Seasonal changes in the uptake capacity of the suprachiasmatic nucleus for ^{3}H-serotonin[1]

D. C. Meyer[2] and W. B. Quay

Endocrinology-Reproductive Physiology Program, Department of Zoology and The Waisman Center on Mental Retardation and Human Development, University of Wisconsin, 2605 Marsh Lane, Madison (Wisconsin 53706, USA),
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Summary. The suprachiasmatic nucleus, a hypothalamic center important in mediation of circadian and estrous cycles, is shown in adult rats to have seasonal changes in its uptake capacity in vitro for ^{3}H-serotonin.

Circannual rhythms, especially those involving endocrine functions, have been demonstrated in various vertebrate species. Hibernating bats show peak levels of plasma testosterone during July and August[3] and in the laboratory rat peak plasma LH and testosterone occur during March and April[4]. Rams maintained in an outdoor environment show peak plasma LH levels during June and July, and peak plasma testosterone levels from September through November[5]. Caged, indoor Rhesus monkeys also show peak testosterone levels during the fall and winter seasons[6,7].

Circannual rhythms in aspects of monoamine metabolism and transport have received less attention. Brain concentrations of serotonin (5-hydroxytryptamine, 5-HT) and 5-hydroxyindole-3-acetic acid (5-HIAA), its principal metabolite, peak during the summer[8] while ^{3}H-5-HT uptake in rat striatum is greatest in October. Uptake of ^{3}H-dopamine has been reported highest in May[9]. These findings pertain to studies under laboratory conditions in various countries in the north temperate zone. In the authors' research, data accumulated during 1974–1976 provides further evidence for a seasonal variability in the in vitro uptake of ^{3}H-5-HT in the suprachiasmatic nucleus of male rats.

Materials and methods. Male rats (Rattus norvegicus), 40–70 days old were maintained in artificially illuminated rooms on a 12L:12D photoperiod and a temperature range of 22 °C ± 1 in Madison, Wisconsin. Rats were allowed access to food and water ad libitum. In one experiment they were maintained on a 2L:22D photoperiod to simulate a seasonal change in lighting. Following 2 weeks acclimation to these conditions they were quickly sacrificed during the first hour of light. The fresh tissue sections obtained of the suprachiasmatic nucleus were incubated in Krebs-Henseleit buffer containing pargyline (monoamine oxidase inhibitor) and nanomolar ^{3}H-5-HT for 20 min[10]. Results are expressed in terms of tissue/medium ratios (cpm ^{3}H-5-HT per mg tissue/cpm per ml medium). Statistical evaluations of differences employed analysis of variance and the Student Newman-Keuls test for multiple comparison among means.

Results and discussion. Resulting data, summarized in the figure, suggest a seasonal pattern of uptake with maximum t/m ratios during the months of July and August, and minimum values during the winter and early spring

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2 Present address: Dr Donald C. Meyer, Wisconsin Regional Primate Center, University of Wisconsin, Madison, Wisconsin 53706, USA.

3 A. W. Gustafson and M. Shemesh, Biol. Reprod. *15*, 9 (1976).

4 E. J. Mock, F. Kamel, W. W. Wright and A. I. Frankel, Nature *256*, 61 (1975).

5 L. M. Sanford, W. M. Palmer and B. E. Howland, Can. J. Anim. Sci. *54*, 247 (1974).

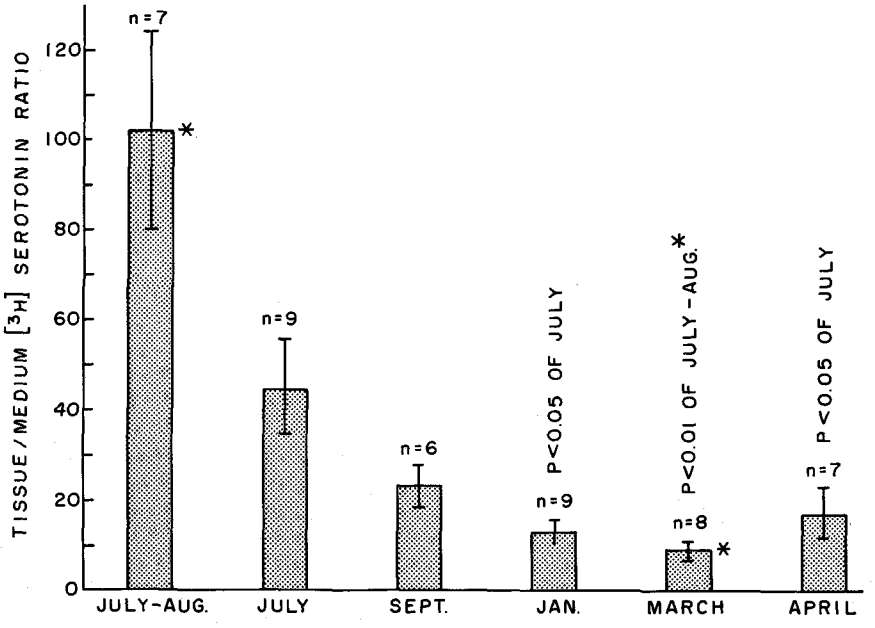
6 T. M. Plant, D. Zumpe, M. Sauls and R. P. Michael, J. Endocr. *62*, 403 (1974).

7 J. A. Robinson, G. Scheffler, S. G. Eisele and R. W. Goy, Biol. Reprod. 13, 203 (1975).

8 S. Garattini and L. Valzelli, in: Serotonin. Elsevier, Amsterdam 1965.

9 A. Wirz-Justice, Experientia *30*, 1240 (1974).

10 D. C. Meyer and W. B. Quay, Endocrinology *98*, 1160 (1976).



Summary of results showing differences in uptake of ^{3}H-serotonin by the male rat's suprachiasmatic nucleus according to time of year. All animals were sampled during the first hour of daily artificial light, in a 12L : 12D photoperiod, except for the starred groups, which were in a 2L : 22D photoperiod. Means ± SE are plotted.

($p < 0.05$). Interestingly, animals maintained on a 2L:22D photoperiod also show this seasonal difference ($p < 0.01$), suggesting that it is not dependent on length of daily photoperiod. The work of Wirz-Justice[9], also using an in vitro uptake model, implies support for our results by showing maximum monoamine uptake in brain slices during October and minimum uptake during June. The fact that these seasonal differences persist in the laboratory environment as well as in field conditions suggests either an endogenous generator mechanism or perhaps a subtle geophysical mechanism. One current molecular theory attributes rhythms to membrane changes which, via an ion transport system, could influence the conformational state of the membrane[11]. This may have important implications for changes in monoamine reuptake sites, synaptic vesicles, and control factors in neuronal activity.

Yet, environmental lighting is the major and most consistent external variable and Zeitgeber to affect both the suprachiasmatic nucleus[12] and the pineal[13] via direct neuronal links with the retina. Furthermore pinealectomy, which removes the hormonal output of this gland, has been shown to induce cytochemical changes in certain cells within the suprachiasmatic nucleus suggesting modulation of suprachiasmatic neuronal or secretory activity[14]. Reiter has demonstrated that the pineal is critical for the annual reproductive capability of the golden hamster[15], and others have suggested that the pineal may function as an integrating device between the primary synchronizer of environmental photoperiod and various regional brain indole rhythms. The roles of the various serotonergic, catecholaminergic and other chemical mediators in the physiology of the suprachiasmatic nucleus are not completely understood, but it does appear very likely now that the importance of this nucleus in mediating light information for central nervous system periodicity is widespread among mammals[12]. The importance of monoamine periodicity in the suprachiasmatic nucleus, and of related circannual endocrine rhythms, may stem, at least in part, from adaptations to appropriately timed reproductive trophic and steroid hormones for optimal reproductive success and thus, species survival.

11 T. V. Driessche, Biosystems *6*, 188 (1975).
12 R. Y. Moore and V. B. Eichler, Psychoneuroendocrinology *1*, 265 (1976).
13 R. Y. Moore, A. Heller, R. J. Wurtman and J. Axelrod, Science *155*, 220 (1967).
14 A. R. Smith and J. A. Kappers, Brain Res. *86*, 353 (1975).
15 R. J. Reiter, J. exp. Zool. *191*, 111 (1975).

Experimentally induced otitis and audiogenic seizure in the mouse

M. M. Niaussat

Laboratoire de Physiologie Acoustique C. N. R. Z., F-78350 Jouy-en-Josas (France), 26 April 1976

Summary. Audiogenic seizures can be induced in genetically non-susceptible 17-day-old mice (Rb/3 strain) with various results. Priming only induces 9% of seizures, auditory insulation 3,8%, while experimental otitis leads to 79%. The hypothesis concerning disuse supersensitivity subsequent to acoustic deprivation was not confirmed by the experiment. However, modification of acoustic transmission at middle ear level induced by otitis or ear physical damage during the maturation period, exposes the upper nervous centers to intense stimulation to which the reaction is a recruiting response.

Recent studies have demonstrated that audiogenic seizures (A.S.) could be induced in genetically non-susceptible mice following acoustic priming[1,2], tympanic membrane perforation[3], or plugging of the external meatus[4], during a critical period of postnatal development (17–25 days). It was hypothesized that the acoustic deprivation resulting from such modifications produces a subsequent hyperreactivity (disuse supersensitivity) of the CNS auditory centers. However, on one hand, the effects of acoustic priming on A.S. susceptibility are different in 2 genetically resistant strains of mice (17% A.S. in the C57 BL Charles River and only 9% A.S. in the Rb/3) while the auditory impairment measured by cochlear potential shifts are identical[5]. (Rb: Swiss albinos has 2 substrains: Rb/1 genetically susceptible to A.S. Rb/3 genetically resistant [Mouse News Lett. *1959*, Companion issue No. 21, p. 42].) Therefore, mechanisms other than that of disuse supersensitivity must be involved in the induction of seizure susceptibility.

On the other hand, spontaneous middle ear infections enhance A.S. sensitization following acoustic priming by an electric bell in genetically non-susceptible mice[6]. Another priming (10 kHz, 120 dB) gave a 62% seizure rate in the Rb/3 resistant strain, but 23% of these animals had spontaneous middle ear infection. 1 year later, however, identical experiments produced significantly different results: 23% A.S. and 5% otitis (Niaussat, unpublished data). Considering these findings, a study was undertaken to define the exact influence of otitis on A.S. sensitization, in genetically non-susceptible mice. Irritative otitis was chemically induced in such mice and an investigation was conducted to determine the possible relationship between varying degrees of ear damage and the nature of the resulting seizures.

Materials and methods. The otitis induced was to be irritative but non-infectious to avoid introduction of a pathological reaction of the organism to infection. After preliminary trials with various chemicals: e.g., silver nitrate, trichloracetic acid, formol, B-methyl-metacrylate, the latter was found to be most irritative to the external and middle ear skin and mucosa. B-methyl-metacrylate is a dental cement catalyzer which was discovered to be irritative to mouse skin when used in excess during implantation of chronic electrodes (personal observation). 17-day-old mice from the non-susceptible Rb strain were

1 K. R. Henry, Science *158*, 938 (1967).
2 W. B. Iturrian and G. B. Fink, Fed. Proc. *26*, 736 (1967).
3 C. S. Chen, G. R. Gates and G. R. Bock, Exp. Neurol. *39*, 277 (1973).
4 M. D. McGinn, J. F. Willott and K. R. Henry, Nature New Biol. *244*, 255 (1973).
5 M. M. Niaussat, C. r. Soc. Biol. *168* (8-9), 937 (1974).
6 M. M. Niaussat, C. r. Soc. Biol. *164* (1), 57 (1970).