Activation of ACR-7 nicotinic receptors by acetylcholine in the nematode *C. elegans*.

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Introduction

Schizophrenia is a debilitating disorder that affects roughly 1% of people in the U.S. The disorder is characterized by an altered perception of reality, which heavily affects decision-making and management of emotions. These effects of perception make it difficult for afflicted individuals to discern what is real. Schizophrenics experience delusions, hallucinations, lack of both emotional expression and interest in the world around them.[1] Severity of schizophrenia spans a wide spectrum, and there are therefore many resulting treatments. However, some cases, known as treatment-refractory schizophrenia are resistant to most forms of treatment.

Clozapine is currently the most effective antipsychotic drug (APD) available for patients with treatment-refractory schizophrenia. While powerful, this drug causes debilitating side-effects, including the development of metabolic syndrome, agranulocytosis, hypersalivation, fatigue, and memory problems. The way clozapine works, that is, its mechanism of action, is currently unknown; this hampers the development of improved compounds.

The nematode *C. elegans* is a well-studied model organism. This simple worm provides a system that will allow us to identify clozapine's cellular target. Buttner et al.[2] recently used *C. elegans* to identify a clozapine target very similar to nicotinic acetylcholine receptors (nAChRs) in humans.

Feeding clozapine to wild-type *C. elegans* inhibits their pharyngeal pumping, resulting in larval arrest. Because the pharynx is continuously contracted , the worms are unable to eat and grow. Buttner and colleagues found that knocking out production of the ACR-7 protein in *C. elegans* rescued pharyngeal pumping despite the presence of clozapine. They found that loss of function in the ACR-7 receptor partially suppresses clozapine's inhibition of feeding by restoring pharyngeal pumping. Our investigation of the mechanism of action in clozapine at the ACR-7 receptor will facilitate the development of therapeutics with reduced toxicity for patients with schizophrenia. As a first step towards understanding the mechanism of action in clozapine, we are investigating the functional properties of the ACR-7 receptor activated by acetylcholine.

We use single channel patch clamp methods to track the activation of individual receptors on pharyngeal cells. Adapting the dissection protocol of Shtonda and Avery[3], we created both an optimized dissection and enzyme preparation protocol to harvest pharynx muscle tissue for physiological recordings. We are enthusiastic that our method will permit further receptor recordings in various cellular solutions to potentially extend our receptor target study beyond clozapine alone.

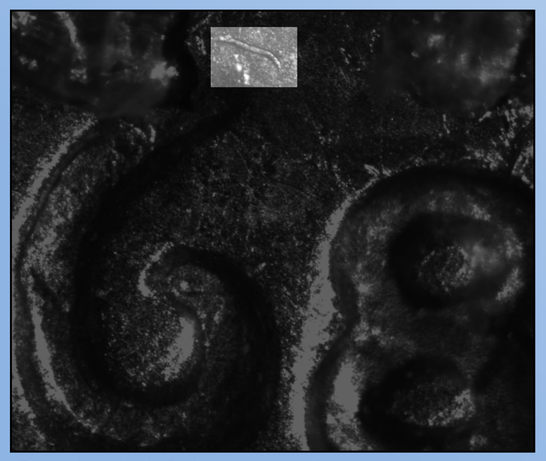


Figure 1. A single *C. elegans* nematode on a penny.

Methods

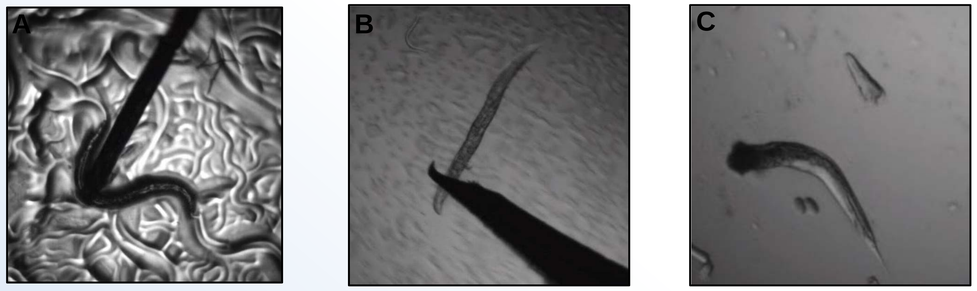
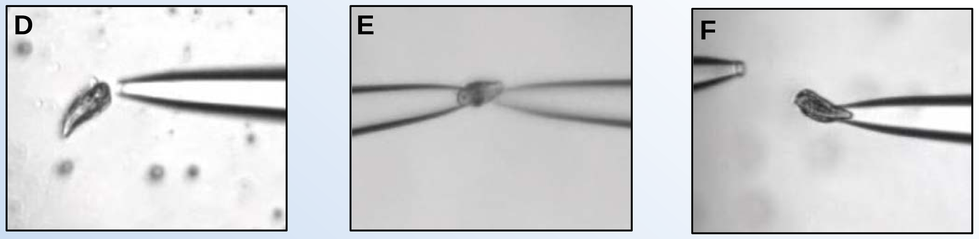


Figure 2. **(A-C)** Individual *C. elegans* were transferred from nematode-growth-media to dissection chambers using an eyelash brush. Worms were then moved to an ice-filled flask prior to dissection. This served to chill the dissection chamber and reduce worm mobility in preparation for head cutting. Heads were removed using a handheld 25-gauge syringe needle. Cuts were made at the level of the terminal bulb on the pharyngeal-intestinal valve. The removal of the head was accomplished with a horizontal incision made under a 4x objective. Extractions were performed using glass micropipettes mounted on manipulators and attached to 10 mL syringes to provide suction.



(D-F) The mouth-end of the head was secured using right pipette, while the left pipette was used to remove excess cuticle from the pharynx. This exposes the terminal bulb. Applying gentle suction, the left pipette grabs and holds the terminal bulb.

(G-I) After firmly gripping the terminal bulb, suction from the left pipette is locked and held constant. The right pipette is then used to grasp the cuticle near the anterior end of the terminal bulb. The right pipette is then moved away from the terminal bulb, pulling away the cuticle. The cuticle is then fully inverted to prevent it from again enveloping the pharynx.

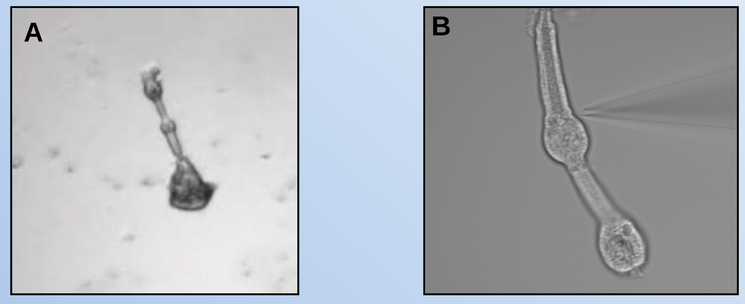


Figure 3. (A) After extraction, the pharynx was released into solution. The pharynx was then placed onto new cover glass. Pharynxes are bathed in 50-µL of digestion mix for 5-7 minutes to digest the basement membrane and expose muscle tissue for patching. Following digestion, the pharynx was transferred to a new coverslip where it was rinsed in 100 µL low Ca^{++} Dent's solution. After a second rinse, the pharynx was moved to the recording chamber. After securing the pharynx to the bottom of the chamber, 3 mL of 3mM Ca^{++} Dent's solution was added to the recording chamber. **(B)** Pharynx in recording chamber with patch pipette nearby.

Electrophysiology and Solutions

Bath and pipette solutions were a modified Dent's solution in (mM): 140 mM NaCl, 6 mM KCl, 1 $MgCl_2$, 3 $CaCl_2$, 5 HEPES, and 45 D-mannitol adjusted to pH 7.3 (345 mOsm kg l^{-1}). Low Ca^{++} Dent's solution has the same concentrations for all components with the exception of $CaCl_2$, which is present at 1 x 10^{-5} mM. Enzyme treatments consisted of (in U/mL): 1300 trypsin, 13 thermolysin, 1 chitinase, 600 protease, and 25 collagenase. Patching pipettes were pulled from thick-walled borosilicate glass to resistances of 7 to 12 MΩ, and coated with Sylgard for noise reduction. Pipettes were front and back filled with Dent's solution containing 10 µM ACh. Patches were voltage-clamped at -20 mV with a HEKA EPC-10 using PatchMaster software. Idealized records and distributions were constructed in QuB.

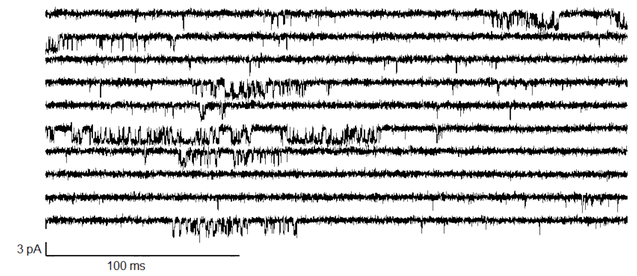


Figure 4. Continuous sweeps from 3 s segments of the data record is shown in the presence of 10 µM ACh.

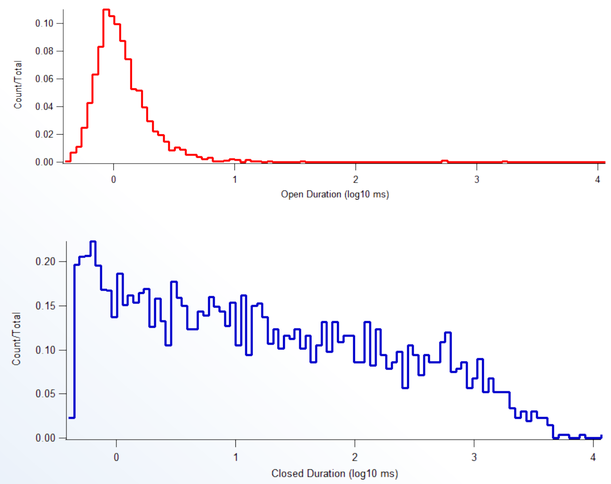


Figure 5. Distributions open and closed duration distributions for the receptor shown in Figure 4, above. The distribution for open durations (top) suggest that the receptor exhibits one predominant open state with a lifetime of approximately 1 ms. Closed duration distributions (bottom) were complex.

Conclusions

We have developed an efficient method for extraction of the pharynx from *C. elegans,* facilitating the single-molecule studies that are required to determine the mechanism of drug action. We have obtained preliminary data that constitutes a first step in understanding the functional properties of ACR-7 and the mechanism of action of clozapine.

References:

1. National Institute of Mental Health. (2009). Schizophrenia. Retreived from http://www.nimh.nih.gov/health/publications/schizophrenia/index.shtml

2. Buttner et al. (2013). "A genome-wide RNAi screen in *Caenorhabditis elegans* identifies the nicotinic acetylcholine receptor subunit ACR-7 as an antipsychotic drug target." PLoS Genet., 9, p. e1003313.

3. Shtonda B, Avery L. 2005. CCA-1, EGL-19 and EXP-2 currents shape action potentials in the Caenorhabditis elegans pharynx. J Exp Biol. 208:2177–2190.