Variation of Nuclear Size of Neurons of the Ventromedial Nucleus in Relation to Spermatogenesis in the Squirrel Monkey (*Saimiri sciureus*)

The hypothalamic ventromedial nucleus (VMN) has been implicated in the regulation of growth hormone[1], adrenal steroids[2], gonadal steroids[3] and of food intake[4] and hemorrhagic stress response[5]. The VMN together with the arcuate nucleus is known as a 'tonic center' for control of gonadotrophin releasing hormone secretion[6]. Stimulation in the VMN induces far-reaching changes in the tubules and Leydig cells[7] and the VMN is one of the areas where testosterone concentrating neurons are found[8]. On the other hand, testicular function is impaired after destruction of the VMN[9].

The participation of VMN in spermatogenesis is not well understood. Therefore, as part of a study investigating morphological sex differences in the limbic and hypothalamic structures involved in reproductive activity[10], nuclear size of neurons in the VMN, lateral septum (LS) and ventral hippocampus (VH) was measured in normal male and ovariectomized female squirrel monkeys on replacement estrogen/progesterone therapy. Both the LS and VH have also been described as being involved in reproductive activity[3]. The average nuclear diameters of 200 cells from VM, LS and VH were compared with endometrial activity in females and the stage of spermatogenic activity in males. The parietal part of the cerebral cortex (CC) was selected as a control area without any known relevant endocrine activity.

Materials and methods. Squirrel monkeys (*Saimiri sciureus*) were housed at 25° ± 1 individually for at least 3 weeks prior to study and were fed monkey chow and given water, ad libitum. 5 mature females were ovariectomized and left undisturbed for 1 week. They were then treated with a daily i.m. dose of 2.5 mg estradiol benzoate and 12.5 mg of progesterone[11] for 10 days. These females and 10 normal males were left undisturbed for 17 h prior to sacrifice. To avoid an alteration in nuclear size due to a stress response[5], the animals were quickly heparinized and then decapitated in less than 1 min after the room was entered. Brain perfusion with saline through the carotid artery and later fixation with Bouin's solution via the same route was finished in 5 to 6 min after death in order to protect against nuclear changes due to hypoxia[12]. The brains were embedded in paraffin and modified SZENTHAGOTHAI et al.[9] method[13] was used for measurement of the cell nucleus diameter in Hematoxylin-eosin stained slides.

All animals were weighed and the uterus or the testis and epididymis were removed and fixed in formalin. The stage of the endometrium and the number of sperm cells in the testes and the epididymis were estimated in hematoxylin-eosin stained slides. The average nucleus diameters of the VMN cells in the male were arranged in order according to spermatogenic activity (if more sperm cells were found in the testes than in the epididymis – high spermatogenesis, if reverse – low spermatogenesis[14] and were also compared with body weight. Higher body weight has been found to be a characteristic feature of the male squirrel monkey during the breeding season[11]. Correlation coefficients were calculated between the nuclear size and spermatogenic activity and between the nuclear size and body weight[15].

Results. A remarkable inverse relationship was found between the nuclear size of the VMN neurons in the male and the stage of spermatogenic activity ($r = 0.84, p < 0.05$). The animals with the smallest nuclear diameter (number 272, 286, 296) were found to have the highest spermatogenic activity. In addition, these animals exhibit very high body weight ($r = 0.72$, $p < 0.05$) (Figure 1). In the ovariectomized females kept on estrogen/progesterone therapy, the endometrium was found to be in the secretory phase in all animals. The VMN cell diameter in females showed no significant differences between individual animals and also no correlation with body weight. In the lateral septal area (Figure 2), in the ventral hippocampus (Figure 3) and in the cerebral cortex[10], no significant differences between sexes or between individual animals were observed. The medial septal area and the dorsal hippocampus were investigated in a pilot study and found to be unsuitable for karyometric evaluation. Each of these areas has a very heterogeneous population of cells with a large range of nuclear diameters and probably contains more than one cell population.

Discussion. In the rat, a significant difference in the nuclear size of hypothalamic VMN neurons has been reported between the intact male (continuously in rut)

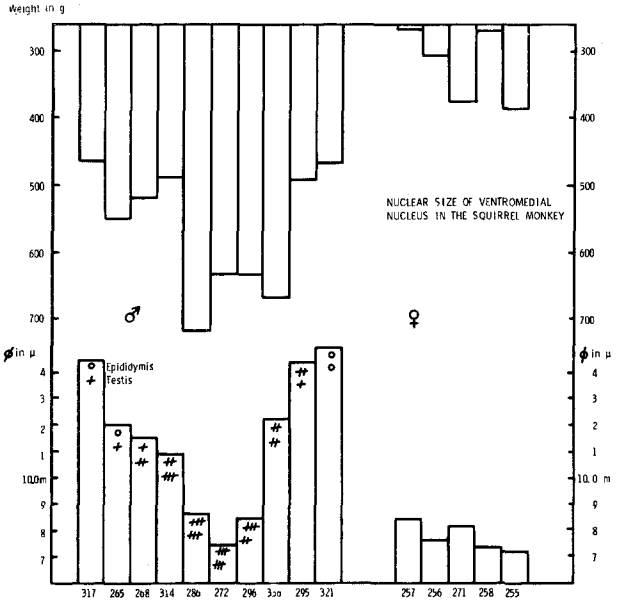

Fig. 1. Nuclear size of ventromedial nucleus in the squirrel monkey.

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and in the intact or castrate female[16]. Castration or testosterone treatment in the adult male rat[9] as well as castration in the female[16] does not influence the nucleus size of VMN neurons. Our findings in the male squirrel monkey shows a clear inverse relationship between the nucleus size of the VMN cells and the spermatogenic activity. In the ovariectomized female squirrel monkey kept on estrogen/progesterone replacement therapy, the average nuclear diameters of the VMN cells in 5 animals were not significantly different and were almost equal to the nucleus size of VMN cell diameter of the male with the strongest spermatogenic activity. This disagreement with the findings of DÖRNER and STAUDT[16] who found the VMN cell size in the male rat with active spermatogenesis smaller than in the female may be related to the different type of male breeding (as the rat is a continuous breeder while the squirrel monkey is a seasonally breeding animal). Another but less likely possibility is that nuclear volume changes in VMN in the female squirrel monkey were induced by the estrogen/progesterone treatment. In the female rat, changes of estrogen/progesterone levels during the estrous cycle does not influence the nuclear size of VMN cells[17].

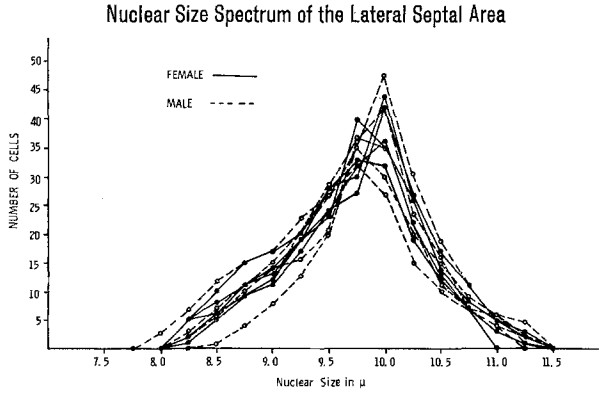

Fig. 2. Nuclear Size Spectrum of the Lateral Septal Area.

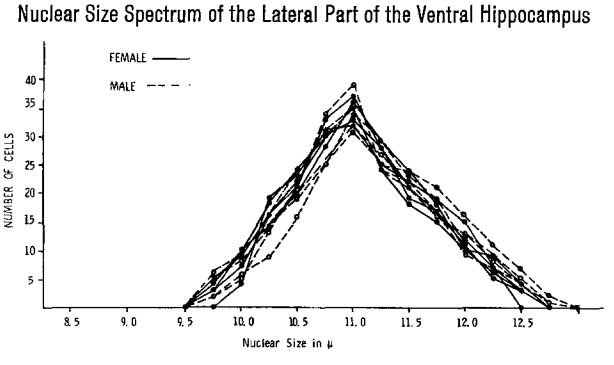

Fig. 3. Nuclear Size Spectrum of the Lateral Part of the Ventral Hippocampus.

A sex-dependent neural control of reactivity to electric footshock has been found after destruction of the VMN but not after lesions in the septum. In male rats, destruction of the VMN increases sensitivity to electric shocks but in the female no changes were found[18]. Unfortunately, as the endocrine activity of these animals was not examined, it is possible that a decrease of testosterone level increased the sensitivity to electric shock[19].

Stimulation of VMN cells induced a marked increase of activity in mitosis of spermatogonia and spermatocytes in the seminiferous tubules[7] showing that the VMN exerts some direct influence on spermatogenesis. The findings of SZENTHAGOTHAI et al.[9] that spermatogenesis persists after VMN destruction indicate that the VMN influence on testicular activity is not essential. Unilateral destruction of VMN in man has been used as a therapy in the cases of psychological deviation (exhibitionism, sex offenders, etc.) without inducing changes in either libido or spermatogenic activity[20].

The VMN area has very close connections to limbic system structures like the septum, amygdala and hippocampus[3]. Therefore, it might be possible that the limbic system which modulates the activity of the neurons that are responsible for the secretion of gonadotropin releasing hormones[6] influences the hypothalamic centers through VMN neurons with which it has direct connections[21]. Of the limbic system structures studied karyometrically, only the medial amygdala exhibits sex dimorphism[10] but not the LS or VH.

It seems probably that the VMN has a multiple role in the regulation of endocrine activity and that the different subunits of this nucleus may have different functions[17] regarding GH[1], ACTH[9] and gonadotrophic secretion[6]. We can conclude that in the squirrel monkey, a seasonally breeding animal, the nuclear size of the VMN cells exhibits changes which correlate with spermatogenic activity.

Zusammenfassung. Nachweis, dass bei ausgewachsenen Totenkopfaffen (*Saimiri sciureus*) die Zellkerndurchmesser im Nucleus ventromedialis hypothalami in der Periode der hochaktiven Spermiogenese kleiner sind als zu Beginn der Brunstperiode. Da das limbische System mit diesem Kern zusammenhängt, sind direkte Einflüsse nicht auszuschliessen.

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Increased Cardiac Turnover of Noradrenaline after Chronic Administration of Guanethidine in the Rat

Guanethidine, which is used in the treatment of hypertension, has been shown to cause a depletion of the peripheral stores of noradrenaline[1, 2]. This drug is taken up and stored in the sympathetic ganglion nerve cells[3, 4]. However, the gross depletion of noradrenaline is not caused by a direct replacement reaction[5]. Several authors could show that chronic treatment with guanethidine caused histological damage of the adrenergic system in