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Investigation of Role of Melatonergic System on the Modulation of Compulsive Behavior Using Agonist and Antagonist

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Abstract

Obsessive Compulsive Disorder (OCD) is a disorder of the brain and behavior. OCD causes severe anxiety in those affected. OCD involves both ***obsessions and compulsions*** *that take a lot of time and get in the way of important activities the person values. One of the recent drugs used for the treatment of depression is agomelatine which happen to be a nonselective melatonin agonist. It has high-affinity for MT1/MT2 melatonin receptors and is also a 5-HT2C serotonin receptor antagonist. Agomelatine does not directly affect the uptake of serotonin, norepinephrine, or dopamine. By inhibiting 5HT-2C receptors, however, it secondarily increases norepinephrine and dopamine in the frontal cortex of the brain. In the present study the role of melatonergic system on the modulation of compulsive behavior was studied using agomelatine and luzindole Agomelatine does not bind to adrenergic, cholinergic, or histamine receptors. In contrast to this luzindole, a Selective melatonin receptor (MT-1 and MT-2) antagonist has also been used, which provoke the stress and anxiety level and promote obsessive compulsive disorder symptoms.In view of these evidences, it appears that compulsive behavior might be modulated by OCD melatonergic system and hence this study aims to investigate the role of melatonergic system on the modulation of compulsive behavior in rodents.*

Key words: *Obsessive Compulsive Disorder (OCD), Agomelatine, Luzindole, Marble, Buying Behavior (MBB), Melatonergic System.*

Introduction:

Obsessive compulsive disorder (OCD) involves severe alterations in thought processes and behavior. Its core features are intrusive and persistent thoughts that cause distress (obsessions), and compulsions, which are performed in order to alleviate this distress. Obsessions and compulsions are typically egodystonic, that is, the person is consciously aware that they are abnormal, yet cannot control them.

Functional neuroimaging of subjects with OCD has revealed abnormalities in corticostriatal-thalamo-cortical circuits, involving the orbitofrontal cortex (OFC), anterior cingulated gyrus, insula, striatum and thalamus. These studies are consistent with the existence of distinct OCD symptom dimensions, as contamination/washing, checking and hoarding subgroups show different patterns of brain activity.[1]

It is now recognized that OCD affects almost 3% of the world's population and is a major worldwide health problem .Recent studies on the epidemiology of OCD estimate that almost 50 million patients suffer from OCD worldwide, thus making it a global problem. The prevalence of OCD is 2.4% in person aged 18 to 54 years and 1.5% in those older than age 55 years. OCD usually begins early in life with 20% of cases occurring in childhood, 29% in adolescence and 49% of cases occurring by age 20. The onset of illness is earlier in men than women.[1]

In obsessive-compulsive disorder (OCD), only the potent 5-HT reuptake inhibitors (SRIs) are effective namely, fluoxetine, fluoxamine, paroxetine, sertraline, and citalopram have been demonstrated to significantly attenuate OCD in placebo-controlled trials. Furthermore, the TCA clomipramine, which is a potent 5-HT reuptake inhibitor, also produces an anti-OCD effect, but relapse takes place when patients are switched in a double-blind fashion to the TCA desipramine.[2]

Recently, 5-HT7 receptor has also been implicated in the pathogenesis of OCD. It was observed that inactivation of the 5-HT7 receptor leads to decreased burying behaviour in the marble burying test in mice after treatment with a selective 5-HT7 receptors antagonist. Agomelatine, a novel antidepressant with an innovative pharmacological profile, had received a marketing authorization by the European Medicines Agency for the treatment of major depressive disorder. One of the recent drugs used for the treatment of depression is agomelatine which happen to be a nonselective melatonin agonist. It has high-affinity for MT1/MT2 melatonin receptors and is also a 5-HT2C serotonin receptor antagonist.[2]

Melatonin Receptors

Melatonin receptors are named and classified on the basis of operational and structural criteria. The receptors were named for their endogenous ligand melatonin, which is abbreviated as "MT" using capital letters, and each particular type of receptor was denoted by a numerical subscript (i.e., MT_1, MT_2).

Melatonin receptors as therapeutic targets

Melatonin receptor agonists currently on the market or in advanced stages of development are all MT1/MT2-nonselective melatonin receptor agonists. These agonists are indicated or being developed for a number of conditions ranging from insomnia and circadian entrainment to depression and seasonal affective disorder. Melatonin and the synthetic melatonin agonists are generally devoid of the common side effects frequently observed with sleep medication (e.g., impairment of learning, memory or motor function).[3]

Results from clinical trials with agomelatine, a melatonergic receptor agonist (MT1/MT2) and 5HT2C receptor antagonist, have shown that it is efficacious in both the acute phase and the continuation phase of treatment of depression. Agomelatine seems to have a favorable balance between efficacy and tolerability profile. It also lacks the prominent side effects shown by most classes of antidepressants, such as sexual dysfunction, gastrointestinal reactions, and discontinuation symptom.[4,5]

Luzindole in contrast to agomelatine is a Selective melatonin receptor antagonist and 5HT2C receptor agonist and it provoke the stress and anxiety level which is associated with seemingly purposeful ritualistic behavior (compulsions) and obsession. Beyond this agomelatine might contribute to its antidepressant activity. In view of these evidences, it appears that compulsive behavior might be modulated by OCD melatonergic system and hence this study aims to investigate the role of melatonergic system on the modulation of compulsive behavior in rodents.

Material and methods

Adult male albino Swiss mice (22-25 g) were used for the present study. The animals were breed from an original stock purchased from Veterinary College, Mhow, India. The animals were group housed [mice (n=6)] under a standard 12h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55-65%). All animals were acclimatized to laboratory conditions for at least seven days before carrying out the experiments, which were carried at 08.00 to 15.00 h daily. Separate group of mice (n=6/12) was used for each set of experiments. Agomelatine and Luzindole were purchased from Sigma-Aldrich. Agomelatine was dissolved in 1% hydroxyl ethyl cellulose while luzindole was dissolved in Di methyl Sulphoxide solution.

Volume of drug administration

The volume of administration of drug vehicle was calculated based upon the body weight of mice i.e 10 ml/kg body weight of mice.

Apparatus: Marble-burying behaviour test apparatus

It consisted of plastic cages (40 × 28 × 14 cm) containing 5 cm thick wood dust bedding. Twenty small glass marbles (~10 mm), were arranged on the bedding evenly spaced in four rows of five each. The cage was covered by transparent plastic lead with line markings (2 × 2) and the apparatus is placed 1.5-2.0 m below a video camera in the experiment room with bright light (100 lux).

Figure 1. Marble burying behaviour of mice

Experimental methods

Assessment of marble-burying behavior and motor activity in mice

The marble-burying behavior and locomotor of mice was recorded as reported by Umathe et al., earlier with slight modifications. In brief, mice were individually placed in marble-burying behavior apparatus with 20 glass marbles for 30 min. The behavior of the mice during the test session was recorded by a video camera. At the end mice were removed, and unburied marbles were counted. A marble was considered 'buried' if its two-third size was covered with saw dust. The total number of marbles buried was considered as an index of obsessive-compulsive behavior. The video recording was analyzed to determine the number of line crossings made by the animal during a test session. Total number of line crossings measured during 30 min was considered as locomotor counts for the animals.[6,7]

Treatments

Experiment 1: Acute study

Mice were randomly assigned to treatment conditions (n=6/12) in which Agomelatine (10,20,30,40,50mg/kg,i.p.) and Luzindole (10, 3, 5 mg/Kg, i.p.) were administered. Marble-burying behaviour was tested for 30 min after the administration of drugs.

Experiment 2:Chronic study

Mice were randomly assigned to treatment conditions (n=6/12) in which agomelatine (20, 30 mg/kg,i.p.) and Luzindole (1, 3, mg/Kg,i.p.) were administered for 10 days. Marble-burying behavior was tested on day 11, 30 min after the last treatment.

Influence of acute drug treatment on MBB and Locomotor count

Agomelatine

One-way ANOVA revealed that acute administration of Agomelatine in different doses had a significant effect on the MBB of male mice [F (5, 53)=6.835,P<0.0001] .

Post hoc analysis

Further the dunnett multiple comparison test revealed that agomelatine had a significant effect at 20mg/Kg (P<0.01), 30mg/Kg (P<0.05) and at 50mg/kg (P<0.001) however the lower dose found to be non significant at 10mg/Kg(P>0.05). On studying locomotor activity, agomelatine has shown decrease in locomotor count in the dose dependent manner. At following doses of 20 and 30 mg/kg locomotor count has shown insignificant.

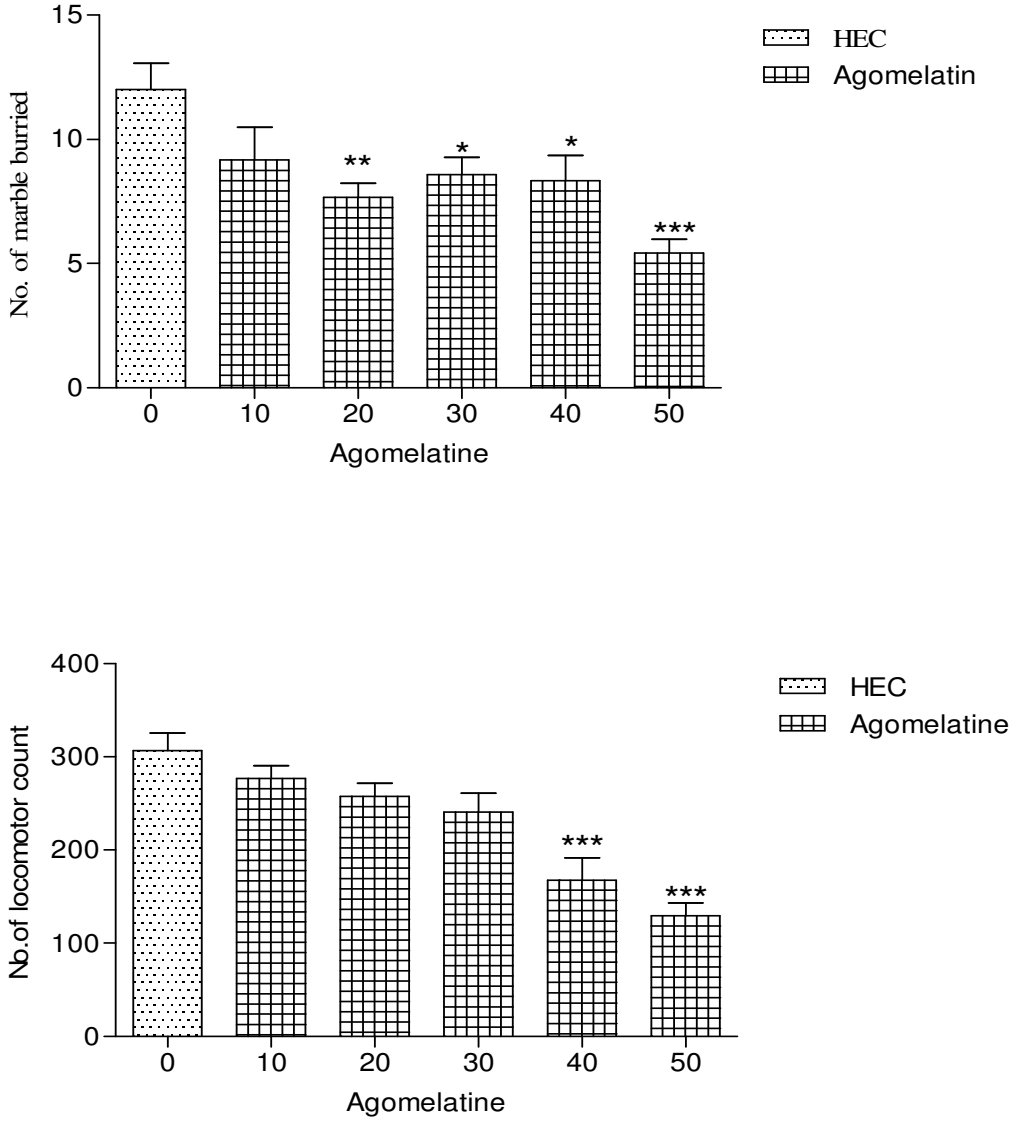

Figure 2. Influence of agomelatine on anticompulsive activity

Luzindole

One-way ANOVA revealed that acute administration of Diazepam in different doses had a significant effect on the MBB of male mice [F (5, 30) = 1.844, P = 0.1344].

Post hoc analysis

Further the dunnett multiple comparison test revealed that luzindole had a significant effect at 1 mg/Kg and 3 mg/Kg (P<0.001). However, the lower dose found to be non-significant at 0.25 mg/Kg (P>0.05). On studying locomotor activity luzindole showed significant effect on 1mg/Kg (P<.01) and 3mg/Kg (P<.05).

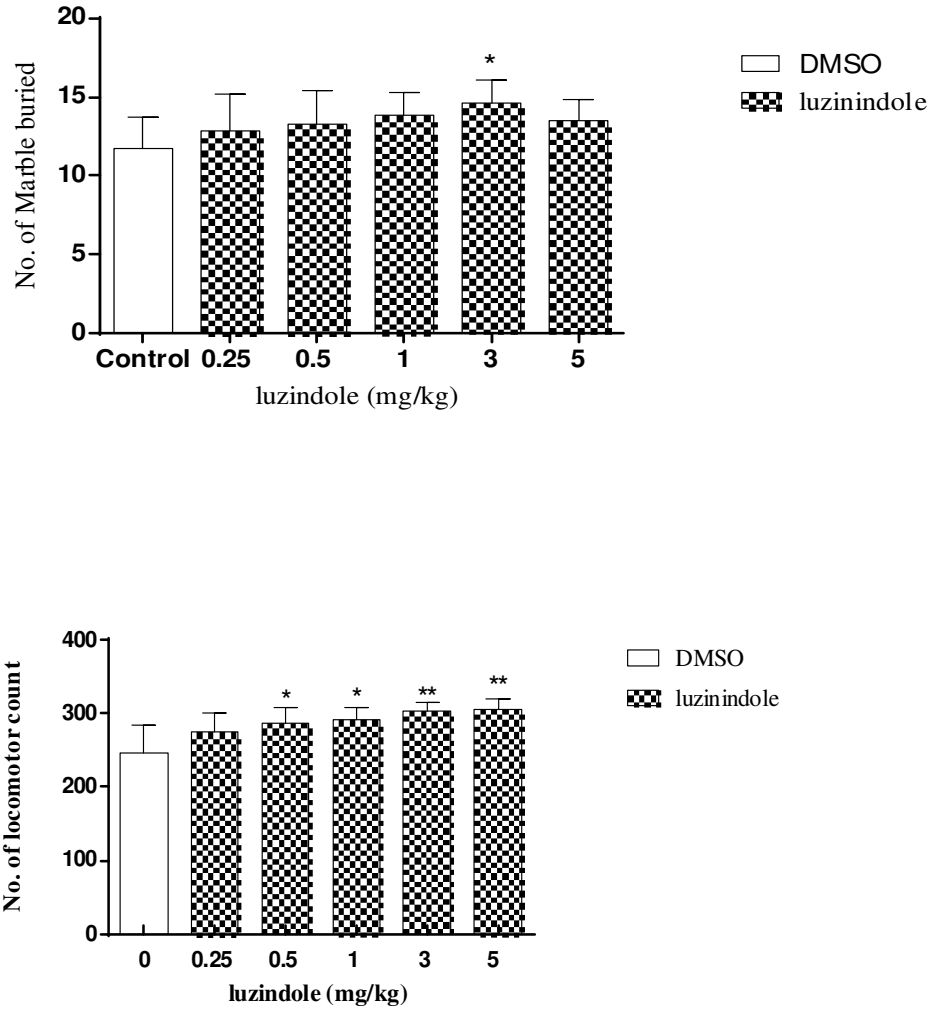

Figure 3. Influence of luzindole on anticompulsive activity

Experiment 2: Chronic study

Influence of chronic drug treatment on Marble Burying Behaviour and Locomotor Count

Effect of chronic drug treatment on the marble-burying behavior of mice was studied. One-way ANOVA indicated a significant influence of both the drug treatments on the marble-burying behavior of mice [F (7, 47) = 20.25; $P <$ 0.0001]. Tukey's post-hoc test indicated that chronic administration of agomelatine (20, 30 mg/kg) significantly ($P <$ 0.001) inhibited the marbe-burying behavior and in contrast to this luzindole promote the marble burying behavior, which indicate the high level of stress and anxiety.

However, the locomotor activity was also significantly affected [F (7, 47) = 18.50; $P < 0.0001$]. The post hoc analysis indicated that agomelatine (20 mg/kg), treatment significantly ($P < 0.001$) reduced the locomotor activity and in contrast to this luzindole promote the marble burying behavior, which indicate the high level of stress and anxiety. The comparative study of chronic drug treatment on MBB and LC between agomelatine (20, 30 mg/kg) and luzindole (1 mg/Kg and 3 mg/Kg) was done to prove the intermittent role of melatonergic receptors in the treatment of OCD.

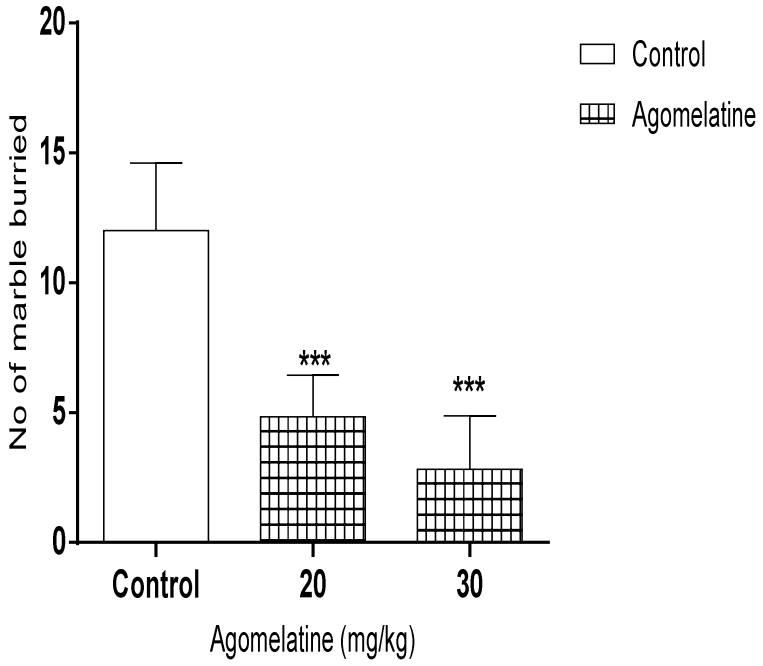

Figure 4. Influence of chronic dose of agomelatine on no. of marble buried

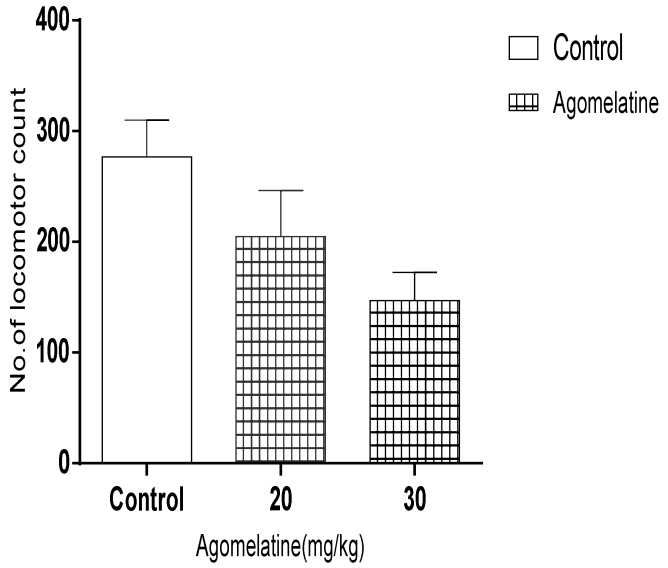

Figure 5. Influence of chronic dose of agomelatine on no. of locomotor count

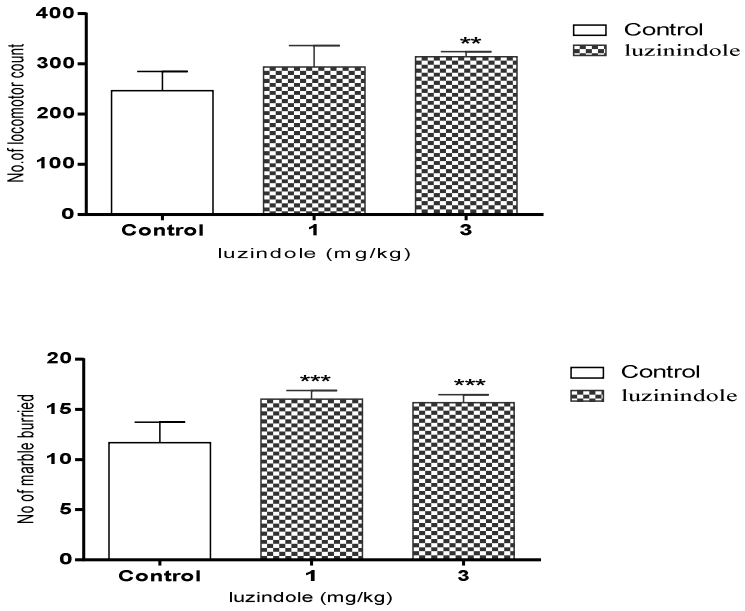

Figure 6. Influence of chronic dose of Luzindole on anticompulsive activity

Conclusion

On studying the comparative study of agomelatine and luzindole on the rodents, agomelatine shows the less no. of locomotor count and marble buried count, which indicate the decrement in stress and anxiety level which is related to

the treatment of OCD and in contrast to this luzindole promote the marble burying behavior, which indicate the high level of stress and anxiety.

In the present work agomelatine a novel melatonergic analogue dose-dependently attenuated marble-burying behavior in mice, an effect that was comparable with that of Luzindole. In addition, this effect of agomelatine was maintained after its administration for 10 days.

It is observed that mice do not avoid marbles when given the opportunity to do so, indicating their non-aversive property. In addition, repeated exposure to marbles does not induce habituation, suggesting that this behaviour is not related to novelty or fear however, because the marbles are nonreactive, they cannot provide the animal with the necessary stimuli to a natural ending of the investigation, and this 'frustrated' investigation leads to compulsive burying.

Hence, although inhibition of object burying was originally suggested as a screening test for anxiolytic activity, the above findings and the reduction in burying behavior by serotonin reuptake inhibitors suggest that this behavior may be related to obsessive–compulsive disorder. For these reasons, we selected marble-burying behavior as a paradigm to screen the anticompulsive effect of agomelatine. It is a still a matter of debate whether marble burying measures anxiety or compulsivity; however, it is clear that marble burying is decreased by both anxiolytic drugs and anticompulsive drugs. Therefore, our results suggest that agomelatine may have anti-anxiety or anti-compulsive activity.

Finally, although the marble-burying model may at times fail to discriminate between anxiolytic and anticompulsive agents after acute administration, all drugs that modulate marble-burying behavior after repeated exposure without altering locomotor activity have been found to be clinically useful in the treatment of obsessive–compulsive disorder The anxiolytic actions of agomelatine are well documented; hence, the observed effect of agomelatine on marble burying observed in the present study indicates its anticompulsive potential and prompts further evaluation in other animal models of compulsivity.

Obsessive-compulsive disorder has a major impact on quality of life and affects all aspects of the individual's daily life. The etiology and pathophysiology of OCD is still far from being clear. Also the treatment of OCD is viewed as difficult and unsatisfactory, as almost half of the OCD patients do not respond to pharmacotherapies established so far. Available data indicates that agomelatine are involved in numerous neuropsychiatric disorders, and have modulatory role on neurotransmitters. Accordingly, the present study was designed to test the hypothesis of modulatory role of agomelatine in obsessive-compulsive disorder.

Major findings of the present investigations are that agomelatine reduced marble-burying behavior in mice, which was comparable to control. These studies were carried out by employing marble-burying behavior test in mice, which is widely employed to screen the effects of various agents on compulsive behavior. Marble-burying behavior is an unconditioned species-specific defensive reaction in rodents, which is not associated with physical danger, and does not habituate upon repeated testing). In male mice it is markedly attenuated by acute administration of SSRI and tricyclic antidepressants despite the acute anxiogenic properties of these drugs .These observations suggest that the burying behavior in male mice models the compulsive behavior rather than anxiety. In view of these evidences, it appears that compulsive behavior might be modulated by OCD melatonergic system and hence this study aims to investigate the role of melatonergic system on the modulation of compulsive behavior in rodents.

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