from 2-cell stage eggs developed in vitro, as well as the zona-intact ones, into normal blastocysts at the expected time. The size of blastocysts formed from these embryos was similar to or slightly smaller than that of normal blastocysts. By contrast, both the one-quarter embryos (¼-embryos) with and without the zona showed a marked drop of developmental potential, and formed various abnormal embryos at the blastocyst stage, which were similar to those described in a previous report[12]. The lack of developmental potential of ¼-embryos might be due to the inadequacy of the number of cells composing the embryos developed from ¼-embryos, as Rossant[13] has suggested; the volume of most blastocysts from ¼-embryos was far smaller than that of ordinary ones. Kelly[14] reported that ¼- and ⅛-embryos of mouse could give rise to embryos which could be born normally as chimaeras when combined with other blastomeres to restore the normal cell numbers.

The rate of development into blastocysts was slightly lower in the zone-intact blastomeres than the zona-free ones. The debris of destroyed blastomeres remaining inside the zona might affect the development of the intact blastomeres.

It was reported that the zona-free embryos did not undergo any further cleavage when transferred to the oviduct because they adhered to the wall of the oviduct[7,8]. When naked embryos were inserted into an empty zona or embedded in agar and transferred to recipient oviducts, they produced live young[6,15]. On the other hand, naked morulae or blastocysts which were transferred into the uterus gave rise to normal conception[16]. However, the possible significance of the zona in normal development in vitro of single blastomeres is not well known. In this experiment, there was no difference in developmental potential in vitro between blastomeres with and without the zona. We therefore conclude that the presence of the zona pellucida is not essential for the development of single mouse blastomeres in vitro.

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Neuron numbers in hypothalamic nuclei of young, middle-aged and aged male rats

J.L. Sartín[1] and A.A. Lamperti[2]

Institute of Aging and Department of Anatomy, Temple University, School of Medicine, Philadelphia (Pennsylvania 19140, USA), 29 February 1984

Summary. Morphologic analysis of nine hypothalamic areas revealed significant decreases in the number of neurons per unit area in the ventral medial and arcuate nuclei. These data suggest that altered neuron numbers in the VMW and perhaps the ARC may participate in the well documented reductions in endocrine and neuroendocrine function observed in aging rats.
Key words. Hypothalamus; neuron number; aging; neuroendocrinology.

Several studies have suggested that morphologic alterations in the hypothalamus occur with advancing age. Glees et al.[3] found degenerative changes in mitochondria in the hypothalamus of aging monkeys. Age-related changes, which included a deterioration and loss of dendritic surfaces, were also found in hypothalamic neurosecretory nuclei of old mice[4]. Detailed studies of the arcuate nucleus (ARC) of the hypothalamus have shown a decrease in axosomatic and axo-dendritic synapses in old male rats[5]. There is a loss of neurons in the medial preoptic area (mPOA), anterior hypothalamic area (AHA) and the ARC in aging female rats[6]. Each of these studies provides morphologic data which can be correlated with well documented age-related physiological changes in endocrine and neuroendocrine function[7,8].

Aging male Sprague-Dawley rats are often used to study a wide variety of endocrine and neuroendocrine dysfunctions during senescence[9–12]. Since there are relatively few reports concerning any morphological changes in the hypothalamus of this animal model, it became imperative to determine whether changes in neuron number occurred in specific hypothalamic nuclei of aging male Sprague-Dawley rats.

Materials and methods. Male Sprague-Dawley rats, ages 3, 12 and 24 months (young, middle-aged and aged, respectively), were obtained from Charles River Breeding Laboratories (Wilmington, MA). These rats were Cesarian derived and raised behind specific pathogen free barriers. Animals were housed five per cage and provided food and water ad libitum in a temperature (24 °C) and photoperiod controlled (12 h light – 12 h dark, lights on at 06.00 h) animal room. Normal aging pathology and requirements for short-term handling have been reported elsewhere[9,13].

The brains of five young, five middle-aged and six aged rats were removed following decapitation and fixed in 10% neutral buffered formalin, dehydrated and embedded in paraffin. The hypothalami were sectioned at 9 µm and stained with cresyl violet. The mPOA, suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN), ventral medial nucleus (VMN), dorsal medial nucleus (DMN), ARC, subventricular portion of the ARC (SV) and the medial mammillary nucleus (MM) were studied according to the following procedure which was previously reported[14]. The numbers of neurons in these nuclei were counted in every fifth section under an AO microscope

equipped with an eyepiece micrometer in a 10 × ocular with a 1.7 × zoom lens. When a 40 × objective was used, the area under the grid of the micrometer was calculated to be 0.01 mm^2. The average number of neurons per unit area was obtained for each nucleus in all age groups. In addition the number of tissue sections which data were obtained from each nucleus was recorded. This information helped to determine any changes in the rostral-caudal extent of each nucleus. The diameters of these nuclei were also obtained in every fifth section with the use of the eyepiece micrometer.

Data were analyzed by analysis of variance and Student's t-test. A p-value less than 0.05 was considered significant.

Results. The number of neurons per unit area, diameters and rostral-caudal extent of 9 hypothalamic nuclei are shown in the table. There was a significant reduction in the number of neurons per unit area in the ARC ($p < 0.05$), VMN ($p < 0.01$) and the SV ($p < 0.001$) in 24-month-old rats. Similar reductions also occurred when data was compared to middle-aged rats. The rostral-caudal extent of the ARC of aged rats was significantly increased ($p < 0.05$) when compared to young rats but not statistically significant when compared to middle-aged rats. Diameters of these nuclei did not change with age.

Discussion. There is progressive decline in endocrine and neuroendocrine function with age[7,15]. In male Sprague-Dawley rats there is a diminished adrenal response to starvation[9], reduced levels of circulating thyroxine and triiodothyronine[10], and a diminished insulin secretory response to glucose[11]. Neuroendocrine studies have reported a progressive loss of the thyrotropin circadian rhythm as rats age from 2 to 24 months[10], an enhanced inhibition of luteinizing hormone (LH) and follicle stimulating hormone (FSH) release by testosterone, and a diminished secretion of LH and FSH in response to gonadotropin releasing hormone (GnRH)[12]. Studies in other rat models have likewise suggested alterations in neuroendocrine function during aging. For example, Fisher 344 rats demonstrated decreased immunoreactive GnRH and somatostatin content of the median eminence[16]. Also, the pulsatile release of growth hormone (GH) was decreased in old male Long Evans rats[17]. Many of these age-related alterations in hypothalamic function have been suggested to result from altered neurotransmitter levels in the hypothalamus[18].

Several authors have suggested that chemical lesions of the ARC result in altered secretion of GH, LH and FSH[19–23]. At first glance, our data suggest that the loss of neurons in the ARC could explain the altered secretion of GH, LH and FSH observed in old rats[12,17,18,24]. However, the apparent loss of neurons in the ARC is offset by an increase in the rostral-caudal extent of the ARC. Therefore, altered hormone secretion may not necessarily be due to a loss of neurons in this nucleus, but rather due to a loss of hypothalamic neurohormones and/or neurotransmitters. It is of interest to note that the growth curve of the aging male Sprague-Dawley rat tends toward mild obesity[13] and has been associated with hyperglycemia, hyperinsulinemia[25] and large multinodular pancreatic islets of Langerhans[26]. Since chemical lesions of the VMN produce hyperinsulinemia and obesity in mice and rats[27,28], the loss of neurons in the VMN, without a corresponding increase in rostral-caudal length, may contribute to specific growth characteristics as well as to altered insulin regulation observed during aging in this rat model.

The data in this study are similar to the recent article by Peng and Hsu[29] who found no loss of neurons in the hypothalamus of aging male Sprague-Dawley rats. However, we did observe a loss of neurons in the VMN. There are two possible explanations for this apparent discrepancy. First, the method used to count neuron numbers differs between the two studies. Peng and Hsu[29] obtained total neuron number by multiplying the volume of each area by the neuron density, whereas we calculated the number of neurons per unit area. Secondly, the source of rats, normal aging pathology, and housing conditions were unspecified by those authors. Sprague-Dawley rats in this study were maintained behind specific pathogen free barriers at Charles River. Nutrition and housing conditions have been rigorously defined and normal aging pathology documented[9,13].

In conclusion, it is plausible to suggest that changes in hypothalamic neuron numbers in the VMN as observed in this paper may relate to the altered endocrine and neuroendocrine function in aging Sprague-Dawley rats. Furthermore, since there were no differences between young and middle-aged rats, it is likely that the changes reported in this paper reflect aging rather than maturational changes.

Quantitation of neurons in nine hypothalamic nuclei of male Sprague-Dawley rats (See materials and methods for abbreviations)

Nucleus		Young (3 months) n = 5	Middle aged (12 months) n = 5	Aged (24 months) n = 6
mPOA	a	12.6 ± 0.8	14.7 ± 1.9	9.9 ± 0.8
	b	16.5 ± 4.5	10.0 ± 1.7	10.3 ± 1.7
	c	0.59 ± 0.05	0.59 ± 0.05	0.60 ± 0.09
SCN	a	40.7 ± 3.4	38.5 ± 2.1	32.8 ± 2.9
	b	10.8 ± 1.2	9.8 ± 1.3	8.5 ± 1.8
	c	0.35 ± 0.03	0.34 ± 0.01	0.36 ± 0.04
SON	a	7.8 ± 0.6	8.6 ± 0.6	7.7 ± 0.9
	b	27.3 ± 1.3	31.8 ± 2.5	28.5 ± 6.6
	c	0.44 ± 0.03	0.40 ± 0.02	0.44 ± 0.06
PVN	a	12.8 ± 0.9	13.6 ± 1.4	11.3 ± 0.6
	b	10.5 ± 1.6	10.2 ± 1.0	9.5 ± 1.4
	c	0.62 ± 0.03	0.57 ± 0.02	0.56 ± 0.02
VMN	a	15.0 ± 1.3	13.6 ± 0.6	10.0 ± 0.7 (e,h)
	b	21.8 ± 1.1	21.2 ± 0.5	26.3 ± 2.9
	c	0.81 ± 0.03	0.78 ± 0.03	0.77 ± 0.08
DMN	a	14.7 ± 1.7	13.5 ± 0.2	12.0 ± 2.1
	b	13.8 ± 1.7	12.8 ± 1.1	14.5 ± 2.9
	c	0.77 ± 0.01	0.72 ± 0.03	0.77 ± 0.07
ARC	a	19.7 ± 2.2	16.7 ± 0.7	13.4 ± 0.6 (d,g)
	b	23.1 ± 1.1	26.2 ± 1.5	38.5 ± 7.0 (d)
	c	0.43 ± 0.01	0.42 ± 0.01	0.49 ± 0.03
SV	a	13.8 ± 0.9	13.8 ± 0.6	7.8 ± 0.8 (f,i)
	b	3.3 ± 0.8	2.8 ± 0.4	3.5 ± 0.3
	c	0.47 ± 0.03	0.44 ± 0.03	0.45 ± 0.05
MM	a	15.4 ± 0.8	14.8 ± 0.8	13.0 ± 0.6
	b	16.0 ± 2.9	14.8 ± 2.4	19.6 ± 1.9
	c	0.84 ± 0.05	0.86 ± 0.04	0.81 ± 0.11

a, neurons per unit area (0.01 mm^2); b, numbers of sections counted (every fifth 9μm section); c, diameters of nuclei (mm). Comparisons of young vs aged, d, $p < 0.05$; e, $p < 0.01$; f, $p < 0.001$; comparisons of middle aged vs aged: g, $p < 0.01$; h, $p < 0.005$; i, $p < 0.001$.

1 Present address: J.L.S., Department of Physiology and Pharmacology, Auburn University, School of Veterinary Medicine, Auburn University (AL 36849, USA).
2 Reprint requests: A.A.L., Department of Anatomy, Temple University, School of Medicine, 3420 North Broad Street, Philadelphia (PA 19140, USA).

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Moth ocellar interneurons show abnormal development in the absence of receptor innervation

J.L. Eaton and M.M. Sprint

Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg (Virginia 24061, USA), 26 April 1984

Summary. Unilaterally ocellate adult moths were produced by cauterization of one of the pair of ocellar primordia in fifth instar larvae. The remaining ocellar nerves and associated interneurons of the adult moths were subjected to cobalt infiltration and Timm's intensification. Two interneurons from the ablated ocellus were observed to grow into the synaptic region of the remaining ocellus and presumably made functional connections with ocellar receptor cell axons.
Key words. Development; interneurons; moth; ocellus; *Trichoplusia ni;* Lepidoptera: Noctuidae.

Developing ocellar interneurons of locusts grow out along the pathway pioneered by retinula cell axons and form a peripheral synaptic region at the base of the ocellus[2]; in moths these ocellar interneurons remain within the brain[3]. In locusts and in moths development of ocellar neuropile follows invasion of the brain by developing retinula cell axons[2,3]. This development of interneurons along specific pathways has been explained by the newly proposed 'labeled pathways', hypothesis which suggests that developing interneuron growth cones can recognize and extend upon the surfaces of preexisting labeled axons to reach their normal sites of innervation[4]. Unilateral removal of auditory afferents result in alterations of the growth patterns of a cricket identified auditory interneuron producing a dendritic field contralateral to its normal position in the prothoracic ganglion[5]. Similarly, unilateral removal of cercal afferent innervation during interneuron development in locust embryos results in a reduction in interneuron dendrite development in denervated neuropile regions[6]. These results suggest that normal dendrite development involves an interaction between sensory axons and interneurons.

Cabbage looper moths (*Trichoplusia ni,* Lepidoptera: Noctuidae) have two dorsal ocelli which develop in the pupal stage from larval primordia[7]. Parallel with ocellar development, three ipsilaterally, two contralaterally and one bilaterally projecting large ocellar interneurons arise from neuroblasts in the pars intercerebralis and develop in the protocerebrum of the brain[4,8]. By carefully using a cauterization technique, ocellar primordia of fifth instar larvae were unilaterally ablated, producing unilaterally ocellate adult moths[7]. The ocelli of control and unilaterally ocellate moths were infiltrated with cobalt chloride, for 19–20 h at 5°C. After cobalt precipitation with 0.01% ammonium sulfide, heads were fixed in Bouin's, dehydrated, embedded in paraffin, sectioned, and subjected to Timm's intensification to reveal infiltrated neurons[8,9]. Comparison of the results of infiltrations of control (fig. 1, A, B) and unilaterally ocellate moths (fig. 2, A, B) revealed two additional infiltrated interneurons extending into the synaptic region (O) of the remaining ocellus in the unilaterally ocellate moths. These neurons were the two contralaterally projecting interneurons from the ablated ocellus (C'). We are certain that these interneurons are not displaced ipsilateral interneurons from the intact ocellus since portions of two ipsilateral interneurons are visible in figure 2A and are also shown in figure 3. The ipsilaterally projecting interneurons (I') from the ablated ocellus were not infiltrated by cobalt chloride but did develop (fig. 3). Application of the 'labeled pathways' hypothesis to the development of moth ocellar neuropile leads to the proposition that the portion of the ocellar interneuron from which the dendritic terminals arise develops along a labeled pathway provided by ocellar retinula cell axons. Our findings suggest a bilateral symmetry in pathway labeling such that in the absence of a normal labeled pathway, an alternative, similarly labeled, pathway may be followed if it is close enough for contact by filopods of developing interneurons[5]. The failure of the ipsilaterally projecting interneurons of the denervated side to grow toward the axon of the contralateral ocellus may be due to the distance separating these developing cells from the contralateral pathways or perhaps to the use of a differently labeled pathway by these interneurons.

An important question raised by our findings is whether these abnormally developing interneurons make functional connections with ocellar receptor cell axons. A tentative answer is yes, due to the fact that these interneurons were infiltrated by cobalt passing into them in the synaptic region from the pro-