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Study the Effects of some Neuroleptic Drugs on the Haloperidol-Induced Tardive Dyskinesia in Male Mice

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ABSTRACT

Tardive dyskinesia (TD) is a neurological iatrogenic disorder and is one of the neurodegenerative movement syndromes affecting mainly orofacial region which includes vacuous chewing movements (VCMs), tongue protrusion (TP), and facial jerking (FJ), resulting from chronic neuroleptic treatment of schizophrenia. The aims of this paper are to evaluate the effects of some a typical neuroleptic drug (risperidone, olanzapine, and aripiprazole) to reduce the TD in mice and study the effects of these drugs on dopamine levels in the brain, see scheme 1.

Adult, albino mice were enrolled in this experiment. The animals were randomly divided into five groups, each group has 10 mices . Each mouse of group1 received normal saline in equal volume to the haloperidol dose intraperitoneally (IP) for 21 days. Each mouse of group 2,3,4 and 5 received haloperidol 2 mg/kg IP for 21 days. mices of group 3,4 and 5 received risperidone 1 mg/kg, olanzapine 2.5 mg/kg, aripiprazole 3 mg/kg orally by gastric tube respectively for 3 days. On the 25th day, each mouse was placed in the glass box for 10 minutes, and VCM was recorded by video camera. Then each mouse of the whole groups was decapitated, and the brain were removed from the skull for measurement of dopamine (DA).

Dopamine level decreased significantly in group 2 as compared with group1 (P<0.05), there were significant increase in the DA levels between group 3 and group 4 as compared with group 2 (P>0.05), but there were no significant differences between group 5 as compared with group 2 (P>0.05). VCM increased significantly in group 2 as compared with group1 (P<0.05). VCM decreased significantly in group 3 and 4 as compared with group 2 (P<0.05), but there were no significant differences between group 5 as compared with group 2 (p>0.05).

Keywords: Tardive dyskinesia, vacuous chewing movements, neuroleptic drugs

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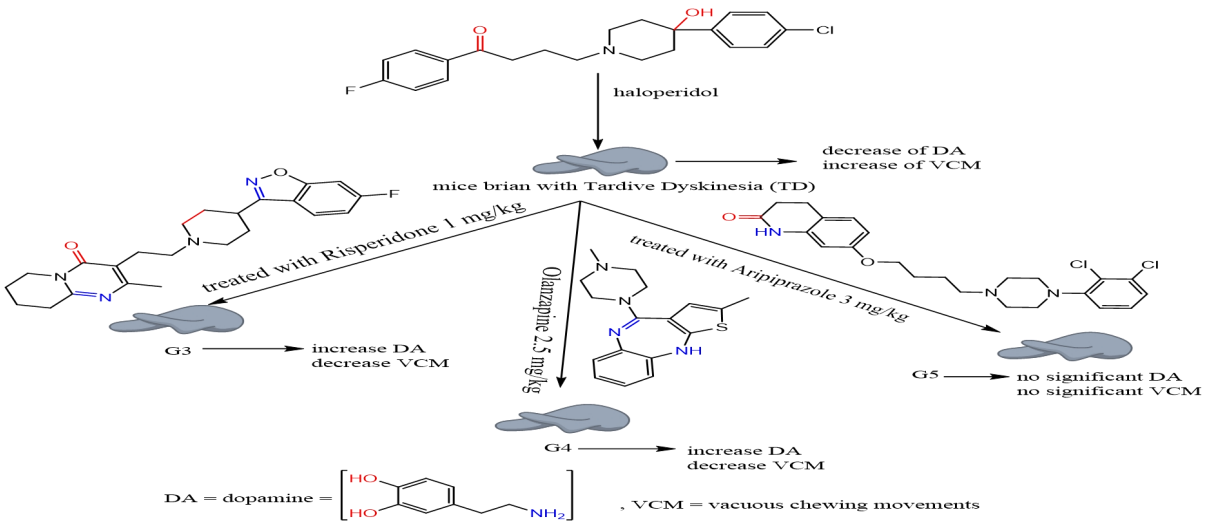


Scheme1 :Schematic diagram about the Effect of neuroleptic drug on provoked Taridive Dyskinesia in mice

INTRODUCTION

The term dyskinesia refers to involuntary muscle movements that can range from slight tremor to uncontrollable movement of the entire body. TD form of dyskinesia gets its name from the slow-or tardive-onset of involuntary movements of the face, lips, tongue, trunk, and extremities [1] [2].

The first generation "typical" neuroleptics with high dopamine D_2 receptor occupancy have been reported to have a higher risk of causing TD than the second- or

third-generation medications, often referred to as ''atypical'' antipsychotics, with low D_2 receptor occupancy, such as clozapine and quetiapine. TD is also associated with a wide variety of other medications [1] [2].

The pathophysiology of TD lacks a universally accepted theory and mechanism. Several hypotheses have been proposed that include prolonged blockade of postsynaptic DA receptors leading to DA receptor supersensitivity, gammaaminobutyric acid (GABA) depletion, cholinergic deficiency, oxidative stress, and neurotoxicity [3] [4].

With regard to the oxidative stress hypothesis, antidepressants block DA receptors, increasing DA synthesis and metabolism. The result of increased DA metabolism is an increase in the production of free radicals. The basal ganglia, subcortical nuclei comprised of several brain regions including the striatum and substantianigra, are highly innervated by DA neurons and are therefore especially at risk for oxidative stress and the occurrence of TD [3] [4].The aim of this paper was to evaluate the effect of some neuroleptic drugs on Tardive Dyskinesia ,dopamine and dopamine receptor levels in brain mice.

Experimental work:

A. Animals

Fifty male, adult, albino mice were enrolled in this expermints. Their weights were 20–44 g. The mice were housed in the Animal House of the College of Medicine/Babylon University, and kept on 25°C and 12 hr_s light and 10 h_s light-dark cycles with water and food ad libitum.After two weeks of adaptation, the animals were randomly divided into 10 mice in each group.

Procedure

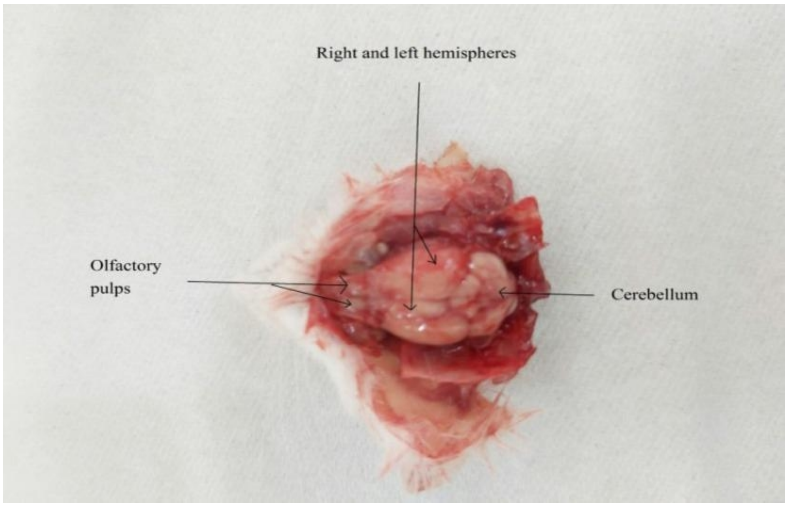
Each mouse of group1 received normal saline in equal volume to the haloperidol dose IP for 21 days. Each mice of group 2,3,4 and 5 received haloperidol 2 mg/kg for 21 days. Then mice of group 3,4 and 5 received risperidone 1 mg/kg, olanzapine 2.5 mg/kg, aripiprazole 3 mg/kg orally by gastric tube respectively for 3 days. On the 25th day, each mouse was placed in the glass box for 10 minutes, then in the open field box for 5 minutes, then on the narrow beam for 5 minutes and all behaviors were recorded by video camera. Then each mice of the whole groups were decapitated, and the brain were removed from the skull for chemical examination.

B. Brain dissection

On the 25th days of treatment**, the** animals were sacrificed, and the brains were removed after dissection of skull from foramen magnum posteriorly. Olfactory pulps and cerebellum were removed, and the brain removed gently from the skull and the mid and forebrain were taken and dissected out and rinsed in isotonic saline and weighted.

C. Steps of preparation of sample

Tissue homogenates: residual blood removed by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Tissue homogenized after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue). Generally speaking, 9mL PBS would be appropriate to 0.5-gram tissue pieces. Protease inhibitors were added into the PBS with a glass homogenizer on ice (adding at 1: 100 (v/v) dilution to 1 solution samples before assaying). For further breaking the cells, sonication has been done with an ultrasonic cell disrupter or subjection the suspension to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to get the supernatant.



Picture 1: Skull dissection of the mouse.

D. Assessment of dopamine using ELISA kit

DA was measured by enzyme linked immunosorbent assay (ELISA), for more information see the reference [19].

RESULTS

A.Dopamine level

DA level decreased significantly in group 2 (haloperidol 2 mg/kg), group 3 (risperidone 1 mg/kg), group 4 (olanzapine 2.5 mg/kg) and group 5 (aripiprazole 3 mg/kg) as compared with group1 (normal saline) ($P<0.05$) (**Table 1** and **Figure 2**).

There were significant differences in the DA levels between group 3 (risperidone 1 mg/kg) and group 4 (olanzapine 2.5 mg/kg) as compared with group 2 (haloperidol 2 mg/kg) ($P>0.05$), but there were no significant differences between group 5 (aripiprazole 3 mg/kg) as compared with group 2 (haloperidol 2 mg/kg) ($P>0.05$) (**Table 1** and **Figure 2**).

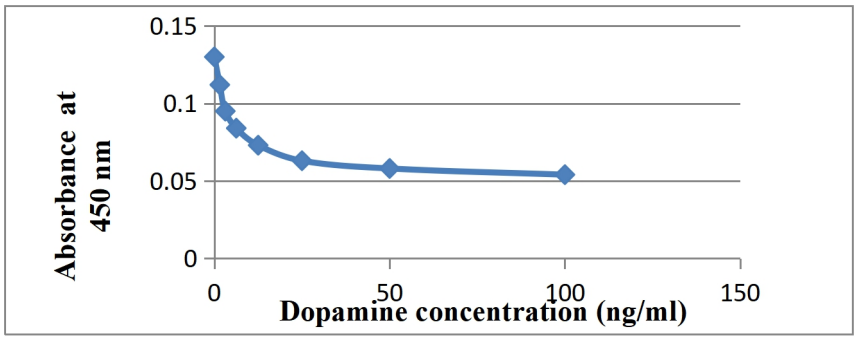
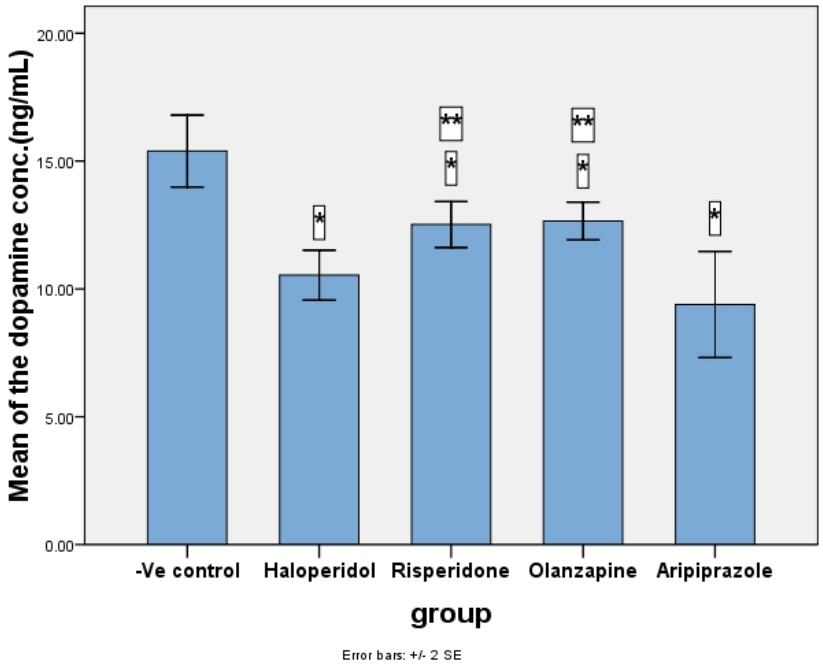


Figure 1: Standard curve of dopamine.

table 1: A comparison of the mean differences of DA level between different groups (group 1: control group; group 2: haloperidol 2 mg/kg; group 3: risperidone 1 mg/kg; and group 4: olanzapine 2.5 mg/kg; group 5: aripiprazole 3 mg/kg).

***P<0.05 significant**

Dopamine level Mean	Control 1	Haloperidol 2	Risperidone 3	Olanzapine 4	Airpiprzole 5
Control (1)	X	4.8*	2.8*	2.7*	6*
Group (2)	-4.8*	X	-1.9*	-2.1*	1.1
Group (3)	-2.8*	1.9*	X	-0.1	3.1*
Group (4)	-2.7*	2.1*	0.1	X	3.2*
Group (5)	-6*	-1.1	-3.1*	-3.2*	X



*: significant differences (P<0.05) between 2,3,4,5 groups as compared with group1
**: significant differences (P<0.05) between 3,4,5 groups as compared with group2
Figure 2: Means of the DA concentration of all groups; group1 (control group), group2 (haloperidol 2 mg/kg), group3 (risperidone 1 mg/kg), group4 (olanzapine 2.5 mg/kg) and group5 (aripiprazole 3 mg/kg),(No. of animal=10 for each group).

B. Vacuous chewing movement

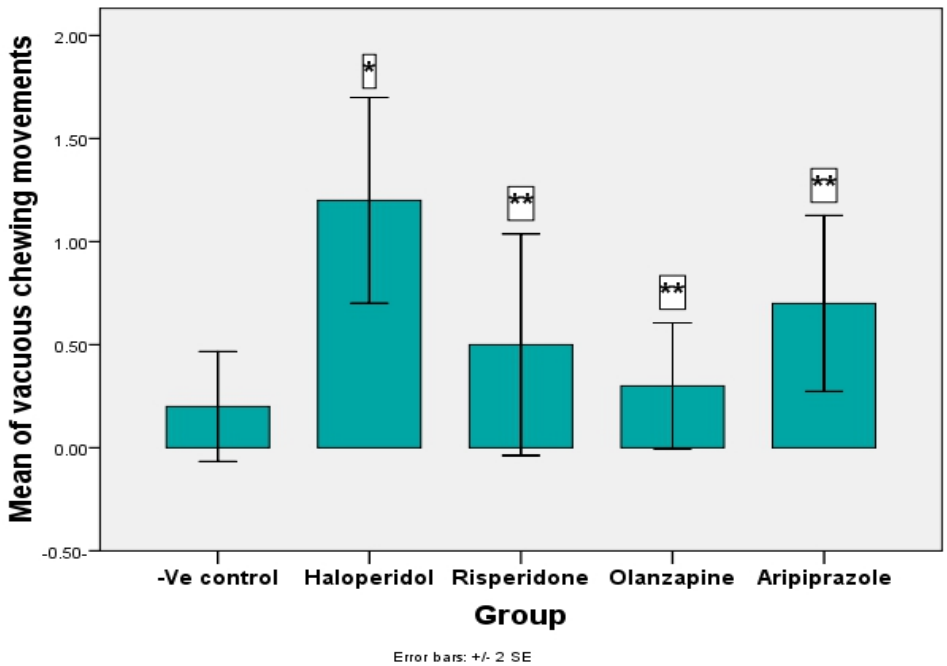
No. of VCM increased significantly in group 2 (haloperidol 2 mg/kg) as compared with group1 (normal saline) (P<0.05). While there were no significant differences between group 3 (risperidone 1 mg/kg), group 4 (olanzapine 2.5 mg/kg) and group 5 (aripiprazole 3 mg/kg) as compared with group1 (P>0.05) (Table 2 and Figure 3).

No. of VCM decreased significantly in group 3 (risperidone 1 mg/kg) and group 4 (olanzapine 2.5 mg/kg) as compared with group 2 (haloperidol 2 mg/kg) (P<0.05), but there were no significant differences between group 5 (aripiprazole 3 mg/kg) as compared with group2 (p>0.05) (Table 2 and Figure 3).

Table 2: A comparison of the mean differences of the No. of vacuous chewing movements between different groups (group 1: control group; group 2: haloperidol 2 mg/kg; group 3: risperidone 1 mg/kg; and group 4: olanzapine 2.5 mg/kg; group 5: aripiprazole 3 mg/kg).

Mean of VCM	control 1	Haloperidol 2	Risperidone3	Olanzapine4	Airpiperizole 5
Group 1	X	-1*	-0.3	-0.1	-0.5
Group 2	1*	X	0.7*	0.9*	0.5
Group 3	0.3	-0.7*	X	0.2	-0.2
Group 4	0.1	-0.9*	-0.2	X	-0.4
Group 5	0.5	-0.5	0.2	0.4	X

***P<0.05 significant**



***: significant differences (P<0.05) between 2,3,4,5 groups as compared with group1**
****: significant differences (P<0.05) between 3,4,5 groups as compared with group2**
Figure 3: Means of the No. of vacuous chewing movements of all groups; group1(control group), group2 (haloperidol 2 mg/kg), group3 (risperidone 1 mg/kg), group4 (olanzapine 2.5 mg/kg) and group5 (aripiprazole 3 mg/kg),(No. of animal=10 for each group).

DISCUSSION

A. Dopamine level:

According to the Data of the present results demonstrated that administration of haloperidol decreases the brain dopamine level in the brain of the mice. This finding agrees with those reported by [12]. The molecular mechanisms by which haloperidol decreased DA are that it acts by chronic blockade of dopamine D_2 receptor in nigrostriatal neurons of the brain lead to increase in DA turnover in basal ganglia and classical neuroleptics such as haloperidol remains bound to dopamine D_2 receptors and increased uptake of DA, especially after withdrawal of antipsychotics, which results in TD [12].

Also the administration of risperidone decreases the brain DA level in the brain of the mice. This finding disagrees with those reported by [6]. But risperidone showed significant increase in DA level in the mice as compared with haloperidol. Also there was convincing evidence that. there was an interaction between serotonergic and dopaminergic neurons in the basal ganglia region. Specifically, serotonin inhibits dopamine release through an interaction with receptors in the axon terminals of the dopaminergic neurons [7]. We can, therefore, consider the hypothesis that atypical neuroleptic drugs (serotonergic/dopaminergic antagonists) such as risperidone could cause less extrapyramidal effects by blocking serotonergic receptors in the axon terminals of the dopaminergic neurons, causing an increase in dopamine release in the nigrostriatal system and reverting the effects of D_2 blockade leading to development of haloperidol-induced oral dyskinesia.

The olanzapine and aripiprazole decrease the brain DA level in the mice. The that significant decreases in the DA synthesis were found following treatment with olanzapine in comparison with the control group, specifically in the medial prefrontal cortex (PFC) and ventral tegmental area (VTA) brain regions of the DA neurotransmitters system. But olanzapine showed significant increase in DA level in the mice as compared with haloperidol[8]. The acute administration of olanzapine significantly reversed haloperidol-induced suppression of striatal nitric oxide synthase (NOS) activity, a well-recognized effect of nitric oxide which is synthesized by NOS is to promote striatal DA release by promoting DA efflux or by inhibiting DA reuptake [9].

On the other hand the administration of aripiprazole decreases the brain DA level in the mice. Also, these findings agree with those reported by [11]. DA synth esis mRNA expression in VTA was decreased after aripiprazole administration. Since tyrosine hydroxylase is the rate-limiting enzyme for the synthesis of DA, this indicates a reduction of DA synthesis in this brain region. The selective effects of aripiprazole on reducing DA production may provide a mechanism to explain its long-term efficacy. A reduction in DA synthesis may be mediated by D_2 autoreceptors reported by [10]. Previously, in-vivo studies have found that aripiprazole has potent agonist activities at DA autoreceptors reported by [11]. As a compensatory mechanism, D_2 autoreceptor synthesis in the VTA may be increased in response to the decrease of DA synthesis and release caused by aripiprazole treatment. Consistent with this, an increase in D_2 receptor mRNA expression was observed as reported by [11].

B. Model of Tardive Dyskinesia in mice

For, the experimental work the haloperidol increases VCM in the mice. This finding agrees with those reported by [12], [13]. [13] reported that chronic administration of typical neuroleptics haloperidol led to significant increase in VCMs which is associated with significant decrease in serotonin, DA and norepinephrine levels where as atypical antipsychotics showed less prevalence of extrapyramidal side effects. [14]Yasmin et al (1996) reported that it appears that VCMs may also develop rapidly, particularly following intraperitoneal or subcutaneous (SC) injection. [14] Yasmin et al (1996) reported that acute VCMs may develop with treatment that rapidly blocks D_2 receptors, such as SC and IP injections. [13] reported that chronic administration of neuroleptics is associated with proliferation of D_2 receptors in caudate putamen and NAc. Also, chronic blockade of dopamine D_2 inhibitory receptor located in the glutamatergic terminals in the striatum leads to persistent and enhanced release of glutamate that damages the striatal output neurons resulting in increased orofacial movements and oxidative damage which are the hallmark of TD.

It is possible typical and atypical antipsychotic differently affects neuronal survival and death and that these effects considerably contribute to the differences in the development of TD as reported by Bishnoi et al [16].

The haloperidol is metabolized by an oxidase which generates large quantities of toxic metabolites that induces oxidative stress. Chronic blockade of dopamine D_2 receptors by neuroleptics in nigrostriatal neurons of the brain leads to an increase in DA turnover in basal ganglia and this may lead to overproduction of free radicals**.** The DA supersensitivity hypothesis proposes that antipsychotic drug treatment causes hypersensitization of dopamine D_2 receptors, via increased density in all dopaminergic pathways [12]. This disturbs DA levels in brain regions responsible for motor symptoms, resulting in motor dysfunction. Classical neuroleptics such as haloperidol remain bound to dopamine D_2 receptors and accumulate in brain tissue. This leads to increased density of dopamine D_2 receptors and increased uptake of DA, especially after withdrawal of antipsychotics, which results in TD. [11] reported that the results further suggest that haloperidol may produce EPS by D_2 receptor blockade and prolonged free DA reuptake in caudate putamen.

This study showed that administration of risperidone inhibits the VCMs which are induced by haloperidol in the mice. This effect may be due to blocking effect of risperidone on 5-HT_2 in the CNS [7]. [13] reported that risperidone treatment resulted in insignificant increase in VCM as compared to control. [7] reported that there is an interaction between serotonergic and dopaminergic neurons in the basal ganglia region. Serotonin inhibits the release of dopamine. Therefore, risperidone could cause less extrapyramidal effects by blocking 5-HT_2 receptor.

[13] reported that short-term (<45 days) treatment studies in rats have reported increased oxidative stress and oxidative (i.e., oxygen free radical-mediated) neural cell injury with typical antipsychotics such as haloperidol, but not with the atypicals such as risperidone. This drug displays different alterations in different brain areas.

Also showed that administration of olanzapine inhibits the VCMs which are induced by haloperidol in the mice. This effect may be due to blocking effect of olanzapine on 5-HT_2 in the CNS**.** This finding agrees with those reported by [17] Mccullumsmith et al (2003). [17] Mccullumsmith et al **reported** that haloperidol induced VCM measured after olanzapine were decreased compared to before olanzapine treatment, suggesting that additional treatment with olanzapine permitted spontaneous recovery from chronic VCM.

[9] reported that the limbic-selective actions, low D_2 receptor occupancy, antagonism of 5-HT2 receptors, or antimuscarinic effects of olanzapine have been used to explain its superior motor side-effect profile. It is [9] suggestion that its efficacy in treating TD may involve another less-recognized site of action, one that is relevant to the underlying neurobiology of TD. [13] reported that short-term (<45 days) treatment studies in rats have reported increased oxidative stress and oxidative (i.e., oxygen free radical-mediated) neural cell injury with typical antipsychotics such as haloperidol, but not with a typical such as risperidone. This drug displays different alterations in different brain areas.

While the administration of aripiprazole shows no significant difference in VCM in the mice. about our knowldgement, there is no previous data about the effects of aripiprazole on VCM. [18] reported that partial DA agonistic activity of aripiprazole may normalize D_2 receptor upregulation, thus resulting in TD remission [18].

Conclusion

Risperidone and olanzapine decreased VCM of TD in male mice. Aripiprazole has no effect on model of TD in mice. Risperidone and olanzapine decreased DA level in brain of mice (but not aripiprazole). see scheme (1)

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