

# CHROMIUM PICOLINAT INFLUENCE ON BRAIN REWARD SYSTEM IN NAÏVE AND MORPHINE – TREATED RATS

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## Abstract

In this study, it was assessed the influence of chromium picolinate (CrPi) on the reward system in rats. For this, we have used the conditioned place preference technique (CPP). We have worked on 6 groups, each of 10 Wistar adult male rats. CrPi was given intraperitoneal, in doses of 0.05 and 0.01 mg/kg b.w., 2 h before conditioning sessions. We have also assessed the CrPi effect on morphine-induced CPP. Our results showed that CrPi significantly increased the time spent in the conditioning chamber in a dose-dependent manner (by  $19.18 \pm 7.67\%$ ,  $p < 0.05$  for CrPi 0.01 mg/kg b.w. and by  $35.20 \pm 12.40\%$ ,  $p < 0.01$  for CrPi 0.05 mg/kg b.w. post-conditioning time vs. pre-conditioning time). In the case of association between morphine and CrPi, both CrPi doses determined a slight but significant increase of morphine stimulating effect on the reward system ( $p < 0.05$ ).

## Rezumat

În acest studiu, s-a determinat efectul picolinatului de crom (CrPi) asupra sistemului cerebral al recompensei la șobolani, utilizând metoda condiționării preferinței locului (CPL). S-a lucrat pe 6 loturi, fiecare a câte 10 șobolani masculi Wistar adulți. Dozele de CrPi utilizate au fost 0,05 mg/kg corp și 0,01 mg/kg corp, intraperitoneal, cu 2 ore înaintea ședințelor de condiționare. De asemenea s-a determinat efectul aceluiași doze de CrPi asupra CPL induse de morfină. Rezultatele obținute au arătat că CrPi crește semnificativ și dependent de doză timpul asociat compartimentului de condiționare (cu  $19,41 \pm 2,01\%$ ,  $p < 0,05$  pentru CrPi 0,01 mg/kg corp și cu  $35,79 \pm 6,58\%$ , în cazul CrPi 0,05 mg/kg corp  $p < 0,01$ , respectiv; post-condiționare vs. pre-condiționare). Ambele doze determină o creștere moderată, dar semnificativă statistic ( $p < 0,05$ ) a acțiunii stimulante a morfinei asupra sistemului de recompensă la animalele luate în studiu.

**Keywords:** chromium picolinate, reward system, morphine, conditioned place preference

## Introduction

Chromium is an essential trace element with multiple roles in the human body. It can be found in bi-, tri- and hexavalent form, and for therapeutic practice it is used mainly the trivalent chromium as picolinate salt. Frequently it is used for reducing insulin resistance and for decreasing hyperglycaemia. Chromium histidinate stimulates also the regenerative potential of cerebral tissue.

The reward system is one of the most important brain systems, with great implications in human behaviour and many substances may either stimulate or inhibit it [12].

The brain structures with the most important involvement in conditioned place preference development are *nucleus accumbens*, prefrontal cortex, ventral tegmental area etc. [4, 11, 15]. There are several neurotransmitters involved for conditioned place preference development. Mesolimbic dopaminergic pathways play, in the opinion of most researchers, a key role in reward and motivational processes [10, 18, 19, 21]. Other neurotransmitters, such as serotonin, glutamate, opioids, substance P are also involved in these kinds of processes [20].

Biological active metals (magnesium, zinc, calcium and others) are involved in brain reward system functions and influence conditioned place preference induced by strongly addictive substances such as morphine, heroin, cocaine and others [9, 13, 14]. There are few data referring to CrPi capacity to influence the reward system.

In this study, we aimed to determine the influence of chromium picolinate (CrPi) on the reward system in naive and morphine treated rats.

## Materials and Methods

### Chemicals

The experiments were performed using morphine chlorhydrate (Sicomed, Romania) and chromium picolinate (Sigma-Aldrich, Germany).

### Animals

The present experiment was performed on adult male Wistar rats (180 - 270 g) hosted in individual cages; water and food were provided *ad libitum* except for the behaviour testing period. Animals were exposed to a 12/12 hour light-dark cycle; the tests were performed during the light period.

This study was approved by the Ethics Committee of “Gr. T. Popa” University of Medicine and Pharmacy, Iași, Romania. All animal procedures were performed according to the European Union law on the Care and Use of Animals for Scientific Purposes and in accordance with the Recommendations of the Helsinki Declaration.

*The assessment of reward system by conditioning preference place technique (CPP)*

The reward system was assessed by using the conditioning place preference (CPP) method. The conditioning apparatus (Panlab, Spain) consists of three chambers: two main chambers, distinguished by different wall and floor patterns, situated to the edges of the apparatus (one with black and the other with white walls), and one smaller intermediary chamber. Communication between chambers may be either open or closed (by inter-changeable guillotine-like separations, suitable for a 180° movement). Illumination conditions were standardized by placing bulbs above each chamber. To induce CPP we used method comprising three steps: pre-conditioning, conditioning and post conditioning step [19].

*Pre-conditioning* (day 1). The rat spent 15 minutes in the apparatus (the communication between chambers was open). Time spent in each chamber was measured; the main chamber, where the animals spent more time, was referred as *preferred* chamber, and the other as *non-preferred*. Prior to pre-conditioning, animals were subjected to habituation sessions (2 sessions of 30 minutes daily, 2 days and one day before pre-conditioning).

*Conditioning* (days 2 - 9). The communication between chambers was closed. Days 2, 4, 6 and 8: the rat was restricted for 40 minutes to the preferred chamber, after 3 mg/kg b.w. intraperitoneal (ip) morphine. Days 3, 5, 7 and 9: alternatively, the rat was restricted for 40 minutes to the non-preferred chamber, immediately after the administration of 1 mL/kg b.w. saline solution.

*Post-conditioning* (day 10). The preference for the main chambers was again measured for 15 minutes (the communication between chambers was open). Pre-conditioning, conditioning and post-conditioning stages took place in standard illumination conditions, during the same period of the day, in the absence of other stimuli that might have an influence on behaviour [3].

Pre-conditioning was initially applied to 80 rats. Animals showing a high preference towards a certain chamber (those spending more than 60% of the pre-conditioning time in any of the main chambers,

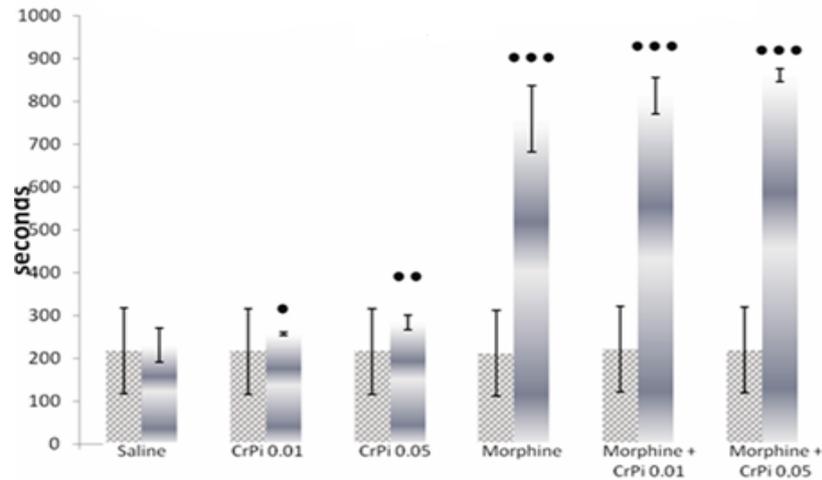
or more time in the intermediary chamber than in any of the main ones) were excluded from further testing. Thus, 12 such animals (15% of the initial test group) were excluded. Of the remaining 68 animals, by applying homogeneity criteria 60 rats divided into 6 groups of 10 rats were selected for further tests.

According to the treatment applied on experiment days 2, 4, 6 and 8 during the conditioning phase, the groups received: Group I – saline solution (NaCl 0.9%), 1 mL/kg b.w. ip; Group II – CrPi 0.01 mg/kg b.w. ip; Group III – CrPi 0.05 mg/kg b.w. ip; Group IV – morphine 3 mg/kg b.w. ip; Group V – morphine 3 mg/kg b.w. ip + CrPi 0.01 mg/kg b.w. ip; Group V – morphine 3 mg/kg b.w. ip + CrPi 0.05 mg/kg b.w. ip. On days 2, 4, 6 and 8 of the experiment, the rats received the previously described treatment, and then were placed in the non-preferred chamber. Morphine was administered intraperitoneally, immediately before placing the animal in the non-preferred chamber; CrPi was ip administered 2 hours before morphine. On experiment days 3, 5, 7 and 9 all animals received intraperitoneally saline solution, 1 mL/kg b.w., and were placed immediately in the preferred chamber. A significant increase in the time spent in the non-preferred (conditioning) chamber (post-conditioning vs. pre-conditioning) indicates the change of preference. In such a case, it is believed that this is due to the fact that the animal associates reward (pleasant, euphoric effect of a substance) to the chamber in which the substance was administered. The obtained results were statistically assessed by p-value, ANOVA test paired to compare post-conditioning vs. pre-conditioning time in each group and also to compare different animal groups.

## Results and Discussion

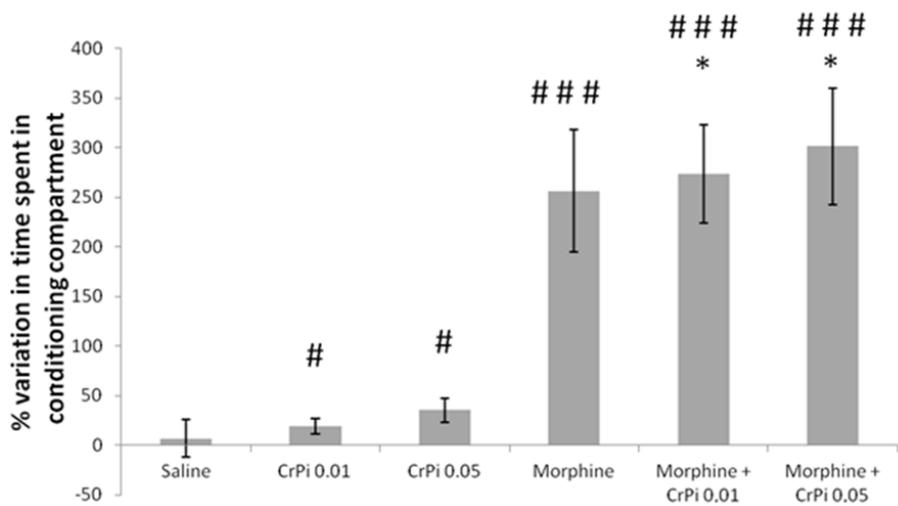
The obtained results showed a moderate, but statistically significant stimulation of CPP by both the doses of CrPi used in the study (Figure 1). Both CrPi doses determined a slight rewarding effect ( $p = 0.046$  in lower-dose,  $p = 0.007$  in higher-dose - post-conditioning vs. pre-conditioning time, where the increase in the time spent in conditioning chamber was  $35.20 \pm 12.40\%$ ).

Morphine CPP was achieved in all morphine-treated groups. Time spent in the conditioning chamber increased by  $256.19 \pm 61.67\%$  in the morphine treated group (post-conditioning vs. pre-conditioning). Also, both CrPi doses augmented the rewarding effect of morphine (Figure 2).



**Figure 1.**

Time spent in the conditioning chamber - in seconds (pre-conditioning and post-conditioning)  
 •p < 0.05 post-conditioning vs. pre-conditioning; ••p < 0.01 post-conditioning vs. pre-conditioning; •••p < 0.001 post-conditioning vs. pre-conditioning; ▨ pre - conditioning   ■ post - conditioning



**Figure 2.**

The percentage variations in the time spent in the conditioning chamber (post-conditioning vs. pre-conditioning)  
 #p < 0.05 vs. saline; ###p < 0.001 vs. saline; \*p < 0.05 between morphine only-treated vs. morphine + CrPi treated groups

CPP represents a technique which allows the investigation of the influence induced by different substances on animal's brain reward system. A significant increase of the place preference is determined by substances with addictive potential, such as opioids. In CPP development, a series of endogenous neurotransmitters such as dopamine, endogenous opioids, epinephrine, glutamate and serotonin are involved [12, 20]. Existing data show that dopamine is the main neurotransmitter involved in CPP development in case of morphine, cocaine, amphetamine and other substances [19, 23].

CrPi administration has various involvement pathways in behaviour. In stressed Swiss male albino rats, CrPi produced a significant antidepressant effect [2]. The immobility time in forced swimming test of adult rats in experimental chronic stress was reduced

by CrPi 16 µg/kg b.w./day administrated in the drinking water. Meanwhile, it is shown that CrPi decreased the plasma concentration of cortisol and increased the serotonin level. These influences on cortisol and serotonin levels could be involved in the improvement of symptoms of depression caused by stress [7].

Chronic stress is associated with anhedonia and anxiety. CrPi administration also determines an anxiolytic effect. It is also possible that K<sup>+</sup> channels would also be at least partially involved in antidepressant and anti-diabetic effect determined by CrPi [11]. This cation modulates the expression of neuronal plasticity markers in experimental hypoglycaemia - induced brain damages in rats [17]. A series of endogenous active substances reduce the potency of morphine to increase place

preference. So, TNF $\alpha$ , acting on *nucleus accumbens* attenuates morphine stimulation of reward functions evaluated by conditioned place preference in rats [22]. The same effects can be obtained by the blockade of orexin-1 receptors in ventral tegmental area [23]. Genetic deletion of MT1 and MT2 melatonin receptors reduce the rewarding effect and the stimulation of place preference by amphetamine [5]. Neuropeptide Y (NPY), BDNF (brain derived neurotrophic factor), arrestin and neurotensin are also involved in the induction of CPP [3, 6, 16]. This derivate of Cr<sup>3+</sup> determined increased serotonin and norepinephrine levels in rats which is important for its antidepressant effect [8].

Our data showed that CrPi, in the mentioned doses determines a stimulation of CPP when administered alone, and also increases morphine stimulating action on the reward system. Literature data show that CrPi administration determined a significant increase in dopamine, noradrenaline, serotonin, and tryptophan concentration in rats. This effect was accompanied by a decrease of cortisol level after the administration of CrPi 80  $\mu\text{g}/\text{kg}$  b.w./day [8]. Central tryptophan bioavailability and brain serotonin levels are increased by CrPi but not the serotonin receptor sensitivity [1].

### Conclusions

Because tryptophan is the amino acid from which serotonin is synthesized, we consider that most probably, the effect of CPP stimulation induced by CrPi is due to increased brain serotonin concentration. The increased norepinephrine concentration may also be involved in CrPi effect of augmentation in morphine reward stimulating properties. As chromium is used as dietary supplement we consider that its administration should be rational and prudent in psychiatric patients.

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