# *In Vitro* Astrocyte Model to Assess Tetramethylenedisulfotetramine (TETS)-Induced Neuroinflammation

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## ABSTRACT:

Tetramethylenedisulfotetramine (TETS) is a seizurogenic compound and potential chemical warfare agent that poses a significant threat to public health. Although banned worldwide in 1991, it is still available and illegally used for intentional and unintentional poisonings in China and even in the United States. TETS blocks the integral chloride channel necessary to promote $GABA_AR$-mediated central nervous system (CNS) inhibition, which can ultimately lead to fatal convulsive seizures and excitotoxicity. Our lab has shown that acute exposure to TETS dramatically alters $Ca^{2+}$ dynamics in primary hippocampal neuronal cultures; such changes can influence growth, complexity, and function of neuronal networks, which may promote cognitive and memory impairments. While the neurotoxic effects of TETS on neuronal cultures have been investigated, it is unclear how astrocytes influence the acute toxicological endpoints associated with TETS poisoning. Using the Axion Maestro microelectrode array (MEA) system, we demonstrated that TETS (≥0.1 µM) is capable of increasing network activity in neuronal cultures, whereas the effects are abolished in a co-culture with astrocytes. It is well documented that astrocytes become reactive following CNS insult, which may initially confer a neuroprotective role; however, prolonged reactivity has been linked to promotion of further neurotoxicity. Therefore, our initial findings highlight a need to better understand how astrocyte activation determines their dualistic role of neuroprotection and neurotoxicity during prolonged exposure to TETS. To this end, we established an *in vitro* assay utilizing primary astrocyte cultures and ImageXpress high content analysis to investigate if TETS can induce astrogliosis, a biomarker of neuroinflammation, and/or affect cell viability. TETS (30 µM) in the absence of GABA led to increased astrogliosis whereas introduction of GABA mitigated the effects. However, TETS either in the presence or absence of GABA did not affect cell viability. The establishment of this assay will not only allow us to determine whether TETS and related seizurogenic compounds directly induce astrogliosis, but more importantly, present astrocytes as a novel target for therapeutic interventions following intoxication by TETS and other like agents.

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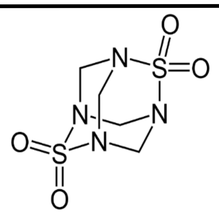
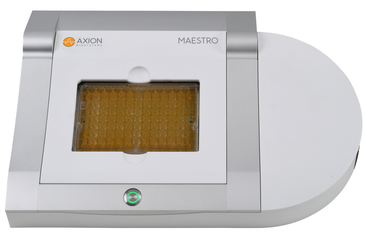


**Figure 1. Structure of tetramethylenedisulfotetramine (2,6-Dithia-1,3,5,7-tetraazadamtane-2,2,6,6-tetraoxide;TETS)**

## METHODS:

- ✔ Maestro MEA System (Axion Biosystems)
  - ✔ Analysis of spike frequency, using Neurometric software, of enriched neuronal culture or anstrocyte-neuronal co-culture in the absence or presence of increasing concentration of TETS




- ✔ ImageXpress High throughput Analysis/Imaging (HCA)
  - ✔ Immunocytochemistry staining of astrocyte cells (GFAP) and their nuclei (DAPI) to quantify astrogliosis and cell viability respectively

- ✔ Statistical Analysis: Computer program GraphPad Prism, using a One-Way ANOVA, followed by post-test Dunnett to determine the level of significance

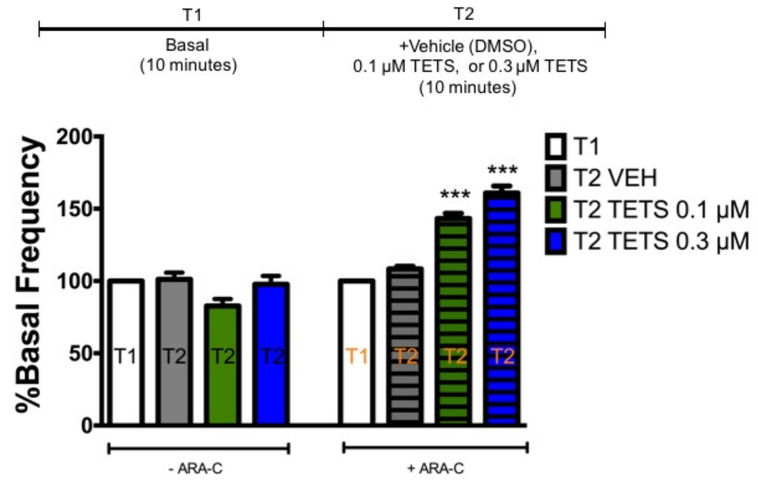



**Figure 2. TETS causes an increase in neuronal spike frequency which can be mitigated by astrocytes.** Enriched neuronal culture established through addition of a mitotic inhibitor (Ara-C) exhibits an increase in spike frequency in the presence of TETS in a concentration-dependent manner. In the presence of astrocytes, spike frequency is unaltered compared to control regardless of TETS concentration.

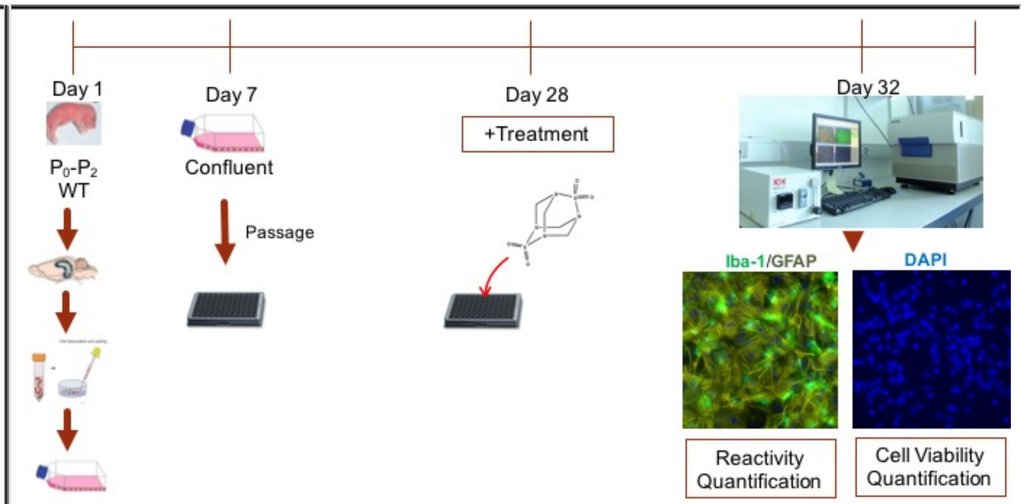



**Figure 3. Dosing paradigm utilizing *in vitro* astrocyte culture model to assess astrogliosis (neuroinflammation) and cell viability following sub-chronic exposure (4 days) to TETS**. Hippocampal astrocytes were isolated from mouse pups and allowed to grow to full confluency before being passaged onto 96-well plates for treatment with TETS in the absence and presence of increasing concentration of GABA. At DIV 4, plates were used for immunocytochemistry staining for GFAP (astrocytes) and DAPI (nuclei) to quantify reactivity/astrogliosis and cell viability, respectively.

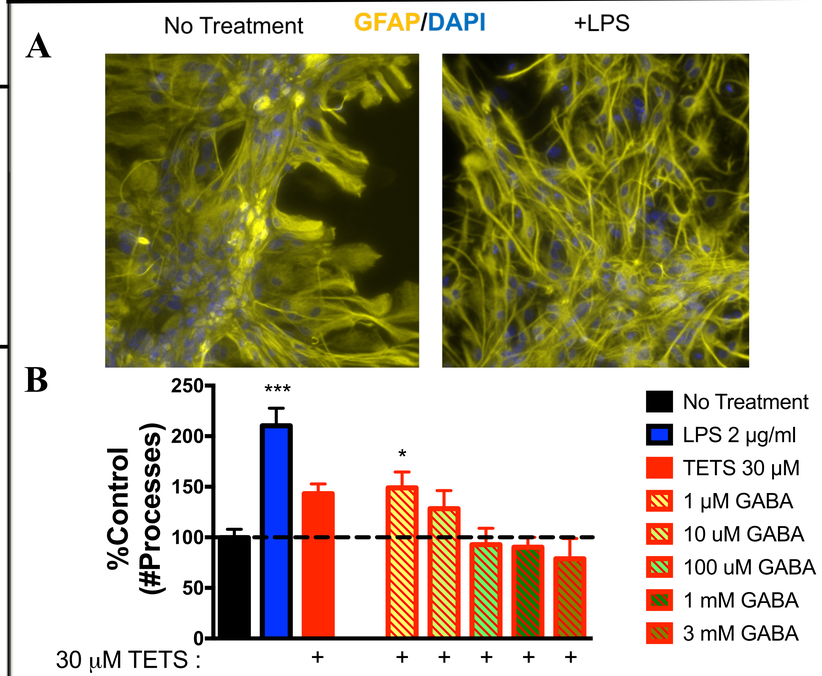



**Figure 4. Sub-chronic exposure (4 days) to TETS increases astrogliosis but is countered in the presence of increasing concentration of GABA**. **(A)** Astrocyte processes were quantified to determine level of astrogliosis. **(B)** Total number of processes for each treatment were normalized to percent control. Increasing concentration of GABA in the presence of TETS (30 µM) depressed astrogliosis. LPS (2 µg/ml) served as a positive control.

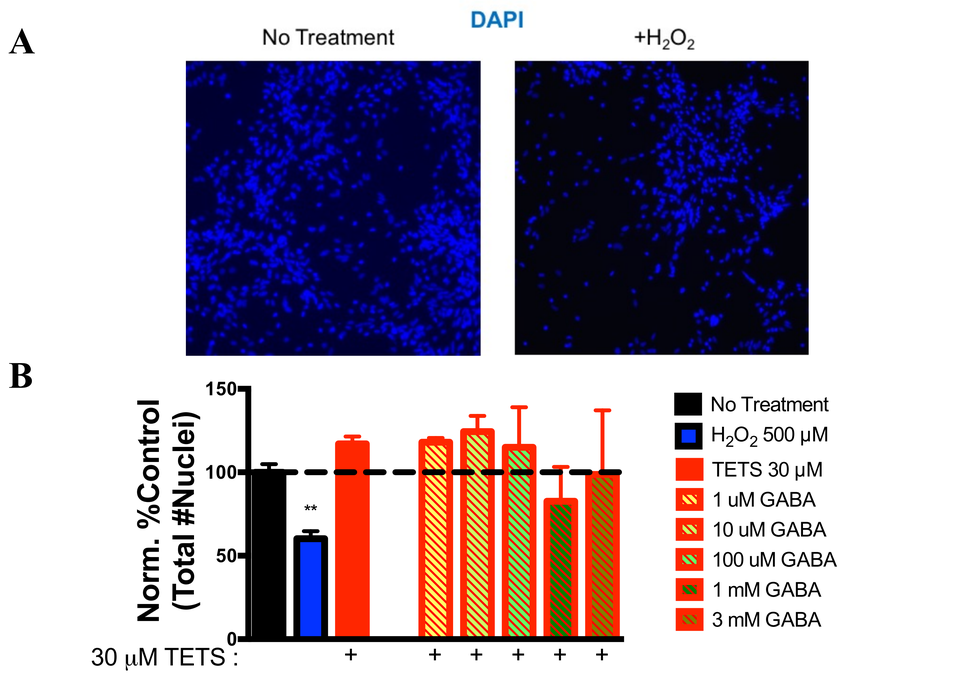



**Figure 5. TETS (30 µM) either in the absence or presence of increasing [GABA] does not affect cell viability**. **(A)** Images of DAPI staining were acquired from ImageXpress, **(B)** quantified and normalized to percent control to determine changes in cell viability. Increasing concentration of GABA in the presence of TETS did not affect cell viability. Hydrogen peroxide (500 µM) served as a positive control.

## CONCLUSIONS:

- ✔ Astrocytes can mitigate the increase in neuronal activity induced by TETS
- ✔ Astrocyte cultures can be used as an *in vitro* model to assess astrogliosis, a biomarker of neuroinflammation, and effects on astrocyte cell viability
- ✔ Sub-chronic exposure (4 days) to TETS (30 µM):
  - ✔ In the absence or presence of increasing concentration of GABA does not affect cell viability
  - ✔ Alone leads to increase in astrogliosis, but is dampened in the presence of increasing concentration of GABA