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Functional Significance of the Innervation of the Gonads

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Gonadal functions are governed by the hypothalamo-hypophysial system. Recent studies have demonstrated the existence of a multisynaptic neural pathway between the brain and the gonads. This review summarizes the morphological and physiological data that suggest the role of the brain–gonadal circuitry in the control of gonadal functions and discusses relevant clinical observations.

Key Words: Gonads; innervation; brain; viral tracing technique; neural control.

Introduction

It is well established that the nervous system controls gonadal functions via the hypothalamohypophysial system. It is also generally accepted that autonomic nerves to and from the gonads exert regulatory action on the vasomotor control of blood vessels. Besides the mentioned neuroendocrine and the peripheral vascular control mechanisms, an increasing number of data indicate that autonomic nerves innervating the gonads also play a physiological role in the control of hormone secretion of the organ. Furthermore, recent studies strongly suggest that cerebral structures connected transneuronally with the gonads control gonadal functions through a pituitary-independent, purely neural mechanism. The direct neural control is considered as one of the fine-tuning control mechanisms. The other one includes local autocrine and paracrine modulatory actions. These mechanisms, no doubt, are minor to the regulatory action of the hypothalamohypophysial–gonadal axis, but are required for the full control of reproductive functions.

It is well known that in both sexes the gonads and other organs of the reproductive system are innervated by sympathetic and parasympathetic efferent (motor) fibers belonging to the autonomic nervous system. In addition, these nerves possess afferent (visceral sensory) fibers that carry information toward the central nervous system (CNS). The nerve fibers to and from the organs, in addition to the classical neurotransmitters of the autonomic nervous system—noradrenalin, adrenalin, and acetylcholine—contain several other neurotransmitters and neuropeptides including serotonin, vasoactive intestinal polypeptide, substance P, calcitonin gene–related peptide, and galanine *(1,2)*. It seems to be very likely that the neural signals transmitted via the autonomic nerves to the gonads are integrated signals that include neuronal impulses from very different sites of the CNS. Apart from the most recent years, very little information was available on which supraspinal brain structures may be connected and probably involved in the neural control of the gonads.

It is clear that the key to understanding the neural control of the gonads includes the knowledge of which spinal cord and brain nuclei are neurally linked to this organ. Apart from recent years, this neuromorphological information was missing, because the tract-tracing techniques available did not permit following a pathway beyond the first synapse. The introduction of the viral labeling technique overcame this problem *(3–6)*. An important and crucial feature of this technique, compared with other methods for neuroanatomical tracing, such as fluorescent dyes or horseradish peroxidase, is that the virus is transmitted from an infected neuron to other neurons via their synaptic connections, thus defining the specific pathways involved in the neuron-to-neuron signaling *(4)*.

This review summarizes the peripheral, spinal, and supraspinal components of gonadal innervation and discusses their functional significance, including both experimental and clinical observations.

Viral Transneuronal Tracing Technique

Neurotropic alpha-herpesviruses are used for transneuronal tracing, i.e., for investigating the structural organization of multisynaptic pathways or circuits of several neurons. The utility of this technique is based on the ability of the virus to invade and replicate in neurons following central and peripheral inoculation and then infect synaptically connected nerve cells. Injection of the virus, in most cases Bartha's strain *(7)* of Aujeszky's disease virus *(8)*, an attenuated vaccine strain of a swine alpha-herpesvirus, known as pseudorabies virus, into a peripheral organ or the brain is followed by the uptake of the virus by nerve endings

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located in the injected area. The virus then travels along the axonal flow to the first-order neurons innervating the organ. After reproductive infection within the nerve cell, the virus passes through the synapse and infects the second-order neuron. Then the infection proceeds to further synaptically linked neurons. Thus, there is a kinetic of propagation of virus in the CNS. It starts with infection of the first-order neurons and perhaps a few second-order neurons. This is followed by prominent infection of second-order neurons, in addition to that of the first-order neurons, and a few third-order neurons. Infection progresses with the prominent infection of the third- and fourth-order neurons. The propagation time greatly varies with the different types of nerve fibers, and it is inversely correlated with the diameter and length of the nerve fibers *(6)*. The kinetic of virus infection implies that the analysis of virus-infected neurons with a well-defined time course is critically important to the interpretation of the data *(5,9)*. Jansen et al. *(10)* have reported that there is a narrow time window in which specific transneuronal labeling occurs. In the case of Bartha's strain, the virus travels primarily retrogradely *(3,4,11)*.

The virus-infected neurons and thus the organization of circuits can be defined by immunocytochemical localization of the virus. Because the virus travels primarily retrogradely, infected neurons in the CNS represent structures that give rise to efferent fibers involved in the innervation of the inoculated organ or brain area.

Testis

Peripheral, Spinal, and Supraspinal Components of Testicular Innervation

The testis is innervated by the superior spermatic nerve and the inferior spermatic nerve. The superior spermatic nerve, the major contributor of testicular innervation, originates from the celiac and aortic plexuses and runs alongside the testicular vessels. The cell bodies of the preganglionic sympathetic fibers are located in the thoracic segments 10 and 11 of the spinal cord. The parasympathetic component of the nerve belongs to the vagus nerve. The inferior spermatic nerve accompanies the ductus deferens, then travels within the epididymis and reaches the testis at its lower pole. Sympathetic fibers of the nerve originate from the inferior mesenteric and hypogastric plexuses, while the parasympathetic ones are branches of the pelvic nerve. The preganglionic neurons of the pelvic nerve are located in the sacral parasympathetic nucleus (for review, see ref. *1*).

With the aid of the transneuronal viral tracing technique, the supraspinal connections of testicular nerves could be revealed *(12,13)*. In addition to the presence of labeled cells in the sympathetic and parasympathetic neurons of the spinal cord that give rise to preganglionic fibers innervating the reproductive organs, several cerebral nuclei contained virus-infected neurons. In the brain stem, virus-infected neurons could be detected, among others in the A1, A5, A7 noradrenergic cell groups, in the caudal raphe nuclei (raphe obscurus, raphe pallidus, raphe magnus), in the locus coeruleus, in Barrington's nucleus, and in the mesencephalic periaqueductal gray. The main diencephalic structure in which we found labeled nerve cells was the parvocellular division of the hypothalamic paraventricular nucleus. In addition, a moderate number of labeled neurons could be detected in the lateral hypothalamus, medial preoptic area, dorsal hypothalamus, periventricular area, and arcuate nucleus. In the telencephalon, the bed nucleus of the stria terminalis, central nucleus of the amygdala, medial portion of the frontal cortex (mainly primary motor area), and a well-defined region of the insular cortex (primarily the posterior part of the agranular insular area) exhibited virus-infected cells. The density of virus-labeled cells was very high in the insular cortex and restricted almost exclusively to pyramidal cells.

The localization of virus-infected neurons in the sympathetic and parasympathetic preganglionic neurons of the spinal cord is consistent with previous data obtained by classical neuromorphological techniques. The time elapsed between pseudorabies virus injection into an organ and the presence of virus-labeled neurons in different cerebral structures, in addition to the distance between the end organ inoculated and the structure in question, suggests the number of synapses. The involvement of the vagus nerve in the direct innervation of the reproductive organs is suggested by the fact that despite the longer course, labeling in preganglionic sympathetic neurons and in the parasympathetic vagal nuclei appears almost simultaneously. Shortly after the detection of infected neurons in the spinal cord, those cerebral structures exhibited labeled cells that are in direct synaptic contact with preganglionic neurons of the spinal cord (Fig. 1). These brain stem nuclei include the paragigantocellular nucleus *(14)*, the caudal raphe nuclei *(15)*, the A5 noradrenergic cell group *(16)*, the Barrington's nucleus *(17)*, the locus coeruleus *(18)*, and the periaqueductal gray of the mesencephalon *(19)*. Similarly, direct descending fibers of hypothalamic nuclei, such as the paraventricular nucleus *(20,21)* and the lateral hypothalamus *(22)*, terminate in preganglionic autonomic neurons of the spinal cord. Structures exhibiting labeling at longer postinoculation time are those that have known synaptic contact with nuclei infected directly via preganglionic neurons of the spinal cord or vagal nuclei. The hypothalamic paraventricular nucleus and the nucleus of the solitary tract have extensive connections with diencephalic and limbic structures *(23–25)* including those which contained infected neurons (medial preoptic area, arcuate nucleus, amygdala, and so on).

Functional Considerations

Experimental Data

Increasing amounts of evidence indicate that neural structures at different levels (periphery, spinal cord, hypothalamus, limbic structures) of the circuit innervating the testis

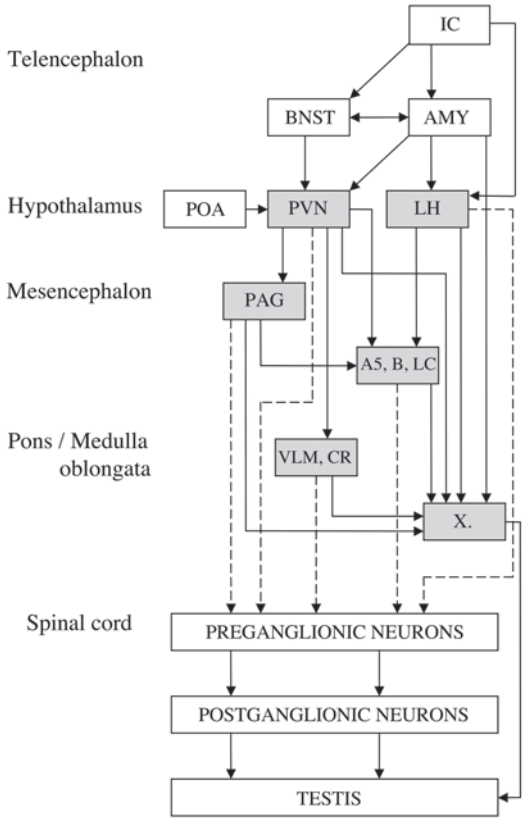

Fig. 1. Simplified schematic drawing illustrating known descending connections between the CNS structures labeled from the testis by viral transsynaptic tracing technique. A5: A5 noradrenergic cell group; AMY: amygdala; B: Barrington's nucleus; BNTS: bed nucleus of the stria terminalis; CR: caudal raphe nuclei; IC: insular cortex; LC: locus coeruleus; LH: lateral hypothalamus; PAG: periaqueductal gray; POA: preoptic area; PVN: paraventricular nucleus of the hypothalamus; VLM: ventrolateral medulla; X: vagal nuclei.

exert regulatory action on the endocrine compartment of the organ. It is well established that transection of nerves to and from the testis interferes with testicular functions. Transection of the superior spermatic nerve induces a decrease in testicular serotonin content *(26)*, a reduction in the number of gonadotropin receptors, and an attenuation of androgen secretion to hCG stimulation *(27)*. In both neonatal and adult rats *(28,29)*, hemivasectomy, which also includes the interruption of the inferior spermatic nerve, prevents the hemicastration response. The significance of sympathetic innervation of the testis is suggested both by in vivo and in vitro studies. In neonatal rats, pharmacological sympathectomy of the testis (local injection of the neurotoxin 6-hydroxydopamine) retards growth and differentiation of the organ and prevents the development of compensatory testicular hypertrophy that follows hemicastration *(30,31)*.

The role of cholinergic transmission in the control of steroidogenesis is suggested by observations which indicate that administration of acetylcholine to the spermatic nerve plexus decreases testosterone secretion in isolated testis–nerves plexus system *(32)*.

Concerning the role of the vagus nerve, bilateral vagotomy has been reported to have a transient suppressive effect on bilateral orchidectomy-induced gonadotropin release *(33)*. In adult rats, unilateral (right-sided) transection of the vagus nerve induces a significant luteinizing hormone rise *(34)*, while, in peripubertal hemicastrated rats, right-sided vagotomy combined with right-sided hemiorchidectomy decreases serum gonadotropin concentrations and testosterone secretion *(35)*.

No experimental data are available on the involvement of the brain stem in the neural control of the testis, except one report which indicates that lesions in the mesencephalic reticular formation cause testicular atrophy *(36)*.

Early studies have indicated that the brain also controls testicular functions by a pituitary-independent, neurally mediated mechanism. These data are based on special experimental models, which allows one to propose the existence of a neural pathway between the testis and the brain whose function is to provide a fine-tuning control of the organ. Consistent with this view are findings which indicate that hemiorchidectomy induces unilateral changes in the uridine incorporation *(37)* and the gonadotropin-releasing hormone (GnRH) content of the hypothalamus *(38)*. Furthermore, unilateral deafferentation of the hypothalamus has been reported to prevent the hemicastration-induced serum follicle stimulating hormone (FSH) rise if the two interventions were on the same (right) side *(39)*. Studies by Turnbull and Rivier *(40)* indicate that intracerebroventricular injection of interleukin-1β suppresses the human chorionic gonadotropin (hCG)–induced testosterone secretion by a catecholamine-dependent neural pathway between the brain and the testis.

Following identification of structures transneuronally connected with the testis, areas that exhibited intensive and consistent virus labeling from the testis and not known to control testicular functions, such as the hypothalamic paraventricular nucleus, amygdala, and the insular cortex, were examined. Electrolytic lesion of the paraventricular nucleus did not influence Leydig cell responsiveness to hCG, but prevented the inhibitory effect of corticotropin-releasing factor or the β-adrenergic agonist on the response *(41)*.

Recent studies indicate that intracerebroventricular injection of ethanol blunted hCG-induced testosterone secretion without altering serum LH concentration or testicular blood flow. The treatment significantly upregulated c-fos mRNA transcripts in the parvocellular portion of the hypothalamic paraventricular nucleus. Lesion of the nucleus partially restored the ethanol-induced reduced testosterone response to hCG *(42)*.

These results indicate that the paraventricular nucleus plays a role in the control of testosterone secretion through a mechanism that is independent of LH secretion and peripherally mediated effects.

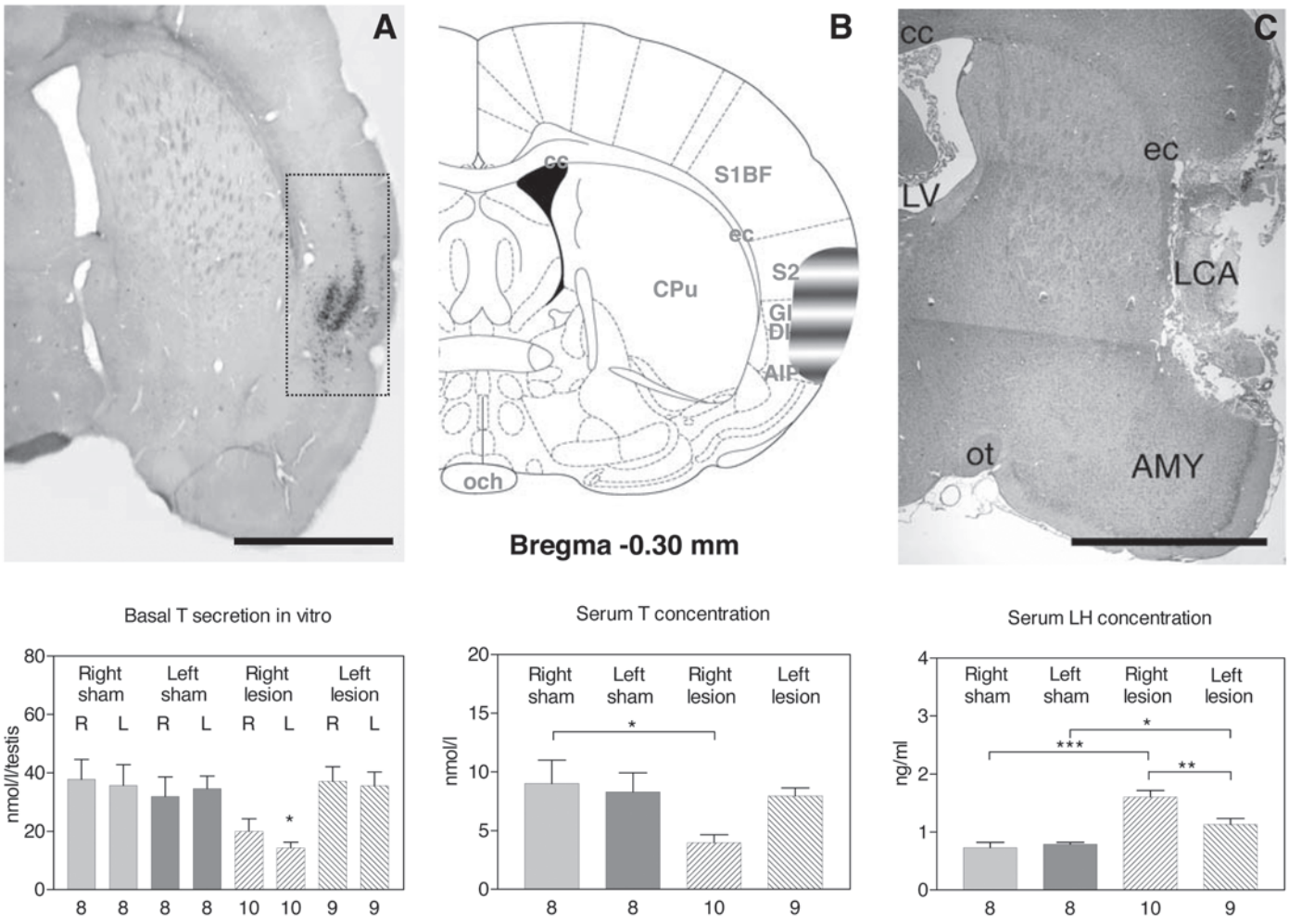

Fig. 2. (**A**) Virus-labeled neurons in the insular cortex following virus administration into the testis. Schematic illustration (**B**) and photomicrograph (**C**) showing histology of the lesion (LCA) of the insular cortex. **Lower panel**: Effect of left- or right-sided lesion of the insular cortex on basal testosterone secretion in vitro, serum testosterone, and LH concentrations. AIP: agranular insular cortex; AMY: amygdala; cc: corpus callosum; CPu: caudal putamen; D1: dysgranular cortex; ec: external capsule; GI: granular insular cortex; L: left testis; LV: lateral ventricle; och: optic chiasm; ot: optic tract; R: right testis; S1BF: primary somatosensory cortex, Barrel field; S2: secondary somatosensory cortex. Asterisks indicate significant difference ($p < 0.05$) and numbers under the horizontal bars animal numbers of the groups. Scale bar: 500 μm.

The role of the amygdala as one of the extrahypothalamic nuclei modulating GnRH secretion is known for decades. Two studies indicate that the cell group is involved also in the pituitary-dependent neural control of testosterone secretion. Unilateral lesion of the amygdala with kainic acid resulted in a significant decrease in basal testosterone secretion in vitro of both testes and in serum testosterone secretion. LH secretion was suppressed following left- but not right-sided lesion *(43)*. These results indicate functional asymmetry of the amygdala concerning the mechanism by which the left (via the hypothalamohypophysial–testicular axis) and the right amygdala (via direct neural route) control testosterone secretion. These data are consistent with the results of previous experiments in which deafferentation of an area in the temporal lobe containing the amygdala was performed in hemicastrated rats *(44)*. Deafferentation on the left side in left orchidectomized rats resulted in a significant decrease in the steroidogenesis of the remaining (right) testis with no change in serum LH level. Any other combination of the two interventions was ineffective to alter the parameters studied.

Unilateral lesion of the insular cortical area, where testicular-related neurons could be detected, resulted in a significant decrease in basal testosterone secretion in vitro and in serum testosterone concentration, when the intervention was on the right side *(45)* (Fig. 2). Lesion on either the left or the right side induced a significant increase in serum LH level. Nevertheless, the hormone level was significantly higher after right- than after left-sided injury. The results indicate that the insular cortex also modulates the final neuroendocrine/neural input to the testis. In addition, data also suggest functional predominance of the right insular cortex over testicular functions.

Clinical Observations

Relatively few clinical data are available on the involvement of neural structures innervating the testis. However, these observations are in accordance with the experimental data mentioned above. Testicular atrophy is common in men with spinal cord lesion in the T12 region, where the sympathetic preganglionic neurons are located *(46)*. It is well documented that reproductive endocrine disorders and reproductive dysfunctions are unusually frequent among men with epilepsy *(47)*, particularly among individuals who have partial seizures of temporal lobe origin *(48)*. In men with temporal lobe epilepsy associated with reproductive endocrine disorders, the hormonal parameters of the hypothalamohypophysial–testicular axis are not uniform. The majority of individuals exhibit testosterone and LH concentrations characteristic of hypogonadotropic hypogonadism. Other subjects have hyperprolactinaemia or hypergonadotropic hypogonadism *(48)*. Furthermore, temporal lobe paroxismal discharges (on the right side) are often associated with a lower serum level of testosterone in human patients *(49)*. Furthermore, semen analysis has revealed a high frequency of decreased sperm count and abnormalities in sperm morphology and motility in men with epilepsy *(50)*.

Ovary

Peripheral, Spinal, and Supraspinal Components of Ovarian Innervation

The ovary is innervated by the superior ovarian nerve and the ovarian plexus. The superior ovarian nerve, located in the suspensory ovarian ligament, enters the organ at the hilum and its fibers are distributed around the hormone-secreting cells of the organ, while the ovarian plexus provides mainly the perivascular innervation *(51)*. The majority of the ovarian nerves are noradrenergic *(52)*. Recent studies indicate that catecholamines are synthesized also within the ovary *(53,54)*. Preganglionic sympathetic fibers are located in the lower thoracic and upper lumbar segments of the spinal cord, while the parasympathetic input is of vagal origin. Perikarya of afferent (sensory) fibers are located in the lower thoracic and upper lumbar dorsal root ganglia and in the nodose ganglion of the vagus.

Using the viral transneuronal tracing technique, we were able to reveal the hierarchical chain of CNS neurons connected with the ovary and presumably involved in the control of ovarian functions *(55,56)*.

After the injection of the pseudorabies virus into the ovary, as one would expect, the first neurons in the CNS exhibiting viral antigen are found in the intermediolateral cell column of the spinal cord. The greatest number of infected cells are present at thoracic spinal cord segments T_9–T_{10} (Fig. 3C). At the early stage of infection the labeling is ipsilateral to virus inoculation. As the infection progresses, the labeling becomes bilateral with ipsilateral predominance and occasional infected neurons can be seen in the central autonomic nucleus (lamina X) and in the dorsal horn. The finding that the first CNS region in which infected cells can be detected following intraovarian virus inoculation is the intermediolateral cell column of the spinal cord, where the preganglionic sympthetic neurons are located, is in accordance with the classical neuromorphological observations.

In the brain stem several nuclei exhibit virus-infected neurons. It is of note that already at the initial stage of infection, when no other cerebral structures are labeled, a few immunopositive neurons can be detected in the nucleus of the solitary tract and the dorsal motor nucleus of the vagus (Fig. 3D). The early occurrence of virus-labeled neurons in the dorsal vagal complex indicates a route other than the spinal cord, i.e., the infection reaches this medullary nucleus through the vagus nerve. Therefore, the intermediolateral cell group and the dorsal vagal complex are the sites of the CNS where other structures are infected from. As the infection progresses in the medulla oblongata and pons, neurons of several nuclei and cell groups (area postrema, nucleus of the solitary tract, dorsal vagal complex, nucleus ambiguus, paragigantocellular nucleus, parapyramidal nucleus, A1, A5 [Fig. 3A], and A7 cell groups, caudal raphe nuclei, locus coeruleus, subcoeruleus nucleus, Barrington's nucleus, Kölliker–Fuse nucleus) were found to be transneuronally labeled. In the mesencephalon, the ventrolateral part of the periaqueductal gray matter contained virus-labeled neurons. In the diencephalon a very intensive cell body labeling was observed in the hypothalamic paraventricular nucleus (Fig. 3B) and a few virus-infected neurons could be detected in the lateral and dorsal hypothalamus; in the arcuate nucleus, zona incerta, perifornical area; and in the anterior hypothalamus. Concerning the telencephalic structures, virus-labeled cells were found in the bed nucleus of the stria terminalis and in the central amygdala nucleus. Because the density of infected neurons in the vagal nuclei was extremely rich, we studied the contribution of the vagal nerve to viral transneuronal labeling of brain structures from the ovary *(57)*. In animals subjected to unilateral vagotomy prior to virus inoculation ipsilateral to vagotomy, the labeling in the spinal cord did not differ from that observed in rats with intact vagi. After unilateral transection of the vagus nerve, no infected cells could be detected in the vagal nuclei and in the area postrema. In addition, in several brain stem nuclei, such as the A1, A5, and the caudal raphe nuclei and in the hypothalamic paraventricular nucleus, the proportion of infected cells was less than in controls. On the basis of these observations it can be assumed that the structures exhibiting infected neurons belong to the cranial parasympathetic system. The finding that the number of labeled cells is reduced in certain cerebral structures suggests that these nuclei, on the one hand, are interconnected with preganglionic sympathetic neurons of the spinal cord, and, on the other hand, that these cell groups also receive ovarian-related fibers via vagal nuclei.

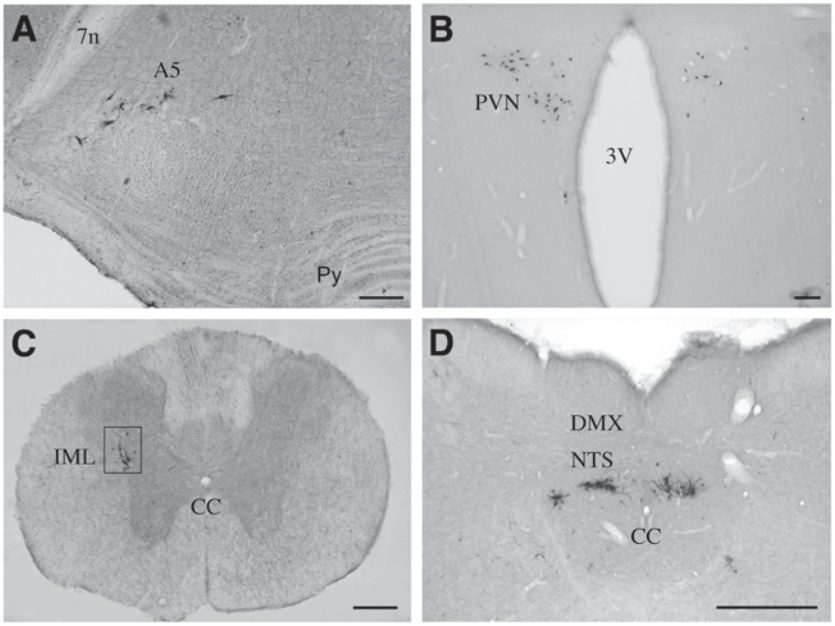

Fig. 3. Photomicrographs illustrating virus-infected neurons in the A5 cell group (**A**), in the hypothalamic paraventricular nucleus (PVN) (**B**), in the intermediolateral cell column (IML) of the spinal cord (**C**), in the dorsal motor nucleus of the vagus (DMX) and in the nucleus of the solitary tract (NTS) (**D**) following injection of neurotropic virus into the ovary. Left side of the picture is the side of injection. 3V: third ventricle; 7n: facial nerve; CC: central canal; PY: pyramid. Scale bars: **A,C,D**: 5 μm; **B**: 100 μm.

In general, the number of CNS neurons infected from the testis is less than from the ovary. This phenomenon is most probably due to the poor innervation of the testicular tissue. Consistent with this view are the data of Card et al. *(58)*, who observed that the infection of transneuronally labeled neurons largely depended on the density of nerve terminals in the area of virus injection. The CNS structures labeled following virus injection into the testis *(12)* show considerable overlap with those observed after virus inoculation of the ovary *(55)*. Only minor differences in the labeling pattern between the male and female gonad can be noted. Virus infection of the testis also results in infected neurons in the sacral parasympathetic nucleus, owing to the parasympathetic innervation of the testis from sacral preganglionic neurons *(1)*. The number of labeled neurons in the vagal nuclei is significantly less after virus injection into the testis compared to the injection into the ovary. This difference is due to the fact that the parasympathetic fibers innervating the testis arise from both the cranial and the sacral parasympathetic system. A cerebral area that exhibits a considerable difference is the insular cortex. At a late stage of infection from the testis, a large number of labeled neurons (mainly pyramidal cells) occur in a well-defined region of the insular cortex. Such localization of transneuronally labeled neurons cannot be observed from the ovary.

Functional Considerations

Experimental Data

Increasing data indicate that all the neural structures (nerves to and from the ovary, brain stem nuclei, hypothalamic and extrahypothalamic cell groups) connected with the ovary exert regulatory action on the gland.

The functional significance of the ovarian innervation is well established. Development of ovarian nerves precedes the onset of folliculogenesis *(59)*. Furthermore, Mayerhofer et al. *(60)* have reported that the ovarian nerves play a role in the induction of FSH receptors in newly formed follicles. In both prepubertal and adult hemiovariectomized rats, unilateral or bilateral section of the superior ovarian nerve interfered with the development of compensatory ovarian hypertrophy and ovulation response to gonadotropins. The effect of intervention depended on the age of the animal at surgery and the side of transection of the superior ovarian nerve *(61–63)*. Furthermore, neonatal transection of the superior ovarian nerve has been reported to disturb cyclic activity of the rats *(64)*. Chemical sympathectomy by 6-hydroxydopamine or guanethidine prevents the development of compensatory ovarian hypertrophy that follows hemiovariectomy *(65)* and interferes with ovarian development, respectively *(66,67)*.

Increasing number of studies indicate that a derangement of sympathetic inputs to the ovary contributes to the development of polycystic ovary syndrome. In our recent study we investigated the distribution of transneuronally labeled CNS neurons from rat polycystic ovary induced by a single injection of estradiol valerate. Interestingly enough, despite the hyperactivation of the sympathetic ovarian nerves, there was almost no viral labeling in the central nervous system *(68)*. Some clinical observation relevant to this issue is briefly described below, while an accompanying article is entirely dedicated to this topic *(69)*.

The functional significance of sensory nerves in the control of ovarian function has been indicated by studies in which capsaicin—a neurotoxin selectively desensitizing a subpopulation of primary sensory neurons acting via a specific membrane-bound vallinoid receptor *(70)*—was administered to neonatal rats *(71)*. The treatment did not interfere with the timing of puberty but the number of follicles of the ovary was less and, in spite of the fact that capsaicin-injected rats copulated, fertility rate was significantly reduced.

As mentioned previously the nucleus of the solitary tract and the dorsal motor nucleus of the vagus are transsynaptically connected with the ovary. The functional significance of the rich vagal innervation in the ovarian control is well documented. Vagotomy has been reported to delay the onset of puberty *(72)*, to decrease steroid secretion *(73)*, to reduce the number of ova shed *(74)*, and to suppress compensatory ovarian hypertrophy *(75)*. Vagotomy performed in estrous or proestrous rats altered the length of estrous cycle and prevented the induction of pseudopregnancy in response to cervical stimulation *(76,77)*. Studies in which unilateral vagotomy was performed indicate functional asymmetry of the nerve, suggesting that the character of information carried by the left and right vagus nerve seems to be different *(78)*. Recent studies indicate that also in prepubertal rats several parameters of ovarian functions (onset of puberty, number of ova shed, serum estradiol and progesterone concentration) can be altered by vagotomy. The efficiency of intervention depends on the age of the animal at surgery and on the fact whether uni- or bilateral vagotomy was performed. Interestingly enough, no vagal asymmetry was present in prepubertal rats *(79)*.

The demonstration of neural connections between the brain and the ovary using the transsynaptic viral-tracing technique has provided the neuromorphological evidence for the existence of a "brain–ovary–brain" neuronal circuit that had already been proposed by physiological studies more than three decades ago. The first observation that pointed in this direction was made by us: unilateral ovariectomy induced unilateral changes in the protein-synthesizing activity *(80)* and the GnRH content of the hypothalamus *(81)*, an effect that could not be explained by neuroendocrine mechanism. The assumption that the control of ovarian functions also involves regulatory actions independent of the pituitary is further supported by studies performed in hypophysectomized animals. In hypophysectomized plus hemiovariectomized animals, the ovarian atrophy is less severe than in rats that solely underwent hypophysectomy *(82)*. In addition, in hypophysectomized rats normal ultrastructural appearance of ovarian interstitial cells has been reported following electrical stimulation of the ovarian plexus *(83)*. Further support for this assumption has been provided by Kawakami et al. *(84)*. They found that, in the acutely hypophysectomized and adrenalectomized female rats, ovarian venous plasma concentrations of estradiol and progesterone were significantly increased by ipsilateral stimulation of some hypothalamic and mesencephalic regions and decreased by stimulation of certain limbic structures, suggesting the existence of direct neural activation of ovarian steroid secretion independent of the pituitary gland. In hypophysectomized immature rats a large lesion of the left preoptic-anterior hypothalamic region resulted in a significant rise in the vasoactive intestinal polypeptide level of the ipsilateral ovary *(85)*.

Physiological studies on the control of different structures of the central nervous system over ovarian functions is in good agreement with the neuromorphological observations obtained with the transneuronal virus-tracing technique. Hemitransection of the spinal cord contra- but not ipsilateral to hemiovariectomy prevented the development of compensatory ovarian hypertrophy *(86)*. Transection of the spinal cord in hypophysectomized and adrenalectomized rats canceled ovarian estradiol secretion induced by hypothalamic stimulation *(84)*. Among brain stem structures transneuronally connected with the ovary, the locus coeruleus has been reported to be involved in the direct neural control of the organ. Right-sided lesion of the locus coeruleus interferes with the development of compensatory ovarian hypertrophy regardless of the side of hemiovariectomy *(86)*.

The majority of physiological data suggesting direct effect of cerebral structures on the ovary include studies in which unilateral lesion of the hypothalamus was performed. Interestingly enough, these data revealed functional asymmetry of the hypothalamus (for review see refs. *87* and *88*). Right-sided lesion of the anterior hypothalamus has been reported to prevent the hemiovariectomy-induced FSH rise *(89)*. Lesion of the anterior hypothalamus *(90)* on the right side suppressed the development of compensatory ovarian hypertrophy that follows unilateral ovariectomy. Similarly, right-sided lesion of the preoptic area *(91)* resulted in a decrease in the number of ova shed, and ovulation was completely blocked by implantation of atropine into the right anterior hypothalamus *(92)*. Among limbic structures the amygdala has been reported to be involved in the neural control of ovarian functions. Right- but not left-sided deafferentation of the mediobasal portion of the temporal lobe (the area included the corticomedial amygdaloid nucleus) suppressed the rate of compensatory ovarian hypertrophy regardless of the side of ovariectomy *(93)*. Furthermore, a lesion of the right-sided medial amygdala on diestrus 1 resulted in a decrease in the number of rats ovulating. In ovulating animals the number of ova shed by the left ovary was significantly reduced *(94)*.

Clinical Observations

The involvement of the sympathetic nervous system has been suggested to be involved in the etiology of polycystic ovary syndrome. Increased density of catecholaminergic nerves in the ovary of patients with polycystic ovarian syndrome has been observed *(95)*. In addition, in both adult and adolescent polycystic ovary patients peripheral catechola-

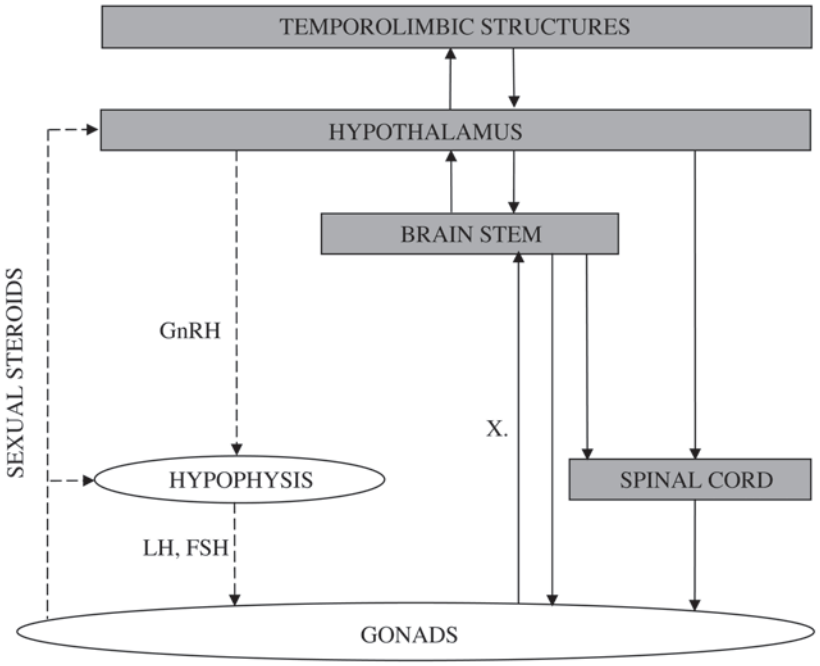

Fig. 4. Simplified schematic drawing illustrating the neuroendocrine (left side, broken lines) and the direct neural (right side, solid lines) control of the gonads. X: vagus nerve.

minergic alterations suggesting a change in noradrenaline deamination and/or uptake have been reported *(96,97)*. The potential contribution of the peripheral sympathetic system to the disease is further suggested by the effectiveness of ovarian wedge resection or laparoscopic laser cauterization to restore ovulation *(98–100)*. This topic is dealt with in more detail in an accompanying article.

Reproductive endocrine dysfunctions are more common among men and women with temporal lobe epilepsy than in the general population or in subjects with general or focal motor seizure disorders *(101)*. In women there is a strong predominance of right-sided seizures of temporal lobe origin with hypogonadotropic hypogonadism. In contrast, polycystic ovarian syndrome in female subjects with left-sided epileptiform discharges of temporal lobe occurred more frequently than in the case of right-sided partial epilepsy *(48, 102)*. Furthermore, women with left temporal foci associated with polycystic ovarian syndrome exhibited more than two times higher average LH pulse frequency than patients with hypogonadal hypogonadism and right-sided temporal focus *(103)*. Recent studies indicate decreased serum estradiol and dihydroepiandrosterone sulfate concentration, greater variability of LH pulse frequency, prolactin pulse amplitude, and FSH level among women with temporal lobe epilepsy than among controls *(104)*. These findings suggest that the amygdala, a frequent site of adult epilepsy due to its rich connection with the hypothalamus, is involved in the promotion of the development of reproductive endocrine dysfunctions. Furthermore, seizure frequency or lifetime number of seizures has been reported to be associated with age at menopause. The timing of cessation of reproductive cycling in women with epilepsy is earlier than in the general population *(105)*.

Conclusions

The data summarized above indicate that the control of gonadal functions is more complex than previously thought. The demonstration of neural connections between the brain and the gonads using the transsynaptic viral-tracing technique has provided the neuromorphological evidence for the existence of the neural pathway.

Some components of the pathway, such as the hypothalamus, amygdala, and insular cortex, appear to be involved in the control of gonadal functions by a pituitary-independent, neural mechanism (Fig. 4). Further experiments are needed to provide data that other structures exhibiting consistent and intensive labeling following virus injection into the testis or ovary play a role in gonadal regulatory processes. It can be expected that further clinical observations will be available which, on the one hand, confirm the experimental data and, on the other hand, contribute to our better understanding of the etiology of some endocrine reproductive dysfunctions.

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