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Galanin mRNA in the Nucleus Basalis of Meynert Complex of Baboons and Humans

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ABSTRACT

Galanin, a 29-amino acid peptide, has been shown by immunocytochemistry to occur in most large acetylcholinergic neurons of the complex that includes the nucleus basalis of Meynert and the nucleus of the diagonal band of Broca in nonhuman primates. In contrast, several studies have reported that most large neurons of the human nucleus basalis of Meynert complex appear to lack galanin immunoreactivity. We investigated this apparent species-difference by hybridization histochemistry for galanin messenger ribonucleic acid (mRNA) in humans and baboons. The results confirm previous immunocytochemical data; very few large neurons of the nucleus basalis of Meynert complex in humans contained detectable galanin messenger RNA, whereas most such cells in baboons were labeled by the oligodeoxynucleotide probe. The few labeled neurons in humans were primarily medial or ventral to the main body of the nucleus basalis of Meynert and corresponded in location to a minor population of relatively intensely labeled cells in baboons. These findings indicate that the indetectability of immunoreactive galanin in most cells of the nucleus basalis of Meynert complex in humans is due to a paucity or an absence of galanin messenger RNA and not to differences in posttranslational processing or transport of the peptide. Inasmuch as the probe labeled neurons in several other nuclei of both species, it is unlikely that differences in galanin messenger RNA sequences underlie the species-related disparity in hybridization in the nucleus basalis of Meynert complex. The indetectability of galanin messenger RNA in most cells of the human nucleus basalis of Meynert complex indicates that the expression of the galanin gene is regulated by as yet unidentified influences that differ in human and nonhuman primates. The varying phenotypes of galanin in primates suggest potentially important species-differences in the function of galanin in neurons of the nucleus basalis of Meynert complex.

Key words: acetylcholine, Alzheimer's disease, in situ hybridization, nucleus of the diagonal band of Broca

Galanin is a widely distributed neuroactive peptide that has been found to coexist with cholinergic markers in neurons of the nucleus basalis of Meynert (nbM) complex (Melander et al., '85, '86; Melander and Staines, '86). The nbM complex is a neuronal continuum formed by the nbM and nucleus of the diagonal band of Broca (ndbB) and termed the "Basalkernkomplex" by Brockhaus ('42). Immunocytochemical studies have indicated some intriguing differences in the distribution of galanin in the nbM complex of different species. In rats, galanin occurs in most large cells of the ndbB/medial septal nucleus but not in the nbM (Melander et al., '86). In monkeys, galanin exists in most magnocellular neurons throughout the nbM complex, including the nbM (Melander and Staines, '86; Kowall and Beal, '89; Walker et al., '89; Kordower and Mufson, '90). In humans, immunocytochemical studies are incongruent; some data indicate that most large cells of the nbM do not contain galanin, although there is a local population of mostly smaller galaninergic cells (Chan-Palay, '88a,b; Kordower and Mufson, '90); other studies report that galanin is present also in the majority of large cells of the human nbM

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This paper is dedicated to the memory of Professor Dr. Dr.h.c. Helmut O. Hofer, 1912–1989.

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complex (Kowall and Beal, '89; Vogels et al., '89). Because of differing results of immunocytochemical studies in primates, we have employed hybridization histochemistry for galanin mRNA as a sensitive and specific marker for galaninergic neurons in the nbM complex of humans and baboons.

MATERIALS AND METHODS

Subjects

Tissues containing the nbM complex were taken at autopsy from four male humans ranging in age from 16 to 61 years, with a mean postmortem interval of 9 hours (Table 1). All subjects had died acutely, and complete autopsies did not disclose disorders that would be expected to compromise our analyses. Blocks of tissue were placed on foil-coated glass slides, frozen at −30°C in isopentane, and then stored at −80°C. Four baboons (genus *Papio*), including three females and one male ranging in age from newborn to 5 years, were given an overdose of sodium pentobarbital. Brains were removed immediately, divided into slabs, fresh-frozen on dry ice, and stored at −80°C.

Hybridization histochemistry

Sections (20 μm) were cut on a cryostat-microtome at −20°C and processed for hybridization histochemistry as described previously (Young, '89). Briefly, sections were brought to room temperature, postfixed in 4% formaldehyde in phosphate-buffered saline, treated with 0.25% acetic anhydride, and delipidated in a graded series of ethanols and chloroform. After drying, sections were incubated 20 hours at 37°C in a buffer consisting of 600 mM NaCl, 80 mM Tris-HCl (pH 7.5), 4 mM ethylenediaminetetraacetic acid (EDTA), 0.1% sodium pyrophosphate, 0.2% sodium dodecyl sulfate, 0.2 mg/ml heparin sulfate, 100 mM dithiothreitol (DTT), and approximately 10^6 disintegrations per minute of [^{35}S]-labeled probe per 50 μl (see below). Sections were then washed in a solution of 0.3 M NaCl, 30 mM sodium citrate, and 50% formamide at 40°C. After drying, sections were dipped in Kodak NTB3 nuclear emulsion and exposed for 2–4 months at 4°C.

The three oligodeoxynucleotide probes were 44 or 48 bases in length and made on an Applied Biosystems DNA synthesizer (courtesy of Dr. M.J. Brownstein, National Institute of Mental Health). Probes were purified on 8 M urea/8% polyacrylamide gels and labeled using terminal deoxynucleotidyl transferase (Boehringer-Mannheim, Indianapolis, IN) and [^{35}S] deoxyadenosine 5′-(α-thio)triphosphate (dATP) (> 1000 Ci/mmol, New England Nuclear Corp., Boston, MA) or [^{32}P] dATP (> 300 Ci/mmol) for hybridization histochemistry or Northern analysis, respectively. The galanin probe was directed against bases 228–271 of the rat sequence (Kaplan et al., '88). Northern analysis performed with human total RNA (Chirgwin et al., '79) revealed only a 1.0 kilobase band, slightly larger than that observed in other species (Rökeaus and Brownstein, '86; Kaplan et al., '88). The probe used to control for the specificity of the galanin probe in human tissue was comple-

Abbreviations

AD	Alzheimer's disease
CA	commissura anterior
Cd	nucleus caudatus
ChAT	choline acetyltransferase
CI	capsula interna
DNA	deoxyribonucleic acid
GAL	galanin
GP	globus pallidus
mRNA	messenger ribonucleic acid
nbM	nucleus basalis of Meynert
ndbB	nucleus of the diagonal band of Broca
ndbBv	nucleus of the diagonal band of Broca, ventral component
Put	putamen
RNA	ribonucleic acid
TH	tyrosine hydroxylase
TO	tractus opticus

TABLE 1. Perimortem Data for Human and Baboon Subjects

Human case #	Sex	Age (years)	Cause of death	Postmortem interval (hours)
88-426	male	61	acute myocardial infarction	13
2810	male	16	lacerated lung	12
2814	male	52	asphyxiation	4
2871	male	23	gunshot wound to torso	7
Baboon Case #				
FB-14	female	newborn	Na Pentobarbital overdose	< 1
FB-15	male	2.5	Na Pentobarbital overdose	< 1
FB-18	female	5.0	Na Pentobarbital overdose	< 1
FB-19	female	5.0	Na Pentobarbital overdose	< 1

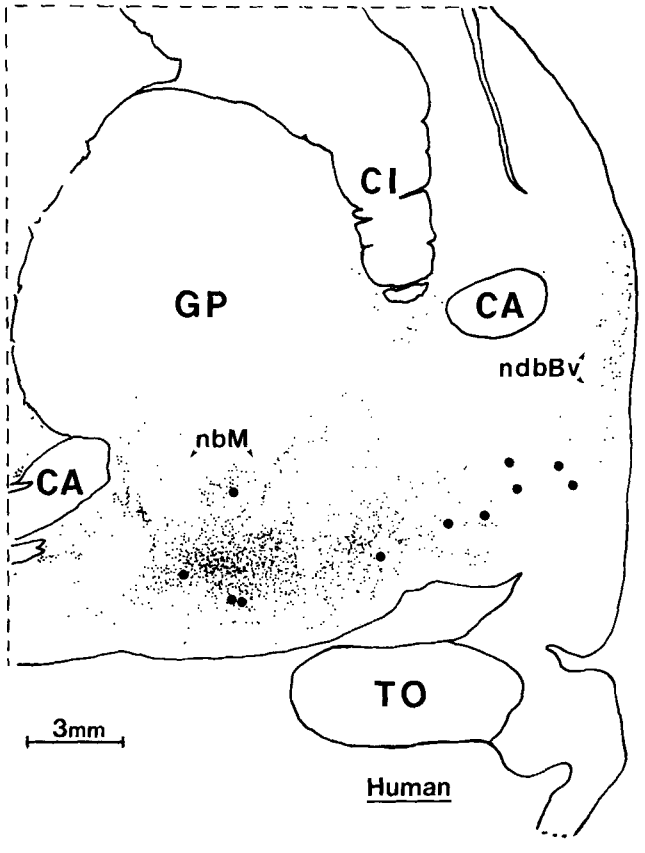

Fig. 1. Semischematic, computer-assisted diagram of the nbM complex of a 52-year-old human showing 1,372 unlabeled magnocellular neurons (small dots) and 11 neurons labeled for galanin mRNA (large dots). The dorsomedialmost neurons may not be associated with the nbM complex proper. Other galaninergic neurons that are clearly outside the nbM complex are not indicated. Compare with the baboon nbM complex in Figure 4, in which all indicated cells contain galanin mRNA.

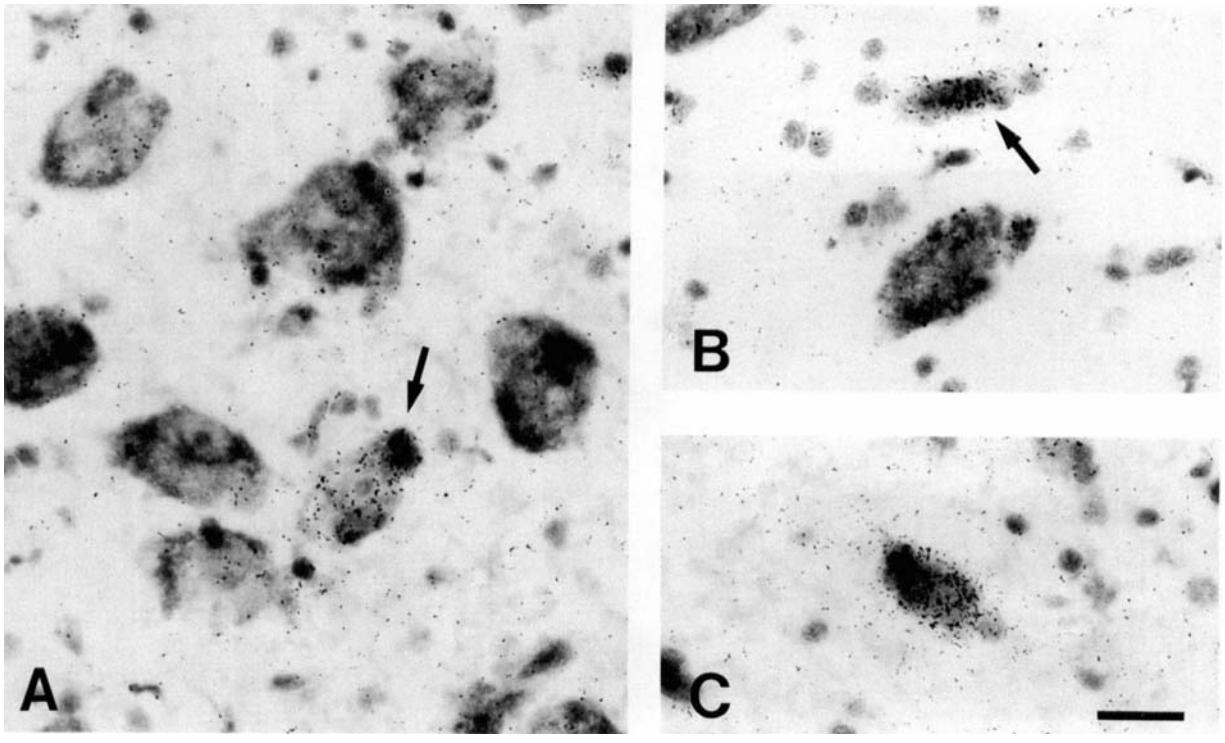

Fig. 2. Hybridization histochemistry for galanin mRNA in nbM complex neurons of a 61-year-old human (bar = 25 μm). **A.** Large, lightly labeled galaninergic neuron (arrow) among unlabeled magnocellular neurons of the anteromedial nbM. **B.** Galaninergic smaller neuron (arrow) in the anteromedial nbM. **C.** Galaninergic neuron in the horizontal limb of the ndbB.

mentary to bases 1496–1543 of human tyrosine hydroxylase (TH) mRNA (Grima et al., '87), and the control probe for baboon tissue coded for the last 16 amino acids of the rat vasopressin preprohormone (Ivell and Richter, '84). All sections were counterstained with toluidine blue.

Analysis of tissue

Neurons were considered to contain galanin mRNA if the density of perikaryal silver grains exceeded three times that of the surrounding neuropil. Although this criterion limited the likelihood of including false-positive cells, some very lightly labeled cells could have been excluded (false negatives). In selected sections, galanin-hybridoreactive neurons were mapped microscopically with a computerized mapping system. The maximum length and width of labeled neuronal somata of the nbM complex were measured using an eyepiece micrometer; only cells with evident nuclei were included. Galaninergic (n = 42) and, for comparison, unlabeled magnocellular (n = 120) neurons were measured in the nbM of the four humans. Galaninergic cells of the ndbB (n = 65) and the anterior nbM (n = 164) also were measured in the 2.5-year-old baboon and one 5-year old baboon. Finally, galanin-labeled neurons were sought in other regions known to contain galanin-immunoreactive cells (Melander et al., '86), particularly in the hypothalamus, to verify the specificity of the probe in humans and baboons. Identification of structures in human brain was aided by several sources (Nauta and Haymaker, '69; Langevin and Iverson, '80; Hedreen et al., '84) and, in the baboon brain, by the atlas of Davis and Huffman ('68).

RESULTS

Humans

In humans, less than 1% of all large neurons of the ndbB and nbM were labeled by the galanin probe (Figs. 1, 2A). The few neurons that were galanin-hybridoreactive were usually small (Fig. 2B,C) and peripheral to the dense clusters of large neurons (Fig. 1), although occasional large, labeled cells were seen (Fig. 2A). The mean size of galanin mRNA-labeled neurons in the human anterior nbM was 16.8 × 26.6 μm, whereas the mean size of the unlabeled magnocellular neurons was 27.5 × 41.0 μm.

In the hypothalamus of humans, galanin mRNA was present in numerous neurons, including those in the anterior hypothalamic nucleus (Fig. 3A). As expected, the TH probe labeled cells in certain hypothalamic nuclei, including the paraventricular nucleus (Fig. 3B), but most magnocellular neurons of the nbM complex were negative (Fig. 3C).

Baboons

Galanin mRNA-containing neurons were abundant in both the ndbB and the nbM (Figs. 4, 5, 6A,B) of all four baboons. Most (> 90%) of the large, hyperchromic neurons of the nbM were labeled by the galanin probe (Fig. 5A), and a somewhat lesser percentage of large cells in the ndbB also contained galanin mRNA (Figs. 5B,C, 6A). Some smaller cells within the nbM complex were labeled with the galanin probe (Fig. 5D). The average size of galanin mRNA-containing neurons in the ndbB was 20.3 × 36.1 μm and, in the anterior nbM, 21.1 × 35.9 μm. A few (< 5%) neurons

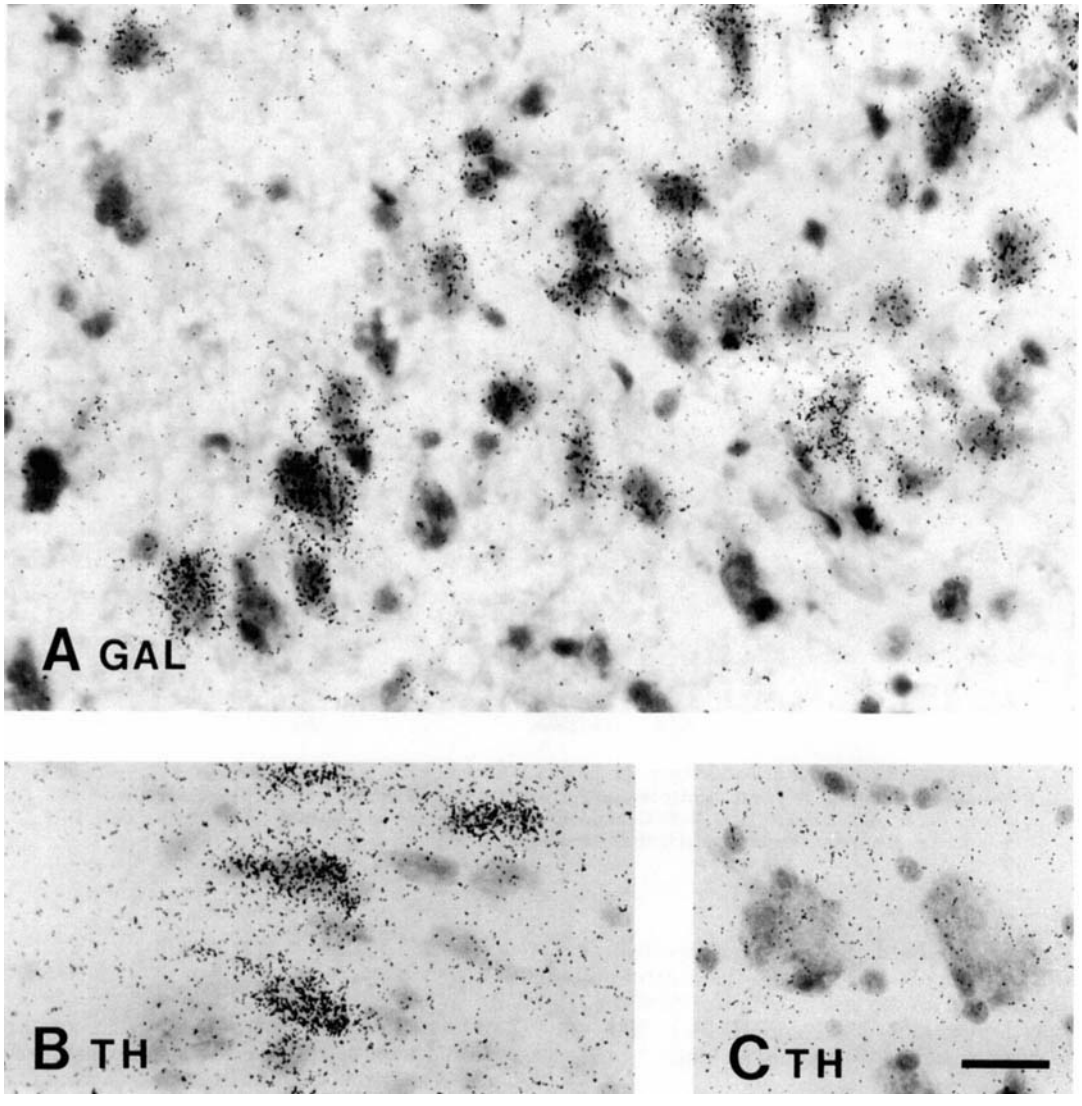

Fig. 3. Hybridization histochemistry for galanin mRNA (A) or TH mRNA (B,C) in a 61-year-old human (bar = 25 μm). **A.** Positive control: galanin mRNA-labeled neurons in the anterior hypothalamus. **B.** Known TH-producing neurons of the hypothalamic paraventricular nucleus are labeled by the TH probe. Compare with nbM complex neurons in Figure 3C. **C.** Negative control: the TH probe did not label most magnocellular neurons of the nbM. These neurons are in the same section as the labeled cells of the paraventricular nucleus shown in Figure 3B.

were more intensely labeled than most magnocellular neurons (Fig. 6A,B), and the heavily labeled cells were usually on the periphery of the nbM complex. In the anterior nbM complex, intensely labeled neurons formed a broken strip traversing the ventralmost nbM and the horizontal component of the ndbB (Fig. 4).

The galanin probe also labeled known galaninergic neurons in the hypothalamus of baboons, including those in the anterior hypothalamic nucleus (Fig. 6C). The sense vasopressin probe (control) did not label neurons in any region.

DISCUSSION

Our findings indicate that the distribution of galanin mRNA in neurons of the nbM complex differs in baboons and humans. Specifically, in baboons, galanin mRNA is localized in most large cells of the nbM complex, particularly in the nbM proper, whereas, in humans, nearly all large neurons throughout the nbM complex appear to be devoid of detectable galanin mRNA. The presence of galanin mRNA thus corresponds to the distribution of galanin immunoreactivity in the nbM complex of nonhuman primates (Melander and Staines, '86; Kowall and Beal, '89; Walker et al., '89) and humans (Chan-Palay, '88a,b; Kordower and Mufson, '90).

In baboons and humans, the galanin probe robustly labeled neurons in hypothalamic nuclei that are known from immunocytochemical studies to contain galanin-immunoreactive somata (Skofitsch and Jacobowitz, '85; Melander et al., '86; Kordower and Mufson, '90). Furthermore, some neurons in and around the nbM complex were labeled in both species. Thus the lack of labeling in most large cells of the human nbM complex is likely due to the absence—or at least the indetectability—of galanin mRNA

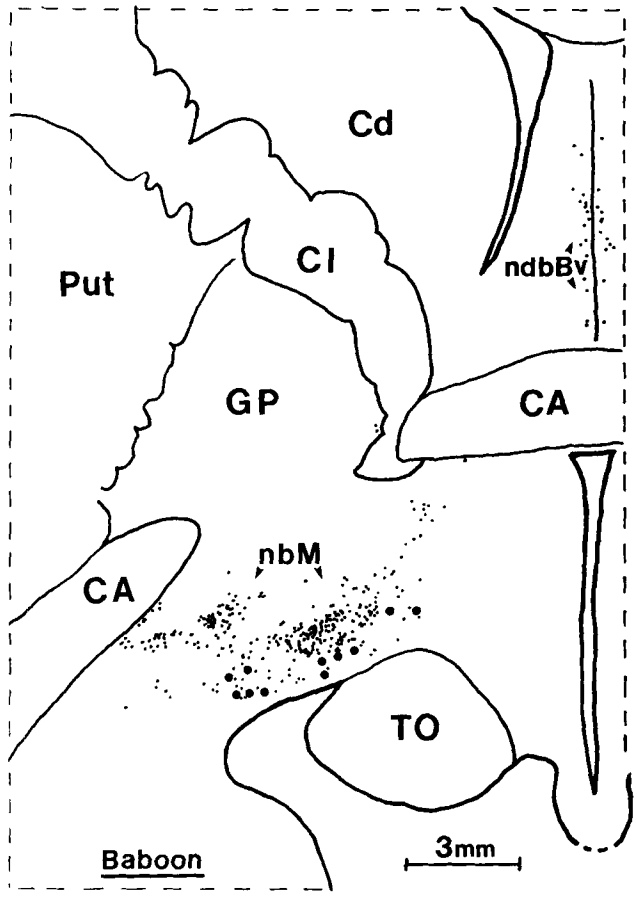

Fig. 4. Semischematic, computer-assisted diagram of neurons containing galanin mRNA in the nbM complex of a 2.5-year-old baboon. Each small dot represents one of 392 relatively lightly labeled neurons (see Fig. 5A); each large dot represents one of 11 more heavily labeled neurons (see Fig. 6A,B). Greater than 90% of all magnocellular neurons contained galanin mRNA in baboons, which was not the case in humans (see Fig. 1). Note that the heavily labeled galaninergic cells are generally medial and/or ventral to the typical dense clusters of magnocellular neurons. Galanin-hybridoreactive neurons outside the nbM complex are not indicated.

in these neurons, and not to species-differences in mRNA or to the effects of differing postmortem delays. Differences in gender do not appear to account for our findings; all four humans studied were males, but the 2.5-year-old baboon also was male and demonstrated unequivocal labeling of magnocellular neurons (Fig. 5A). Although the humans as a group were relatively older than the baboons, the youngest human, at 16 years of age, was approximately equivalent in stage of life to the 5-year old baboons, yet the species-difference in galanin mRNA expression was evident in these subjects.

Immunocytochemical studies have found either an absence (Chan-Palay, '88a,b; Kordower and Mufson, '90) or presence (Kowall and Beal, '89; Vogels et al., '89) of galanin immunoreactivity in most human magnocellular neurons. One hypothetical explanation for this discrepancy is that the immunocytochemical methods of the latter investigators were more sensitive than those employed by the former. However, both Chan-Palay ('88a,b) and Kordower and Mufson ('90) used sensitive immunocytochemical methods with intensification of the reaction product, and both were able to detect a subpopulation of galanin-immunoreactive neurons in the nbM. Furthermore, Chan-Palay ('88a,b) used tissue that was perfusion-fixed, in some cases after short postmortem intervals, which would enhance tissue preservation and minimize postmortem artefact. Our hybridization histochemical analysis supports the finding that galanin is scarce or absent in most large cells of the human nbM complex but is present in smaller neurons (Chan-Palay, '88a; Kordower and Mufson, '90) and indicates that the lack of immunostaining results from the reduced or absent expression of the galanin gene rather than from posttranslational events such as the rapid transport of the peptide away from the somata. A previous analysis using autoradiographic film also has indicated a paucity of galanin mRNA in the human nbM (Palacios et al., '89).

Studies of the levels of galanin in Alzheimer's disease (AD) are consistent with the concept that galanin is largely absent in magnocellular neurons of the human nbM. The loss of cholinergic markers in neocortices of humans with AD (Bowen et al., '76; Davies and Maloney, '76) is related to the dysfunction and eventual death of large, cortically projecting neurons of the nbM complex (Hirano and Zimmerman, '62; Whitehouse et al., '82; Arendt et al., '83; Candy et al., '83; Pearson et al., '83). However, a recent study found no decline in neocortical galanin immunoreactivity in AD, despite a significant loss of choline acetyltransferase (ChAT), a specific marker for cholinergic neurons (Beal et al., '88). Although levels of ChAT in the nbM also are reduced in AD, galanin levels are increased in this region (Beal et al., '90), presumably due to the persistence and hypertrophy of local circuit galaninergic neurons (Chan-Palay, '88a). The uncoupling of cholinergic and galaninergic markers in AD is consistent with our observation that cholinergic neurons projecting to neocortex (primarily the large cells of the nbM) do not produce significant quantities of galanin mRNA in humans.

In the human nbM, neurons that produce galanin mRNA are similar in location to the intensely labeled galaninergic neurons in the baboon nbM. If these cells represent the same subpopulation in both species, then the levels of galanin mRNA are clearly lower in labeled human cells. One possible implication of this observation is that galanin mRNA levels in most magnocellular neurons of the human nbM may be below the threshold of detectability for our methods. It might be fruitful to consider the possibility that the phenotype for galanin differs in the nbM complex of humans and baboons because of different species-specific modulatory influences on these cells. Differences among individuals in the modulation of nbM complex neurons also might account for within-species variations in the detectability of galanin. There is evidence that variations in innervation can alter the transmitter phenotype of postsynaptic neurons in peripheral ganglionic neurons (Walicke, '77) and in the brain (Baldino et al., '88). In fact, the expression of the gene for galanin in hypothalamic supraoptic neurons is increased by the interruption of certain afferents to the nucleus, whereas the expression of the gene for vasopressin in the same neurons is unchanged by the lesions (Young et al., in press). Furthermore, a variety of hormonal changes may influence the levels of putative neurotransmitters and their mRNAs (for review, see Young and Zoeller, '87). Consequently, it would be informative to determine whether the synaptic (and other) inputs to neurons of the nbM complex differ between humans and nonhuman primates.

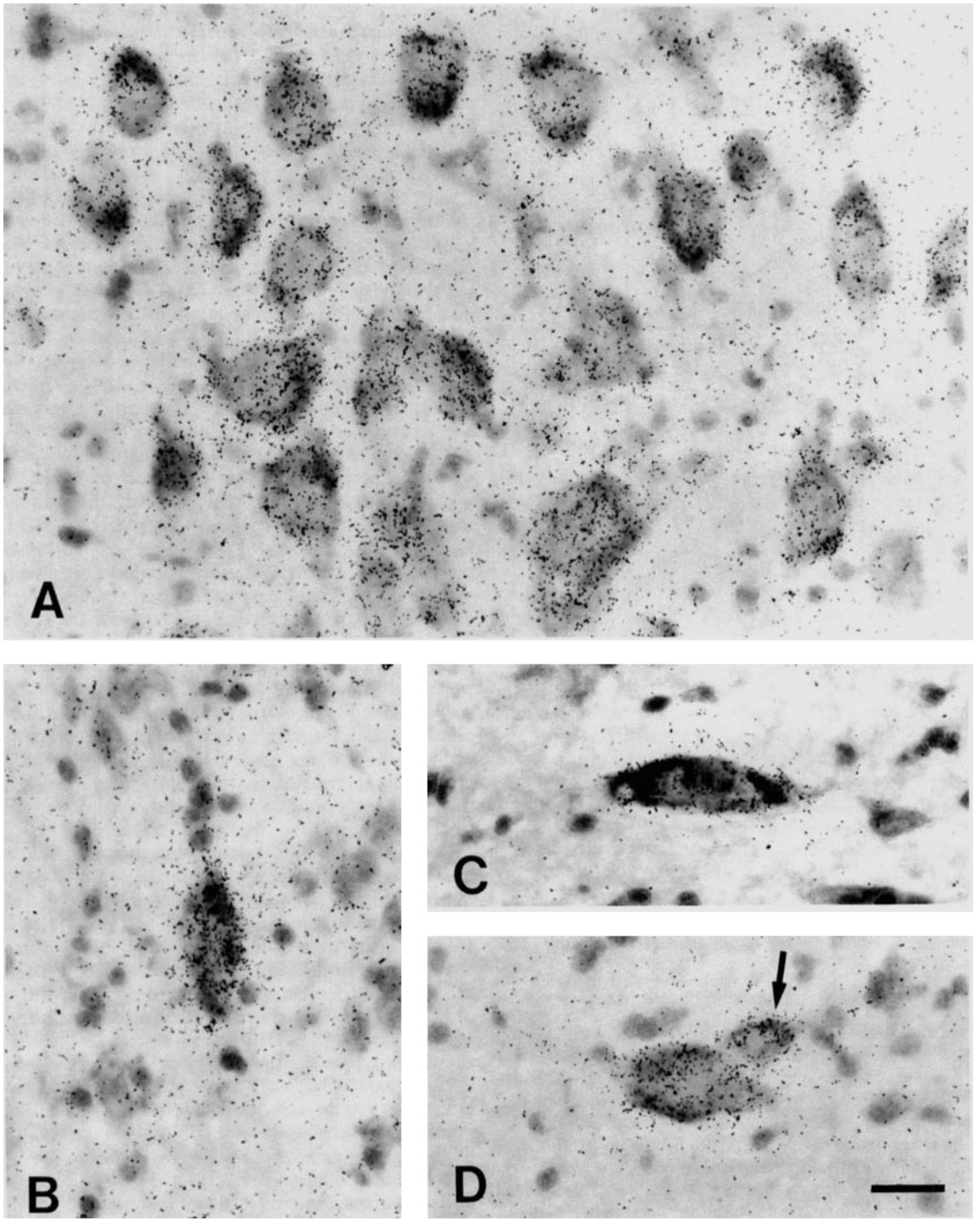

Fig. 5. Hybridization histochemistry for galanin mRNA in nbM complex neurons of baboons (bar = 25 μm). **A.** Cluster of galaninergic neurons in the anteromedial nbM of a 2.5-year-old baboon. **B.** Galaninergic neuron in the vertical limb of the ndbB of a 5-year-old baboon. **C.** Galaninergic neuron in the horizontal limb of the ndbB of a 2.5-year-old baboon. **D.** Small galaninergic neuron (arrow) next to a large labeled neuron in the anteromedial nbM of a 2.5-year-old baboon.

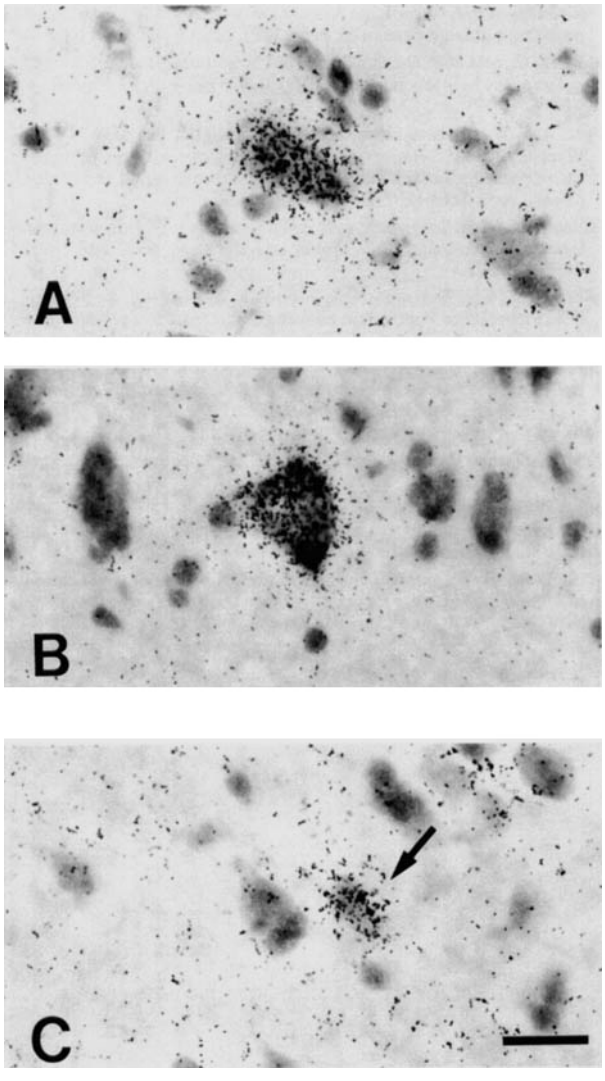

Fig. 6. Hybridization histochemistry for galanin mRNA in the nbM complex and hypothalamus of baboons (bar = 25 μm). **A.** Relatively heavily labeled galaninergic neuron in the horizontal limb of the ndbB of a 5-year old baboon. **B.** Relatively heavily labeled galaninergic neuron in the ventral nbM of a 2.5-year old baboon. **C.** Positive control: labeled galaninergic neuron (arrow) next to an unlabeled cell in the anterior hypothalamus of a 2.5-year old baboon.

It is possible that the relatively profuse network of galaninergic axons and terminals in the human nbM (Kordower and Mufson, '90) is responsible for the down-regulation of galanin in magnocellular neurons. The clarification of extrinsic factors regulating the production of galanin might suggest a variety of indirect means by which the function of the peptide in the nbM complex could be modified pharmacologically.

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