

## The Acute Effects of Different doses of Tramadol on Neuronal Activity of Medial Prefrontal Cortex in Rats

### Abstract

**Background:** Tramadol is an opioid analgesic with monoamine reuptake inhibitory effects. Although tramadol has been widely used to control pain, there is controversy about the risk of abuse. Therefore, in the present study, the acute effects of tramadol on neuronal activity in the medial prefrontal cortex (mPFC), which is one of the important centers of the reward system, were investigated electrophysiologically. **Materials and Methods:** Tramadol was injected interperitoneally (12.5 and 25 mg/kg) or subcutaneously (40 mg/kg) and its effect on the firing of mPFC neurons was investigated, using *in vivo* extracellular single unit recording. **Results:** Tramadol could not significantly affect neural activity in mPFC, suggesting no acute and rapid effect on mPFC. **Conclusions:** The present results showed that neural activity in mPFC was not rapidly affected by acute application of tramadol. Since the role of mPFC in tramadol addiction has been elucidated, it can be concluded that these effects may be due to delayed responses or chronic use of tramadol.

**Keywords:** Electrophysiology, neurons, prefrontal cortex, tramadol

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### Introduction

Pain is an unpleasant sensation that occurs mainly due to tissue damage. It is influenced by behaviors, thinking, outlook, and community factors and causes emotional and psychological distress.<sup>[1]</sup> Pain management with safe medications with fewer side effects is very important, therefore, analgesics change rapidly to have fewer side effects, tolerance, and dependence and are especially important in the management of chronic pain.<sup>[2]</sup>

Opioids are one of the most widely used analgesics<sup>[1,2]</sup> that affect the central nervous system and cause addiction.<sup>[3]</sup> Tramadol is an opioid used to treat acute or relatively severe pain.<sup>[4]</sup> Tramadol has a long-lasting analgesic effect and is a relatively ideal drug for the treatment of chronic pain.<sup>[5]</sup> It has ten times less analgesic power than morphine but is preferred because it has less respiratory depression, gastrointestinal disorders, and addiction. However, in therapeutic doses or overdose, dangerous side effects such as seizures, serotonin syndrome, and poisoning have been observed.<sup>[6]</sup> Although different mechanisms have been proposed for the effects of

tramadol, the exact mechanism of its effects, especially in relation to its addiction, needs further study. Tramadol can mimic the effects of opioid, but its complex side effects, as well as its addictive complexity, appear to be related to its opioid and nonopioid effects.<sup>[2]</sup> Tramadol appears to exert its analgesic effect by binding to the  $\mu$ -opioid receptor (MOR) and modulating the noradrenergic, serotonergic activities as a serotonin-norepinephrine reuptake inhibitor, and also, gamma-aminobutyric acid (GABA) -ergic system.<sup>[7,8]</sup> These multiple effects on different neurotransmitter systems can complicate the effects of tramadol and its addiction.

The medial prefrontal cortex (mPFC) is a part of the reward system that has strong modulatory effects on the mesocorticolimbic dopaminergic system and its role in addiction and especially in tramadol addiction has been shown.<sup>[9,10]</sup> The mPFC receives input from other areas of the reward circuitry, same to ventral tegmental area and nucleus accumbens. Because these areas can be directly or indirectly affected by serotonergic and adrenergic neurotransmitter systems, they are likely to be affected by tramadol.<sup>[11,12]</sup>

It has been demonstrated that addiction to tramadol is accompanied to structural and

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Received: 25 January 2021

Revised: 17 April 2021

Accepted: 22 June 2021

Published: 30 March 2022

### Access this article online

Website: [www.advbiores.net](http://www.advbiores.net)

DOI: 10.4103/abr.abr\_17\_21

### Quick Response Code:



**How to cite this article:** Hasanpour Razmanjani N, Reisi P. The acute effects of different doses of tramadol on neuronal activity of medial prefrontal cortex in rats. *Adv Biomed Res* 2022;11:24.

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functional changes in mPFC.<sup>[3]</sup> However, it is not known whether these changes are due to the immediate effects of tramadol on mPFC; or it may be due to delayed direct responses or possibly indirectly due to chronic use and through affecting other areas of the brain. Therefore, our aim was to evaluate the acute and potential direct effects of tramadol on mPFC neuronal activity.

## Materials and Methods

### Animals

Experiments have been done on male Wistar rats, weighing 200–250 g (School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran). Animals were maintained in an animal house, under controlled temperature and scheduled illumination conditions (12-h light/12-h dark cycle, lights on at 07:00 am) with water and food available *ad libitum*. All experiments were approved by the Animal Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.MED.REC.1398.608) and performed in strict accordance with the directive, regarding care and use of animals for experimental procedures and the use of laboratory animals (National Institutes of Health, Publication No. 85-23), revised 2010. We tried to minimize animal suffering and the number of animals was minimized to achieve statistically significant results.

The animals were randomly divided into four groups ( $n = 6-8$ ): the control, the tramadol 12.5 mg/kg (i. p.), 25 mg/kg (i. p.), and 40 mg/kg (s. c.), respectively (Alborz Darou CO. Iran).<sup>[13]</sup>

### Single-unit recordings and data collection

Rats were deeply anesthetized by injection of urethane (1.6 g/kg, i.p)<sup>[14]</sup> and placed in a stereotaxic apparatus. Animal body temperature was continuously monitored and maintained at 37°C, using an electrically controlled heating pad. Surgery was performed and a hole (roughly 3 mm in diameter) was made to permit positioning of a one-barrel micropipette (recording electrode) into the right mPFC (AP = +3.5 mm; L = ±0.5 mm; DV = -3.5 mm).<sup>[15]</sup>

Single-unit activities of neurons of mPFC were recorded extracellularly with fine tip (1–3 μm) glass micropipettes, filled with 2 M sodium chloride solution. Micropipettes were gently pulled into the mPFC, using a manual microdrive. Recorded signals were presented as a rate histogram. We recorded the extracellular electrical activity of one to three neurons from each animal. Recorded extracellular signals were filtered (300 Hz to 3 kHz bandpass), and single-unit firings digitized, using a commercial analog to the digital data acquisition system. Data analysis was performed by the related software tools, eLab (Science Beam Institute, Iran). The neurons were isolated based on the firing rate and waveform characteristics; neurons

with a firing rate <10 Hz, and spike duration more than 500 μs were chosen, therefore, according to the previous studies,<sup>[16,17]</sup> we assumed that our target neurons were pyramidal neurons [Figure 1]. When steady firing rate was identified, the baseline was recorded for 15 min, and then, tramadol/placebo was injected and the neuronal response was recorded for 30 min. Examinations were done on 12–18 neurons in 6–8 rats, in each experimental group.

### Histological verification

After each experiment, rats were kept anesthetized and perfused transcardially with normal saline, followed by 10% buffered formalin. Then, brains were removed and sectioned coronally at 55 μm thickness, and recording and injection sites were histologically verified under a microscope, and compared to the rat brain Atlas [Figure 1].<sup>[15]</sup>

### Data analysis

Data were analyzed, using the SPSS version 23 for windows (IBM Corporation). The spontaneous firing rate over 15 min was defined, as the baseline firing rate (in spikes/second). An increase/decrease of firing rates beyond the mean ± two-fold of the standard deviation of the baseline firing rate was considered as an excitatory/inhibitory response, respectively.<sup>[18]</sup> The percent changes of the firing rate concerning the baseline firing rate between the groups were analyzed, using the one-way analysis of variance, followed by a *post hoc* Tukey test and unpaired Student's *t*-test, and the Chi-squared test for comparing cells with excitatory or inhibitory responses, between different groups. Data are expressed as mean ± standard error of the mean ( $n = 6-10$  rats).  $P < 0.05$  were considered statistically significant.

## Results

The results of statistical comparison of the number of neurons with the response of increase, decrease or no change, according to Table 1 shows that tramadol with different doses and also different method of injection (ip or sc) was not significantly different from the control group.

Furthermore, according to Figure 2, although after the injection of tramadol, the overall neuronal activity in mPFC decreased in all doses; in none of them, this decrease was significant.

## Discussion

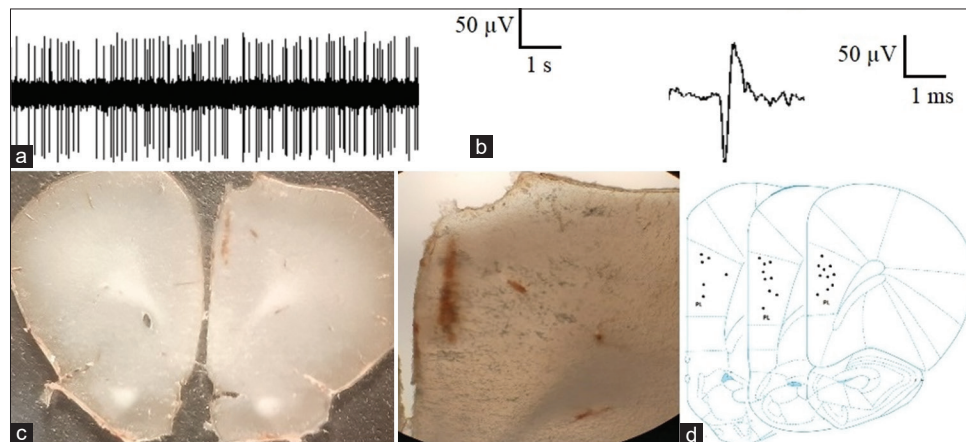
The results of the present study showed that systemic injection of tramadol cannot acutely change the neuronal activity in the mPFC. According to the characteristics of the selected neurons,<sup>[16,17]</sup> this response was related to the pyramidal neurons in this area.

It has been demonstrated that the role of mPFC in reward is not uniform. The mPFC appears to be important for

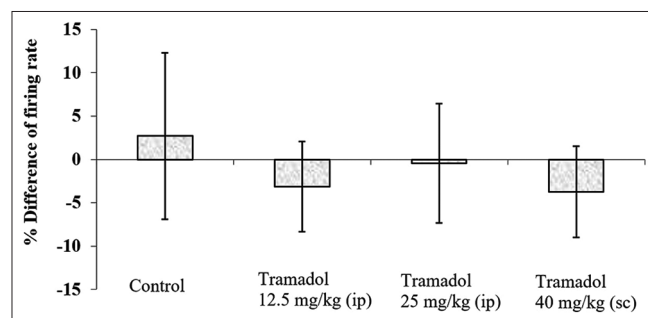
**Table 1: Effects of tramadol on the neuronal activity in medial prefrontal cortex**

Percentage of changes in neuronal firing	Group			
	Control	Tramadol 12.5 mg/kg (IP)	Tramadol 25 mg/kg (IP)	Tramadol 40 mg/kg (SC)
Increase	33.3	16.1	21.4	8.3
Decrease	25	6.5	3.6	16.67
Unchanged	41.7	77.4	75	75
Pearson $\chi^2$		5.423	5.769	3.143
<i>P</i>		0.066	0.056	0.208

SC: Subcutaneous, IP: Interperitoneal. The data show the percentage of changes in neuronal firing compared to baseline in each group after treatments. The criterion for increasing/decreasing activity was changes in firing rates beyond the mean  $\pm$  two-fold of the SD of the baseline firing rate. The statistical results presented for each tramadol group are compared to the control group



**Figure 1:** (a) A representative pattern of baseline neuronal electrical activity recorded from the medial prefrontal cortex. (b) An expanded waveform of a spike recorded from a medial prefrontal cortex neuron. (c) A representative image, displaying the microinjection site in the medial prefrontal cortex; prelimbic, (d) Schematic illustrations of coronal sections of the rat brain, adapted from an Atlas (15), show the approximate microinjection sites in the medial prefrontal cortex



**Figure 2:** Effects of tramadol systemic administration on the percentage difference of firing rate of neurons within the medial prefrontal cortex. Data are expressed as mean  $\pm$  standard error of the mean

cocaine-rewarding actions, whereas it is not important for amphetamines.<sup>[19]</sup> The mPFC is made up of subregions and receives a variety of inputs from different areas of the brain<sup>[20]</sup> and appears to mediate different aspects of addiction.<sup>[21]</sup> One of the challenges of accurately understanding mPFC activity is the diversity of excitatory and inhibitory neurons<sup>[22]</sup> and the complex pharmacological and neurochemical aspect.<sup>[23]</sup> It has been suggested that some drugs may induce reward directly by affecting mPFC, while others, even if they do not directly stimulate reward in mPFC, require mPFC function for their rewarding effects.<sup>[9]</sup>

Tramadol is generally a weak  $\mu$ -receptor agonist and also exerts some of its effects through other neurotransmitter systems, such as serotonergic, noradrenergic, and gamma-aminobutyric acid (GABAergic) systems.<sup>[4,24]</sup> The exact mechanism of how tramadol affects each of these systems is unclear and few studies have been performed in this field.

Substances and drugs that are used systemically and can cross the blood-brain barrier and have receptors in any region of the brain can directly affect the activity of that region, and depending on the type of receptor, create rapid or delayed responses. In this study, we investigated the effects of tramadol on mPFC using doses and injection routes that have been shown to be effective in suppressing pain.<sup>[13]</sup> The results of this study showed that tramadol could not rapidly and acutely affect neuronal activity in mPFC until about 30 min after injection.

However, it has been demonstrated that addiction to tramadol is accompany to structural and functional changes in mPFC.<sup>[3,10,25]</sup> These changes can be caused by chronic effects and repeated use of tramadol.<sup>[3,26]</sup> However, some studies have compared the acute and chronic effects of tramadol on MOR, transcription factor  $\Delta$ FosB and cAMP response element-binding protein (CREB) gene and protein expressions and showed that acute exposure to

tramadol does not affect the level of  $\Delta$ FOSB in PFC, but increases the levels of MOR and p-CREB in mPFC in acute and chronic exposure to tramadol.<sup>[25]</sup> These effects were appeared at least 1 h after tramadol application.<sup>[25]</sup> Therefore, it can be concluded that tramadol cannot cause rapid reactions such as changes in membrane permeability and neuronal excitability in mPFC and affect neuronal activity in this region.

## Conclusions

The present results showed that the rapid response of neuronal activity in mPFC, which can be due to direct or indirect effects on neuronal excitability in this area, is not affected by acute application of tramadol. Since the role of mPFC in tramadol addiction has been identified, it can be concluded that tramadol may cause functional and structural changes in mPFC, either directly or by affecting the areas projecting to mPFC. These effects are likely through the development of delayed responses that are associated with gene and protein expressions.

## Acknowledgments

This research was funded by a grant (398902) from the Isfahan University of Medical Sciences, Isfahan, Iran.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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