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Original Article

# The Modulating Effect of Glucocorticoids and Opioid System on Anxiety Related Behavior in Young and Adult Rats

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## Abstract

One of the main components of the stress system is hypothalamus- pituitary-adrenal (HPA) axis. Acute activation of μ-opioid receptors increases the activity of the HPA axis, leading to release of ACTH and corticosterone. Glucocorticoids can change behaviors, depend on age but there were no evidences about the interaction between age, opioid system and glucocorticoids. In this experiment, effects of dexamethasone (1mg/kg) and RU486 (20mg/kg) as an agonist and antagonist of glucocorticoid receptors, morphine (5mg/kg) and naloxone (20mg/kg) as an agonist and antagonist of the opioid system on anxiety in young and adult male Wistar rats were examined. The percentage of time in the open arms of plus maze was evaluated for anxiety behavior also percentage of the number of entries in closed arms was evaluated for locomotor activity. The results showed that morphine (5mg/kg) and dexamethasone (1mg/kg) had an anxiolytic effect on both young and adult rats while just in young rats reduced locomotor activity. RU486 could prevent the anxiolytic effect of morphine, and the anxiolytic effect of dexamethasone had been inhibited by naloxone in young but it wasn't seen in adult rats. These results show an interactive effect between glucocorticoids and the opioid system on mediating anxiety that can be influenced by age.

*Keywords*: anxiety, glucocorticoid, mice, opioid, RU486.

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## 1. Introduction

The stress system coordinates the adaptive responses of the organism to any kind of stress [1, 2, 3]. The main components of the stress system are the corticotrophin-releasing hormone (CRH)

and locous-ceruleus-norepinephrine (LC/NE), autonomic nervous systems and hypothalamic- pituitary-adrenal (HPA) axis [1, 2,]. Activation of the stress system leads to behavioural and peripheral changes that improve the ability of organism to adjust homeostasis and increase its chances for survival [2, 3]. The end hormones of the HPA axis, glucocorticoids, have multiple roles [3].

There are evidences that disregulation of the HPA axis is implicated in the pathophysiological disorders like anxiety [4, 5]. Some studies have shown acute injection of glucocorticoids such as dexamethasone (DEX) in lower doses reduced anxiety while at higher doses increased anxiety in animals [6].

It has been demonstrated that opioid receptor agonists and antagonist such as morphine and naloxone can influence anxiety in animals [7, 8, 9]. On the other hands some reports show that opioid system activity can affect HPA axis activity [6, 10, 11].

It has been shown that dexamethasone is able to modify the effects of opioids in analgesia and anxiety that indicating an important interaction between glucocorticoids and opioid system activity [6, 12, 13].

Acute activation of opioid receptors increases the activity of the HPA axis, leading to release of ACTH and corticosterone in the rat [12, 14]. The pituitary-adrenocortical response to mild stress was markedly increased in juveniles exposed to morphine [15]. Morphine may either increase the perceived severity of stress or decrease sensitivity to the negative feedback effects of stress levels of corticosterone in juvenile males [15]. There is a striking shift in morphine's effects on the pituitary-adrenocortical axis across development [15]. Opioid receptor antagonist like naloxone can inhibit the anxiolytic effect of glucocorticoids like dexamethasone [6].

Several variables, such as age, are known to influence anxiety [16, 17, 18]. It has been shown that anxiety in adolescents is less than adults [19, 20]. There are some evidences that glucocorticoids can change behaviors like anxiety, depend on age [21, 22, 23] but there were no evidences about the interaction between age and anxiolytic effect of opiate or glucocorticoids.

In this study was investigated the interaction between opioidergic system activity and glucocorticoids in anxiety related behaviors in young and adult male rats.

## 2. Materials and Methods

### *2.1. Animals and Treatments*

The experiments were performed with 98 male Wistar rats: 49 young rats with one month age, weighing 40-45 gr and 49 adult

rats with three month age, weighing 170-180 gr. Animals were kept in constant temperature (22± 2˚c) with a 12-h light/dark cycle (lights on from 7:00 a.m. to 7:00 p.m.) with free access to food and water. They had been handled 4-5 days before the test. All experiments were done in light phase (9-11 AM).

Animals were divided into 14 groups in both age groups that receiving: saline, morphine (5mg/kg)[8], DEX (1mg/kg)[24], morphine (2.5mg/kg) + DEX (1mg/kg), morphine (5mg/kg) + DEX (1mg/kg), RU486 (Mifepristone glucocorticoid antagonist) (20mg/kg) [25] + morphine (5mg/kg) , naloxone (2mg/kg)(26) + DEX (1mg/kg). The use of a single effective dose of medication in this study was based on previous studies.

All experimental procedures were carried out in accordance with international and institutional guidelines for animal care and use.

*2.2. Drugs*

Morphine (Temad Co. Iran) and dexamethasone (Iran Hormone Co. Iran) were dissolved in saline. Naloxone (Tolidaru Co. Iran) and RU486 (Sigma Co.UA) were dissolved in saline. All drugs were injected intraperitoneal in a volume of 5 CC saline/ kg.

*2.3. Elevated Plus Maze*

The elevated plus maze test was made of wood with two open arms (50 x 5 cm) and opposite closed arms of the same size but with 40-cm high walls. The arms were connected by a central square (10×10 cm), and thus formed a plus sign. The apparatus was elevated 50 cm above the floor. Each rat was placed in the central square of the plus maze facing an enclosed arm. The time spent in enclosed and open arms was scored for 5 min. An arms entry was defined as an animal entering the arms with all four feet and the number of entries into open and enclosed arms was scored [27] (time spent in open arms /total ratio) ×100, was measured as anxiety index and closed arms entries/ total ratio × 100 was measured for locomotor activity.

*2.4. Statistical Analysis*

All results are presented as mean ± S.E.M. for seven animals per group. A two-tailed student's t-test was used to compare the mean frequency of the behaviors between groups. Some data were assessed by analysis of variance (ANOVA). Following post-hoc analyses (LSD test) were performed in assessing specific group comparisons and differences, where $P<0.05$ was considered statistically significant. Calculations were performed by using the SPSS (version 19) statistical package.

## 3. Results and Discussion

### *3.1. Treatment of Young and Adult Male Rats with Morphine and/or DEX*

As shown in figure 1-A, the percentage of time in open arms was increased (P<0.001) by morphine (5mg/kg) and also by dexamethasone (DEX) (1mg/kg) (P<0.001) in the younger group, also in adult group it increased (P<0.05) by morphine (lesser than the young) and dexamethasone (P<0.001) that show anxiolytic effects of them. Also, there is significant difference in time between the open arms between control groups (P<0.05) and between receiving morphine groups of young and adult rats (P<0.05) this means that the anxiety in adult rat is greater than the young and morphine is more effective in young rats.

Closed arms entries as an index of locomotor activity decreased in young rats that receiving morphine (5mg/kg) or DEX (1mg/kg) in comparison with control group (P<0.05), (Figure 1-B).

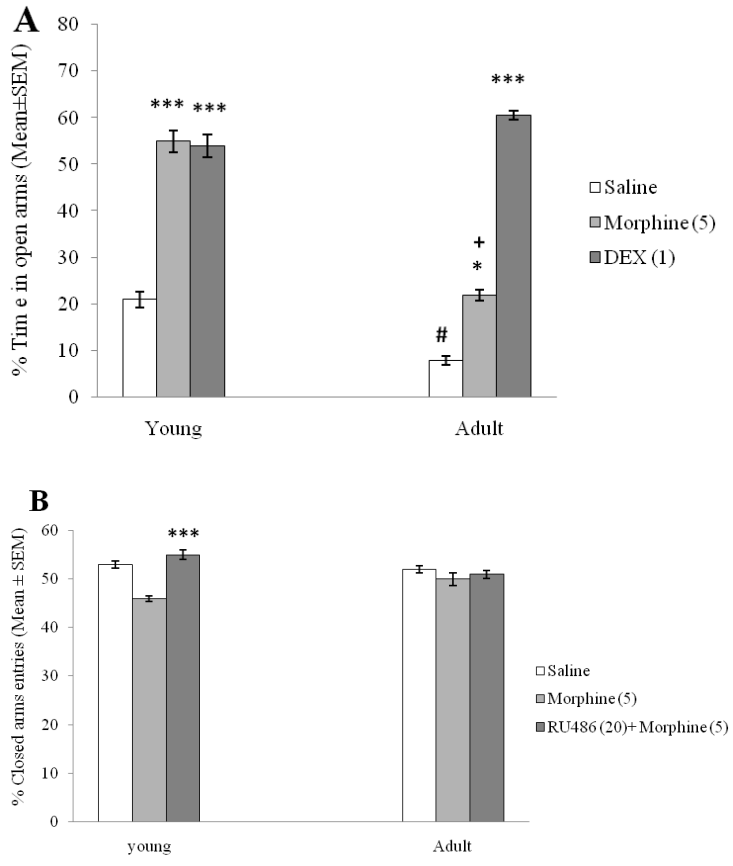


**Figure1**. Effect of morphine (5mg/kg) and dexamethasone (1 mg/kg) on anxiety (A) and locomotor activity (B) parameters in young and adult rats: all drugs injected intraperitoneally. *P<0.05, ***P<0.001 compared to subjects receiving saline. #P<0.05 comparisons between young and adult rats. +P<0.05 comparisons between young and adult rats receiving morphine. n=7

*3.2. The Effect of Co-Injection of Morphine and DEX in Young and Adult Male Rats*

Figure 2-A shows, there was no significant difference between young rats that receiving 5 mg/kg with DEX 1mg/kg in comparison with morphine 5 mg/kg alone in anxiety parameter. But, there was a significant difference between morphine 5 mg/kg alone and co-administration of morphine 5mg/kg + DEX 1mg/kg (P<0.001) in the adult group. Closed arms entries or locomotor activity did not change in young rats that received morphine 5 mg/kg + DEX 1mg/kg in comparison with morphine 5mg/kg alone while in adult rats significantly increased (P<0.01) (Figure 2-B).

*3.3. Anxiolytic Effect of Morphine in Presence of RU486 in Young and Adult Rats*

There were significant differences in both of time percentage in open arms and closed arms entries (locomotor activity) between the group that received RU486 (20mg/kg) + morphine (5mg/kg) in

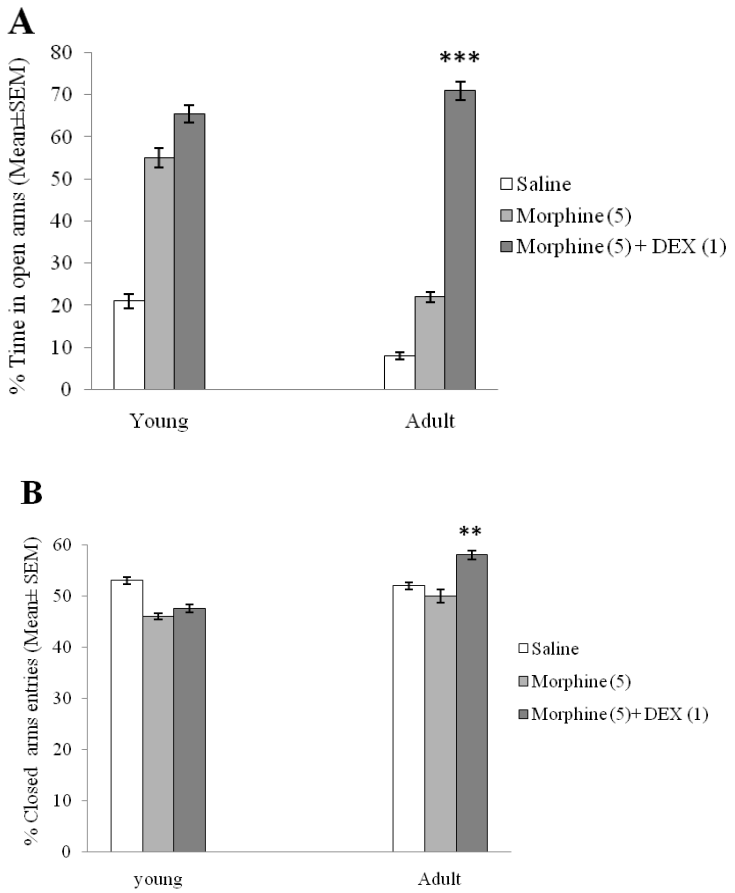


**Figure 2**. The effect of co-injection of morphine (5mg/kg) and dexamethasone (1mg/kg) in young and adult male rats on anxiety (A) and locomotor activity (B) parameters. **P<0.01, in comparison with morphine 5 mg/kg. n= 7

comparison with morphine (5mg/kg) alone in young rats (P<0.001). There were no differences between these two groups in adult rats (Figure 3). This means that the anxiolytic effect of morphine was prevented by RU486 only in young rats.

### *3.4. Anxiolytic Effect of DEX in Presence of Naloxone in Young and Adult Rats*

There were significant differences in percentage of time in open arms and locomotor activity between the group that received naloxone 2mg/kg + DEX 1mg/kg in comparison with DEX (1mg/kg) in young rats (P<0.001). There were no differences between these two groups in adult rats (Figure 4).

This study showed the highest level of anxiety in adult rats, which is in agreement with previous studies that age-related changes in anxiety indexes in male rat at elevated plus maze and light/dark test, that show anxiety in adult rats was higher than young rats [27, 28, 29].

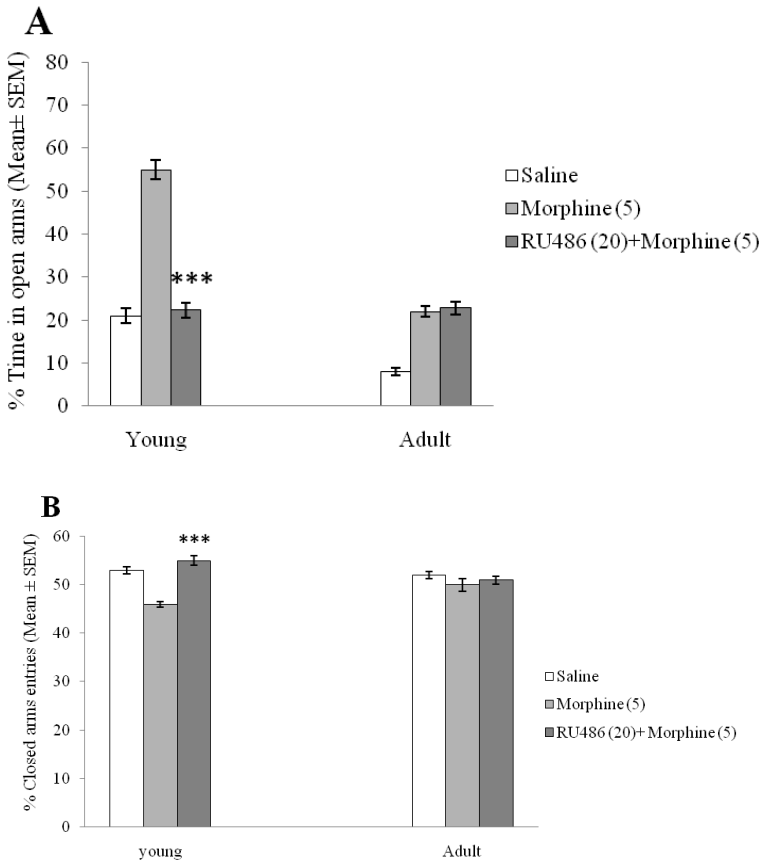


**Figure 3**. Anxiolytic effect of morphine (5mg/kg) in presence of RU486 (20mg/kg) in young and adult rats on anxiety (A) and locomotor activity (B) parameters. ***P<0.001 in comparison with morphine 5mg/kg. n= 7.

The present results also show that morphine (5mg/kg) and also dexamethasone (1mg/kg) induced a significant increase in the percentage of time in the open arms, which clearly indicates an anxiolytic effect for both of them (Figure 1). In young rats, morphine and dexamethasone reduced locomotor activity while didn't change it in adult rats. Previous studies have shown that these doses of morphine and dexamethasone have anxiolytic effects in adult animals that confirm our results [6, 29].

There were significant differences between co-administration of morphine and DEX in comparison with morphine alone on anxiety behavior in adult rats, but it was not seen in young rats. In adult rats when morphine co-injected with DEX increased locomotor activity too (figure 2-B).

In previous studies, morphine has been shown to induce clear corticosterone responses at a dose of 20-30 mg/kg, whereas no significant effect of morphine on corticosterone levels was found at doses of 5 or 10 mg/kg [30], these results are consistent with our results and it seems that a part of the combination of two antianxiety drugs related to the change in locomotor activities.

Results also showed that morphine in the presence of RU486 increase anxiety with decrease the percentage of time spent in open arms in young rats also increase locomotor activity in them but it doesn't show effect in adult rats (Figure 3). It seems, RU486 has prevented the anxiolytic effect of morphine. It has been indicated that the inhibitory effect of RU486 was dose-dependent and linked to a decrease of the affinity of labeled dihydromorphine to opioid receptors [31]. Kinetic experiments have shown that RU486 induces a decrease in the association rate constant of dihydromorphine[31]. RU486 also proved able to dissociate the dihydromorphine-μ-opioid receptor complex [31] RU486 inhibits the binding of labeled dihydromorphine to μ-opioid receptors present on membrane preparations derived from rat and mouse brain, as well as from human neuroblastoma cells [31]

In addition, it has been demonstrated that impairment of the receptor-dependent glucocorticoid action in neonatal brain resulted in long-lasting hormonal stress responses, reduced number of locomotion, and increases the anxiety level in adulthood [32]. This discordance the results with our results in adult rats can be related to the difference between the additional protocols work.

The results of the present study showed that naloxone inhibited the anxiolytic effect of dexamethasone and increase locomotor activity in young rats, but this wasn't seen in adult rats (Figure 4). This result is in agreement with the study showed that there

were no detectable increases in plasma cortisol levels following neither naloxone nor saline administration and indicating an effective opioid blockade at the level of hypothalamic-pituitary unit occurred [6,33]. This finding indicates involvement of naloxone sensitive pathway in mediating the influences of glucocorticoids on anxiety. There are two possible explanations for an interaction between dexamethasone and naloxone on anxiety. First, it is likely that dexamethasone activates the endogenous opiate system and then that mediates their influences on anxiety. Although, there are some evidences indicating that the effects of DEX on some behaviors such as analgesia and emotional memory mediated, at least in part, by the endogenous opiate system [26] but to our knowledge there are no reports in literature concerning with such mediation on anxiety. Another explanation for these results might be that naloxone interacts with stress and anxiety induced glucocorticoids or dexamethasone in the plasma membrane of target neurons in brain [34. 11].

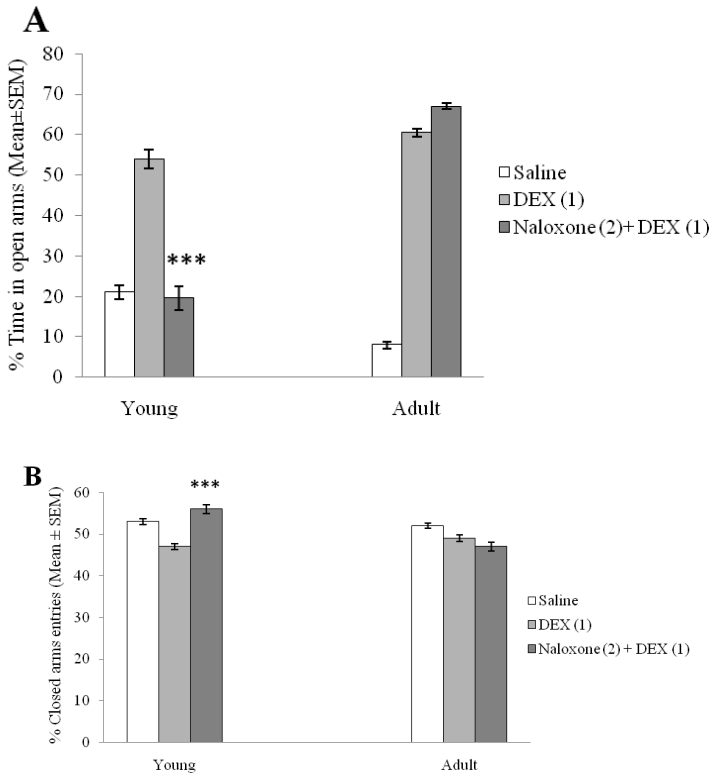


**Figure 4**. Anxiolytic effect of dexamethasone (1mg/kg) in presence of naloxone (2 mg/kg) in young and adult rats on anxiety (A) and locomotor activity (B) parameters. ***P<0.001 in comparison with DEX 1mg/kg. n= 7.

## 4. Conclusion

In conclusion present study shows that opioid and glucocorticoid receptor agonists are effective to reduce anxiety in young and adult rat. Blockage of these receptors influenced anxiety in young rats more than adults that this may be related to the different neural and hormonal system activity in young and adult animals.

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