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Central Levels of Noradrenaline, 3-Methoxy-4-hydroxyphenylethyleneglycol and Cyclic AMP in the Rat After Activation of *Locus coeruleus* Neurons: Influence of Single and Repeated Neuroleptic Treatment

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Abstract. The influence of single and repeated neuroleptic treatment on noradrenaline (NA) metabolism in two areas of the central nervous system with different neuronal organizations (forebrain and spinal cord) was studied. The doses of the neuroleptics studied were chosen because of their maximum effects on turnover of dopamine in various brain areas.

The endogenous levels of 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG) in the forebrain and spinal cord of the rat were markedly increased by single intraperitoneal doses of clozapine (15 mg/kg), haloperidol (1.0 mg/kg) or chlorpromazine (2.0 mg/kg). The levels of noradrenaline (NA) in the hippocampus were decreased by a single dose of clozapine and haloperidol. Fluphenazine (0.1 mg/kg) and sulpiride (40 mg/kg) caused only slight increases of MOPEG in the forebrain and none in the spinal cord. Following repeated treatment with either clozapine or haloperidol tolerance to the stimulatory effects on NA turnover developed more rapidly in the spinal cord than in the forebrain (within 4 and 15 days respectively). After 4 days of repeated treatment the initial decrease in hippocampal NA levels had disappeared.

Unilateral electrical stimulation of the *locus coeruleus* (LC) after a single dose of either clozapine or haloperidol induced smaller reductions of hippocampal NA (ipsilateral versus contralateral) than in saline treated control animals. In subchronically clozapine or haloperidol treated rats, LC-stimulation induced an ipsilateral decrease of NA similar to that in controls. The levels of MOPEG after LC-stimulation were elevated compared to untreated stimulated rats both in the ipsilateral and contralateral forebrain.

Neither single nor repeated treatment with clozapine or haloperidol altered basal cyclic AMP levels or inhibited the cyclic AMP response to LC-stimulation.

This study is evidence: (1) that neuroleptics decrease NA by release of the amine from a rapidly releasable pool (2) that even when the influence of subchronic neuroleptic treatment on cerebral NA metabolism has ceased, such treatment has a lasting influence on NA-neurons (3) that in vivo the formation of cyclic AMP is not influenced by neuroleptic treatment.

Key words: Noradrenaline – 3-Methoxy-4-Hydroxyphenylethyleneglycol – Cyclic AMP – *Locus coeruleus* – Neuroleptic treatment – Clozapine – Haloperidol

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Various neuroleptic drugs affect neurotransmission mediated by noradrenaline (NA), either at the presynaptic or postsynaptic level (see Carlsson 1978). Thus the inhibitory effects of NA and stimulation of the *locus coeruleus* (LC) on cerebellar Purkinje cells were antagonized by simultaneous iontophoretical or parenteral administration of antipsychotic drugs (Freedman and Hoffer 1975; Freedman 1977). Intraperitoneal administration of the neuroleptic drug clozapine significantly enhanced the spontaneous activity of NA neurons in the LC (Souto et al. 1979).

In mouse and rat brain, utilization and turnover of NA and the formation of its metabolite, normetanephrine, and 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG e.g. Schanberg et al. 1968) were accelerated by various neuroleptic compounds (Carlsson and Lindqvist 1963; Keller et al. 1973; Bartholini et al. 1973; Berridge and Sharman 1974; Bürki et al. 1975; Hyttel 1975; Swahn and Wiesel 1976; McMillen and Shore 1978; Adèr and Korf 1979). These effects are

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presumably due to a blockade of central NA-receptors (Carlsson and Lindqvist 1963; Andén et al. 1970).

In agreement with the view that at least some of the central NA-receptors are linked to an adenylate cyclase system (Blumberg et al. 1976) the in vitro induction of cyclic AMP by NA in brain slices and homogenates is inhibited by antipsychotic drugs (Palmer et al. 1971, 1972; Uzunov and Weiss 1971; Palmer and Manian 1974; Blumberg et al. 1975, 1976; Horn and Phillipson 1976). Whether these in vitro effects are related to an impairment of NA-receptor efficacy in vivo is open to question.

Prolonged treatments with neuroleptics are often prescribed, so that the question arises as to whether acute and chronic treatment with neuroleptics have similar effects upon NA-neurotransmission in the brain. This is of interest because some actions of neuroleptics (e.g. sedation and extrapyramidal side-effects) cease upon prolonged treatment (see Carlsson 1978). The biochemical effects of repeated administration of neuroleptics on NA-synthesis and turnover have been the subject of only a relatively small number of studies. The stimulation of NA turnover in the rat brainstem by various neuroleptics diminished within seven days upon repeated administration (Bürki et al. 1974). In mouse brain, endogenous levels and synthesis of NA rapidly returned to normal upon continuous exposure to teflutixol either by daily dosage or by a depot preparation (Hyttel 1975).

These results suggest that the effects of neuroleptics on cerebral NA-neurotransmission may only be transient at least when the LC-neurons are in a relatively low state of activity.

We now report the effects of single and repeated administration of neuroleptics on noradrenergic mechanisms in vivo in the rat. To do this we measured the levels of MOPEG and cyclic AMP after stimulation of the LC. Free MOPEG is a metabolite of NA in the rat brain which is markedly increased by electrical stimulation of the LC (Adèr et al. 1978). Recently Korf and Sebens (1979) described a biochemical approach to the study of cerebral NA-neurotransmission in vivo. Electrical stimulation of the LC was found to produce an activation of the noradrenergic cyclic AMP generating system in the rat cerebral cortex. This technique allows study of the interference of psychotropic drugs with the efficiency of cerebral NA-neurotransmission in vivo as far as this is mediated by cyclic AMP (Korf et al. 1979).

Materials and Methods

Stimulation

Male albino Wistar rats (170–200 g weight, TNO, Zeist, The Netherlands) were anaesthetized with chloral hydrate (400 mg/kg, IP) and placed in a Kopf stereotaxic instrument (nose bar 2.5 mm beneath the interaural line). Monopolar stainless steel electrodes (diameter 0.2 mm, exposed tip 1 mm) were stereotaxically placed in the *locus coeruleus* as previously described (Adèr et al. 1979). Maximal formation of MOPEG in the brain was induced by stimulation of the LC by passing 300 μA of current for 4 min at a frequency of 30 Hz through the electrodes (Korf et al. 1973a). The animals were decapitated 5 min after stimulation. Maximal elevations of cyclic AMP in the cerebral cortex were obtained by application of biphasic pulses (2 ms duration; current 500 μA; frequency 50 Hz) for 15 s (Korf and Sebens 1979). Immediately after stimulation the rats were removed from the stereotaxic apparatus and killed by microwave exposure for 8 s in a Litton 70/50 microwave oven with a focussed output of 2 kW at a frequency of 2.45 gHz.

Biochemical Assays

Cyclic AMP. Frontal parts of the cerebral cortex (30–60 mg tissue) were dissected, frozen on dry ice, weighed and stored at −80°C. The assay used has been described by Korf and Sebens (1979). Briefly: tissue was homogenized in 7% trichloracetic acid (TCA). After centrifugation TCA was removed by ether extraction. The nucleotide was isolated from the aqueous solution (buffered with 50 mM Tris + 4 mM EDTA, pH 7.5) on BioRad AG50W × 4. Part of the eluate (in water) was dried at 40°C under a stream of air. The dry residue was dissolved in 100 μl Trismaleate buffer pH 7.4. The nucleotide was determined with a protein binding method (Dinnendahl 1974; Korf and Sebens 1979).

NA and MOPEG. Brain (without cerebellum and brainstem) and the whole spinal cord were removed. Both the left and right hippocampus were dissected (60–90 mg tissue) and the rest of the brain was divided into two halves by a sagittal section. The tissue samples were immediately frozen on dry ice, weighed and stored at −80°C. NA in the hippocampus was isolated on Sephadex G10 and determined in a continuous flow analysis system equipped with a filter fluorometer (Westerink and Korf 1977). MOPEG in the rest of the brain and spinal cord was determined in a similar analysis system after isolation by successive chromatography on Sephadex G10 and DEAE Sephadex A25 in the borate form (Adèr and Korf 1979).

Drugs and Statistics

The following drugs and doses were used for either single or multiple IP injections: clozapine (Sandoz, 15 mg/kg) was dissolved in a drop of acetic acid and diluted with saline (0.9%). Haloperidol was obtained as a commercially prepared solution (Janssen Pharmaceutica, 1.0 mg/kg). Chlorpromazine (Specia, 2.0 mg/kg), Sulpiride (Delagrange, 40 mg/kg) and Fluphenazine (Labaz, 0.1 mg/kg) were dissolved in physiological saline.

These dosages produce maximal increases in the metabolism of dopamine in various brain areas (e.g. Westerink et al. 1977).

Subchronic treatment consisted of 15 consecutive daily doses. Stimulation procedures and killing were performed 2 h after a single or last daily injection with the respective neuroleptic drug.

Control rats received saline during the whole experiment. Neither acute nor subchronic treatment with saline altered the endogenous levels of NA and MOPEG and the response of MOPEG and cyclic AMP upon *locus coeruleus* stimulation. The significances of the differences of the data were computed with the two-tailed non-parametric Mann-Whitney *U*-test. The significance of the differences between ipsilateral and contralateral levels of cyclic AMP in the cerebral cortex was calculated with the Wilcoxon matched pairs signed ranks test.

Results

A single dose of either clozapine or haloperidol or chlorpromazine increased endogenous levels of

MOPEG in forebrain and spinal cord (Table 1). Fluphenazine and sulpiride at the doses used caused only slight increases of MOPEG in the forebrain and none in the spinal cord. Clozapine and haloperidol were selected to test whether after repeated administration tolerance to the stimulatory effects upon NA-metabolism would develop. Such tolerance was dem-

Table 1. Concentration of 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG) in rat forebrain and spinal cord 2 h after IP injection of various neuroleptic compounds

Compound	MOPEG (ng/g wet weight tissue ± SEM)				
	N	Forebrain	*P*	Spinal cord	*P*
Saline	9	19.6 ± 1.0		32.4 ± 1.9	
Clozapine	5	36.1 ± 3.3	< 0.01	67.6 ± 7.6	< 0.01
Haloperidol	5	28.1 ± 2.0	< 0.01	53.7 ± 6.1	< 0.01
Chlorpromazine	5	26.3 ± 2.6	< 0.03	46.7 ± 7.8	< 0.01
Fluphenazine	5	22.1 ± 1.0	< 0.03	27.4 ± 2.3	NS
Sulpiride	5	21.7 ± 2.4	< 0.05	23.3 ± 1.4	NS

N: number of animals
P values (versus saline) calculated with the Mann-Whitney *U*-test
Doses are mentioned in Materials and Methods

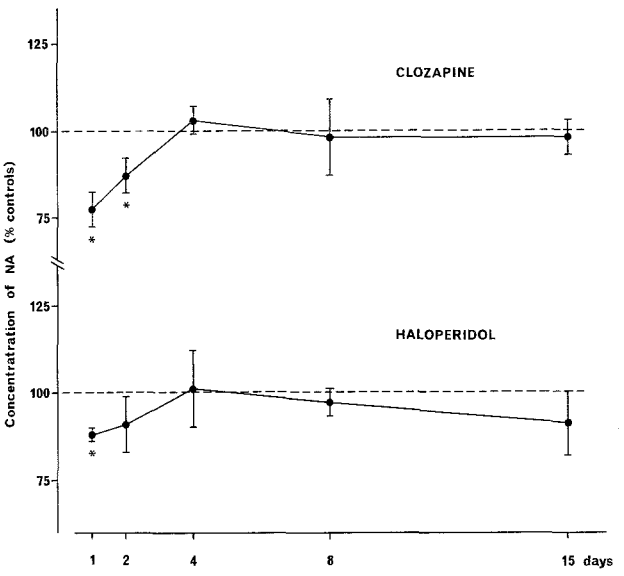

Fig. 2. Concentration of noradrenaline (*NA*) in rat hippocampus (% of controls) 2 h after the last daily injection of clozapine (15 mg/kg, IP) or haloperidol (1.0 mg/kg, IP). $*P < 0.05$ (Mann-Whitney *U*-test). Control value of *NA*: 297 ± 13 ng/g wet weight tissue ± SEM ($N = 30$)

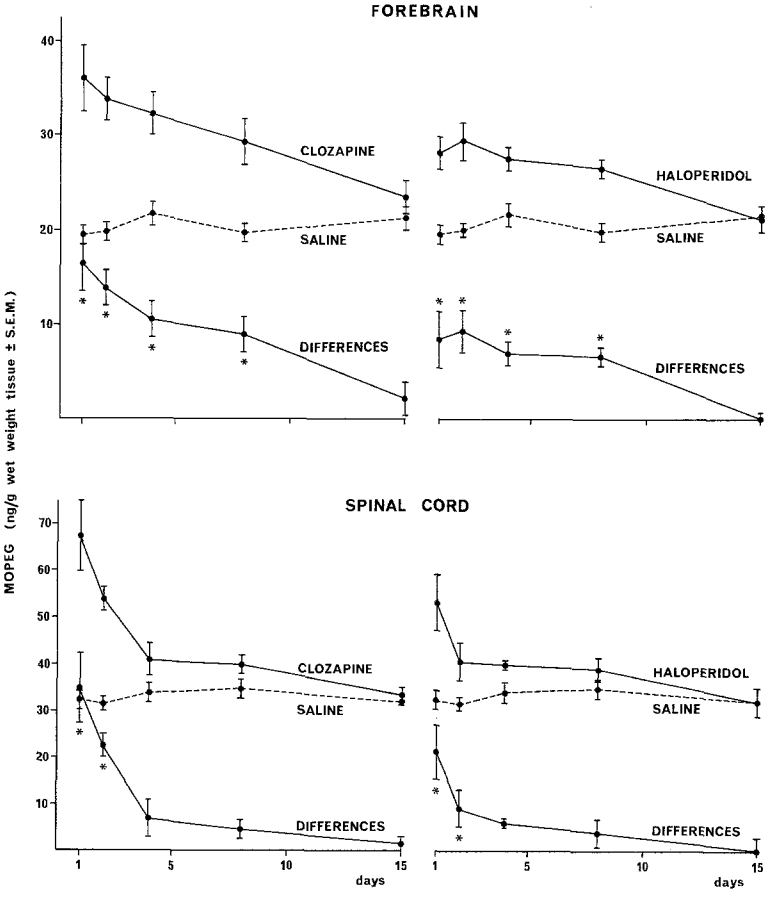

Fig. 1
Concentration of 3-methoxy-4-hydroxy-phenylethyleneglycol (*MOPEG*) in rat forebrain and spinal cord 2 h after the last daily injection of clozapine (15 mg/kg, IP), haloperidol (1.0 mg/kg, IP) or saline. $*P < 0.02$ (Mann-Whitney *U*-test)

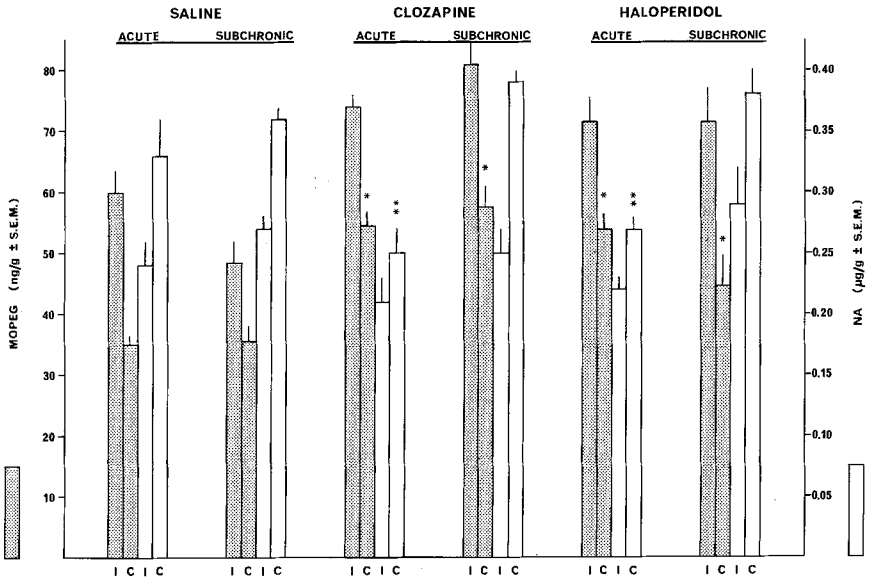

Fig. 3. Concentration of forebrain 3-methoxy-4-hydroxyphenylethyleneglycol (*MOPEG*) and hippocampal noradrenaline (*NA*) 5 min after unilateral electrical stimulation of the *locus coeruleus* in the ipsilateral (*i*) and contralateral (*c*) brain side of rats, treated with clozapine 15 mg/kg, IP), haloperidol (1.0 mg/kg, IP) or saline. Subchronic treatment was for 15 days. Before electrical stimulation animals received chloral hydrate anaesthesia (400 mg/kg, IP). Significance of *i* versus *c*: $*P < 0.02$; $**P < 0.05$ (versus saline)

Table 2. Effect of acute and repeated administration of clozapine and haloperidol on the increase in cyclic AMP-levels in the rat cerebral cortex following electrical stimulation of the *locus coeruleus*

	N	Levels of cyclic AMP (pmol/g ± SEM)			% Difference
		Ipsilateral i	Contralateral c	Difference i − c	
Acute					
Controls	6	1,553 ± 117	1,061 ± 51	493 ± 72	45.7 ± 5.2
Clozapine	6	1,263 ± 15	958 ± 48	306 ± 54	32.9 ± 6.4
Haloperidol	6	1,455 ± 86	987 ± 48	468 ± 89	48.7 ± 10.3
Subacute					
Controls	5	1,263 ± 72	949 ± 20	314 ± 67	32.9 ± 6.0
Clozapine	5	1,365 ± 97	1,034 ± 33	332 ± 83	32.0 ± 7.9
Haloperidol	5	1,425 ± 154	957 ± 68	468 ± 126	40.0 ± 7.9

Clozapine and haloperidol were given at doses of 15 mg/kg/day and 1.0 mg/kg/day respectively. Subacute treatment was for 15 days. *N* = number of animals

onstrated by comparing the increases of forebrain and spinal MOPEG after single and multiple application of clozapine and haloperidol (Fig. 1).

Whilst endogenous hippocampal NA levels returned to normal after 4 days of treatment (Fig. 2), the increased activity of NA neurons, as indicated by MOPEG levels in the forebrain only disappeared after about 15 days of treatment. Spinal MOPEG levels did not significantly differ from controls from the fourth day of treatment onwards (Fig. 1).

As shown in Fig. 3 electrical stimulation of the LC caused significant decreases of hippocampal NA and increases of forebrain MOPEG (ipsilateral versus contralateral) in animals treated with either saline, clozapine or haloperidol (acute and subchronic). The stimulation-induced reductions of NA in the hippocampus after a single dose of clozapine or haloperidol were significantly smaller than in saline treated rats.

After repeated treatment LC-stimulation produced higher levels of MOPEG both in the ipsilateral and contralateral forebrain when compared to saline treated stimulated animals.

After comparison of the cyclic AMP levels in the cerebral cortex contralateral to the stimulated LC of neuroleptic- and saline treated rats (Table 2) it was

concluded that neither acute nor subchronic treatment with clozapine or haloperidol altered the formation of cyclic AMP. Likewise, the cyclic AMP response to LC stimulation did not show significant differences between the experimental groups.

Discussion

The present experiments indicate that, if NA-neurons are activated, neurotransmission processes are not impaired either by acute or subchronic treatment with neuroleptics, so far as the parameters considered are relevant. The relevance of the parameters used may be evident from the following considerations. In the rat brain, endogenous levels of NA and those of its metabolite MOPEG are respectively reduced and enhanced after electrical stimulation of the LC and these alterations are dependent upon the position of the electrode tip, duration and frequency of stimulation (Korf et al. 1973a, b; Adèr et al. 1978, 1979). A neuroleptic compound like clozapine, which enhances the firing rate of LC-neurons (Souto et al. 1979) similarly induces alterations of the endogenous levels of the amine and its metabolite (Keller et al. 1973). Thus, alterations of NA and MOPEG levels are considered to be indices of release of NA from noradrenergic nerve terminals.

The formation of cyclic AMP in the rat cerebral cortex is increased by electrical stimulation of the LC (Korf and Sebens 1979). The cyclic AMP response was frequency dependent and not seen after positioning of the electrode outside the LC, after blockade of both α- and β-adrenoceptors, or in the absence of catecholamines (Korf and Sebens 1979). Thus, cyclic AMP in the rat cerebral cortex appears to be involved in the neurotransmission of noradrenergic neurons originating in the LC.

From results of several studies there appears to be an interrelationship between catecholamine turnover, receptor blockade and behavior (e.g. Andén et al. 1970; Andén and Strombom 1974). Of the various neuroleptics tested we have chosen haloperidol and clozapine in our further experiments. It may be of interest that these two neuroleptics have different affinities towards dopamine receptors. Whilst haloperidol is considered to be a potent blocker of DA2, clozapine is a more potent blocker of DA 1 receptors (Kebabian and Calne 1979; Miller et al. 1974; Clement-Cormier et al. 1974; Westerink and Korf 1975).

After repeated administration of either clozapine or haloperidol tolerance developed to both the increased formation of MOPEG and the reduction of NA in the rat brain. Tolerance to the latter effect developed more rapidly than to the formation of MOPEG, which can be explained by a compensatory effect on synthesis and/or storage of NA. On the other hand it must be noted that the level of free MOPEG is a more sensitive index of changes in NA turnover than the level of the amine itself (Adèr et al. 1978). The acute effects of the neuroleptic drugs are similar in the brain and spinal cord, which is in accordance with the effects of some non-neuroleptic drugs on spinal and cerebral NA metabolism (Adèr and Korf 1979). Tolerance to the formation of MOPEG more rapidly developed in the spinal cord than in the brain. This may point to a difference of response between LC-neurons, which are the exclusive source of noradrenergic nerve terminals in the forebrain, and other bulbospinal noradrenergic neurons, which contribute substantially to the noradrenergic innervation of the spinal cord. Indeed we established that about 30% of NA and 50% of MOPEG in the spinal cord originates in nerve terminals, belonging to the *coeruleo*-spinal projection (Adèr et al 1979; Adèr and Korf 1979).

Treatment with the neuroleptic drugs (acute or subchronic) did not alter the levels of cyclic AMP in non-stimulated rats. This is in accordance with in vitro studies (Blumberg et al. 1975, 1976; Horn and Phillipson 1976). These findings are not necessarily in conflict with the observation by Berndt and Schwabe (1973) of a transient elevation (about 2 min) of cyclic AMP content in the forebrain in vivo after a single IV administration of chlorpromazine. In psychotic patients spinal fluid cyclic AMP is decreased by some antipsychotic drugs (Biederman et al. 1977; Bowers and Heninger 1979). Such a response may not be related to noradrenergic mechanisms and may be significant only in psychotic patients.

After a single dose of neuroleptic, the stimulation-induced reductions of hippocampal NA (ipsilateral versus contralateral) were significantly smaller than those in saline treated animals. From this we conclude that clozapine and haloperidol decrease endogenous NA mainly by release of the amine from a rapidly releasable pool of NA in LC-neurons.

Significantly higher levels of MOPEG compared to stimulated saline treated rats in both the ipsilateral and contralateral forebrain were observed during subchronic treatment with neuroleptics. Thus subchronically administered neuroleptics have a lasting influence on central NA-neurons.

From in vitro studies Farnebo and Hamberger (1971) concluded that neuroleptics enhanced the outflow of NA from brain slices after K^+-depolarization. The present study on the stimulus coupled metabolism of NA corroborates these findings. From various studies it has been concluded that in vitro neuroleptics block NA-receptors linked to an adenylate cyclase (Blumberg et al. 1975, 1976; Horn and Phillipson 1976). In our in vivo experiments no evidence for such

blockade was found. The apparent lack of effect of clozapine and haloperidol is in contrast to the effects of antidepressants. After subchronic treatment with various tricyclic and tetracyclic antidepressant drugs a marked diminution of the LC-stimulation induced cyclic AMP accumulation was found (Korf et al. 1979) in agreement with in vitro studies (e.g. Schultz 1976, 1979).

As we observed different effects on cyclic AMP after activation of the LC in vivo in neuroleptics and antidepressants treated rats, we conclude, that our system discriminates between the actions of these two classes of drugs.

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