Modulation of neuronal electrical excitability by a common flame retardant

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Background

Incorporating chemical flame retardants (FRs) into many consumer products is required by law[1], but due to the adverse health and environmental effects from legacy FRs, such as polybrominated diphenyl ethers (PDBEs)[2,3], and corresponding changes in safety regulations, there has been a shift toward using novel flame retardants, such as 1,2-dibromo-4-(1,2-dibromoethyl) cyclohexane (TBECH)[4]. Although relatively new, TBECH is pervasive in the environment[5] and bioaccumulative in animals[6]. Recent studies indicate TBECH is an endocrine disruptor[7], affects reproductive physiology[8] and is an obesogen[9]. TBECH has recently been demonstrated to decrease the frequency of spontaneous action potentials in mammalian Purkinje neurons, although the mechanism is unknown.[10]

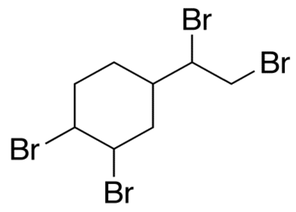

Figure 1. Molecular structure of TBECH

In order to assess in detail the neurobiological activity of TBECH, we have examined its effects on electrical activity of dissociated *Lymnaea stagnalis* neurons using current clamp and voltage clamp electrophysiology. By understanding the effects of TBECH on electrical excitability, we can apply these findings to other organisms, including humans, to assess its safety.

Objectives

To determine whether acute TBECH treatment of dissociated *Lymnaea stagnalis* neurons alters electrical excitability.

To investigate the mechanism behind the effects of TBECH.

Methods

Animals and neuron isolation Great pond snails (*Lymnaea stagnalis*) were anesthetized and ganglia rings were removed. Ganglia were incubated in 0.4 mg/mL Sigma Type XIV protease for 16 minutes, in 1.0 mg/mL papain for 50 minutes, mechanically denuded and gently triturated to dissociate neurons. Neurons were plated on 35mm glass bottom dishes and visualized with Zeiss IM 35 microscope equipped with phase contrast optics and Carl Zeiss PH2 Neofluar 40/0.75 objective.

Electrophysiology
Whole cell patch-clamp of dissociated neurons was conducted in current clamp and voltage clamp configurations. External recording solution (ERS) for current clamp and voltage clamp experiments consisted of (in mM) 4.1 $CaCl_2$, 1.5 $MgCl_2 \cdot 6H_2O$, 5.0 HEPES, 4.0 KCl, 51.3 NaCl, 5.0 D-glucose, pH 7.9 with NaOH, 110-120 mOsm. Data were acquired using HEKA EPC 10 amplifier and PatchMaster V2x90 software.

TBECH
Technical-TBECH was dissolved and diluted in 0.1% dimethyl sulfoxide (DMSO) and added to ERS.

Literature

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Results

TBECH alters the electrical excitability of neurons

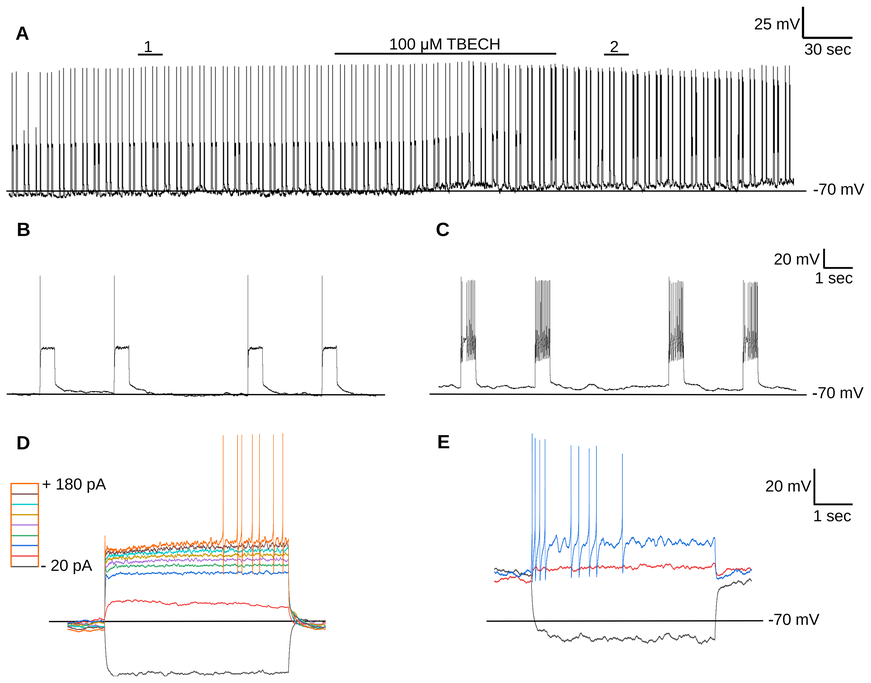

Figure 2. A. Representative current clamp trace of a dissociated *L. stagnalis* neuron held at -70 mV in whole-cell configuration exposed to 100 µM TBECH for 2 minutes. Action potentials were elicited by +210 pA current injections for 0.5 seconds. Black bars 1 and 2 represent points which are enlarged in Figures 1.B. and 1.C. **B.** Representative action potential before and **C.** after TBECH treatment. **D**. Voltage traces representing action potential frequency before and **E.** after TBECH treatment by current injections starting at -20 pA and increasing in 25 pA increments.

TBECH changes the electrical properties of neurons

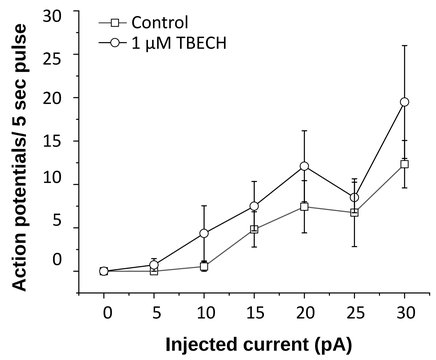

Figure 3. Effect of 1 µM TBECH on number of action potentials in *L. stagnalis* neurons during 5 second current injections in 5 pA increments.

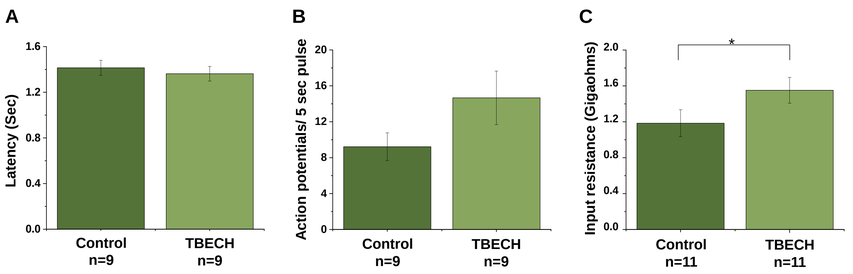

Figure 4. Changes in **A.** latency to the first action potential, **B.** number of action potentials during 5 second current injection, and **C.** input resistance caused by 1 µM TBECH washed over *L. stagnalis* neurons for 2 minutes.

Results

TBECH blocks voltage-gated K⁺ currents

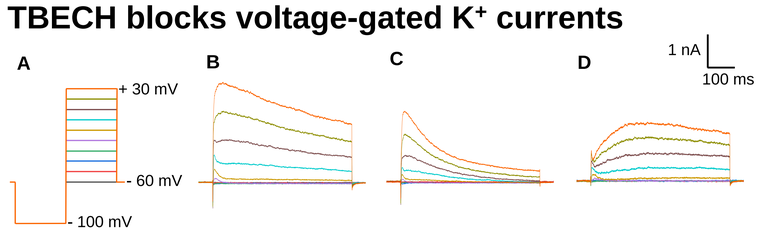

Figure 5. A. Protocol used to elicit K^+ currents, consisting of a hyperpolarising pre-pulse at -100 mV followed by 10 0.5 second depolarising voltage steps from -60 mV and increasing in 10 mV increments. **B.** Representative trace of K^+ currents elicited from a *L. stagnalis* neuron. **C.** Representative trace from the same neuron 100µM TBECH exposure. **D.** Difference trace representing TBECH sensitive current.

TBECH blocks voltage-gated K⁺ currents in a dose-dependent manner

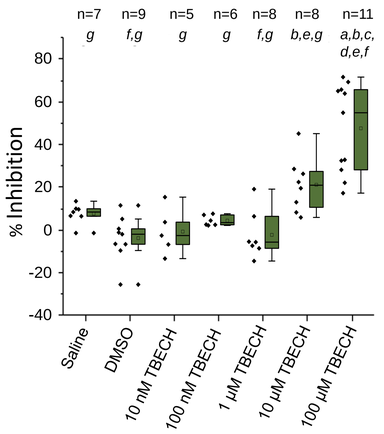

Figure 6. TBECH dose-dependently inhibits voltage-gated K^+ currents in *L. stagnalis* neurons. Box plot extends to the 25th and 75th percentile, whiskers extend to the 5th and 95th percentile, line represents the median and inset square represents the mean (one-way ANOVA, followed by *post-hoc* Tukey comparisons).

a - Statistically different from Saline; b – DMSO; c - 10 nM TBECH; d - 100 nM TBECH; e – 1 µM TBECH; f – 10 µM TBECH; g – 100 µM TBECH.

TEA and TBECH block different populations of K⁺ Currents

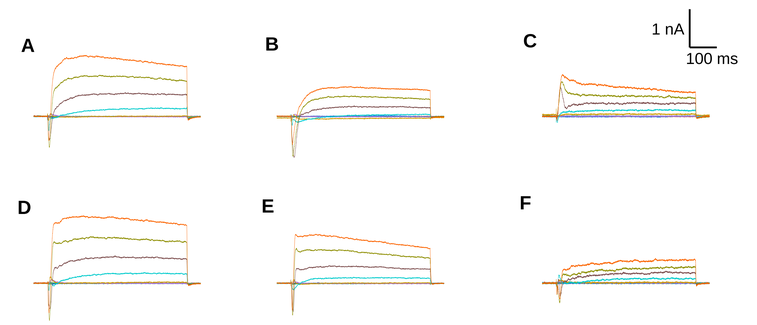

Figure 7. Voltage-gated K^+ currents from a dissociated *L. stagnalis* neuron treated with TEA, followed by washout, and then treated with 100 µM TBECH. Current traces elicited as above (Fig 5.A). **A.** Current traces before TEA treatment. **B.** Current traces after TEA treatment. **C.** Difference current representing TEA-sensitive current. **D.** Current traces showing good recovery from TEA treatment. **E.** Current traces after TBECH treatment. **F.** Difference current representing TBECH-sensitive current.

Conclusions

1. TBECH alters the electrical excitability of neurons by decreasing latency to the first action potential, increasing action potential frequency and increasing input resistance. Many cells experienced a depolarization of RMP.
2. The mechanism by which TBECH modulates electrical activity includes blocking voltage-gated K^+ currents in a dose-dependent manner.
3. TEA and TBECH block different subsets of voltage-gated K^+ currents

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