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Cholinergic Mechanisms Involved in Head-Shaking of Infant Rats

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HOLMGREN, B. AND R. URBA-HOLMGREN. *Cholinergic mechanisms involved in head-shaking of infant rats.* PHARMAC. BIOCHEM. BEHAV. 7(6) 493–499, 1977. – Central cholinergic mechanisms involved in D-amphetamine induced head-shaking (H-S) were explored in 9-day-old albino rats using anticholinergic, anticholinesterase and cholinomimetic drugs. Scopolamine (5 mg/kg, IP) blocks both spontaneous and D-amphetamine induced H-S. Physostigmine (0.10 mg/kg, IP), but not neostigmine, increases D-amphetamine induced H-S up to 400%. Pilocarpine (1-10 mg/kg, IP) per se induces H-S and strongly potentiates the amphetamine H-S effect. Cholinergic – catecholaminergic interactions in the CNS are discussed in relation to the expression of this motor item.

Head-shaking Amphetamine Stereotyped behavior Scopolamine Cholinergic mechanisms
Physostigmine ACh-CA interactions Neostigmine Infant rats Pilocarpine

A HIGH proportion of infant albino rats (5 to 12-day-old) when injected with D-amphetamine, exhibit frequent episodes of head-shaking (H-S) [22]. This motor item is displayed very seldom in noninjected rats, even if it seems to be a part of the rat's normal motor repertoire [44]. These H-S episodes are characterized by quite regular rocking movements of the head, with clear predominance of rotatory components around the sagital axis, the oscillatory frequency of which increases with age from approximately 5–10 cps. [22].

Some authors [21, 36, 43, 46] have included head-shaking or very rapid and persistent small head movements or stereotyped movements of the head, generically among other stereotyped movements induced by amphetamine or dopaminergic agonists. Because of chronological and quantitative differences in H-S induced by D-amphetamine or apomorphine, and to the fact that the alpha-adrenergic receptors' blocking drug phenoxybenzamine can inhibit D-amphetamine induced H-S [23] but does not influence other stereotyped movements [1,23], head-shaking would appear as a motor item with a mixed dopaminergic-noradrenergic background, while perseverative oral or stereotyped sniffing motor patterns would seem to be more purely dopaminergic [2, 13, 48, 51].

From several lines of research suggestions have been advanced that distinct balances or interactions between catecholaminergic and cholinergic systems mediate different functions of the brain.

Thus, Aprison and Hintgen [3] have conceived that the level of behavioural excitation results from changes in balance between a telencephalic cholinergic system and mesencephalic adrenergic influences. Similar ideas have been used to explain anticholinergic potentiation of adrenergic arousal in developing rats [10,15]. An adequate function of the extrapyramidal motor system seems to depend on a balanced function of monoaminergic and cholinergic influences [4, 5, 26]. The theory of a cholinergic-dopaminergic imbalances (due to dopamine decrease) in the explanation of Parkinsonism, view first advanced by McGeer, Boulding, Gibson and Foulkes [40] and Barbeau [6], has nowadays many adepts (for recent reviews, see Klawans [30,31]). A imbalance in the opposite direction has been assumed for stereotyped movements induced by amphetamine, on which acetylcholine seems to exert a certain inhibitory effect, since anticholinergic agents enhance the amphetamine effect or restore already vanished stereotyped activity [16].

As head-shaking induced by D-amphetamine is a motor item that appears mixed together with stereotyped behavior, but by its tremorous characteristics might also be interpreted as an extrapyramidal disorder, it appeared logical to investigate if central cholinergic mechanisms are also somehow involved in it. If so, the administration of anticholinergic or cholinomimetic drugs might modify the quantitative expression of this motor pattern in opposite directions.

MATERIALS AND METHOD

The procedure used has been extensively described in previous papers [22,23]. As the maximal amphetamine induced H-S effects were observed at the 9th postnatal day, in this work only 9-day-old albino rats (Wistar strain) were used. The results are based on 80 litters, each of which was reduced to 8 rats 24–36 hr after birth. The experiments were performed in morning hours (8–10 a.m.) and the animals tested only once. After being injected, groups of two to four rats were placed in boxes (25 × 40 × 10 cms)

with vinyl plastic floors and observed by simple visual inspection during one hour. The following data were quantified: H-S occurrence, H-S episodes' duration, and total H-S time during the observation period.

Freshly prepared solutions in saline (sodium chloride, 0.9%) of the following drugs were used: D-amphetamine sulphate (Rhone-Poulenc) physostigmine salicylate (Sigma Chemical Co.)., neostigmine methylsulfate (Medexport), scopolamine hydrobromide (Merck), and pilocarpine hydrochloride (C. H. Boehringer). Controls were injected with saline. All drugs, except D-amphetamine sulphate, are expressed in mg/kg of the free base. Injections were carried out intraperitoneally, at a standard volume of 0.01 ml/g bodyweight. Animals from every litter were distributed at random between control and experimental groups. Statistical procedures are mentioned with the results.

RESULTS

A. *Experiments with Scopolamine*

When scopolamine is injected simultaneously with D-amphetamine in 9-day-old rats, a reduction in the head-shaking inducing effect of this last drug is observed. The antagonistic dose-effect curve is illustrated in Fig. 1, which also shows an inhibitory effect of scopolamine on spontaneous H-S. Although these results are statistically significant, as approximately a 50% of D-amphetamine injected rats do not present H-S, we felt that more clear-cut results should be obtained from animals with evident head-shaking behavior, induced by the injection of D-amphetamine 15 min before. The effect of scopolamine with this type of experimental design is shown in Fig. 2. With 5 mg/kg a significant restraining effect of scopolamine on H-S was observed. Higher doses, up to 10 mg/kg, did not increase this effect. A lower dose, of 2.5 mg/kg, was ineffective.

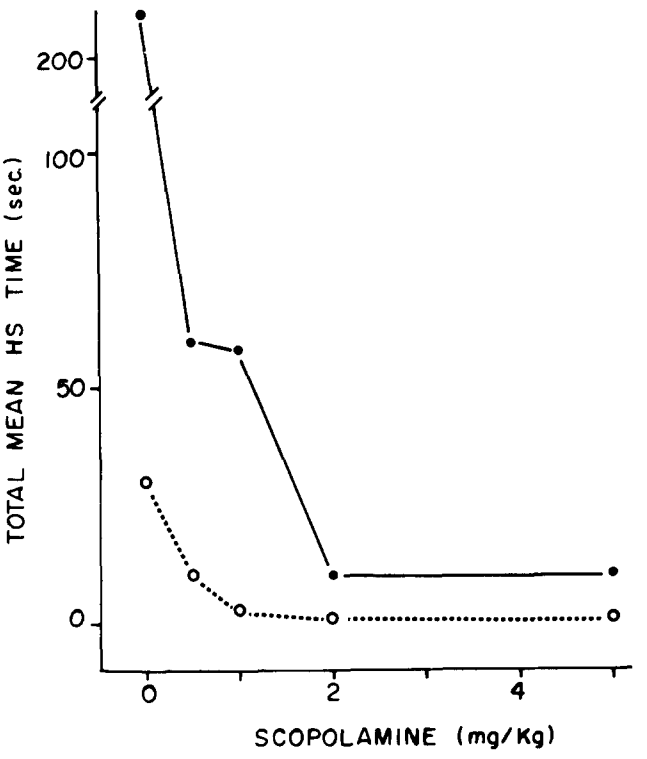

FIG. 1. Depressing action of scopolamine on spontaneous and amphetamine induced H-S. Dose-effect curves. Ordinate: H-S time in seconds during 1 hour. Abscissa: scopolamine dose in mg free base/kg bodyweight. ○-----○ scopolamine injected rats; ●—● rats injected with scopolamine + D-amphetamine (5 mg/kg). Each point corresponds to a mean value obtained from 25 nine-day-old rats. For spontaneous H-S the only statistically significant depression was obtained with 2 and 5 mg/kg ($p<0.01$ - Mann-Whitney U Test). For amphetamine induced H-S all scopolamine doses produced significant effects, as follows: 0.5 and 1 mg/kg, $p<0.05$; 2 mg/kg, $p<0.01$; 5 mg/kg, $p<0.001$.

B. *Experiments with Anticholinesterase Drugs*

A first series of experiments was performed in order to find an adequate dose of physostigmine for further more detailed analysis of its effect on H-S. A total of 70 rats, from 9 litters, were distributed in five groups, of 14 animals each. The control groups in this particular case were two: one was injected only with D-amphetamine (5 mg/kg), the other exclusively with physostigmine (0.10 mg/kg). The three test groups received physostigmine in doses equivalent to 0.05, 0.10 and 0.20 mg/kg simultaneously with D-amphetamine (5 mg/kg). The animals were observed only during 30 min after being injected. Table 1 shows the results obtained. Total H-S time is more than doubled with an adequate dose of physostigmine (0.10 mg/kg), with which slight muscle fasciculations may already be observed. With higher doses, rigidity and tremor apparently interfere with H-S, thus explaining the decrease in total mean H-S time observed. Physostigmine per se does not increase the spontaneous H-S observed in animals injected with saline.

When the effect of physostigmine (0.10 mg/kg) was followed during one hour, as shown in the experiment illustrated in Fig. 3, A, a potentiating effect on amphetamine induced H-S of the order of 400% is observed. Simultaneity in the administration of D-amphetamine and physostigmine seems to be the best condition for the elicitation of this potentiating effect. If the anticholinesterase is injected 15 min before or 15 min after the administration of D-amphetamine, the effect is not so dramatic, the increase in H-S time being only of the order of 100%.

To be sure that the effect of physostigmine on head-shaking results from its action on cholinergic synapses in the central nervous system, and not in the periphery, some experiments with neostigmine were performed, since it is well known that this last drug does not pass the blood-brain barrier in significant amounts [8, 14, 50]. In these experiments, Fig. 3, B, neostigmine (0.10 mg/kg) was injected simultaneously with amphetamine, while controls, from the same litters, received only amphetamine. No differences in H-S occurrence, nor in total mean H-S time were observed. With a higher dose of neostigmine (0.20 mg/kg) similar results were obtained.

C. *Experiments with Pilocarpine*

Pilocarpine is a well known cholinergic drug, with predominant muscarinic actions, which readily passes the blood brain barrier [24,32]. When 9-day-old rats are injected with pilocarpine alone, head-shaking stands out as a quantitatively significant motor item, but other behavioural elements, isolated or in rather stereotyped sequences

TABLE 1

PHYSOSTIGMINE POTENTIATION OF D-AMPHETAMINE INDUCED H-S

Drug	Total Mean H-S Time (sec/30 min)
1. D-amphetamine sulphate (5 mg/kg)*	191
2. Physostigmine (0.10 mg/kg)†	20§
3. D-amphetamine + physostigmine (0.05 mg/kg)	321‡
4. Idem + physostigmine (0.10 mg/kg)	467§
5. Idem + physostigmine (0.20 mg/kg)	217

N=14 rats (9-day-old in each group).
*Refers to the dose of the salt; †Refers to the dose of the base. Significantly different from the controls (Group 1) by the Mann-Whitney U Test: ‡at the $p<0.05$ level; §at the $p<0.01$ level.

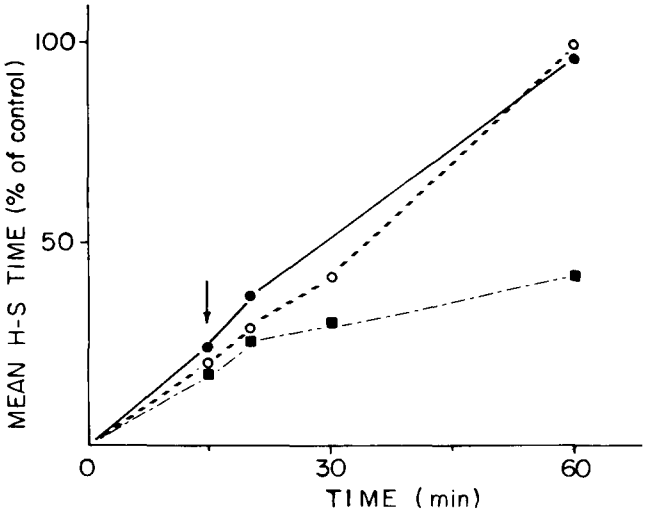

FIG. 2. Scopolamine effect on amphetamine induced H-S. Ordinate: H-S Time expressed as % of H-S Time of controls at 60 min. Abscissa: observation time in minutes. All animals were injected with D-amphetamine sulphate (5 mg/kg) at 0 time; the arrow indicates the injection of saline or scopolamine at 15 minutes. ○----○ controls injected with saline; ●——● scopolamine (2.5 mg/kg); ■—·—·—·—■ scopolamine (5 mg/kg). Differences between controls and scopolamine (5 mg/kg) injected rats were significant (Mann-Whitney U Test, $p<0.001$).

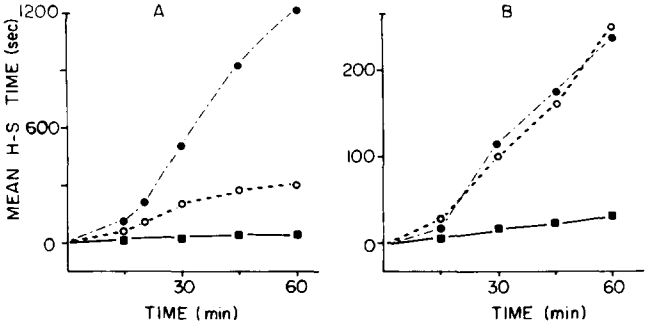

FIG. 3. Effect of anticholinesterase drugs on amphetamine induced H-S. A. *Physostigmine* (0.10 mg free base/kg); B. *Neostigmine* (0.10 mg free base/kg). Ordinate: Mean H-S Time in sec. Abscissa: observation time, in minutes. ○-----○ controls, injected with D-amphetamine (5 mg/kg) at 0 time; ●——● D-amphetamine + anticholinesterase; ■——■ anticholinesterase alone. N = 12 nine-day-old rats in each group. Physostigmine increases amphetamine induced H-S Time significantly as follows: at 20 min, $p<0.025$; at 60 min, $p<0.001$. Neostigmine does not change amphetamine induced H-S (Mann-Whitney U Test).

are also quite conspicuous. One often observes chewing or gumming, licking of the forelimbs followed by cleaning movements of the snout, the sequence ending in distinct yawning. Intense and frequent scratching, especially with the higher doses tested, is particularly impressive. Figure 4 shows the dose-effect curve for the pilocarpine H-S effect, in the 1–10 mg/kg range. The differences in H-S time for pilocarpine injected rats are statistically significant in relation to control animals at all doses tested.

Once established that pilocarpine per se induces H-S in infant rats, the possible existence of drug synergism between pilocarpine and D-amphetamine in regard to head-shaking was explored. The results of such an experiment are illustrated in Fig. 5. While saline injected controls practically did not shake their heads at all (an average of 8 sec in one hour), pilocarpine injected rats had a total mean H-S time of 245 seconds, amphetamine injected rats reached 695 sec, and rats receiving a mixture of amphetamine (5 mg/kg) and pilocarpine (4 mg/kg) had a total mean H-S time of 2,378 sec, i.e. this last group of rats spent two thirds of the observation time shaking their heads.

DISCUSSION

The above reported results point towards a significant participation of cholinergic influences in spontaneous and amphetamine induced head-shaking of infant rats. The administration of adequate doses of scopolamine (2–5 mg/kg), drug which blocks muscarinic cholinoceptive receptors, produces a complete suppression of the H-S effect (Fig. 1). On the other hand, a three to four-fold potentiation of the duration of amphetamine induced head-shaking is obtainable with centrally acting cholinomimetic drugs, as pilocarpine, which has predominant muscarinic actions [33], or the anticholinesterasic agent physostigmine (Figs. 3 and 5, respectively). These results clearly demonstrate that H-S in infant rats differs from other amphetamine-induced stereotypies in adult animals because the latter "are prolonged and enhanced by anticholinergics and weakly antagonized by cholinergics" [47].

In the particular motor item which we have used as a

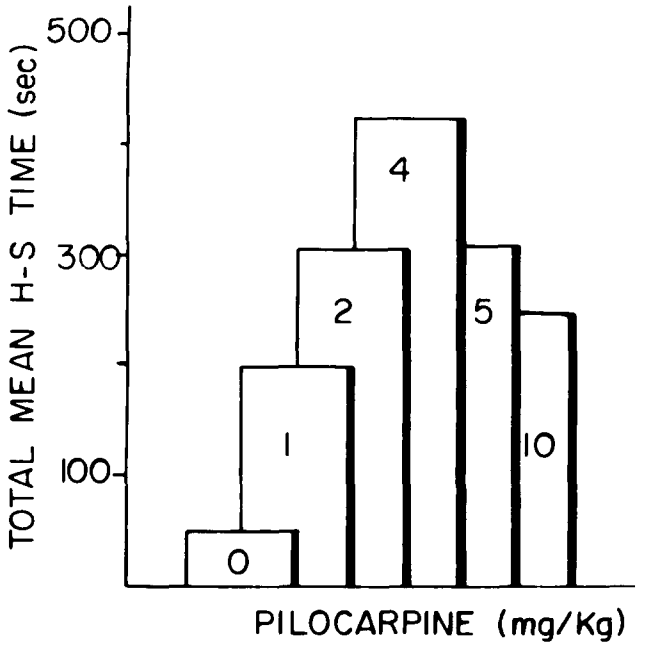

FIG. 4. Pilocarpine-induced head-shaking. Dose-effect curve. Ordinate: Total Mean H-S Time, in seconds, during 1 hr of observation; Abscissa: pilocarpine doses (mg free base/kg), indicated in each bar. N = 12 nine-day old rats in each group. All doses produced significant effects when compared with the controls (pilocarpine 0 mg/kg) as follows: 1 mg/kg, $p<0.02$; 2 mg/kg, $p<0.01$; 4 mg/kg, $p<0.001$; 5 and 10 mg/kg, $p<0.01$. (Mann-Whitney U Test).

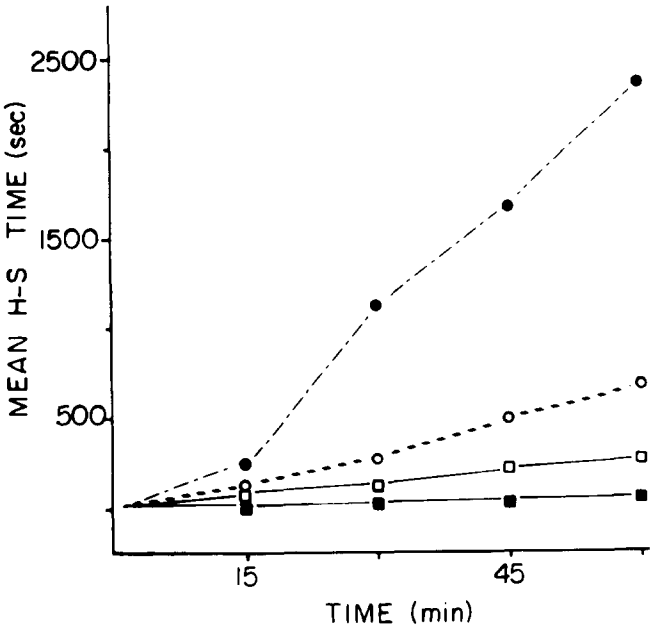

FIG. 5. Potentiation of H-S effects induced by D-amphetamine and pilocarpine. Ordinate: Mean H-S Time, in seconds. Abscissa: observation time, in minutes. All injections performed at 0 time. ○-----○ D-amphetamine sulphate (5 mg/kg); ●-----●, D-amphetamine (5 mg/kg) + pilocarpine (4 mg/kg); ○——○ pilocarpine (4 mg/kg); ■——■ saline. Differences between pilocarpine and D-amphetamine + pilocarpine injected rats were significant as follows: at 15 minutes, $p<0.01$; from 30–60 min, $p<0.001$ (Mann-Whitney U Test). N = 10 nine-day-old rats in each group.

model for our studies, catecholaminergic and cholinergic influences are powerfully concurrent, and not antagonistic, as seems to be the case with stereotyped behaviour [16, 38, 47], and is most generally accepted for the control of extrapyramidal function, [4, 6, 26, 30, 31, 32, 40].

We have formerly suggested that head-shaking in infant rats might be initiated by excitation of striatal neurons through dopaminergic afferents [23]. This possibility is based on the assumption that excitatory dopaminosensitive receptors in the striatum may mature earlier than the inhibitory ones, and thus predominant during a certain period, until the development of the latter conceal their action, if explored with drugs injected systemically. It may be recalled that although several authors have observed more inhibitory effects on caudate-putamen neurons to microionophoretically applied dopamine [11, 17, 41, 42, 53] or to the stimulation of the nigrostriatal pathways [11,17] other reports indicate that excitatory effects are practically as frequent or even more than inhibitory ones [7, 29, 56]. These considerations seem pertinent, because the most recent hypothetical models of organization of the nigrostriatal system, which according to Creese and Iversen [12] is the anatomical substrate of the amphetamine locomotor and stereotyped responses in the rat, conceive that inhibitory afferent dopaminergic fibres from the substantia nigra establish synaptic contacts with cholinergic interneurons in the striatum [5, 18, 27, 35, 39, 49, 52, 55]. The effects of ionophoretically applied acetylcholine on striatal neurons is predominantly excitatory (91% of the neurons tested by Bevan, Bradshaw and Szabadi, [7]). As no concurrent effects could be obtainable by increase in dopaminergic tone and cholinergic activity in a nervous pathway in which the first link were inhibitory and the second excitatory, our results do not fit in the above mentioned models. They are neither consistent with the previous more simple and schematical idea that cholinergic and dopaminergic influences are acting as "two independent antagonistic systems regulating the activity of a common neuron in the striatum". Following this quotation from Stadler, Lloyd *et al.* [52]: "they rather suggest the existence in this structure of an interdependent cholinergic-dopaminergic neuronal network", and come in line with other data that, in the words of Bevan, Bradshaw and Szabadi [7] "argue against the claim that dopamine and acetylcholine have opposite effects in the caudate nucleus".

One should also have in mind the possibility that cholinergic mechanisms might be operating by a Burn and Rand effect [9], in which acetylcholine liberated locally at the dopamine terminals might induce increased liberation of the catecholamine from the presynaptic endings, or by its modified version [25] based on the proximity of aminergic and cholinergic fibre terminals, in which it is postulated that acetylcholine could influence the release of dopamine (and vice versa) from adjacent nerve terminals.

When studying the differential development of caudate enzymes in the neonatal rat, McGeer, Fibiger and Wickson [38] have shown that while tyrosinehydroxylase activity is already at a level of 50% of the adult's value by the 10th postnatal day, choline acetylase activity is only slightly above 10%, and acetylcholinesterase activity around 60%. Pharmacological data existed in the literature indicating that, in relation to arousal, amphetamine was effective in 10-day-old rats, while scopolamine and pilocarpine, which depend upon cholinergic mechanisms, are not active until

about the twentieth day [10,15]. In relation to stereotyped gnawing behaviour induced by amphetamine on which scopolamine has a synergistic action, McGeer, Fibiger and Wickson [38] were unable to demonstrate this synergism in 10 day-old rats, while it was very significant in the 30 day-old rats, even if the former group appeared to be more sensitive to amphetamine. They therefore conclude that: "10-day-old rats have no functioning cholinergic mechanisms to counteract the dopaminergic effects initiated by amphetamine". Our experiments limit the validity of the above quoted conclusions to cholinergic mechanisms eventually involved in stereotyped gnawing, or in adrenergic arousal, because in 9-day-old rats, D-amphetamine induced head-shaking is significantly reduced by scopolamine and intensely potentiated by cholinomimetic drugs. These results suggest that cholinergic elements, maturing perhaps earlier than those involved in adrenergic arousal or in stereotypedactivity, participate in the expression of D-amphetamine induced head-shaking, or may, by their independent activity, evoked this motor item (experiments with pilocarpine, Fig. 4.) Until now we have assumed that the interactions between cholinergic and dopaminergic neural elements take place in the striatum. But other possibilities may be considered. According to Olivier, Parent *et al.* [45] "the outflow from the striatum to the pallidum and substantia nigra is nearly, if not exclusively cholinergic". A crossed pallido-reticular pathway which seems to be cholinergic has been described [19]. Its activation produces contraversive turning of the head, and contralateral increase in muscle tone, the rigidity being easily reduced by anticholinergic drugs. If rotation of the head around the sagital axis is under pallidal influences, and the mesencephalic controlling centers responsible for this movement reciprocally organized, the tegmental mesencephalic level might well be critical for the production of head-shaking in infant rats. It should be recalled that tremorine-tremor, which is a cholinergic tremor, seems to have a pacemaker in the tegmental ponto-mesencephalic reticular formation [54].

Head-shaking in infant rats, although apparently produced by an increase in dopaminergic and noradrenergic influences [23], has in common with the tremor observed in Parkinson's disease, or in drug-induced parkinsonism, that both are reduced by anticholinergics and aggravated by cholinomimetics [30,31]. Therefore we feel it is appropiate to comment on a model proposed by Kuczenski, Segal and Mandell [34] for the interpretation of Parkinsonian tremor. They believe that the cholinergic system, being phasic and excitatory, may be responsible for the phasic activation of flexors and extensors, while the dopaminergic system, which is tonic and inhibitory, would impose a higher threshold of excitability for the emergence of the oscillatory tendency of the reciprocally innervated flexor and extensor muscles, as well as a diminution of their resting tonus. The decrease in dopaminergic influences resulting from the pathological process or the drug actions would thus produce both rigidity and tremor. The scheme proposed by these authors might also be applicable to head-shaking in infant rats, if the assumption is tenable that in the course of postnatal development some dopaminergic facilitatory influences were active before the maturation of dopaminergic inhibitory mechanisms.

A complex interaction between monoaminergic and cholinergic influences could also be conceived at the rhombencephalic level, where a neuronal loop involving the pontine reticular formation, the inferior olive, the cerebellum, reticulospinal and/or vestibulospinal neurones has been presented as the neuroanatomical substrate of harmaline tremor in the cat [37]. Drug-induced rhythmic activity, at a frequency of 6 to 11 cps in the inferior olivary complex has also very recently been demonstrated in the rat [20]. Furthermore, as vestibular units, either directly, or more probably through the reticular formation, are inhibited by muscarinic blocking agents, and strongly excied by physostigmine [28], a cholinoceptive modulation of rhythmical descending and reciprocally organized vestibulospinal inputs on motoneurones innervating head and neck muscles could be a reasonable hypothetical explanation of our observations on the effects of these same drugs on head-shaking.

As a final summarizing comment, we feel that it is remarkable that an apparently so simple motor activity, as head-shaking in infant rats, may have such a complex neurohumoral background, in which at least central dopaminergic, noradrenergic and cholinergic links or elements have up to now been disclosed.

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