A circannual rhythm in bovine pineal serotonin[1]

R. Philo[2] and R.J. Reiter[3]

Department of Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio (Texas 78284, USA), 3 September 1979

Summary. Bovine pineal serotonin (5-HT) was analyzed at the time of the solstices and equinoxes from December, 1975 until June, 1978. The highest values of 5-HT were detected at the winter solstices and lowest values at the summer solstices of each year examined. The peaks in bovine pineal 5-HT correspond with a lessened fertility in cattle reported during the winter months.

There is considerable morphological evidence indicating that the function of the pineal gland in mammals changes with different seasons of the year[4-7]. Additionally, the pineal gland has been implicated in the control of annual reproductive cycles of a variety of mammals suggesting a physiological change in the gland throughout the year[8,9]. The environmental variable which impels the seasonal changes in the pineal gland is the photoperiod. Information about the photoperiodic environment reaches the pineal gland by a circuitous neural pathway which includes both the retinas and the peripheral sympathetic nervous system[10].

1 compound in high concentration within the pineal gland is serotonin (5-hydroxytryptamine or 5-HT)[11,12]. This substance is a precursor compound of melatonin[13], a factor which is important in determining seasonal changes in reproductive capability[9]. The purpose of the present study was to investigate a possible seasonal change in the serotonin content of the bovine pineal gland. This species was selected since a constant supply of young cattle raised in feed lots under ambient lighting and temperature conditions was available for use at a local meat packing house. The mean daylengths in southern Texas range from just over 10 h at the winter solstice to near 14 h at the summer solstice. Seasonal temperature varied between 0 and 35 °C depending on time of year.

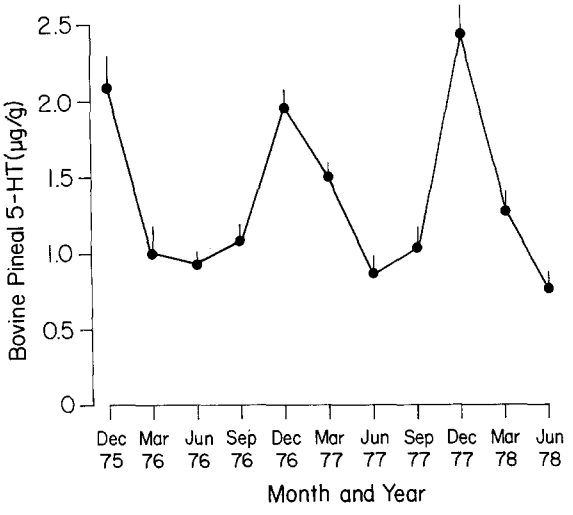
Methods and materials. The skulls of 20–30-month-old heifers of Hereford stock were split mid-sagittaly within 20 min of death using a hydraulic press. Pineal glands were collected between 08.00 and 11.00 h on the solstices and equinoxes in order to sample at periods of extremes in photoperiod length, and at those times when daylength and nightlength were equal. At each sampling time, beginning on December 21, 1975, and ending June 21, 1978, 12 pineal glands were collected. The amine serotonin (5-HT) was isolated on amberlite ion-exchange resin and quantified according to a method previously described[14]. The statistical analyses included an ANOVA followed by a Student-Newman-Keuls multiple rank test.

Results. The serotonin content was greater in bovine pineals at the times of winter solstice (figure) than at the equinoxes or summer solstice ($p < 0.01$). The only exception was the 5-HT content on the vernal equinox of 1977 when values were not at the level of those of previous or subsequent years. Within each year studied, the lowest levels occurred at the time of summer solstice (figure). Generally, the values at the winter solstice were twice those at mid-June.

Discussion. Jovan et al.[12] have reported the pineal concentration of 5-HT to be about 2.7 µg/g while Giarman et al.[11] have reported values on the order of 0.4 µg/g. The values reported herein are closer to those reported by the former author; it is possible, however, that Giarman et al.[11] may have collected their pineals during the summer months when the 5-HT concentrations are at their lowest point. In the present study the lowest concentration of 5-HT was reported to be 0.9 µg/g and was observed in 2 consecutive summers.

A very strong point suggesting the seasonal changes in pineal 5-HT content are real is that the rhythm was reproducible throughout a 2.5 year period. The pineal glands were always collected in the morning hours, 2–4 h after sunrise. This collection schedule was rigidly adhered to since there may be circadian variations in the level of this constituent within the pineal of cattle. Certainly in other species, 5-HT values fluctuate throughout a 24-h-period with daytime levels often being several fold higher than concentrations measured at night[13]. The major question relates to the significance of the seasonal rhythm in pineal 5-HT levels. Increased concentrations of 5-HT could mean that indoleamine synthesis (e.g., melatonin) is depressed with a resultant accumulation of the precursor. Conversely, greater precursor availability, i.e., higher concentrations of 5-HT, could result in more melatonin being formed. Without additional studies, the significance of the observed changes remains unknown.

Mercier and Salisbury[15,16] studied records of fertility in 125,000 cows and found a high correlation between fertility and daylength. Lowest fertility occurred in the winter with highest fertility in the fall. The same authors also noted that young cattle (such as used in the present investigation) were more easily influenced by daylength than older animals. In addition, Sweetman[17] reports that artificially lengthening the day results in an increase in conception rates in Alaskan cattle during the winter months. Whether the bovine pineal gland is involved in these seasonal fluctuations in reproduction, as has been shown for other species [8,9], remains to be established.



Mean (± SD) concentration of serotonin (5-HT) in bovine pineal glands collected at the solstices and equinoxes from December, 1975, to June, 1978. Each point represents the mean of 12 pineals. Values for Dec. 75, Dec. 76, March 77, and Dec. 77 differ from all other values with $p < 0.01$.

The results of the present study indicate a marked rhythm occurs in the bovine pineal amine serotonin which corresponds with observed changes in cattle fertility. In the cow the pineal may be involved in alterations in fertility, but only in concert with other factors, as the year long breeding capabilities of cattle are well known[18].

1 Supported by NSF grant PCM 77-05734.
2 Present address: Department of Reproductive Medicine and Biology, The University of Texas Medical School at Houston, Houston, TX 77025.
3 The authors wish to thank Roeglein's Provision Company, Beef Processing Division and especially H. Schmidt, Foreman.
4 W.B. Quay, J. Morph. *98*, 471 (1956).
5 R.K.H. Mogler, Z. Morph. Ökol. Tiere *47*, 267 (1958).
6 Lj. Nesíc, Acta anat. *49*, 376 (1962).
7 M. Roux, M.P. Richoux and J.L. Cordonnier, J. neural Transm. *41*, 209 (1977).
8 R.J. Reiter, Chronobiologia *1*, 365 (1974).
9 R.J. Reiter, Progr. Reprod. Biol. *4*, 169 (1978).
10 R.Y. Moore, Progr. Reprod. Biol. *4*, 1 (1978).
11 N.J. Giarman and M. Day, Biochem. Pharmac. *1*, 235 (1959).
12 P.Jovan, S. Samperez, J. Boissezu and A. Dupois, Revue roum. Endocr. *5*, 161 (1968).
13 W.B. Quay, Pineal Chemistry. Charles C. Thomas, Springfield 1974.
14 R. Philo and R.J. Reiter, Epilepsia *19*, 133 (1978).
15 E. Mercier and G.W. Salisbury, J. Dairy Sci. *30*, 747 (1947).
16 E. Mercier and G.W. Salisbury, J. Dairy Sci. *30*, 817 (1947).
17 W.J. Sweetman, J. Dairy Sci. *33*, 391 (1950).

Reciprocal connections between substantia nigra and medullary reticular formation in the rat[1]

Betty Pardey-Borrero and J.A. Gonzalez-Vegas

Escuela de Bioanalisis, Facultad de Medicina, Universidad Central, Caracas (Venezuela), 23 July 1979

Summary. Electrophysiological analysis shows reciprocal connections between substantia nigra and medullary reticular formation (nucleus reticularis giganto celullaris). The nigro-reticular connection appears to be monosynaptic, as shown by antidromic activation, and comprises an ipsi and a contralateral component. Its effect is mainly inhibitory. The reticulo-nigral component produces mainly excitatory effects and includes fibres from nucleus giganto celullaris and nucleus parvocelullaris in the medullary reticular formation.

It has been demonstrated that stimulation of the substantia nigra (SN) can induce changes in spinal reflexes[2] and produce motor responses[3]. As there are no known connections between the SN and the spinal cord, it has been proposed that these effects could be mediated either via nigro-tecto-spinal or reticulo-spinal pathways. There is anatomical[4] and electrophysiological[5] evidence of nigro-tectal connections, but evidence concerning nigro-reticular connections is more scarce. The present work was designed to obtain electrophysiological evidence of the presence of nigro-reticular connections, as the reticular formation (RF) has a well known action on spinal activity[6], and there exists anatomical support for the presence of such connections[7]. The presence of reticulonigral fibres was also investigated.

Materials and methods. Experiments were carried out in Sprague-Dawley rats (200–300 g) anaesthetized with urethane (1 g/kg IP), and positioned in a stereotaxic frame according to the atlases of Albe-Fessard et al.[8] and Abad-Alegría[9]. In 1 set of experiments, the SN was stimulated by means of bipolar electrodes (square wave pulses of 0.5 msec duration, repeated every 2 sec with intensities up to 300 μA) at coordinates AP 3.2; L 2; V 3, and recordings were made using glass microelectrodes filled with Pontamine sky blue, in order to mark the recording site[10]. Recordings were made at coordinates AP −3.5; L 1; V 0, taken from Abad-Alegría, in the region of the nucleus reticularis giganto celullaris (n.r.g.c.). In another set of experiments, the situation was reversed: the stimulating electrode was positioned in the region of the n.r.g.c. or more laterally in the nucleus parvocelullaris (n.p.c.), and recordings were made in the SN and adjacent ventral tegmental area (VTA). In all cases post-stimulus time histograms were made with a computer in order to assess the effects of stimulation in 100 responses. Recording and stimulating sites were histologically checked after the experiments.

Results and discussion. Stimulation of the SN produced mainly inhibitory responses on spontaneously active RF units. 22 out of 37 units showed periods of inhibition ranging in duration from 20 to 120 msec, in some cases followed by excitation. The latency of this inhibition had a mean value of 12.5 msec, 9 units were excited and in 2 this excitation was followed by inhibition (figure 1 a, b). Some units showed reverberatory activity (cycles of excitation and inhibition) after SN stimulation. Histological analysis confirmed that all responsive units were located within the boundaries of the n.r.g.c. In some experiments recordings were made contralaterally, with similar results.

Stimulation of the n.r.g.c. evoked responses in SN and VTA units. 13 cells were excited with a mean latency of 17.5 msec., and 3 were inhibited out of a total of 40 neurons. Stimulation of the n.p.c. produced inhibition in 3 units and

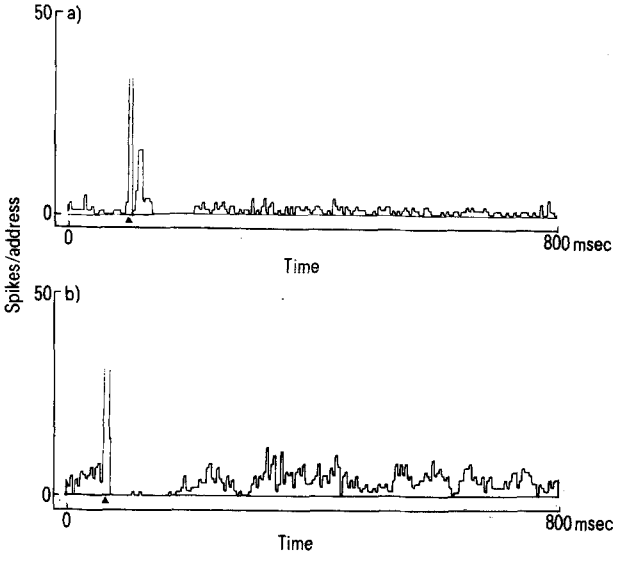


Fig. 1. Post-stimulus – time histograms of 2 neurons in the medullary reticular formation after stimulation of the SN. a) excitation followed by inhibition. b) inhibition followed by reverberatory activity. ▲, stimulus artefact. 100 responses; 4 msec/bin; 200 bins.