Korean J Physiol Pharmacol
Vol 13: 147–151, June, 2009

Role of Dopamine Receptors on Electroencephalographic Changes Produced by Repetitive Apomorphine Treatments in Rats

Hwan Soo Jang[1,3,*], Ji Young Kim[1,*], Sang Heon Kim[2], and Maan-Gee Lee[1,3]

Departments of [1]Pharmacology and [2]Psychiatry, School of Medicine, [3]Brain Science and Engineering Institute, Kyungpook National University, Daegu 700-422, Korea

Repeated psychostimulants induce electroencephalographic (EEG) changes, which reflect adaptation of the neural substrate related to dopaminergic pathways. To study the role of dopamine receptors in EEG changes, we examined the effect of apomorphine, the dopamine D1 receptor antagonist, SCH-23390, and the D2 receptor antagonist, haloperidol, on EEG in rats. For single and repeated apomorphine treatment groups, the rats received saline or apomorphine for 4 days followed by a 3-day withdrawal period and then apomorphine (2.5 mg/kg, i.p.) challenge after pretreatment with saline, SCH-23390, or haloperidol on the day of the experiment. EEGs from the frontal and parietal cortices were recorded. On the frontal cortex, apomorphine decreased the power of all the frequency bands in the single treatment group, and increased the theta (4.5 ~ 8 Hz) and alpha (8 ~ 13 Hz) powers in the repeated treatment group. Changes in both groups were reversed to the control values by SCH-23390. On the parietal cortex, single apomorphine treatment decreased the power of some frequency bands, which were reversed by haloperidol but not by SCH-23390. Repeated apomorphine treatment did not produce significant changes in the power profile. These results show that adaptation of dopamine pathways by repeated apomorphine treatment could be identified with EEG changes such as increases in theta and alpha power of the frontal cortex, and this adaptation may occur through changes in the D1 receptor and/or the D2 receptor.

Key Words: Apomorphine, Dopamine D1 receptor, Dopamine D2 receptor, SCH-23390, Haloperidol, EEG, Rat

INTRODUCTION

Psychostimulants such as amphetamine and cocaine are widely abused drugs. On acute exposure they evoke hyperactivity and mind-altered states. On repeated exposure they enhance their locomotor stimulant effects and stereotypies, so called "behavioral sensitization" (Segal and Mandell, 1974; Post and Rose, 1976; Robinson and Becker, 1986; Vezina and Stewart, 1989; Damianopoulos and Carey, 1993; Stewart and Badiani, 1993). This phenomenon has been proposed to play a critical role in psychostimulant addiction (Robinson and Berridge, 1993; De Vries et al., 1998; Deroche et al., 1999; Robinson and Berridge, 2000).

Some studies demonstrate good correlations between some EEG activity and stereotyped behavior during sensitization and conditioning with the psychostimulants amphetamine (Ferger et al., 1994; Stahl et al., 1997) and cocaine (Ferger et al., 1996), and the dopamine receptor agonist apomorphine (Kropf et al., 1989; 1991). Electroencephalographic (EEG) spectral patterns and field potential are a sensitive tool for time-course studies of responses to different compounds acting on neurotransmitter systems (Ferger and Kuschinsky, 1995; Binienda et al., 2000). The sensitization is particularly associated with dopaminergic systems (Vanderschuren and Kalivas, 2000; Steketee, 2003), which can be evaluated with EEG activity. For example, repeated administration of psychostimulants specifically increases the power of the alpha-1 and alpha-2 bands (Ferger et al., 1996; Stahl et al., 1997).

To investigate whether the EEG changes after repeated stimulation of dopaminergic pathways measure adaptive changes in dopaminergic pathways, we used the dopaminergic receptor agonist apomorphine for the stimulation and selective dopamine D1 and D2 receptor antagonists, SCH 23390 and haloperidol, respectively for differential block of the adaptational changes.

Received March 18, 2009, Revised April 10, 2009,
Accepted April 22, 2009

Corresponding to: Maan-Gee Lee, Department of Pharmacology, School of Medicine, Kyungpook National University, 2-101, Dongin-dong, Jung-gu, Daegu 700-422, Korea. (Tel) 82-53-420-4832, (Fax) 82-53-426-4703, (E-mail) mglee@knu.ac.kr
*These authors contributed equally to this work.

METHODS

Animals and surgery

Thirty male Sprague-Dawley rats (Hyochang Science, Daegu, Korea) weighing 280 ~ 300 g on the day of surgery were used in this study. The animals were kept in 12 h

ABBREVIATIONS: APO, apomorphine; EEG, electroencephalogram; HAL, haloperidol; SAL, saline; SCH, SCH-23390.

light-dark cycles (light on from 07:00 to 19:00) and housed individually in stainless steel cages (23×40×18 cm). The ambient temperature was maintained at 21~25°C. Except during experiments, standard laboratory pellet food and tab water were available ad libitum. The experiments followed the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2003).

Epidural screw electrodes for EEG recording were implanted in the rats under anesthesia with a cocktail of ketamine and xylazine (37.5 mg ketamine and 1.9 mg xylazine in 1 ml saline, 2 ml/kg i.p.). The rat was fixed in a stereotaxic apparatus. The scalp was locally anesthetized with a subcutaneous injection of a mixture of 2% lidocaine and epinephrine. Then, the midline of the scalp was incised, the periosteum was removed, and the skull surface was cleaned, after which 6 small holes were drilled into the frontal bones (2.5 mm anterior to bregma and 2.5 mm lateral to midline), the parietal bones (2.5 mm posterior to bregma and 2.5 mm lateral to midline) bilaterally, and the interparietal bone (2.5 mm posterior to lambda and 2.5 mm lateral to midline), without perforating the dura. Gold-plated stainless steel screws (tip diameter 1.2 mm) were inserted into the holes. Two screw electrodes over the cerebellum (on the interparietal bones) served as reference and ground electrodes. Pins connected to screw electrodes with enamel-coated copper wires were arranged together in 3×2 matrices and fixed over the skull with dental acrylic.

Drugs

SCH-23390 HCl and apomorphine HCl (Research Biochem., Natick, MA) were dissolved in physiological saline. Haloperidol (Research Biochem., Natick, MA) was dissolved with a small amount of 0.1 N HCl and then the solution was diluted with physiological saline. The concentration of each drug solution was adjusted so that the volume injected was 1 ml/kg body weight. All drugs were administered i.p.

Experimental procedures

The animals were allowed to recover from surgery for at least one week. The animals were divided into either single treatment (S: n=15) or repeated treatment (R: n=15) groups. In the S group, saline (1 ml/kg) injection was repeated once a day for 4 days followed by a 3-day withdrawal period before the apomorphine challenge (2.5 mg/kg). In the R group, apomorphine (2.5 mg/kg) injection was repeated once a day for 4 days followed by a 3-day withdrawal period before the apomorphine challenge. High doses of apomorphine produce behavioral sensitization (Castro et al., 1985; Braga et al., 2008). Each group was subdivided into 3 groups by pretreatment drugs on the day of the experiment: (1) 1 ml/kg of saline (SAL(S): n=5; SAL(R): n=5); (2) 0.04 mg/kg of SCH-23390 (SCH(S): n=5; SCH(R): n=5); and (3) 0.5 mg/kg of haloperidol (HAL(S): n=5; HAL(R): n=5).

On the day of the apomorphine challenge, the animals were put on a Plexiglas cage (28 cmW×42 cmD×18 cmH) and connected with a swivel system. The rats were acclimated to the recording setup in the recording chamber for about 30 min, followed by EEG recording. Control recording for 30 min was followed by injection of a pretreatment drug and subsequent recording for 30 min, and finally by apomorphine challenge and recording for 60 min.

EEG measurements

Four-channel EEG signals from the left and right frontal and parietal cortices were recorded monopolarly with respect to the reference electrode via a bioelectric amplifier (Model 1700, A-M system, Inc., USA; CyberAmp 380, Axon instruments Inc., USA). Signals were amplified 10,000× and filtered with a range of 1 to 60 Hz. They were sampled by an AD converter (DigiData 1200A, Axon instruments Inc., USA) at a sampling rate of 200 Hz. Digitized data were stored into hard disks and analyzed off-line.

Raw EEG signals were inspected prior to the analysis, and only the artifact-free signals of a 5-min period immediately before each injection and those of a 5-min period from 25 to 30 min (APO30) and 55 to 60 min (APO60) after apomorphine challenge injection were used to calculate power spectra. Signals were converted to power spectra by the fast Fourier transform algorithm and the power spectra of 4-sec sweeps in a 5-min period were averaged to give the power spectra of a 5-min period by customized Matlab programs (Matlab R11 version 5.3, The MathWorks, USA). The power spectra were divided into 7 frequency bands: Delta1, 1~2.5 Hz; Delta2, 2.5~4.5 Hz; Theta, 4.5~8 Hz; Alpha, 8~13 Hz; Beta1, 13~20 Hz; Beta2, 20~30 Hz; and Gamma, 30~50 Hz. The log transformed values of the powers are given in figures and used for statistical analysis.

Statistical analysis

We analyzed unilateral EEG signals from the one frontal and one parietal cortices because bilateral power spectra are similar. All data are expressed as mean±S.E.M. Statistical analysis for control, single and repeated treatments, or among different pretreatments was performed with one-way ANOVA and Dunnett's t-tests (SPSS 12.0K, Datasolution, Korea), and $p<0.05$ was considered statistically significant.

RESULTS

EEG of the frontal cortex

We first tested the effects of apomorphine (2.5 mg/kg, i.p.) challenge on EEG band powers in the frontal cortex of the S and R groups (Fig. 1). Apomorphine significantly decreased the powers of Delta1, Delta2, Beta1, and Beta2 in the S group, but increased Alpha power in the R group compared to controls. Band power profiles produced by apomorphine were similar 30 min and 60 min after administration, though the magnitude decreased with time. The Theta and Alpha powers were significantly higher in the R group than in the S group.

We then examined effects of the dopamine antagonists, SCH-23390 and haloperidol, on the power changes produced by apomorphine challenge (Fig. 2). SCH-23390 blocked the decreased power in Delta1, Delta2, Beta1, and Beta2 bands in the S group and reduced the increased power in the Theta and Alpha bands in the R group, as well as decreasing Delta2 and Beta1 power bands in the R group compared to controls (Fig. 2A and 2B). Haloperidol reversed the decrease in the powers of Delta1, Delta2, Beta1, and Beta2 bands by apomorphine and increased the power of Delta1 band in the S group above controls (Fig. 2C).

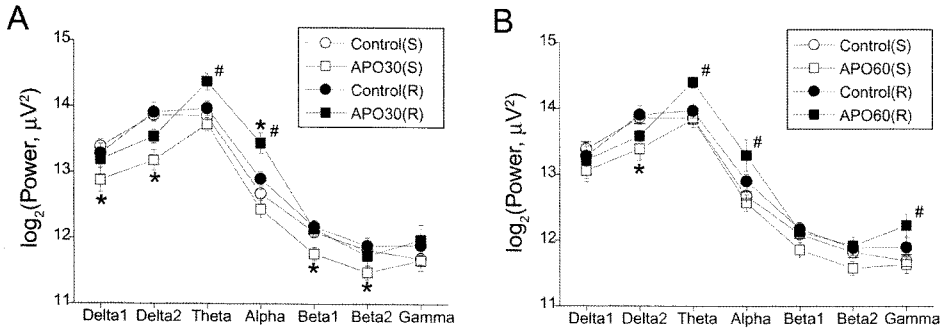
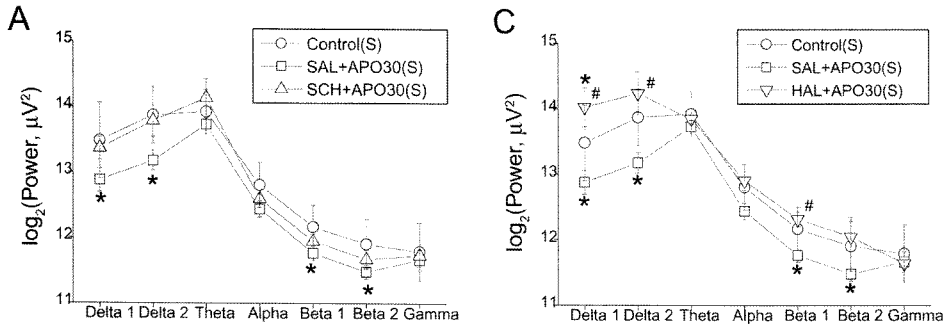


Fig. 1. EEG band powers of the frontal cortex after apomorphine (APO) treatment in single (S) and repeated (R) apomorphine treatment groups. (A) Band powers in the control state and 25~30 min after APO injection; (B) Band powers in the control state and 55~60 min after APO injection. Each point and bar represent the mean±S.E.M. *Significantly different from the control state; #Significantly different between R and S groups ($p<0.05$ by ANOVA, t-test).



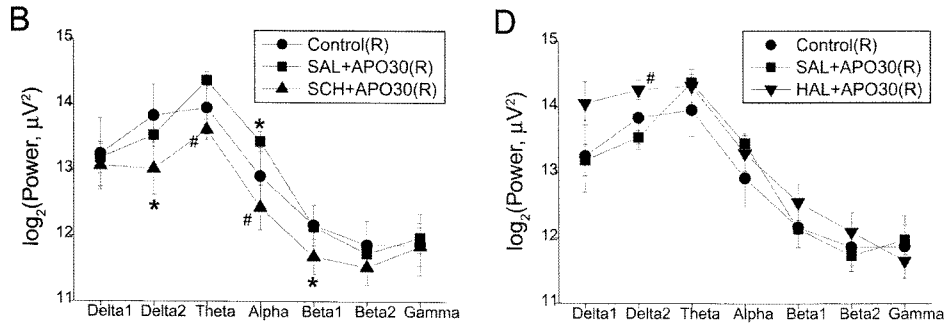


Fig. 2. EEG band powers of the frontal cortex 25~30 min after apomorphine (APO) injection in single (S, upper panel, A and C) and repeated (R, lower panel, B and D) treatment groups pretreated with: SCH-2390 (SCH, A and B) and haloperidol (HAL, C and D). Each point and bar represent the mean±S.E.M. *Significantly different from the control state; #Significantly different from saline (SAL) pretreatment ($p<0.05$ by ANOVA, t-test).

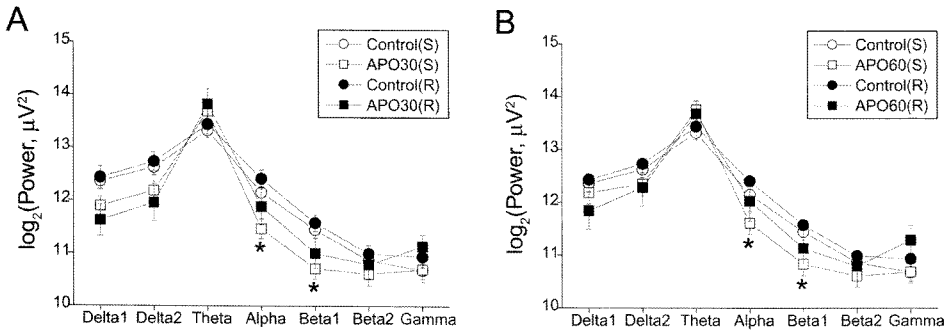


Fig. 3. EEG band powers of the parietal cortex after apomorphine (APO) treatment in single (S) and repeated (R) apomorphine treatment groups. (A) Band powers in the control state and 25~30 min after APO injection; (B) Band powers in the control state and 55~60 min after APO injection. Each point and bar represent the mean±S.E.M. *Significantly different from the control state ($p<0.05$ by ANOVA, t-test).

Haloperidol significantly increased the power of the Delta2 band in the R group over the S group (Fig. 2D).

EEG of the parietal cortex

We similarly tested the effects of apomorphine challenge on EEG band powers in the parietal cortex (Fig. 3). Apomorphine only decreased the powers of Alpha and Beta1 bands, but Delta1 and Delta2 bands, in the S group. Apomorphine did not increase the power of Alpha band of the parietal cortex in the R group. SCH-23390 did not affect apomorphine-induced changes in the S and R groups (Fig. 4A and 4B), but haloperidol reversed all apomorphine-induced decreases in the powers of Alpha and Beta1 bands in the S group and increased further the power of the Delta bands (Fig. 4C). Haloperidol reversed apomor-

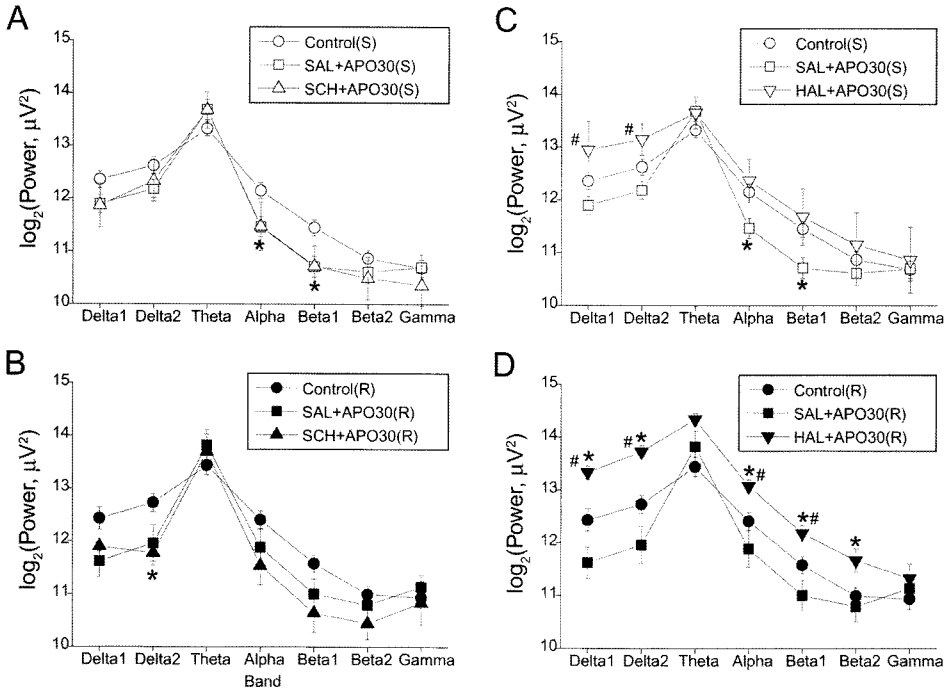


Fig. 4. EEG band powers of the parietal cortex 25~30 min after apomorphine (APO) injection in single (S, upper panel, A and C) and repeated (R, lower panel, B and D) treatment groups pretreated with: SCH-2390 (SCH, A and B) and haloperidol (HAL, C and D). Each point and bar represent the mean±S.E.M. *Significantly different from the control state; #Significantly different from saline (SAL) pretreatment ($p<0.05$ by ANOVA, t-test).

phine-induced power decreases and further increased the powers of all frequency bands except Gamma over controls in the R group (Fig. 4D).

DISCUSSION

Chronic apomorphine treatment caused different changes than acute treatment in the EEG band power profile of the frontal cortex, but not the parietal cortex. These frontal cortex changes could be reversed by the dopamine D1 receptor antagonist, SCH-23390, but not by the dopamine D2 receptor antagonist, haloperidol. In the parietal cortex, haloperidol decreased the power of all frequency bands but SCH-23390 had no effect.

Acute apomorphine treatment decreased the power of all frequency bands in the frontal cortex, whereas chronic treatment increased Theta (4.5~8 Hz) and Alpha (8~13 Hz) bands. These results are similar to changes induced by 0.6 mg/kg amphetamine (Stahl et al., 1997) and 20 mg/kg cocaine (Ferger et al., 1996). Lower doses of amphetamine or cocaine do not cause changes in either acute or chronic treatment (Ferger et al., 1996; Stahl et al., 1997), indicating that neural adaptation requires strong stimulation of dopamine receptors.

SCH-23390 could block the acute apomorphine-induced decreases in band power, as shown with amphetamine (Ferger et al., 1994), and decrease the theta and alpha powers below even control levels. Apomorphine directly activates dopamine receptors, whereas amphetamine and cocaine act indirectly on dopamine release or other receptor pathways. Chronic apomorphine treatment may increase the contribution of D1 receptors through adaptation. Haloperidol could also block acute apomorphine activity but not after chronic exposure, indicating a diminishing D2 receptor component. However, activation of D2 receptors after acute and/or repeated administration of (large doses of) psychostimulants leads to the activation of alpha1 band (7.0~9.5 Hz) and alpha2 band (9.75~12.5 Hz) that overlap with Alpha (8~13 Hz) in this study (Kropf et al., 1989; Kropf and Kuschinsky, 1993; Ferger et al., 1994; Ferger and Kuschinsky, 1995; Ferger et al., 1996; Stahl et al., 1997). Delta1 and Delta2 band powers showed consistent increases unrelated to apomorphine treatment. Haloperidol increased power in the Delta bands, which may be associatedwith sedative effect (Kwon et al., 2005), but did not affect adaptation to apomorphine treatment.

Here, chronic dopamine stimulation increased Theta and Alpha power. Conversely, chronic dopamine hypostimulation in rats with a 6-hydroxydopamine lesion of the medial forebrain bundle showed notable decreases in theta and alpha bands and an increase in the fast wave band power (Vorobyov et al., 2003). Therefore, changes in Theta and Alpha activities may reflect adaptation of dopamine neurotransmission.

Acute apomorphine administration decreased the power of some frequency bands in the parietal cortex, which could be reversed by the D2 receptor antagonist, but chronic treatment did not. The frontal cortex, particularly the prefrontal cortex, has more afferents from the mesocortical dopamine pathway than the parietal cortex. Adaptation may not occur in the parietal cortex because of insufficient dopaminergic innervation.

Dopamine receptors, particularl D1 receptors, influence behavioral sensitization (Castner and Williams, 2007). Here, a D1 antagonist could block the changes in theta and alpha bands induced by chronic apomorphine. However, acute apomorphine decreased the same band powers that were increased by chronic treatment, whereas behavioral sensitization such as hyperlocomotion and stereotypy occurs after acute apomorphine treatment and is potentiated by repeated apomorphine treatment. These band power

changes may still reflect changes in dopamine neurotransmission, as apomorphine challenge was required to change band power profiles in both naïve rats and apomorphine-treated rats. This finding suggests that the resting state is similar in both groups, but stimulation of dopaminergic activity reveals the altered neural substrate through abnormal behavior and EEG changes.

In conclusion, repeated apomorphine administration could change EEG band power as a reflection of dopaminergic adaptation with or without sensitization. These changes included increases of Theta and Alpha power, as well as behavioral changes, from hyperstimulation of the D1 receptor and/or the D2 receptor in the mesolimbic-mesocortical dopaminergic pathway. The results suggest that stimulating dopamine neurotransmission reveals the underlying alterations in function that occur in addiction to psychostimulants.

ACKNOWLEDGEMENTS

This work was supported by Kyungpook National University Research Fund (2008) and the Brain Korea 21 Project.

REFERENCES

Binienda Z, Beaudoin MA, Thorn BT, Sadovova N, Skinner RD, Slikker W, Jr., Ali SF. Application of electrophysiological method to study interactions between ibogaine and cocaine. *Ann N Y Acad Sci* 914: 387−393, 2000.

Castner SA, Williams GV. From vice to virtue: insights from sensitization in the nonhuman primate. *Prog Neuropsychopharmacol Biol Psychiatry* 31: 1572−1592, 2007.

Damianopoulos EN, Carey RJ. Apomorphine sensitization effects: evidence for environmentally contingent behavioral reorganization processes. *Pharmacol Biochem Behav* 45: 655−663, 1993.

De Vries TJ, Schoffelmeer AN, Binnekade R, Mulder AH, Vanderschuren LJ. Drug-induced reinstatement of heroin- and cocaine-seeking behaviour following long-term extinction is associated with expression of behavioural sensitization. *Eur J Neurosci* 10: 3565−3571, 1998.

Deroche V, Le Moal M, Piazza PV. Cocaine self-administration increases the incentive motivational properties of the drug in rats. *Eur J Neurosci* 11: 2731−2736, 1999.

Ferger B, Kropf W, Kuschinsky K. Studies on electroencephalogram (EEG) in rats suggest that moderate doses of cocaine or d-amphetamine activate D1 rather than D2 receptors. *Psychopharmacology (Berl)* 114: 297−308, 1994.

Ferger B, Kuschinsky K. Effects of morphine on EEG in rats and their possible relations to hypo- and hyperkinesia. *Psychopharmacology (Berl)* 117: 200−207, 1995.

Ferger B, Stahl D, Kuschinsky K. Effects of cocaine on the EEG power spectrum of rats are significantly altered after its repeated administration: do they reflect sensitization phenomena? *Naunyn Schmiedebergs Arch Pharmacol* 353: 545−551, 1996.

Kropf W, Kuschinsky K. Effects of stimulation of dopamine D1 receptors on the cortical EEG in rats: different influences by a blockade of D2 receptors and by an activation of putative dopamine autoreceptors. *Neuropharmacology* 32: 493−500, 1993.

Kropf W, Kuschinsky K, Krieglstein J. Apomorphine-induced alterations in cortical EEG activity of rats. Involvement of D-1 and D-2 dopamine receptors. *Naunyn Schmiedebergs Arch Pharmacol* 340: 718−725, 1989.

Kropf W, Kuschinsky K, Krieglstein J. Conditioning of apomorphine effects: simultaneous analysis of the alterations in cortical electroencephalogram and behaviour. *Naunyn Schmiedebergs Arch Pharmacol* 343: 559−567, 1991.

Kwon JS, Kim KM, Chang SU, Kim CY, Chung TH, Choi BJ, Lee MG. Differential effects of typical and atypical antipsychotics on MK-801 induced EEG changes in rats. *Korean J Physiol Pharmacol* 9: 17−22, 2005.

Post RM, Rose H. Increasing effects of repetitive cocaine administration in the rat. *Nature* 260: 731−732, 1976.

Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Res* 396: 157−198, 1986.

Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev* 18: 247−291, 1993.

Robinson TE, Berridge KC. The psychology and neurobiology of addiction: an incentive-sensitization view. *Addiction* 95 Suppl 2: S91−117, 2000.

Segal DS, Mandell AJ. Long-term administration of d-amphetamine: progressive augmentation of motor activity and stereotypy. *Pharmacol Biochem Behav* 2: 249−255, 1974.

Stahl D, Ferger B, Kuschinsky K. Sensitization to d-amphetamine after its repeated administration: evidence in EEG and behaviour. *Naunyn Schmiedebergs Arch Pharmacol* 356: 335−340, 1997.

Steketee JD. Neurotransmitter systems of the medial prefrontal cortex: potential role in sensitization to psychostimulants. *Brain Res Brain Res Rev* 41: 203−228, 2003.

Stewart J, Badiani A. Tolerance and sensitization to the behavioral effects of drugs. *Behav Pharmacol* 4: 289−312, 1993.

Vanderschuren LJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology (Berl)* 151: 99−120, 2000.

Vezina P, Stewart J. The effect of dopamine receptor blockade on the development of sensitization to the locomotor activating effects of amphetamine and morphine. *Brain Res* 499: 108−120, 1989.

Vorobyov VV, Schibaev NV, Morelli M, Carta AR. EEG modifications in the cortex and striatum after dopaminergic priming in the 6-hydroxydopamine rat model of Parkinson's disease. *Brain Res* 972: 177−185, 2003.