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Pharmacological characterisation of spontaneous or drug-associated purposeless chewing movements in rats

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Abstract. Continuous administration of haloperidol, sulpiride, or *cis*-flupenthixol, but not of domperidone or apomorphine, to Wistar rats for up to 3 weeks caused an increase in spontaneous purposeless chewing movements. Treatment with physostigmine and pilocarpine, but not neostigmine, for up to 3 weeks increased chewing, whilst scopolamine decreased chewing. Metergoline and cyproheptadine, but not quipazine, increased chewing after only 1 and 7 days but not thereafter.

Chewing was not altered following treatment with compounds acting on GABA or noradrenaline systems or by a range of non-neuroleptic agents inducing dystonia in man.

The enhancement of chewing induced by neuroleptic and cholinomimetic drugs was reduced by acute treatment with scopolamine, and reverted to control levels following drug withdrawal.

Neuroleptic-induced purposeless chewing in Wistar rats appears to be primarily influenced by cerebral dopamine and acetylcholine function and may resemble acute dystonia, rather than tardive dyskinesia.

Key words: Dopamine – Acetylcholine – Acute dystonia – Peri-oral behaviour – Rat

Treatment of rats for weeks or months with neuroleptic drugs such as haloperidol, *cis*-flupenthixol, trifluoperazine, or fluphenazine causes an increase in perioral behaviours such as purposeless chewing jaw movements (Clow et al. 1979; Iversen et al. 1980; Waddington et al. 1983; Rupniak et al. 1983). Chewing movements in rats treated orally with neuroleptic drugs subside rapidly after withdrawal from neuroleptic treatment (Clow et al. 1980). They are reduced by concurrent administration of anticholinergic agents, and are exacerbated by cholinomimetic drugs, such as physostigmine (Yamada and Furukawa 1980; Gunne et al. 1982; Rupniak et al. 1983).

These findings correlate well with the characteristics of neuroleptic-induced acute dystonia observed in man and other primate species (Ayd 1961; Cochlin 1974; Gunne and Barany 1976, 1979; Meldrum et al. 1977; Casey et al. 1980; Liebman and Neale 1980; Porsolt and Jalfre 1981). However, in other respects, chewing behaviour in rats does not resemble neuroleptic-induced acute dystonia. Most strikingly, purposeless chewing merely represents an exaggeration of a normal rat's behavioural repertoire; bizarre postures and muscle spasms typical of acute dystonia do not appear to be induced by neuroleptic drugs in rodents. In addition, the administration of cholinomimetic agents, such as physostigmine, also increases chewing movements in naive rats (Rupniak et al. 1983); such compounds are not known to induce dystonia in man.

In the present study we have further examined the pharmacological similarity between purposeless chewing in rats and acute dystonia in man. We have examined chewing movements in Wistar rats treated with a wide range of compounds acting on either dopamine, acetylcholine, serotonin, GABA, or noradrenaline systems. In addition, we have examined whether treatment with other non-neuroleptic agents known to cause acute dystonia in man can also increase chewing behaviour in rats. Such compounds include the anticonvulsant diphenylhydantoin (Chadwick et al. 1976), antihistamines resembling diphenhydramine (Thach et al. 1975) and the antimalarial chloroquine (Bhargava et al. 1973; Akindele and Odejide 1976; Singhi et al. 1977; Umez-Eronini and Eronini 1977). Animals were observed over a 3-week period during continuous drug administration. The effects of acute treatment with the anticholinergic agent scopolamine and of drug withdrawal for 2 weeks on drug-induced chewing behaviour were examined in order to assess the similarity between these movements and acute dystonia. Our findings indicate that the pharmacological manipulation of chewing in Wistar rats closely resembled that for acute dystonia and that this behaviour is predominantly affected by alterations in cerebral dopamine and acetylcholine function.

Methods

Protocol. Seventy female Wistar rats (277 ± 16 g; Bantin and Kingman Ltd., Hull) were housed in groups of three to five and received one of 25 compounds acting on different neurotransmitter systems via their daily drinking water for a continuous period of 3 weeks. Drugged drinking water solutions were prepared fresh daily. Drugs known to be sensitive to photo-oxidation were presented in water bottles painted black. All animals had free access to food, and were housed and observed under standard laboratory conditions of temperature (21 ± 3° C) and lighting (12 h light/dark cycle). Housed alongside drug-treated animals were control rats which received distilled water alone.

Between 7 and 20 rats were treated with each individual drug. After 1, 7, 14, and 21 days of continuous drug treatment, animals were examined in comparison to

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untreated control rats for the prevalence of purposeless chewing movements. When chewing behaviour was altered following 3 weeks continuous drug treatment, the effect of a single acute treatment with scopolamine or of drug withdrawal also was examined.

All rats were maintained drug-free for at least 4 weeks following initial drug treatment and then were randomly reallocated to new drug treatment groups. Prior to the further drug treatment of each group, baseline chewing activity was assessed to ensure that it did not differ from that in the untreated control animals. Each rat received a total of three or four periods of 3-week treatment, but was always naive to the drug under investigation.

Assessment of chewing behaviour. Rats were observed individually on a clean table area measuring 45 × 15 cm between 8.00 and 18.00 h. Following a 2-min acclimatisation period, the number of individual chewing jaw movements was recorded during a 5-min test period. Movements were recorded only if they appeared to be purposeless, that is, if they were not directed towards specific objects.

Drugs administered to manipulate purposeless chewing movements: dopamine receptor agonists and antagonists. Rats were treated with the neuroleptic drugs haloperidol (2 mg/kg/day; Janssen Pharmaceutica), sulpiride (100 mg/kg/day; SESIF) or *cis*-flupenthixol hydrochloride (1 mg/kg/day; Lundbeck). Target doses were in ratio to the therapeutic doses used in the control of schizophrenia (see Titeler and Seeman 1980), increased by an arbitrary factor of five times. The peripheral dopamine receptor antagonists domperidone (2 mg/kg/day; Janssen Pharmaceutica) was given in a dose which, when given acutely, antagonises apomorphine-induced hypothermia in rats (Arneric et al. 1982). The dopamine agonist apomorphine hydrochloride (5 mg/kg/day; Sigma) was used in a dose which when given acutely induces intense sterotyped behaviour in rats (Ernst 1967).

Haloperidol and domperidone were dissolved in a minimum quantity of glacial acetic acid and diluted with distilled water to give a stock solution of 10 mg/ml. Sulpiride was dissolved in a minimum volume of 2% v/v sulphuric acid, and diluted with distilled water to give a stock solution of 50 mg/ml. The pH of each stock solution was brought to 5.5–7.0 using 2 N sodium hydroxide. Stock solutions were further diluted with distilled water and presented to rats as drinking water. *cis*-Flupenthixol hydrochloride and apomorphine hydrochloride were dissolved directly in distilled water. An amount of sodium metabisulphite equivalent to 20% of the weight of the drug was added to apomorphine solutions to retard oxidation.

Drugs acting on cholinergic systems. The anticholinesterase physostigmine salicylate (0.2 mg/kg/day), the muscarinic agonist pilocarpine (4 mg/kg/day), and an anticholinesterase penetrating poorly into the brain, neostigmine bromide (0.2 mg/kg/day), were studied. Decreased cholinergic activity was produced by treatment with the antimuscarinic agent scopolamine hydrobromide (0.5 mg/kg/day). Target drug doses were based on the activities of these compounds on yawning in rats, a behaviour under central cholinergic control (Yamada and Furukawa 1980). All drugs were obtained from Sigma, and were dissolved directly in distilled water.

Drugs acting on serotonin systems. The serotoninergic antagonists metergoline (Farmitalia) and cyproheptadine hydrochloride (Sigma) and the agonist quipazine maleate (Miles Scientific) were all given in a target dose of 10 mg/kg/day, derived from the activities of these compounds on myoclonus in guinea pigs (Luscombe et al. 1981).

Metergoline was mixed with an equal weight of tartaric acid and dissolved in a minimum volume of 70% alcohol prior to dilution with distilled water. Cyproheptadine hydrochloride and quipazine maleate were dissolved directly in distilled water.

Drugs acting on noradrenaline receptors. The α-adrenergic agonist clonidine hydrochloride (Sigma) was given in a target dose of 0.1 mg/kg/day, an amount capable of activating the rat cardiodepressor reflex. The α-antagonist phentolamine mesylate (Ciba) was administered in a target dose of 2 mg/kg/day which acutely blocks the effect of clonidine in the rat cardiodepressor reflex (Kobinger and Pichler 1975). The non-selective β-adrenergic antagonists propranolol hydrochloride (Inderal; Burroughs Wellcome), the β_1 selective antagonist metoprolol tartrate (Astra Pharmaceuticals), the β_2 selective antagonist butoxamine hydrochloride (Burroughs Wellcome), and the cardioselective β_1 antagonist atenolol (Burroughs Wellcome) were all administered in a target dose of 20 mg/kg/day, based on their centrally-induced hypotensive activity (Garvey and Ram 1975).

Clonidine hydrochloride, propranolol hydrochloride, atenolol, butoxamine hydrochloride, and metoprolol tartrate were all freely soluble in distilled water. Phentolamine mesylate was obtained in 10 mg/ml ampoules (Rogitine) and diluted in distilled water.

Drugs acting on GABA systems. The GABA antagonist picrotoxin (Sigma) was administered in a subtoxic target dose of 2 mg/kg/day, assessed from its ability to induce seizures in mice following acute systemic administration (Schechter and Tranier 1977). The GABA agonist muscimol (Sigma) was given in a target dose of 0.1 mg/kg/day by reference to its cataleptogenic effect in mice (Biggio et al. 1977). Diazepam (5 mg/kg/day; Roche Products Ltd.) was administered to enhance GABA function in a dose effective in the rotorod test in mice (Krall et al. 1978).

Picrotoxin and muscimol were dissolved directly in distilled water. Diazepam was obtained in 10 mg/ml ampoules (Valium) and diluted with distilled water.

Other drugs. The anticonvulsant phenobarbitone (May and Baker) and diphenylhydantoin (Parke, Davis) were administered in a target dose of 40 mg/kg/day. This dose is effective in the rotorod test in mice (Krall et al. 1978). The antihistamine diphenhydramine hydrochloride (Parke, Davis) was given in a target dose of 20 mg/kg/day which is capable of reversing haloperidol-induced suppression of self-stimulation in rats (Carey 1982). The antimalarial chloroquine diphosphate (20 mg/kg/day; Sigma) was given in a dose previously shown to penetrate into rat brain (Osifo 1979).

Phenobarbitone was obtained in ampoule form (200 mg/ml; Gardenal sodium) and diluted with distilled

water. Diphenylhydantoin was obtained in ampoules of 250 mg/5 ml (Epanutin) and diluted in distilled water. Diphenhydramine hydrochloride (Benadryl) and chloroquine diphosphate were dissolved directly in distilled water.

Reversal of drug-induced chewing behaviours. When drug treatment for 3 weeks altered the frequency of chewing movements, by comparison to control rats, chewing behaviour was further examined in some rats after 23 days of continuous drug treatment immediately before and 50 min following systemic administration of scopolamine hydrobromide (0.5 mg/kg IP). Other rats were withdrawn from drug treatment and given normal drinking water for a further 2 weeks prior to re-assessment of chewing behaviour by comparison to untreated control rats.

Statistical analysis. Overall group differences between control and drug-treated animals were determined using the Kruskal-Wallis analysis of variance of ranks. In cases where the resulting H scores were associated with a probability of less than 5%, groups were then compared by paired Mann-Whitney U-test. Results are expressed as the mean number of chewing jaw movements (± 1 SEM) during a 5-min test period for a minimum of seven rats per drug treatment.

Results

Drug intake. Drug solutions were readily acceptable to rats as drinking water. Mean drug intakes achieved varied from 75% to 150% of the target drug dose. Drug intakes based on the daily consumption of drinking water by rats during the 3-week period are listed in Table 1. After 3 weeks continuous treatment with the various drugs, rats did not differ in body weight from control animals and appeared in good health.

Drugs acting on dopamine receptors. Treatment of rats with haloperidol (1.5–1.9 mg/kg/day), sulpiride (104–150 mg/kg/day) or *cis*-flupenthixol (0.8–1.2 mg/kg/day) for between 1 and 21 days caused an approximate doubling in the frequency of purposeless chewing jaw movements by comparison to untreated control animals (Fig. 1). In contrast, treatment with domperidone (2.6–3.6 mg/kg/day), did not alter chewing behaviour. Similarly, apomorphine (5.5–5.9 mg/kg/day) did not alter chewing behaviour at any time by comparison to control rats (Fig. 1).

Drugs acting on cholinergic systems. Administration of physostigmine (0.27–0.35 mg/kg/day) or pilocarpine (4.2–5.6 mg/kg/day) for between 1 and 21 days caused more than a doubling in the frequency of chewing jaw movement (Fig. 2). In contrast, neostigmine (0.21–0.25 mg/kg/day) did not affect chewing movements at any time (Fig. 2). Scopolamine (0.42–0.46 mg/kg/day) generally decreased chewing movements below control levels, although this only reached statistical significance after 1 and 7 days of treatment (Fig. 2).

Drugs acting on serotonin receptors. Administration of metergoline (7.2–12.6 mg/kg/day) or cyproheptadine (7.2–8.2 mg/kg/day) for 1 and 7 days increased chewing movements. After 14 and 21 days of treatment, values remained higher than those observed in control rats, but this no longer reached statistical significance (Table 2). Administration of quipazine (9.8–11.4 mg/kg/day) for up to 3 weeks did not alter chewing movements by comparison to control rats (Table 2).

Table 1. Estimated intakes of various compounds administered to rats via daily drinking water continuously for 3 weeks

Neurotransmitter system and drug	Target dose (mg/kg/day PO)	Drug intake achieved (mg/kg/day PO)
Dopamine		
Haloperidol	2	1.5 – 1.9
Sulpiride	100	104 –150
cis-Flupenthixol	1	0.8 – 1.2
Domperidone	2	2.6 – 3.6
Apomorphine	5	5.5 – 5.9
Acetylcholine		
Physostigmine	0.2	0.27– 0.35
Pilocarpine	4	4.2 – 5.6
Neostigmine	0.2	0.21– 0.25
Scopolamine	0.5	0.42– 0.46
Serotonin		
Metergoline	10	7.2 – 12.6
Cyproheptadine	10	7.2 – 8.2
Quipazine	10	9.8 – 11.5
Noradrenaline		
Clonidine	0.1	0.07– 0.09
Phentolamine	2	1.8 – 2.5
Propranolol	20	15.7 – 17.9
Metoprolol	20	22.3 – 29.7
Butoxamine	20	14.9 – 25.9
Atenolol	20	21.9 – 27.1
GABA		
Picrotoxin	2	2.1 – 2.3
Muscimol	0.1	0.10– 0.12
Diazepam	5	5.6 – 5.7
Other		
Phenobarbitone	40	46.1 – 50.7
Diphenylhydantoin	40	32.8 – 45.2
Diphenhydramine	20	18.9 – 21.9
Chloroquine	20	19.9 – 23.1

Achieved drug intakes are mean ± 1 SEM values obtained over a 3 week period. Values are derived from volumes of drugged drinking water consumed per cage of three to five rats over a 24-h period

Drugs acting on noradrenaline receptors. Administration of clonidine (0.07–0.09 mg/kg/day) or phentolamine (1.8–2.5 mg/kg/day) did not alter purposeless chewing movements over the 3-week period. Similarly, propranolol (15.7–17.9 mg/kg/day) failed to significantly alter chewing behaviour, although values tended consistently to be higher than those in control rats over the 3 weeks of treatment. Metoprolol (22.3–29.7 mg/kg/day), butoxamine (14.9–25.9 mg/kg/day) or atenolol (21.9–27.1 mg/kg/day) administration for up to 21 days did not alter chewing movements (Table 3).

Drugs altering GABA function. Treatment of rats for up to 3 weeks with either picrotoxin (2.1–2.3 mg/kg/day), mus-

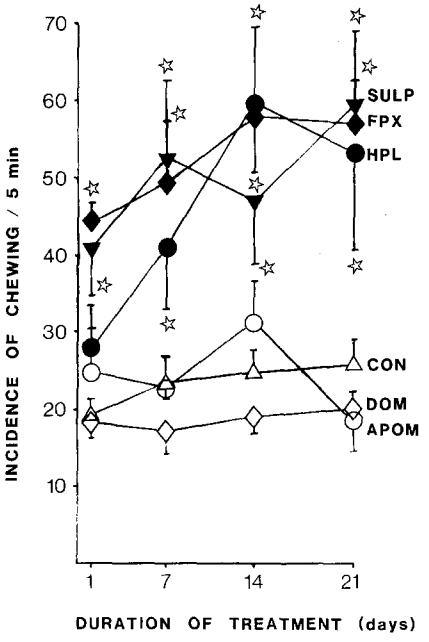

Fig. 1. Chewing jaw movements in rats treated continuously via daily drinking water with compounds acting on dopamine systems for up to 3 weeks, compared to control animals. Values are the mean ± 1 SEM for observations during a 5-min test perod. n = 7–17. Animals received either haloperidol (*HPL*), sulpiride (*SULP*), *cis*-flupenthixol (*FPX*), domperidone (*DOM*), or apomorphine (*APOM*) via their daily drinking water continuously for 3 weeks. See Table 1 for drug consumption over the 3-week period. Control rats (*CON*) received distilled drinking water alone. Overall group differences were determined using the Kruskal-Wallis analysis of variance of ranks. The following H scores and associated probabilities were obtained: 1 day, H = 20.49, $P < 0.01$, 7 days, $H = 20.32$, $P < 0.01$; 14 days, $H = 24.32$, $P < 0.001$; 21 days, $H = 29.03$, $P < 0.001$. In cases where H scores were associated with $P < 0.05$, groups were compared by Mann-Whitney U-tests. *$P < 0.05$ vs control, Mann-Whitney U-test

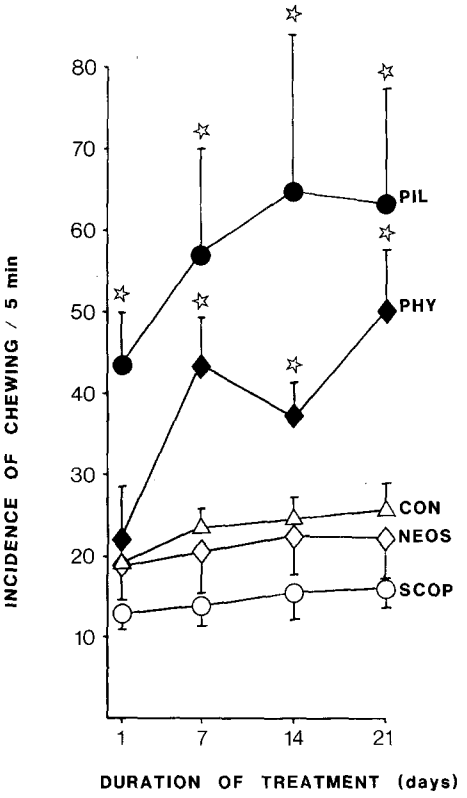

Fig. 2. Chewing jaw movements in rats treated continuously via daily drinking water with compounds acting on acetylcholine systems for up to 3 weeks compared to control animals. Values are the mean ± 1 SEM for observations during a 5-min test period. n = 6–17. Animals received either physostigmine (*PHY*), pilocarpine (*PIL*), neostigmine (*NEOS*) or scopolamine (*SCOP*) via their daily drinking water continuously for 3 weeks. See Table 1 for drug consumption over the 3-week period. Control rats (*CON*) received distilled drinking water alone. Overall group differences were determined using the Kruskal-Wallis analysis of variance of ranks. The following H scores and associated probabilities were obtained: 1 day, H = 12.43, $P < 0.05$; 7 days, $H = 17.40$, $P < 0.01$; 14 days, H = 18.48, $P < 0.001$; 21 days, $H = 20.12$, $P < 0.001$. In cases where resulting H scores were associated with $P < 0.05$, groups were compared by Mann-Whitney U-tests. *$P < 0.05$ vs control, Mann-Whitney U-test

Table 2. Chewing jaw movements in rats treated continuously via daily drinking water with compounds acting on serotonin systems for up to 3 weeks compared to control animals

Chronic treatment	Incidence of chewing/5 min and duration of treatment (days)			
	1	7	14	21
Control	18.3 ± 2.1	21.9 ± 2.6	23.2 ± 3.0	26.7 ± 4.1
Metergoline	41.4 ± 8.4*	36.3 ± 7.4*	35.3 ± 7.2	38.0 ± 7.4
Cyproheptadine	35.1 ± 4.9*	37.1 ± 3.8*	30.3 ± 3.7	32.1 ± 4.4
Quipazine	18.1 ± 2.8	28.3 ± 4.6	21.7 ± 3.9	21.4 ± 5.5
H score	13.75	9.13	4.16	4.42
P	< 0.01	< 0.05	< 0.05	< 0.05

Values are the mean ± 1 SEM for observations during a 5-min test period. n = 7–12. See Table 1 for drug consumption over the 3-week period. Control rats received distilled drinking water alone. Overall group differences were determined using the Kruskal-Wallis analysis of variance of ranks. In cases where resulting H scores were associated with $P < 0.05$, groups were compared by Mann-Whitney U-tests

* $P < 0.05$ vs control, Mann-Whitney U-test

cimol (0.10–0.12 mg/kg/day) or diazepam (5.6–5.7 mg/kg/day) was without effect on chewing jaw movements compared to control animals (Table 4).

Other drugs. Treament of rats for up to 3 weeks with phenobarbitone (46.1–50.7 mg/kg/day), diphenhydramine (18.9–21.9 mg/kg/day) or chloroquine (19.9–23.1 mg/kg/day) had no effect on chewing movements by comparison to control values (Table 5). Administration of diphenylhydantoin (32.8–45.2 mg/kg/day) tended to increase chewing behaviour, but this effect reached statistical significance only on day 1 but not thereafter (Table 5).

Reversal of drug induced perioral movements; (a) acute anticholinergic treatment. The increased frequency of chewing movements observed in rats treated for 23 days

Table 3. Chewing jaw movements in rats treated continuously via daily drinking water with compounds acting on noradrenaline systems for up to 3 weeks compared to control animals

Chronic treatment	Incidence of chewing/5 min and duration of treatment (days)			
	1	7	14	21
Control	17.5 ± 1.6	22.6 ± 2.5	23.9 ± 2.8	23.5 ± 3.4
Clonidine	15.9 ± 2.2	43.1 ± 7.5	29.6 ± 4.7	30.0 ± 2.9
Phentolamine	25.1 ± 8.5	22.3 ± 5.6	18.2 ± 5.0	19.3 ± 5.4
Propranolol	34.1 ± 6.4	39.9 ± 6.4	39.8 ± 5.2	32.7 ± 4.7
Metoprolol	23.6 ± 4.4	31.1 ± 5.2	23.9 ± 2.9	30.6 ± 4.4
Butoxamine	25.7 ± 5.2	29.9 ± 7.6	23.7 ± 5.9	28.9 ± 7.3
Atenolol	24.0 ± 5.2	25.7 ± 3.3	25.1 ± 5.2	25.7 ± 5.5
H score	7.56	10.04	9.72	7.22
P	> 0.05	> 0.05	> 0.05	> 0.05

Values are the mean ± 1 SEM for observations during a 5-min test period. See Table 1 for drug consumption over the 3-week period. Control rats received distilled drinking water alone. No overall group differences were obtained using the Kruskal-Wallis analysis of variance of ranks

Table 4. Chewing jaw movements in rats treated continuously via daily drinking water with compounds acting on GABA systems for up to 3 weeks compared to control animals

Chronic treatment	Incidence of chewing/5 min and duration of treatment (days)			
	1	7	14	21
Control	18.3 ± 2.1	21.9 ± 2.6	23.2 ± 3.0	26.7 ± 4.1
Picrotoxin	30.0 ± 2.1	28.4 ± 7.2	28.6 ± 5.9	31.0 ± 4.2
Muscimol	20.1 ± 4.5	25.9 ± 3.4	35.0 ± 7.8	21.4 ± 2.6
Diazepam	25.9 ± 6.5	31.1 ± 6.2	23.1 ± 4.0	31.1 ± 5.4
H score	2.73	1.68	2.47	2.60
P	> 0.05	> 0.05	> 0.05	> 0.05

Values are the mean ± 1 SEM for observations during a 5-min test period. $n = 7–12$. See Table 1 for drug consumption over the 3-week period. Control rats received distilled drinking water alone. No overall group differences were obtained using the Kruskal-Wallis analysis of variance of ranks

Table 5. Chewing jaw movements in rats treated continuously via daily drinking water with compounds acting on various neurotransmitter systems for up to 3 weeks compared to control animals

Chronic treatment	Incidence of chewing/5 min and duration of treatment (days)			
	1	7	14	21
Control	18.2 ± 1.9	24.1 ± 2.5	24.9 ± 2.5	25.1 ± 2.5
Phenobarbitone	14.5 ± 2.9	25.2 ± 4.8	32.5 ± 6.0	32.9 ± 4.5
Diphenylhydantoin	35.1 ± 4.0*	30.5 ± 4.0	36.5 ± 4.2	31.8 ± 3.6
Diphenhydramine	28.1 ± 5.2	38.1 ± 6.1	30.4 ± 3.5	32.4 ± 3.4
Chloroquine	18.4 ± 1.9	20.3 ± 3.7	23.7 ± 4.6	18.6 ± 5.0
H score	18.41	6.83	5.39	8.92
P	> 0.01	> 0.05	> 0.05	> 0.05

Values are the mean ± 1 SEM for observations during a 5-min test period. $n = 7–20$. See Table 1 for drug consumption over the 3-week period. Control rats received distilled drinking water alone. Overall group differences were determined using the Kruskal-Wallis analysis of variance of ranks. In cases where resulting *H* scores were associated with $P < 0,05$, groups were compared by Mann-Whitney *U*-test
*$P < 0.05$ vs control, Mann-Whitney *U*-test

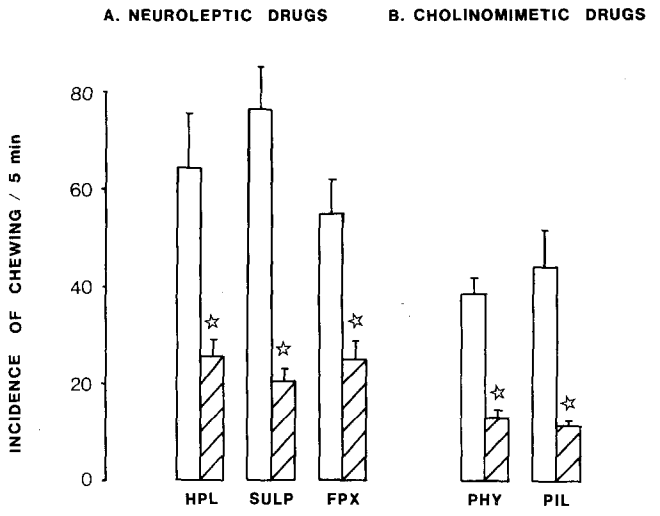

Fig. 3. Chewing jaw movements in rats treated with *A* neuroleptic or *B* cholinomimetic drugs continuously for 23 days before and after acute challenge with scopolamine (0.5 mg/kg IP). Values are the mean ± 1 SEM for observations during a 5-min test period. $n = 7$. See Table 1 for drug consumption over a 3-week period. The same animals were observed before (*open bars*) and 50 min after administration of scopolamine (0.5 mg/kg IP) (*hatched bars*). Overall differences before and after scopolamine treatment were determined using the Kruskal-Wallis analysis of variance of ranks. The following H scores and associated probabilities were obtained: neuroleptic drugs, $H = 27.91$, $P < 0.001$; cholinomimetic drugs, $H = 19.90$, $P < 0.001$. In cases where H scores were associated with $P < 0.05$, groups were compared by Mann-Whitney U-test. $^*P < 0.05$, before vs following scopolamine treatment, Mann-Whitney U-test

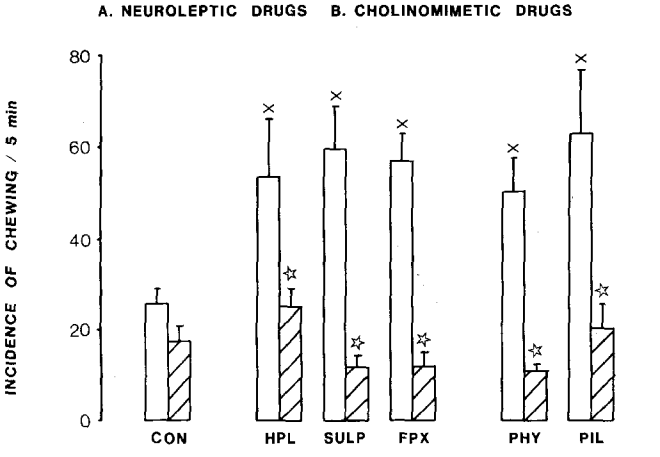

Fig. 4. Chewing jaw movements in rats treated with *A* neuroleptic or *B* cholinomimetic drugs continuously for 21 days and following drug withdrawal for 14 days compared with control animals. Values are the mean ± 1 SEM for observations during a 5-min test period. $n = 6-15$. See Table 1 for drug consumption during the 3-week period. Animals were observed after 21 days drug treatment (*open bars*) and following drug withdrawal for 14 days (*hatched bars*). Overall group differences were determined using the Kruskal-Wallis analysis of variance of ranks. The following H scores and associated probabilities were obtained: on drug, $H = 21.96$, $P < 0.001$; off drug, $H = 8.24$, $P > 0.05$. Neuroleptic drugs (on and off drug), $H = 31.39$, $P < 0.001$; cholinomimetic drugs (on and off drug), $H = 18.68$, $P < 0.001$. Where H scores were associated with $P < 0.05$, groups were compared by Mann-Whitney U-tests. $^*P < 0.05$ vs control, Mann-Whitney U-test. x $P < 0.05$ on drug vs following drug withdrawal, Mann-Whitney U-test

with haloperidol, sulpiride, *cis*-flupenthixol, physostigmine, or pilocarpine was reversed by acute administration of scopolamine (0.5 mg/kg IP, 50 min previously). Drug-induced chewing movements were reduced by more than 50% of their previous levels in all drug treatment groups (Fig. 3).

(b) Drug withdrawal. The exaggeration of chewing movements in rats treated for 3 weeks with haloperidol, sulpiride, *cis*-flupenthixol, physostigmine, or pilocarpine was not maintained following 2 weeks withdrawal from drug treatment. After withdrawal, perioral behaviour did not differ in these animals from values in untreated control rats (Fig. 4).

Discussion

In agreement with previous studies (Glassman and Glassman 1980; Gunne et al. 1982; Rupniak et al. 1983), we find that administration of the neuroleptic drugs haloperidol, sulpiride, and *cis*-flupenthixol for up to 3 weeks increased the frequency of purposeless chewing movements in normal rats. In the present study the increased number of movements was apparent within 1 or 7 days of commencing neuroleptic treatment. Previously, a single intraperitoneal dose of haloperidol has been reported to increase chewing movements in normal rats (Glassman and Glassman 1980). In contrast, administration of the peripheral dopamine receptor antagonist domperidone did not alter perioral behaviour, indicating that neuroleptic-induced movements are mediated via an action on cerebral dopamine receptors. As has been reported previously, the neuroleptic-induced chewing did not persist following drug withdrawal (Clow et al. 1980) and was reversed following acute treatment with the anticholinergic agents atropine or scopolamine (Gunne et al. 1982; Rupniak et al. 1983). These findings resemble the known time course and pharmacology of neuroleptic-induced acute dystonia in man and other primates (Ayd 1961; Cochlin 1974; Gunne and Barany 1976, 1979; Meldrum et al. 1977; Liebman and Neale 1981; Porsolt and Jalfre 1981).

The mechanisms by which neuroleptic drugs induce acute dystonia are not known, but have been related to the compensatory increase in dopamine release following postsynaptic receptor blockade (see Meldrum et al. 1977, but see Neale et al. 1984, for the opposite view, which is discussed below). Thus, pretreatment of baboons with a combination of the amine depletor reserpine and the synthesis inhibitor alpha-methyl-para-tyrosine reduced the severity and duration of neuroleptic-induced acute dystonia (Meldrum et al. 1977). Increased cerebral dopamine release is further implicated in the genesis of acute dystonia by observations that L-dopa treatment of Parkinsonian patients (Parkes et al. 1976) and primates (Paulson 1973) may provoke acute dystonia, and by the ability of focal intrastriatal injections of dopamine in primates to induce dystonia (Cools et al. 1974). Similarly, in rats treated for up to 1 month with L-dopa, Pycock et al. (1982) reported an increase in chewing movements, although this behaviour was not quantified. However, in the present study, oral administration of apomorphine did not alter chewing behaviour in rats. These findings may be related to the short half-life of this compound since, in a previous study, acute IP administration of apomorphine (0.5–4.0 mg/kg) to

rats induced chewing within 20 min (Yamada and Furukawa 1980). Acute systemic administration of apomorphine is also reported to exaggerate neuroleptic-induced chewing movements (Glassman and Glassman 1980; Waddington and Gamble 1980). Thus, an increase in dopaminergic activity may be related to the appearance of neuroleptic-induced dystonia in man and other primates, and to neuroleptic-induced chewing in rats. However, other evidence has recently emerged in primate studies that a dopamine deficiency, rather than excess, may underlie acute dystonia. Thus, the administration of the amine depleting agents, alpha-methyl-para-tyrosine and tetrabenazine, like neuroleptic drugs, could induce acute dystonia in squirrel monkeys (Neale et al. 1984). Further characterisation of the role of central dopamine function in the elicitation of the these movements is, therefore, required.

Like neuroleptic drugs, administration of the cholinomimetic agents physostigmine and pilocarpine, but not the peripheral anticholinesterase neostigmine, induced an exaggeration of chewing movements in rats as previously reported (Rupniak et al. 1983). These movements were also reversed following 2 weeks' drug withdrawal or by acute concurrent treatment with scopolamine. The frequency of chewing movements in rats appears to be directly related to alterations in cholinergic function, since treatment of naive rats with scopolamine also decreased chewing behaviour as we have previously observed following a single dose (Rupniak et al. 1983). An overactivity of cholinergic function has also been implicated in the genesis of acute dystonia (Neale et al. 1984). Although there is no direct evidence to indicate that cholinomimetics induce acute dystonia in man, such agents may induce dystonia when given systemically in susceptible primates (Neale et al. 1984) or following intrastriatal application in primates (Murphey and Dill 1972; Cools et al. 1974). It is of considerable interest that the intrastriatal injection of carbachol or acetylcholine in rats induced purposeless chewing movements (Dill et al. 1968). Moreover, peripheral co-administration of cholinomimetic agents exaggerates both neuroleptic-induced chewing in rats (Yamada and Furukawa 1980; Gunne et al. 1982; Rupniak et al. 1983) and neuroleptic-induced acute dystonia in primates (Meldrum et al. 1977; Casey et al. 1980). These findings suggest that an increase in both dopamine and acetylcholine function in the brain are directly involved in the manifestation of acute dystonic reactions and chewing movements in rats.

In general, manipulation of other neuronal systems had little consistent effect on perioral chewing movements. Exceptionally, the serotonin antagonists metergoline and cyproheptadine, but not the agonist quipazine, increased chewing after 1 and 7 days of treatment. However, these movements were less intense than with neuroleptic or cholinomimetic agents, and were not maintained. These findings are perhaps not surprising in view of the close interaction between serotonin and dopamine systems in the brain (Carter and Pycock 1981). These agents are not known to induce dystonia in man and intrastriatal administration of serotonin in rats does not elicit dyskinetic movements (Dill et al. 1968). Agents acting on noradrenaline receptors were also largely without effect on mouth movements, with the exception of propranolol which tended to increase chewing by comparison to control rats. This effect appears unlikely to be mediated by a direct action on noradrenaline systems, but may be related to the action of propranolol on serotonin systems which is not observed with β_1 or β_2-selective antagonists (Costain and Green 1978). Thus, the intrastriatal application of noradrenaline was not reported to induce chewing in rats (Dill et al. 1968). In addition, the noradrenergic agonist clonidine and antagonist propranolol did not alter haloperidol-induced acute dystonia in primates (Neale et al. 1984).

Similarly, administration of agents acting on GABA systems (diazepam, picrotoxin, and muscimol) did not affect perioral chewing in naive rats. Likewise, administration of diazepam did not induce acute dystonia in primates (Neale et al. 1984). Movements also were not affected by administration of phenobarbitone, indicating that chewing may not be induced as a result of sedative actions of drugs. In contrast, compounds enhancing GABA transmission, such as gabaculline, baclofen, and diazepam may reduce neuroleptic-induced dystonia in man and primates (Ramsden and Frogatt 1972; Neale et al. 1983). Such effects may be related to the alteration of dopamine metabolism in the brain (see Biggio et al. 1977).

Finally, administration of the non-neuroleptic agents chloroquine and diphenhydramine which have on rare occasions been observed to induce acute dystonic reactions in man (Bhargava et al. 1973; Thach et al. 1975) did not alter chewing in rats. The mechanisms by which these agents induce dystonia in man are not known, but appear not to be related to an action on dopamine systems. Diphenylhydantoin, an agent also inducing dystonia in man (Chadwick et al. 1976), did transiently increase chewing in rats. Of this group of compounds, dipenylhydantoin is the only one known to possess dopamine antagonist activity in rodents, although this is weak (Elliot et al. 1977).

In summary, the manipulation of central dopamine and acetylcholine function, as assessed in the present study, may influence neuroleptic-induced chewing movements in Wistar rats in a manner comparable to acute dystonia in primate species. In general, agents acting on other neurotransmitter systems did not induce persistent alterations of chewing behaviour. These findings indicate that this behaviour may represent a rodent model in which the mechanisms of neuroleptic-induced *acute dystonia* might be further investigated.

The conclusions of the present study are at variance with some other similar investigations. There are reasons for regarding the neuroleptic-induced perioral movements examined by Waddington et al. (1983) and Gunne et al. (1982) as being distinct from those measured in the present study. There is agreement between the groups of Waddington and Gunne (personal communication) that the movements they have observed are essentially the same. These comprise side-to-side chewing movements, involving audible teeth grinding, which are accompanied by mouth opening only in severe cases. This contrasts with the results of our present and previous study (Rupniak et al. 1983) and that of Glassman and Glassman (1980) which found that teeth grinding was not a frequent component of neuroleptic-induced perioral behaviour, but that chewing (with the mouth open or closed) was the commonest behavioural component. There are also differences in the normal incidence of various types of movements in the rat population. All Wistar rats in our population exhibited purposeless chewing movements prior to drug treatment,

and these were exacerbated by neuroleptic administration. In contrast, teeth grinding was infrequent in control animals in the studies by Gunne's and Waddington's groups. This may to some extent relate to rat strain; in particular the studies of Waddington and colleagues have utilised Sprague Dawley rats whereas we employed the Wistar strain. Differences also exist in the time course over which the perioral movements were observed in these various studies. In our investigations the increase in mouth movements was of rapid onset and did not persist following drug withdrawal (Rupniak et al. 1983; Clow et al. 1980; present study). In contrast, both Waddington et al. (1983) and Gunne et al. (1982) found the mouth movements observed in their studies to increase only after some weeks or months of neuroleptic treatment, and to persist following drug withdrawal. As in the present investigation, Gunne et al. (1982) employed Wistar rats, but they used depot neuroleptic preparations which may have contributed to the persistence of chewing movements.

These observations suggest that the specific components of perioral behaviour examined in different studies (chewing versus teeth grinding) differ in their response to neuroleptic treatment. The characteristics of the neuroleptic-induced teeth grinding studies by Waddington et al. (1983) and Gunne et al. (1982) have led these authors to suggest that their protocols provide a potential animal model for the investigation of *tardive dyskinesia* rather than acute dystonia. Some discrepancies are, however, apparent. Thus, Waddington et al. (1983) did not find an exacerbation of teeth grinding in haloperidol treated rats, in contrast to other neuroleptics. Also, Gunne et al. (1982) found the perioral behaviour in their study to be decreased by anticholinergic drugs.

In conclusion, various perioral movements induced by chronic neuroleptic treatment in rodents may represent spontaneous correlates of some neuroleptic-induced extrapyramidal side-effects in man, but further critical analysis of the details of these behaviours is required to establish whether they are representative of acute dystonia or chronic tardive dyskinesia.

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References

Akindele O, Odejide AO (1976) Amodiaquine-induced involuntary movements. Br Med J 6029: 213–215

Arneric SP, Collins ED, Bhatnagar RK, Lond JP (1982) Is domperidone a selective peripheral dopamine receptor antagonist in vivo? Neuropharmacology 21: 1317–1321

Ayd FJ (1961) A survey of drug-induced extrapyramidal reactions. J Am Med Assoc 175: 1054–1060

Bhargava RK, Parakh LK, Hakim A, Gori MN, Bhandari NC (1973) Extrapyramidal syndrome after antimalarials. J Assoc Physicions India 21: 969–973

Bibbio G, Casu M, Corda MG, Vernaleone F, Gessa GL (1977) Effect of muscimol, a GABA-mimetic agent, on dopamine metabolism in the mouse brain. Life Sci 21: 525–532

Carey RJ (1982) A comparison of atropine, benztropine and diphenhydramine on the reversal of haloperidol-induced suppression of self-stimulation. Pharmacol Biochem Behav 17: 851–854

Carter CJ, Pycock CJ (1981) The role of 5-hydroxytryptamine in dopamine-dependent sterotyped behaviour. Neuropharmacology 20: 261–265

Casey DE, Gerlach J, Christensson E (1980) Dopamine, acetylcholine and GABA effects in acute dystonia in primates. Psychopharmacology 70: 83–87

Chadwick D, Reynolds EH, Marsden CD (1976) Anticonvulsant-induced dyskinesias: a comparison with dyskinesias induced by neuroleptics. J Neurol Neurosurg Psychiatr 39: 1210–1218

Clow A, Jenner P, Marsden CD (1979) Changes in dopamine-mediated behaviour during one year's neuroleptic administration. Eur J Pharmacol 57: 365–375

Clow A, Theodorou A, Jenner P, Marsden CD (1980) Cerebral dopamine function in rats following withdrawal from one year of continuous neuroleptic administration. Eur J Pharmacol 63: 145–157

Cochlin DL (1974) Dystonic reactions due to metoclopramide and phenothiazines resembling tetanus. Br J Clin Pract 28: 201–202

Cools AR, Hendriks G, Korten J (1974) The acetylcholine-dopamine balance in the basal ganglia of rhesus monkeys and its role in dynamic dystonic, dyskinetic and epileptoid motor activities. J Neural Transm 36: 81–105

Costain DW, Green AR (1978) β-adrenoceptor antagonists inhibit the behavioural responses of rats to increased brain 5-hydroxytryptamine. Br J Pharmacol 64: 193–200

Dill RE, Nickey WM, Little MD (1968) Dyskinesias in rats following chemical stimulation of the neostriatum. Texas Reports Biol Med 26: 101–106

Elliot PNC, Jenner P, Chadwick D, Reynolds E, Marsden CD (1977) The effect of diphenylhydantoin on central catecholamine containing neuronal systems. J Pharm Pharmacol 29: 41–43

Ernst AM (1967) Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. Psychopharmacology 10: 316–323

Garvey HL, Ram V (1975) Central induced hypotensive effects of β-adrenergic blocking drugs. Eur J Pharmacol 33: 283–294

Glassman RB, Glassman HN (1980) Oral dyskinesia in brain-damaged rats withdrawn from a neuroleptic: implication for models of tardive dyskinesia. Psychopharmacology 69: 19–25

Gunne L-M, Barany S (1976) Haloperidol-induced tardive dyskinesia in monkeys. Psychopharmacology 50: 237–240

Gunne L-M, Barany S (1979) A monitoring test for the liability of neuroleptic drugs to induce tardive dyskinesia. Psychopharmacology 63: 195–198

Gunne L-M, Growdon J, Glaeser B (1982) Oral dyskinesia in rats following brain lesions and neuroleptic drug administration. Psychopharmacology 77: 134–139

Iversen SD, Howells RB, Hughes RP (1980) Behavioural consequences of long-term treatment with neuroleptic drugs. Adv Biochem Psychopharmacol 24: 305–313

Kobinger W, Pichler L (1975) The central modulatory effect of clonidine on the cardiodepressor reflex after suppression of synthesis and storage of noradrenaline. Eur J Pharmacol 30: 56–62

Krall RL, Penry JK, White BG, Kupferburg HJ, Swinyard EA (1978) Anti-epileptic drug development: II. Anticonvulsant drug screening. Epilepsia 19: 409–428

Liebman J, Neale R (1980) Neuroleptic-induced acute dyskinesias in squirrel monkeys: correlation with propensity to cause extra-pyramidal side effects. Psychopharmacology 68: 25–29

Luscombe G, Jenner P, Marsden CD (1981) Pharmacological analysis of the myoclonus produced by 5-hydroxytryptamine in the guinea pig suggests the presence of multiple 5-hydroxytryptamine receptors in the brain. Neuropharmacology 20: 819–831

Meldrum BS, Anlezark GM, Marsden CD (1977) Acute dystonia as an idiosyncratic response to neuroleptics in baboons. Brain 100: 313–326

Murphey DL, Dill RE (1972) Chemical stimulation of discrete brain loci as a method of producing dyskinesia models in primates. Exp Neurol 34: 244–254

Neale R, Gerhardt S, Liebman JM (1984) Effects of dopamine agonists, catecholamine depletors, and cholinergic and GABAergic drugs on acute dyskinesias in squirrel monkeys. Psychopharmacology 82: 20–26

Osifo NG (1979) Drug-related transient dyskinesias. Clin Pharmacol Ther 25: 767–771

Parkes JD, Bedard P, Marsden CD (1976) Chorea and torsion in Parkinsonism. Lancet 1: 155

Paulson GW (1973) Dyskinesias in monkeys. In Barbeau A, Chase TN, Paulsin GW (eds) Huntington's Chorea. Raven Press, New York. Adv Neurol 1: 647–650

Porsolt RD, Jalfre M (1981) Neuroleptic-induced acute dyskinesias in rhesus monkeys. Psychopharmacology 75: 16–21

Pycock C, Dawbarn D, O'Shaughnessy C (1982) Behavioural and biochemical changes following chronic administration of L-dopa to rats. Eur J Pharmacol 79: 201–215

Ramsden PD, Frogatt DL (1972) Idiosyncratic responses to phenothiazines. Br Med J 1: 246

Rupniak NMJ, Jenner P, Marsden CD (1983) Cholinergic manipulation of perioral behaviour induced by chronic neuroleptic administration to rats. Psychopharmacology 79: 226–230

Schechter PJ, Tranier Y (1977) Effect of elevated brain GABA concentrations on the actions of bicuculline and picrotoxin in mice. Psychopharmacology 54: 145–148

Singhi S, Singhi P, Singh M (1977) Chloroquine-induced involuntary movements. Br Med J 6085: 520

Thach BT, Chase TN, Bosma JF (1975) Oral facial dyskinesia associated with prolonged use of antihistamine decongestants. New Eng J Med 293: 486–487

Titeler M, Seeman P (1980) Radioreceptor labeling of pre- and postsynaptic dopamine receptors. In: Cattabeni F, Racagni G, Spano PF, Costa E (eds) Long-term effecs of neuroleptics. Raven Press, New York. Adv Biochem Psychopharmacol 24: 159–165

Umez-Eronini E, Eronini EA (1977) Chloroquine-induced involuntary movements. Br Med J 6066: 945–946

Waddington JL, Cross AJ, Gamble SJ, Bourne RC (1983) Spontaneous orofacial dyskinesia and dopaminergic function in rats after 6 months of neuroleptic treatment. Science 220: 530–532

Yamada K, Furukawa T (1980) Direct evidence for involvement of dopaminergic inhibition and cholinergic activation in yawning. Psychopharmacology 67: 39–43

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