrane (BLM) of the identified neuron VD1, a neuron visible from the dorsal side of the visceral ganglion of *L. stagnalis* [2]. Within seconds of adding DMF to the solution in which the central nervous system (CNS) of the snail was bathed, the regular beating pattern of VD1 changed to a bursting pattern.

## II. MATERIALS AND METHODS

DMF is a hydrophilic solvent which can be used to dissolve peptides. For this research, 10mM DMF (*N,N*-Dimethylformamide, Sigma-Aldrich) was used as a control in evaluating the effects of gramicidin on the BLM. A volume of 200 $\mu$ L of DMF was added to a Petri dish containing the CNS of *L. stagnalis* and 30mL of snail saline (NaCl 51.3 mM, KCl 1.7 mM, CaCl<sub>2</sub> 4.1 mM, MgCl<sub>2</sub> 1.5 mM, with an adjusted pH of 7.4).

The dissection of *L. stagnalis* begins with the administration of 0.36M MgCl to anesthetize the animal. The snail is removed from its shell and pinned to a Sylgard lined Petri dish in a solution of snail saline. Micro dissection techniques are used to expose the CNS, and the ganglia are removed intact and transferred to a smaller Sylgard lined Petri dish. The structure is stretched and pinned securely for ease of insertion of the glass microelectrode [3].

The neuron VD1 was identified by visual inspection (Fig. 2) and was impaled with a glass sharp microelectrode filled with 3M KCl.

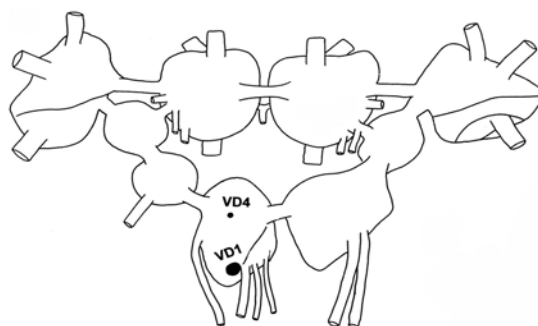
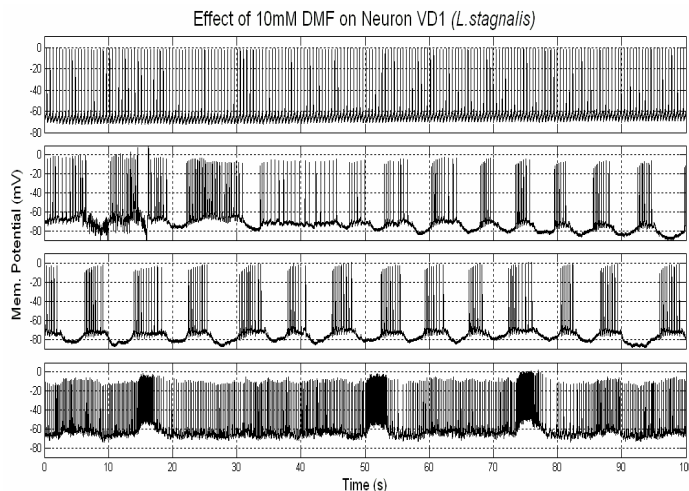


Figure 2. The image is a diagrammatic representation of the ring ganglia of the CNS of *L. stagnalis* [2]. The far left and right ganglia are the cerebral ganglia and have been separated to spread out the ring. This is the dorsal view of the CNS and the neuron VD1 is located at the base of the visceral ganglion.



**Figure 3.** The top trace shows the steady beating output of the neuron VD1 in the visceral ganglion of *L. stagnalis*. The next trace (below) shows the effects of DMF on the neuron. DMF was added at approximately 6 seconds after the beginning of the recording and almost instantly, the pattern becomes bursting. Two minutes later (next lower trace) the neuron has settled into a rhythmic bursting pattern. The bottom trace shows the output of the cell after rinsing the DMF with snail saline.

Time series recordings are made using a Gene Clamp 500 (Axon Instruments) amplifier/stimulator and a PMD-1608FS USB Analog to Digital (A/D) converter (Measurement Computing). MATLAB® (Mathworks, Inc., Natick, MA) was used as the A/D converter interface language as well as the graphing tool. A 1mL sample of 10mM was added to the 30mL vessel containing snail saline and the CNS of *L. stagnalis*. Time series recordings were made before the addition of DMF, while adding DMF, and after 2 minutes of DMF.

### III. RESULTS

The top recording in fig. 3 shows a baseline recording from the identified neuron VD1 from *L. stagnalis*. The neuron is known to be a steady beating neuron under normal conditions [2]. The neuron VD4 in the same ganglion was used for the evaluation of gramicidin in DMF. The effect observed in the recording from VD1 was not observed from VD4. More research is necessary before a plausible theory can be supported as to the reasons for this.

The next recording (immediately below) shows the onset of bursting activity approximately 6 seconds after the addition of DMF to the snail saline. DMF was added directly to the snail saline using a micropipette. No agitation was used as this would be manifested as a mechanical stimulation of the neuron due to the nature of sharp microelectrode recording.

The third recording from top shows that the regular beating pattern has changed to a bursting pattern. What is most noteworthy is the speed at which this reaction happens. Notice in the middle recording that a steady bursting pattern has established itself within 60s of adding the DMF. The

bursting pattern continued until the recording was stopped, some 30 minutes after adding DMF to the saline.

The bursting pattern was stopped by flushing the DMF with snail saline (bottom trace, Fig. 3). There are still some lasting effects from the DMF on the cell, but clearly the steady bursting has reverted to a steady beating pattern

### IV. DISCUSSION

The effects of DMF on the beating pattern of a neuron in the visceral ganglion of the pond snail *L. stagnalis* have been presented. It is unclear what the application of this discovery may be. However, bursting cells have an important role in many invertebrate physiological functions including movement and feeding as well as the reciprocal inhibitory oscillator. It is possible that complex physiological processes can be altered by the addition of DMF to the normal saline environment. Further research will investigate such a hypothesis as well as the dose response associated with the observed effect.

There is some concern over the issue that there appears to be no reference to this phenomenon in literature. In fact, a thorough literature search revealed no reference to any similar effect, only that DMF is used as a solvent for proteins and peptides. Some research has indicated that DMF increases cellular permeability [5]. There may be a connection between this action and the mechanisms that allow for the bursting activity of the cell. It may also be that DMF is affecting either another cell or the neural tissue of the CNS of the snail and the bursting pattern is a response to those effects. Research will continue to help address these theories.

### ACKNOWLEDGEMENT

The authors would like to thank Dr. Robert Hill, research professor at the University of Rhode Island, for establishing the network of researchers for us to draw upon. Without his generous support, both in laboratory equipment and teaching, the successes of this research would be few to be sure. We would also like to thank Dr. Ken Lukowiak from the University of Calgary, for providing the initial colony of *Lymnaea stagnalis*.

### REFERENCES

- [1] M. Rack, S. Hu, N. Rubly, C. Waschow. "Effects of chemical modification of amino and sulfhydryl groups on the voltage-clamped frog node of Ranvier," *Pflügers Archiv European Journal of Physiology*, Vol. 400, April, 1984.
- [2] Lukowiak, K. Central pattern generators: some principles learned from invertebrate model systems. *J Physiol (Paris)*. 85(2):63-70, 1991.
- [3] NI Syed and W Winlow. Respiratory behavior in the pond snail *Lymnaea stagnalis*. II. Neural elements of the central pattern generator (CPG). *J Comp Physiol [A]* 169:557-568, 1991.
- [4] <http://en.wikipedia.org/wiki/Dimethylformamide>
- [5] M. Rack, S. Hu, N. Rubly, C. Waschow. "Effects of chemical modification of amino and sulfhydryl groups on the voltage-clamped frog node of Ranvier," *Pflügers Archiv European Journal of Physiology*, Vol. 400, April, 1984.