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# Possible Genetic Factors Underlying the Pathophysiology of Tardive Dyskinesia

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ROSENGARTEN, H., J. W. SCHWEITZER AND A. J. FRIEDHOFF. *Possible genetic factors underlying the pathophysiology of tardive dyskinesia.* PHARMACOL BIOCHEM BEHAV **49**(3) 663–667, 1994.—Rates of spontaneous and drug-induced repetitive jaw movements (RJM) in rats vary widely. Low and high RJM responders were isolated and genetically selected. At each generation mean RJM responses (spontaneous or SKF 38393-induced) of the two types of rats were found to differ significantly, whereas neither apomorphine-induced stereotypic responses nor $D_1$ and $D_2$ receptor numbers and affinities differed. A significant increase in cAMP production was evident in SKF 38393-stimulated striatal homogenates of high RJM responders as compared with low responders. Animals subjected to 8-months exposure to fluphenazine exhibited RJM that were about twice as great as that of controls, 2 months after the last treatment, with a prevalence of about 75%. Similarities between RJM observed in rats and neuroleptic-induced tardive dyskinesia suggest that the two are strongly related.

Genetics Tardive dyskinesia RJM Rats

THE INTRODUCTION of neuroleptics to clinical practice in 1952 was followed by numerous reports describing a syndrome of abnormal involuntary dyskinetic movements in 10–20% of patients so treated (12,15,17,18,29). Further studies implicated the dopaminergic system in neuroleptic-induced TD (3,8,14,26). On the basis of initial studies with rats, it was suggested that dopamine receptor supersensitivity was responsible for this disorder (16,22). This hypothesis, however, presents a number of problems, the chief one being that following even short-term neuroleptic treatment, supersensitivity of $D_2$ receptors occurs to about the same extent in all rats (6), whereas TD occurs in some patients only, and generally only after prolonged treatment. On this basis, several investigators have concluded that neuroleptic treatment may precipitate TD only in those patients who already are predisposed to development of this disorder (31).

It has been reported that long-term neuroleptic treatment in rats (4,10,11,25,31) and monkeys (20) can induce increases in the frequency of spontaneous orofacial movements both during treatment and withdrawal. In our experience, bursts of repetitive jaw movements (RJM), which correlate with retest scores, vary considerably among rats, suggesting that some animals may be better responders than others (25). A frequency distribution plot of RJM scores, perhaps bimodal (27), fits a pattern of orofacial scores of psychiatric patients on long-term neuroleptic treatment (9).

The symptomatology of the purposeless chewing behaviors observed in rats and monkeys, manifested by side-to-side chewing, tongue protrusions, audible tooth grinding, and bursts of opening and closing of jaws, parallels several features characteristic of TD and corresponds, temporally, during washout, to the onset of TD in humans. Furthermore, not all rats or humans develop such movements in response to chronic neuroleptic treatment (31). Suspecting a genetic link to this disorder, we isolated high and low responders and, by means of selective breeding (7,13,21), developed rats that differ markedly both in spontaneous and $D_1$ agonist-inducible RJM.

The present article describes these efforts and additionally, of effects of 8-month chronic fluphenazine effects in mixed and genetically selected rats.

## METHOD

Rats were housed in the animal facility maintained under a 12-h light-dark cycle at a temperature of 21 ± 1°C.

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*Genetic Selection*

The original foundation was a heterogeneous population of Sprague-Dawley rats consisting of 120 males and females. Before the selection experiment was begun, rats were assessed for spontaneous and SKF 38393 stimulated RJM (see below) according to our previously published method (23,24). The criterion for the selection was the RJM response rating. The selection was bidirectional and was employed to produce divergent lines for the expression of RJM. Eight of the most responsive and least responsive males and females were selected from each group for the breeding procedure. Breeding within the same litter was precluded. In each subsequent generation all offspring were tested for the RJM response and used again for breeding. The same criterion of selection was used for subsequent generations.

*Behavioral Assessments*

*RJM.* To evaluate RJM equal numbers of rats (60 days old for genetic separation studies and 11 or more months old for the chronic fluphenazine studies) were placed individually in wire mesh cages 7 × 7 × 10 inches for 1 h of habituation prior to $D_1$ agonist administration. Episodes of spontaneous RJM were observed for 10 1-min periods over a period of 45 min by an investigator blind to group and treatment. In this assessment each (rapid) burst (episode) of mouth movements, with or without tongue protrusion, is given a score of 1. Each animal is observed in this way for 1 min, 10 times, to yield RJM/10 min. SKF 38393, 20 mg/kg [Research Biochemicals International; (RBI)] was then administered and RJM assessments were again initiated 15 min later. Results were analyzed by appropriate statistical tests (generally ANOVA followed by Newman-Keuls test).

N-*propylapomorphine (NPA)-induced stereotypy.* NPA (RBI), 0.1 mg/kg, was administered SC in the region of the neck and stereotypy responses (5) were recorded for 1 min, five evenly spread times over a 50-min period. Scores for each animal were summed over time and divided by the number of observation periods.

*Fluphenazine exposure.* Prior to the initiation of the genetic studies, a large group of rats was treated with fluphenazine decanoate (25 mg/kg/3 weeks) or saline for 7 months followed by fluphenazine hydrochloride, 1 mg/kg, daily for 1 month. At various time points during washout, the animals were assessed for RJM and stereotypy (Fig. 4). The time table chosen for the behavioral tests in the following chronic study are based on that initial investigation.

Equal numbers of 3-month-old high and low RJM responders from the eighth generation were treated with fluphenazine decanoate (Princetown Pharmaceutical) 25 mg/kg or vehicle, IM, every 3 weeks for 7 months, and as before, at the beginning of the eighth month the neuroleptic treated group was injected daily IP for 1 month with fluphenazine hydrochloride, 1 mg/kg. Rats were then given an 8-week washout period before weekly behavioral testing was begun.

*Biochemical assessments.* Striata were dissected and stored at −80°C. $D_1$ and $D_2$ receptor densities and affinities were determined in each generation in striatal tissue of 60-day-old drug naive rats. For the determination of $D_1$ receptor binding to striatal homogenates, $^3$H-SCH 23390 (NEN, S.A. 70.3 mCi/mol) was used as described by Billard et al. (1), and for $D_2$ receptors, $^3$H -Spiperone (NEN, S.A. 20.3 mCi/mol) as described by List et al. (19). $B_{max}$ and $K_d$ values for each rat were estimated by Scatchard analysis and means were statistically analyzed by Student's *t*-test.

Basal- and SKF 38393-stimulated cAMP levels in striatal homogenates of drug naive low and high responders of the eighth generation prepared from fresh (nonfrozen) tissue were carried out as described by Traficante et al. (28), using the protein binding procedure developed by Brown et al. (2) for the measurement of cAMP. Results were analyzed by two-way ANOVA followed by Newman-Keuls test.

## RESULTS

Two divergent lines of rats were developed with respect to RJM and were identified as RJML and RJMH for low and high responders, respectively. Significant differences both for spontaneous and SKF 38393-stimulated RJM between the two types of responders were already evident by the second generation (Fig. 1) and remained so across subsequent generations [three-way ANOVA (spontaneous): overall $F(1, 224) = 916.91$, $p < 0.0001$, Newman-Keuls, $p < 0.05$; SKF 38393-induced RJM: $F(1, 224) = 1178.8$, $p < 0.0001$, Newman-Keuls, $p < 0.05$]. In contrast, NPA-induced stereotypy scores did not differ between groups or across generations (Fig. 2), nor were there differences in $D_1$ and $D_2$ receptor densities or dissociation constants between these groups (Table 1).

Striatal homogenates of eighth generation rats were exposed to SKF 38393 and analyzed for cAMP levels. The mean cAMP level stimulated by $10^{-4}$ M SKF 38393 in RJM responders was significantly higher than that of the RJM group (Fig. 3) [two-way ANOVA: $F(1, 24) = 22.8$, $p < 0.001$], Newman-Keuls analysis: $p < 0.05$. cAMP levels were also lower in basal and $10^{-5}$ M SKF 38393-stimulated tissue of RJML animals, but these were not significantly different from the corresponding RJMH group.

A large group of rats was subjected to 7 months fluphenazine decanoate followed by 1 month of fluphenazine hydrochloride and monitored for RJM and stereotypy during washout. Spontaneous RJM (Fig. 4) peaked at 8 weeks after the initiation of washout. Three-way ANOVA yielded statistically significant differences between the two groups, $F(1, 112) = 70.99$, $p < 0.0001$, Newman-Keuls, $p < 0.05$ with the 8-week point being higher than all other washout periods, New-

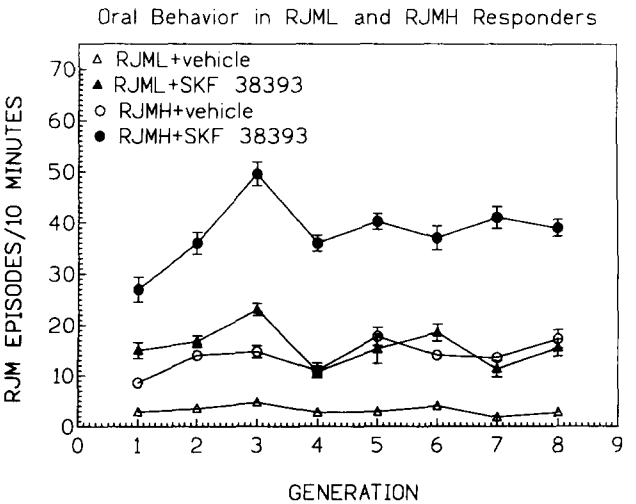


FIG. 1. Significant differences were obtained for spontaneous RJM between RJML and RJMH populations at each of the generations. Similar findings were obtained following acute SKF 38393 administration.

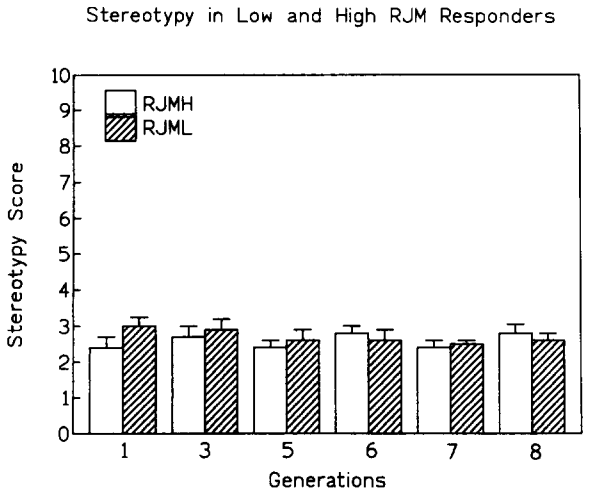


FIG. 2. *N*-propylapomorphine-induced stereotypy scores for RJML and RJMH groups were not significantly different within or between generations.

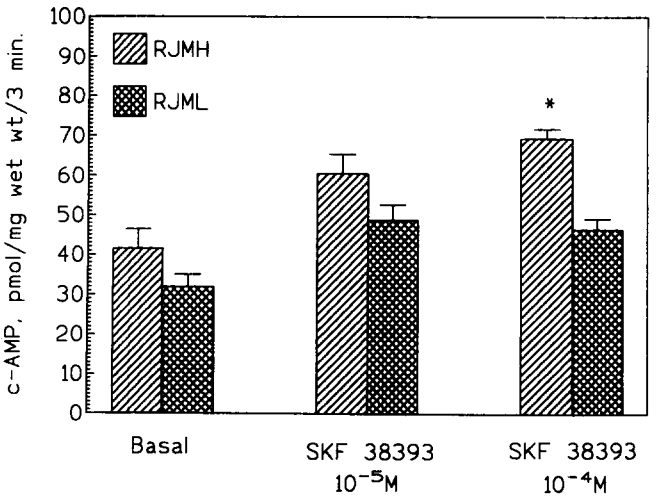


FIG. 3. Striatal homogenate cAMP accumulations. A significant difference between RJML and RJMH responders was observed at $10^{-4}$ M SKF 38393 [two-way ANOVA, $F(1, 24) = 22.8$; Newman-Keuls, $p < 0.05$].

man-Keuls, $p < 0.05$. There was also a significant increase in the prevalence of RJM (Fig. 5) in the fluphenazine treated group vs. vehicle, Mann-Whitney, $p < 0.001$, which also peaked at 8 weeks of washout. A significance difference in response to SKF 38393 between vehicle and fluphenazine treated rats was observed at 8 weeks of washout, two-way ANOVA, $F(1, 28) = 29.44$, $p < 0.001$, Newman-Keuls, $p < 0.05$ (Fig. 6). At that time, mean RJM scores for the neuroleptic groups were about double that of their respective vehicle treated controls. An 8 month fluphenazine study was also carried out with RJML and RJMH rats with similar results. Results at 8 weeks of washout are shown in Fig. 7. The acute administration of SKF 38393 induced a much greater elevation in the RJMH groups than in the RJML groups [two-way ANOVA, $F(1, 56) = 30.15$, Newman-Keuls, $p < 0.05$], and at 8 weeks of washout mean RJM scores for the neuroleptic groups were more than double that of their respective vehicle-treated controls. In addition, the mean spontaneous RJM score for the RJMH-treated group was about twofold higher than that of the RJML group [two-way ANOVA: $F1(1, 56) = 29.88$, $p < 0.001$, Newman-Keuls, $p < 0.05$] (Fig. 5), and the acute administration of SKF 38393 induced a much greater elevation of RJM in the RJMH groups than in the RJML groups [two-way ANOVA; $F2(1, 56) = 30.15$, $p < 0.001$, Newman-Keuls, $p < 0.05$].

## DISCUSSION

There are several important findings in this study. First, that the magnitude of the RJM response can be manipulated by genetic selection. Exploring pathways responsible for this

TABLE 1

SCATCHARD ANALYSIS OF STRIATAL $D_1$ AND $D_2$ RECEPTORS IN RJML AND RJMH

| | RJML | | RJMH | |
|---|---|---|---|---|
| | $B_{max}$, pmol/g | $K_d$, nM | $B_{max}$, pmol/g | $K_d$, nM |
| $D_1$ receptors generations | | | | |
| 1st | 89.2 ± 2.9 | 0.97 ± 0.04 | 90.2 ± 2.7 | 0.95 ± 0.06 |
| 2nd | 103.4 ± 6.3 | 0.91 ± 0.05 | 103.6 ± 3.0 | 0.89 ± 0.02 |
| 5th | 102.9 ± 5.0 | 0.89 ± 0.05 | 101.3 ± 6.9 | 0.91 ± 0.04 |
| 6th | 102.6 ± 5.7 | 0.91 ± 0.17 | 108.0 ± 6.2 | 0.98 ± 0.06 |
| 8th | 101.0 ± 2.7 | 0.69 ± 0.07 | 106.6 ± 1.4 | 0.69 ± 0.07 |
| $D_2$ receptors generations | | | | |
| 1st | 37.4 ± 2.8 | 0.08 ± 0.02 | 36.7 ± 1.1 | 0.07 ± 0.01 |
| 2nd | 38.8 ± 2.7 | 0.12 ± 0.01 | 38.7 ± 1.2 | 0.13 ± 0.02 |
| 5th | 40.7 ± 3.1 | 0.11 ± 0.01 | 38.0 ± 2.2 | 0.11 ± 0.02 |
| 6th | 39.2 ± 2.1 | 0.14 ± 0.02 | 41.2 ± 1.7 | 0.12 ± 0.06 |
| 8th | 44.2 ± 3.2 | 0.16 ± 0.03 | 45.8 ± 2.3 | 0.12 ± 0.02 |

All values are expressed ± SEM. There were no significant differences between $K_d$ values for RJML and RJMH or between $B_{max}$ values for RJML and RJMH within generations. $N = 8$ rats/group.

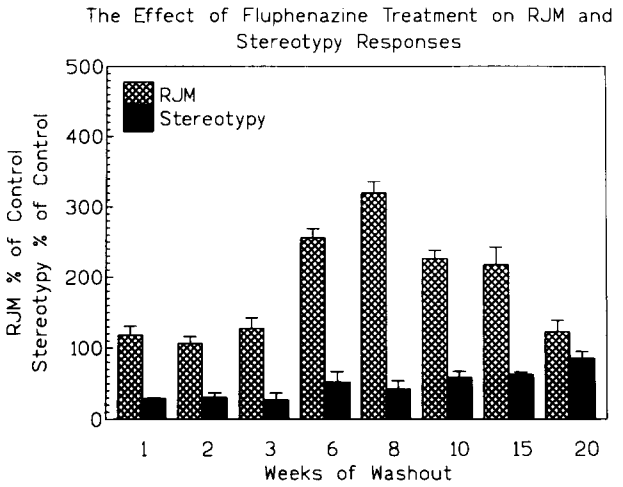


FIG. 4. A nongenetically selected population of rats was treated with fluphenazine or vehicle for 8 months and then monitored for RJM and *N*-propylapomorphine-inducible stereotypy weekly. Three-way ANOVA revealed statistically significant differences in spontaneous RJM between vehicle and fluphenazine treated rats during washout, $F(1, 112) = 7.1.0$, $p < 0.0001$, which was confined largely to the eighth week of washout (Newman-Keuls, $p > 0.05$). Stereotypy scores, initially decreased due to $D_2$ receptor blockade, gradually returned to near normal values by 20 weeks of washout.

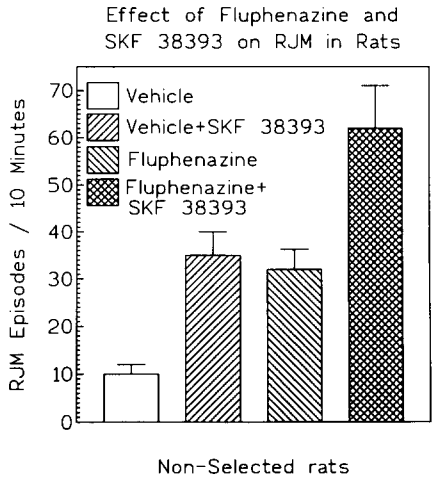


FIG. 6. An examination of RJM in a genetically nonselected population, at 8 weeks of washout following 8 months of treatment with fluphenazine indicates that the increase in spontaneous RJM in the fluphenazine group can be additionally augmented by a specific $D_1$ agonist, an effect similarly obtained in a naive (vehicle) group, providing evidence that spontaneous and neuroleptic-induced RJM are pharmacologically similar.

observation, it was found that SKF 38393-stimulated cAMP elevations in striatal tissue homogenates were significantly elevated in high RJM responders with respect to low responders, in support of previous findings from this laboratory, implicating dopamine $D_1$ receptor pathways in the mediation of RJM (23-25). The present results also suggest a postreceptor modification in neurotransmission (at the G-protein site?) because binding data (see the Results section) failed to uncover differences between RJML and RJMH rats. Next, it was observed that the mean RJM score in RJMH animals was more than double that of the RJML group following neuroleptic treatment. Thus, a genetic disposition to high spontaneous RJM scores in drug-native rats was further greatly augmented by chronic exposure.

An examination of RJM in a genetically nonselected population, at 8 weeks of washout following 8 months of treatment with fluphenazine, indicated that the increase in spontaneous RJM found in the fluphenazine group could be further significantly augmented by the administration of the specific $D_1$ agonist, SKF 38393, an effect similarly found, though to a lesser degree, in the neuroleptic-naive group, to provide evidence that spontaneous and neuroleptic-induced RJM are pharmacologically similar.

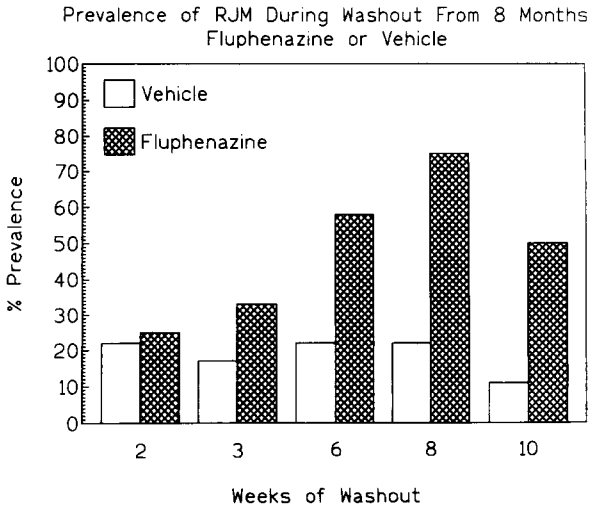


FIG. 5. The prevalence of RJM during washout from fluphenazine peaked at 8 weeks (Mann-Whitney rank sum test, $p < 0.001$).

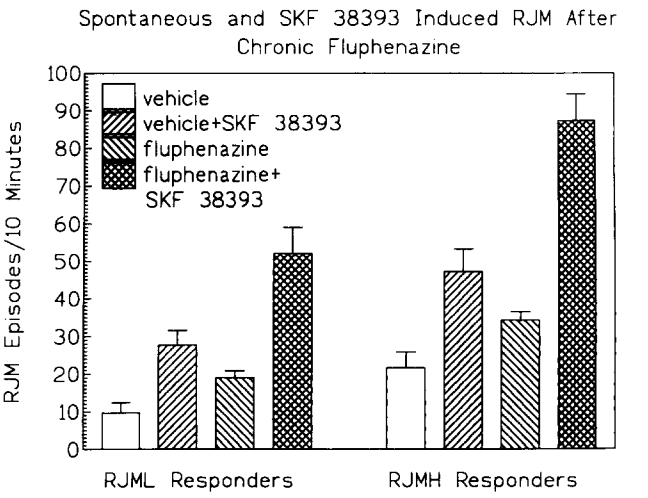


FIG. 7. See legend to Fig. 6. Here, RJML and RJMH animals were studied.

RJM behavior shares several phenomenological similarities with neuroleptic-induced involuntary dyskinetic oral behavior in humans. It is induced by chronic neuroleptic treatment and is associated with tongue thrusting and purposeless chewing movements, behaviors also seen in TD. Therefore, on the basis of this study, we propose that there is, very likely, a genetic disposition to oral TD and perhaps to other neurological side effects of chronic neuroleptic exposure.

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## REFERENCES

1. Billard, W.; Rupeto, V.; Crosby, G.; Iorio, L. C.; Barnett, S. A. Characterization of the binding of $^3$H-SCH 23390, a selective $D_1$ receptor agonist ligand in rat striatum. Life Sci. 35:1885–1893; 1984.
2. Brown, B. L.; Albano, J. D. M.; Ekins, R. P.; Sgherzi, A. M.; Tampion, W. A simple and sensitive saturation assay method for the measurement of adenosine-4,5′-cyclic monophosphate. Biochem. J. 121:561–562; 1971.
3. Burt, D. R.; Creese, I.; Snyder, S. H. Anticholinergic drugs: Chronic treatment elevates dopamine receptor binding in brain. Science 196:326–328; 1977.
4. Clow, A.; Jenner, P.; Marsden, C. D. Changes in dopamine mediated behavior during one year's neuroleptic administration. Eur. J. Pharmacol. 57:365–375; 1979.
5. Costal, B.; Naylor, R. B. The involvement of dopaminergic systems with the stereotyped behavior patterns induced by methylphenidate. J. Pharmacol. 26:30–33; 1974.
6. Creese, I.; Burt, D. R.; Snyder, S. H. Dopamine receptor binding predicts pharmacological potencies of antischizophrenic drugs. Science 192:481–483; 1976.
7. Falconer, D. S. Introduction to quantitative henetics. New York: Wiley and Sons Publishers; 1989.
8. Friedhoff, A. J.; Bonnet, K.; Rosengarten, H. Reversal of two manifestations of dopamine receptor supersensitivity by administration of L-dopa. Res. Commun. Chem. Pathol. Pharmacol. 116:411–423; 1977.
9. Gardos, G.; Sokol, M.; Cole, J. S.; Shiffin, C. Eye color and tardive dyskinesia. Psychopharmacol. Bull. 12:7–10; 1976.
10. Glassman, R. B.; Glassman, H. N. Oral dyskinesia in brain damaged rats withdrawn from a neuroleptic: Implications for tardive dyskinesia. Psychopharmacology (Berlin) 69:19–25; 1980.
11. Gunne, L. M.; Growdon, J.; Glaeser, B. Oral dyskinesia in rats following brain lesions and neuroleptic drug administration. Psychopharmacology (Berlin) 77:134–139; 1982.
12. Hall, R. A.; Jackson, R. B.; Swain, J. Neurotoxic reactions resulting from chlorpromazine administration. JAMA 2:214–218; 1955.
13. Jenner, P.; Marsden, C. D. Is the dopamine hypothesis of tardive dyskinesia completely wrong? Trends Neurosci. 259; 1986.
15. Kane, J. M.; Smith, J. M. Tardive dyskinesia: Prevalence and risk factors 1959–1979. Arch. Gen. Psychiatry 39:473–481; 1982.
16. Klawans, H. L.; Rubovits, R. An experimental model of tardive dyskinesia. J. Neural Transm. 32:235–246; 1972.
17. Klawans, H. L.; Carvey, P.; Tannerm C. M.; Goetzm C. G. The pathophysiology of tardive dyskinesia. J. Clin. Psychiatry 46:38–41; 1985.
18. Kullenkampf, C.; Tarnow, G. Ein Eigentuliches Syndrome i Ovalen bereich bei Megaphenaplikation. Nervenarzt 27:178–180; 1976.
19. List, S. J.; Seeman, P. Resolution of dopamine and serotonin components of $^3$H-spiperone binding to rat brain regions. Proc. Natl. Acad. Sci. USA 78:2620–2624; 1980.
20. Lubin, H.; Gerlack, J. Behavioral effects of dopamine $D_1$ and $D_2$ receptor agonists in monkeys previously treated with haloperidol. Eur. J. Pharmacol. 153:239–245; 1988.
21. McLean, G. E.;, Kakihana, R. Development of animal models as pharmacogenetic tools. US Dept. Health and Human Services Monograph-6 Publ. No. (ADM); 1981:81–113.
22. Muller, P.; Seeman, P. Brain neurotransmitter receptor after long term haloperidol. Life Sci. 21:1751–1758; 1977.
23. Rosengarten, H.; Schweitzer, J. W.; Friedhoff, A. J. Induction of oral dyskinesia in naive rats by $D_1$ stimulation. Life Sci. 33: 2479–2482; 1983.
24. Rosengarten, H.; Schweitzer, J. W.; Egawa, M.; Friedhoff, A. J. Diminished $D_2$ dopamine receptor function and the emergence of repetitive jaw movements. Adv. Exp. Med. Biol. 235:159–167; 1988.
25. Rosengarten, H.; Schweitzer, J. W.; Egawa, M.; Friedhoff, A. J. Prolonged fluphenazine decanoate treatment augments oral movements in rats: Mediation by $D_1$ receptor. Sixth Int. Catecholamine Symposium Abstracts; 1988:14.
26. Rupnak, N. M. J.; Jenner, P.; Marsden, C. D. The effects of chronic neuroleptic administration on cerebral dopamine receptor function. Life Sci. 32:2289–2323; 1983.
27. Schweitzer, J. W.; Rosengarten, H.; Friedhoff, A. J. Apparent bimodality of a dopamine receptor mediated behavior in the rat. IVth World Congress Biol. Psychiatr. Abstr.; 1985.
28. Traficante, L. J.; Friedman, E.; Oleshansky, M. A.; Gershon, S. Dopamine-sensitive adenylate cyclase and cAMP phosphodiesterase in substantia nigra and corpus striatum of rat brain. Life Sci. 19:1061–1066; 1976.
29. Uhrband, C.; Faurbye, A. Reversible and irreversible dyskinesia after treatment with perphenazine, chlorpromazine, reserpine and electroconvulsive therapy. Psychopharmacology (Berlin) 408–418; 1960.
30. Waddington, J. L. Tardive dyskinesia in schizophrenia and other disorders: Associations with aging, cognitive dysfunction and structural brain pathology in relation to neuroleptic exposure. Hum. Psychopharmacol. 2:11–22; 1987.
31. Waddington, J. L.; Gamble, S. J. Emergence of vacuous chewing during 6 months continuous treatment with fluphenazine decanoate. Eur. J. Pharmacol. 68:387–388; 1980.