Seasonal changes in the levels and the turnover of brain serotonin and noradrenaline in the European hamster kept under constant environment

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Summary. Seasonal changes in the content and the turnover of noradrenaline and serotonin are shown in various parts of the brain of the European hamster kept under constant conditions of light and temperature.

Circannual rhythms of b. wt, hibernation and reproduction are present in many species of hibernators kept under constant conditions of light and temperature[1-4]. However, circannual rhythms of the metabolism of the monoamines noradrenaline (NA) and serotonin (5HT), which are involved in the neural control of thermoregulation[5] and regulation of b. wt.[6], have received less attention. Recently, Meyer and Quay[7] have shown a seasonal change in the uptake capacity in vitro for 3H-serotonin in the suprachiasmatic nucleus of adult male rats with peak values during summer. We present here some data on regional brain 5HT and NA metabolism obtained on a hibernator, the European hamster, kept under constant conditions of light and temperature which provide evidence that there is a seasonal rhythm in the level and turnover of these monoamines.

Material and methods. 84 male, adult, European hamsters (*Cricetus cricetus*) were utilized. After trapping, animals were kept in individual cages under constant conditions until the end of the experiment: light/dark 12/12 (150 lux:0), 15 °C (±1 °C). Animals were allowed access to food and water ad libitum.

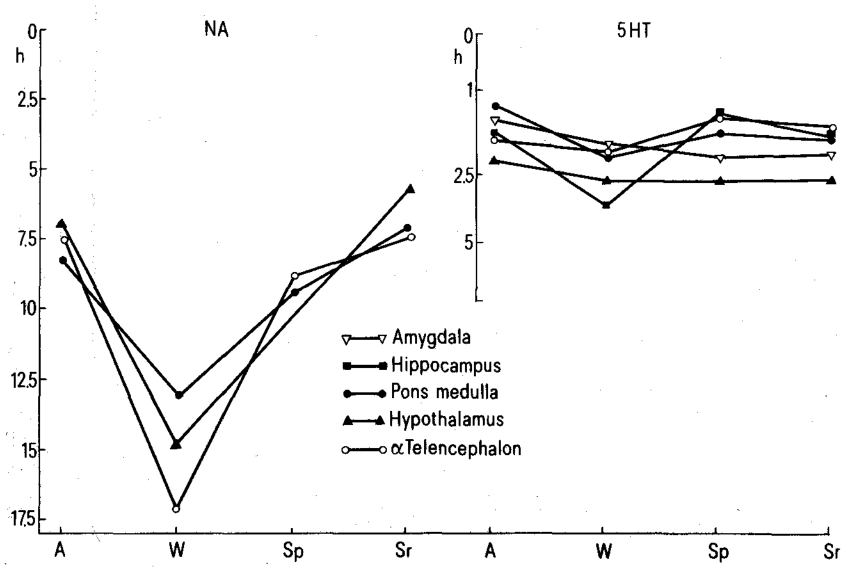
Under these conditions, animals exhibit a marked b.wt and food intake rhythm but hibernation does not occur[8].

Experiments were performed at the 2 solstices and the 2 equinoxes each time at the same hour of the day to prevent possible circadian variations[9]. Brain monoamines were estimated in brain stem, hypothalamus, anterior telencephalon, amygdala and hippocampus according to a procedure previously described[10] after 3 min of light anaesthesia with nitrogen protoxyde. After dissection, samples were frozen and stored in liquid nitrogen. NA[11] and 5HT[12] were estimated fluorometricaly. Turnover parameters were determined by blocking either synthesis or degradation of the transmitters and following changes in their levels as already described[13]. Each experiment required 21 animals: 7 for determination of the steady state levels of NA and 5HT (NA_0 and $5HT_0$), 7 for determination of turnover time (Tt) and turnover rate (TR) of NA after i.p. injection of methyltyrosine (300 mg/kg), the last 7 for determination of Tt and TR of 5HT after i.p. injection of pargyline (75 mg/kg).

Results and discussion. The resulting data, summarized on the figure and the table, strongly suggest a seasonal pattern of Tt and TR both for NA and 5HT. Concerning NA, the levels are stable during the year particularly in the Pons medulla, the main localization of the NA-containing cells. The most important fact is the slow turnover in winter and spring in all parts of the brain as compared with the high values in summer.

Concerning 5HT, the levels are less stable, especially in spring where they are higher than in the other seasons in all the regions particularly in the hypothalamus in which the value, 1421 ng/g, is significantly different ($p \leqslant 0.05$) from the values found in autumn and winter. The winter decrease in 5HT TR and Tt is less pronounced in Pons medulla, well expressed in hippocampus, not always seen in amygdala, telencephalon and hypothalamus. Maximum values are generally seen in spring and autumn.

Patterns of the 2 monoamines differ also in certain regions. For example, in anterior telencephalon there is no difference between autumn and winter in the turnover of 5HT while there is a marked reduction in the metabolism of NA. It is the 1st time, to our knowledge, that a seasonal rhythm of brain monoamines metabolism has been shown. The fact that these seasonal differences persist under a constant environment suggest an endogenous mechanism. The importance of monoamine rhythms in various parts of the brain of the hamster may be related to the circannual rhythms of hibernation and b. wt. Serotonin has been shown to be involved in hibernation in *Cricetus cricetus* and



Turnover time of brain NA and 5TH during the year in the European hamster. Same conditions as in the table. A, autumn; W, winter; Sp, spring; Sr, summer.

Citellus lateralis. In the later species, lesions of the raphe nucleus discrupt hibernation and brain serotonin levels decrease during entrance into hibernation[14]. In *Cricetus cricetus,* 5HT levels of various parts of the brain are lower during winter in the hibernating animal than in the active one[10]. The high synthesis of 5HT we show in autumn may perhaps be related to its influence in provoking a preparation for hibernation which takes place during that season. NA is implicated in b. wt regulation[6]. At 15 °C, food intake of *Cricetus cricetus* is lower in autumn and winter than in spring and summer[8]. NA metabolism is also lower in autumn and winter than in spring and summer (table). The role of serotoninergic and noradrenergic pathways are not yet clearly understood, but in view of the role of this neurotransmitters in the b. wt regulation[6] and in normal sleep[15], the seasonal changes in NA and 5HT metabolism do appear important for the comprehension of the circannual rhythms of many physiological functions of the hibernators.

Levels of noradrenaline (NA_0) and serotonin ($5HT_0$) in various parts of the brain of the European hamster kept under constant conditions of light (light/dark 12/12) and temperature (15 °C)

	Autumn	Winter	Spring	Summer
Pons medulla				
NA_0 (ng/g)	640 ± 42	651 ± 71	583 ± 45	642 ± 41
Tt (h)	8.20	13.10	9.40	7.0
TR (ng/h)	78 ± 11.4	50 ± 17	62 ± 12.7	81 ± 18
$5HT_0$ (ng/g)	964 ± 127	984 ± 90	1072 ± 94	942 ± 93
Tt (h)	1.25	2.18	1.71	1.82
TR (ng/h)	620 ± 111	450 ± 41	628 ± 45	550 ± 75
Hypothalamus				
NA_0 (ng/g)	939 ± 103	899 ± 105	785 ± 85	849 ± 62
Tt (h)	7.0	14.90	10.10	5.65
TR (ng/h)	134 ± 28	60 ± 26	78 ± 27	150 ± 24
$5HT_0$ (ng/g)	1113 ± 87	1099 ± 93	1421 ± 167	1223 ± 155
Tt (h)	2.20	2.60	2.60	2.60
TR (ng/h)	508 ± 86	429 ± 49	550 ± 72	478 ± 70
Anterior telencephalon				
NA_0 (ng/g)	371 ± 18	373 ± 26	405 ± 38	433 ± 37
Tt (h)	7.50	17.30	8.85	7.25
TR (ng/h)	49 ± 4.9	21 ± 8.7	46 ± 11	60 ± 15.7
$5HT_0$ (ng/g)	586 ± 81	613 ± 45	636 ± 45	565 ± 53
Tt (h)	1.88	2.08	1.44	1.60
TR (ng/h)	313 ± 49	295 ± 57	441 ± 37	353 ± 46
Amygdala				
$5HT_0$ (ng/g)	853 ± 181	803 ± 112	918 ± 100	886 ± 120
Tt (h)	1.53	2.03	2.2	2.12
TR (ng/h)	558 ± 93	396 ± 53	417 ± 68	418 ± 57
Hippocampus				
$5HT_0$ (ng/g)	497 ± 37	568 ± 22	548 ± 46	502 ± 69
Tt (h)	1.76	3.05	1.44	1.78
TR (ng/h)	281 ± 37	186.6 ± 26	381 ± 52	283 ± 47

Tt, turnover; TR, turnover rate. Each value is the mean ± SE of 7 animals.

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Induction of electrical excitability in crustacean muscle by 4-cyclopentene-1,3-dione[1]

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Summary. 4-Cyclopentene-1,3-dione induces electrical activity in inexcitable crustacean muscle. This effect is blocked by previous treatment with p-chloromercuribenzoic acid. These results suggest that crustacean muscle becomes excitable when certain -CH_2-SH side chains are converted to thioethers having carbonyl groups.

Studies of the effects of sulfhydryl reagents on the functional properties of excitable membranes led to the general conclusion that blocking free SH groups causes the loss of electrical excitability[2-9].

Recently we investigated the action of the SH reagent NEM on inexcitable crustacean muscle fibres and our results were opposed to those previously reported[10,11]. Electrical excitability was induced in this tissue by treatment with NEM (1-2 mM; 5-10 min). This effect is not simply the result of binding free SH groups, since organic mercurials did not induce excitability, though they prevented the subsequent effect of NEM. In an attempt to determine the structural features of NEM necessary to induce excitability, we found that the ethylene chain attached to the N was not needed, since other N-maleimide derivatives also exerted the same action[11]. This suggested that the 2 symmetrical carbonyl groups or the tertiary nitrogen may be more important. To study the role of these groups, we performed experiments with 4-CPD, a compound similar to maleimide but with a methylene group instead of the nitrogen. Although it has not been used as an SH reagent, it has a double bond capable of reacting with free SH groups[12].

Materials and methods. Experiments were performed on the ventroabdominal flexor muscles of *Atyas occidentalis.* The muscles were fixed on a Petri dish provided with a layer of Sylgard and filled with Van Harreveld's saline[13]. The