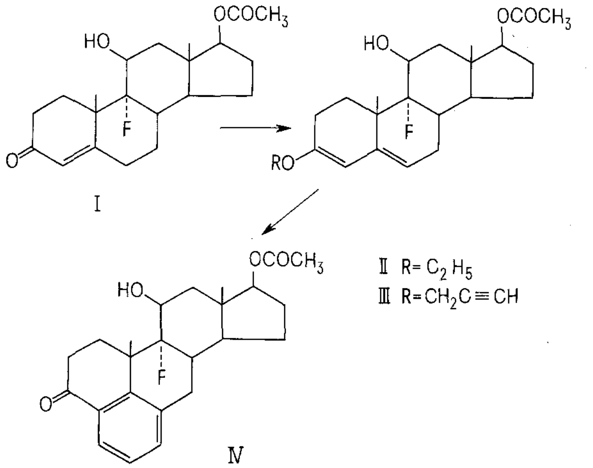
parabiotic rats[7] revealed that FBTA produces the same effect as TP with doses 20 times greater, yet scarcely androgenic. Also, 10 mg of FBTA proved to be less uterotrophic than 0.4 µg of estrone and as anti-uterotrophic as 20 mg of TP in the mouse test[8].

Tested in rats bearing hormone-dependent mammary tumors induced by 7,12-dimethylbenz[a]anthracene (DMBA)[9], FBTA after 30 days of s.c. treatment at daily doses of 2 and 4 mg decreased the death rate from 50% of the controls to 11 and 25% respectively. Tumor regression, evaluated as the ratio between the average areas of treated and untreated control tumors, was 0.05 and 0.13, respectively. The lack of a dose-related effect is not explainable; this observation has been frequently reported with other compounds used in anti-tumor screening[10,11]. The anti-tumor effect was still evident 30 days after discontinuing treatment.



FBTA is biologically very similar to its analog, BTA, except for a higher antigonadotrophic and anti-tumor potency at low doses. The hypothesis is proposed that the antigonadotrophic and anti-estrogenic activities make FBTA a very effective inhibitor of experimental hormone-dependent mammary tumors of rats.

Riassunto. È stato preparato un nuovo analogo pentaciclico del testosterone e ne sono state saggiate le proprietà ormonali e l'attività nel test del tumore mammario indotto dal DMBA nel ratto. Il conposto ha dimostrato un'elevata attività antitumorale e una scarsa attività androgena.

G. Briziarelli[12], P. P. Castelli[13], R. Vitali and R. Gardi

Warner-Vister Steroid Research Institute, I–22064 Casatenovo (Como, Italy), 19 October 1972.

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[12] G. Briziarelli: present address Warner-Lambert Research Institute, Morris Plains, New Jersey, USA.
[13] P. P. Castelli: present address Istituto Lark per le Ricerche Mediche, San Giuliano Milanese, Italy.

Morphologic Sex Differences in Primate Brain Areas Involved in Regulation of Reproductive Activity

The major region regulating sexual behavior and secretion of gonadotrophins in mammals is the hypothalamus[1]. However, an equally important area in this control is the 'limbic system', an area mediating the connections between the old an new brain structures. The amygdala plays a particularly important role in the regulation or modification of both sexual behavior[2] and gonadal function[3]. RNA metabolism in both the hypothalamus and amygdala shows marked alterations in response to neonatal androgen administration as compared to other parts of the brain[4]. To determine whether morphological sex differences could be found in these areas in the primate brain, nuclear size measurements were performed on neurons in the nucleus medialis amygdalae (NMA), the suprachiasmatic nucleus (SCH), medial preoptic area (MPOA), arcuate nucleus (ARN) and cerebral cortex (CC) in normal male and ovariectomized female squirrel monkeys on replacement estrogen/progesterone therapy.

Material and methods. Five mature females were treated for 10 days (beginning 1 week after ovariectomy) with an i.m. dose of 2.5 mg of estradiol benzoate and 12.5 mg of progesterone[5]. These females and 5 normal males were left undisturbed for 17 h prior to sacrifice. To avoid an alteration in nuclear size due to a stress response[6], 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 was finished in 5 to 6 min after death in order to protect against nuclear changes due to hypoxia[7]. The brains were embedded in paraffin and for measurement of the cell nucleus diameter in Hematoxylin-Eosin slides a modified Szenthagothai et al method was used[8]. In each described area, 200 cell nuclei were measured.

Results. A marked sex difference was found in the nuclear diameter of neurons in the NMA. The mean diameter of the cell nuclei in the NMA in the female group was 8.74 µm ± S.E. 0.028 and was significantly smaller ($P \leq 0.01$) than in the male group (Figure 2) which averaged 9.57 µm ± 0.054. The SCH area together with the neighboring MPOA are known to be involved in the

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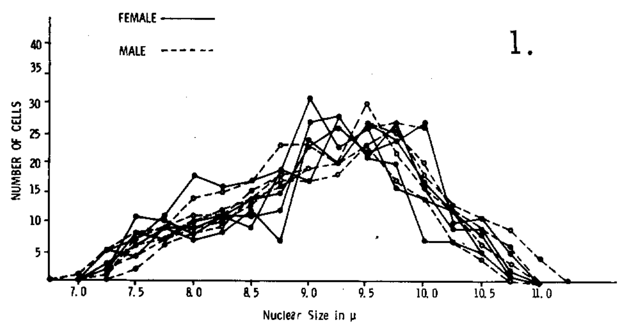
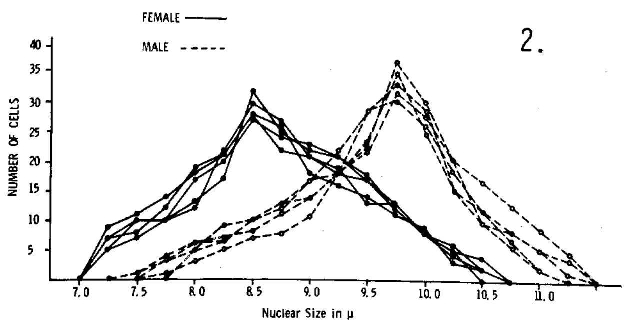
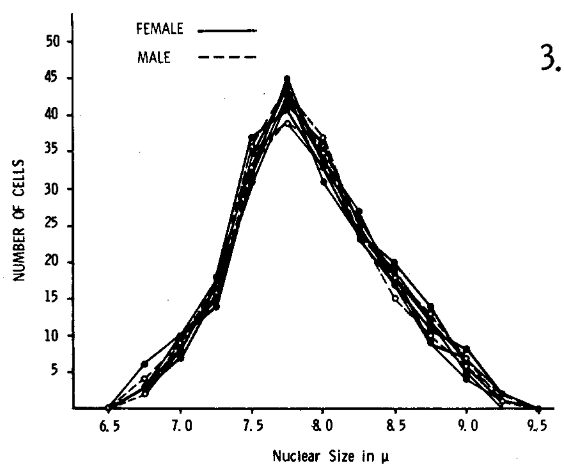
Nuclear Size Spectrum of the Medial Preoptic Area



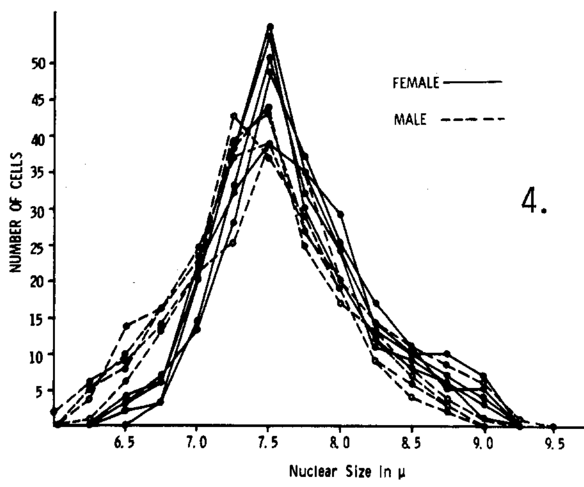
Nuclear Size Spectrum of the Medial Amygdala



Nuclear Size Spectrum of the Cerebral Cortex



Nuclear Size Spectrum of the Suprachiasmatic Nucleus



control of gonadotropins[1] and sexual behavior[9] while the ARN is involved in gonadotropin control. The SCH cells have a small range of nuclear diameter, a clear-cut peak and probably only one population of cells are morphologically distinguishable with our methods (see Figure 4). The MPOA cell population was found to be very heterogenous (Figure 1) as was the ARN. The cells have a large range of nuclear diameter, no clear-cut peak, probably contain more than one cell population and therefore could not be statistically evaluated. No sexual dimorphism was evident in the SCH. (Female mean diameter 7.67 μm $\pm$ S.E. 0.017, male 7.47 μm $\pm$ S.E. 0.016, $P > 0.05$).

As a control area without any relevant endocrine activity, the parietal part of the CC was selected (Figure 3). The mean diameter of the cell nuclei in the CC in the male group (7.91 μm $\pm$ S.E. 0.0046) did not differ from the female group (7.88 μm $\pm$ S.E. 0.0094, $P > 0.05$).

Discussion. There are at least 3 possible mechanisms which might produce such a male/female difference. First, constitutional male/female differences may exist which are independent from the level of circulating hormones. Sex differences have been reported in the nuclear size of the cells in the medial preoptic area between postpubertally castrated male and female[10] animals which should have no difference in hormone levels at the time of study. Secondly, the difference may be due to the different hormone environments in males and females[11]. Changes in the nuclear size during the estrous cycle in the female have been described in the anterior hypothalamic area[12], and are presumably related to changing hormone levels. The third possibility is a combination of the above 2. Some constitutional differences could exist which could be further modified by the level of circulating hormones.

The difference in nuclear size found in medial amygdala neurons may be related to sex differences in pituitary gonadotropins which seem to be modulated by the amygdala. Changes of LH levels in plasma registered after electrochemical stimulation (ES) in the MA[13] is dependent on the prepubertal sexual differentiation[14,15].

The SCH-MPOA also seems to have a role in the preovulatory LH surge[15,16] but it appears that the activity of the MA may be more strictly sex dependent.

The sex difference in the MA may also be related to the fact that the NMA contains a very high number of estradiol-concentrating neurones[17,18] while in the SCH no such neurons are found[18]. All the above studies were performed in the rat. It is possible that differences exist between primates and rodents.

In conclusion, significant sex differences were found in the nuclear size of neurons in the medial amygdala but not in neurons of the suprachaismatic nucleus or in the cerebral cortex in the squirrel monkey.

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Zusammenfassung. Der Durchmesser der Zellkerne von Neuronen im Nucleus Medialis Amygdalae (NMA) der normalen männlichen Totenkopfaffen ist grösser als derjenige der ovarektomierten Weibchen mit Östrogen/Progesteron-Substitution. Keine Unterschiede wurden jedoch im Nucleus suprachiasmaticus and cerebralen Cortex festgestellt. Diese morphologischen Unterschiede können auf eine unterschiedliche funktionelle Bedeutung der Amygdala bei Männchen und Weibchen bezogen werden.

G. A. Bubenik and G. M. Brown

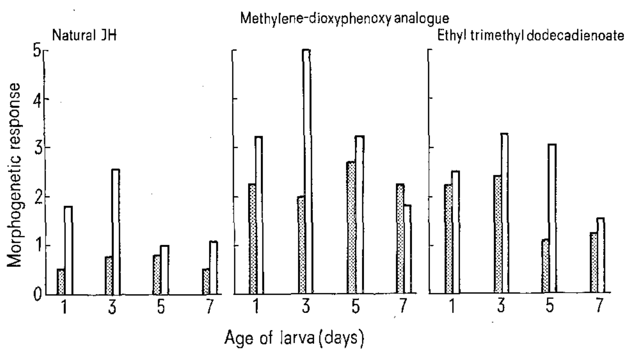
Neuroendocrine Research Section, Clarke Institute of Psychiatry, 250 College Street, Toronto M5T JR8 2b (Ontario, Canada), 30 October 1972.

Effect of Diet on Response to Juvenile Hormone in *Galleria mellonella* Larvae

In the course of a study of the morphogenetic activity and metabolism of juvenile hormone (JH) and its analogues in last instar larvae of *Galleria mellonella*, we found that the morphogenetic response of wax moth larvae to JH varied with the diet. Larvae raised on a diet containing beeswax, honey, glycerin and Gerber's mixed cereal[1] showed a higher morphogenetic response to a standard dose of JH than larvae raised on a similar diet lacking beeswax.

Galleria larvae were raised on a diet consisting of Gerber's mixed cereal, glycerin, honey and beeswax in the proportion 12 :1 :1 : 0.5 by volume or on a similar diet but without beeswax. The larvae raised on the diet containing beeswax will be referred to as wax-fed larvae while the other larvae will be referred to as wax-deprived larvae. The larvae were raised from the first instar in plastic containers with unlimited food supply and were maintained at 29 $\pm$ 1°C and 70% r.h. Freshly molted last instar larvae can be recognized by their weight, the size of the head capsule[2] and, in this strain of *Galleria*, by pigmentation of the larval integument. The term larva in this communication refers to the last instar larva. The last larval stadium in *Galleria* under these conditions spans a period of 9 to 10 days, of which the last 2 days represents the pharate pupal stage.

Natural juvenile hormone[3] (methyl 12,14 dihomo-juvenate), and methylenedioxyphenoxy 6-epoxy 3-ethyl 7-methyl 2-nonene[4] (Hoffmann La Roche, Nutley, N.J.) and ethyl 3,7,11, trimethyl 2,4 dodecadienoate[5] (Zoecon Corp. Palo Alto, California) were used in this study. These compounds are mixed isomers and possess potent juvenile hormone activity in *Tenebrio* bioassay[6]. Morphogenetic response of the larvae to JH was determined according to Sehnal and Meyer[7] modification of a procedure described by Piepho[8]. For this purpose, 25 µg of natural JH, 5 µg of methylenedioxyphenoxy analogue or 2 µg of ethyl trimethyl dodecadienoate were injected into the larvae in 1 µl of peanut oil. Control larvae were injected with 1 µl of peanut oil. Morphogenetic response of the larvae to JH was estimated by determining the extent of retention of larval characters at the succeeding ecdysis which normally occurred within 10 to 12 days. Some of the additional treated larvae pupated later. However, several larvae treated with ethyl trimethyl dodecadienoate failed to pupate, even 35 days after treatment with this analogue. A score of 5 was assigned for larvae which underwent an additional larval molt, and a score of 0 was assigned if the larvae metamorphosed into perfect pupae. At least 30 larvae were used for each determination and thus the score presented in the graph represents an average response of 30 to 50 insects.



Effect of presence of beeswax in the diet on the morphogenetic response of last instar *Galleria* larvae to JH and analogues.
▨ wax-free diet; □ diet with beeswax.
Dose applied: Natural JH, 25 µg per larva; Methylenedioxyphenoxy analogue, 5 µg; Ethyl trimethyl dodecadienoate, 2 µg.

Since the response of *Galleria* larvae to juvenile hormone varies with the age of the larva[7], larvae of diverse ages, 1, 3, 5 and 7-days-old were used in this investigation. All control larvae (wax-fed as well as those raised on wax-free diet) except some 1-day-old larvae, metamorphosed into perfect pupae. The larval molting of 10 to 14% of the peanut oil injected 1-day-old control larvae is attributed to injury which induces molting in these larvae[9]. The results recorded in the Figure show that wax-fed larvae are more sensitive to JH than the larvae on wax-free diet. This difference in sensitivity to JH is most conspicuous in 3-day-old larvae when compared to the other ages. Except for 7-day-old larvae treated with methylenedioxyphenoxy analogue, the wax-fed larvae always showed a greater morphogenetic response than the larvae raised on wax-free diet. In addition, the data also show that the response to JH in wax-fed larvae varied with age; 3-day-old larvae being most sensitive, confirming the earlier observations of Sehnal and Meyer[7]. Larvae raised on wax-free diet did not show conspicuous age-related changes in their response to JH although there are some minor fluctuations. The morphogenetic response of 3-day-old wax-fed larvae to

[1] Gerber mixed cereal, Gerber Products Co. Freemont, Michigan Composition: Protein, 11.7%; Fats, 4.5%; Carbohydrates, 73%; Crude fibre, 1.1%; Ash, 2.7%; Moisture 0.7% and vitamins.
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