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APOMORPHINE-INDUCED UPREGULATION OF SEROTONIN 5-HT_{2A} RECEPTORS IN MALE RATS IS INDEPENDENT FROM DEVELOPMENT OF AGGRESSIVE BEHAVIOUR

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The [^{3}H]ketanserin binding characteristics in the apomorphine-induced aggressive and nonaggressive adult male Wistar rats were studied. Repeated apomorphine (0.5 mg/kg, once daily) treatment gradually induced aggressive behaviour in sixteen animals from twenty. Thereafter the animals were retrospectively divided into apomorphine-induced aggressive and nonaggressive group. The maximal number of the [^{3}H]ketanserin binding sites was increased in the apomorphine-treated animals in the frontal (233.9 ± 26.5, 364.6 ± 31.7, and 367.0 ± 34.8 fmol/mg protein for the vehicle, apomorphine-nonaggressive, and apomorphine-aggressive group, respectively) and cerebral cortex (164.2 ± 6.7, 289.7 ± 29.3, and 249.0 ± 15.4 fmol/mg protein for the vehicle, apomorphine-nonaggressive, and apomorphine-aggressive group, respectively). In conclusion, our experiments demonstrate that repeated apomorphine treatment upregulates the maximal number of the 5-HT_{2A} receptors in rat frontal and cerebral cortex as measured by [^{3}H]ketanserin binding and this phenomenon is independent from the development of aggressive behaviour.

Key words: *apomorphine, aggressive behaviour, 5-HT_{2A} receptors, [^{3}H]ketanserin binding, rat.*

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) receptors have been proposed to play an important role in the mediation of aggressive behaviour (1, 2), whereas the main interest has been focused to the 5-HT_{1A} and 5-HT_{2A} receptors (2—4). Thus, the 5-HT_{1A} receptor agonists have been found to elicit antiaggressive effects in some paradigms of aggressive behaviour (5, 6). Similar findings have been done with the 5-HT_{2A} receptor antagonists, though there exist some controversies between the results obtained using different methods of aggressive behaviour (2). In some preclinical studies it has been found that the

selective serotonin reuptake inhibitors (SSRIs) such as sertraline, fluoxetine, femoxetine and fluvoxamine, which are widely used clinically as antidepressant and antianxiety drugs, may elicit weak antiaggressive effects on isolation- induced aggression in mice (6). Furthermore, in our previous works we have proposed that the antiaggressive effect of SSRIs is not mediated by the regulation of the 5-HT transporter in itself. Thus, the SSRI-treatment causes up/downregulation of the 5-HT receptors that leads to antiaggressive effect of drugs acting at the 5-HT transporter (7, 8).

On the other hand, the most widely used groups of antiaggressive drugs in psychiatric patients are still the classic neuroleptics that act as dopamine D_2 receptor blockers. *Vice versa*, the selective D_2 as well as unselective dopamine receptor agonists may intensify the aggressive behaviour both in animals and humans (9—11). Repeated treatment with small doses of an unselective dopamine D_1 and D_2 receptor agonist apomorphine (0.5—2.0 mg/kg, s.c., once or twice daily) has been found to induce aggressive behaviour in rodents (12—19). Because the apomorphine-induced aggressiveness is effectively antagonised by neuroleptics, D_2 receptor blockers, morphine, and NMDA receptor antagonists, this phenomenon has been proposed to be an equivalent to human pathology of aggressive behaviour or even schizophrenia (15, 16). In our previous study, we have found that the SSRIs were ineffective in the apomorphine-induced aggressiveness test, but the 5-HT_{1A} receptor partial agonist buspirone, in nonsedative dose range, blocked the aggressive postures of animals (7). The role of the 5-HT_{2A} receptors in the apomorphine-induced aggressiveness is not known. We have found previously that repeated apomorphine treatment may downregulate the 5-HT transporter binding characteristics in the frontal cortex but not in other brain regions (7).

The main objective of the present study was to investigate the 5-HT_{2A} receptor binding using the [^{3}H]labelled ketanserin. [^{3}H]ketanserin is a relatively selective 5-HT_{2A} receptor ligand with only a weak affinity to the 5-HT_{2C} receptors (20, 21). Since the apomorphine-induced aggressiveness test is a time-consuming method and usually in 10—20% of the animals the aggressivenss will not develop, we have used a sophisticated test design. Recently we have found that the apomorphine-induced aggressive and non-aggressive animals differ in the [^{3}H]raclopride-sensitive dopamine D_2 receptor binding sites (22). Therefore, in our experiments the animals were divided retrospectively into apomorphine-induced aggressive and nonaggressive group. All animals exhibited similar stereotyped behaviour and the only difference was the presence or absence of the aggressive behaviour. Thus, we measured the number of the maximal apparent 5-HT_{2A} receptor binding sites (B_{max}, fmol/mg protein) and the affinity of the 5-HT_{2A} receptor binding sites

(K_d, nM) in four rat brain regions (frontal cortex, cerebral cortex, hippocampus, and hypothalamus) in apomorphine-induced aggressive, nonaggressive, and vehicle-treated rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (from Kuopio National Animal Center, Kuopio, Finland) weighing at least 300 g were used in all experiments. The animals were housed separately (one per cage) under standard laboratory conditions; tap water and standard commercial food pellets (R70, Lactamine, Stockholm, Sweden) were available *ad libitum*. The animal room had controlled temperature (20 °C ± 2 °C) and light/dark cycle (light on from 8.00 a.m. to 8.00 p.m.).

Drugs and drug administration

In the experiments of aggressive behaviour, apomorphine in a form of commercially available substance for clinical use (from Reakhim, Krasnoyarsk, Russian Federation) was used.

Apomorphine were dissolved in distilled water containing 0.5% ascorbic acid and stored as stock solution at 4 °C for no more than five days. Prior a test, the stock solution was diluted up to 0.5 mg/ml and injected subcutaneously in a volume of 1 ml/kg body weight (0.5 mg/kg body weight). The apomorphine treatment lasted for 14 days.

The vehicle-treated animals received daily an injection of distilled water.

Behavioural experiments

The measurement of aggressive behaviour was performed in specially designed cages (transparent plastic side walls (35 × 35 × 55 cm, length × width × height) and stainless steel floor, covered with aspen chips (4 × 4 × 1 mm)). Between the test sessions, the animals were housed in home cages that were placed into standard racks. For a test, the animals were picked from neighbour cages and immediately after apomorphine injection, the animals were put pairwise to the test cage and observed for (1) the time of the latency (the time before the first attack or the first aggressive posture) and (2) the intensity of aggressive behaviour. The animals were observed during 15 min and the rating of aggressive behaviour was scored on the 0—3 point scale (modified from (13)):

0.) no aggressive manifestations;
1.) intermittent mild aggressive posture or attack with other rat, no vocalisations;
2.) intermittent intensive upright aggressive posture or attack or boxing with other rat, vocalisations, but no biting or continuous fighting;
3.) continuous fighting or attempts to bite the opponent rat, loud vocalisations.

In the case of the development of the highest score of aggressive behaviour, the test was cancelled to avoid the suffering of the animals.

[^{3}H]ketanserin binding

In the radioligand binding experiments, the following chemicals were used: [^{3}H]ketanserin (66.4 Ci/mmol) from NEN, Brussels, ketanserin tartrate, from RBI, Natick, MA, USA; Tris HCl from Sigma, St. Louis, MO, USA; all other chemicals were of analytical grade from local commercial sources.

Rats were moved from the animal department to the laboratory, decapitated and the brains were quickly dissected on ice. This procedure took no more than five minutes. The brain samples were stored in polypropylene tubes at −82°C until assayed.

The [^{3}H]ketanserin binding studies were performed as described previously by Chaouloff *et al.*, (23) with some modifications. Thus, the homogenate was centrifuged at 15,000 X*g* for 15 min. The buffer was discarded, the pellet was rehomogenised in 50 mM Tris HCl buffer, centrifuged at 15,000 X*g* for 15 min, thereafter the homogenate was incubated 15 min at 37°C with subsequent recentrifugation (15 min, 15,000 X*g*). The buffer was discarded, the pellet was stored overnight at −28°C. On the next day, the pellet was resuspended in the incubation buffer using a Kinematica Polytron homogeniser, setting 5, 5 sec, the final concentration of the homogenate was adjusted to 15 mg wet weight tissue/ml which yielded ca 0.9—1.1 mg of protein/ml. The binding was performed on standard 96 hole microplates at room temperature in a total volume of 300 µl. [^{3}H]ketanserin was used in concentrations from 0.2 to 5 nM; 5 µM unlabeled ketanserin tartrate was used as displacer. The probes were measured in duplicate. The incubation (15 min, at room temperature) was terminated by rapid filtration through the 48-channel Brandell cell harvester (Whatman GF/B glass-fibre filters). The filters were washed five times with 0.5 ml 50 mM TrisHCl washing buffer and dried. The dried filters were left overnight in Wallac High Safe III scintillation cocktail and assayed in a Wallac β-scintillation counter.

Protein concentration was measured by the classic Lowry method (24).

Statistics

For statistical analysis of the results from behavioural experiments Mann-Whitney U test was used. The data obtained from biochemical experiments were subjected to one-way ANOVA, and when appropriate, for post hoc data comparison, Fisher's LSD test was used. The probability levels $p<0.05$ were always considered statistically significant.

RESULTS

Development of the apomorphine-induced aggressive behaviour

In total, 20 animals were included into this study. In sixteen of them apomorphine-induced aggressive behaviour developed, whereas the other four did not exhibit any signs of aggressiveness (*Fig. 1*). Other behavioural phenomena induced by apomorphine, i.e. stereotyped behaviour, motor hyperactivity *etc.* were similar in all animals. For the subsequent neurochemical analysis, four most aggressive animals from the apomorphine-induced aggressive group (intensity score 3.0), four non-aggressive as well as four vehicle-treated animals were chosen.

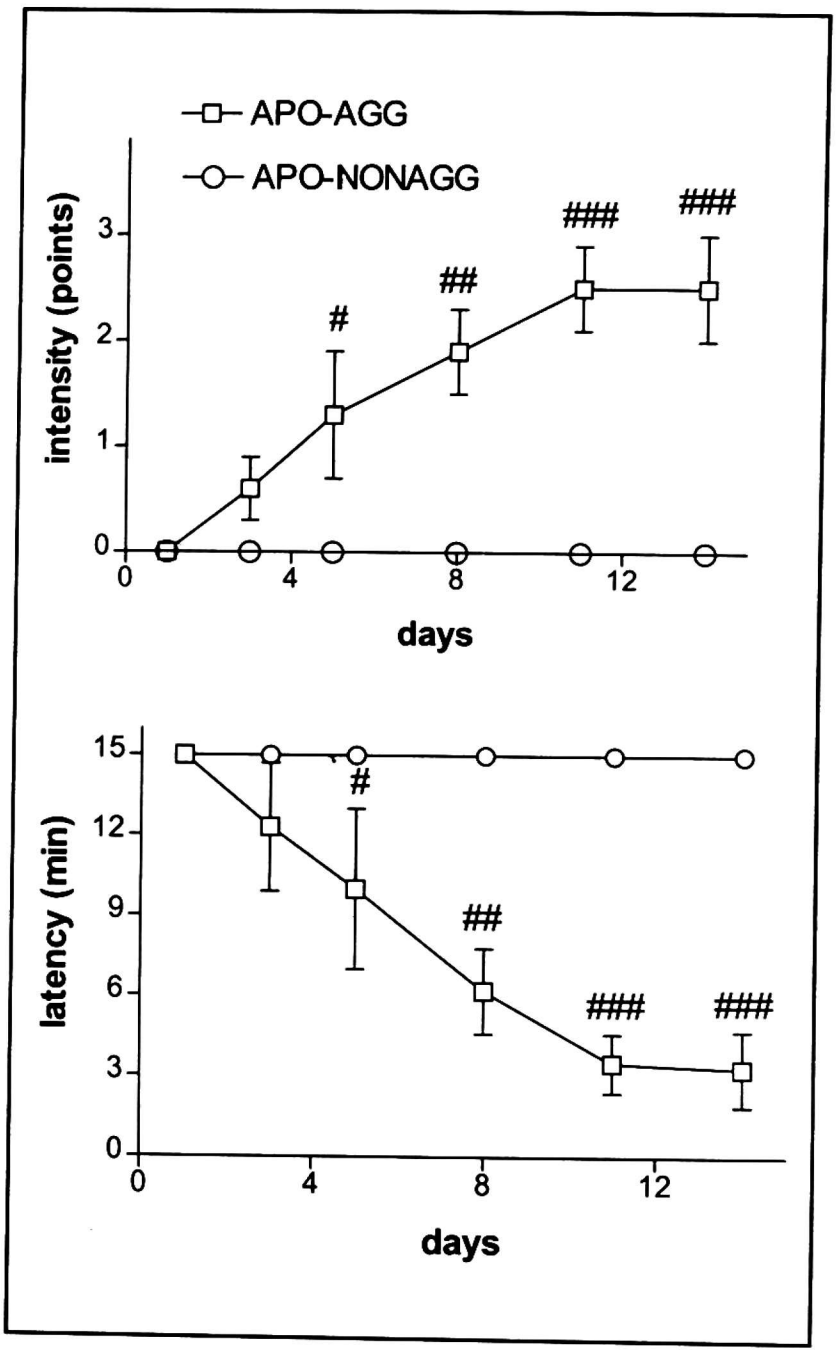

Fig. 1. Development of aggressive behaviour in apomorphine-treated male Wistar rats. All animals (n = 20) were retrospectively divided into apomorphine-treated nonaggressive (APO-NONAGG; n = 4) and aggressive (APO-AGG; n = 16) group. Data are expressed as means ± S.E.M. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ as compared with APO-NONAGG group (Mann-Whitney U test).

[^{3}H]ketanserin binding in rat brain of the apomorphine-induced aggressive, nonaggressive, and vehicle-treated animals

Repeated apomorphine treatment induced significant changes in the number of the maximal apparent binding sites (B_{max}) in frontal cortex, $F(2, 9) = 5.94$, $p < 0.05$ and cerebral cortex, $F(2,9) = 10.74$, $p < 0.01$ (*Fig. 2*). Applied Fisher's LSD test revealed a significant difference between the vehicle-treated group and apomorphine-induced aggressive or nonaggressive group, whereas there was no difference between the two latter groups. The K_d value vas unchanged in both regions. In the hippocampus and hypothalamus no changes either in the B_{max} or K_d value were found (*Table 1*).

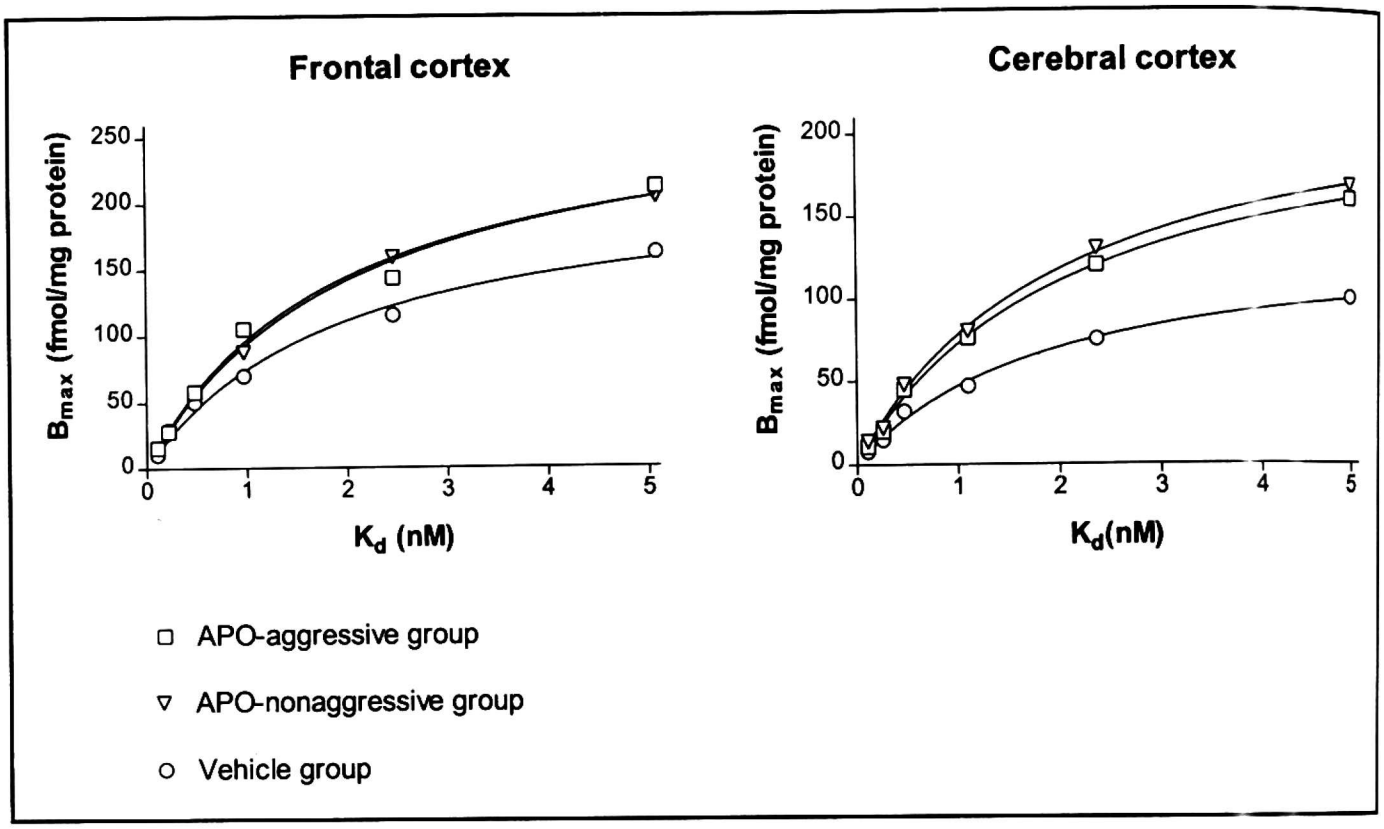

Fig. 2. Saturation curves of [^{3}H]ketanserin binding in the rat frontal cortex and cerebral cortex in apomorphine-induced aggressive, nonaggressive, and vehicle-treated rats. Each point presents the mean of duplicate determinations. Each binding curve is a randomly selected example from the respective group.

Table 1. 5-HT_{2A} receptor binding characteristics in the rat frontal cortex, cerebral cortex, hippocampus, and hypothalamus after repeated apomorphine administration

Group	B_{max} (fmol/mg protein)	K_d (nM)
Frontal cortex		
Vehicle group	233.9 ± 26.5	1.469 ± 0.239
Apomorphine-nonaggressive group	364.6 ± 31.7$^{\#}$	1.809 ± 0.263
Apomorphine-aggressive group	367.0 ± 34.8$^{\#}$	1.825 ± 0.319
Cerebral cortex		
Vehicle group	164.2 ± 6.7	1.891 ± 0.194
Apomorphine-nonaggressive group	289.7 ± 29.3$^{\#\#\#}$	2.001 ± 0.066
Apomorphine-aggressive group	249.0 ± 15.4$^{\#}$	1.880 ± 0.118
Hippocampus		
Vehicle group	101.3 ± 12.4	2.046 ± 0.284
Apomorphine-nonaggressive group	98.7 ± 14.5	1.991 ± 0.165
Apomorphine-aggressive group	95.9 ± 10.7	2.100 ± 0.297
Hypothalamus		
Vehicle group	82.6 ± 10.4	2.087 ± 0.187
Apomorphine-nonaggressive group	96.2 ± 8.6	2.145 ± 0.248
Apomorphine-aggressive group	94.9 ± 7.8	2.159 ± 0.024

All values are data (±S.E.M.) obtained from [^{3}H]ketanserin binding experiments, that were subjected to one-way ANOVA followed by Fisher's LSD test. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ drug treatment group vs. corresponding vehicle group, Fisher's LSD test after significant ANOVA.

DISCUSSION

The present study indicates, that the repeated administration of moderate doses of apomorphine is capable to induce aggressive behaviour. Similar findings have been done previously by Lang *et al.*, (15), who found that the aggressiveness develops gradually and starting from the eighth day of the experiment, most of the animals will be aggressive. In this regard, our study is in good agreement with the previous experiments (7, 8, 12—17). Furthermore, the finding that eighty per cent of the animals became aggressive is in exact line with our previous report where a similar study design was used (22). Thus, the apomorphine-induced aggressiveness experiments are fairly reproducible within one stock of animals. However, there should be born in mind that the aggressiveness in animals, likewise the human one, is a symptom that is dependent from several factors such as motivational, emotional, environmental, and phenotypical (1). Therefore, the stock-differences should always be considered in this context.

In the earlier works, the emphasis has been focused to the 5-HT_{1A} receptors. Thus, we have found that the 5-HT_{1A} receptor agonist buspirone blocks the apomorphine-induced aggressive behaviour at doses 2.5 and 5.0 mg/kg (7, 8). Sanchez *et al.*, (6), found that the isolation-induced aggressiveness in male mice may be mediated *via* the 5-HT_{1A} receptors. Finally, it has been reported that 5-HT_{1A} receptor expression in forebrain regions of aggressive house mice is enhanced (25). On the other hand, there is no much information available on the role of the 5-HT_{2A} receptors in the models of aggressive behaviour though this receptor subtype is also implicated to the aggressiveness. Our results indicate, that the repeated administration of apomorphine, that in itself does not have any affinity to the 5-HT_{2A} receptors, may upregulate the [^{3}H]ketanserin binding in the frontal cortex and cerebral cortex (in our experiments the cerebral cortex is defined as whole brain cortex minus frontal cortex), but has no effect on the other brain regions studied. However, it should be emphasised that though the 5-HT_{2A} receptors are presented in all brain regions, their distribution is unequal. Furthermore, in the frontal cortex and cerebral cortex the distribution of the 5-HT_{2A} receptors is quite constant, but in hippocampus the 5-HT_{2A} receptors have been found in high density in strata radiatum and oriens of the CA3 field. In other fields close to that, i.e. CA2 and CA4, the receptors have been found with much lower density (26). In hypothalamus, the density of the 5-HT_{2A} receptors is low, too (26). It can be speculated that even if the latter two brain regions have a role in the mediation of the aggressiveness, the [^{3}H]ketanserin binding method is not the tool of choice to elucidate such fine differences. As a consequence, it can be proposed that repeated apomorphine treatment upregulates the maximal number of the apparent [^{3}H]ketanserin binding sites only in frontal cortex and cerebral

cortex. This effect of apomorphine is evidently due to the dopamine D_1 receptors. This idea is evidenced by two facts. First, the serotonin terminals own D_1 receptors (27, 28) and secondly, our experiments demonstrate that there is no difference between the B_{max} value of the apomorphine-induced aggressive and nonaggressive animals. The apomorphine-induced aggressiveness, as assumed above, is mediated *via* the D_2 receptors while other behavioural phenomena such as hyperlocomotion, licking, and stereotyped body movements, are probably mediated *via* the D_1 receptors (29). Thus, the repeated administration of the moderate doses of apomorphine upregulates the cortical 5-HT_{2A} receptors whether or not the aggressiveness will develop. The apomorphine-induced aggressiveness seems to be mediated *via* other, yet not known, neurobiological pathways than a simple dopamine-serotonin interaction (30). Ketanserin administration has been reported to have only a weak effect on NMDA-receptor antagonist-precipitated monoaminergic hyperlocomotion (31) that provide further evidence that the link between the dopamine D_1 and D_2 and serotonin 5-HT_{2A} receptors is rather complicated [for a review regarding the human data, see (32)]. Thus, the intimate mechanism of this link remains unclear.

In conclusion, our experiments demonstrate that repeated apomorphine treatment upregulates the maximal number of the 5-HT_{2A} receptors in rat frontal and cerebral cortex as measured by [^{3}H]ketanserin binding.

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REFERENCES

1. Miczek KA. The psychopharmacology of aggression. In LL Iversen, SD Iversen, Snyder SH (eds). Handbook of psychopharmacology, Vol. 19. New directions in behavioral pharmacology. New York, Plenum Press, 1987, pp. 183—328.
2. Miczek KA, Weerts EM, Vivian JA, Barros HM. Aggression, anxiety and vocalisations in animals: $GABA_A$ and 5-HT anxiolytics. *Psychopharmacology* 1995; 121: 38—56.
3. Winslow JT, Miczek KA. Habituation of aggression in mice: Pharmacological evidence of catecholaminergic and serotoninergic mediation. *Psychopharmacology* 1983; 81: 286—291.
4. Delini-Stula A, Hunn C. Effects of single and repeated treatment with antidepressants on apomorphine-induced yawing in the rat: The implication of α-1 adrenergic mechanisms in the D—2 receptor function. *Psychopharmacology* 1990; 101: 62—66.
5. Sanchez C, Arnt J, Hyttel J, Moltzen EK. The role of serotoninergic mechanisms in inhibition of isolation-induced aggression in male mice. *Psychopharmacology* 1993; 110: 53—59.
6. Sanchez C, Hyttel J. Isolation-induced aggression in mice: effects of 5-hydroxytryptamine uptake inhibitors and involvement of postsynaptic 5-HT_{1A} receptors. *Eur J Pharmacol* 1994; 264: 241—247.

7. Matto V, Allikmets L, Skrebuhhova T. Apomorphine-induced aggressiveness and [^{3}H]citalopram binding after antidepressant treatment in rats. *Pharmacol Biochem Behav* 1998; 59: 747—752.
8. Matto V, Skrebuhhova T, Allikmets L. The effect of antidepressants on rat aggressive behaviour in the electrick footshock and apomorphine induced aggressiveness paradigms. *Methods Find Exp Clin Pharmacol* 1998; 20: 329—337.
9. Allison K, Paetsch PR, Baker GB, Greenshaw AJ. Chronic antidepressant drug treatment attenuates motor-suppressant effects of apomorphine without changing [^{3}H]GBR 12935 binding. *Eur J Pharmacol* 1993; 249: 125—131.
10. Pucilowski O, Kostowski W. Diltiazem suppresses apomorphine-induced fighting and pro-aggressive effect of withdrawal from chronic ethanol or haloperidol in rats. *Neurosci Lett* 1988; 93: 96—100.
11. Puglisi-Allegra S, Mack G, Oliverio A, Mandel P. Effects of apomorphine and sodium di-n-propylacetate on the aggressive behaviour of three strains of mice. *Prog Neuropsychopharmacol* 1979; 3: 491—502.
12. Allikmets LH, Stanley M, Gershon S. The effect of lithium on chronic haloperidol enhanced apomorphine aggression in rats. *Life Sci* 1979; 25: 165—170.
13. Allikmets LH, Vasar E. Sensitization of male rats to aggressive behavior. *Zh Vyssh Nerv Deiat* 1982; 32: 130—135.
14. Kostowski W, Valzelli L, Baiguerra G. Effect of chronic administration of alprazolam and adinazolam on clonidine- or apomorphine-induced aggression in laboratory rodents. *Neuropharmacology* 1986; 25: 757—761.
15. Lang A, Harro J, Soosaar A *et al.* Role of N-methyl-D-aspartic acid and cholecystokin receptors in apomorphine-induced aggressive behaviour in rats. *Naunyn-Schmiedeberg's Arch Pharmacol* 1995; 351: 363—370.
16. Lang A, Soosaar A, Koks S *et al.* Pharmacological comparison of antipsychotic drugs and sigma antagonists in rodents. *Pharmacol Toxicol* 1994; 75: 222—227.
17. Lang A, Vasar V, Soosaar A, Harro J. The involvement of sigma and phencyclidine receptors in the action of antipsycotic drugs. *Pharmacol Toxicol* 1992; 71: 131—138.
18. Nikulina EM, Kapralova NS. Role of dopamine receptors in the regulation of aggression in mice; relationship to genotype. *Zh Vyssh Nerv Deiat* 1991; 41: 734—740.
19. Puglisi-Allegra S, Mandel P. Effects of sodium n-dipropylacetate, muscimol hydrobromide and (R,S) nipecotic acid amide on isolation-induced aggressive behavior in mice. *Psychopharmacology* 1980; 70: 287—290.
20. Aghajanian GK. Central 5-HT receptor subtypes: Physiological responses and signal transduction mechanisms. In: Central serotonin receptors and psychotropic drugs. CA Marsden, DJ Heal (eds). Oxford, Blackwell Scientific Publications, 1992, pp. 39—55.
21. Glennon RA, Ducat M. Serotonin receptor subtypes. In: Psychopharmacology. The Fourth Generation of Progress. FE Bloom, DJ Kupfer (eds). New York, Raven Press, Ltd, 1995; pp. 1349—1360.
22. Matto V, Allikmets L. Apomorphine-induced aggressive and nonaggressive rats differ in [^{3}H]raclopride-sensitive D_2 receptor binding characteristics. *Med Sci Res* 1998; 26: 499—501.
23. Chaouloff F, Kulikov A, Mormede P. Repeated DOI and SR 43649B treatments do not affect elevated plus-maze anxiety despite opposite effects on cortical 5-HT_{2A} receptors. *Eur J Pharmacol* 1997; 334: 25—29.
24. Lowry OH, Rosebrough NJ, Farr AL, Randall RT. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265—275.
25. Mechiel Korte S, Meijer OC, de Kloet ER *et al.* Enhanced 5-HT_{1A} receptor expression in forebrain regions of aggressive house mice. *Brain Res* 1996; 736: 338—343.

26. Palacios JM, Waeber C, Mengod G, Pompeiano M. Molecular Neuroanatomy of 5-HT receptors. In Serotonin: Molecular Biology, Receptors and Functional Effects, JR Fozard, PR Saxena, (eds). Basel, Birkhäuser Verlag, 1991, pp. 5—20.
27. Whitaker-Azmitia PM, Azimitia EC. Serotonin trophic factors in development, plasticity and aging. In Serotonin: Molecular Biology, Receptors and Functional Effects, JR Fozard, PR Saxena, (eds). Basel, Birkhäuser Verlag, 1991, pp. 43—49.
28. Arnt J, Overo KF, Hyttel J, Olsen R. Changes in rat dopamine- and serotonin function in vivo after prolonged administration of the specific 5-HT uptake inhibitor, citalopram. *Psychopharmacology* 1984, 84: 457—465.
29. Rowlett JK, Mattingly BA, Bardo MT. Locomotor activity and dopamine synthesis following 1 and 15 days of withdrawal from repeated apomorphine treatments. *Pharmacol Biochem Behav* 1997; 57: 13—18.
30. Young KA, Zavodny R, Hicks PB. Effects of serotoninergic agents on apomorphine-induced locomotor activity. *Psychopharmacology* 1993; 110: 97—102.
31. Maj J, Rogoz Z, Skuza G, Wedzony K. The synergistic effect of fluoxetine on the locomotor hyperactivity induced by MK-801, a non-competitive NMDA receptor antagonist. *J Neural Transm* 1996; 103: 131—146.
32. Staley JK, Malison RT, Innis RB. Imaging of the serotoninergic system: Interactions of neuroanatomical and functional abnormalities of depression. *Biol Psychiatry* 1998; 44: 534—549.

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