

# P-18 Nicotine Differentially Cross-talk with Alcohol and Marijuana

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## Abstract

**Introduction:** The prevalent assumption is that nicotine's co-intake with alcohol or marijuana either potentiates or lessens these drugs' response.

**Objective:** To understand behavioural addictive drugs cross-talk, using Zebrafish as the animal model.

**Methodology:** Zebrafish of equal size and caudal fin length, displaying swimming coordination were selected. Fish were treated with 0.5-1.5mg/L nicotine (N-TF), 0.25-1.5% alcohol (A-TF) and 25-200mg/L marijuana tea extract (MTE-TF) or in combination of nicotine (1.0mg/L)-alcohol (0.25-1.00%)(N-A-TF) or nicotine (1.0mg/L)-MTE (25m-100mg/L)(N-MTE-TF). Caudal-tail-flickering (CTF) were recorded after immobilization of fish with free caudal fin projection. ImageJ software was used to evaluate different discrete velocity swimming (DVS), total distance travel (TDT), swimming coordination (Sm-cord), escape behaviour and caudal tail flickering (CTF).

**Results:** N-TF, A-TF and MTE-TF showed time and concentrations dependent effects, resulting in different pattern of DVS and CTF; all potentially disturb the Sm-cord. Nicotine lowered the alcohol dependent swim burst through incorporation of nicotine CTF and alcohol CTF. In contrast for N-MTE-TF, both drugs reduced each other's CTF producing stiff CTF causing lower DVS. Nicotine in co-treatment induced speedy recovery in alcohol after drug withdrawal. The effect are dose dependent, effective only at lower doses but higher doses produced increase mortality. Therefore, co-consuming these drugs is more dangerous to health than consuming each drug alone.

Key words: Nicotine; Alcohol; Marijuana, cross-talk, Zebrafish

## Introduction & Objectives

### Introduction

Tobacco is a popularly consumed drug in the Jamaican population, with a national survey done in 2010 finding that 30% of adults (15 years and older) consumed tobacco products. More astoundingly, the same study showed that tobacco usage in youths between the ages of 13-15 years is at a staggering 56%. This is extremely problematic considering that nicotine, the psychoactive component of tobacco, can increase users' predisposition to respiratory illness and cardiovascular disease.

The problems associated with tobacco usage may be exacerbated when it is used in combination with other social drugs particularly marijuana and alcohol. It was reported, by frequent users of these drugs in combination, co-consumption of nicotine with marijuana or alcohol increases the intensity of euphoria experienced when compared to using nicotine alone.

This study seeks to investigate whether marijuana and alcohol potentiate or lessen the effects of nicotine using zebrafish (*Danio rerio*) as an animal model. The information garnered from this study will add to an already existing body of knowledge and can be used in drug abuse campaigns to deter usage of these dangerous drugs either by themselves or in combination.

The genomic similarity as well as the similarity in drug response between zebrafish and humans makes it an effective model for this study. It is rising in popularity for drug pharmacodynamics and has been used in countless other studies.

### Objectives:

- To investigate the effect of nicotine, alcohol, MTE, N-A and N-MTE on DVS, TDT, Sm-cord, escape behaviour and CTF.
- To determine if alcohol and marijuana respectively potentiates or lessens the effects of nicotine.

## Methods

### Materials:

Vaporfi E-Liquid Nicotine (1.8% mass/vol), Overproof Rum (63% vol/vol), marijuana buds (14%THC, <1% CBD), adult zebrafish.

### Preparation of Analytes:

- The stock marijuana tea extract (MTE) was prepared by boiling 5g of marijuana buds in 1000ml of distilled water for 20mins, and then allowed to cool.
- The stock nicotine solution was prepared by dissolving 5ml of E-Liquid nicotine in 995ml of distilled water.
- The alcohol stock solution was prepared by dissolving 250ml of White overproof Rum in 750ml of distilled water.
- Dilutions of the stock solutions were made to the required concentration and administered to the pair fish in each treatment tank.

### Administration of Analytes:

- A pair of fish with similar size and caudal fin length were selected and placed in treatment tanks, to which the prepared analytes administered.
- A webcam was used to record the swimming pattern of the fish for 20mins.
- Fish were allowed to recover from treatment for 60mins and their swimming pattern was recorded.

### Analysis of Data:

- Analysis of the discrete velocity swimming (DVS), total distance travel (TDT), swimming coordination (Sm-cord), escape behaviour and CTF was done using ImageJ software.

## Results

Fig. 1. Determination of Swim Coordination and Discrete Velocity Swimming

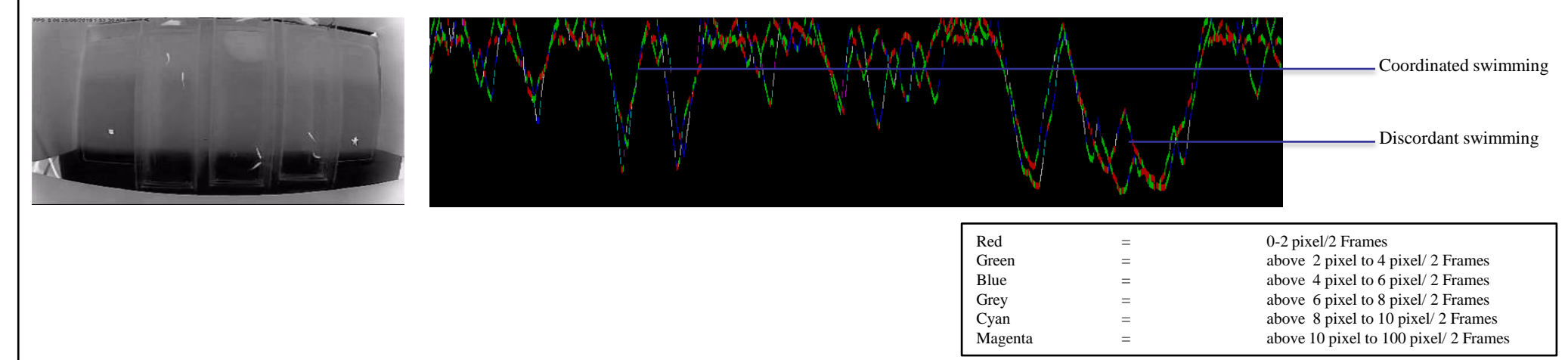


Fig. 2. Effect of Single Addictive drugs on DVS of Zebrafish

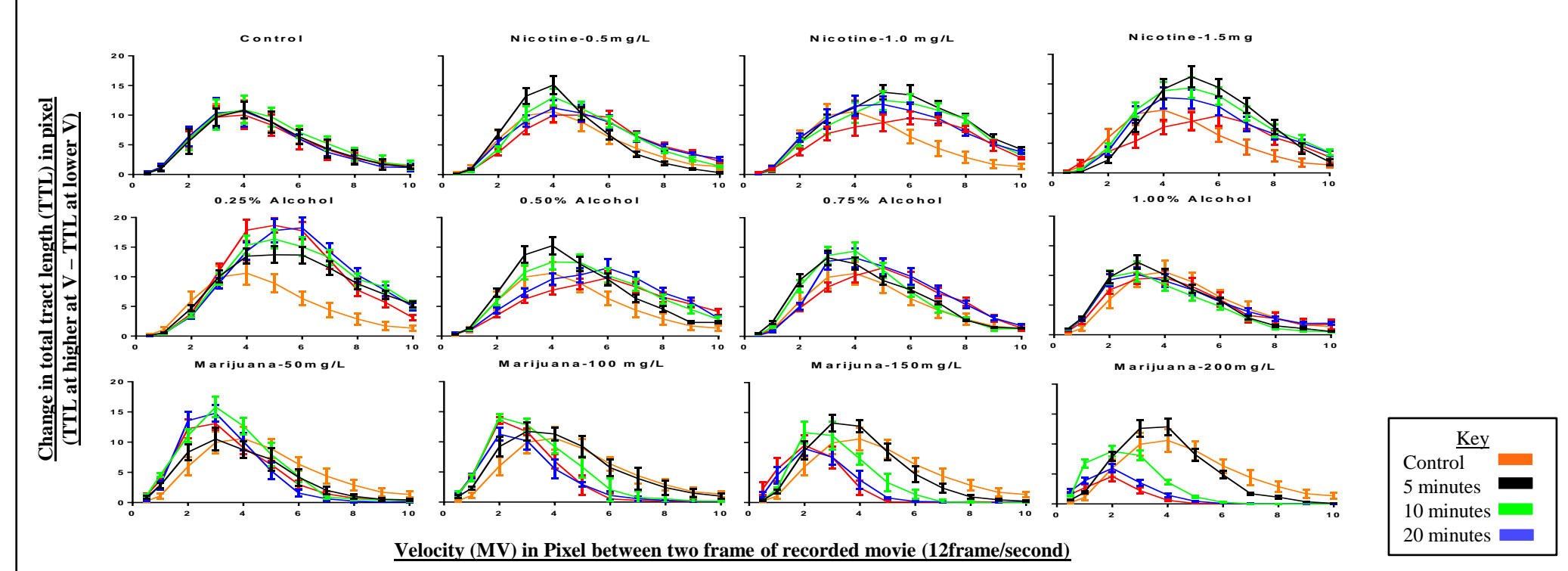


Fig. 3. Effect of Single Addictive drugs on Sm-Cord and Escape Behaviour of Zebrafish

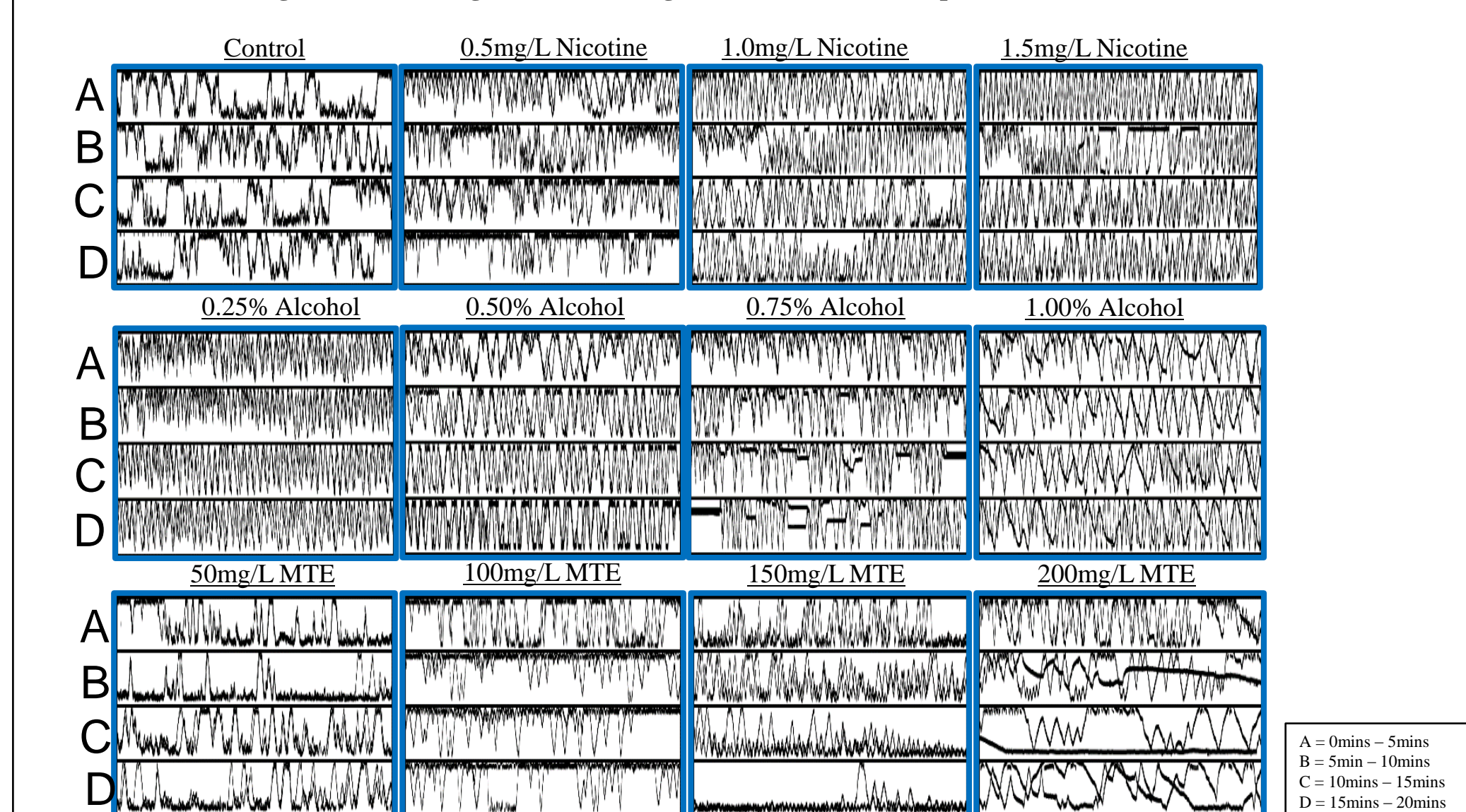


Fig. 4 Comparison of DVS and Sm-Cord for Nicotine and Various Alcohol with Nicotine Treatments

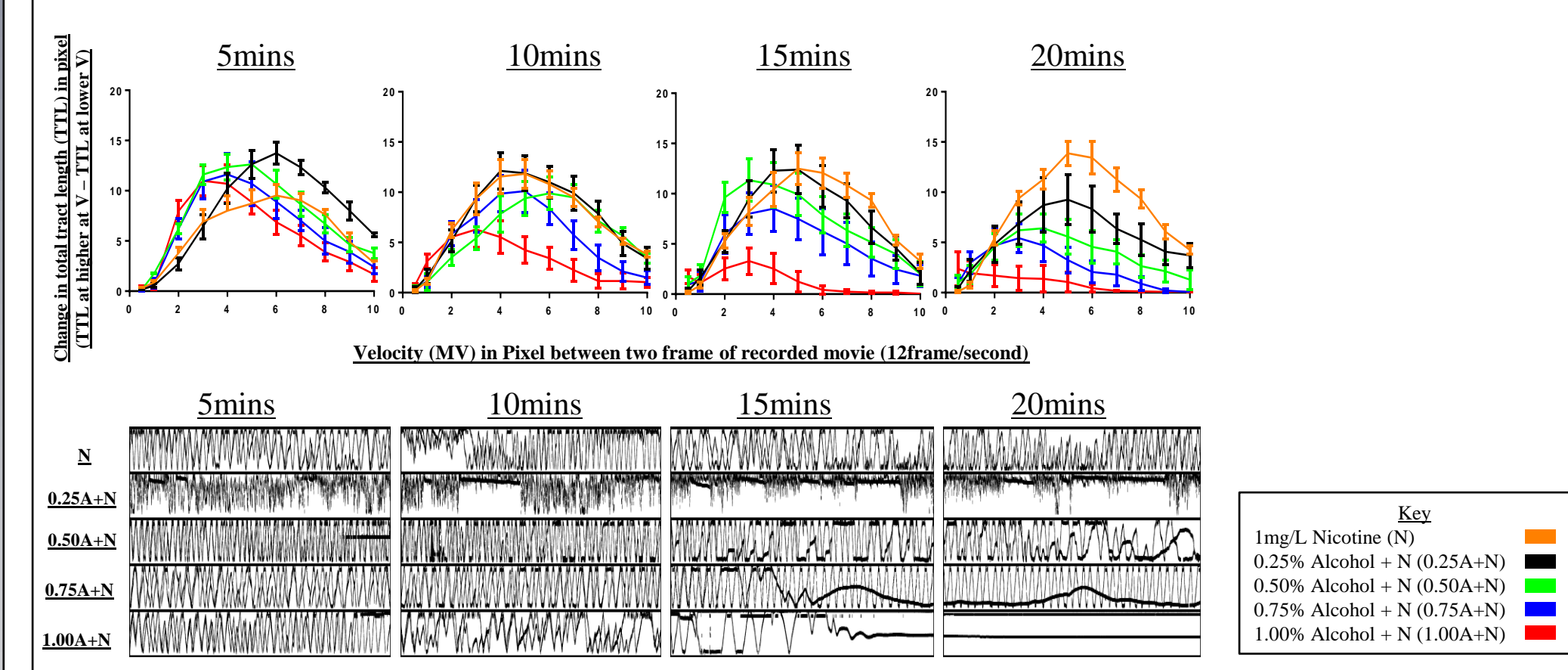


Fig. 5 CTF Pattern in Zebrafish After Nicotine and Alcohol Treatment

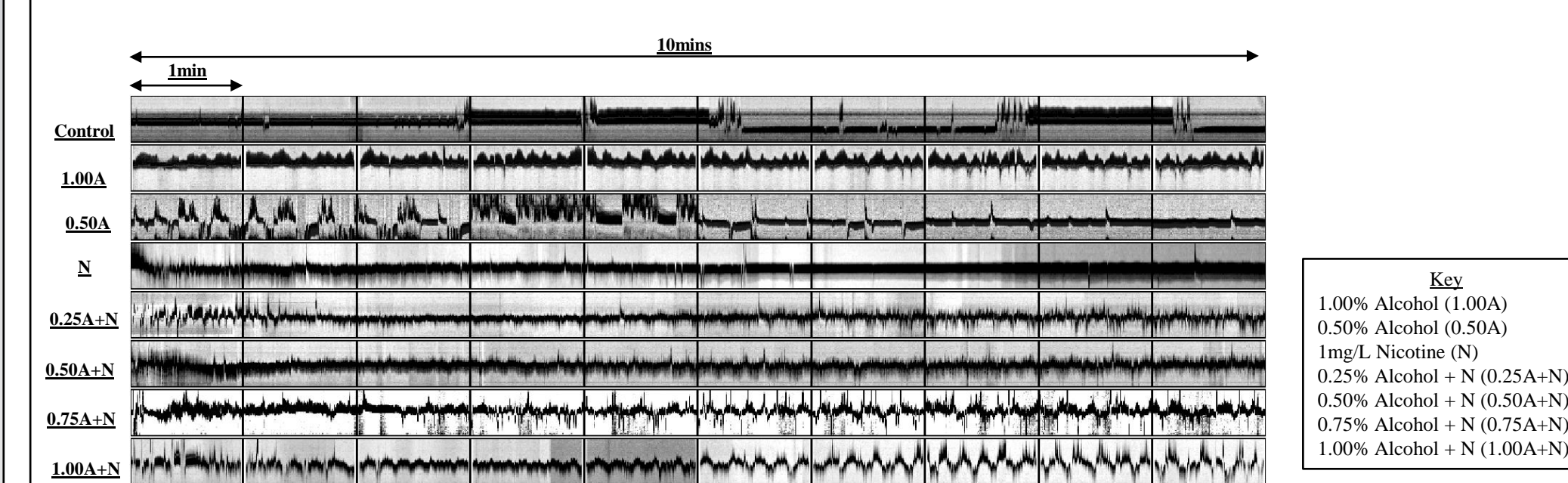


Fig. 6 Comparison of DVS and Sm-Cord for Nicotine and Marijuana treatments at Five Minute Intervals

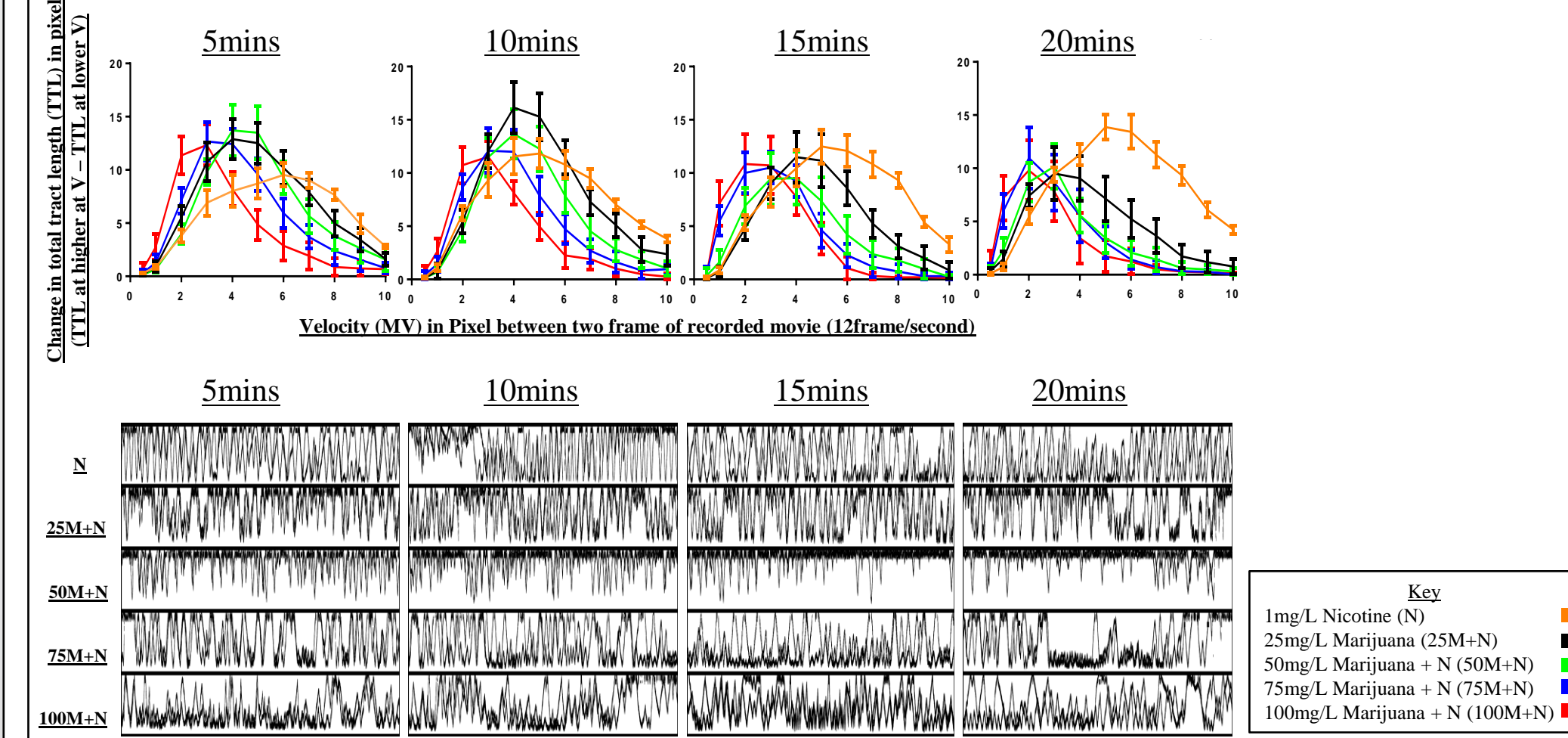


Fig. 7. CTF from Marijuana treatments for First Five Minutes

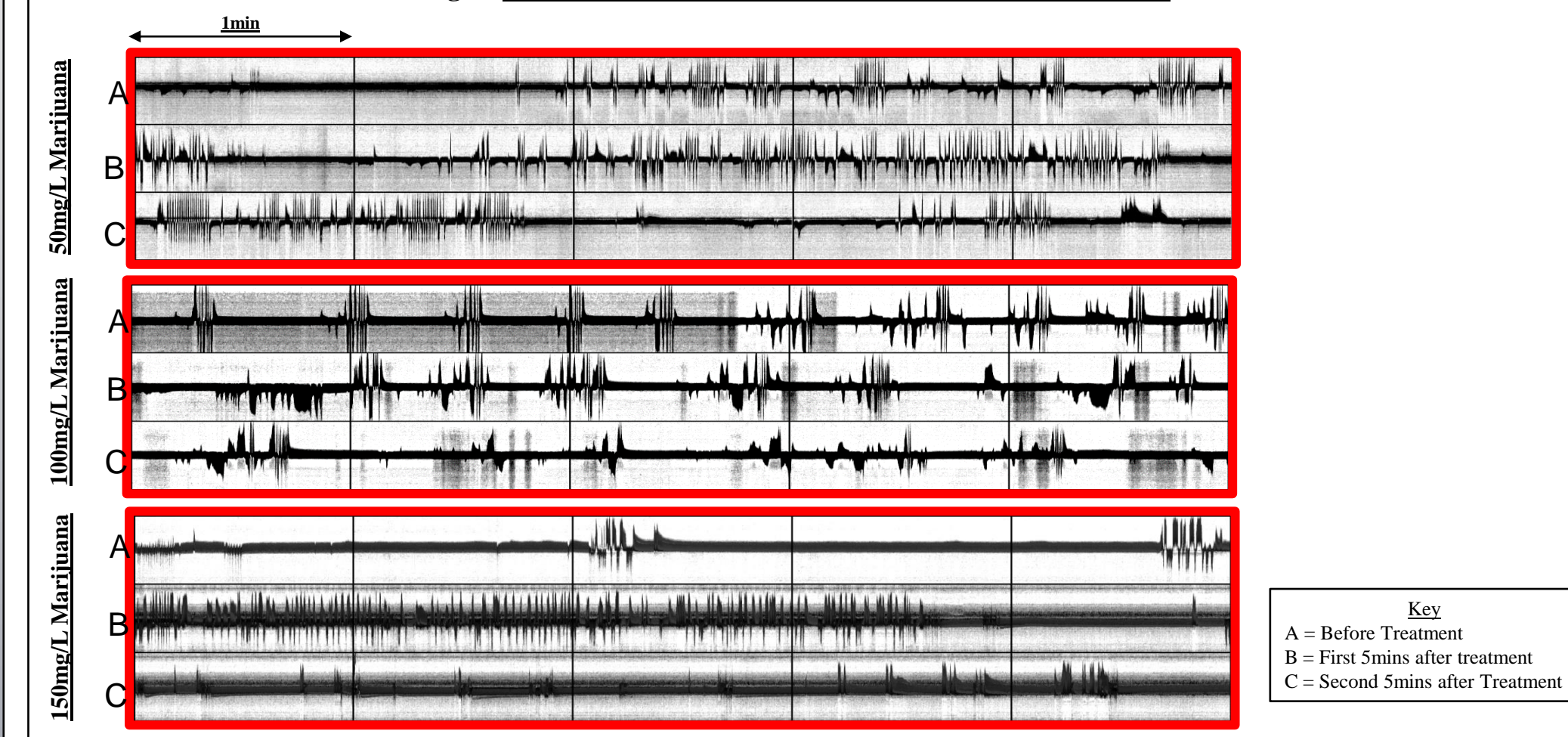


Fig. 8 CTF from Marijuana and Alcohol Treatments for First Ten Minutes

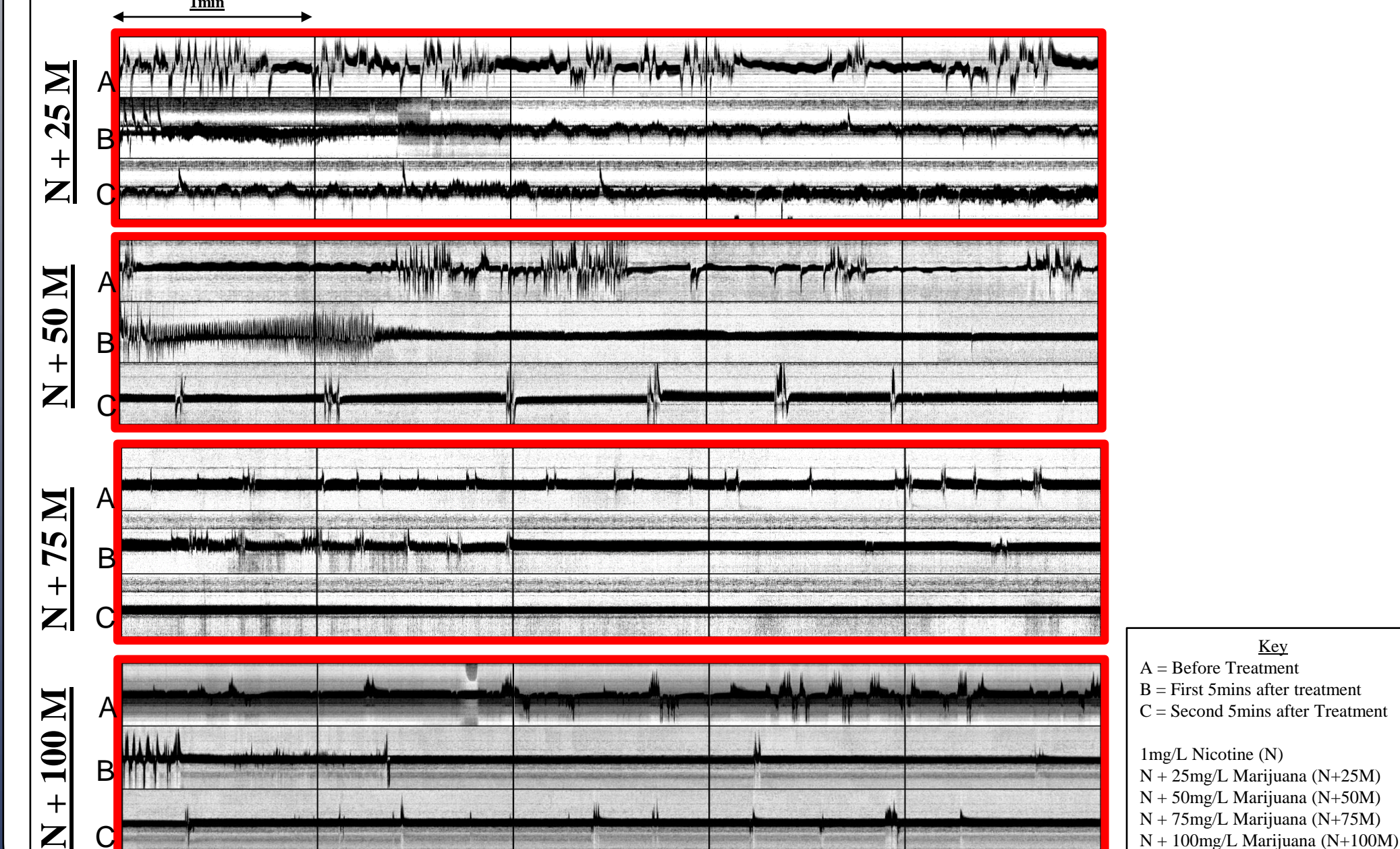


Fig. 9 The Effect of Pre-Exposure with MTE and Nicotine with MTE on Drug Response

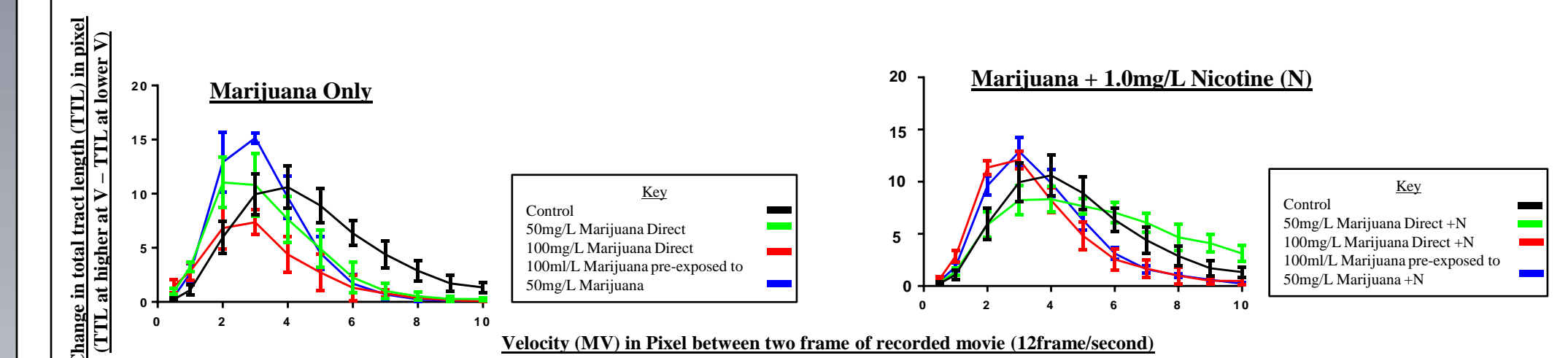
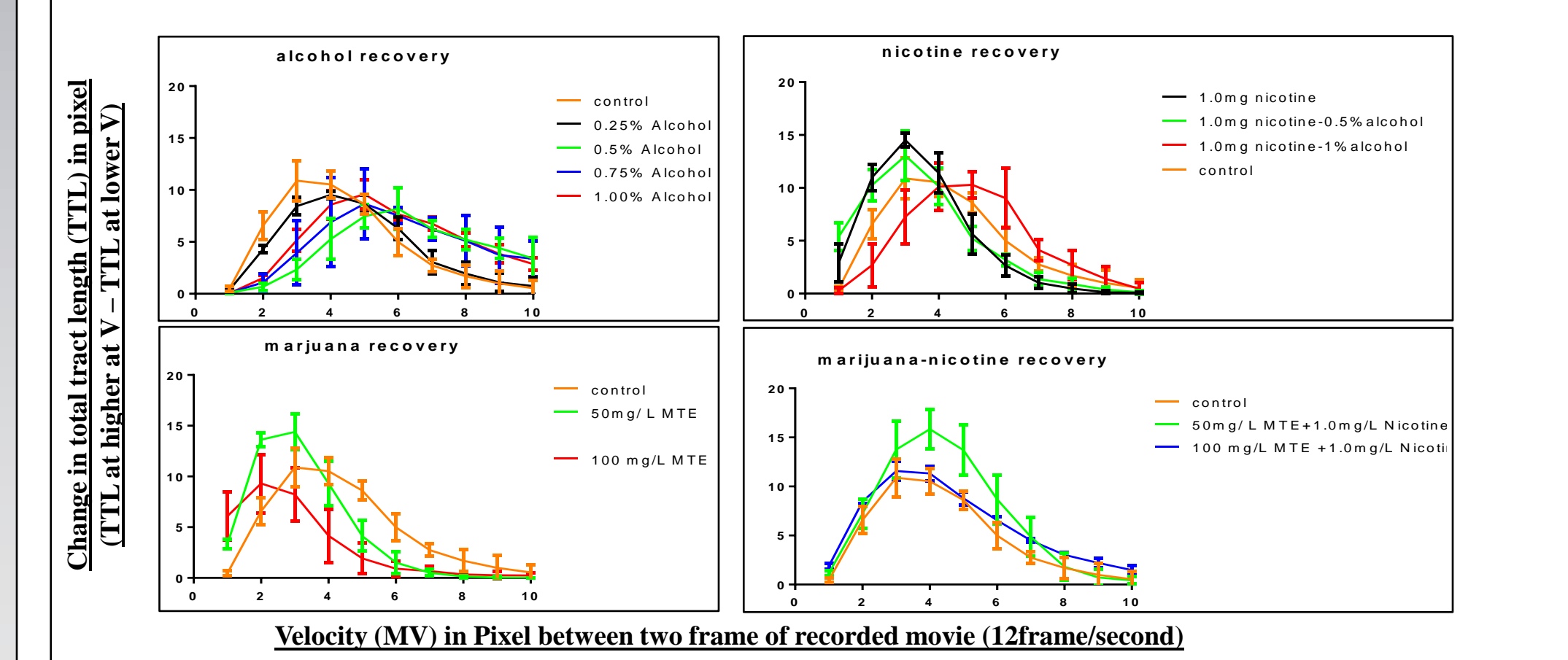


Fig. 10 Recovery DVS of Zebrafish After Drug Administration



## Discussion

- Nicotine, a known stimulant of the central nervous system, caused a consistent and dose-dependent increase in DVS of the zebrafish in comparison to the control. This was because of sustained frantic swimming up and down the length of the tank caused by brisk CTF vibrations. The intensity of the vibrations increases with increasing nicotine concentration. At the lowest concentration of nicotine, the stimulation caused a sustained display of escape behaviour indicating the onset of anxiety. The overstimulation leads to loss of synergy between the fish and progressive loss of Sm-cord.
- The depressant effects of alcohol manifest themselves as low anxiety (no escape behaviour). At the lowest concentration, a swim burst was observed as the fish utilized the alcohol for metabolic energy, but as the concentrations increased, the fish become increasingly disoriented. This caused a lack of stability and coordination because of a slow, side to side, swaying of the caudal fin. With increasing alcohol concentration comes slower and wider swaying on the caudal fin, thereby decreasing stability in a concentration dependent manner.
- The sedative effects of marijuana manifest themselves as fish shifting to lower DVS and lower TTL with increasing drug concentration. This is because marijuana causes a stiffening in the caudal fin and recovery from this stiffness proves more difficult for the fish with increasing MTE concentration. This thereby decreases motility and stability which greatly disrupts Sm-cord. The onset of escape behaviour is seen at lower concentrations and is sustained through to higher concentrations indicating anxiety.
- Simultaneous use of alcohol and nicotine causes progressive lowering of the TTL meaning that the fish and moving around much less in the tank compared to nicotine alone. This is because both the CTF patterns of nicotine and alcohol are being expressed. This means that the excitatory behaviour of the nicotine is being counteracted by the depressant behaviour of the alcohol.
- Concurrent use of marijuana and nicotine leads to concentration dependent lowering of DVS compared to nicotine alone because of the stiffening of the caudal fin which was observed in the marijuana treatment. In the case of low MTE, the caudal fin vibration characteristic of nicotine is seen first, and the stiffening of the caudal fin follows. As the MTE concentration increases, the vibrating CTF is no longer observed, instead there is just a persistent stiffening of the caudal fin. MTE therefore masks the effects of nicotine, while its own effect is dominant. The stiff CTF becomes more difficult to reverse as the MTE concentration increases, which restricts acceleration and Sm-cord.
- Pre-exposure to 50 mg/L MTE before treatment with 100 mg/L MTE resulted in a less severe drug reaction compared to direct treatment with 100 mg/L MTE. In contrast, pre-exposure to 50 mg/L MTE+1 mg/L nicotine before treatment with 100 mg/L MTE+1 mg/L nicotine showed as strong a drug reaction as the fish exposed to 100 mg/L MTE+1 mg/L nicotine directly. It is inferred that nicotine increases the cycling of cannabinoid receptors however further work is needed to confirm.
- Fish treated with nicotine and alcohol recover faster than fish treated with just nicotine due to their opposing effects while nicotine prolongs the effect of marijuana, leading to delayed recovery since nicotine was found to potentiate the effect of marijuana.

## Conclusion

Nicotine lowers the alcohol dependent swim burst through the incorporation of both the nicotine induced caudal tail flickering and alcohol induced caudal tail flickering. In contrast, with nicotine & marijuana treated fish, the marijuana induced caudal tail flickering dominates the alcohol induced caudal tail flickering. This causes a stiff caudal tail resulting in lower discrete velocity. Nicotine in nicotine & marijuana treated fish induced a speedy recovery after drug withdrawal. In both nicotine & alcohol treated fish and nicotine & marijuana treated fish, the effects were dose dependent, nicotine is effective only at lower doses while higher doses resulted in increased mortality. Therefore, co-consuming of these drugs is far more dangerous to health than consuming each drug alone.

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