G PROTEINS IN THE BASAL GANGLIA

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ABSTRACT

G proteins are alpha-beta-gamma heterotrimers in the resting state, bound to GDP and complexed with the unbound receptor. Once the receptor becomes occupied, the alpha subunit exchanges GDP for GTP, becomes activated, and dissociates from the receptor and can stimulate or inhibit many intracellular activities such as phosphorylation and channel conductance. For example, Gs and Golf alpha subunits stimulate and Gi alpha subunits inhibit adenylyl cyclase. Go alpha subunits are abundant in brain, but are of unknown function.

cDNAs for the alpha subunit have been cloned. In order to examine the relative distributions of G proteins in the brain, we used in situ hybridization with radiolabelled synthetic oligonucleotide probes. By using a tyrosine hydroxylase antibody, we found that the dopaminergic neurons of the substantia nigra and the noradrenergic neurons of the locus ceruleus express mRNA for the alpha subunits for each of Gi, Go, and Gs. We noted a paucity of Gs mRNA in the striatum. This was surprising because the basal ganglia contain a dopamine-stimulated adenylyl cyclase activity which has been assumed to be transduced by Gs. Also, immunohistochemistry, immunoblotting, and cholera ADP-ribosylation indicated a very high level of Gs alpha-like protein in the striatum. In order to ascertain which specific G protein we were detecting, we made probes to a new G protein previously identified in the olfactory system. Golf is a stimulatory G protein with size and sequence characteristics similar to those of Gs. The cholera toxin ADP-ribosylation site and C-terminal region to which the antibody was made are identical. We made oligonucloetide probes to the translated and untranslated portions of Golf alpha. High levels Golf mRNA and protein were detected in the striatum and nucleus accumbens, in addition to the expected high levels in the olfactory tubercle. Northern blot studies indicated that Golf transcripts are approximately ten-fold more abundant than Gs alpha transcripts in the striatum. These data indicate that Golf in not an olfactory-specific G protein. It is also the major stimulatory G protein in the basal ganglia. The selective expression of high levels of Golf in dopamine-rich forebrain areas suggest that it may couple D1 dopamine receptors to adenylyl cyclase. The role of Golf in dopaminergic neurotransmission and neuropsychiatric disease should be considered.

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The basal ganglia is a group of bilateral sub-cortical nuclei which exert dopamine-mediated control over motor function. The main components consist of the caudate, the putamen, the pallidum, the subthalamic nucleus and the substantia nigra. The striatum (caudate and putamen divided by internal capsule in humans) is composed of cells derived from the telencephalon and serves as the major input nucleus. Cells of both the substantia nigra pars reticulata and the internal pallidum (rodent entopeduncular nucleus) are derived from the diencephalon and are the major output structures (Parent, 1990).

Input to the basal ganglia is predominantly glutamatergic from the cortex and synapses on striatal neurons. The rostral cortex projects to the rostral striatum and the more posterior cortical areas project progressively to more posterior parts of the striatum (Parent, 1990). Thalamostriate fibers project from the rostral intralaminar thalamic nuclei and parafascicular nucleus to the caudate nucleus. The centromedian nucleus of the thalamus projects to the putamen. The medial, dorsal, and ventral nuclei of the thalamus also project to the striatum (Carpenter, 1989). The substantia nigra also provides input to the striatum. This dopaminergic projection, though only 15% of striatal synapses, is critical to motor function. These nigral neurons give off ipsilateral fibers which travel rostrally to the striatum with additional processes to the amygdala and cortical areas (Albin, 1989).

Output from the striatum arises from numerous the GABAergic medium spiny neurons (Freund et al., 1984). This cell type comprises over 90% of the striatal neuronal population (Kemp, 1971). Medium spiny striatal neurons project to the globus pallidus (Albin, 1989), the entopeduncular nucleus and the substantia nigra pars reticulata (Beckstead, 1985).

A very good marker for nigrostriatal neurons is tyrosine hydroxylase, an enzyme necessary to the formation of the monoamines dopamine and noradrenaline. Patients exhibiting Parkinsonism show a drastically reduced population of tyrosine-positive nigrostriatal dopaminergic neurons. Pharmacologically, this striatal dopamine deficiency can be rectified by supplementing additional precurser, such as L-dopa. This is clinically routine, but is only a temporary relief because it has permanent and degenerative side effects.

Predictably, drugs that block the effect of dopamine can exacerbate Parkinsonism, but they have been found to ameliorate the symptoms of schizophrenia (Creese et al., 1976; Seeman et al., 1976). Kebabian and Calne (1976) found that ergot derivatives, which have a relatively high affinity for dopamine sites, could affect the production of an intracellular messenger when applied to brain slices. They found that the rate of cAMP production by adenylyl cyclase was not directly correlated with drug affinity. They came to the conclusion that there were at least 2 dopamine receptor types. The D1 receptor, when activated, increases the levels of cAMP by stimulating adenylyl cyclase. Conversely, when the D2 receptor is activated, adenylyl cyclase activity is either inhibited (striatum and pituitary) or unchanged (limbic forebrain) (Kelley et al., 1987) The D1 receptor binding sites appear postsynaptic on the substance P and dynorphin-producing cell bodies of the striatonigral projection (Graybiel, 1990). The D2 receptors, however, are located both postsynaptically on medium spiny enkephalinergic striatopallidal cell bodies (Beackstead, 1988), and as autoreceptors on nigrostriatal dopaminergic neurons (Filloux et al., 1988).

What these two dopamine receptor types have in common, is their transduction mechanism to achieve their physiological effect. It is postulated that these receptors complex with members of a family of intermediaries called the guanine nucleotide binding proteins (G proteins). Receptors able to couple to G-proteins typically have 7 stretches of 20 to 28 hydrophobic amino acids (Bunzow et al., 1988) which form alpha helices through the cell membrane. They all have potential glycosylation sites at the amino terminus and potential phosphorylation sites at the carboxy teminus (Iyengar, 1990, p328-329).

G proteins are transmembrane intermediaries which can communicate the extracellular neurotransmitter signals to the intracellular millieu. In the resting state, they are heterotrimers bound to GDP and complexed to the unbound membrane receptor (see Gilman (1987) for review). Of the alpha, beta and gamma subunits, the former is the largest and has the most clearly defined functions. In most systems including the dopaminergic pathways, the alpha subunit determines the unique characteristics of the receptor-effector interactions.

The alpha subunits of G proteins are part of a large multi-gene family. The various alpha subunits are characterized by their molecular size, the receptor to which they couple and the downstream action they have on effectors. For example, Gs stimulates and Gi inhibits adenylyl cyclase. Not only do alpha subunits control the reaction rates of adenylyl cyclase, but these 39 to 52 kDa subunits can also control ion channel conductances, intracellular structural proteins and a spectrum of cytosolic and membrane-bound enzyme activities (Kim, 1989). All alpha subunits demonstrate GTPase activity. Most types of alpha subunits can be ADP-ribosylated, which alters the ability to switch between active and inactive states by changing nucleotide binding or cleaving activities.

The other two subunits, beta and gamma, form a tightly complexed dimer that is relatively small (35-36 kDa) (Gilman 1897). The function of the beta-gamma subunits in the transduction process is poorly defined. Since they are considerably more lipophilic than the alpha family, it is possible that the beta-gamma component acts as a tether to anchor the more labile alpha subunits. The dimer is not known to bind nucleotides, but beta-gamma subunits are interchangeable between most of the known alpha subunits and receptors (Kim et al., 1989). In sharp contrast to the alpha subunits, there are few subtypes of these subunits.

Once a receptor becomes occupied by its ligand, the complexed G protein releases GDP, which is the rate limiting step, in exchange for GTP. This causes a conformational change in the G-protein to the active state which initiates the second messenger cascade. Dissociation of the ligand-bound receptor from the activated G-protein opens up the docking site of the receptor allowing further G-protein-GDP complexes to be activated. Thus a single molecule of hormone can activate multiple G protein alpha subunits. As the lifetime of activated G-protein-GTP complex (once free from the receptor) is many seconds, there is a great capacity for amplification of the signal. The activated alpha subunit can activate one or more types of effectors. Endogenous GTPase activity which cleaves the terminal phosphate of GTP to form GDP, returns the alpha subunits to the resting state. Alteration of this GTPase activity has great potential as a tool in modulation of the physiological impact that a given amount of

neurotransmitter has (Ingeyar and Birnabaumer 1990).

Because of the vital role of G proteins in neurotransmission, we sought to explore the distribution of G-protein alpha subunits and their mRNA in noradrenergic and dopaminergic neurons in rat brain.

It has previously proven difficult to identify cells which produce G-proteins because most of the transduction events occur at the synapses. Antibodies are ideal for localizing protein in tissue sections, but not the cell body where the protein in question originated. Therefore several techniques were used to localize G proteins in the brain. *In situ* hybridization takes advantage of a necessary step to protein expression. DNA of a cell must be transcribed first into RNA, which then migrates into the cytoplasm where it is translated into protein. It is possible to synthesize complimentary oligonucleotide sequences which will hybridize to the natural mRNA in the cytoplasm. If the oligonucleotide is tagged with radioactivity, exposure of the sections to photographic emulsion quantitatively reveals the relative abundance of the particular sequence in question.

The combination of immunohistochemistry and *in situ* hybridization forms a powerful double labeling technique to localize accurately the neurons expressing particular G-protein mRNA at the cellular level.

In order to understand better the mechanisms of neurotransmitter-related disorders, the individual elements of signal processing must first be understood. As a first step, anatomical distribution of these elements can be very indicative of function. The following study was designed to identify G protein components of the dopamine-stimulated adenylyl cyclase activity in the striatum.

EXPERIMENT 1

G Protein mRNA Expression in Immunohistochemically Identified Dopaminergic and Noradrenergic Neurons in the Rat Brain

In the present study, radiolabelled oligonucleotide probes were used to examine the distributions of the mRNAs for the alpha subunits of Gi, Go and Gs in the rat brain. One observation of particular interest was the high level of expression noted in the substantia nigra and the locus ceruleus. These cell groups have been the focus of extensive physiological studies which have indicated that G proteins are involved in the synaptic responses of the dopaminergic and noradrenergic neurons in these areas (Aghajanian and Wang, 1986; 1987; Innis and Aghajanian, 1987; North et al., 1987; Lacey et al., 1988; Simson et al., 1988; Wang and Aghajanian, 1987a; 1987b; 1988). Therefore, we have sought evidence for the expression of G protein mRNA in these catecholamine neurons by first identifying them immunohistochemically, and then examining the expression of the mRNAs for the various G proteins in these areas at the cellular level.

MATERIALS AND METHODS

Materials. Synthetic 39 base oligonucleotide probes to the alpha subunits of Gi, Go and Gs which were 3'-end labelled with [^{35}S]-dATPaS by terminal deoxynucleotidyl transferase were from NEN Research Products (Boston, MA). Rabbit anti-tyrosine hydroxylase antiserum was from Eugene Tech Intl. (Allendale, NJ), and the rabbit ABC-kit from Vector Laboratories (Burlingame, CA).

Northern Blotting. Total RNA was extracted from whole rat brain by the guanidine thiocyanate-CsCl method (Chirgwin et al., 1979). The RNA (10.5 ug/lane) was electrophoresed in 1% agarose-1.0 M formaldehyde gel and subsequently electrophoretically transferred to a nylon membrane (Gene Screen Plus, NEN Research Products). The membrane was baked under vacuum at 80^{o}C for two hr, and then pre-incubated in hybridization buffer, consisting of 1% SDS, 50% formamide, 10X Denhardt's (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin), 4X Standard Saline Citrate (SSC), 10% dextran sulfate, for 1 hr at 21^{o}C. The membrane was then incubated overnight at 37^{o}C with 1.8 X 10^{6} cpm/mL of ^{35}S-labelled oligonucleotide probe in the same buffer containing 0.25 mg/ml tRNA (Sigma, Type V) and 40 mM b-mercaptoethanol. After hybridization, the blots were washed at 21^{o}C for 8 X 15 min in 1X SSC, for 4 X 15 min with 0.5X SSC and for 4 X 15 min at 50^{o}C in 0.5X SSC, and dried. The blots were then exposed to X-ray film (Kodak RP) for 9 days at -80^{o}C using NENhance (NEN Research Products). Size was determined by staining the gel with ethidium bromide to visualize E. coli (Pharmacia) and mammalian ribosomal RNA as markers.

In situ Hybridization and Immunohistochemistry. Solutions were pretreated with 0.1% diethylpyrocarbonate (DEP) and autoclaved prior to use. Adult male Wistar rats (200 gm) were given an overdose of pentobarbital and perfused transcardially with 50 ml of saline followed by 300 ml of 4% paraformaldehyde in 0.1 M sodium phosphate (pH 7.4). The brain was removed and placed in phosphate buffered 15% sucrose for 48 h at 4^{o}C. Coronal sections

were cut at 30 microm thickness on a freezing microtome and collected in sterile saline. Some of the sections were mounted onto acid-washed slides that had been double coated with chrom-alum, and treated with DEP. These were processed for *in situ* hybridization immediately. Other sets of sections were processed for immunohistochemistry prior to mounting and *in situ* hybridization.

The avidin-biotin-complex method was used for the immunohistochemical localization of catecholamine neurons (Vincent, 1988). The free-floating sections were incubated in the primary antibody (rabbit anti-tyrosine hydroxylase), diluted 1:50 in Tris-buffered saline (TBS) containing 0.3% Triton X-100, for 48 hr at $4^{o}C$. They were then rinsed 3 X 20 min in TBS, and incubated in 0.5% biotinylated goat anti-rabbit IgG for 1 hr at room temperature. After rinsing again in TBS (3 X 20 min), the sections were incubated in 1% avidin-biotinylated horseradish peroxidase complex for 1 hr at room temperature, and then rinsed 3 X 20 min in TBS. Peroxidase activity was demonstrated with 0.025% 3,3'-diaminobenzidine, 0.01 mM imidazole and 0.0075% H2O2 in 50 mM Tris buffer, pH 7.4. After staining, the sections were washed in TBS, mounted and allowed to dry before processing for *in situ* hybridization.

The *in situ* hybridization was performed using standard procedures for synthetic oligonucleotide probes (Lewis et al., 1988). The mounted sections were dipped into 4% paraformaldehyde for 5 min at $21^{o}C$, rinsed 4 X 15 min in saline, and the excess liquid then blotted off. The sections were treated with prehybridization buffer consisting of 10 X Denhardt's, 4X SSC, 0.1% SDS and 50% formamide at $21^{o}C$ for 1 hr (500 uL/slide). This solution was blotted off and the sections hybridized overnight at $37^{o}C$ in 4X SSC, 50% formamide, 1 X Denhardt's, 0.25 mg/ml tRNA, 10% dextran sulfate, 40 mM b-mercaptoethanol and the [^{35}S]-labelled probe (8.8 X 105 cpm/200 uL/slide). After hybridization, the sections were rinsed as described for the Northern blotting, and then dehydrated through a graded series of ethanol diluted with 0.3 M ammonium acetate (pH 7), followed by two changes in xylene, and a final rinse in ethanol. The slides were air dried and dipped into Kodak NTB-2 emulsion (diluted 1:1 with 0.3 M ammonium acetate) at $42^{o}C$, dried

and exposed for 1 to 4 weeks at 4°C. The slides were developed with Kodak D-19 for 3 min at 16°C, rinsed with water, fixed and washed in water. The sections were then counterstained with cresyl violet, and examined and photographed under light- and dark-field illumination.

In addition to the G protein probes, an ^{35}S-labelled oligonucleotide probe to somatostatin, a neuropeptide not known to be present in the substantia nigra or locus ceruleus catecholamine neurons, was used as a control. Hybridization with this probe labelled many neurons throughout the brain, consistent with the distribtuion of somatostatin immunoreactive neurons. However, the tyrosine hydroxylase-immunoreactive neurons of the locus ceruleus and substantia nigra were not labelled with this probe.

RESULTS

Northern blot analysis of total RNA from whole rat brain was performed at the same stringency used for *in situ* hybridization. The Gi alpha probe recognized one major mRNA species of 2300 bases. The Go alpha probe hybridized to two distinct bands corresponding to mRNA species of 4300 and 3300 bases. Hybridization of total brain RNA with the Gs alpha probe produced the strongest signal, corresponding to an mRNA of 1900 bases (Figure 1).

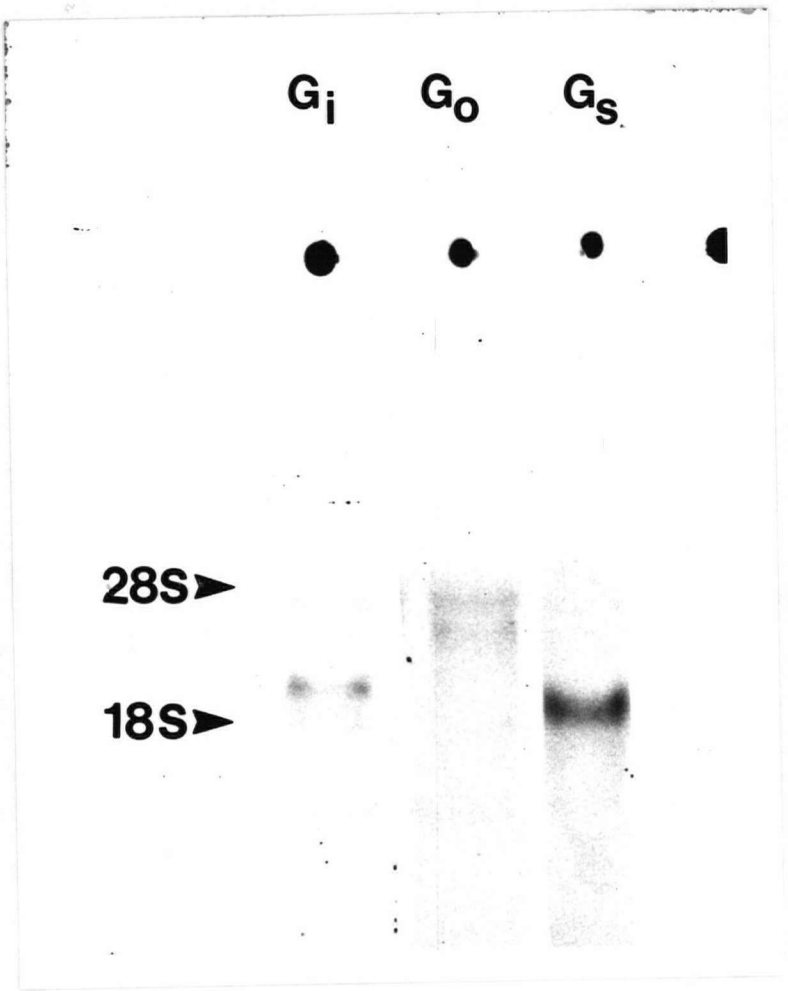
The *in situ* hybridization studies indicated that the Gi alpha, Go alpha and Gs alpha probes each had a widespread and unique distribution in the brain. Gi alpha gave the weakest signal with Northern analysis, but expression was noted in the piriform cortex, the granule and pyramidal cells of the hippocampal formation, the basal forebrain, septum and bed nucleus of the stria terminalis. The supraoptic, paraventricular and arcuate nuclei and the subfornical organ were well labelled. A very high signal was observed in the subthalamic nucleus, the lateral mammillary nucleus and the periventricular thalamus. The pars compacta of the substantia nigra, and the ventral tegmental area were also well labelled (Figure 2A). The dorsal raphe (Figure 5), locus ceruleus (Figure 3B), pontine nuclei and the dorsal vagal complex were also clearly labelled.

The neocortex, the pyramidal and granule cells of the hippocampal formation and the

endopiriform nucleus displayed high levels of Go alpha message. A very high signal was detected over the organum vasculosum of the lamina terminalis and the subfornical organ. The magnocellular basal forebrain, bed nucleus of the stria terminalis and magnocellular neurosecretory neurons were heavily labelled. Strong labelling was observed over the arcuate nucleus and the medial habenula. The substantia nigra pars compacta, ventral tegmental area and dorsal raphe nucleus showed very high grain densities relative to the rest of the midbrain (Figure 2C). Very strong labelling was seen over the entire pontine tegmentum, including the locus ceruleus (Figure 3D). The Purkinje cell layer of the cerebellar cortex was clearly marked.

The Gs alpha probe gave the strongest signal in the brain. Forebrain regions showing particularly strong labelling included the pyramidal cells of the piriform cortex and hippocampus, and the magnocellular basal forebrain. The supraoptic and paraventricular nuclei were especially well labelled. High grain densities were also noted in the subthalamic nucleus and medial habenula. The substantia nigra (Figure 2E), dorsal raphe

Fig. 1. Northern blot analysis of whole rat brain RNA with synthetic oligonucleotide probes for the α subunits of G_i, G_o and G_s. The G_i probe recognized one major band corresponding to 2300 bases. The G_o probe hybridized to two bands of 3300 and 4300 bases, and the G_s probe recognized a single band of 1900 bases. Note the high levels of G_s mRNA present, compared to G_i and G_o. Ribosomal RNA size markers are indicated by arrows, and the origins by ^{35}S-labelled spots.



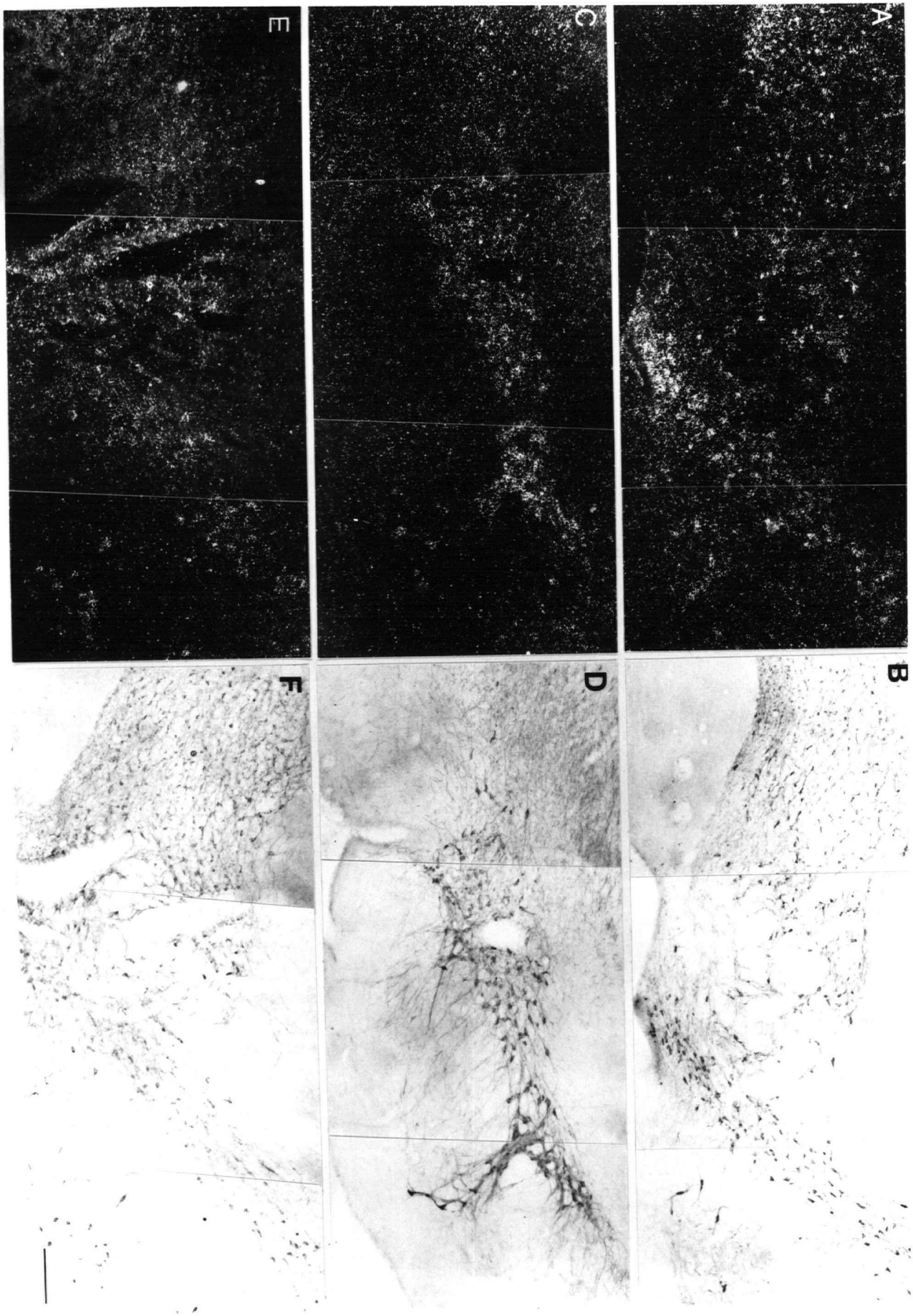

A
C
E
B
D
F

Fig. 2. The localization of the mRNA for the alpha subunits of G_i (**A**), G_o (**C**) and G_s (**E**) in the substantia nigra of the rat in dark-field micrographs following *in situ* hybridization using ^{35}S-labelled oligonucleotide probes. The same sections were immunohistochemically stained with antibodies to tyrosine hydroxylase, prior to *in situ* hybridization, to identify the dopaminergic neurons (**B**,**D**,**F**). Scale bar indicates 200 μm for all figures.

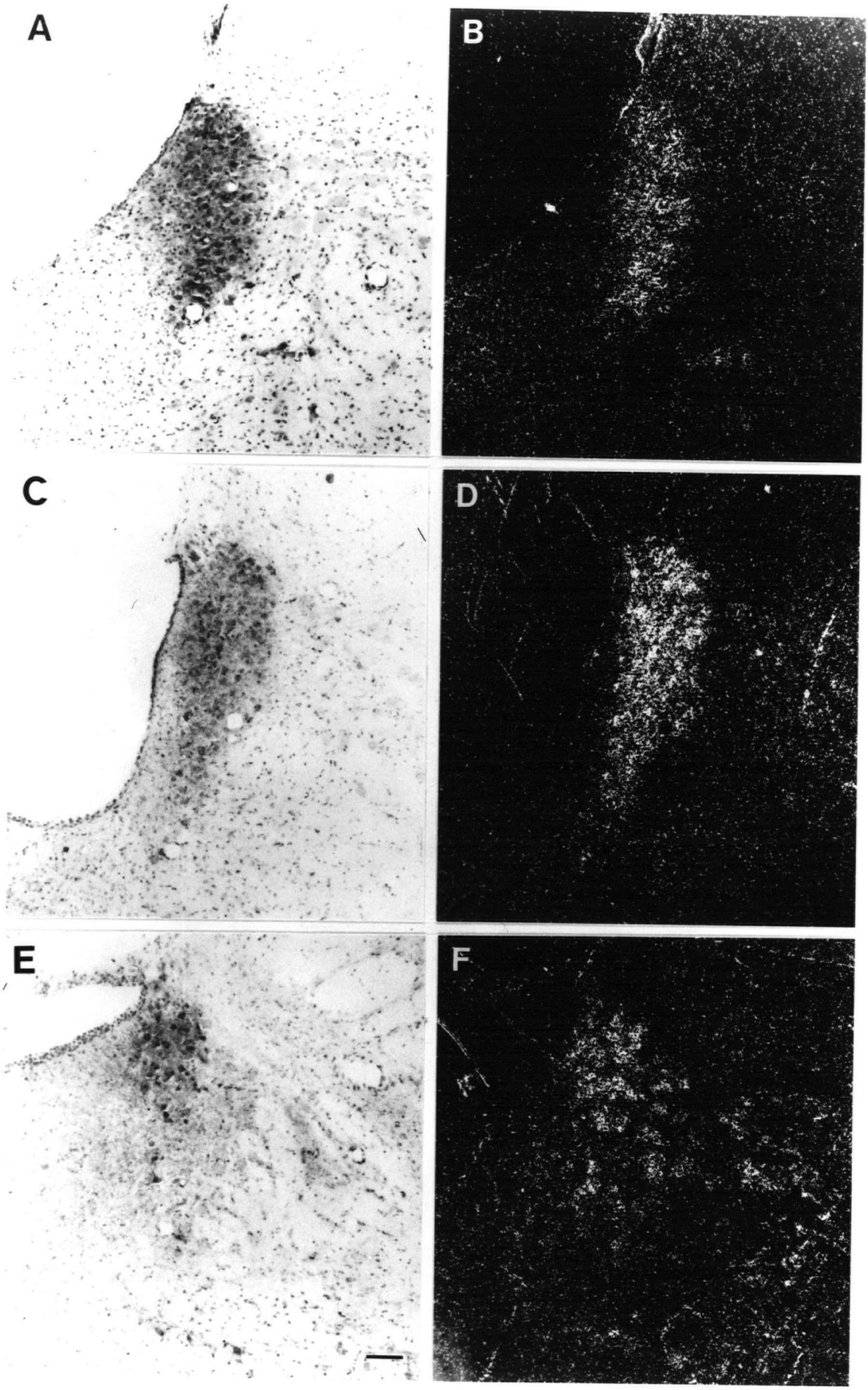

A
B
C
D
E
F

Fig. 3. *In situ* hybridization localization of the expression of the alpha subunit mRNAs for G_i (**B**), G_o (**D**) and G_s (**F**) in the locus ceruleus of the rat under dark-field illumination. The same sections are shown under bright-field illumination (**A,C,E**) to illustrate the noradrenergic neurons identified using tyrosine hydroxylase immunohistochemistry. Scale bar indicates 200 μm for all figures.

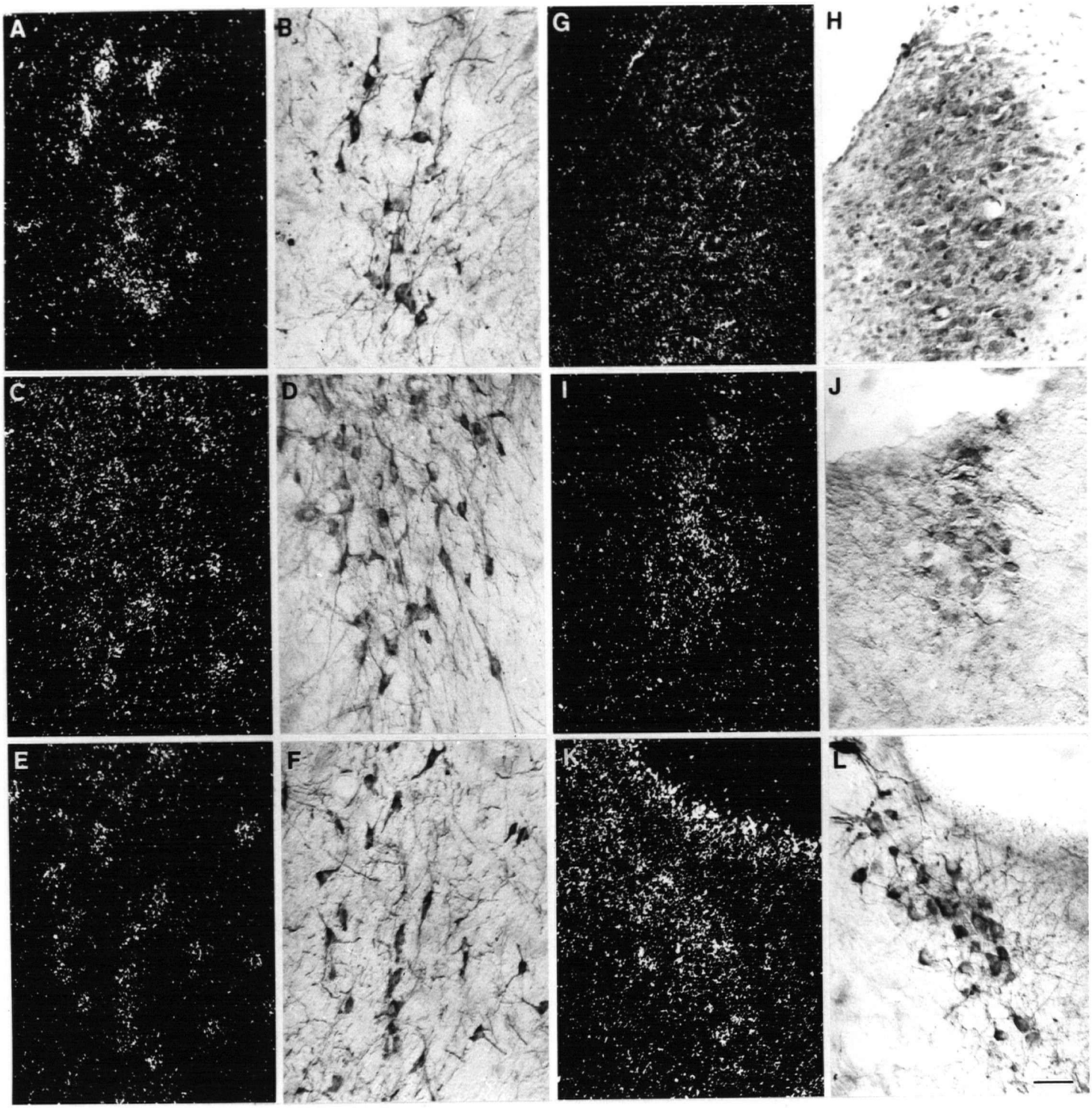

A
B
G
H
C
D
I
J
E
F
K
L

Fig. 4. Cellular distribution of mRNA for the alpha subunits of G_i (**A,G**), G_o (**C,I**) and G_s (**E,K**) in the dopaminergic neurons of the substantia nigra (**A,C,E**) and the noradrenergic neurons of the locus ceruleus (**G,I,K**) is illustrated under dark-field illumination following *in situ* hybridization. Tyrosine hydroxylase immunoreactivity is shown under bright-field illumination in the same sections in (**B,D,F**) for the substantia nigra, and (**H,J,L**) for the locus ceruleus. Scale bar indicates 50 μm for all figures.

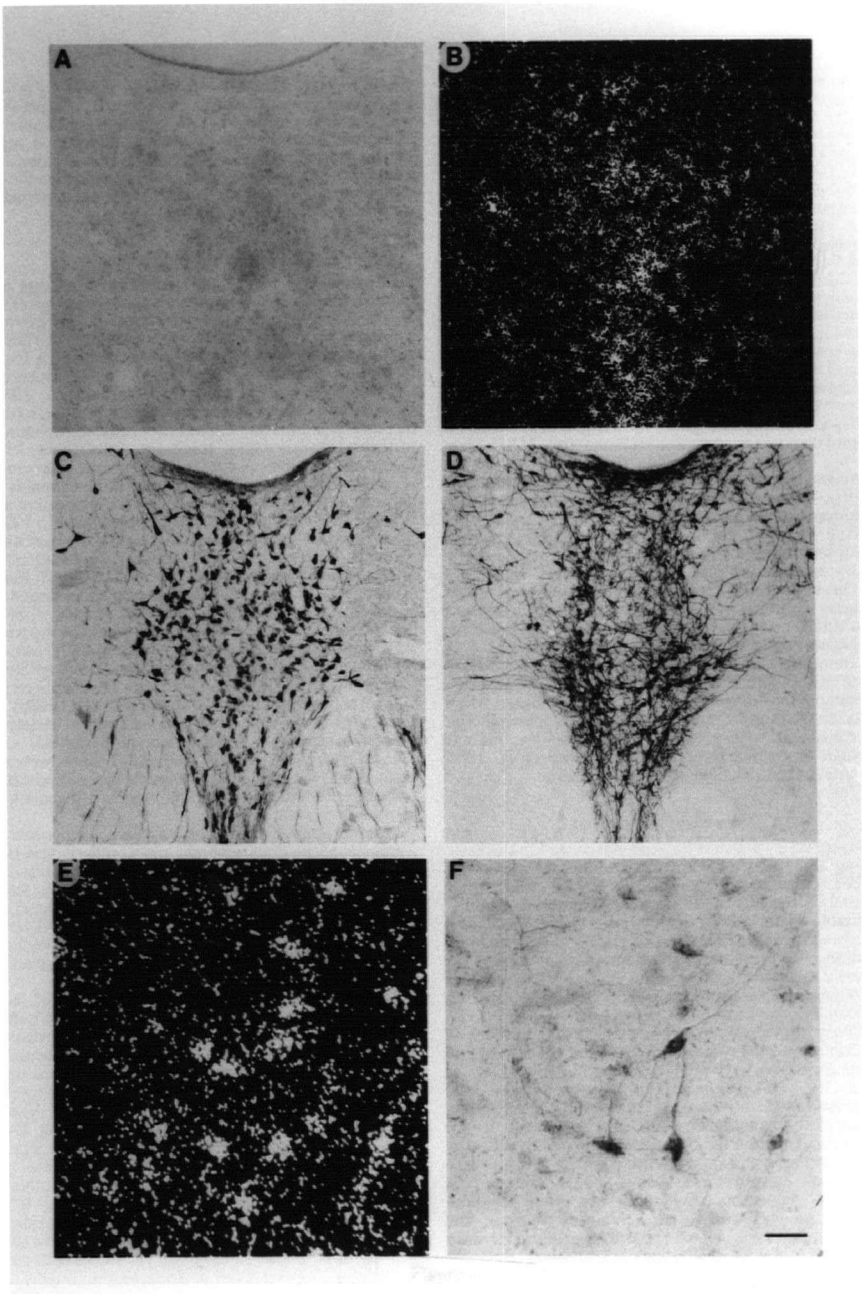

A
B
C
D
E
F

Fig. 5. High levels of G_{i}_α mRNA expression are observed in the dorsal raphe nucleus under bright (**A**) and dark-field (**B**) illumination. Both serotonin (**C**) and tyrosine hydroxylase (**D**) immunoreactive neurons are present in this area. When the same sections were immunohistochemically stained with antibodies to tyrosine hydroxylase, prior to *in situ* hybridization, both dopaminergic (arrows) and non-dopaminergic (arrowheads) neurons were labelled following hybridization with the ^{35}S-labelled probe (**E,F**). Scale bar indicates 100 μm for A-D and 25 μm for E,F.

and locus ceruleus (Figure 3F) were clearly labelled. The dorsal vagal complex, and the ventrolateral medulla contained particularly high grain densities. The striatum expressed surprisingly low levels of Gs message.

When sections through the substantia nigra were first immunohistochemically stained for tyrosine hydroxylase and then processed for *in situ* hybridization, the presence of the various G protein mRNAs in the dopaminergic neurons could be directly examined. Using this approach, individual dopaminergic neurons were identified and found to display high levels of G protein mRNA (Figure 4A-F). Essentially all of the tyrosine hydroxylase-immunoreactive neurons in the nigral sections showed high grain densities following *in situ* hybridization with each of the G protein probes. This indicates that the nigral dopaminergic neurons express all three of these G proteins.

A similar approach was used to examine G protein expression in the locus ceruleus. Again, the tyrosine hydroxylase immunoreactive noradrenergic neurons were clearly labelled by all three probes following *in situ* hybridization (Figure 4G-L).

High levels of expression for all three G protein mRNAs was noted in the dorsal raphe nucleus. Although this nucleus is often considered serotoninergic, it also contains a large population of dopamine neurons. Therefore immunohistochemical studies with tyrosine hydroxylase antibodies were combined with *in situ* hybridization to determine the cellular localization of G protein mRNA expression in this area (Figure 5). These double-labelling experiments clearly indicated that the dopamine neurons in the dorsal raphe nucleus expressed high levels of Gi alpha, Go alpha and Gs alpha mRNA. Other neurons in the dorsal raphe which were not tyrosine hydroxylase-positive were also heavily labelled with these probes.

DISCUSSION

Specificity of the oligonucleotide probes. It is now well-known that multiple isozymes of G protein alpha subunits exist in mammalian tissues (Lochrie and Simon, 1988). Thus far, three distinct forms of Gi alpha, designated Gi-1a (Nukada et al., 1986; Bray et al., 1987), Gi-2a (Itoh et al., 1986) and Gi-3a (Jones and Reed, 1987; Itoh et al., 1988) have been characterized and cloned. The nucleotide sequences of Gi-1a and Gi-3a at the sites complementary to the oligonucleotide probe used here are 74% and 82% identical to the Gi-2a mRNA against which the oligonucleotide probe was designed. The single mRNA species recognized by the Gi probe in the present study was the same size as Gi-2a mRNA (Brann et al., 1987; Didsbury et al., 1987; Jones and Reed, 1987). The high stringency wash was performed well above the theoretically determined Tm (50% melting temperature) for hybridization between the probe and Gi-1a (42oC) but near that for Gi-3a (54oC). No mRNAs corresponding in length to Gi-1a or Gi-3a (Nukada et al., 1986; Jones and Reed, 1987; Codina et al., 1988) were recognized by the probe using Northern blot analysis. Previous Northern analyses have indicated that Gi-3a mRNA is poorly expressed in rat brain (Jones and Reed, 1987; Codina et al., 1988), and Gi-3a protein is reported to be present in very low or undetectable levels in brain (Goldsmith et al., 1988b). However, a recent study has demonstrated the presence of Gi-3a-like immunoreactivity in brain, in particular in nigral dopaminergic neurons (Cortes et al., 1988). Thus in our studies it is possible that Gi-3a mRNA was detected in nigral neurons by *in situ* hybridization, but in the whole brain extract not enough was present to be detected by Northern analysis.

Approximately equal amounts of two mRNA species, with lengths of 4300 and 3300 bases were detected with the Go probe. Previous Northern blot analyses of rat brain have also detected more than one Go alpha mRNA species (Brann et al., 1987; Jones and Reed, 1987), and multiple forms of Go protein have been detected in brain (Goldsmith et al., 1988a; Kanaho et al., 1988).

There are four forms of Gs alpha which arise from alternate mRNA splicing in humans (Bray et al., 1986). Two forms of cDNA correspond to the Mr 52,000 kD polypeptide and the other two to the 45,000 kD polypeptide, the former being more efficient at reconstituting S49 cyc- adenylyl cyclase activity. Since our Gs probe is designed against the ADP-ribosylation site, all forms would be recognized. The Gs probe recognized one major mRNA of about 1900 bases, which was the most prevalent G protein mRNA detected. This is consistent with previous work in bovine, rat and human brain (Harris et al., 1985; Bray et al., 1986; Brann et al., 1987; Jones and Reed, 1987).

In situ Hybridization. Go alpha and beta-gamma proteins have been localized in the brain using immunohistochemical techniques (Worley et al., 1986; Asano et al., 1987; 1988; Chang et al., 1988; Terashima et al., 1988). Because G proteins are, presumably, associated with synaptic membranes, such studies provide information primarily about the localization of G proteins in the neuropil. The aim of the present study was to determine which cells are capable of synthesizing G proteins, and for this, *in situ* hybridization is the method of choice.

The results of the present study regarding the general distributions of G protein mRNAs in brain extend previous general discriptions (Brann et al., 1987; Largent et al., 1988; Mengod et al., 1988). Largent et al. (1988) described moderate amounts of Gs alpha in the pars compacta of the substantia nigra, and high levels in the locus ceruleus and dorsal raphe. However, low resolution mapping studies such as this cannot distinguish the type of cell, neuron or glia, that is labelled. Therefore, in order to determine unambiguously whether these mRNAs are localized in catecholamine neurons, the dopaminergic neurons of the substantia nigra were identified immunohistochemically, and simultaneously shown to express high levels of Gs alpha mRNA by *in situ* hybridization. Using a similar strategy, Gs alpha expression was also demonstrated within immunohistochemically identified dopaminergic neurons in the dorsal raphe and noradrenergic neurons in the locus ceruleus. In addition, we have presented the first evidence for the expression of Go alpha and Gi alpha mRNA in these dopaminergic and noradrenergic cells.

While attention has focused upon the ability of alpha subunits to activate second messenger systems and ion channels, it has recently been shown that the beta-gamma complex has actions as well (Kim et al., 1989), although considerably higher concentrations are required (Neer and Clapham, 1988). The co-expression of multiple G proteins in catecholamine neurons raises the intriguing possibility that simultaneous occupation of multiple receptors coupled to all three G protein subtypes may liberate sufficient beta-gamma subunits to have actions on its own.

Dopaminergic nigral and noradrenergic locus ceruleus neurons possess somatodendritic receptors of the D2 and alpha-2 types, respectively (Groves et al., 1975; Svensson et al., 1975). The D2 receptor is a member of a family of G protein-coupled receptors possessing seven transmembrane domains (Bunzow et al., 1988), which can interact with both Gi and Go (Ohara et al., 1988). Electrophysiological evidence suggests that G proteins mediate dopaminergic activation of potassium channels in the substantia nigra (Innis and Aghajanian, 1987; Lacey et al., 1988). A similar mechanism may couple m-opiate and alpha-2 receptors to potassium channels in noradrenergic locus ceruleus neurons (Aghajanian and Wang, 1986; North et al., 1987; Simson et al., 1988). Although this phenomenon is blocked by pertussis toxin, it appears to be independent of alterations in intracellular cAMP (Aghajanian and Wang, 1987; North et al., 1987) and is thus likely not to be mediated by either Gs or Gi-2 acting on adenylyl cyclase. A more likely candidate for these responses would be a pertussis toxin sensitive G protein which directly couples these receptors to potassium channels. Recent evidence indicates that all three Gi alpha subtypes can couple receptors to potassium channels (Yatani et al., 1988b). In addition, Go has been shown to activate a variety of potassium channels in hippocampal pyramidal cells (Vandongen et al., 1988). Thus any of these pertussis toxin-sensitive G proteins may couple these somatodendritic receptors to potassium channels in catecholamine neurons.

Autoreceptors of the D2 and alpha-2 type are present on terminals of the dopaminergic and noradrenergic neurons, where they mediate agonist-induced inhibition of transmitter

synthesis and release, via pertussis toxin-sensitive mechanisms (Allgaier et al., 1985; Bean et al., 1988). Thus the pertussis sensitive G proteins, Gi or Go, may be involved in autoreceptor function. We have found that both these types of G proteins can be synthesized in these catecholamine neurons.

Although the alpha-2 and m opiate responses of locus ceruleus neurons appear to be independent of cAMP, our evidence indicates that locus ceruleus neurons express both Gs alpha and Gi-2a mRNA. These G proteins were originally defined by their opposing actions upon adenylyl cyclase activity. It is therefore interesting that a cAMP-dependent inward current has been demonstrated in these neurons (Aghajanian and Wang, 1987; Wang and Aghajanian, 1987b), and vasoactive intestinal polypeptide appears to be able to activate this current (Wang and Aghajanian, 1988). Presumably, agonist-induced elevation of intracellular cAMP levels is mediated via activation of Gs. Gi-2 may antagonize this by mediating inhibition of adenylyl cyclase in these neurons in response to some, as yet uncharacterized, transmitter. We have found that nigral dopaminergic neurons also express Gs alpha and Gi-2a. The effect of cAMP on ionic currents in these cells is not known. However, recent evidence indicates that serotonin receptors are negatively coupled to adenylyl cyclase in the substantia nigra (Bouhelal et al., 1988; Schoeffter et al., 1988).

A dihydropyridine-sensitive (L type) calcium current has been demonstrated in locus ceruleus neurons (Williams and North, 1985; Cherubini and Williams, 1988). Gs can stimulate this type of current (Yatani et al., 1988a), and may thus mediate the activation of calcium channels in locus ceruleus neurons in response to some, as yet unidentified, neurotransmitters. Noradrenaline can reduce an L type calcium current in locus ceruleus cell bodies by acting on a site distinct from the alpha-2 receptor (Williams and North, 1985; Cherubini and Williams, 1988). Although the effect of dopamine on calcium currents in nigral neurons is not known, dopamine can reduce calcium channel activity in other neurons (Marchetti et al., 1986). Some evidence indicates that Go may mediate agonist-induced inhibition of the L-current via protein kinase C (Hescheler et al., 1987; Rosenthal et al., 1988). The results of the present

study demonstrate that Go is expressed in high amounts in both noradrenergic locus ceruleus neurons and dopaminergic nigral neurons. Thus, in the locus ceruleus, and perhaps also the substantia nigra, Go may mediate the effects of noradrenaline and dopamine on both potassium and calcium conductances.

This study is the first report of the co-expression of the alpha subunits of Gi, Go and Gs within the same cells. The activities of dopaminergic and noradrenergic neurons are well known to be specifically affected by various drug treatments. For example, chronic neuroleptic administration results in an absence of impulse activity in dopaminergic neurons (Bunney and Grace, 1978). Chronic morphine treatment leads to tolerance to the opioid-induced potassium conductance increase (Aghajanian, 1978; Christie et al., 1987), and increases the amount of Gi alpha and Go alpha protein in the locus ceruleus (Nestler et al., 1989). Also chronic antidepressant treatment appears to result in a functional uncoupling of adrenergic receptors from adenylyl cyclase (Okada et al., 1986; 1988). Thus it will be of particular interest to examine the effects of such treatments on the expression of G protein mRNAs in these cell groups.

EXPERIMENT II

In the previous *in situ* hybridization studies on the localization of G protein mRNA (experiment I), the very low abundance of Gs alpha mRNA in the rat striatum, accumbens and olfactory tubercle was of particular interest. Although a number of forms of Gs alpha have been shown to arise by alternate mRNA splicing (Robishaw et al., 1986), all known forms from the same gene would be detected by our probe which corresponds to the site of ADP-ribosylation by cholera toxin. Other groups, using different probes, have also noted very low levels of Gs alpha mRNA in the rat striatum in both *in situ* and Northern blotting experiments (Largent et al., 1988). This relative lack of Gs alpha message together with the dopamine-sensitive cyclase activity as evidence for abundant Gs alpha-like protein, suggests that a novel G protein may mediate adenylyl cyclase stimulation in the basal ganglia.

Golf is a newly characterized G protein which has 88% sequence homology with Gs alpha and can also stimulate adenylyl cyclase (Jones and Reed, 1989). The two major forms of Gs are 42 and 52 kDa, and Golf is intermediate in molecular size (Pace and Lancet, 1986; Jones et al., 1990). Although originally isolated from olfactory sensory neurons, recent studies using the polymerase chain reaction have identified a portion of the Golf sequence in the brain (Strathman et al., 1989). Golf is recognized by the RM/1 antibody (Simonds et al., 1989) which was used to detect Gs alpha, and is also ADP-ribosylated by cholera toxin (Jones et al., 1990). In order to test the hypothesis that Golf is expressed in the basal ganglia, prepares an oligonucleotide probe to the region of the Golf cDNA sequence previously detected in brain (Strathman et al., 1989), and used in *in situ* hybridization to localize it in the rat brain.

MATERIALS AND METHODS

Materials. Synthetic 39 base oligonucleotide probes to the alpha subunit of Golf, constructed by Dr. T. P. Snutch, were 3'-end labelled with [^{32}P]-dATPaS by terminal deoxynucleotidyl transferase from NEN Research Products (Boston, MA). Rabbit anti Gs RM/1 (Simonds et al., 1989) was from New England Nuclear, and the rabbit ABC-kit from Vector Laboratories (Burlingame, CA).

The probe sequences are the following :

Gs (coding):

5'-TCCAGAGGTCAGGACACGGCAGCGAAGCAGGTCCTGGTC-3',

Golf (coding):

5'-AACACCAACAGACTCCGGGAGTCGCTGGACCTGTTTGAA-3'

3'-untranslated portion of Golf:

5'-GGCTGGGGATATGGACTAGCTGTAGAGTGCTTCCCTAGC-3')

5'-untranslated protion of Golf:

5'-CTCCGATCAGGCAAGTAGGAAGGCGCACCAGAGCCAGCG-3')

Western Blotting: Brain regions were dissected on ice and homogenized in 9 volumes of 0.1 M Tris pH 6.8, containing 2% SDS, 50% glycerol, boiled 5 minutes, electrophoresed on 9% acrylamide gel, electrophoretically blotted onto immobilon (Millipore) and incubated in primary antibody diluted to 1:1000. Blots were then incubated with alkaline phosphatase-conjugated goat-anti-rabbit IgG (Bio-Rad), and detected using 5-bromo-4-chloro-3-indoly phosphate (BCIP) and NBT.

Immunohistochemistry: Cryostat sections 25 microns thick were prepared from brains that had been perfused with 4% paraformaldehyde and 0.15% picric acid. The sections were incubated for 48 h at 4oC in primary antibody diluted 1:2000 in PBS containing 0.3% triton X100 and 2% normal goat serum. They were then processed using an immunoperoxidase kit (Vector Laboratories) and detected with a nickel-enhanced diaminobenzidine procedure.

Cholera Toxin: : A crude membrane preparation was incubated with 10 ug of preactivated cholera toxin (List biochemicals) with 20 uM ^{32}P-NAD following standard protocols (Mickevicius et al., 1986).

Northern Blotting. RNA was extracted from rat brain regions by the lithium chloride-urea method (Auffray C. and Rougeon F. 1980). The RNA (30 ug/lane) was electrophoresed through a 1.1 % agarose gel containing 1.1 M formaldehyde. After capillary transfer to a nylon membrane (Hybond, Amersham), the RNA's were hybridized with radiolabelled oligonucleotide probe for Golf alpha subunit (3' noncoding region). Following exposure to X-ray film, the same membrane was rehybridized with the Gs alpha probe (coding region).

In situ Hybridization. Where appropriate, solutions were pretreated with 0.1% diethylpyrocarbonate (DEP) and autoclaved prior to use. Adult male Wistar rats were given an overdose of pentobarbital and perfused transcardially with 50 ml of saline followed by 300 ml of 4% paraformaldehyde in 0.1 M sodium phosphate (pH 7.4). The brain was removed and placed in phosphate buffered 15% sucrose for 48 h at 4oC. Coronal sections were cut at 25 mm thickness on a freezing microtome and were mounted onto acid-washed slides that had been double coated with chrom-alum. These were processed for *in situ* hybridization. This consisted of a 37oC incubation overnight in 4X SSC, 10X Denhardts, 10% dextran sulfate, and 10^6 cpm/mL probe (as per experiment I). The sections were washed to a final stringency of 0.5% SSC at 50oC. The slides were dried and arranged in X-ray film cassettes and exposed from 1 to 10 days. The X-AR2 film was developed with Kodak GBX for 5 min at 16oC, rinsed with water for 1 min, fixed for 10 min and washed in water.

RESULTS

Western blotting and Immunohistochemistry: The strongest RM/1 antibody tissue staining was in the striatum, nucleus accumbens, olfactory tubercle and substantia nigra (figure 6). These are structures which correspond to those with high levels of D1 binding and dopamine-stimulated adenylyl cyclase. The antibody binding pattern correlated well with the western blot analysis. Figure 7a) demonstrates the presence of Gs-like immunoreactivity in extracts of olfactory tissue (CR: cribiform plate, OB: olfactory bulb) and the striatum (CP). The major immunoreactive protein present in the striatum and substantia nigra was 45 kD although, the other regions of the brain displayed a major immunoreactive protein of 52 kD (Figure 7a). However, the relative abundance of the two bands emphasized the uniqueness of the basal ganglia with respect to the rest of the CNS.

Cholera Toxin: Similar to the western results, a dissociation was seen with regard to the ADP-ribosylation of Gs alpha in different brain regions with cholera toxin. In the cortex, the major labelled protein was again 52 kD, while in the striatum, an additional more heavily labelled 46 kD band was present (Figure 7b). This is consistent with other studies where the cholera-dependent ADP-ribosylation or immunoblotting of striatal G proteins has been noted (Mickevicius et al., 1986; Nestler et al., 1989).

In situ hybridization: Probes to the translated, 3'untranslated and 5' untranslated regions of the Golf alpha mRNA produced very heavy labelling in areas receiving dopaminergic input: the striatum, nucleus accumbens and olfactory tubercle (Figure 8b). The cortex and lateral septum labelling was faint. Indeed, the pattern of hybridization observed was the complement to that seen with the Gs alpha probe (Figure 8). The Gs mRNA labelled preferentially the areas which do not show D1 binding sites, which confirms the results of experiment 1. The bulk of the cortex, medial septum, habenula and cingulate gyrus had high Gs grain densities. Interestingly, the piriform cortex was potently labelled by Gs and all Golf probes.

Northern blot: In all regions, except striatum, the probes to Golf mRNA labelled two major bands of 3.5 and 4.1 kilobases (kb) and a minor of 3.2 (Figure 9). In contrast, in the striatum

the major Golf transcripts are 4.0 and 3.2 kb, while the 4.1 and 3.5 kb transcripts observed in other regions represent minor messages.

The relative distribution of Gs alpha transcripts in the rat brain was compared with that of Golf. A Gs message of about 2.0 kb was detected in all areas of the brain, but was least abundant in the striatum. In the latter structure, there was about ten fold more Golf than Gs transcript. The Golf transcripts detected in striatum are larger than those reported in the olfactory cilia (Jones and Reed, 1989), but are similar to those previously detected in brain (Strathman et al., 1989).

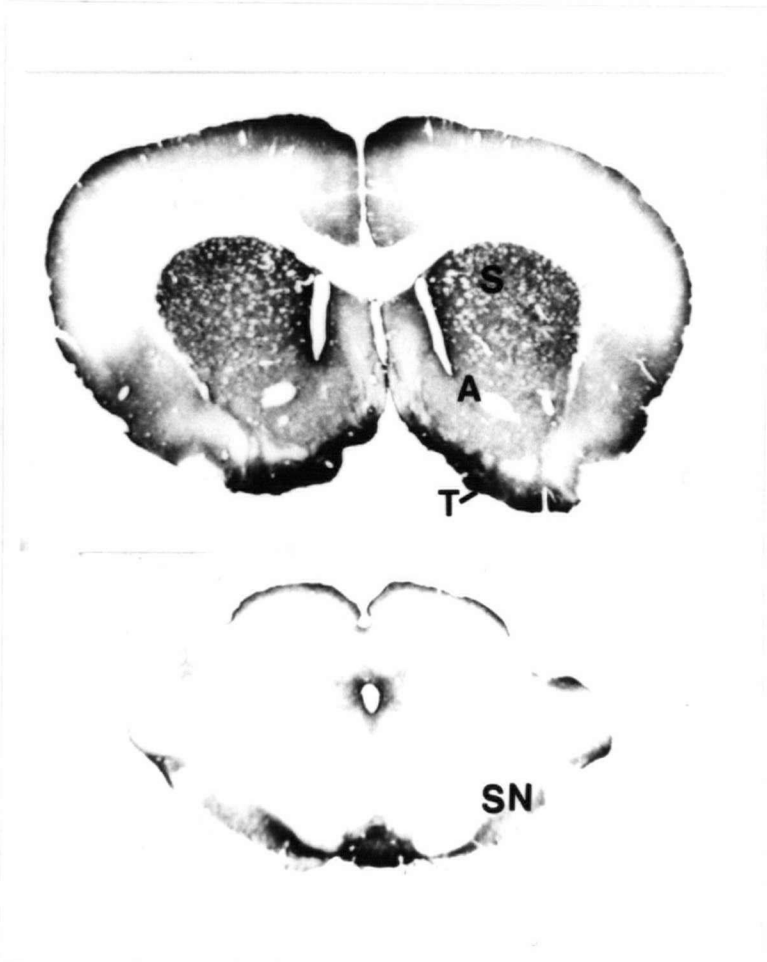
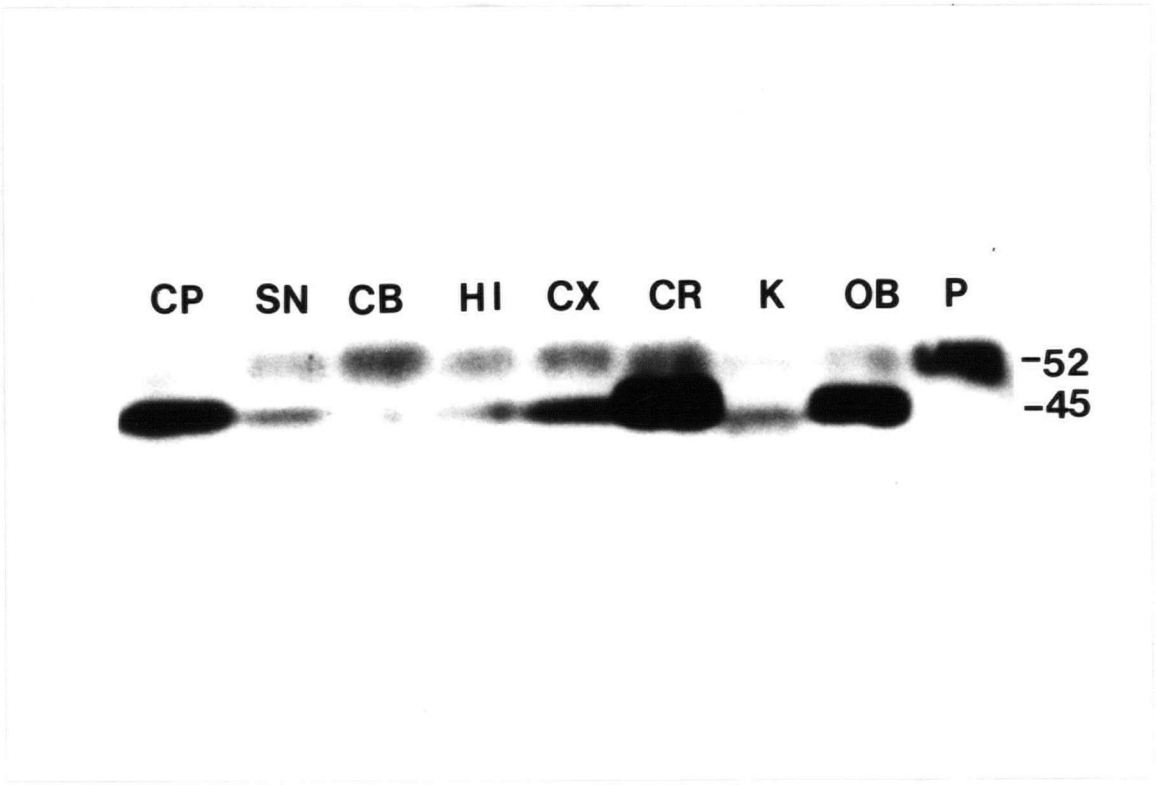


Fig. 6. Immunohistochemical localization of $G_{s\alpha}$-like immunoreactivity in the rat basal ganglia using an antibody raised against the the C-terminal decapeptide of $G_{s\alpha}$ (RM/1, New England Nuclear). This antibody also recognizes G_{olf}, which has an almost identical C-terminal sequence. Note the heavy staining in the striatum (S), nucleus accumbens (A), olfactory tubercle (T) and substantia nigra pars reticulata (SN).


CP
SN
CB
HI
CX
CR
K
OB
P
-52
-45

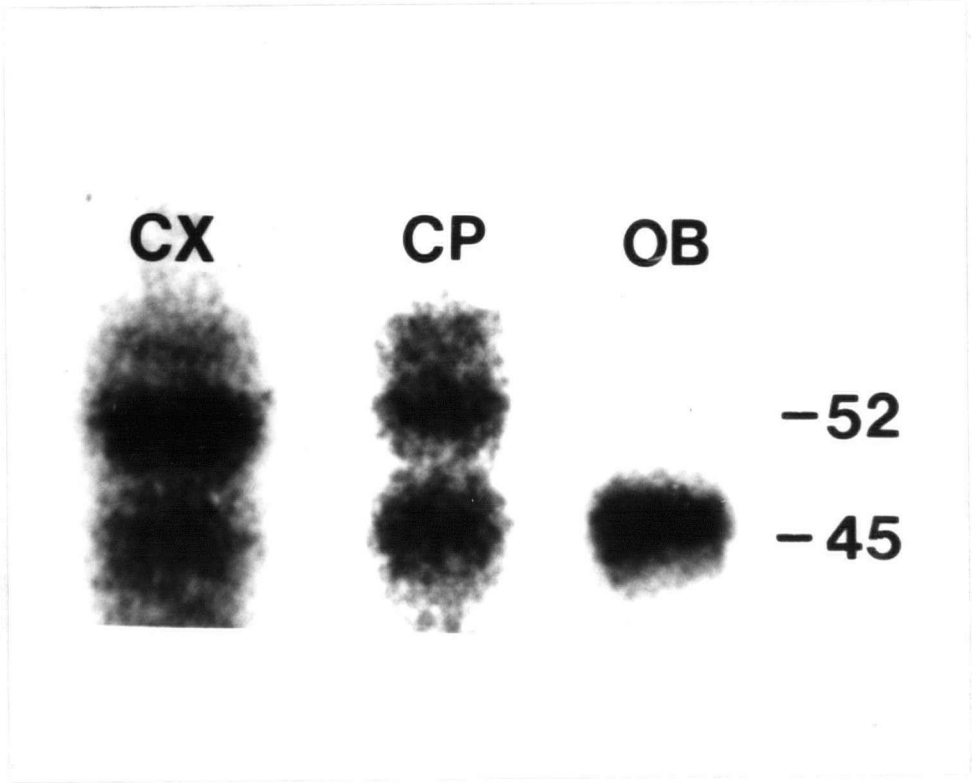

CX
CP
OB
-52
-45

Fig. 7. Characterization of $G_{s\alpha}$-like proteins in rat brain. (**A**) Immunoblot of proteins from various brain regions detected with an antiserum recognizing $G_{s\alpha}$ and G_{olf} (RM/1). Abbreviations are:(CP) striatum, (SN) substantia nigra, (CB) cerebellum, (HI) hippocampus, (CX) cortex, (CR) cribiform plate, (K) kidney, (OB) olfactory bulb, (P) pituitary. (**B**) Cholera toxin-dependent ADP-ribosylation of proteins from cortex, striatum, and olfactory bulb. Units are in kDa.

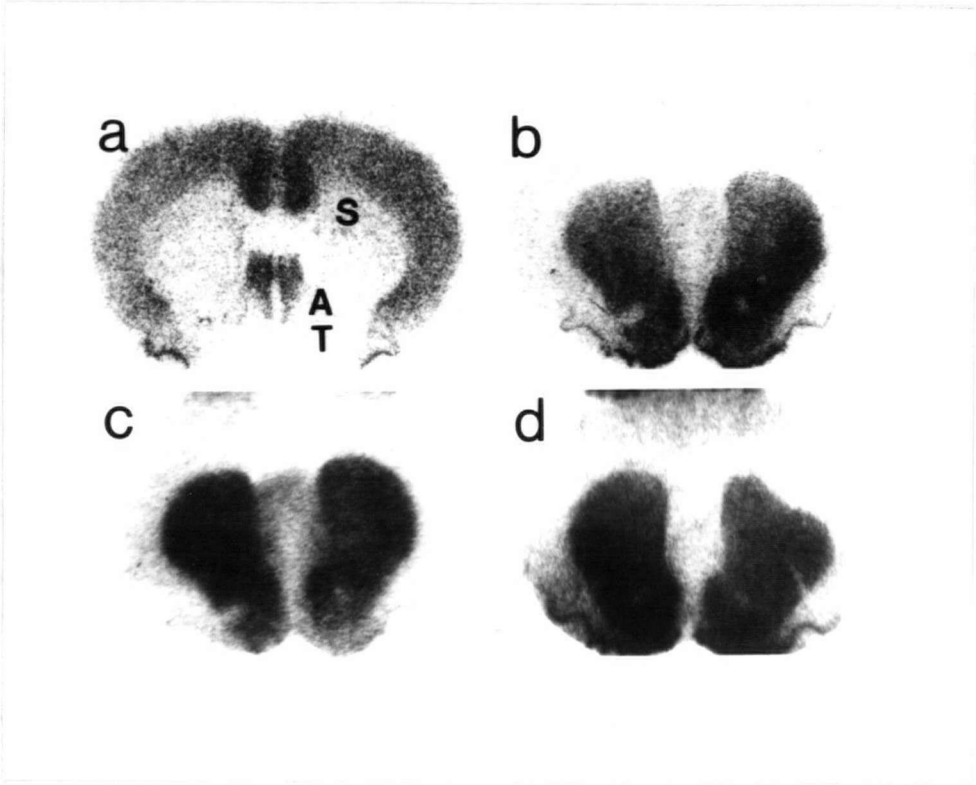


Fig. 8. *In situ* hybridization of rat forebrain using ^{32}P-labelled oligonucleotide probes for G_{sa} (**A**, 5'-TCCAGAGGTCAGGACACGGCAGCGAAGCAGGTCCTGGTC-3'), G_{olf} (**B**, 5'-AACACCAACAGACTCCGGGAGTCGCTGGACCTGTTTGAA-3'), a 3'-untranslated portion of G_{olf} (**C**, 5'-GGCTGGGGATATGGACTAGCTGTAGAGTGCTTCCCTAGC-3') and a 5'-untranslated protion of G_{olf} (**D**, 5'-CTCCGATCAGGCAAGTAGGAAGGCGCACCAGAGCCAGCG-3').

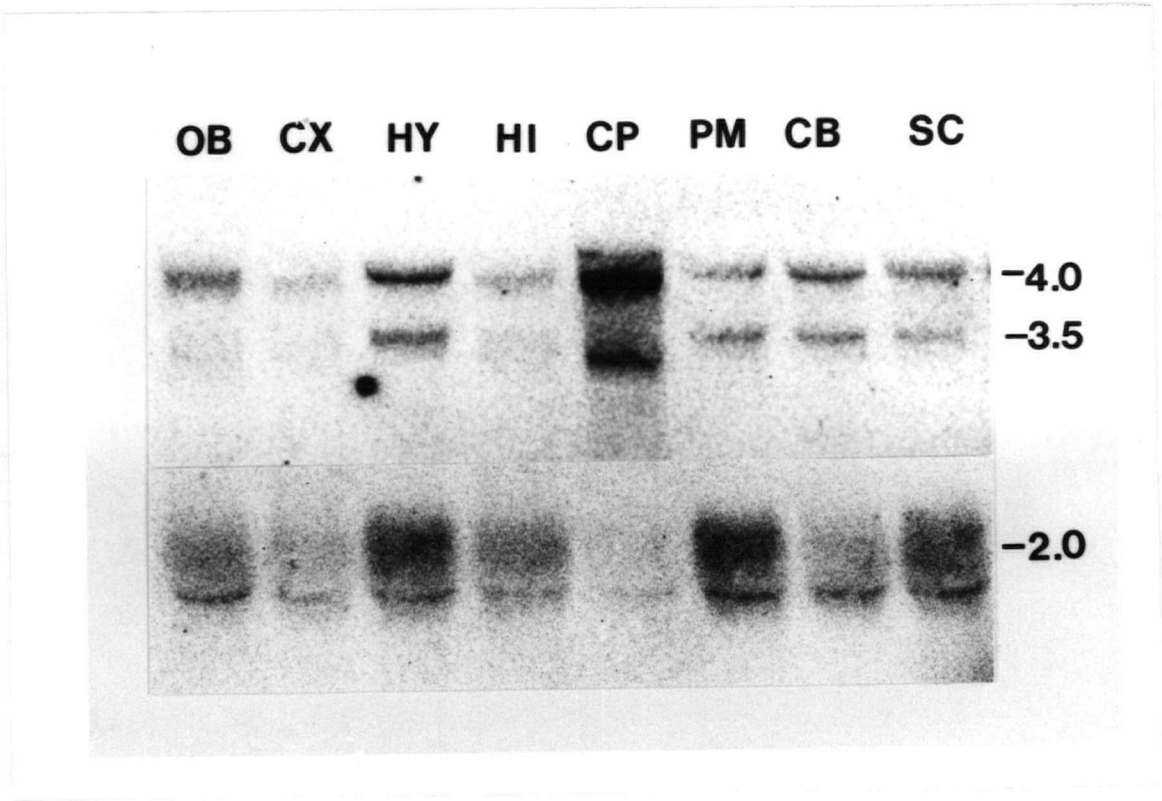


Fig. 9. Expression of Golf and Gs alpha in the rat central nervous system. RNA sizes are determined using synthetic RNA markers (BRL) and are in kilobases. (OB) olfactory bulb, (CX) cortex), (HY) hypothalamus, (HI) hippocampus, (CP) caudate putamen, (CB) cerebellum.

GENERAL DISCUSSION

Some dopamine receptors are linked to a unique striatal adenylyl cyclase which is present only in low amounts in cortex (Kebabian et al., 1972; Mollner and Pfeuffer, 1988) which is regulated separately from other cyclases in the brain. The D1 type of dopamine receptor has been assumed to couple to adenylyl cyclase via Gs (Seeman, 1987; Missale et al., 1989; Andersen et al., 1990). Given the abundance of D1 receptors, *in situ* and Northern studies would be expected to reveal a strong Gs presence in the striatum. On the contrary, there was a surprising paucity of Gs mRNA in the striatum. Paradoxically, immunohistochemistry, immunoblotting and cholera toxin ADP ribosylation clearly showed the present of a Gs alpha-like protein. We sudied this phenomenon in an attempt to clarify which G protein mRNAs were expressed in the striatum and identified a high level of expression of Golf mRNA. Striatal transcripts for Golf mRNA were approximately 10-fold more abundant than those for Gs. These results indicate that Golf mRNA is expressed in the rat brain, and is not an olfactory-specific G protein.

The D1 receptor is very similar to the beta adrenergic receptor in that they both contain conserved sequences for G protein coupling (Dreary et al.,1990; Zhou et al., 1990; Sunahara et al., 1990). When transfected into S49 cyc-kin- cells which lack endogenous Gs alpha, Golf can couple with the beta adrenergic receptor and stimulate adenylyl cyclase (Jones et al., 1989; Jones et al., 1990). Through evolution, Golf may have diverged from Gs alpha to couple olfactory receptors in olfactory cilia and D1 receptors in striatonigral neurons to adenylyl cyclase. A distinct adenylyl cyclase of 180 kD has recently been described in rat olfactory cilia (Pfeuffer et al., 1989). *In situ* hybridization of rat brain sections showed that the Golf distribution matches that of D1 binding sites. We propose that the D1 receptor type which stimulates adenylyl cyclase does so via the Golf G protein.

In addition to the dopamine receptor, the enkephalin (Abbracchio et al., 1989), substance P (Hershey and Krause., 1990), vasoactive intestinal peptide (Wang and Aghajanian, 1989) and opiate (Tirone etal., 1987) receptors are G protein-mediated and have been located in the basal

ganglia. These are clearly a potential source of G protein activity and must be taken into consideration when examining striatal transduction systems.

Harris et al. (1985) were the first to report the isolation of a partial cDNA clone for the alpha subunit of Gs. Subsequently, several laboratories (Robishaw et al., 1986; Nukada et al., 1986; Itoh et al., 1986) deduced the sequence of the entire coding region. A second form of Gs alpha was cloned by Robishaw et al (1986) that differed from the 52kD form by a 42 nucleotide deletion causing a loss of 14 amino acids and a change in the two flanking residues. It has been suggested that alternative splicing of a single gene transcript produces two small and two large forms of the protein (Kozasa et al., 1988). The Gs gene does not appear to have the potential to generate any further alternatively spliced products (Bray et al., 1986). The Gs probe used in this experiment was designed against the cholera-toxin dependent ADP ribosylation site and is specific to all four subtypes of Gs.

The Golf probes were constructed against the 5' or 3' untranslated portions of the sequence as a precaution against Gs/Golf cross reaction. Based on DNA sequence analysis, Golf has been found to be coded by an open reading frame of 381 amino acids which predicts a size of 44kD (Jones and Reed, 1989). Golf lacks the alternatively-spliced exon of Gs, and Jones and Reed have found no evidence that Golf mRNA undergoes alternative splicing.

The striatum is not devoid of Gs. Higher than background levels in northern and *in situ* hybridization studies attest to the presence of Gs in that structure. The concept of neurotransmitter receptors interacting with multiple stimulatory G proteins presents a less likely but interesting scenario. The nucleotide sequences of Gs and Golf are indeed very similar, but Jones et al. (1990) suggest that the Golf GTPase rate may be faster than that of Gs. Since the duration of the activated alpha is the timer which determines the extent of the physiological effect, Golf may be transducing a signal with different characteristics and which would extinguish more quickly than if transduced by Gs. Since at least their putative receptor binding domains are different, this has vast import to disorders which are the result of inappropriate dopaminergic tone. An increase in the proportion of Gs to Golf subunits, or a

wholesale increase in Golf expression alone could be sufficient to prolong the dopamine signal and cause an effect like that found in schizophrenic brains. This change could possibly be mediated by the predominant species of G-protein. Intrastriatal cholera injection, an example of extraordinarily potent stimulator-effector coupling, did in fact cause a behaviour syndrome similar to that produced by dopaminergic agonists (Miller et al., 1988). A revealing future study could involve double labelling tissue with Golf and Gs probes to verify colocalization.

No regulation studies on Golf mRNA have been performed, although it is known that the protein is first expressed in olfactory axons on E15 and in cillia on E16 concurrent with the appearance of action potentials (Mania-Farnel et al., 1990). The current understanding of Golf expression and function in vivo is limited at the present.

With the advent of the cloning of a type of D1 receptor (Dearry et al., 1990; Zhou etal., 1990; Sunahara et al., 1990) and a D3 receptor (Sokoloff et al.,1990) , it should be possible to compare directly the coupling of single dopamine receptor types to Gs or Golf. This will also enable testing of pharmacological agents which could potentially manipulate striatal-specific dopamine imbalances. For example, the GTP-dependent coupling of D1 receptors to a stimulatory G-protein is deficient in Huntingtons disease (DeKeyeser et al., 1989). Alloxan diabetic rats suffered overstimulation of striatal adenylyl cyclase and a significant increase in striatal met-enkephalin (Abbracchio et al., 1989). Perhaps these diseased states can help the pursuit of a better understanding of how molecular events contribute to normal and pathological CNS function. Cholera toxin probably ADP-ribosylates Arg 188 in Golf, thereby destroying the intrinsic GTPase activity of the protein, resulting in its constitutive activation. It has now been documented that point mutations of the corresponding Arg 201 in Gs alpha, results in autonomous cAMP synthesis in human growth hormone-secreting pituitary tumours (Vallar et al., 1987; Landis et al., 1989). Thus one would predict that a similar point mutation in Golf would result in constitutive agonist-independent cAMP production in both olfactory sensory cells and striatonigral neurons. It is of interest in this regard that constitutive activation of cAMP production by local cholera toxin injections of the nucleus

accumbens results in a behavioural syndrome similar to that produced by dopaminergic agonists (Miller and Kelley, 1975). Other mutations in Golf could prevent GTP-dependent, receptor-mediated activation of adenylyl cyclase, as shown for Gs alpha (Miller et al., 1988). Altered G protein function has been shown to be responsible for certain disorders. For example, compromised stimulatory G proteins have been implicated in pseudohypoparathyroidism (Weistock et al., 1986), Albrights Hereditary Dystrophy (Levine et al., 1988) and pituitary tumours (Landis et al., 1989).

Since Golf expression appears to be restricted to only a few cell types, such molecular lesions would probably not be lethal, and their possible role in the etiology of neuropsychiatric diseases should be explored. G protein-dependent activation of adenylyl cyclase has been reported to be selectively enhanced in dopamine rich regions of schizophrenic brains (Memo et al., 1983). Since Golf is abundantly expressed in just these regions, its role in this process warrants consideration. There has been a report that the GTP-dependent coupling of D1 receptors to a stimulatory G protein is deficient in Huntington's disease (DeKeyeser et al., 1989). Similarly, a functional G protein-dependent link between D1 and D2 receptors has been found to be reduced in post-mortem striatum from Huntington's disease and schizophrenia (Seeman et al., 1989).

The findings outlined above indicate that Golf is not an olfactory-specific G-protein. It is especially expressed in the forebrain regions receiving major dopaminergic innervation. This is the first report of Golf in the basal ganglia and these data support the notion that Golf is the major G-protein stimulating adenylyl cyclase in the basal ganglia.

BIBLIOGRAPHY

Abbracchio, M.P., DiLuca, M., Di Giulio, A.M., Cattabeni, F. (1989) Denervation and hyperinnervation in the nervous system of diabetic animals. *Encephalopathy*, 24:517-23.

Aghajanian, G.K. (1978) Tolerance of locus coeruleus neurons to morphine and suppression of withdrawal response by clonidine. *Nature (Lond.)*, 276:186-8.

Aghajanian, G.K., Wang, Y.-Y. (1986) Pertussis toxin blocks the outward currents evoked by opiate and a_2-agonists in locus coeruleus neurons. *Brain Res.*, 371:390-4.

Aghajanian, G.K., Wang, Y.-Y. (1987) Common a_2 and opiate effector mechanisms in the locus coeruleus: intracellular studies in brain slices. *Neuropharmacology*, 26:793-9.

Allgaier, C., Feuerstein, T.J., Jackish, R., and Hertting, G. (1985) Islet-activating protein (pertussis toxin) diminishes a_2-adrenoceptor mediated effects on noradrenaline release. *Naunyn-Schmiedebergs Arch. Pharmac.*, 331:235-9.

Andersen, P.H., Braestrup, C. (1986) Evidence for different states of the dopamine D1 receptor: clozapine and fluperlapine may preferentially label an adenylate cyclase-coupled state of the D1 receptor. *J. Neurochem.*, 47:1822-31.

Andersen, P.H., Ginrich, J.A., Bates, M.D. (1990) Dopamine receptor subtypes: Beyond the D1/D2 classification. *T.I.P.S.*, 11:231-6.

Anholt, R.R.H., Petro, A,E., Rivers, A.M. (1990) Identification of a group of novel membrane proteins unique to chemosensory cilia of olfactory receptor cells. *Biochemistry*, 29:3366-73.

Asano, T., Semba, R., Kamiya, N., Ogasawara, N., Kato, K. (1988) G_o, a GTP-binding protein: immunochemical and immunohistochemical localization in the rat. *J. Neurochem.*, 50:1164-9.

Asano, T., Semba, R., Ogasawara, N., Kato, K. (1987) Highly sensitive immunoassay for the a subunit of the GTP-binding protein G_o and its regional distribution in the bovine brain. *J. Neurochem.*, 48:1617-23.

Bean, A.J., Shepard, P.D., Bunney, B.S., Nestler, E.J., Roth, H.R. (1988) The effects of pertussis toxin on autoreceptor-mediated inhibition of dopamine synthesis in the rat striatum. *Mol. Pharmacol.*, 34:715-8.

Bouhelal, R., Smounya, L., Bockaert, J. (1988) 5-HT_{1B} receptors are negatively coupled with adenylate cyclase in rat substantia nigra. *Eur. J. Pharmacol.*, 151:189-96.

Brann, M.R., Collins, R.M., Spiegel, A. (1987) Localization of mRNAs encoding the a-subunits of signal-transducing G-proteins within rat brain and among peripheral tissues. *FEBS Lett.*, 222:191-8.

Bray, P., Carter, A., Guo, V., Puckett, C., Kamholz, J., Spiegel, A., Nirenberg, M. (1987) Human cDNA clones for an a subunit of G_i signal-transduction protein. *Proc. Natl. Acad. Sci. U.S.A.*, 84:5115-9.

Bray, P., Carter, A., Simons, C., Guo, V., Puckett, C., Kamholz, J., Spiegel, A., Nirenberg, M. (1986) Human cDNA cones for four species of G_{as} signal transduction protein. *Proc. Natl. Acad. Sci. U.S.A.*, 83:8893-7.

Bunney, B.S., Grace, A.A. (1978) Acute and chronic haloperidol treatment: comparison of effects on nigral dopaminergic cell activity. *Life Sci.*, 23:1715-28.

Bunzow, J.R., Van Tol, H.H.M., Grandy, D.K., Albert, P., Salon, J., Christie, M., Machida, C.A., Neve, K.A., Civelli, O. (1988) Cloning and expression of a rat D_2 receptor cDNA. *Nature*, 336:783-7.

Carpenter, M.B. (1989) Connectivity patterns of thalamic nuclei implicated in dyskinesia. *Stereotactic & Functional Neurosurgery*, 52:72-119.

Carpenter, M.B. (1981) V.B. Brooks ed, Handbook of physiology vol 2, Amer. Phyl Soc Bethesda Md., 947-95.

Chang, F.H., Bourne, H.R. (1987) Dexamethason increases adenynyl cyclase activity and expression of the alpha subunit in GH3 cells. *Endocr*, 121:1711-5.

Chang, K-J., Pugh, W., Blanchard, S.G., McDermed, J., Tam, J.P. (1988) Antibody specific to the a subunit of the guanine nucleotide-binding regulatory protein Go: developmental appearance and immunocytochemical localization in brain. *Proc. Natl Acad. Sci. U.S.A.*, 85:4929-33.

Cherubini, E., Williams, J.T. (1988) Nifedipine-sensitive calcium current in rat locus coeruleus neurones. *J. Physiol.*, 401:66.

Chirgwin, J.M., Przyblyal, A.E., MacDonald, R.J., Rutter, W.J. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*, 18:5294-9.

Christie, M.M., Williams, J.T., North, R.A. (1987) Cellular mechanisms of opioid tolerance: studies in single brain neurons. *Mol. Pharmacol.*, 22:633-8.

Codina, J., Olate, J., Abramowitz, J., Mattera, R., Cook, R.G., Birnbaumer, L.J. (1988) a_i-3 cDNA encodes the a subunit of G_k, the stimulatory G protein of receptor-regulated K^+ channels. *J. Biol. Chem.*, 263:6746-50.

Cortes, R., Hökfelt, T., Schalling, M., Goldstein, M. Goldsmith, P., Spiegel, A., Unson, C., Walsh, J. (1988) Antiserum raised against residues 159-168 of the guanine nucleotide-binding protein G_{i3}-a reacts with ependymal cells and some neurons in the rat brain containing cholecystokinin- or cholecystokinin- and tyrosine 3-hydroxylase-like immunoreactivities. *Proc. Natl. Acad. Sci. USA*, 85:9351-5.

Creese, I., Burt, D.R., Snyder, S.H. (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science*, 192:481-3.

Dawson, T.M., Barone, P., Sidhu, A., Wamsley, J.K., Chase, T.N. (1988) The D1 dopamine receptor in the rat brain: quantitative autoradiographic localization using an iodinated ligand. *Neuroscience*, 26:83-100.

DeKeyser, J., De Backer, J-P., Ebinger, G., Vauquelin, G. (1989) Coupling of D1 dopamine receptors to the guanine nucleotide binding protein G_s is deficient in Huntington's disease. *Brain Res.*, 496:327-30.

Dearry, A., Ginrich, J.A., Falardeau, P., Fremeau, R.T., Bates, M.D., Caron, M.G. (1990) Molecular cloning and expression of the gene for human dopamine D1 receptor. *Nature*, 347:72-6.

DeKeyeser, J., Diercks, R., Vanderheyden, R., Ebinder, G., Vauquelin, G. (1988) D1 dopamine receptors in putamen frontal cortex and calf retina. *Brain Res.*, 443:77-84.

Didsbury, J.R., Ho, Y-S., Snyderman, R. (1987) Human G_i protein a-subunit: deduction of amino acid structure from a cloned cDNA. *FEBS Lett.*, 211:160-4.

Dunlap, K., Holz, G.G., Rane, S.G. (1987) G proteins as regulators of ion channel function. *Trends Neurosci.*, 10:241-4.

Filloux, F., Dawson, T.M., Wamsley, J.K. (1988) Localization of nigrostriatal dopamine receptor subtypes and adenylate cyclase. *Brain Res. Bull.*, 20:447-59.

Gilman, A.G. (1987) G proteins: Transducers of receptor-generated signals. *Annu. Rev. Biochem.*, 56:615-50.

Gnegy, M., Treisman, G. (1981) Effect of calmodulin on dopamine-sensitive adenylate cyclase activity in rat striatal membranes. *Molec. Pharmacol.*, 19:256-63.

Gnegy, M.E., Uzunov, P., Costa, E. (1976) Regulation of dopamine stimulation of striatal adenylate cyclase by an endogenous Ca^{++}-binding protein. *Proc. Natl. Acad. Sci. USA*, 73:3887.

Goldsmith, P., Rossiter, K., Carter, A., Simonds, W., Unson, C.G., Vinitsky, R., Spiegel, A.M. (1988b) Identification of the GTP-binding protein encoded by G_{i3} complementary DNA. *J. Biol. Chem.*, 263:6476-9.

Goldsmith, P., Backlund, P.S., Jr., Rossiter, K., Carter, A., Milligan, G., Unson, C.G., Spiegel, A. (1988a) Purification of heterotrimeric GTP-binding protein from brain: identification of a novel form of G_o. *Biochem.*, 27:7085-90.

Graybiel, A.M. (1990) Neurotransmitters and neuromodulators in the basal ganglia. *T.I.N.S.*, 13:244-54.

Granneman, J.G., Bannon, M.J. (1989) Neural control of the alpha-subunit of G_s messenger ribonucleic acid in rat brown adipose tissue. *Endocrinology*, 125:2328-34.

Groves, P.M., Wilson, C.J., Young, S.J., Rebec, G.V. (1975) Self-inhibition by dopaminergic neurons. *Science*, 190:522-9.

Harris, B.A. (1988) Complete cDNA sequence of a human stimulatory GTP-binding protein alpha subunit. *Nuc. Acid Res.*, 16:3585.

Harris, B.A., Robishaw, J.D., Mumby, S.M., Gilman, A.G. (1985) Molecular cloning of complementary DNA for the alpha subunit of the G protein that stimulates adenylate cyclase. *Science*, 229:1274-7.

Harrison, J.K., Hewlett, G.H.K., Gnegy, M.E. (1989) Regulation of calmodulin-sensitive adenylate cyclase by the stimulatory G-protein, Gs. J. Biol. Chem., 264:15880-5.

Hescheler, J., Rosenthal, W., Trautwen, W., Schultz, G. (1987) The GTP-binding protein, G_0, regulates neuronal calcium channels. *Nature (Lond.)*, 325:445-7.

Huff, R. M., Axton, J.M., Neer, E.J. (1985) Physical and immunological characterization of a guanine nucleotide-binding protein purified from bovine cerebral cortex. J. Biol. Chem., 269:10864-71.

Ingeyar, R., Birnbaumer L. (1990) G-Proteins. Academic Press Inc., Toronto.

Innis, R.B., Aghajanian, G.K. (1987) Pertussis toxin blocks autoreceptor-mediated inhibition of dopaminergic neurons in rat substantia nigra. Brain Res., 411:139-43.

Itoh, H., Kozasa, T., Nagata, S., Nakamura, S., Katada, T., Ui, M., Iawi, S., Ohtuska, E., Kawasaki, H., Suzuki, K., Kaziro, Y. (1986) Molecular cloning and sequence determination of cDNAs for alpha subunits of the guanine nucleotide-binding proteins Gs, Gi and Go from rat brain. Proc. Natl. Acad. Sci. U.S.A., 83:3776-80.

Itoh, H., Toyama, R., Kozasa, T., Tsukamoto, T., Matsuoka, M., Kaziro, Y.J. (1988) Presence of three distinct molecular species of G_i protein a subunit. *Biol. Chem.*, 263: 6656-64.

Johansen, P.A., White, F.J. (1988) D1/D2 dopamine receptor interactions in the nucleus accumbens. *Society For Neurocsi. Abstr.*, 377:7.

Jones, D.T., Masters, S.B., Bourne, H.R., Reed, R.R. (1990) Biochemical characterization of three stimulatory GTP-binding proteins. The large and small forms of G_s and the olfactory-specific G-protein, G_{olf}. *J. Biol. Chem.*, 265:2671-6.

Jones, D.T., Reed, R.R. (1987) Molecular cloning of five GTP-binding protein cDNA species from rat olfactory neuroepithelium. *J. Biol. Chem.*, 262:14241-9.

Jones, D.T., Reed, R.R. (1989) G_{olf}: an olfactory neuron specific-G protein involved in odorant signal transduction. *Science*, 244:790-5.

Kanaho, Y., Stadel, J.M., Huang, C-K., Becker, E.L. (1988) Biochemical discrimination of the predominant pertussis toxin substrate of rabbit neutrophils from brain G_i and G_0: isoelectric focusing improves resolution of pertussis toxin substrates. *Biochem. Intl.*, 16:879-86.

Kebabian, J.W., Calne, D.B. (1979) Multiple receptors for dopamine [Review]. *Nature*, 277:93-6.

Kebabian, J.W., Petzold, G.L., Greengard, P. (1972) Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the "dopamine receptor" *Proc. Natl. Acad. Sci. USA*, 69:2145-9.

Kelley, E., Nahorski, S.R. (1987) Mono and divalent cations modulate the affinities of brain D1 and D2 receptors for dopamine by a mechanism independent of receptor coupling to G proteins. *Naunyn Schmeidebergs Arch Pharmacol.*, 335:508-12.

Kemp, J.M., Powel, T.M.P. (1971) The structure of the caudate nucleus of the cat. *Philos. trans. R Soc. (London)*, 262:383-401.

Kim, D., Lewis, D.L., Graziadei, L., Neer, E.J., Bar-Sagi, D., Clapham, D.E. (1989) G-protein bq-subunits activate the cardiac muscarinic K^+-channel via phospholipase A_2. *Nature*, 337:557-60.

Lacey, M.G., Mercuri, N.B., North, R.A. (1988) On the potassium conductance increase activated by $GABA_B$ and dopamine D_2 receptors in rat substantia nigra neurones. *J. Physiol.*, 401:437-53.

Landis, C.A., Masters, S.B., Spada, A., Pace, A.M., Bourne, H.R., Vallar, L. (1989) GTPase inhibiting mutations activate the alpha chain of G_s and stimulate adenylyl cyclase in human pituitary tumours. *Nature* 340:692-6.

Langer, S.Z., Pimoule, C.Z., Reynolds, G.P., Shoemaker, H. (1986) Dopaminergic denervation does not affect SCH23390 binding in rat striatum. *Br. J. Pharmacol.*, 87:161.

Largent, B.L., Jones, D.T., Reed, R.R., Pearson, R.C.A., Snyder, S.H. (1988) G protein mRNA mapped in rat brain by in situ hybridization. *Proc. Natl. Acad. Sci. USA*, 85:2864-8.

Lewis, M.E., Krause, R.G. II, Roberts-Lewis, J.M. (1988) Recent developments in the use of synthetic oligonucleotides for in situ hybridization histochemistry. *Synapse*, 2:308-16.

Lochrie, M.A., Simon, M.I. (1988) G protein multiplicity in eukaryotic signal transduction systems. *Biochemistry*, 27:4957-65.

Mahan, L.C., Burch, R.M., Monsma, F.J. Jr., Sibley, D.R. (1990) Expression of striatal D1 dopamine receptors coupled to inositol phosphate production and Ca2+ mobilization in Xenopus oocytes. Proc. Natl. Acad. Sci. USA, 87:2196-200.

Mailman, R.B. (1986) Breese, G.R., Creese, I. eds., Neurobiology of Central D1 Receptors. Plenum Press, 53-72.

Mania-Farnell, B., Farbman, A.I. (1990) Immunohistochemical localization of guanine nucleotide binding proteins in rat olfactory epithelium during development. *Developmental Brain Research*, 51:103-12.

Marchetti, C., Carbone, E., Lux, H.D. (1986) Effects of dopamine and noradrenaline on Ca channels of cultured sensory and sympathetic neurons of chick. *Pfluegers Arch.*, 406:104-11.

Masters, S.B., Sullivan, K.A., Beiderman, B., Lopez, N.G., Ramachandran, J., Bourne, H.R. (1988) The carboxy terminal domain of G_s alpha specifies the coupling of receptors to the stimulation of adenylyl cyclase. *Science*, 241:448-51.

Masters, S.B., Stroud, R.M., Bourne, H.R. (1986) A family of G protein alpha chains. *Prot. Eng*, 1:47.

Mattera, R., Graziano, M.P., Yatani, A., Zhou, Z., Graf, R., Codina, J., Birnbaumer, L., Gilman, A.G., Brown, A.M. (1989) Splice variants of the alpha subunit of the G protein G_s activate both adenyl cyclase and calcium channels. *Science*, 243:804-7.

Memo, M., Lovenberg, W., Hanbauer, I. (1982) Agonist-induced subsensitivity of adenylate cyclase coupled with a dopamine receptor in slices from rat corpus striatum. *Proc. Natl. Acad. Sci. USA*, 79:4456-60.

Memo, M., Kleinman, J.E., Hanbauer, I. (1983) Coupling of dopamine D1 recognition sites with adenylate cyclase in nuclei accumbens and caudatus of schizophrenics. *Science*, 221:1304-7.

Mengod, G., Palacios, J.M., Probst, A., Harris, B. (1988) Regional distribution of the expression of a human stimulatory GTP-binding protein a-subunit in the human brain studied by in situ hybridization. Mol. Brain Res., 4:23-29.

Mickevicius, C.K., Harrison, J.K., Gnegy, M.E. (1986) Effect of cholera toxin on the activation of adenylate cyclase by calmodulin in bovine striatum. *Molec. Pharmacol.*, 30:469-75.

Miller, R.J., Kelly, P.H. (1975) Dopamine-like effects of cholera toxin in the central nervous system. *Nature*, 235:163.

Miller, R.T., Masters, S.B., Sullivan, K.A., Beiderman, B., Bourne, H.R. (1988) A mutation that prevents GTP-dependent activation of the alpha chain of G_s. *Nature*, 334:712-5.

Missale, C., Nisoli, E., Liberini, P., Memo, M., Bironamici, M., Rossi, A. (1989) Repeated reserpine administration upregulates the transduction mechanisms of D1 receptors without changing density of SCH23390 binding. *Brain Res.*, 483:117-22.

Mochly-Rosen, D., Chang, F.H., Cheever, L., Kim, M., Diamond, L., Gordon, A.S. (1988) Chronic ethanol causes heterologous desensitization of receptor by reducing alpha s mRNA. *Nature*, 333:848-50.

Neer, E.J., Clapham, D.E. (1988) Roles of G protein subunits in transmembrane signalling. *Nature*, 333:129-34.

Nestler, E.J., Erdos, J.J., Terwilliger, R., Duman, R.S., Tallman, J.F. (1989) Regulation of G proteins by chronic morphine in the rat locus coeruleus. *Brain Res.*, 476:230-9.

North, R.A., Williams, J.T., Suprenant, A., Christie, M.J. (1987) m and w receptors belong to a family of receptors that are coupled to potassium channels. *Proc. Natl. Acad. Sci. USA*, 84:5487-91.

Nukada, T., Mishima N. Numa S. (1987) Functional expression of cDNA encoding the alpha subunit of adenylyl cyclase stimulating G protein. *J. Biol. Chem.*, 263:10394-400.

Nukada, T., Tanabe, T., Takahashi, H., Noda, M., Haga, K., Haga, T., Ichiyama, A., Kangawa, K., Hiranaga, M., Matsuo, H., Numa, S. (1986) Primary structure of the a-subunit of bovine adenylate cyclase-inhibiting G-protein deduced from the cDNA sequence. *FEBS Lett.*, 197:305-10.

Ohara, K., Haga, K., Berstein, G., Haga, T., Ichiyama, A., Ohara, K. (1988) The interaction between D-2 dopamine receptors and GTP-binding proteins. *Molec. Pharmacol.*, 33:290-6.

Okada, F., Tokumistsu, Y., Ui, M. (1988a) Desensitization of b-adrenergic receptor-coupled adenylate cyclase in cerebral cortex after in vivo treatment of rats with desipramine. *J. Neurochem.*, 47:454-9.

Okada, F., Tokumistsu, Y., Ui, M. (1988b) Possible involvement of pertussis toxin substrates (Gi, Go) in desipramine-induced refractoriness of adenylate cyclase in cerebral cortices of rats. *J. Neurochem.*, 51:194-9.

Pace, U., Lancet, D. (1986) Olfactory GTP-binding protein: signal-transducing polypeptide of vertebrate chemosensory neurons. *Proc. Natl. Acad. Sci. USA*, 83:4947-51.

Parent, A. (1986) Comparative neurobiology of the basal ganglia. Wiley and Sons in Toronto, 199-226.

Pfeuffer, E., Mollner, S., Lancet, D., Pfeuffer, T. (1989) Olfactory adenylyl cyclase. Identification and purification of a novel enzyme form. *J. Biol. Chem.*, 264:18803-7.

Rall, T., Harris, B.A. (1987) Identification of the lesion in the stimulatory GTP binding protein of the uncoupled S49 lymphoma. *FEBS Lett*, 244:365-71.

Roberts-Lewis, J., Rosebloom, P.H., Iwaniel, L., Gnegy, M.E. (1986) Differential down-regulation of D1 stimulates adenylyl cyclase activity in rat forebrain after in vivo amphetamine treatment. *J. Neurosci.*, 6:2245-51.

Robishaw, J.D., Smigel, M.D., Gilman, A.G. (1986). *J. Biol. Chem.*, 261:9587.

Rosenthal, W., Hescheler, J., Trautwein, W., Schultz, G. (1988) Control of voltage-dependent Ca^{2+} channels by G protein-coupled receptors. *FASEB J.*, 2:2784-90.

Sakai, Y., Sekiguchi, M., Okamoto, K., Kuwano, R., Takahashi, Y. (1990) Dopamine receptors expressed in the Xenopus oocytes injected with bovine striatal mRNA. *Molec. Brain Res.*, 7:183-7.

Schambron, D.L., Joyce J. N. Molinoff P.B. (1987) Differential regulation of dopamine receptors in caudate putamen and nicleus accumbens following chronic admin of reserpine. *Soc. Neurosci. Abstr*, 13:199.

Schoeffter, P., Waeber, C., Palacios, J.M., Hoyer, D. (1988) The 5-hydroxytryptamine 5-HT_{1D} receptor subtype is negatively coupled to adenylate cyclase in calf substantia nigra. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 337:602-8.

Seeman. P. (1987) Dopamine receptors and the dopamine hypothesis of schizophrenia. *Synapse*, 1:133-52.

Seeman, P., Lee, t., Chau-Wong, M., Wong, K. (1976) Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature*, 261:717-9.

Seeman, P., Niznik, H.B., Guan, H-C., Booth, G., Ulpian, C. (1989) Link between D1 and D2 dopamine receptors is reduced in schizophrenia and huntington diseased brain. *Proc. Natl. Acad. Sci. USA*, 86:10156.

Simonds, W.F., Goldsmith, P.K., Woodard, C.J., Unson, C.G. Spiegel, A.M. (1989) Receptor and effector interactions of G_s. Functional studies with antibodies to the alpha s carboxl-terminal decapeptide. *FEBS Lett.*, 249:189-94.

Simson, P.E., Cierpial, M.A., Heynaman, L.E., Weiss, J.M. (1988) Pertussis toxin blocks the effects of a_2-agonists and antagonists on locus coeruleus activity in vivo. *Neurosci. Lett.*, 89:361-6.

Snyder, S. H. (1990) The dopamine connection. *Nature*, 347:121-2.

Sokkolof, P., Giros, B., Martres, M.P., Bouthene, M.L., Schartz, J.C. (1990) Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature*, 347:146-51.

Sternweis, P.C., Robishaw, J.D. (1984) Isolation of two proteins with high affinity for guanine nucleotides from membranes of bovine brain. *J. Biol. Chem.*, 259:13806-13.

Strathmann, M., Wilkie, T.M., Simon, M.I. (1989) Diversity of the G-protein family: sequences from five additional alpha subunits in the mouse. *Proc. Natl. Acad. Sci. USA*, 86:7407.

Sunahara, R.K., Niznik, H.B., Weiner, D.W., Stormann, T.M., Brann, M.R., Kennedy, J.L., Gelernter, J.E., Rozmahel, R., Yang, Y., Israel, Y., Seeman, P., O'Dowd, B.F. (1990) Human dopamine D1 receptor encoded by an introless gene on a chromosome5. Nature, 347:80-83.

Svensson, T., Bunney, B.S., Aghajanian, G.K. (1975) Inhibition of both noradrenergic and serotonergic neurons in brain by the a-adrenergic agonist clonidine. *Brain Res.*, 92:291-8.

Terashima, T., Katada, T., Oinuma, M., Inoue, Y., Ui, M. (1988) Immunohistochemical analysis of the localization of guanine nucleotide-binding protein in the mouse brain. *Brain Res.*, 442:305-11.

Tirone, F., Vigano, A., Gropetti, A., Parenti, M. (1987) Effects Of The Desensitization by Morphine of The Opiate-Dependant Adenylate Cyclase System In The Rat Striatum. Bioch. Pharmacol. 37:1039-44.

Vallar, L., Spada, A., Giannattasio, G. (1987) Altered G_s and adenylate cyclase activity in human GH-secreting piyuitary adenomas. *Nature*, 330:566-8.

VanDongen, A.M.J., Codina, J., Olate, J., Mattera, R., Joho, R., Birnbaumer, L., Brown, A.M. (1988) Newly identified brain potassium channels gated by the guanine nucleotide binding protein G_o. *Science*, 242:1433-7.

Vincent, S.R. (1988) Distributions of tyrosine hydroxylase, dopamine-b-hydroxylase, and phenylethanolamine-N-methyltransferase-immunoreactive neurons in the brain of the hamster (*mesocricetus auratus*) *J. Comp. Neurol.*, 268:584-99.

Vincent, S.R., Hope, B.T., Drinnan, S.L., Reiner, P.B. (1990) G protein mRNA expression in immunohistochemically identified dopaminergic and noradrenergic neurons in the rat brain. *Synapse*, 6:23-32.

Wang, YY., Aghajanian, G.K. (1987a) Intracellular GTPqS restores the ability of morphine to hyperpolarize rat locus coeruleus neurons after blockade by pertussis toxin. *Brain Res.*, 436:396-401.

Wang, YY., Aghajanian, G.K. (1987b) Excitation of locus coeruleus neurons by an adenosine 3',5'-cyclic monophosphate-activated inward current: extracellular and intracellular studies in rat brain slices. *Synapse*, 1:481-7.

Wang, YY., Aghajanian, G.K. (1988) VIP excitation of locus coeruleus neurons: evidence for a G-protein-mediated inward current. *Neurosci. Abstr.*, 14:147.

Williams, J.T., North, R.A. (1985) Catecholamine inhibition of calcium action potentials in rat locus coeruleus neurones. *Neurosci. 14*:103-9.

Worley, P.F., Baraban, J.M., Van Dop, C., Neer, E.J., Snyder, S.H. (1986) Go, a guanine nucleotide-binding protein: immunohistochemical localization in rat brain resembles distribution of second messenger systems. *Proc. Natl. Acad. Sci. USA*, 83:4561-5.

Yatani, A., Imoto, Y., Codina, J., Hamilton, S.L., Brown, A.M., Birnbaumer, L. (1988a) The stimulatory G protein of adenylyl cyclase, G_s, also stimulates dihydropyridine-sensitive Ca^{2+} channels. *J. Biol. Chem.*, 263:9887-95.

Yatani, A., Mattera, R., Codina, J., Graf, R., Okabe, K., Padrell, E., Iyengar, R., Brown, A.M., Birnbaumer, L. (1988b) The G protein-gated atrial K^+ channel is stimulated by three distinct G_ia-subunits. *Nature*, 336:680-3.

Zhou, Q.Y., Grandy, D.K., Thambi, L., Kushner, J.A., VanTol H.H.M., Cone, R., Pribnow, D., Salon, J., Buznow, J., Civelli, O. (1990) Cloning and expression of human and rat D1 dopamine receptors. *Nature*, 347:76-9.