



## **The Effects of Dopamine and Serotonin on Yawning Behavior in the Rat Model of Social Isolation**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

A sample of 80 Male rats (21-day post weaning) were chosen, and were put for 6 weeks in separate cages with black plastic buffers. Eight rats were put in one group of 8 rats in a single cage (the control group) and the rest were put in individual cages: one male rat in each cage. In group 1 or the control group (social conditions) 8 rats were put in one cage. They received saline carrier and their yawning behavior was recorded for 60 minutes. Group 2 (n=8; in separate cages) (social isolation conditions) received no treatment with serotonin and dopamine agonist and antagonist and were kept in separate cages with one rat in each cage. Their yawning behavior was also recorded for 60 minutes. Group 3 (n=8; in separate cages) included the rats that received Apomorphine (dopamine agonist) at a dose of 0.08 mg/kg via subcutaneous injection (SC), and their yawning behavior was recorded for 60 minutes. Rats in group 4 (n=8; in separate cages) received serotonin agonist (m-CPP) at a dose of 0.5 mg/kg via subcutaneous injection, and their yawning behavior was recorded for 60 minutes. Group 5 (n=8; in separate cages) included rats that received Serotonin Antagonist (Mianserin) at a dose of 0.2 mg/kg via subcutaneous injection, and their yawning behavior was recorded for 60 minutes. Group 6 (n=8; in separate cages) included

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rats receiving dopamine antagonist (haloperidol) at a dose of 0.1 mg/kg via Intraperitoneal (IP) injection, and their yawning behavior was recorded for 60 minutes. Group 7 (n=8; in separate cages) included rats receiving Serotonin antagonist (Mianserin) at a dose of 0.2 mg/kg via subcutaneous injection 15 minutes before injection of apomorphine (dopamine agonist) and their yawning behavior was recorded for 60 minutes. Rats in group 8 (n=8; in separate cages) received dopamine antagonist (haloperidol) at a dose of 0.1 mg/kg via intraperitoneal injection (IP) 15 minutes before the injection of serotonin agonist (m-ccp), and their yawning behavior was recorded for 60 minutes. Rats in group 9 (n=8; in separate cages) received Apomorphine (dopamine agonist) at a dose of 0.08 mg/kg and Serotonin agonist (m-CPP) injected subcutaneously (SC) at a dose of 0.5 mg/kg and their yawning behavior was recorded for 60 minutes. Group 10 (n=8; in separate cages) included rats that received dopamine antagonists (haloperidol) at 0.1 mg/kg via intraperitoneal injection (IP) and antagonist serotonin (Mianserin) at 0.2 mg/kg injected subcutaneously and their yawning behavior was recorded for 60 minutes. Dopamine agonist (apomorphine) and serotonin antagonist (Mianserin) induce yawning in the social conditions and injection of haloperidol (dopamine antagonist) before serotonin agonist (m-ccp) reduces yawning in social conditions. Yawning is different in social conditions and social isolation conditions. Using Meta-Chlorophenylpiperazineserotonin (serotonin agonist), Mianserin (serotonin antagonist), apomorphine (dopamine agonist), haloperidol (dopamine antagonist) the role of serotonin and dopamine in yawning, fear, erection etc. can be investigated as a model for human studies.

*Keywords: Yawning; penile erection; dopamine; serotonin.*

## 1. INTRODUCTION

Yawning is a phylogenetically old, stereotyped event that happens under different conditions alone or associated with stretching and/or penile erection with a low frequency in humans, in animals from reptiles to birds and mammals [1,2]. In rats and non-human primates yawning is androgen-dependent and sexually dimorphic, with more common in males than females. Dopamine is one of the most studied neurotransmitters involved in the control of penile erection and sexual [3]. It is understood that cortisol acts to protect our body against stress, both physical and psychological stress loadings. It also regulates the other hormones released within the HPA-axis. It is suggested that, as part of its stress protection and stress-response, cortisol elicits yawning by increasing the electrical activity of the nerves in the muscles around the jaw line, giving rise to yawning [4]. Several neurological disorders have been studied to date in order to discern commonality in mechanisms and neurological pathways. In particular, symptoms have commonality between some disorders which may arise because they share dysfunction in neurological pathways or in the regulation of neurotransmitters between synaptic junctions. For example, serotonin is implicated in both depressive disorders as well as in Parkinson's disease and Alzheimer's disease and the symptoms of mood change are often exhibited [5]. Yawning occurs associated

with increased electroencephalographic activity of the cortex, it has been also suggested that yawning is an ancestral vestige survived through evolution that occurs when attention is low and arousal needs to be increased [6]. Social deprivation may be deleterious not only during childhood and adolescent period but also during adulthood in male mice. Moreover, our results are consistent with previous data showing that stress induces hyperactivity in rodents in response to exposure to novel environments, such as an open field [7]. Rearing animals in isolation is a relevant paradigm for studying early life stress and for understanding the development of certain neurological and psychiatric diseases. It is widely accepted that early social isolation may affect the behavior of adult animals and their responses to psychotropic drugs prevented the reinforcing properties of amphetamine & morphine [8]. Therefore, there is a significant relationship between stress and yawning, and since social isolation is also stressful, social isolation is associated with yawning [9]. Social isolation for a long period causes severe brain neurological degenerations as indicated by the biochemical and the histopathological changes as well as DNA fragmentation [10]. Neurobiological mechanisms underlying the altered brain-derived neurotrophic factor (BDNF) expression as well as the involvement of signaling pathways downstream of BDNF in chronically isolated animals [11]. The role of CLOCK protein and  $\beta$ -

catenin in concert with Gonadotropin-inhibitory hormone (GnIH) neuronal activity may point toward a circadian component in the maintenance of regular neuronal activity under chronically stressful conditions, such as that induced by social isolation [12].

## 2. MATERIALS AND METHODS

### 2.1 Drugs and Peptides

Apomorphine-HCl, haloperidol, mianserin, 1-(3-chlorophenyl) piperazine, were purchased from Sigma (Germany). All other reagents were from available commercial sources.

### 2.2 Animals

Ninety 21-day-old male rats (250-300 g) were randomly allocated in groups of 3-4 under-controlled conditions of  $23 \pm 1^\circ\text{C}$  temperature and 12 h light/dark cycle (lights on at 08:00), with ad libitum access to food and water. All experimental procedures were carried out between 09:00-13:00 h and were approved by the Animal Experimentation Ethics Committee of the University. Every effort was made to minimize animal suffering and the number of animals used.

### 2.3 Interventions

Apomorphine, mianserin were dissolved in normal saline. Haloperidol was dissolved in a drop of acetic acid, diluted with distilled water and adjusted to pH=4.5-5.0. All the compounds was given intraperitoneally (IP) in a volume of 0.5 ml per animal with the exception of apomorphine that were given subcutaneously (SC) in a volume of 0.2 ml per animal. The controls received the same amount of vehicle as SC.

### 2.4 Behavioral Studies

A sample of 80 Male rats (21-day post weaning) were chosen, they were put for 6 weeks in separate cages with black plastic buffers. Eight rats were put in one cage as the control group and the rest were put in individual cages: one male rat in each cage. In group 1 or the control group (social conditions) 8 rats were put in one cage and received saline carrier. The rest of the groups each had 8 rats in separate cages as follows: Group 2 or the placebo group (social isolation conditions) received no treatment with serotonin and dopamine agonist and antagonist and were kept in separate cages with one rat in

each cage. Other groups included: the rats that received Apomorphine (dopamine agonist) at a dose of 0.08 mg/kg via subcutaneous injection (SC); the rats that received serotonin agonist (m-CPP) at a dose of 0.5 mg/kg via subcutaneous injection; the rats that received Serotonin Antagonist (Mianserin) at a dose of 0.2 mg/kg via subcutaneous injection; the rats receiving dopamine antagonist (haloperidol) at a dose of 0.1 mg/kg via Intraperitoneal (IP) injection; the rats receiving Serotonin antagonist (Mianserin) at a dose of 0.2 mg/kg via subcutaneous injection 15 minutes before injection of apomorphine (dopamine agonist); the rats that received dopamine antagonist (haloperidol) at a dose of 0.1 mg/kg via intraperitoneal injection (IP) 15 minutes before the injection of serotonin agonist (m-CPP); the rats that received Apomorphine (dopamine agonist) at a dose of 0.08 mg/kg and Serotonin agonist (m-CPP) injected subcutaneously at a dose of 0.5 mg/kg; and the rats that received dopamine antagonists (haloperidol) at 0.1 mg/kg via intraperitoneal injection (IP) and antagonist serotonin (Mianserin) at 0.2 mg/kg injected subcutaneously 15 minutes before saline injection.

Individual Plexiglas cages (30 cm × 30 cm × 30 cm) were observed for the entire duration of the experiment (60 min) at 10 min intervals in order to count penile erection, yawning episodes and the time spent genital grooming. Penile erections were scored when the penis emerged from the penile sheath which was usually accompanied by penile grooming and hipflexions. Yawning episodes were defined as an opening of the mouth of at least 1-3 s of duration occasionally accompanied by stretching. Behavioral responses were recorded by an observer who was not aware of the treatments done.

### 2.5 Statistical Analysis

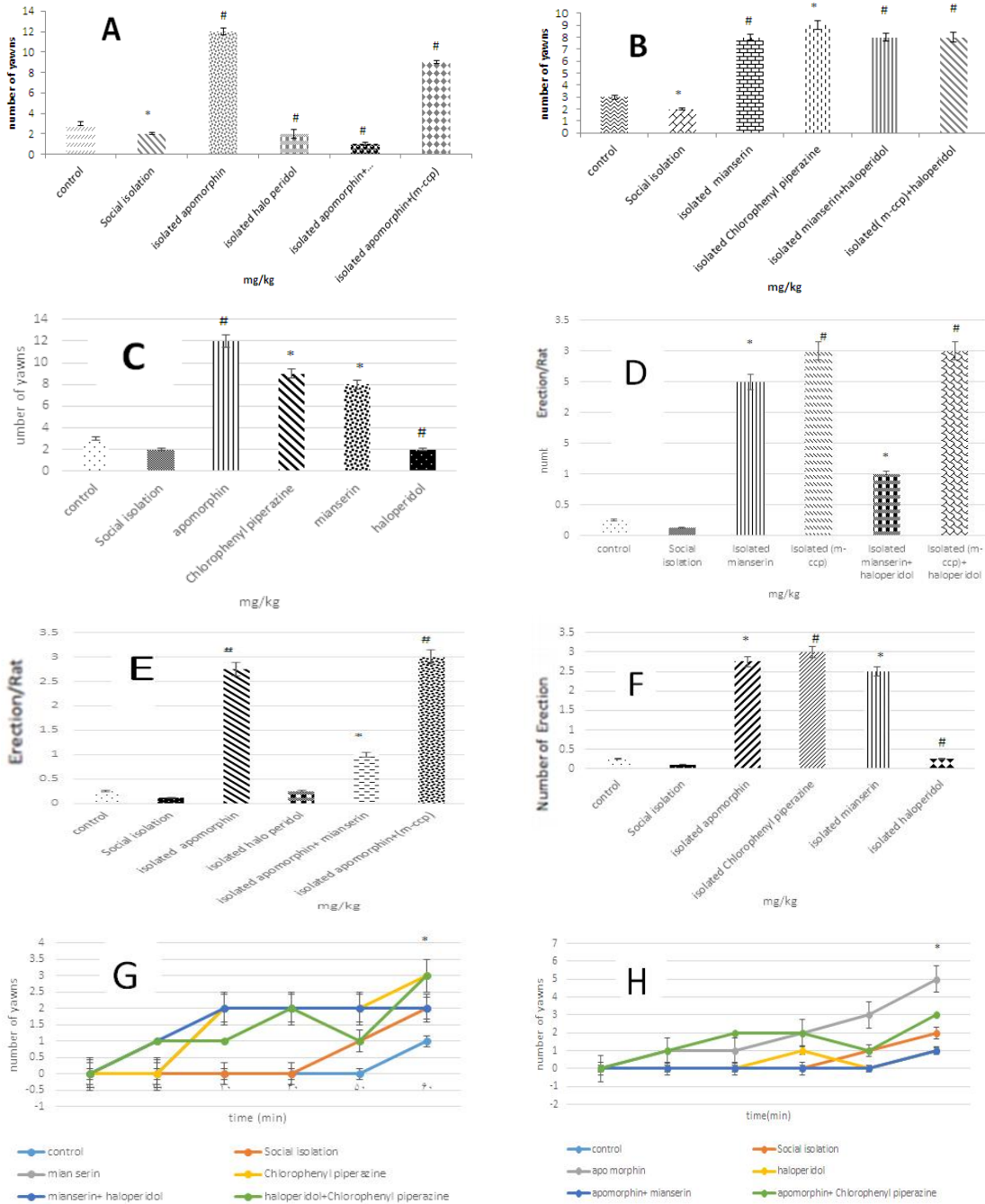
The data were expressed as mean  $\pm$  standard error of the mean. The parameters were evaluated by analysis of variance (ANOVA), followed by Tukey's test. The differences among the groups were assessed by Student's t test, with a significance level of  $P < 0.05$ .

## 3. RESULTS

Diagram A shows Tukey's post-hoc test comparing the groups receiving medicines. The number of yawning in the group receiving apomorphine ( $n = 8$ ;  $P \leq 0.05$ ) was significantly

higher than that of the control group and social isolation group ( $n = 8$ ;  $P \leq 0.05$ ). There was also a significant decrease in the number of yawns in the groups receiving haloperidol ( $n = 8$ ;  $P \leq 0.001$ ); Apo-Morphine + Mianserin ( $n = 8$ ;  $P \leq 0.05$ ); and Apomorphine + Chlorophenyl-Piperazine ( $n = 8$ ;  $P \leq 0.001$ ) as compared to those receiving apomorphine. (The symbols # and \* indicate the significance of the difference between the groups at the level of  $P \leq 0.05$  and  $P \leq 0.001$ ). Diagram B shows the number of yawns in the groups under study. There was a significant increase in the number of yawns in the group receiving Mianserin ( $n = 8$ ;  $P \leq 0.001$ ) compared to the control group ( $n = 8$ ). There was no significant increase in the number of yawns in the groups receiving Chlorophenyl-Piperazine compared to the Mianserin + Haloperidol group ( $n = 8$ ;  $P \leq 0.05$ ). The Chlorophenyl-Piperazine + haloperidol group showed a significant increase in comparison with social isolation and control groups ( $n = 8$ ;  $P \leq 0.001$ ). The symbols # and \* indicate a significant difference between the groups studied at  $P \leq 0.001$  and  $P \leq 0.05$ . Diagram C shows the number of yawns in control and social isolation groups as well as in apomorphine, Chlorophenyl-Piperazine, Mianserin, and Haloperidol. The number of yawns in the group receiving apomorphine ( $n = 8$ ;  $P \leq 0.001$ ) was significantly higher than that of other groups ( $n = 8$ ). Also, a significant increase was observed in the number of yawns in the groups receiving Chlorophenyl-Piperazine ( $n = 8$ ) in comparison with the control group and social isolation group ( $n = 8$ ). This variable in Mianserin group was significantly higher ( $n = 8$ ;  $P \leq 0.001$ ) than the control group and social isolation group. The symbols # and \* indicate a significant difference between the groups studied at  $P \leq 0.001$  and  $P \leq 0.05$ . Diagram D shows that the mean  $\pm$  SD of the number of Erections caused by administration of Mianserin, Chlorophenyl-Piperazine, Mianserin + Haloperidol, and Chlorophenyl-Piperazine + haloperidol showed significant changes after injection compared with the control group of citrate buffer ( $2\mu$  1/rat). The Mianserin group showed a significant increase compared to the control group. And the Chlorophenyl-Piperazine group showed a significant increase compared to the control group and social isolation group. Also, the group receiving haloperidol + Chlorophenyl-Piperazine had a significant increase in the number of erections compared to Mianserin + Haloperidol group and the control group ( $n = 8$ ;  $P \leq 0.05$ ). The symbols # and \* indicate a significant difference

between the groups studied at  $P \leq 0.001$  and  $P \leq 0.05$ . Diagram E shows that mean  $\pm$  standard deviation of the number of Erections caused by administration of apomorphine, haloperidol, apomorphine + Mianserin, and apomorphine + Chlorophenyl-Piperazine showed a significant change after injection compared to control group of citrate buffer ( $2\mu$  1/rat). The apomorphine group had a significant increase compared to other groups, and the apomorphine + Mianserin group showed a significant increase compared to the control group and social isolation group. Also, the group receiving apomorphine + and Chlorophenyl-Piperazine showed a significant increase compared to other groups in the number of erections. The symbols # and \* indicate a significant difference between the groups studied at  $P \leq 0.001$  and  $P \leq 0.05$ . Diagram F shows that the mean  $\pm$  standard deviation of the number of erections showed a significant increase in the control group and social isolation group and after administration of apomorphine, Chlorophenyl-Piperazine, Mianserin, and haloperidol compared to the citrate buffer ( $2\mu$  1/rat) in social conditions. The group receiving apomorphine showed significant changes in the number of erection in ( $n = 8$ ;  $P \leq 0.001$ ) compared to other groups ( $n = 8$ ). Also, a significant increase was observed in the number of erections in the groups receiving Chlorophenyl-Piperazine ( $n = 8$ ) compared to the control group and social isolation group ( $n = 8$ ). The Mianserin group showed a significant increase compared to the control group and social isolation group ( $n = 8$ ;  $P \leq 0.001$ ). The symbols # and \* indicate a significant difference between the groups studied at  $P \leq 0.001$  and  $P \leq 0.05$ . Diagram G shows the mean and standard deviation of the number of yawning due to the administration of Mianserin, Chlorophenyl-Piperazine, Mianserin + Haloperidol, and Chlorophenyl-Piperazine + haloperidol at a 10-minute interval for a total duration of 60 minutes compared to the control group ( $n = 8$ ;  $P \leq 0.05$ ). The symbols # and \* indicate a significant difference between the groups studied at  $P \leq 0.001$  and  $P \leq 0.05$ . Diagram H shows the mean and standard deviation of the number of yawning due to administration of apomorphine, haloperidol, apomorphine + Mianserin, and apomorphine + Chlorophenyl-Piperazine at a 10-minute interval for a total duration of 60 minutes compared to the control group ( $n = 8$ ;  $P \leq 0.05$ ). The symbols # and \* indicate a significant difference between the groups studied at  $P \leq 0.001$  and  $P \leq 0.05$ .



**Fig. 1. Effect of the prior administration of apomorphine, haloperidol, mianserin and (m-cpp) on yawning, penile erection with control. Apomorphine (Apo, 0.08 mg/kg SC) and Haloperidol (Hal, 0.1 mg/kg), (m-CPP) (0.5 mg/kg) and (Mianserin) (Main, 0.2 mg/kg) were given**

#### 4. DISCUSSION

Social isolation rearing from early post-weaning weeks may cause behavioral alteration in the mature animals [9]. It is known that yawning

occurs in every human even at pre-term [13] and also in a variety of non-humans such as vertebrates [14]. This study focuses on the effects of social isolation on yawning. 1-(3-Chlorophenyl) piperazine (m-CPP) (serotonin

agonist), Mianserin (serotonin antagonist), apomorphine (dopamine agonist), and haloperidol (dopamine antagonist) were used and their effect on yawning were investigated. The administration of 1-(3-Chlorophenyl) piperazine (m-CPP) (serotonin agonist) increased the number of yawns in social isolation conditions [15]. Mianserin increased yawning in social conditions. Serotonin + apomorphine increased yawning in social isolation conditions. Administration of serotonin before haloperidol increased yawning while haloperidol alone reduced yawning. Previous studies have shown that the systemic administration of the 5-HT agonist serotonin like piperazine (m-CPP) increase the dose -dependent increase in yawns, and that unlike apomorphine and Oxytocin, the injection of piperazine (m-CPP) and Trifluoromethylphenylpiperazine (TFMPP) to the Paraventricular nucleus (PVN) does not induce yawning and penile erections. Thus, the upward serotonergic pathway from the raphe nuclei to the PVN nucleus does not show induction in the responses related to piperazine (m-CPP) and Trifluoromethylphenylpiperazine (TFMPP) involved [16,17]. This study suggests that different serotonin receptors are likely to have different effects on yawning and other behaviors. The findings show that receptor adjustment or decreased stimulation may play a role in this issue. Serotonergic neurons (5-HT) play a role in controlling the sexual behavior of humans and animals and 5-HT pathways have an inhibitory effect on male sexual behavior in social conditions [18]. However, these pathways may have inhibitory or stimulating effects depending on the receptor in different parts of the nervous system. Also, the effects may vary in different species [19]. There are neural nodes with 5-HT throughout the central nervous system, so that neurons with 5-HT can be found in the raphe nuclei and the abdominal lattice, including the paragigantocellular, as well as the lumbosacral region of the spine in relation to the somatic and autonomic nerves towards the pelvis [20].

## 5. CONCLUSION

Neuropharmacological studies revealed that various neurotransmitters and neuropeptides are effective on yawning. The best known are adrenocorticotropin,  $\alpha$ -melanocyte stimulating hormone (MSH) and related peptides, acetylcholine, dopamine, serotonin, excitatory amino acids, oxytocin, gamma-amino butyric acid (GABA) and opioid peptides [1]. In this study, yawn-increasing compounds such as

apomorphine (dopamine agonist) increased the number of yawns in social and social isolation conditions Dopamine in turn increased central oxytocinergic neurotransmission, leading to penile erection and yawning Serotonin, acting via 5-HT1AR, suppresses nNOS expression in the hippocampus. This modification leads to phosphorylated cAMP response element-binding protein CREB activation, thereby modulating anxiety-related Behaviors metabolism of 5-HT was especially marked in the medial prefrontal cortex (mPFC), after conditioned fear stress (CFS), regarded as psychological stress [15]. The decrease in the amount of 5-HT by synthesis-inhibitor of serotonin, (Para-Chlorophenylalanine), is usually associated with the destruction of axons containing 5,7-dihydroxy- tryptamine [20]. In this study, one of the factors that caused yawning was the amount of corticosterone that increases slightly after stress. Social stress is also stressful. Therefore, in normal conditions without medication, during the time that the rats are in social isolation conditions, the number of yawns is expected to be less than that of social conditions. It is interesting to know the reasons behind yawning in social isolation that can be considered as a warning about neurological issues, especially in social isolation situations. Further studies on the interactions between transmission factors and moderators in social isolation conditions can form the basis of new combination therapies.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

All experimental procedures were carried out between 09:00-13:00 h and were approved by the Animal Experimentation Ethics Committee of the University. Every effort was made to minimize animal suffering and the number of animals used.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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