



<b>Title</b>	<b>Ketamine abuse and apoptosis in the cortex in monkeys and mice</b>
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**P-06.08** Tiagabine modulates the effect of cocaine sensitization on  $\alpha 1$ -adrenoceptors

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**Objective:** Cocaine indirectly stimulates catecholaminergic systems and its repeated administration sensitizes their responses. While interactions between GABA-ergic and dopaminergic neurotransmission in a mechanism of cocaine action were widely described, those concerning the noradrenergic system were less explored. As we recently reported that the cocaine-induced behavioral sensitization is accompanied by changes in the  $\alpha 1$ -adrenergic receptors ( $\alpha 1$ -ARs) density in some regions of rat brain, we presently have investigated how those effects are modulated by activation of GABA-ergic system – a procedure used to depress symptoms of cocaine dependence.

**Methods:** To obtain cocaine sensitized rats (CSR) the animals received cocaine (10 mg/kg) for 5 consecutive days (D1-D5), and remained drug-free for the next four days (D6-D9). Cocaine naive rats (CNR) received saline. On D10 the rats were injected with saline or tiagabine (10 mg/kg) and after 120 min received cocaine injection (10 mg/kg). They were decapitated 24 h later. The cerebral  $\alpha 1$ -ARs density was measured by quantitative *in vitro* autoradiography of [<sup>3</sup>H]prazosin in the absence (total  $\alpha 1$ -ARs) or presence of 10 nM WB4101 to dissect the  $\alpha 1B$  AR subtype.

**Results:** Tiagabine treatment decreased the total and  $\alpha 1B$  pools of  $\alpha 1$ -ARs in structures involved in behavioral effects of cocaine: nucleus accumbens (NAc) and primary motor cortex (M1), while it increased the  $\alpha 1B$  receptor in amygdala and hippocampus. Cocaine in CNR increased  $\alpha 1B$  density in NAc and thalamus, but sensitization abolished this effect. Cocaine decreased the total pool of  $\alpha 1$ -ARs in cingulate and M1 of CNR, but this effect was also abolished in CSR. On the other hand sensitization resulted in a decrease of  $\alpha 1$ -ARs in subcortical areas – the thalamus, amygdala, and hippocampus, not observed in CNR. The modulatory effect of tiagabine appeared in amygdala and hippocampus of CSR, where tiagabine decreased  $\alpha 1B$ , and in NAc and amygdala, where tiagabine annulled the effects of sensitization on the total pool of  $\alpha 1$ -ARs.

**Conclusion:** Tiagabine increased  $\alpha 1$ -ARs density in brain structures involved in cocaine-induced aggressiveness and memory enhancement, while decreased it in structures associated with reward and hypermotility. Effects induced by cocaine in CNR were generally changed in opposite direction in CSR. Tiagabine pretreatment attenuated the effects of cocaine sensitization on  $\alpha 1$ -ARs in NAc and amygdala.

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**P-06.09** Implication of dopamine D1A and D2 receptors in hippocampus in the conditioning of the rewarding effect of cocaine

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**Objective:** The hippocampus is important for contextual conditioning. The conditioned place preference (CPP) to drugs of abuse is a form of associative process, thus it can be assumed to reflect hippocampal-dependent processes. Dopamine is the one that has been more extensively implicated in the mechanism of drug addiction. However, little if any is known about the functional and direct implication of hippocampal dopamine receptors in the development of a cocaine-induced rewarding effect. In the present study, we investigated whether dopamine D1A and D2 receptors in hippocampus are implicated in the development of cocaine-induced rewarding effect using a CPP paradigm in rats.

**Methods:** Rats were divided into four groups by session frequency: Group 0 underwent no conditioning session, pre- and post-test only; Group 1, underwent pre-test, one pair of conditioning session (1 day for cocaine at 15 mg/kg, s.c., and 1 day for saline) and post-test; Group 2 underwent two pairs of conditioning sessions once a day and Group 3 underwent three pairs of conditioning sessions between pre- and post-test.

**Results:** Preference levels for the cocaine-associated box were frequency-dependently increased. The mRNA of dopamine D1A and D2 receptors significantly increased in the hippocampus of Group 2 only.

**Conclusion:** These results indicate that dopamine D1A and D2 receptors in the hippocampus may be one of the critical mediators of the neuronal changes necessary for the induction of this cocaine-induced rewarding effect. The dynamic synaptic events regulated by neurotransmitters and their cognate receptors underlie long-lasting changes that contribute to the intensity and persistence of the drug in memory.

**P-06.10** Vigabatrin and tiagabine abolish the expression of cocaine sensitization

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**Objective:** Agents enhancing GABA transmission are very promising in treatment of cocaine dependence. However, the results concerning clinical efficacy of some of them, particularly of tiagabine, are discrepant. We investigated therefore the effects of two compounds elevating GABA level: tiagabine (a GABA reuptake inhibitor), and vigabatrin (a GABA transaminase inhibitor) on expression of cocaine sensitization, a phenomenon regarded as indicative of cocaine dependence.

**Methods:** Male Wistar rats weighing 230–270 g and housed 8 to a cage, received in their home cages saline or cocaine (10 mg/kg) for 5 consecutive days (D1-D5), and remained drug-free for the next four days (D6-D9), but on D8 were placed in Opto-Varimex cages for 45 min habituation and then injected with saline and returned to the actometer for 60 min of activity recording. On D10 the rats were placed in activity cages at –165 min, and injected with saline, vigabatrin (75 or 150 mg/kg, or tiagabine (10 mg/kg) at –120 min for activity recording. At time 0 min the rats were briefly removed from the cage for cocaine injection (10 mg/kg) and immediately returned to the measuring device for recording motor activity for 60 min. The activity was analyzed using Auto-track software. The vehicle of gabergic drugs (0.5% Tween 80) was given at the corresponding times to controls.

**Results:** Cocaine elevated the locomotor activity of naive mice approximately 14-fold (from 510±155 to 6,897±1,406 beam crosses per hour). Given to rats receiving cocaine during days 1–5, cocaine produced an effect 2.2-fold stronger (15,329±2,575). Pretreatment with both doses of vigabatrin or with tiagabine brought down the cocaine-induced motor stimulation in sensitized animals to the level observed after cocaine administration to cocaine-naive animals (5,585±1,210, 7,735±1,696, and 7,843±1,430). The gabergic drugs used in the same doses and time schedule did not inhibit the locomotor stimulation induced by a single dose of cocaine in naive rats.

**Conclusion:** Clinical aspects of cocaine dependence are complex and possibly not all of them are reflected in particular animal models. The present results suggest that those aspects of cocaine dependence that undergo sensitization (e.g., paranoia and mood elevation, but not drug wanting) might be effectively reduced by both vigabatrin and tiagabine.

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**P-06.11** Ketamine abuse and apoptosis in the cortex in monkeys and mice

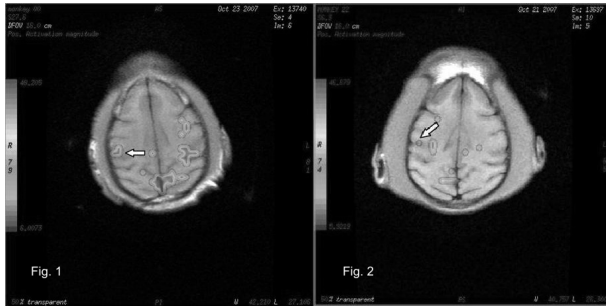
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**Objective:** Ketamine, a noncompetitive antagonist at the glutamatergic N-methyl-D-aspartate (NMDA) receptor, is currently used in human and animal medicine as an injectable anesthetic. Ketamine is also a controlled substance, illegally used as a recreational drug (“Special K”, “Vitamin K”). ketamine, as an NMDA antagonist, can inhibit the reuptake of serotonin, dopamine, and norepinephrine, although the mechanism underlying this action is not entirely clear. There are, however, fewer evidences of the neurobiological or neurochemical alterations according to symptoms of ketamine abuse. No evidence shows the long-term effects in young adults with ketamine abuse. Therefore in the present study, we attempt to examine whether apoptosis is a potential mechanism for the ketamine abuse-related loss of function in the brain (in particular the cortex). Caspases-dependent pathway is one of the most studied apoptosis pathways. Activation of the caspase-9, which in turn activates caspase-3, is a central pathway for apoptosis. Caspase-6 is activated for fragmentation of the nucleus, which leads to downstream events of apoptosis. In addition, we try to use functional magnetic resonance imaging (fMRI) to explore the neuronal activities in cortical and subcortical brain areas in the ketamine abuse animal model.

**Methods:** Establish long-term ketamine abuse models in Cynomolgus Monkeys (1 mg/kg i.v. ketamine for 14 days) and in mice (1 mg/kg i.p. Ketamine for 14 days). Test neuronal activities and metabolism in ketamine abuse monkeys by using fMRI. In this study, we raised the monkey's right legs during the stimulation periods under fMRI. Investigate protein levels of pro-apoptotic (caspase-3, -6 and -9) in the cortex in ketamine abused mice by using western blotting.

**Results:** It is interesting to find an obviously decreased blood oxygenation level dependent (BOLD) signal of the sensation area in the cortex in Ketamine abused monkeys (see Figure 1 and 2). The expression of caspase-3 and -9

significantly increases in the cortex of the ketamine abused mice, however, there is no difference of caspase-6 expression in the cortex in the ketamine abused mice.



Figures 1,2. fMRI studies of the cortex in monkeys. In this study, we moved the monkeys' right legs up and down 5 times during the stimulation periods under fMRI. The white arrow indicates the sensation area of the cortex for the response of leg movements; figure 1: monkeys administered vehicle for 14 days; figure 2: monkeys administered 1 mg/kg ketamine for 14 days.

**Conclusion:** Administration of ketamine for long time could decrease neuronal activities. Caspases-dependent apoptosis in central nervous system (CNS) may involve in this alteration. But further relations between caspases-dependent apoptosis and neuronal activities in ketamine abuse models need to be investigated.

#### P-06.12 Expression of mRNA for corticotropin and vasopressin in hypothalamus and amygdala of rats following administration of psychoactive drugs

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**Objective:** The purpose was to evaluate the expression of mRNA for corticotropin and vasopressin in hypothalamus and amygdala of rats following administration of psychoactive drugs.

**Methods:** Wistar rats were injected intraperitoneally within 4 days in elevated doses with: 1) physiological saline (control; 0.1–0.2–0.4–0.8 ml/rat), 2) amphetamine (0.5–1.0–2.0–4.0 mg/kg); 3) fentanyl (0.00625–0.0125–0.025–0.05 mg/kg), 4) ethanol 40% solution (0.5–1.0–2.0–4.0 g/kg), 5) sodium ethaminal (2.5–5–10–20 mg/kg) or 6) dexamethasone (0.5–1.0–2.0–4.0 mg/kg). The forced regimen of drug administration led to gradual load of the organism and prevented drug tolerance. This method was actively used for formation of drug dependence (or its features) from different narcogens.

**Results:** The biggest mRNA expression for corticotropin was registered in amygdala after administration of dexamethasone (0.46 units compared with  $\beta$ -actin), and the minimal one was after sodium ethaminal (0.07) and fentanyl (0.037). In hypothalamus, sodium ethaminal produced the elevated mRNA expression (0.8 unit), then were ethanol (0.37) and fentanyl (0.039). Amphetamine did not activate mRNA expression for corticotropin nor in hypothalamus, nor in amygdala for all of the drugs studied. The mRNA expression for vasopressin did not register for all drugs both in hypothalamus and amygdala.

**Conclusion:** Therefore, the reinforcing system of hypothalamus supports the typical reaction on narcogens administration, where as the extended amygdala includes both the proper reinforcement and stress reactivity elements.

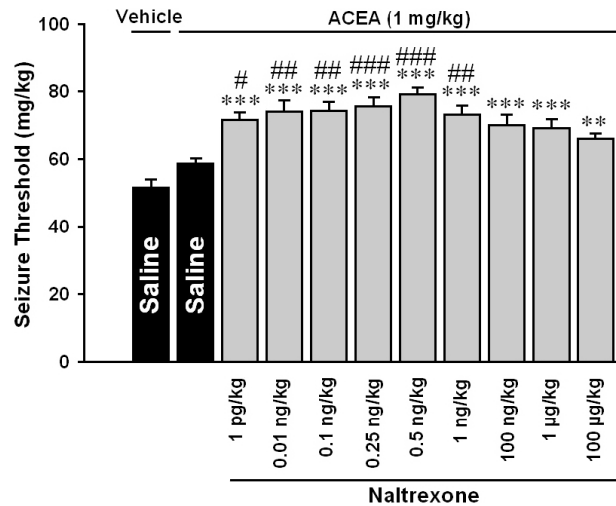
#### P-06.13 Ultra-low dose opioid antagonist naltrexone potentiates cannabinoid anticonvulsant effects in the pentylenetetrazole-induced seizure in mice

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**Objective:** It is widely accepted that cannabinoid compounds are anticonvulsant since they have inhibitory effects at micromolar doses, which are mediated by activated receptors coupling to Gi/o proteins. Surprisingly, both the analgesic and anticonvulsant effects of opioids are enhanced by ultra-low doses (nanomolar to picomolar) of the opioid antagonist naltrexone and as opioid and cannabinoid systems interact, it has been shown that ultra-low dose naltrexone also enhances cannabinoid-induced antinociception. Concerning the seizure modulating properties of both classes of receptors, this study investigated whether the ultra-low dose opioid antagonist naltrexone influences cannabinoid anticonvulsant effects.

**Methods:** The clonic seizure threshold was tested in separate groups of male NMRI mice following injection of vehicle, the cannabinoid selective agonist arachidonyl-2-chloroethylamide (ACEA) and ultra-low doses of the opioid receptor antagonist naltrexone and a combination of ACEA and naltrexone doses in a model of clonic seizure induced by pentylenetetrazole (PTZ).

**Results:** Systemic administration of ultra-low doses of naltrexone (1  $\mu$ g/kg–1 ng/kg, i.p.) significantly potentiated the anticonvulsant effect of ACEA (1 mg/kg, i.p.). Moreover, the very low dose of naltrexone (0.5 ng/kg) unmasked a strong anticonvulsant effect for very low doses of ACEA (10 and 100  $\mu$ g/kg). A similar potentiation by naltrexone (0.5 ng/kg) of anticonvulsant effects of non-effective dose of ACEA (1 mg/kg) was also observed in the generalized tonic-clonic model of seizure.



**Conclusion:** The present data indicate that the interaction between opioid and cannabinoid systems extends to ultra-low dose levels and ultra-low doses of opioid receptor antagonist in conjunction with very low doses of cannabinoids may provide a potent strategy to modulate seizure susceptibility.

#### P-06.14 The effects of ascorbic acid on morphine withdrawal symptoms in rats

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**Objective:** Recent studies indicate that the glutamatergic and Dopaminergic systems are also involved in morphine tolerance and dependence on morphine and in morphine withdrawal syndrome. Ascorbic acid (ascorbate) which is an antioxidant vitamin released from glutamatergic neurons and modulate the synaptic action of dopamine and glutamate as well as behavior. Since Ascorbate modulate the synaptic action of dopamine and glutamate, in this study the effect of Ascorbate on morphine withdrawal syndrome in rats has been investigated. Objective: to determine the effects of Ascorbic acid on morphine withdrawal syndrome.

**Methods:** 30 Male rats (250–300gr) were tested in this study in two groups. The first group as the control group received 3% sucrose in tap water (n=6) and the second group as the dependent group received morphine (0.1, 0.2, 0.3, 0.4 mg/ml each one for 48h, and 0.4 mg/ml remaining days to 21th days) and 3% sucrose in tap water (n=24), this group divided in to 4 sub groups: (1) morphine group, (2,3,4) morphine-Ascorbic acid groups which received AA (100, 500, 1000 mg/kg I.P) every 48 h and in the end (21th day) 30 min before naloxone administration for evaluation effects of AA on withdrawal signs.

**Results:** Our results show that: Ascorbate (100, 500, 1000 mg/kg I.P) can greatly attenuate most of morphine withdrawal syndrome (but not all) dose dependently.

#### P-06.15 Sigma-1 receptor chaperones at the ER regulate dendritic arborization and NMDA/AMPA receptor anchorings in primary hippocampal neurons

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**Objective:** To examine if the sigma-1 receptor chaperone at the endoplasmic reticulum may regulate the development and maturation of dendrites and the anchorings of NMDA/AMPA receptors in developing neurons.