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REGULAR ARTICLE

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Identification of growth-hormone- and prolactin-containing neurons within the avian brain

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Abstract Prolactin (PRL)- and growth-hormone (GH)-containing perikarya and fibers independent of the anterior pituitary gland have been reported to exist in the central nervous system of several mammalian species. The specific distributions of PRL- or GH-like neurons in the avian forebrain and midbrain, however, have not been reported. The objective of the study was to identify GH- and PRL-containing neurons in the hypothalamus and a few extrahypothalamic areas of two avian species. Brain and peripheral blood samples were collected from laying and broody turkey hens and ring doves. Broody turkey hens and doves had significantly higher plasma PRL concentrations compared with laying hens. Coronal brain sections were prepared and immunostained using anti-turkey GH and anti-chicken synthetic PRL antibodies. In turkey hens, the most dense GH-immunoreactive (ir) perikarya and fibers were found in hippocampus (Hp), periventricular hypothalamic nucleus, paraventricular nucleus, inferior hypothalamic nucleus, infundibular hypothalamic nucleus, medial and lateral septal area, and external zone of the median eminence (ME). In the ring dove, a similar pattern of distribution of GH-ir neurons was noticed at the brain sites listed above except that GH-ir fibers and granules were found only in the internal zone of ME and not in the external zone. In both turkeys and doves, the most immunoreactive PRL-ir perikarya and fibers were found in the medial and lateral septal area, Hp (turkey only), and bed nucleus of the stria terminalis pars magnocellularis. There were no apparent differences in the staining pattern of GH- or PRL-ir neurons between the laying and broody states in either species. However, the presence of GH-ir- and PRL-ir perikarya and fibers in several hypothalamic nuclei indicates that GH and PRL may influence parental behavior, food intake, autonomic nervous system function, and/or reproduction.

Key words Neuropeptides · Hypothalamus · Pituitary · Lactotroph · Somatotroph · Broodiness · Turkey · Dove

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Introduction

Prolactin (PRL) and growth hormone (GH) have traditionally been thought to be synthesized only in cells of the adenohypophysis in vertebrates. However, PRL- and GH-containing neurons and fibers that are independent of the anterior pituitary gland, PRL- or GH- cells, have been reported to exist in the central nervous system of several mammalian species.

Pacold et al. (1978) reported that cells of the rat amygdaloid nucleus grown in tissue culture produced a material that was immunologically, chromatographically, and biologically identical to pituitary GH. In the rat and primate brains, GH-like immunoreactive materials were extracted from amygdaloid nucleus, hippocampus, and thalamus (Hojvat et al. 1982). The amount of GH-like material in the brain extracts did not decline in hypophysectomized animals. The brain origin of GH was confirmed by detecting GH mRNA in the rat brain basal cortex, hippocampus, and caudate putamen by in situ hybridization studies (Dihl et al. 1987).

Prolactin-like substances were located in the arcuate (ARC), ventromedial (VMN), premammillary (PMM), supraoptic (SON), and paraventricular (PVN) nuclei in young or adult male and female rats (Toubeau et al. 1979a, 1979b). Prolactin-like immunoreactive (ir) mate-

rial was widely distributed throughout the rat brain and its distribution remained unaffected following hypophysectomy (Paut-Pagano et al. 1993). The de novo synthesis of PRL was evidenced by the existence of PRL mRNA in hypothalamic tissues of male and female rats (Schachter et al. 1984).

In domestic fowl, immunoreactive GH-like proteins were detected by radioimmunoassay and immunoblotting in hypothalamic and extrahypothalamic tissues (Render et al. 1995), in which the GH immunoreactivity was similar in size and antigenicity to pituitary GH. Furthermore, pituitary GH mRNA sequences were found in hypothalamic and extrahypothalamic tissues of chicken brain, where GH-ir proteins are abundantly located (Render et al. 1995). In Japanese quail, PRL-ir neurons were detected in the mediobasal hypothalamus (Berghman et al. 1992).

Prolactin appears to influence reproductive function by a direct action on the central nervous system in several vertebrate species (reviewed in Buntin 1993). Avian species such as turkeys and ring doves exhibit parental behavior and incubation behavior characterized by hyperprolactinemia, nesting, and care of young poults or nestlings. In turkeys, as in many other avian species, incubation behavior results in termination of egg laying. A temporal relationship between hyperprolactinemia and incubation/parental behavior exists in many avian species. For instance, intracranial PRL infusion to laying turkey hens results in expression of incubation behavior, and a decrease in pituitary PRL content (Youngren et al. 1991). In ring doves, intracranial infusion of ovine PRL or turkey GH was found to inhibit gonadotropin secretion (Buntin et al. 1988), and to facilitate food intake (Buntin and Figge 1988). Similarly, intracranial administration of ovine PRL to ring doves was found to stimulate parental behavior (Buntin et al. 1991).

By employing autoradiography, PRL receptors were mapped in the ring dove brain (Buntin et al. 1993). Prolactin receptors were found in the choroid plexus, preoptic area, tuberoinfundibular region, lateral hypothalamic area, and the suprachiasmatic, paraventricular, and ventromedial nuclei. Although PRL receptors identified in these sites may serve to bind blood-borne PRL derived from the anterior pituitary gland, it is possible that brain-derived PRL may also act on these PRL receptors. It is not clear how PRL of pituitary and brain origin interact with each other and with other neurochemical systems to alter reproductive functions or feeding behavior in avian species.

Prolactin- or GH-containing neurons in the turkey or ring dove brain have not been reported in the past. Therefore, we attempted to locate PRL- and GH-containing neurons in hypothalamus and adjacent extrahypothalamic areas of the female turkey and ring dove brains. In addition, we used both laying and incubating turkey hens and doves in the study in order to ascertain if any obvious anatomical difference in the distribution of PRL-, and GH-containing neurons occurred between the two reproductive states.

Materials and methods

Animals

Large white turkey hens (Nicholas strain) were housed in floor pens under a 14 h light and 10 h dark photoperiod. Feed and water were available at all times. Each floor pen was provided with trap nests for use by the hens to lay eggs. A trap nest allows a bird to enter a nest to lay an egg, but a door closes preventing her from leaving until a caretaker collects the egg, identifies and releases the bird. The nest boxes were checked 4 times each day between 0700 hours and 1300 hours at 2-h intervals in order (1) to identify and release trapped hens and (2) to ascertain if the enclosed hen laid an egg. A record of daily nesting frequency and egg production was maintained for every hen. Egg-laying hens and incubating hens (n=3 each) were selected based on nesting frequency and egg production as follows: (1) hens that visited the nest box 1 or 2 times a day and consistently laid an egg every 1–2 days were considered laying hens and (2) hens that were found in the nest box at all nest checks without laying an egg for at least 10 days prior to sacrifice were classified as incubating hens.

The ring doves (*Streptopelia risoria*) were obtained from the breeding colony maintained at the University of Wisconsin-Milwaukee. Birds were housed with breeding partners in acrylic breeding cages under constant photoperiod and temperature conditions (14L:10D; 20°C) with food, grit, and water available at all times. All females had previous breeding experience that consisted of successfully rearing at least one squab to an independent age. Laying females (n=4) were sampled on the 1st or 2nd day of incubation, with day 1 of incubation being defined as the day the first egg of the two-egg clutch was discovered in the nest. Broody females (n=4) were sampled on the 2nd day after their first squab hatched.

Collection of brain

Brains were collected from laying and incubating turkey hens as previously described (Ramesh et al. 1995). Briefly, birds were anesthetized with sodium pentobarbital and their heads were perfused with physiological saline followed by 4% paraformaldehyde solution. The ring doves were sacrificed with an overdose of chloropent and perfused with saline and 4% paraformaldehyde solution. Brains were blocked in a stereotaxic apparatus (Kopf Instruments Inc., Tujunga, CA) into roughly two (forebrain and hindbrain for doves) or three large regions (roughly, fore-, mid-, and hindbrain for turkeys) and postfixed in 4% paraformaldehyde for 2 h, and then stored in 30% sucrose solution until they sank to the bottom. Using a sliding microtome (American Optical Co., NY), frozen tissue sections (40 µm thick) of the diencephalon in the coronal plane were sliced and stored in 0.1 M phosphate buffer solution in 12-well tissue culture wells (Corning Inc., NY) at 4°C until used for immunohistochemistry.

Prolactin and GH immunohistochemistry

The tissue sections cut from anterior to posterior end of forebrain and midbrain regions were collected from each female and immunostained using mouse anti-chicken synthetic PRL peptide (Berghman et al. 1992) or rabbit anti-turkey GH (Bakst 1993). Tissue sections were first treated with 3% hydrogen peroxide solution in methanol to neutralize endogenous peroxidase activity and then equilibrated with a solution of 0.01 M TRIS-HCl and 0.15 M NaCl, pH 7.4 (TBS). Non-specific background staining was reduced by treating tissue sections with 1% goat normal serum (for GH staining) or 1% horse normal serum (for PRL staining) in TBS containing 1% Triton X (Sigma). Tissue sections were incubated with anti-turkey GH antibody (1:15,000) or anti-chicken synthetic PRL peptide (1:40,000) in eight-well culture plates at 4°C for 36–48 h under mild shaking. Following washes in TBS, a biotinylated anti-rabbit IgG (for GH immunostaining) or biotinylated

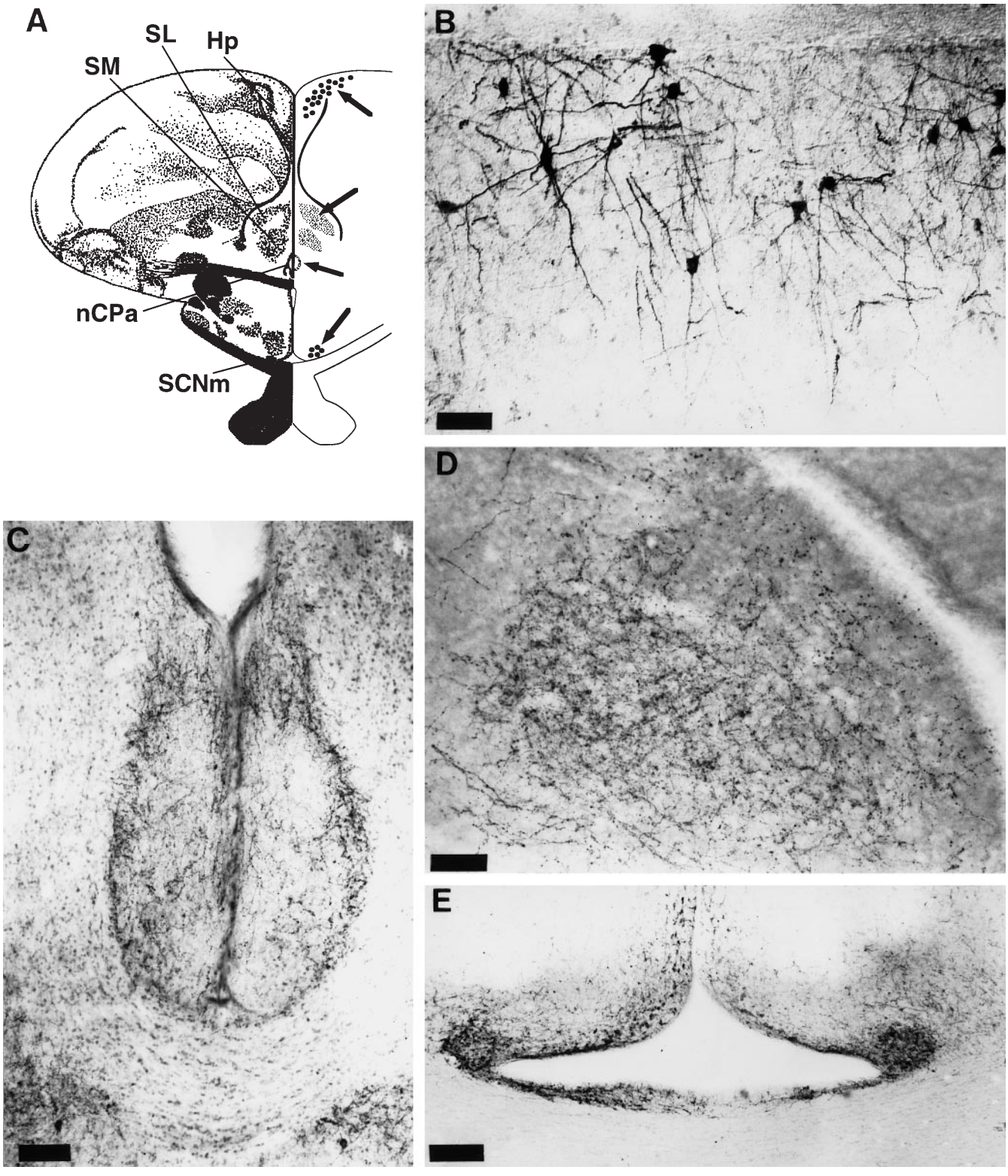


Fig. 1A–E Distribution of growth-hormone (GH)-immunoreactive (ir) perikarya and fibers at the level of the anterior hypothalamus in the turkey brain. **A** Diagram at the level of anterior hypothalamus. *Left side of the diagram* is reproduced from a chick brain atlas (Kuenzel and Masson 1988), while the *right side* is drawn to depict immunostained cell bodies (*arrows and dots*) and fibers (*stippled area*). **B** GH-ir neurons in hippocampus. **C** GH-ir fibers in bed nucleus of the pallial commissure. **D** GH-ir fibers at the level of medial septum. **E** GH-ir perikarya and fibers in the medial suprachiasmatic nucleus (*Hp* hippocampus, *nCPa* bed nucleus of the pallial commissure, *SM* medial septum, *SL* lateral septum, *SCNm* suprachiasmatic nucleus). *Scale bars* 50 µm **(B)**, 100 µm **(C,D)**, 200 µm **(E)**

anti-mouse IgG (for PRL immunostaining) was applied to the tissue sections at 1:400 concentration and incubated at room temperature for 1 h. Subsequently, the tissue sections were washed in TBS and treated with avidin-peroxidase (Vector ABC-Elite kit) at a concentration of 1:200 for 2 h at room temperature. A color reaction was carried out using 0.001% 3,3'-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) and 0.0003% hydrogen peroxide to visualize GH or PRL immunoreactivity as a brown-colored reaction product. The tissue sections were then dehydrated using alcohol and cleared in Americlear (Fisher Scientific) or xylene and coverslipped using Cytoseal (Stephens Scientific, Riverdale, NJ) mounting media. The immunostained tissue sections were studied using a Zeiss Axioplan microscope (Carl Zeiss, NY) at different magnifications in order to locate GH-, or PRL-immunoreactive neurons and fibers in the hypothalamus and some forebrain and midbrain regions. The stereotaxic atlas of chick brain (Kuenzel and Masson 1988) was used to describe specific brain locations showing GH or PRL immunoreactivity.

Plasma hormones

Plasma PRL and GH concentrations (ng/ml) in egg-laying and incubating turkey hens were measured by radioimmunoassay (Proudman and Opel 1981; Proudman 1984). Plasma PRL or GH concentrations of ring doves used for immunohistochemistry were not measured. However, plasma PRL concentrations were measured concurrently in other birds in the breeding colony at the same breeding age by Nb2 lymphoma bioassay (Buntin et al. 1996). Plasma PRL or GH concentrations between laying and incubating states were compared by Student's *t*-test using SAS (1987).

Results

Plasma hormone concentrations

Plasma PRL concentrations of incubating turkey hens (162±17.2 ng/ml) were significantly higher ($P<0.01$) than that of egg-laying hens (36±4.5 ng/ml). Likewise, plasma PRL concentrations in broody ring doves (5.58±0.61 ng/ml; $n=9$) were significantly higher ($P<0.01$) than that of laying ring doves (0.22±0.02 ng/ ml; $n=9$). Plasma GH concentrations in laying and incubating turkey hens were not different (2.98±0.45 vs 3.85±0.42; $P>0.05$). At necropsy, laying turkey hens were found to have functional ovaries bearing a normal hierarchy of preovulatory follicles while the incubating hens had completely regressed ovaries. Broody ring doves had heavier crop sacs (4350.8±725.7 vs 605.2±70.6 mg; $P=0.029$) and smaller oviducts (532.7±98.0 vs 1356.1± 378.9 mg; $P=0.029$) compared with laying doves.

Immunohistochemistry

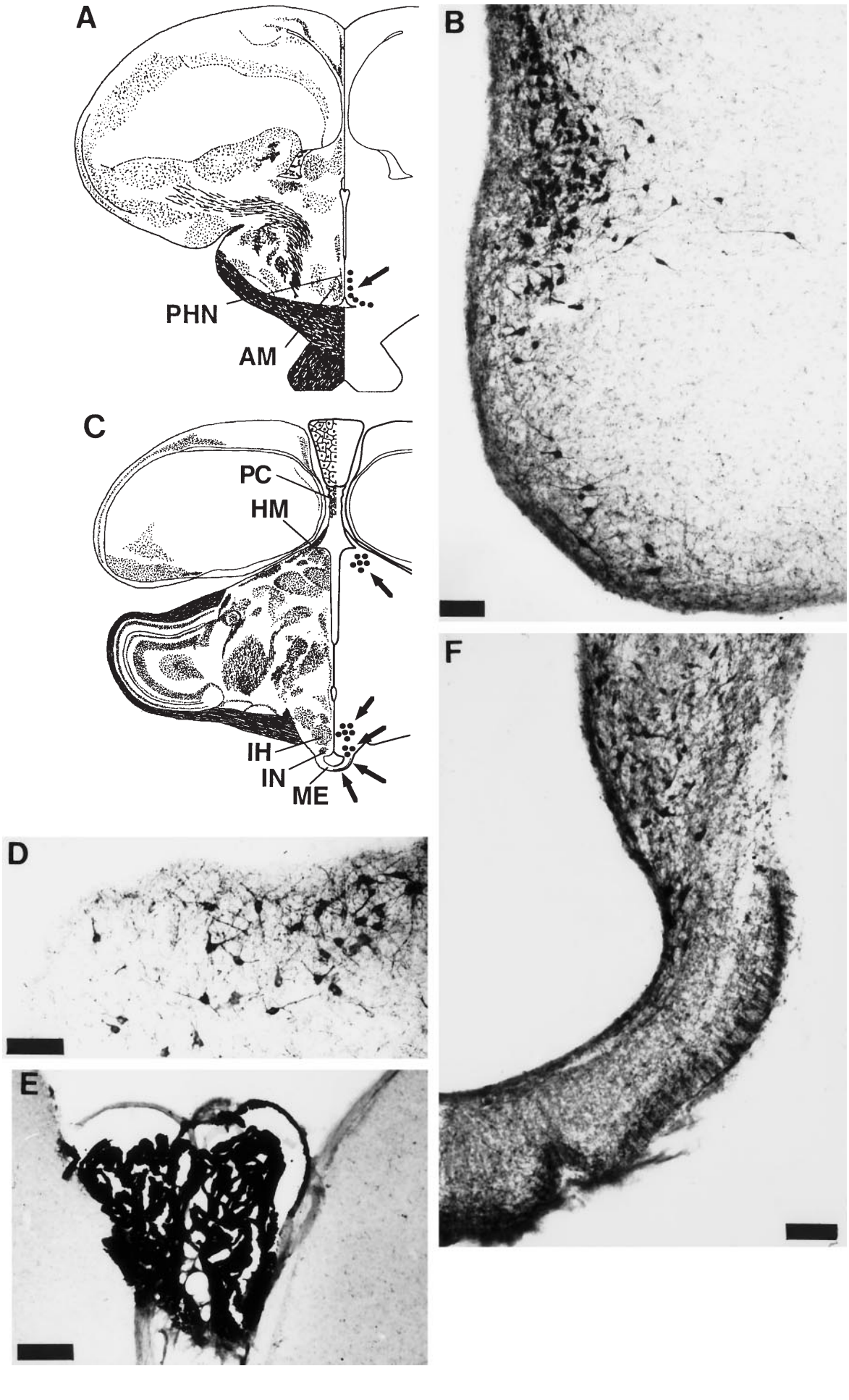
Growth-hormone-ir and PRL-ir perikarya and fibers were found throughout the hypothalamus and in a few extrahypothalamic sites in both turkey and ring dove brains. There were no obvious differences in the distribution of GH-ir or PRL-ir neurons between laying and broody hen brains of either species. Figures 1, 2, 3, 4 and 5 show some of the brain sites where GH-ir or PRL-ir neurons were found in the present study. In addition, a summary of brain sites where GH-ir or PRL-ir were identified in the turkey and ring dove brain tissues is provided in Table 1.

Growth hormone

In the turkey brain, GH-ir granules were found in the cytoplasm of perikarya, while in fibers or neurites they were arranged in a continuous bead-like fashion. Densely stained GH-containing polydendritic neurons were found in the hippocampus (Hp, Fig. 1A,B). The mean diameter of these large multipolar neurons measured 21±3.2 µm. A few GH-ir cell bodies and dense fibers were found in the nucleus of pallial commissure (nCPa, Fig. 1A,C). Other hypothalamic areas that showed the strongest immunoreactive GH-containing perikarya and/or fibers included: (1) septal area (SM, Fig. 1D), (2) supraoptic nucleus, (3) paraventricular nucleus, (4) anterior hypothalamic nucleus (Fig. 2A,B), (5) periventricular hypothalamic nucleus (Fig. 2A,B), (6) medial suprachiasmatic nucleus (SCNm, Fig. 1A,E), (7) medial habenular nucleus (HM, Fig. 2C,D), (8) lateral hypothalamic nucleus, and (9) infundibular (IN, arcuate) nucleus and inferior hypothalamic nucleus (IH, Fig. 2C,F). GH-ir granules in neurons or cells, and fibers were found in the following circumventricular organs: (1) choroid plexus (PC, Fig. 2C,E), (2) paraventricular organ, (3) pineal gland (not shown), and (4) median eminence (ME, external zone only; Fig. 2C,F).

In the ring dove, a similar pattern of distribution of GH-ir neurons was noticed (Fig. 3) at all the brain sites listed above except for the following: (1) inferior hypothalamic nucleus did not contain GH-ir perikarya, (2) GH-ir fibers and granules were found only in the internal zone of median eminence and not in the external zone (ME, Fig. 3E,G), and (3) GH-ir fibers were found in the lateral septal area. Clear immunoreactive perikarya were seen in the hippocampus (Hp, Fig. 3A,B), periventricular hypothalamic nucleus (PHN, Fig. 3A,C), and paraventricular nucleus (Fig. 3D). The choroid plexus was

Fig. 2A–F Distribution of growth-hormone (GH)-immunoreactive (ir) perikarya and fibers at the level of anterior and tuberal hypothalamus in the turkey brain. **A** Diagram at the level of anterior hypothalamus. *Left side of the diagram* is reproduced from a chick brain atlas (Kuenzel and Masson 1988), while the *right side* is drawn to depict immunostained cell bodies (*arrows and dots*) and fibers. **B** GH-ir perikarya and fibers found in the ventral portion of periventricular hypothalamic nucleus and anterior medial hypothalamic nucleus. **C** Diagram at the level of tuberal hypothalamus. **D** GH-ir perikarya and fibers found in medial habenular nucleus. **E** GH-ir granules in choroid plexus at the habenular level. **F** GH-ir perikarya and fibers found in the infundibular (arcuate) nucleus, and in the external zone of median eminence [*PHN* periventricular hypothalamic nucleus, *AM* anterior medial hypothalamic nucleus, *HM* medial habenular nucleus, *PC* choroid plexus at the habenular level, *IN* infundibular (arcuate) nucleus, *IH* inferior hypothalamic nucleus, *ME* external zone of median eminence]. *Scale bars* 200 µm **(B)**, 100 µm **(D–F)** ▶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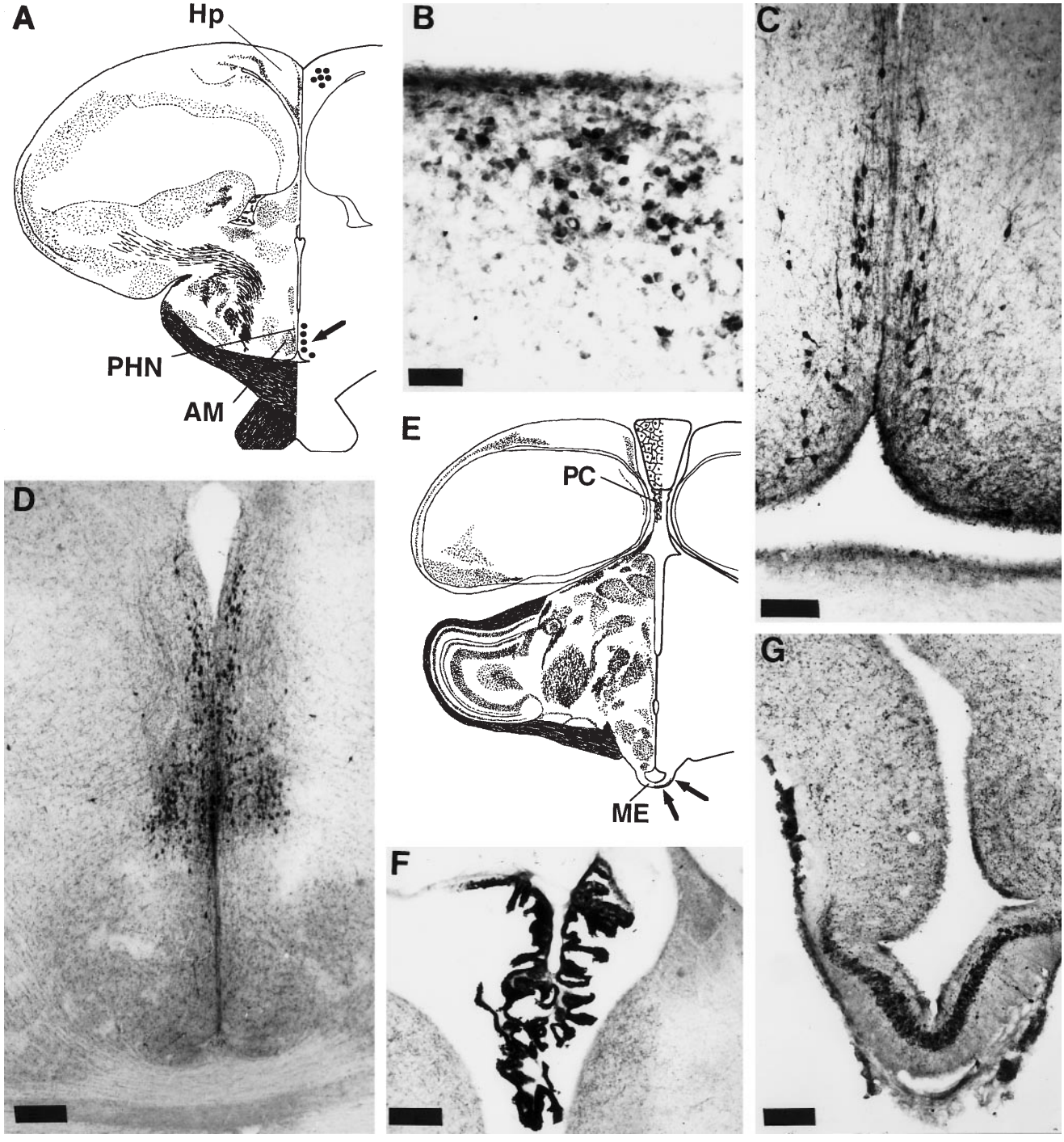


Fig. 3A–G Distribution of growth-hormone (GH)-immunoreactive (ir) perikarya and fibers in the dove brain. **A** Diagram at the level of anterior hypothalamus. *Left side of the diagram* is reproduced from a chick brain atlas (Kuenzel and Masson 1988), while the *right side* is drawn to depict immunostained cell bodies (*arrows and dots*) and fibers. **B** GH-ir parvocellular perikarya found in hippocampus. **C** GH-ir perikarya and fibers found in periventricular hypothalamic nucleus pars ventralis. **D** GH-ir perikarya and fibers found in paraventricular nucleus. **E** Diagram at the level of tuberal hypothalamus. **F** GH-ir granules in the choroid plexus within the III ventricle. **G** GH-ir fibers found in the internal zone of median eminence (*Hp* hippocampus, *PHN* periventricular hypothalamic nucleus pars ventralis, *PC* choroid plexus within the III ventricle, *ME* internal zone of median eminence). *Scale bars* 100 µm **(B,C)**, 200 µm **(D,F,G)**

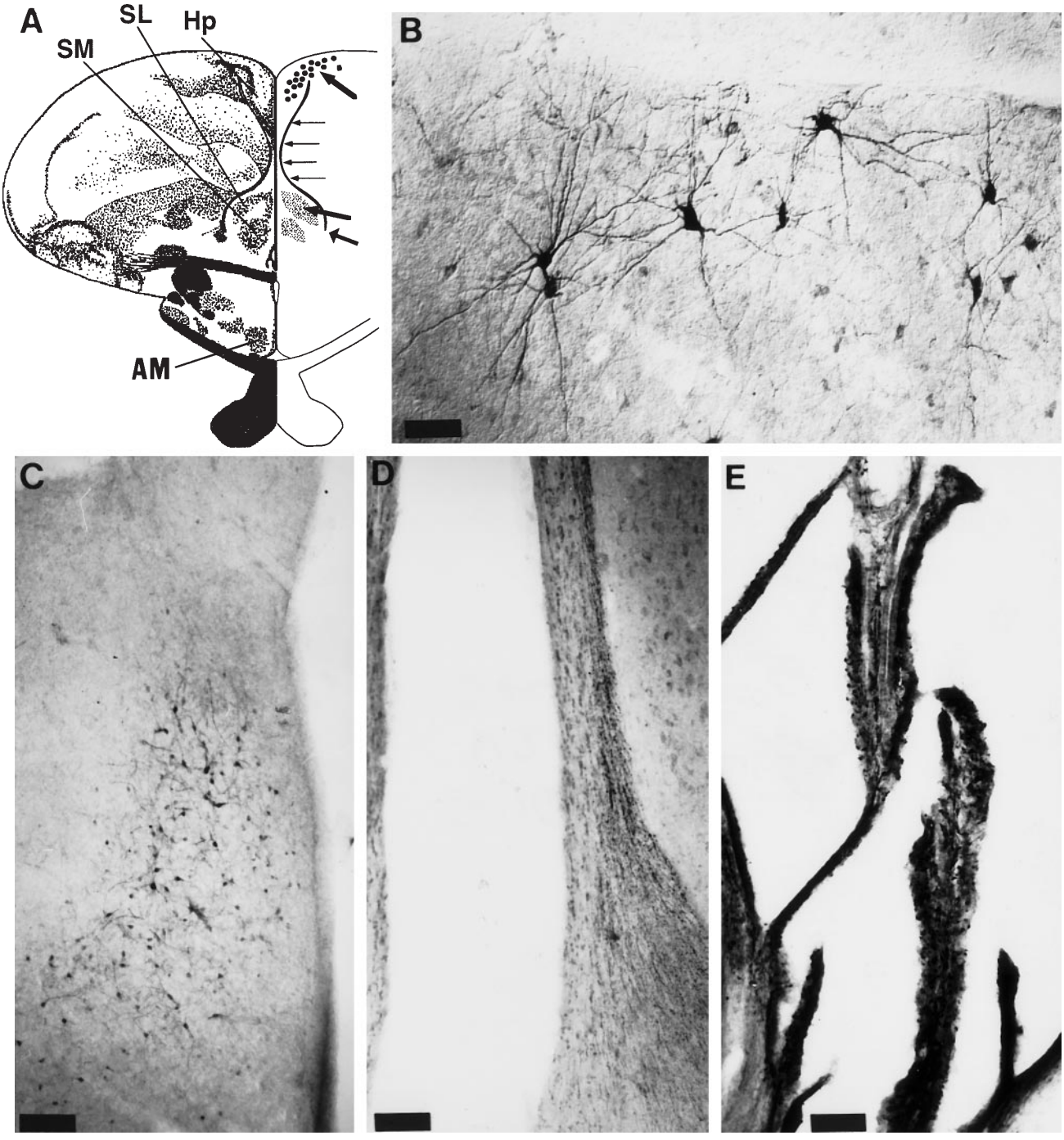
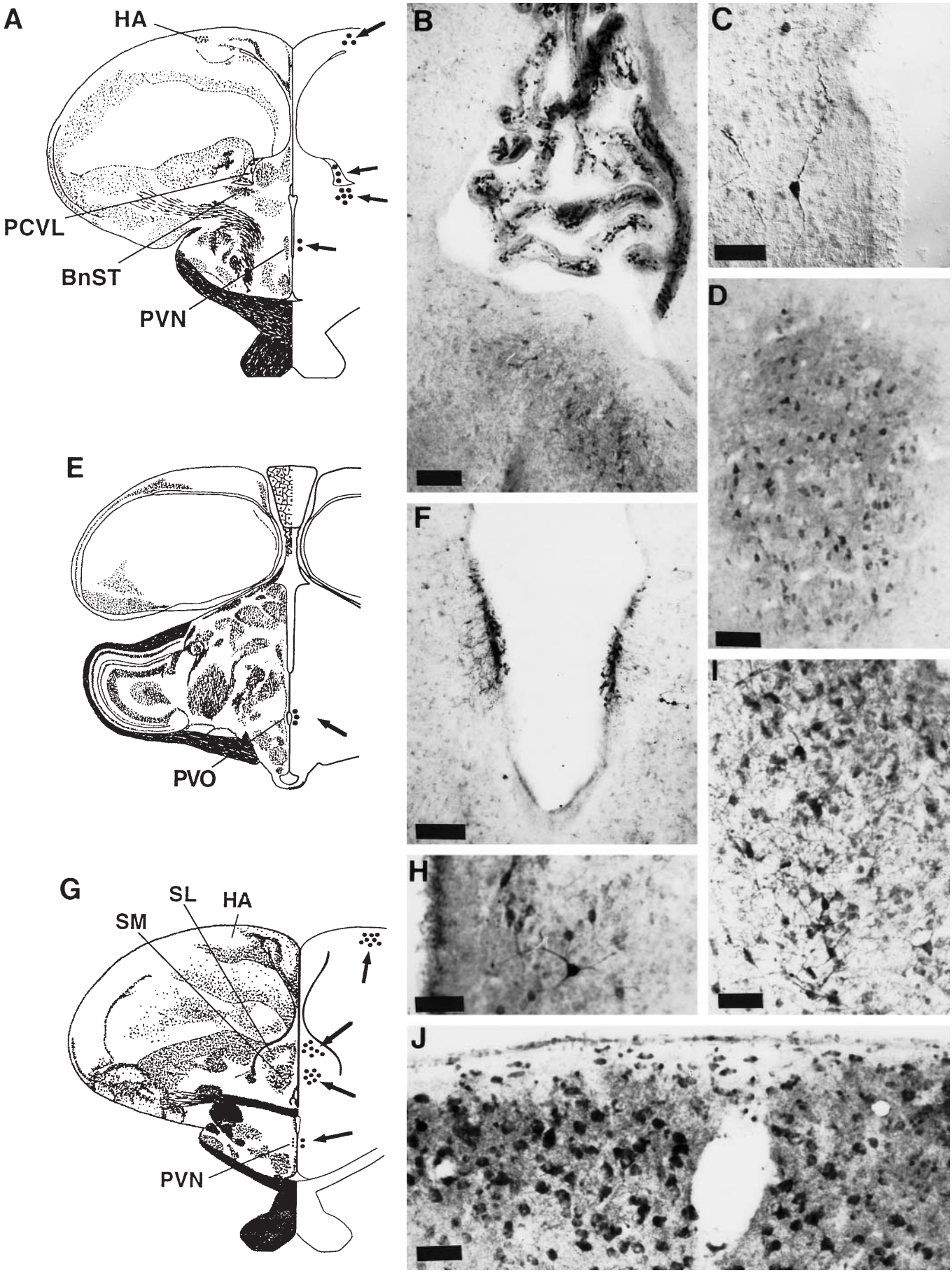


Fig. 4A–E Distribution of prolactin (PRL)-immunoreactive (ir) perikarya and fibers at the level of anterior hypothalamus in the turkey brain. **A** Diagram at the level of anterior hypothalamus. *Left side of the diagram* is reproduced from a chick brain atlas (Kuenzel and Masson 1988), while the *right side* is drawn to depict immunostained cell bodies (*arrows and dots*) and fibers (*stippled area*). *Thin arrows* represent immunoreactive fibers which appear to originate from the hippocampus and project to the septal area. **B** PRL-ir neurons in hippocampus. **C** PRL-ir neurons in the medial preoptic area lying in front of the anterior medial hypothalamic nucleus (*AM*) shown in **A** above. **D** PRL-ir granules and neurons in the medial and lateral septum. **E** PRL-ir granules in the choroid plexus lining the lateral ventricle (*Hp* hippocampus, *AM* anterior medial hypothalamic nucleus, *SM* medial septum, *SL* lateral septum). *Scale bars* 50 µm **(B,E)**, 100 µm **(D)**

densely immunostained (Fig. 3E,F). Of comparative interest was that the hippocampal GH-containing neurons in the turkey (Fig. 1B) were significantly larger and displayed clearly distinguished neurites compared with Hp perikarya in the dove (Fig. 3B).

Since the infundibular (arcuate) nucleus is known to regulate anterior pituitary gland functions, GH-ir neurons in the infundibular nucleus were counted in immunostained tissue sections and compared between laying and incubating turkey hens. A quantitation in the case of dove brain was not possible because of lack of clear GH-ir perikarya in the infundibular (arcuate) nucleus.


A
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PCVL
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PVN
B
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Table 1 Summary of hypothalamic and extrahypothalamic sites where GH or PRL immunoreactivity (ir) was observed in the turkey hen and ring dove (***robust ir, **clear ir, *just detectable ir, *n.* nucleus)

Location	GH-ir perikarya		GH-ir fibers		PRL-ir perikarya		PRL-ir fibers	
	Turkey	Dove	Turkey	Dove	Turkey	Dove	Turkey	Dove
Hippocampus	***	*	***	**	***		***	
Accessory hyperstriatum					**	**	**	*
Medial preoptic area					**	**	*	*
Bed n. of pallial commissure		*	***	***	**	**	**	**
Medial septum			***	***	**	**	***	***
Lateral septum			***	***	**	**	***	***
Bed n. of stria terminalis pars magnocellularis	*	*	**	**	**	**	***	***
Periventricular hypothalamic n.	***	***	**	**	*	*	**	**
Lateral hypothalamic n.	**	*	**	**				
Suprachiasmatic n. (medial)	***	**	**	**				
Paraventricular n.	***	***	***	***	**	**	**	**
Habenular n.	***	*	***	*				
Choroid plexus			***[a]	***[a]			***[a]	***[a]
Pineal gland			**[a]	**[a]			**[a]	**[a]
Supraoptic n.	***	***	**	**	**		*	*
Paraventricular organ	*	*	**	**	*	*	**	**
Infundibular n.	***	*	***	***				
Inferior hypothalamic n.	*	*	***	***				
Median eminence								
External zone	**	*	***	*	*		*	
Internal zone	*	**	*	***				

[a] Presence of immunoreactive granules

There were no differences in the number of GH-ir perikarya between laying (65.0±16.4 neurons/tissue section; three sections per bird; *n*=3) and incubating turkey hen (52.0±12.8 neurons/tissue section; three sections per bird; *n*=3).

Prolactin

Prolactin-ir neurons and fibers were found distributed in hypothalamus and a few extrahypothalamic areas in both dove and turkey brains. Overall staining intensity of PRL-ir neurons or fibers in the brain areas studied was not as robust and clear compared with GH-ir. Nonetheless, striking immunoreactivity was seen in the hippocampus (Hp, Fig. 4A,B) and paraventricular organ (PVO, Fig. 5E,F). The antibody used (anti-PRL) on the brain tissue sections has been successfully used to stain lactotrophs in the turkey anterior pituitary gland (Ramesh et al. 1998). It is possible that all pituitary PRL epitopes may not be present in the brain PRL (Harlan and Scammel 1991). Furthermore, the biological activity of brain-derived PRL in rats was reported to be significantly lower than that of pituitary-derived PRL in Nb2 lymphoma cell assay (Emanuele et al. 1987).

In the turkey brain, clearly stained prolactin-ir cell bodies or fibers were detectable in the following hypothalamic and extrahypothalamic locations: (1) medial preoptic area (Fig. 4C), (2) lateral septum (LS, Fig. 4A,D), (3) medial septum, (4) bed nucleus of stria terminalis pars magnocellularis (BnST, Fig. 5A,B), (5) ventral perihypothalamic nucleus, (6) paraventricular nucleus (PVN, Fig. 5A,C), (7) supraoptic nucleus, and (8) accessory hyperstriatum (HA, Fig. 5A,D). Prolactin-ir fibers appeared to originate from the hippocampus and project to the septal area (Fig. 4A,D). Cells containing PRL-like granules in the cytoplasm were found surrounding the median eminence. In addition, portal blood vessels traversing the median eminence and connecting pars distalis were darkly immunostained with PRL-like material. In the same way, ependymal cells lining the choroid plexus within lateral ventricles (PCVL, Figs. 4E, 5A,B) and third ventricle were darkly immunostained as was the choroid plexus beneath the pineal gland.

In the dove brain, clearly stained prolactin-ir cell bodies and fibers were found in the following hypothalamic

◀ **Fig. 5A–J** Distribution of prolactin (PRL)-immunoreactive (ir) perikarya and fibers in the turkey and the dove brain. **A** Diagram at the level of anterior hypothalamus. *Left side of the diagram* is reproduced from a chick brain atlas (Kuenzel and Masson 1988), while the *right side* is drawn to depict immunostained cell bodies (*arrows and dots*) and fibers. **B** PRL-ir neurons in the turkey bed nucleus of stria terminalis magnocellularis. PRL-ir granules in the choroid plexus within the lateral ventricle. **C** A few PRL-ir neurons in the turkey paraventricular nucleus. **D** PRL-ir neurons in the turkey accessory hyperstriatum. **E** Diagram at the level of tuberal hypothalamus. **F** PRL-ir neurons in the paraventricular organ contacting cerebrospinal fluid in the III ventricle. **G** Diagram at the level of anterior hypothalamus. **H** PRL-ir neurons in the paraventricular nucleus. **I** PRL-ir neurons in the medial and lateral septal area. **J** PRL-ir neurons in the accessory hyperstriatum (*BnSTmc* bed nucleus of stria terminalis magnocellularis, *PCVL* choroid plexus within the lateral ventricle, *PVN* paraventricular nucleus, *HA* accessory hyperstriatum, *PVO* paraventricular organ contacting cerebrospinal fluid in the III ventricle, *SM* medial septal area, *SL* lateral septal area, *HA* accessory hyperstriatum). *Scale bars* 100 μm **(B–D,F)**, 50 μm **(H–J)**

and extrahypothalamic locations: (1) medial preoptic area, (2) medial and lateral septum (SM and SL, Fig. 5G,I), (3) bed nucleus of stria terminalis pars magnocellularis, and (4) paraventricular nucleus (PVN, Fig. 5G,H). There was a striking absence of PRL-ir in hippocampus; however, a discrete nucleus of PRL-ir neurons was found in the accessory hyperstriatum (HA, Fig. 5G,J). Similar to turkeys, choroid plexus within the lateral ventricles and third ventricle showed dark PRL-ir granules.

Immunohistochemical control

Prolactin and GH antibodies were preadsorbed with their respective antigens (turkey PRL at 0.06 µg/ml or turkey GH at 6.67 µg/ml) for 48 h at 4°C and centrifuged at 100,000 *g* for 20 min. When the supernatant was used in place of the primary antibody as control, there was no staining of tissue sections.

Discussion

In the present study, we identified GH- and PRL-ir neurons and fibers throughout the hypothalamus as well as a few extrahypothalamic areas of turkey and ring dove brain tissues by utilizing immunohistochemical techniques.

In the anterior pituitary gland, there seems to be a close interaction between cells that produce PRL and GH. Prolactin and GH are closely related hormones (Niall et al. 1971) and have evolved from common ancestral genes. In the turkey hen anterior pituitary gland, PRL cells replace GH cells in the caudal lobe when the turkey hen shifts from a continuous laying physiological state into incubation behavior or broodiness characterized by hyperprolactinemia (Ramesh et al. 1996). Furthermore, there is an increased abundance of mammosomatotrophs that colocalize GH and PRL during hyperprolactinemia associated with incubation behavior in turkey hens (Ramesh et al. 1998). However, when broodiness is disturbed, a reversal of cellular changes occurs leading to loss of PRL cells, and emergence of GH cells was noticed (unpublished data). Based on the foregoing and from the hypothalamic areas where GH- and PRL-ir neurons and fibers were found (Table 1), it is logical to expect a close interaction between GH- and PRL-like neurons in the central nervous system. We did not find, however, any change in the number of GH-ir neurons in the infundibular (arcuate) nucleus in broody turkey hens despite hyperprolactinemia. It should be noted that our sample size was quite small (n=3/treatment). Similarly, there were no apparent differences in either GH-ir neurons in other sites or PRL-ir neurons or fibers between the two reproductive states in both the species, although we did not attempt to quantify the anatomical data. Therefore, it is not clear how GH-ir or PRL-ir neurons in the brain respond to increased PRL secretion from the anterior pituitary gland.

Growth-hormone-ir neurons found in infundibular (arcuate) nucleus and septal area in both turkey and ring dove brain may possibly be involved in the control of GH secretion from the anterior pituitary gland. There is strong evidence for brain-derived GH to be involved in a short-loop feedback mechanism to regulate GH secretion from the adenohypophysis. For instance, significantly decreased serum GH levels in rats were coupled with a significant increase in GH concentrations in the hypothalamus and amygdala (Hojvat et al. 1986). Recently a growth hormone secretogogue-receptor (GHS-R) mRNA has been located in the rat hippocampus, arcuate and ventromedial nucleus whose concentration is negatively correlated with GH concentration (Bennett et al. 1997). Furthermore, a rostromedial septal area has been found to control pulsatile growth hormone release in the golden hamster (Borer 1987). It is well known that GH secretion from the anterior pituitary gland follows a circadian rhythm and that the hypothalamic suprachiasmatic nucleus has been shown to regulate biological rhythms in several vertebrate species. It is possible that GH-ir neurons found in the avian SCNm may feed back in a manner to integrate internal biological rhythms with external photoperiodic input.

Similar to GH-like neurons possibly regulating somatotrophs, hypothalamic PRL may contribute to the control of lactotrophs. This is evident from a report that axons containing PRL-like peptide were found to project into the perivascular layer of the medial eminence (Alonso et al. 1988). In the present study, we also found PRL-ir granules on the portal blood vessels that surround the external zone of median eminence in the turkey. In turkey hens, a negative correlation between blood PRL concentration and hypothalamic PRL-receptor mRNA transcript concentration (Zhou et al. 1996) provides further evidence for a short-loop feedback mechanism to control pituitary PRL secretion. It has been reported that central or peripheral administration of PRL causes a decrease in pituitary PRL content and blood PRL concentrations in rats (Wardlaw et al. 1997) and in turkeys (Youngren et al. 1991). In addition, central administration of PRL results in expression of maternal behavior in rats when infused into the medial preoptic area (Bridges et al. 1990), and in turkeys by intracerebroventricular (ICV) injections (Youngren et al. 1991). This indicates that the PRL-ir cell bodies found in the turkey infundibular (arcuate) nucleus and medial preoptic area (in both turkeys and doves) may be involved in the regulation of PRL secretion perhaps by modulating VIP secretion (Mauro et al. 1989; Kuenzel and Blähser 1994).

Both PRL and GH have been shown to increase food intake when administered intracerebroventricularly to ring doves (Buntin and Figge 1988). There are data showing that PRL-like neurons present in the rat lateral hypothalamus are involved in regulation of feeding behavior (Bahjaoui-Bouhaddi et al. 1994). In the present study, GH-ir neurons and fibers were identified in several hypothalamic locations where leptin receptor has been reported in rats (Häkansson et al. 1998). As a growth-

regulating molecule, GH in these hypothalamic locations may function to regulate energy balance and feed intake. The presence of GH-ir neurons in the infundibular (arcuate) nucleus raises the possibility that GH may influence GH-releasing hormone (GHRH) neurons (Bloch et al. 1983), VIP neurons (Mauro et al. 1989; Kuenzel and Blähser 1994), or NPY neurons (Kuenzel and Fraley 1995; Walsh and Kuenzel 1997) found in the infundibular nucleus in the avian brain.

Growth hormone-ir fibers and PRL-ir perikarya found in the nucleus of the pallial commissure and medial preoptic area may interact with other neurochemicals, particularly gonadotropin-releasing hormone (GnRH) found in this nucleus (Kuenzel and Blähser 1991; Millam et al. 1993), that regulate gonadotropin secretion from the anterior pituitary gland. It is interesting to note that hypothalamic prolactin was reported to stimulate the release of luteinizing hormone-releasing hormone from rat hypothalamus in vitro (Azad et al. 1990). Furthermore, a subset of PRL-ir neurons in the rat medial basal hypothalamus have been found to selectively accumulate estrogen (Shivers et al. 1989) and probably regulate reproduction.

Prolactin has been found to regulate fluid and electrolyte metabolism by a direct action on the kidney in mammals (Horrobin 1980) and in avian species (Roberts and Dantzler 1992). In addition, PRL may influence blood pressure based on the fact that ir-PRL forms were found to be colocalized with vasopressin in neurons of the hypothalamic paraventricular and supraoptic nuclei and in the rat neurohypophysis (Mejia et al. 1997). In the central nervous system, we identified both PRL- and GH-ir cell bodies in the PVN and SON (turkey only). Presence of GH in SON, PVN, internal zone of ME, and posterior pituitary gland strongly suggests a role of GH in the secretion of posterior pituitary hormones. Dense GH-immunoreactive fibers were observed in the external zone in turkeys and in the internal zone (dove only) of ME. This may indicate transport of GH into the portal blood vessels that carry blood to the anterior pituitary gland in turkeys, while in ring doves GH is possibly transported to the posterior pituitary gland. Both GH and PRL have been found in the nerve endings in the posterior pituitary adult sea lamprey (Wright 1986). Furthermore, PRL-like immunoreactive protein was found to be released from the hypothalamo-neurohypophyseal explants in rats (Torner et al. 1995).

Prolactin- and GH-ir granules were identified in the circumventricular organs in both the turkey and ring dove brain tissues. Several adenohypophyseal hormones have been detected in the cerebrospinal fluid (CSF) in many species (Lenhard and Deftos 1982). In particular, ir-PRL has been located in the rat choroid plexus and in ependymal cells lining the cerebral ventricles (Thompson 1982). Prolactin receptors have been found in the ring dove choroid plexus (Buntin et al. 1993). In rats, a receptor-mediated mechanism has been reported for the transport of PRL from blood to CSF in the choroid plexus (Walsh et al. 1987). Prolactin receptors in the rat choroid plexus were found to be upregulated in response to hyperprolactinemia leading to increased transport of PRL from blood to CSF (Mangurian et al. 1992). Although blood-borne PRL can enter CSF, the hypothalamic areas where PRL-ir neurons have been identified may still secrete PRL into the CSF. It is not known whether GH is transported from the blood to the CSF by a receptor-mediated mechanism in the turkey or ring dove brain. Prolactin- and GH-ir granules were found within the pinealocytes, and in the pineal stalk in the turkey and ring dove. Both GH and PRL have been identified in the ovine pineal gland (Noteborn et al. 1993). It is not known how GH or PRL influences pineal gland function in birds, although PRL has been found to be mitogenic to the chicken pineal gland cells in vivo (Chakraborty and Maiti 1981).

The hippocampus is involved in learning and memory processes and is known to be a target for the neuromodulatory actions of several hormones. We found very darkly stained GH-ir neurons in hippocampus in both avian species and PRL-ir neurons in the turkey. The size of the multipolar neurons (21 μm) and detail of their structure was striking (Figs. 1B, 4B). In the dove brain, a group of parvocellular neurons immunoreactive to PRL was found in a dorsolateral region of the accessory hyperstriatum (Fig. 5J). It might be possible that GH and PRL are involved in learning and acquiring memory about nesting and feeding besides modulating many neuroendocrine functions of hippocampus. Prolactin- and GH-ir neurons and fibers in lateral hypothalamic area are possibly involved in regulating stress behavior in turkeys and doves. Recently, the *GH* gene was found to be expressed in the rat lateral hypothalamus and, in particular, GHRH and stress were found to increase or decrease brain *GH* gene expression, respectively (Yoshizato et al. 1998). Prolactin ir-neurons in the rat lateral hypothalamus have been shown to colocalize dynorphin (Griffond et al. 1993) and preprodynorphin mRNA (Griffond et al. 1994).

In conclusion, we identified PRL- and GH-ir neurons and fibers in specific hypothalamic nuclei and adjacent brain regions of turkey and ring doves. Further studies are needed to confirm the de novo synthesis of PRL or GH within the brain and to elucidate the role of brain-derived PRL and GH in parental behavior, feeding behavior, stress, and reproduction.

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