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Short communication

CIRCADIAN VARIATION OF YAWNING BEHAVIOR

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Abstract. Circadian variation of spontaneous yawning is demonstrated in a line of Sprague–Dawley rats, genetically selected for high frequency of this motor pattern. Yawning is highest in late light and early dark hours, both under natural and artificial illumination. The hypothetical neurotransmitters underlying this physiological rhythm in yawning are discussed.

Yawning is a motor pattern widely represented in the behavioral repertoire among Vertebrates (3, 11) but its physiological significance remains obscure. Apart from its postulated role in social communication in primates (1, 9) and in man (3), common-place knowledge associates yawning with the transitional phases between sleep and the waking state, or with conditions of physical and mental tiredness. But very few solid facts, if any, stand out in relation to its underlying physiological mechanisms. Behavioral pharmacology has nevertheless opened some leads in that direction. As yawning behavior is elicitable in rats both by cholinomimetic drugs (physostigmine and pilocarpine) (24, 29) or by low doses of apomorphine or other dopamine (DA) agonists (13, 17, 26, 29), a tentative model of organization of the central mechanisms controlling yawning has been proposed (13, 26, 29). In this hypothesis (Fig. 1) two sets of DA and cholinergic (ACh) neurons, localized somewhere in the brain, are supposed to be organized "in series", the former tonically inhibiting the latter, which exert a direct excitatory influence on the central pattern generator (CPG) of yawning. Apomorphine in low doses, by activation of DA presynaptic autoreceptors, induces yawning by disinhibition of the cholinergic excitatory neurons. Higher doses of DA agonists directly inhibit the ACh neurons and thus suppress yawning behavior. As negative and positive modulating influences by noradrenergic (NE) and serotonergic (5-HT) mechanisms on yawning have also been demonstrated (12, 16, 25, 30), this apparently simple innate behavior seems to be under the control of a complex constellation of neurotransmitter influences.

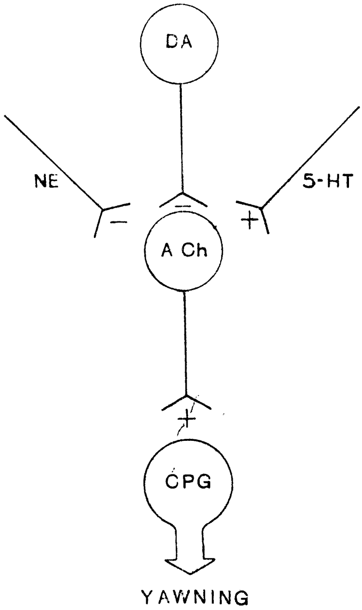

Fig. 1. Hypothetical organization of central neurotransmitter mechanisms regulating yawning behavior. CPG, central pattern generator; DA, dopaminergic neuron; Ach, cholinergic neurons; NE, noradrenergic influence; 5-HT, serotonergic influence.

Being associated to manifestations with so clear circadian rhythmicity as sleep and activity (14), it is to be expected that yawning should also appear with a circadian rhythm, and justified to try to correlate it with circadian variations in activity, reported in the literature, of the neurotransmitter systems postulated as subserving the central control and regulation of yawning. Diurnal variations in brain levels of ACh, DA, NE and 5-HT (7, 10, 21, 27, 31), of their biosynthetic or metabolizing enzymes (5, 27) and of their specific receptors (14, 19, 28) have been vastly explored in the last twenty years (for earlier references see 15, 22). The same is true for different behaviors supposed to be under the influence of these particular neurotransmitters, because those behaviors may be induced or modified by proper pharmacological agonists or antagonists (6, 15, 20, 22). Nevertheless, until 1980, spontaneous and pharmacologically-induced yawning behavior had escaped this detailed scrutiny for diurnal variations (22, 23). By that time Anías (2) described that both apomorphine- and physostigmine-induced yawning in Wistar rats presented a clear circadian rhythm. In these rats, kept under a controlled 12 h–12 h light-dark (LD) cycle (lights on at 7 a.m.), pharmacologically–elicited yawning frequency was lowest between 3 and 8 a.m. and showed a high irregular plateau from 10 a.m. to 23 p.m. Due to the very low spontaneous yawning frequency in Wistar rats (below 1 yawn/hour), no circadian variation in this behavior was noticed in absence of pharmacological manipulations.

As some progress has been made in our Animal House in Puebla in the selective breeding of a "high yawning frequency" line of Sprague–Dawley rats, it is now possible for us to communicate observations on the circadian rhythm of spontaneous yawning.

Our results are based on three groups of young adult male rats, from the F4, F5 and F6 generations, observed during December-January, March–April, and September–October. The first two groups, formed by eight F4 and six F5 animals, were kept and observed in the same laboratory room, under natural illumination (a big 6 m^2 glass window facing East). They were housed in groups of two or three animals, in transparent acrylic boxes (47 × 43 × 20 cm) containing a layer of wood shavings, covered by a galvanized iron wire top, with a depression to serve as food container and to hold a water bottle. For the observation of yawning during dark hours, the animals were illuminated from the side or from above with a 25 W red lamp placed at approximately 40 cm distance. The animals were manipulated only three times a week, when changed to clean cages. They had ad libitum access to food (standard laboratory rodent pellets) and drinking water. The third group, of six F6 rats, was observed in the Animal House of the C.R.I.R.A. (Centro Regional de Investigaciones en Reproducción Animal, Panotla, Tlaxcala, México) where rats were kept under a 14–10 LD schedule, with lights automatically turned on from midnight to 1400, and at an ambient temperature within 19–24°C. This group of rats, when two months old, was accustomed to these new conditions for 25 days before the observation of yawning began.

Yawning occurrence was monitored through the 24 h of the day in twelve to fifteen sessions, irregularly distributed for each group of animals over 1 1/2 months. Observation sessions did not last more than two hours, with two observers sitting on opposite sides of the table on which the cages were placed. Observation implied no direct manipulation of the rats, apart from a discrete movement of the boxes on the table, in the first two groups, and carrying them 5 m from a shelf to the table in the third group. These movements of the cages were performed at least 15 min before beginning the clocking of each yawn during the observation period.

The diurnal distribution of yawning frequency (expressed as average yawns/hour) in the two groups of rats maintained under natural illumination is illustrated in Fig. 2*A*. Apart from the clear peak shown in the afternoon (late light period), a tendency to exhibit additional lower peaks also seems apparent. When the rats were kept and observed under a 14–10 LD schedule, with artificial illumination and sudden transitions from light to dark (Fig. 2*B*), the circadian yawning acrophase was displa-

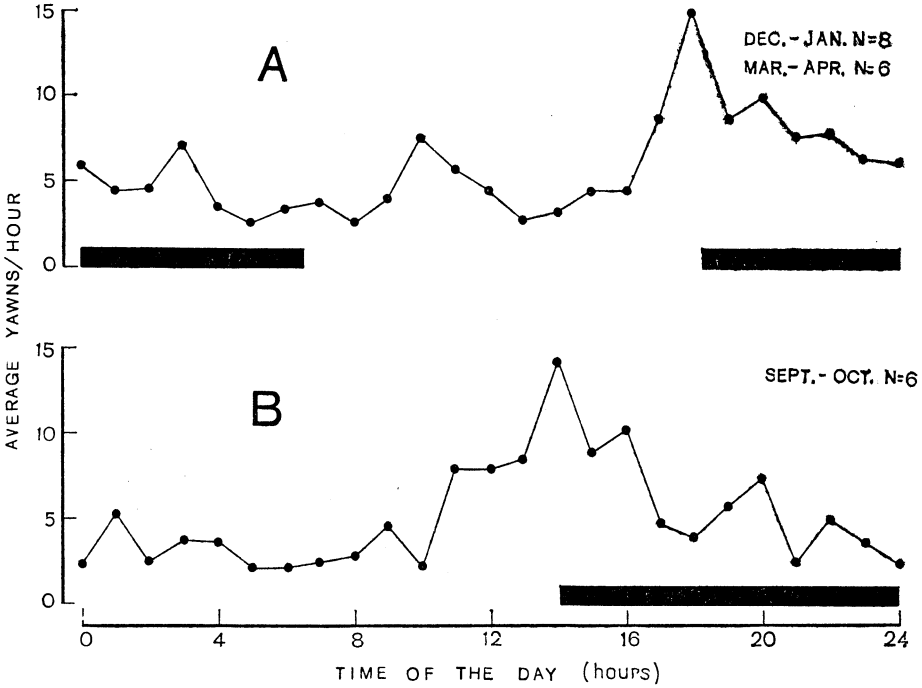

Fig. 2. Circadian variations in spontaneous yawning. Ordinates: average yawning frequency. A, Fourteen Sprague-Dawley rats (8 F4 and 6 F5) observed through the 24 h of the day, during the months of December–January and March–April, under natural illumination. Approximate (± 30 min) sunrise and sunset at the latitude of Puebla, indicated by end (left) and beginning (right) of dark bar. B, Six F6 rats observed during September–October under 14 L-10 D artificial illumination schedule (lights on at midnight). Acrophase yawning frequency has been averaged from three different observations.

ced in the time of the day, but continued to coincide with the last hour of the light period. This result suggests that the light-to-dark transition might be the "primary synchronizer" of the circadian rhythm of yawning, because the higher frequency of yawns around this time results highly significantly different from a uniform distribution ($P < 0.001$), using a directional test based on the coefficient of synchronization (4, 16).

If yawning is a motor pattern under cholinergic activation and subject to DA inhibitory regulation, one might expect that its peak frequency should coincide with hours in the day when cholinergic activity is highest, and dopaminergic activity lowest. It is interesting to note that in two studies in which clear diurnal oscillations of ACh concentrations in the brain have been demonstrated (10, 27), low levels of the neurotransmitter were measured at the latest part of the light period, coincident with our observations of maximal yawning frequency. The generally accepted opinion is that high concentrations of ACh in the brain coincide with low firing rate of the cholinergic neurons, the intracellular neurotransmitter being protected from degradation by ACh-esterase. Perhaps even more suggestive are Cahill and Ehret's (5) results on the circadian variation in DA levels, tyrosine hydroxylase activity and turnover rate of dopamine in the rat brain (see their Fig. 3). The lowest turnover rates of DA were calculated for the late light hours. A decrease in dopaminergic activity would, in our hypothesis, liberate the cholinergic neurons exciting yawning from inhibitory control and thus facilitate the expression of this behavioral pattern. Turnover rates of norepinephrine in the brain stem of the rat, also determined by the same authors (5), show two peaks, at early and late dark period hours, when rats are more active, and according to our results, yawn more unfrequently than in the last hour before the LD transition.

As some evidence exists in favour of a serotonergic facilitation of pharmacologically-induced yawning (17, 25, 30), it is important to consider if serotonergic activity may also contribute to the circadian rhythm of spontaneous yawning. Quay (21) had observed peak 5-HT concentrations in hypothalamus, frontal cortex and lateral portions of the lower brain stem during light hours preceding the LD transition. More recent studies (31) in rats kept in either 12L–12D, or 14L–10D illumination schedules, showed higher 5-HT turnover rates (estimated by 5-HIAA/5-HT ratios) during the dark period, when yawning activity is in our experience lower. But as 5-HT rhythms of different shapes take place in different portions of the brain (21, 31), it may not be altogether surprising to encounter difficulties in correlation with a behavioral pattern of which the neuroanatomical structures involved in its control and regulation are still ignored. On the other hand some doubts exist that total turnover of brain 5-HT may not always reflect the functional serotonergic activity in the brain (8).

A final quantitative comment. Selective breeding of "high yawning frequency" Sprague–Dawley rats, in four to six generations, has brought forth an increase in spontaneous yawning frequency to an average (mesor) above 5 yawn/hour, i.e., an order of magnitude higher than that observed by us (13) and other authors (18, 29) in Wistar rats. Further analysis of the factors involved in such an important increase in yawning frequency may help in the disclosure of the physiological mechanisms underlying this particular behavioral pattern.

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1. ALTMANN, J. 1967. The structure of primate social communication. *In*: S. A. Altmann (ed.). Social communication among primates. Univ. Chicago Press, Chicago, p. 325–362.
2. ANIAS, J. M. 1980. Regulación dopaminérgica del bostezo. Tesis de Especialidad. Instituto Superior de Ciencias Médicas, La Habana, Cuba.
3. BARBIZET, J. 1958. Yawning. J. Neurol. Neurosurg. Psychiatry 21: 203–209.
4. BUDELLI, R. and MACADAR, O. 1979. Statoacoustic properties of utricular afferents. J. Neurophysiol. 42: 1479–1493.
5. CAHILL, A. L. and EHRET, C. F. 1981. Circadian variations in the activity of tyrosine hydroxylase, tyrosine aminotransferase, and tryptophan hydroxylase: relationship to catecholamine metabolism. J. Neurochem. 37: .1109–1115.
6. EVANS, H. L. GHISELLI, W. B. and PATTON, R. A. 1973. Diurnal rhythm in behavioral effects of metamphetamine, *p*-chlorometamphetamine and scopolamine. J. Pharmacol. Exp. Ther. 186: 10–17.
7. FRIEDMANN, A. H. and WALKER, C. A. 1968: Circadian rhythms in rat midbrain and caudate nucleus biogenic amine levels. J. Physiol. (Lond.) 197: 77–85.
8. GRAHAME-SMITH, D. G. 1973. Does the total turnover of brain 5-HT reflect the functional activity of 5-Ht in brain? *In*: J. Barchas and E. Usdin (ed.), Serotonin and behavior. Academic Press, New York–London, p. 5–7.
9. HADIDIAN, J. 1980. Yawning in an Old World monkey, *Macaca nigra* (Primates: *Cercopithecidae*). Behavior 75: 133–147.
10. HANIN, I., MASSARELLI, R. and COSTA, E. 1970. Acetylcholine concentrations in rat brain: diurnal oscillation. Science 170: 341–342.
11. HEUSNER, A. P. 1946. Yawning and associated phenomena. Physiol. Rev. 26: 156–168.

12. HOLMGREN, B. and URBÁ-HOLMGREN, R. 1979. Yawning: a central cholinergic response under monoaminergic modulation. *In*: S. Tucek (ed.), The cholinergic synapse. Prog. Brain Res. 49: 482.
13. HOLMGREN, B. and URBA-HOLMGREN, R. 1980. Interaction of cholinergic and dopaminergic influences on yawning behavior. Acta. Neurobiol. Exp. 40: 633–642.
14. KAFKA, M. S., WIRZ-JUSTICE, A., NABER, D. and WEHR, T. A. 1981. Circadian acetylcholine receptor rhythm in rat brain and its modification by imipramine. Neuropharmacology, 20: 421–425.
15. LUCE, G. G. 1971. Biological rhythms in human and animal physiology. Dover Publ. Inc., New York.
16. MARDIA, K. 1972. Statistics of directional data. Academic Press, New York.
17. MARINI, J. 1981. Serotonergic and dopaminergic effects on yawning. Pharmacol. Biochem. Behav. 15: 711–715.
18. MOGILNICKA, E. and KLIMEK, V. 1977. Drugs affecting dopamine neurons and yawning behavior. Pharmacol. Biochem. Behav. 7: 303–305.
19. NABER, D., WIRZ-JUSTICE, A., KAFKA, M. S. and WEHR, T. A. 1980. Dopamine receptor binding in rat striatum: Ultradian rhythm and its modification by chronic imipramine. Psychopharmacology 68: 1–5.
20. NAGAYAMA, H., TAKAGI, A., YOSHIMOTO, S., MINAMI, H., NISHIWAKI, K. and TAKAHASHI, R. 1982. Circadian rhythm of brain susceptibility to haloperidol during chronic administration. Pharmacol. Biochem. Behav. 16: 311–314.
21. QUAY, W. B. 1968. Differences in circadian rhythm in 5-hydroxytryptamine according to brain region. Am. J. Physiol. 215: 1448–1453.
22. REINBERG, A. and HALBERG, F. 1971. Circadian chronopharmacology. Ann. Rev. Pharmacol. 11: 455–492.
23. RUSAK, B. 1981. Vertebrate behavioral rhythms. *In*: J. Aschoff (ed.), Handbook of behavioral neurobiology, Plenum, New York, Vol. 4, p. 183–213.
24. URBÁ-HOLMGREN, R., GONZÁLEZ, R. M. and HOLMGREN, B. 1977. Is yawning a cholinergic response? Nature 267: 261–262.
25. URBÁ-HOLMGREN, R., HOLMGREN, B., RODRÍGUEZ, R. and GONZÁLEZ, R. M. 1979. Serotonergic modulation of yawning. Pharmacol. Biochem. Behav. 11: 371–372.
26. URBA-HOLMGREN, R., HOLMGREN, B. and ANIAS, J. 1982. Pre- and postsynaptic dopaminergic receptors involved in apomorphine-induced yawning. Acta Neurobiol. Exp. 42: 115–125.
27. WALKER, C. A., CHAICHAERON, D. and WHITWORTH, U. G. 1981. Circadian rhythm in the acetylcholine levels of rat whole brain, caudate nucleus, cerebellum, cortex and midbrain. *In*: C. A. Walker, K. F. A. Soliman and C. M. Winget (ed.), Chronopharmacology and chronotherapeutics. Florida (A and M) University Foundation, Florida, p. 119–127.
28. WIRZ-JUSTICE, A., KAFKA, M. S., NABER, D. and WEHR, T. 1980. Circadian rhythms in rat brain alpha- and beta-adrenergic receptors are modified by chronic imipramine. Life Sci. 27: 341–347.
29. YAMADA, K. and FURUKAWA, T. 1980. Direct evidence for involvement of dopaminergic inhibition and cholinergic activation in yawning. Psychopharmacology 67: 39–43.

30. YAMADA, K. and FURUKAWA, T. 1981. The yawning elicited by α-melanocyte-stimulating hormone involves serotonergic-dopaminergic-cholinergic neuron link in rats. N. S. Arch. Pharmacol. 316: 155–160.
31. YATES, C. A. and HERBERT J. 1979. The effects of different photoperiods on circadian 5-HT rhythms in regional brain areas and their modulation by pinealectomy, melatonin and oestradiol. Brain Res. 176: 311–326.

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