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PRINCIPAL INVESTIGATOR: Yoland Smith, Ph.D.

CONTRACTING ORGANIZATION: Emory University
Atlanta, GA 30322

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14. ABSTRACT

The goal of the work presented in this report was to understand the localization and function of kainate receptors (KARs) in the basal ganglia, a group of brain structures affected in Huntington's and Parkinson's diseases. KARs are part of a family called ionotropic glutamate receptors. Using specific antibodies and glutamate-related compounds, we have achieved successfully a series of studies of the localization and function of these receptors in the striatum and globus pallidus, two key structures of the basal ganglia circuitry. Our findings reveal three main features about KARs: (1) They have a pattern of distribution strikingly different from other ionotropic glutamate receptors, (2) They mediate slow and long-lasting excitation and (3) They are often expressed pre-synaptically where they act as auto- or heteroreceptors to modulate glutamatergic or GABAergic transmission. These findings pave the way for a better understanding of KARs function in the basal ganglia and provide novel targets for therapeutic approaches in movement disorders.

15. SUBJECT TERMS
Glutamate, Parkinson's disease, Huntington's disease, Excitotoxicity, drug development striatum, globus pallidus.

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INTRODUCTION

The basal ganglia are the main targets of various neurodegenerative diseases including Parkinson's and Huntington's diseases. Parkinson's disease is characterized by the progressive death of midbrain dopaminergic neurons in the substantia nigra pars compacta (SNc) while the main pathological feature of Huntington's chorea is the degeneration of striatal projection neurons. Although the etiology of sporadic Parkinson's disease remains unknown, Huntington's disease is the result of an unstable expansion of CAG (trinucleotide) repeats on the gene which encode the protein "Huntingtin" on chromosome 4. The loss of specific neuronal populations in each of these diseases results in dramatic changes in the functional circuitry of the basal ganglia, which leads to an abnormal regulation of thalamocortical activity, thereby, changes in cortical excitability and abnormal control of motor behaviors. Over the past fifteen years, the pathophysiology of Parkinson's disease has been studied in great detail and it is now clear that abnormal glutamatergic transmission at various levels of the basal ganglia circuitry underlies some of the behavioral abnormalities that characterize this disease. Although glutamate is an essential excitatory transmitter in the brain, it can also be harmful if not regulated properly. In fact, glutamate excitotoxicity is involved in cell death of most brain diseases including Parkinson's and Huntington's diseases. For these obvious reasons, the development of drug therapies that could reduce glutamatergic transmission without altering normal brain functions has been the topic of research for many laboratories and pharmaceutical industries. However, data obtained so far have been disappointing since most of the drugs that have been used result in significant detrimental side effects, which limit considerably their clinical relevance for human therapy. Until now, the focus has largely been devoted towards the use of drugs that target the two major members of the ionotropic glutamate receptors family, AMPA and NMDA receptors. These two receptors are found throughout the whole brain and mediate fast excitatory neurotransmission. It is, therefore, not surprising that antagonists for these receptors have detrimental effects on normal brain functioning. Another group of ionotropic glutamate receptors that has been much less characterized are the kainate receptors. Although kainate receptors have long been known, their localization and function remain poorly understood because of the lack of specific tools to differentiate them from AMPA receptors. However, the recent development of specific antibodies and selective AMPA receptor antagonists allowed various groups to characterize in more detail the unique features of kainate receptors in the hippocampus. These findings demonstrate that the physiology and localization of these receptors in the hippocampal network are strikingly different from those of AMPA or NMDA receptors. Kainate receptor subunits are widely distributed in the basal ganglia, but very little is known about their synaptic localization and function. Interestingly, subsets of early onset Huntington's disease patients that cannot be explained by a large number of CAG repeats were found to have mutations in the gene that encodes for the GluR6 subunit of the kainate receptor, suggesting the potential involvement of these receptors in Huntington's pathogenesis. In order to further understand the potential role of kainate receptors in the basal ganglia, we completed a series of immunocytochemical and electrophysiological experiments to elucidate the subcellular and subsynaptic localization of kainate receptor subunits and characterize the regulatory mechanisms of kainate receptors activation on synaptic transmission in the striatum and globus pallidus, two key structures in the functional circuitry of the basal ganglia.

BODY

The work that has been achieved in our laboratory over the past funding period can be presented under the following two main topics:

- **Subcellular and subsynaptic localization of kainate receptors in the monkey striatum.**

The main goal of this study was to characterize the subcellular and subsynaptic localization of kaniate receptor subunits GluR6/7 and KA2 in the monkey striatum. This study was achieved using high resolution electron microscopic immunocytochemical procedures combined with anterograde transport techniques. Results of these experiments have been published in The Journal of Neuroscience and presented in various reviews and abstracts (see Reportable Outcomes section). The main findings and their relevance for Huntington's disease pathogenesis are as follows:

(1) The relative abundance of glutamatergic terminals immunoreactive for kainate receptor subunits does not vary throughout the striatum despite the fact that some striatal regions are more sensitive than others to degeneration in Huntington's disease.
   - ❖ *A differential degree of striatal neurodegeneration has been shown in Huntington's disease (Vonsattel and DiFiglia, 1998). For instance, the nucleus accumbens is the least sensitive striatal region whereas the tail of the caudate nucleus is the most affected part of the striatum in the brains of Huntington patients. Assuming that pre-synaptic kainate receptors might be involved in striatal neurodegeneration, we hypothesized that this cell death variability might be due to a differential expression of pre-synaptic kainate receptors among striatal territories. However, our data show that such is not the case, which suggests that the degree of striatal cell death observed in Huntington's disease is not merely the result of a larger number of glutamatergic terminals that express kainate receptors, but likely involves more complex changes in the functional and pharmacological properties of these receptors.*

(2) Pre- and postsynaptic GluR6/7 and KA2 immunoreactivity is largely expressed intracellularly under basal conditions.

(3) Most of the membrane-bound labelling for GLUR6/7 and KA2 is expressed extrasynaptically though synaptic and perisynaptic labelling of glutamatergic synapses is also seen.

(4) In immunoreactive terminals, GluR6/7 and KA2 labelling is associated with the membrane of vesicular structures which are randomly distributed relative to the pre-synaptic grid of asymmetric synapses.
   - ❖ *The latter three sets of data are interesting and are consistent with the known physiology of kainate receptors observed in other brain regions. In brief, kainate responses are usually slow and necessitate tetanic stimulation of pre-synaptic afferents to be induced (Lerma et al., 1997; Kamiya, 2002). These functional effects resemble much more responses generated by metabotropic glutamate receptors than other ionotropic receptors such as NMDA and AMPA (Anwyl, 1999; Lerma et al., 1997). The fact that these receptors are either intracellular or largely extrasynaptic, a pattern reminiscent of group I mGluRs in the monkey striatum, raises interesting questions regarding their trafficking, synaptic targeting and mechanisms of activation. Regarding Huntington's disease, an interesting possibility could be that the mutation of the GluR6 gene in HD patients alters the trafficking of this subunit, thereby affects the subsynaptic localization of kainate receptors, which eventually leads to an excessive activation of presynaptic receptors and overflow of extracellular glutamate.*

(5) More than half of cortical and thalamic inputs from the primary motor cortex and the centromedian nucleus, respectively, express GluR6/7 and KA2 immunoreactivity in the postcommissural putamen.
   - ❖ *Although the cerebral cortex provides the most massive glutamatergic input to the striatum, we and others have shown that the intralaminar thalamic*

nuclei also contribute substantially to this innervation (Sadikot et al., 1992; Sidibe and Smith, 1996). Here, we demonstrate that both cortical and thalamic afferents express pre-synaptic kainate receptors, which means that an altered regulation of these receptors in HD, due to the mutation of the GluR6 subunit gene, may affect not only the glutamatergic transmission at corticostriatal synapses but also the thalamic influences upon striatal neurons.

- **Localization and Function of Kainate Receptors in the Globus Pallidus**

The functional interactions between the globus pallidus and the subthalamic nucleus are considered as the "pacemaker" of the basal ganglia circuitry (Plenz and Kitai, 1999). The lack of knowledge of kainate receptor functions in these brain regions combined with the fact that glutamate receptor antagonists have beneficial effects in Parkinson's disease (Starr, 1995), most likely through modulation of the overactive excitatory synaptic transmission at subthalamopallidal synapses, set the stage for a deeper understanding of kainate receptor functions in the globus pallidus. In the following account, we will summarize the main findings obtained through this series of studies over the past few years and briefly discuss their relevance to better understand the substrate that underlies synaptic transmission in the basal ganglia circuitry and the development of new therapeutic approaches for basal ganglia diseases.

1. *Localization of GluR6/7 kainate receptor subunits in the monkey globus pallidus*

During the course of the striatal studies described above, we noticed that other basal ganglia nuclei, including both segments of the globus pallidus, were enriched in GluR6/7 immunoreactivity. At the light microscopic level, neuronal cell bodies and the neuropil of the internal (GPi) and external (GPe) pallidum displayed strong immunolabelling. In order to better characterize the exact localization of kainate receptors in the monkey pallidum, we undertook an electron microscopic immunoperoxidase study of GluR6/7 immunoreactivity in the GPe and GPi of rhesus monkeys. Results of this study have been published in Neuroscience and were presented in an abstract form at the Society for Neuroscience in 2003 (see Reportable Outcomes). The main findings of this study is that kainate receptors are strongly expressed pre- and post-synaptically in both pallidal segments. At the pre-synaptic level, both GABAergic and non-GABAergic terminals display GluR6/7 immunoreactivity, which suggest that pre-synaptic kainate receptors can act as auto- or heteroreceptors in the monkey pallidum. The importance of these data regarding basal ganglia pathophysiology of various movement disorders including HD is the potential development of novel drugs that could selectively target GluR6-containing kainate receptors and modulate glutamatergic and GABAergic neurotransmission in GPe and GPi. It is still premature to speculate about a particular treatment strategy at this point without knowing the exact role pre-synaptic kainate receptors play in this system. We, therefore, achieved a series of electrophysiological studies in rat brain slices to elucidate the effects of kainate receptor activation on glutamatergic and GABAergic neurotransmission.

2. *Localization and Functions of Kainate Receptors in the Rat Globus Pallidus*

To make sure that the pattern of subcellular localization of kainate receptors described in monkeys is valid in rats, we carried out an electron microscopic analysis of GluR6/7 immunoreactivity in the rat globus pallidus. The main features of kainate receptor distribution in monkey GPe and GPi are seen in the rodent GP, ie there is heavy GluR6/7 postsynaptic labeling associated with proximal and distal dendritic shafts as well as pre-synaptic labeling in putative GABAergic and glutamatergic axon terminals.

We then tested the role of postsynaptic kainate receptor activation on GP neuronal activity in rat brain slices using whole cell patch clamp recording technique. Results of this study have been published in book chapters and abstracts and a peer-reviewed manuscript is currently under revision for publication in European Journal of Neuroscience (see Reportable Outcomes). In brief, the main results of this study can be summarized as follows:

Functional kainate receptors are expressed on GP neurons. This was demonstrated as follows: (1) bath application of low concentration of kainate evoked inward currents in GP neurons in the presence of AMPA and NMDA receptor antagonists and (2) low concentration of kainate depolarizes GP neurons in the presence of other glutamate receptor antagonists.

Post-synaptic kainate receptors are activated by synaptically released glutamate in the GP: To reach this goal, evoked excitatory post - synaptic currents (EPSCs) were recorded from GP neurons by stimulating the internal capsule (IC) with a bipolar tungsten electrode. Based on previous studies from our laboratory and others, stimulation of the internal capsule medial and ventral to the GP is the most efficient approach to impale ascending glutamatergic projections from the subthalamic nucleus. Although additional minor glutamatergic inputs from the thalamus, cortex and brainstem may have also been recruited by these stimulations, it is well established that the bulk of glutamatergic innervation to the GP arises from the STN. Findings of these experiments demonstrate that stimulation of the internal capsule can elicit a small synaptic component that is resistant to AMPA receptor antagonist, but sensitive to AMPA/KARs antagonist, suggesting that this component is not mediated by AMPA receptors activation. This small EPSC has slower activation and deactivation kinetics than those mediated by AMPA receptors, a typical feature for KARs in other brain regions (Huettner, 2003).

Pre-synaptic kainate receptors activation reduces AMPA and NMDA-mediated EPSCs in the rat GP. We demonstrated that averaged, stimulus-evoked AMPA and NMDA-mediated EPSCs are depressed by 1 μM KA in the rat GP. Furthermore, we provided evidence that this effect is mediated by pre-synaptic mechanisms since kainate application increases paired-pulse facilitation ratio. These findings demonstrate that kainate receptors can act as functional heteroreceptors that modulate glutamatergic transmission at the subthalamopallidal synapse. Knowing the importance of this system in Parkinson's disease pathophysiology, the relevance of KARs as potential targets for future drug therapies in Parkinson's disease should be considered.

Pre-synaptic kainate receptor-mediated effects do not involve activation of other G-protein-coupled receptors: One of the particular features of pre-synaptic KARs-mediated effects that have been shown in the hippocampus is the indirect involvement of G-protein-coupled receptors (Huettner, 2003). To determine whether such receptors were also involved in mediating pre-synaptic KARs effects in the GP, we bath applied various G-protein-coupled receptor antagonists prior to kainate application and measured the impact of these drugs on the kainate-induced inhibition of AMPA EPSCs in GP neurons. We demonstrate that the pre-synaptic functions of KARs on AMPA-mediated EPSCs are not affected by the presence of various G protein-coupled receptor antagonists ruling out the involvement of these receptors in the KAR-mediated pre-synaptic effects in the rat GP.

Together, these findings demonstrate the unique features of kainate receptors localization and function in the rat and monkey striatopallidal complex. The modulatory postsynaptic effect of these receptors combined with their pre-synaptic function provide them unique features essential for the development of future drug therapies aimed at regulating glutamatergic and GABAergic transmission in basal ganglia diseases.

- ***Future Experiments***

1. To test the effects of KARs activation on inhibitory synaptic transmission in the rat GP.

KEY RESEARCH ACCOMPLISHMENTS

The main findings obtained in this project are summarized as follows:

- Kainate receptors are widely expressed pre- and post-synaptically in the striatum and globus pallidus
- In the striatum, post-synaptic kainate receptors are largely extrasynaptic or intracellular, a pattern of subsynapic distribution strikingly different from other ionotropic glutamate receptors which are largely confined to glutamatergic synapses
- Pre-synaptic kainate receptors are expressed in both cortical and thalamic glutamatergic inputs to the striatum.
- Pre- and post-synaptic kainate receptors are strongly expressed in the monkey and rat globus pallidus. Pre-synaptic receptors are associated with both GABAergic and glutamatergic terminals suggesting that their activation may lead to auto- or hetero-regulation of neurotransmitters release.
- Kainate receptor activation evokes EPSC's that are insensitive to AMPA or NMDA receptor antagonists in rat GP neurons, which suggest the functional expression of postsynaptic kainate receptors in pallidal neurons.
- Kainate receptors activation depolarizes GP neurons in the presence of TTX and AMPA/NMDA receptor antagonists.The importance of these data regarding basal ganglia pathophysiology of various movement disorders including HD is the potential development of novel drugs that could selectively target GluR6-containing kainate receptors and modulate glutamatergic and GABAergic neurotransmission in the globus pallidus.
- Kainate receptors activation elicits a non-AMPA/non-NMDA small synaptic component that has slower deactivation kinetics than AMPA receptors. These data provide evidence for synaptically activated post-synaptic KARs in GP neurons.
- Kainate receptors activation reduces excitatory synaptic transmission through pre-synaptic mechanisms in the rat GP. These findings provide novel targets whereby kainate receptor agonists may modulate glutamatergic transmission from the overactive STN-GP synapse in Parkinson's disease.
- The pre-synaptic effects of KARs on glutamatergic transmission are not affected by the blockade of various G protein-coupled receptors in the rat GP. This demonstrates a high degree of specificity in KAR-mediated pre-synaptic effects on glutamate release in the GP.

REPORTABLE OUTCOMES

Peer-reviewed manuscripts

- Kieval, J.Z., A. Charara, J.-F. Paré and Y. Smith (2001) Subcellular and subsynaptic localization of pre- and post-synaptic kainate receptor subunits in the monkey striatum. J. Neurosci. 21: 8746-8757.
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Book Chapters

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- Jin, XT and Y. Smith (2004) Kainate Receptors Mediate Excitatory Synaptic Transmission in the Rat Globus Pallidus. Proc. IBAGS VIII meeting, Crieff, Scotland, abstr P66, p. 73.
- Jin and Smith (2005) Pre-synaptic kainate receptors modulate GABAergic and glutamatergic transmission in the globus pallidus. Abstr 631.8.

CONCLUSIONS

In conclusion, kainate receptors appear as a unique subtype of ionotropic glutamate receptors in the central nervous system. We have demonstrated that their pattern of synaptic localization and function in the striatopallidal complex are strikingly different from AMPA and NMDA receptors. Their extrasynaptic localization, pre-synaptic regulatory function of neurotransmission

and slow kinetic provide these receptors interesting features for the development of novel therapeutic approaches for movement disorders.

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APPENDIX 1

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Ionotropic and metabotropic GABA and glutamate receptors in primate basal ganglia

Yoland Smith [a,b,*], Ali Charara [a], Maryse Paquet [a], Jeremy Z. Kieval [a], Jean-François Paré [a], Jesse E. Hanson [a], George W. Hubert [a], Masaaki Kuwajima [a], Allan I. Levey [b]

[a] *Division of Neuroscience, Yerkes Regional Primate Research Center, Emory University, 954, Gatewood Rd NE, Atlanta, GA 30322 , USA*
[b] *Department of Neurology, Emory University, Woodruff Memorial Building, Suite 6000, PO Drawer V, Atlanta, GA 30322, USA*

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Abstract

The functions of glutamate and GABA in the CNS are mediated by ionotropic and metabotropic, G protein-coupled, receptors. Both receptor families are widely expressed in basal ganglia structures in primates and nonprimates. The recent development of highly specific antibodies and/or cDNA probes allowed the better characterization of the cellular localization of various GABA and glutamate receptor subtypes in the primate basal ganglia. Furthermore, the use of high resolution immunogold techniques at the electron microscopic level led to major breakthroughs in our understanding of the subsynaptic and subcellular localization of these receptors in primates. In this review, we will provide a detailed account of the current knowledge of the localization of these receptors in the basal ganglia of humans and monkeys.

1. Introduction

Although the implication of "the basal ganglia" in the control of motor behaviors has long been known, the exact mechanisms by which these brain regions participate in motor control is still obscure and controversial. Furthermore, it is now clear that the functions of basal ganglia extend far beyond mere sensorimotor integration to include major cognitive and limbic components. The evidence that many neurodegenerative diseases of the basal ganglia often lead to major cognitive impairment accompanied by psychiatric problems strongly support the non-motor functions of these brain regions (Brown and Marsden, 1984; Marsden, 1984; Brown and Marsden, 1988; Sano et al., 1989; Mayeux et al., 1990, 1992). Our knowledge of the anatomy and pathophysiology of primate basal ganglia has increased dramatically over the past 20 yr due to the introduction of highly sensitive chemoanatomical methods, brain imaging techniques and the discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a chemical which selectively kills midbrain dopaminergic neurons in primates and induces Parkinson's disease (PD) (Davis et al., 1979; Langston et al., 1983). The MPTP model of PD is one of the best animal model of neurodegenerative diseases currently available. The use of this animal model has led to major breakthroughs in our understanding of the functional circuitry of the basal ganglia and served as the cornerstone for the development of novel surgical and pharmacological therapies for PD (see Starr, 1995; Blandini et al., 1996; Poewe and Granata, 1997; Vitek, 1997 for reviews).

The work that has been carried out in our laboratory over the past 10 yr has aimed at understanding various aspects of the connectivity and synaptic organization of the basal ganglia in non-human primates (Smith et al., 1998a,b). The recent development of highly sophisticated electron microscopic immunocytochemical approaches allowed us and others to better characterize the subsynaptic and subcellular localization of neurotransmitter receptors involved in mediating synaptic communication at various GABAergic and glutamatergic synapses in the primate basal ganglia (Paquet and Smith, 1996; Paquet et al., 1997; Waldvogel et al., 1998;

* Corresponding author. Tel.: +1-404-7277519; fax: +1-404-7273278.
E-mail address: yolands@rmy.emory.edu (Y. Smith).

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Charara et al., 1999; Hanson and Smith, 1999; Waldvogel et al., 1999; Charara et al., 2000a; Smith et al., 2000a). Unfortunately, due to problems inherent to postmortem tissue with the preservation of ultrastructural features, such studies cannot be carried out in human material. However, due to similarities in the subcortical organization of basal ganglia structures in human and non-human primates, there is a high likelihood that our findings in monkeys can be extrapolated to humans. In this review, we will present some of our most recent data on the subsynaptic localization of metabotropic glutamate receptors and GABA-B receptors in the monkey basal ganglia. We will also give a brief overview of the current knowledge of the localization of various subtypes of glutamate and GABA receptors in the human basal ganglia largely based on data gathered by autoradiographic binding studies, in situ hybridization method, light microscopic immunocytochemical method and PET imaging technique. Finally, we will examine the possibility of using novel drug therapies directed towards specific subtypes of G protein-coupled glutamate and GABA receptors to treat PD.

Because of the scope of the paper, this review will mostly cover data gathered in monkeys and humans. The reader is referred to recent extensive reviews and compendia related to basal ganglia research for a more extensive coverage of literature (Joel and Weiner, 1994; Percheron et al., 1994; Parent and Hazrati, 1995; Chesselet and Delfs, 1996; Gerfen and Wilson, 1996; Ohye et al., 1996; Joel and Weiner, 1997; Levy et al., 1997; Smith et al., 1998a,b; Wilson, 1998).

2. Basal ganglia circuitry

2.1. Striatal afferents

In primates, the basal ganglia are comprised of five tightly interconnected subcortical structures involved in the integration and processing of sensorimotor, cognitive and limbic information. The main entrance of cortical information to the basal ganglia circuitry is the striatum which is comprised of the caudate nucleus (CD), putamen (PUT) and nucleus accumbens (Acc). The glutamatergic corticostriatal projection is highly topographic and imposes a functional compartmentation of striatal regions. The post-commissural PUT receives inputs from the primary motor and somatosensory cortices as well as PM and SMAs whereas the pre-commissural PUT and the CD are the main targets of associative cortical regions. On the other hand, the bulk of cortical afferents to the Acc arise from limbic cortices, amygdala and hippocampus (Heimer et al., 1995). Another level of striatal compartmentation is the patch/matrix organization. This concept, which originally relied upon the heterogeneous distribution of acetylcholinesterase is now considered to be a basic framework of striatal architecture. Most neurotransmitters and neuropeptides as well as major striatal afferent projections and striatal output neurons display a preferential distribution for the patch or the matrix compartment (Graybiel, 1990).

Another major glutamatergic input to the striatum arises from the caudal intralaminar thalamic nuclei, namely the centromedian (CM) and parafascicular (Pf) nuclear complex (Smith and Parent, 1986; Sadikot et al., 1992a,b; Parent and Hazrati, 1995). Projections from the CM terminate preferentially in the sensorimotor striatal territory whereas inputs from Pf innervate the associative and limbic striatal regions (Sadikot et al., 1992b). Finally, the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) are the two main sources of dopamine to the dorsal and ventral striatum, respectively. Additional sources of innervation of the striatum include the external globus pallidus (GPe), the subthalamic nucleus (STN), the dorsal raphe and the tegmental pedunculopontine nucleus (TPP) (Smith and Parent, 1986) (Fig. 1).

The main targets of striatal afferents are the GABAergic medium sized spiny projection neurons which account for more than 90% of the total neuronal population of the striatum (Smith and Bolam, 1990). The glutamatergic inputs from the cortex terminate almost exclusively on the heads of dendritic spines whereas the thalamic afferents from CM/Pf preferentially innervate dendritic trunks (Sadikot et al., 1992a; Smith et al., 1994). Dopamine and cortical inputs often converge at the level of individual spines, which supports the tight functional interaction between dopamine and glutamate in mediating proper basal ganglia functions (Smith et al., 1994). In addition to projection neurons, the striatum is also endowed with various populations of aspiny interneurons recognized by their size and differential content in neurotransmitter, neuropeptides and calcium binding proteins. Four main classes of interneurons have been recognized in the primate striatum: (1) the cholinergic neurons, (2) the parvalbumin-containing neurons, which co-express GABA, (3) the somatostatin-containing neurons which also contain neuropeptide Y and nitric oxide synthase and (4) the calretinin-containing neurons. Albeit less massively innervated than spiny neurons, interneurons also receive direct cortical, thalamic and nigral inputs (Kawaguchi et al., 1995; Sidibé and Smith, 1999; Bolam et al., 2000).

2.2. Direct and indirect striatofugal pathways

Once integrated and processed at the striatal level, the information is conveyed to the basal ganglia output structures, the internal globus pallidus (GPi) and the

substantia nigra pars reticulata (SNr), via two pathways: (1) The direct pathway, which arises from a subset of striatal projection neurons enriched in substance P/dynorphin and D1 dopamine receptors, terminates directly in GPi and SNr or (2) the indirect pathways, which originate from striatal neurons enriched in enkephalin and D2 dopamine receptors, terminate in GPe (Albin et al., 1989; Bergman et al., 1990; Gerfen et al., 1990). In turn, GPe neurons provide GABAergic inputs to the STN, which relays signals to the basal ganglia output nuclei. Imbalance in the activity of these two pathways, in favor of the indirect pathway, underlies some of the motor deficits in PD (DeLong, 1990; Wichmann and DeLong, 1996). The model of direct and indirect pathways as originally introduced was, by necessity, a simplification and only included the major projections of sub-nuclei of the basal ganglia (Albin et al., 1989; Bergman et al., 1990). However, since its introduction there have been many developments in our knowledge and understanding of the anatomical and synaptic organisation of the basal ganglia that have led to reconsideration and updates of

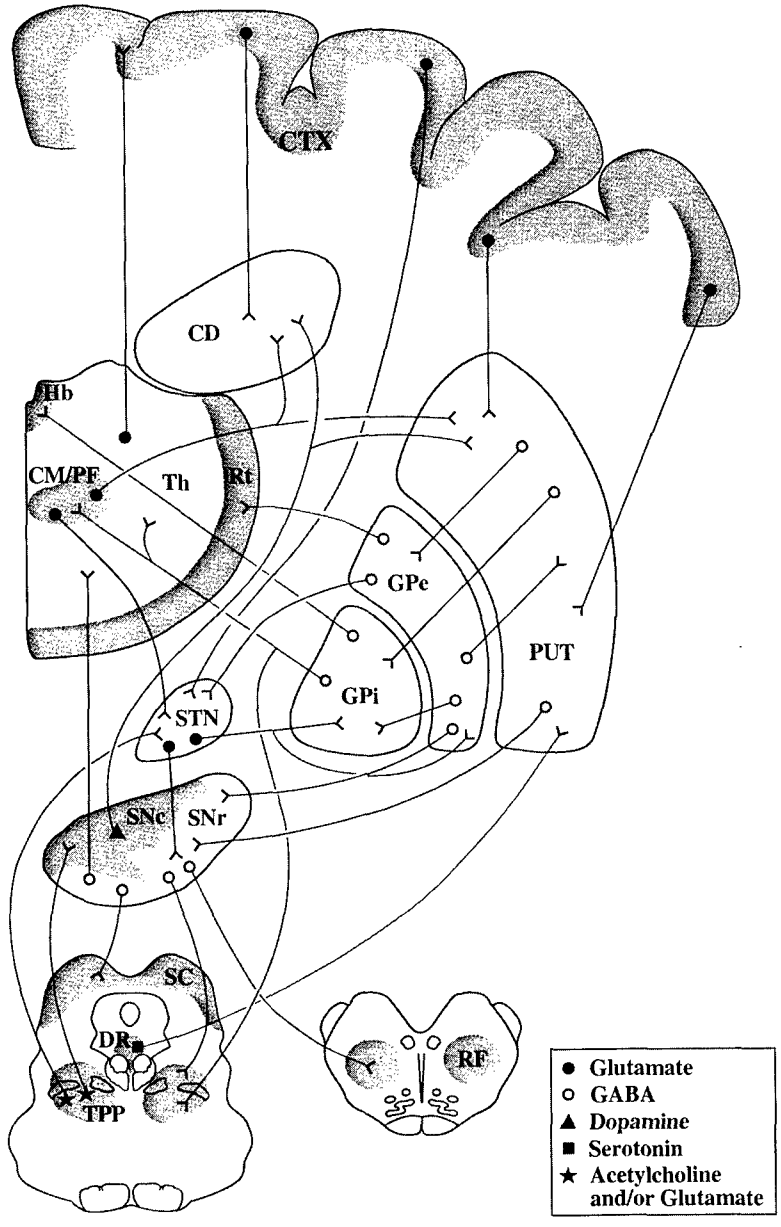

Fig. 1. Connectivity of the primate basal ganglia: schematic representation of the main afferent and efferent connections of basal ganglia structures in primates. For the sake of clarity, some connections have been omitted. Abbreviations: CD: caudate nucleus; CM/Pf: Center median/parafascicular complex; CTX: cerebral cortex; DR: dorsal raphe; GPe: globus pallidus, external segment; GPi: globus pallidus, internal segment; Hb: habenular nucleus; PUT: putamen; RF: reticular formation; Rt: reticular nucleus; SC: superior colliculus; SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; STN: subthalamic nucleus; Th: thalamus, TPP: tegmental pedunculopontine nucleus.

some aspects of the model. One of the most important new finding regarding the anatomical organization of the basal ganglia is the demonstration of multiple indirect pathways of information flow through the basal ganglia. In addition to the classical indirect pathway through the GPe and the STN, it is now well established that the GPe gives rise to GABAergic projections that terminate in basal ganglia output structures (GPi, SNr), the reticular nucleus of the thalamus and the striatum (see Parent and Hazrati, 1995; Smith et al., 1998a,b for reviews) (Fig. 1). Even if the exact functions of these connections remain unknown, it should be kept in mind that the circuitry of the basal ganglia as outlined in the original model of "direct and indirect" pathways is likely to be more complex than previously thought (Smith et al., 1998a). It is noteworthy that molecular and anatomical data showing: (1) a higher degree of co-localization of D1 and D2 dopamine receptors in striatal projection neurons and (2) a higher degree of collateralization of individual "direct" striatofugal neurons recently challenged the concept of direct and indirect pathways (see Gerfen and Wilson, 1996 for a review). Although these findings do not rule out the segregation of striatofugal neurons, they must be kept in mind while considering the functional significance of the direct and indirect striatofugal pathways in normal and pathological conditions.

2.3. Basal ganglia outflow

Once the information has reached the GPi and SNr, it is conveyed to various thalamic and brainstem nuclei which project to motor and pre-motor (PM) cortical areas or to lower brainstem regions. Although both the GPi and SNr project to the ventral anterior/ventral lateral thalamic complex (VA/VL), the nigral and pallidal afferents largely terminate in different subdivisions of the VA/VL nuclei in primates (Ilinsky et al., 1993). Other targets of SNr neurons include the brainstem TPP, the superior colliculus and the medullary reticular formation (Fig. 1). The nigrocollicular fibers, which terminate mainly onto tectospinal neurones in the intermediate layer of the superior colliculus, play a critical role in the control of visual saccades. At thalamic level, inputs from the medial part of the SNr terminate mostly in the medial magnocellular division of the VA (VAmc) and the mediodorsal nucleus (MDmc) which, in turn, innervate anterior regions of the frontal lobe including the principal sulcus (Walker's area 46) and the orbital cortex (Walker's area 11) in monkeys (Ilinsky et al., 1985). On the other hand, neurones in the lateral part of the SNr project preferentially to the lateral posterior region of the VAmc and to different parts of the MD mostly related to posterior regions of the frontal lobe including the frontal eye field and areas of the premotor cortex (see Sidibé et al., 1997 for a review). Another thalamic target of SNr neurons is the caudal intralaminar Pf, which provides a massive feedback projection to the CD (Ilinsky et al., 1985; Smith et al., 2000b).

In addition to the VA/VL and caudal intralaminar thalamic nuclei, the lateral habenular nucleus and the TPP also receive significant inputs from GPi. Efferents from the sensorimotor GPi remain largely segregated from the associative and limbic projections at the level of the thalamus whereas they partly overlap in the TPP (Shink et al., 1997; Sidibé et al., 1997). On the other hand, limbic and associative pallidal projections innervate common nuclei in the thalamus and TPP. In squirrel monkeys, the sensorimotor GPi outputs are directed towards the posterior VL (VLp), whereas the associative and limbic GPi preferentially innervate the parvocellular ventral anterior (VApc) and the dorsal VL (VLd). The ventromedial nucleus receives inputs from the limbic GPi only (Sidibé et al., 1997). These findings, therefore, suggest that some associative and limbic cortical information, which is largely processed in segregated corticostriatopallidal channels, converge at common thalamic nuclei in monkeys (Sidibé et al., 1997). The basal ganglia influences are then conveyed to the cerebral cortex via the VA/VL nuclei. Although it has long been thought that the sensorimotor information from the GPi was conveyed exclusively to the supplementary motor area (SMA), recent anatomical and physiological data in macaques demonstrate that the information from the GPi may also be sent to the primary motor cortex (M1) and the PM cortical area (Rouillier et al., 1994; Hoover and Strick, 1999). Retrograde transneuronal virus studies showed that different populations of GPi neurones project to SMA, M1 and PM (Middleton and Strick, 2000).

Most pallidal neurons which project to thalamic relay nuclei send axon collaterals to the caudal intralaminar nuclei where they are distributed according to a specific pattern of functional organization. Pallidal axons arising from the sensorimotor GPi terminate exclusively in CM, where they form synapses with thalamostriatal neurons projecting back to the sensorimotor territory of the striatum (Smith and Sidibé, 1999). In contrast, associative inputs from the caudate-receiving territory of GPi terminate massively in a dorsolateral extension of Pf (PFdl) which, surprisingly, does not project back to the CD but rather preferentially innervates the pre-commissural region of the PUT. Finally, the limbic GPi selectively innervates the rostrodorsal part of Pf which gives rise to the thalamo-accumbens projection (Sidibé et al., 1997; Smith et al., 1998b; Smith and Sidibé, 1999). Therefore, it appears that the CM/Pf is part of closed and open functional loops with the striatopallidal complex (Smith and Sidibé, 1999). Neurons in Pf that project to the CD do not receive inputs from any functional regions of GPi,

but receive substantial innervation from the SNr (Smith et al., 2000b).

In monkeys, more than 80% of GPi neurones that project to the VA/VL send axon collaterals to the TPP (Parent and Hazrati, 1995). In contrast to the thalamus that conveys the basal ganglia information to the cerebral cortex, the TPP gives rise to descending projections to the pons, medulla and spinal cord as well as prominent ascending projections to the different structures of the basal ganglia, the thalamus and the basal forebrain (Inglis and Winn, 1995; Rye, 1997). The pallidotegmental projection may thus be a route by which information can escape from the basal ganglia-thalamocortical circuitry and reach lower motor and autonomic centers.

3. Glutamate and GABA receptor families in the CNS

Glutamate and GABA receptors are categorized in two main groups based on their structure and mechanisms of actions. The ionotropic receptors are ligand-gated ion channels, which mediate fast synaptic transmission whereas the metabotropic receptors are coupled to G proteins and initiate intracellular signaling cascades.

3.1. Ionotropic and metabotropic glutamate receptors

Two main subtypes of ionotropic glutamate receptors have been identified: the *N*-methyl-D-aspartate (NMDA) receptors and the alpha-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA)/kainate receptors. Numerous subunits and variants constituting the different types of ionotropic glutamate receptors have now been cloned and sequenced. Various factors, including the subunit composition and the relative abundance of these subunits influence the biophysical properties of these receptors. The NMDA receptors consist of eight splice variants (named a–h) of the NMDAR1 subunit and four different NMDAR2 receptor subunits (NR2A, NR2B, NR2C and NR2D), whereas the AMPA receptors are made up of the GluR1-4 subunits. Finally, heteromeric combinations of the high-affinity kainate binding subunits (GluR5–7; KA1–2) form the kainate receptors (Gasic and Hollmann, 1992; Hollmann and Heinemann, 1994; Westbrook, 1994). In general, activation of AMPA and kainate receptors is responsible for primary events in fast glutamatergic transmission since NMDA receptors only become fully activated by glutamate secondarily when their Mg^{+2} block is relieved by depolarization.

The metabotropic glutamate receptor family includes eight different subtypes pooled into three major groups based on their sequence homology, pharmacological properties and transduction mechanisms. In vitro data have shown that the group I mGluRs, which include the splice variants of mGluR1 (a,b,c,d) and mGluR5 (a,b), are positively coupled via Gq to phospholipase C and PI hydrolysis. Activation of these receptors, which are usually found postsynaptically, generally leads to slow depolarization, though presynaptic group I mGluRs were also found in some brain regions (Nakanishi, 1994; Pin and Duvoisin, 1995; Conn and Pin, 1997). Group II mGluRs (mGluR2,3) are negatively coupled via Gi/Go to adenylyl cyclase and inhibit the formation of cyclic AMP following exposure to forskolin or activation of an intrinsic Gs-coupled receptor. Similarly, group III mGluRs (mGluR4,6,7,8) inhibit adenylyl cyclase via a pertussis toxin sensitive G-protein. Group II and group III mGluRs are generally found presynaptically where they act as auto- or hetero-receptors to modulate the release of glutamate or other neurotransmitters (see Cartmell and Schoepp, 2000 for a review). The three groups of mGluRs can be further differentiated pharmacologically by their selective sensitivity to specific agonists (Nakanishi, 1994; Conn and Pin, 1997; Schoepp et al., 1999). However, selective compounds for specific subtypes of receptors in the same group are still missing, except for the recent development of specific antagonists for mGluR1 and mGluR5 (see Schoepp et al., 1999 for a review).

3.2. Three major groups of GABA receptors

The GABA receptors are pooled into three groups. The GABA-A receptors, which are ligand-gated chloride channels, mediate fast inhibitory transmission in the CNS. These receptors are pentameric glycoproteins made up of various subunits which, to date, can be categorized into seven groups based on sequence homology (6α, 4β, 4γ, 1δ, 1ε, 1π, 1θ) (Macdonald and Olsen, 1994; Bonnert et al., 1999; Mehta and Ticku, 1999). The combinational assembly of these subunits (and splice variants of several of them) into a pentameric structure results in diverse receptor subtypes. In vivo, fully functional GABA-A receptors are generally made up of a combination of α, β and γ2 subunits. About 80% of all GABA-A receptors in the CNS are sensitive to benzodiazepines (BZ) and contain the classical BZ binding sites (Möhler et al., 1997; Upton and Blackburn, 1997; Mehta and Ticku, 1999). The γ2 subunit is essential to convey BZ sensitivity to GABA-A receptors which is consistent with the widespread distribution of this subunit in the brain. This BZ sensitivity has been instrumental for the development of various ligands to map the distribution of GABA-A receptor binding sites in the human CNS (see below). Another particular feature of GABA-A receptors is their sensitivity for the highly specific agonist and antagonist, muscimol and bicuculline.

Another major subtype of GABA receptors in the CNS is the GABA-B receptors, which were identified in

the early 1980s as a novel type of bicuculline-insensitive Cl^- -independent GABA receptors (Hill and Bowery, 1981). GABA-B receptors, which are selectively activated by baclofen and insensitive to bicuculline (Bowery et al., 1980; Hill and Bowery, 1981), belong to the family of seven transmembrane domain receptors and are coupled to Ca^{2+} and K^+ channels via G proteins and second messenger systems, e.g. inhibiting adenylate cyclase (Bormann, 1997; Bowery, 1997; Deisz, 1997). GABA-B receptors generate the late inhibitory postsynaptic potentials that are important for the fine tuning of inhibitory neurotransmission (Bettler et al., 1998). During the past few years, an impressive amount of work has been devoted to the mechanisms of GABA-B action in the CNS (see reviews by Kerr and Ong, 1995; Misgeld et al., 1995; Bowery, 1997; Deisz, 1997). The development of potent GABA-B antagonists (Olpe et al., 1990; Bittiger et al., 1993; Froestl et al., 1999; Ong et al., 1999) has greatly facilitated the investigation of the various facets of GABA-B receptors. Recent cloning of GABA-B receptor subtypes revealed extended similarity with metabotropic glutamate receptors. So far, two GABA-B receptor subunits have been identified, GABA-BR1 and GABA-BR2 which assemble into heterodimers to form a functional GABA-B receptor (Bettler et al., 1998; Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Kuner et al., 1999; Makoff, 1999; Martin et al., 1999).

Finally, the last group of GABA receptors are the GABA-C receptors which, like GABA-A receptors, are members of the ligand-gated ion-channel superfamily receptors permeable to chloride ions. However, GABA-C receptors stand as a separate class of receptors due to their lack of sensitivity to bicuculline and baclofen (Johnston, 1997). Another feature that characterizes GABA-C receptors is their lack of sensitivity to BZ and barbiturates. Furthermore, these receptors are activated at lower concentrations of GABA and are less liable to desensitization than most GABA-A receptors. GABA-C receptors are made up of ρ subunits which were cloned in the early 1990s from a human retinal library. To date, three ρ subunit cDNAs have been characterized, but very little is known about their functions and distribution in the primate brain (Johnston, 1997). Although originally thought to be expressed exclusively in the retina, recent in situ hybridization studies revealed a much broader distribution of these receptor subunits in the rodent and human brain (Enz et al., 1995; Wegelius et al., 1998; Enz and Cutting, 1999).

4. Technical developments for the localization of neurotransmitter receptors

The cloning techniques combined with the development of sensitive high resolution electron microscopic immunocytochemical methods led to major breakthroughs in our current knowledge of receptor localization in the CNS. Although autoradiographic ligand binding method has long been a major tool used to visualize receptor binding sites in the brain, the lack of resolution of this approach significantly hampers the interpretation of the exact neuronal localization of receptor subtypes. A better resolution can be obtained using the techniques of light and electron microscopic immunocytochemistry and in situ hybridization. However, due to limitations inherent to postmortem tissue in the preservation of membranes and glycoprotein antigenicity, the immunocytochemical detection of receptors at the electron microscopic level in human brain is limited, which makes the ligand binding methods still the most commonly used tool to study receptor distribution in postmortmem human brain tissue (see below).

The electron microscopic immunogold methods provide a way by which the exact localization and relative abundance of receptors in relation to specific release sites of neurotransmitters can be studied (Van Lookeren Campagne et al., 1991; Nusser et al., 1994; Matsubara et al., 1996). In our laboratory, we use two different immunogold methods to study glutamate and GABA receptors in the monkey basal ganglia, namely the pre-embedding silver-intensified immunogold method and the freeze-substitution post-embedding immunogold technique. These approaches combined with the regular immunoperoxidase method provide complementary information which help characterize the precise localization of a particular receptor in the brain. The main advantage of the immunogold methods over the immunoperoxidase technique is the higher level of spatial resolution of gold particles in comparison to the amorphous diaminobenzidine (DAB) reaction product. However, the immunoperoxidase approach still remains the most sensitive technique to detect low level of receptor proteins. In the pre-embedding immunogold method, a secondary antibody conjugated to 1.4 nm gold particles, rather than the peroxidase complexes, is used to localize the antigenic sites. The size of the gold particles is, then, increased by silver intensification which results in 30–50 nm electron-dense particles. The main problem with this approach is the poor penetration of gold-conjugated antibodies which limits the electron microscopic analysis to the most superficial part of tissue sections and significantly hampers the interpretation of negative data. For that reason, the post-embedding immunogold technique is definitely the only reliable approach to quantify and unequivocally compare receptor densities associated with different synapses on the same section. In order to maintain the antigenicity of GABA and glutamate receptors after embedding, it is necessary to use the technique of fast freezing followed by low temperature dehydration (a process named freeze-substitution) and low temperature

embedding of fixed brain tissue in non-polar resin (Baude et al., 1993; Nusser et al., 1994; Baude et al., 1995; Nusser et al., 1995; Lujan et al., 1996; Matsubara et al., 1996; Bernard et al., 1997; Nusser et al., 1997; Ottersen and Landsend 1997; Bernard and Bolam, 1998; Clarke and Bolam, 1998; Nusser et al., 1998; Nusser, 1999).

5. Glutamate and GABA receptors in the striatum

5.1. Ionotropic glutamate receptors

5.1.1. AMPA receptors

The localization of AMPA receptors in the human striatum has been studied in normal and pathological conditions by means of autoradiographic ligand binding (Dure et al., 1991, 1992; Lee and Choi, 1992; Ball et al., 1994; Noga et al., 1997; Healy et al., 1998; Blue et al., 1999), in situ hybridization (Bernard et al., 1996; Tomiyama et al., 1997; Healy et al., 1998) and light microscopic immunohistochemistry (Meng et al., 1997; Cicchetti et al., 1999). Overall, the whole human striatum is quite enriched in AMPA receptor binding sites but a slightly higher density is found in the matrix compartment (Dure et al., 1992). Analysis of striatal AMPA binding sites in various pathological conditions revealed: (1) either no significant differences (Healy et al., 1998) or increases (Noga et al., 1997) in AMPA binding sites in schizophrenics, (2) significant reductions in AMPA receptor bindings in the PUT of girls with Rett syndrome (Blue et al., 1999) and (3) significant decreases of AMPA binding sites in the CD of Huntington's patients (Dure et al., 1991). More recent studies using in situ hybridization and immunohistochemical methods revealed that the GluR1,2,3 subunits are widely expressed in both projection neurons and large interneurons in the human striatum whereas GluR4 is confined to a small population of large- and medium-sized neurons (Bernard et al., 1996; Meng et al., 1997; Tomiyama et al., 1997). Subpopulations of large and medium CR-containing interneurons are endowed with GluR1, GluR2 and GluR4 AMPA receptor subunits (Cicchetti et al., 1999). It is noteworthy that GluR4 mRNA and immunoreactivity is confined to interneurons in the rat striatum (Tallaksen-Greene and Albin, 1994; Chen et al., 1996; Bernard et al., 1997). No significant changes in GluR1–4 mRNA expression was found in the striatum of parkinsonian patients (Bernard et al., 1996).

In monkeys, GluR1,2,4 immunoreactivities are found in medium-sized projection neurons in both the CD and PUT (Martin et al., 1993). In contrast, large cholinergic interneurons, which partly co-localize with CR in humans (Cicchetti et al., 1998), express GluR4 immunoreactivity but are devoid of GluR1 and GluR2/3 labeling (Martin et al., 1993). GluR1, but not GluR2/3 or GluR4 immunoreactivity, is more intense in the ventral striatum than the dorsal striatum. In the CD, GluR1 is preferentially expressed in medium sized spiny neurons in patches whereas the matrix contains large GluR4-containing cholinergic interneurons (Martin et al., 1993). Recent data showed an upregulation of the AMPA GluR1 subunit in the striatal patch compartment of MPTP-treated parkinsonian monkeys (Betarbet et al., 2000). Most intrinsic dopaminergic neurons in the monkey striatum, which increase substantially in number after MPTP treatment, express GluR1, but not GluR2/3 immunoreactivity (Betarbet and Greenamyre, 1999).

At the electron microscopic level, the GluR1 subunit is enriched in dendritic spines (Martin et al., 1993), which is consistent with recent immunogold data showing that the bulk of AMPA receptor subunit immunoreactivity is confined to asymmetric axo-spinous synapses in the rat striatum (Bernard et al., 1997).

5.1.2. Kainate receptors

Due to the lack of specific markers that could differentiate kainate from AMPA receptor binding sites, very little is known about the localization of kainate receptors in the human striatum. Using in situ hybridization approach, Bernard et al. (1996) showed that the GluR6, GluR7 and KA2 receptor subunits are detected in about 50–60% of striatal medium-sized neurons whereas the KA1 labeling is restricted to 20–30% of these neurons. Less than 2% of striatal neurons express the GluR5 subunit mRNA (Bernard et al., 1996).

We recently carried out a detailed analysis of the subsynaptic localization of kainate receptor subunit immunoreactivity in the monkey striatum using antibodies raised against the GluR6/7 and KA2 kainate receptor subunits (Fig. 2). One of the major finding of this study was that kainate receptor subunits are expressed presynaptically in glutamatergic axon terminals forming asymmetric axo-spinous and axo-dendritic synapses (Charara et al., 1999; Kieval et al., 2000) (Fig. 2A–B). To determine the source of these terminals, the anterograde transport of biotinylated dextran amine (BDA) was combined with GluR6/7 or KA2 immunostaining. Following BDA injections in the CM thalamic nucleus or the M1, more than half of anterogradely labelled boutons in the postcommissural PUT displayed GluR6/7 and KA2 immunoreactivity (Fig. 2C), which indicate that kainate receptors may act as pre-synaptic autoreceptors to control glutamate release from the thalamus and the cerebral cortex in the primate striatum. A particular feature of kainate receptor subunit immunoreactivity is their strong intracellular expression under basal conditions (Fig. 2B–D). Using pre- and post-embedding immunogold methods, most of the GluR6/7 and KA2 immunoreactivity is, indeed, associ-

ated with intracellular organelles rather than being bound to the plasma membrane (Fig. 2B–D). In immunoreactive axon terminals, kainate receptor subunit immunoreactivity is attached to the membrane of large vesicles which, in some cases, are located in the active zone of asymmetric synapses (Fig. 2D–E). Postsynaptic labelling of asymmetric postsynaptic specializations is also seen (Fig. 2F). Although the functions

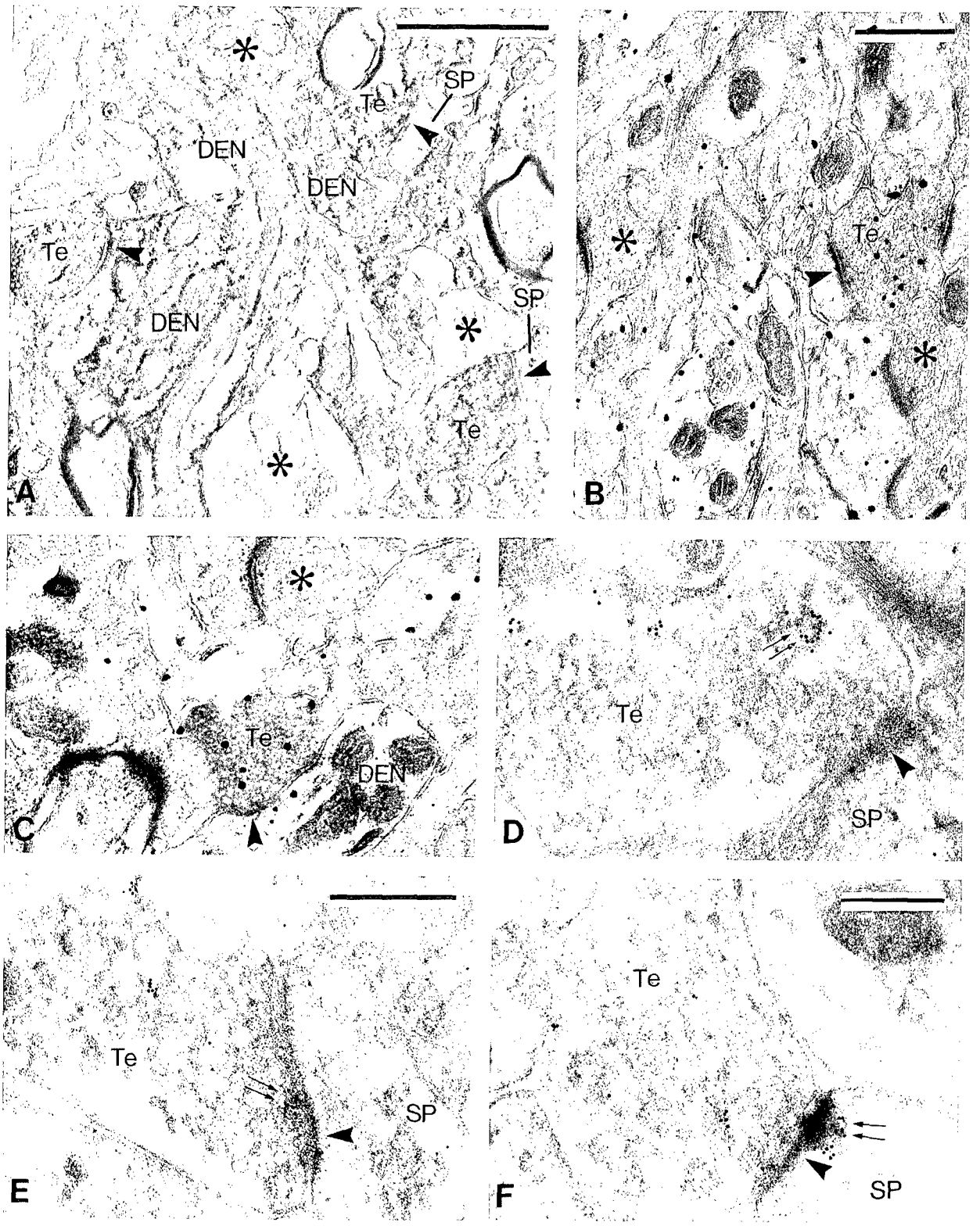

Fig. 2.

of kainate receptors in the striatum are still obscure due to the lack of specific compounds to modulate these receptors, the recent development of specific AMPA antagonists (Partenain et al., 1995) should help further our knowledge of the role of these receptors in the functional circuitry of the basal ganglia. Another promising research avenue that should definitely be explored over the next few years is the potential role of presynaptic kainate receptors in the excitotoxic phenomenon involved in the death of striatal projection neurons in Huntington's disease. The recent findings that the age of onset of Huntington's disease could, in some cases, be attributed to the genotype variation of the GluR6 kainate receptor subunit in humans strongly suggest that these receptors may, somehow, be involved in the neurodegenerative process in Huntington's patients (Rubinztein et al., 1997; MacDonald et al., 1999).

5.1.3. NMDA receptors

Irrespective of the approach used, all data agree that the human striatum is enriched in NMDA receptors. Ligand binding studies show dense NMDA binding sites throughout the whole extent of the CD, PUT and Acc, with a tendency to be slightly higher in the matrix than the patch compartment (Dure et al., 1992; Lee and Choi, 1992). As discussed above for the AMPA receptors, NMDA binding is affected in various brain diseases (Dure et al., 1991; Ulas et al., 1994; Noga et al., 1997; Blue et al., 1999). Data obtained in postmortem parkinsonian brains are controversial; both increases (Ulas et al., 1994) and decreases (Gerlach et al., 1996; Meoni et al., 1999) of NMDA binding sites have been reported in the CD and PUT of these patients. Despite these changes in binding density, no major difference in NMDAR1 mRNA expression is found in the striatum of parkinsonian patients (Meoni et al., 1999). On the other hand, significant reduction in NMDA binding was found in the striatum of Huntington's patients (Dure et al., 1991) whereas an increased density of NMDA receptors was reported in the PUT of schizophrenics (Aparicio-Legarza et al., 1998). Recent in situ hybridization data provide clear evidence for a differential distribution of various NMDA receptor subunits among the two populations of striatal projection neurons and interneurons (Kosinski et al., 1998; Küppenbender et al., 2000). In brief, those data indicate: (1) intense NMDAR1 and NMDAR2B signal over all striatal neurons, (2) strong NMDAR2A signal over GAD67-immunoreactive neurons, intermediate labeling over substance P-containing projection neurons, low labeling over enkephalin-positive projection neurons but no signal over somatostatinergic and cholinergic interneurons which, on the other hand, express moderate signals for NMDAR2D (3) weak NMDAR2C signal over all striatal neurons except for the moderate labeling of cholinergic interneurons and (4) low NMDAR2D labeling over GAD67- and substance P-containing neurons and no labeling over enkephalin-positive projection neurons. Furthermore, only 25% of intrastriatal dopaminergic neurons express NMDAR1 immunoreactivity in MPTP-treated monkeys (Betarbet and Greenamyre, 1999). These data highlight the fact that, although all striatal neurons express NMDA receptors, their subunit composition may significantly differ among the various neuronal populations. This provides a basis for therapeutic development aimed a targeting glutamatergic synapses associated with specific NMDA receptor subtypes in neurodegenerative diseases (see below).

5.2. Metabotropic glutamate receptors

As mentioned above, three main groups of mGluRs have been cloned. Antibodies have now been generated against most of these receptor subtypes which allow to study their neuronal and subsynaptic localization at the electron microscopic level. To our knowledge, data on the localization of mGluRs in the human striatum are limited to a few binding studies (Dure et al., 1991; Blue et al., 1999) and a recent immunocytochemical analysis of the distribution of mGluR2 (Phillips et al., 2000). The neuropil in both the dorsal and ventral striatum displays strong mGluR2 immunoreactivity, but no cells or recognizable neuronal processes could be seen. This supports recent data showing that mGluR2/3 immunoreactivity in the rat striatum is mostly associated with cortical axon terminals (Testa et al., 1998).

We used polyclonal antisera raised against mGluR1a and mGluR5 (a,b) to study the subsynaptic distribution of group I mGluRs in the dorsal striatum of monkeys

Fig. 2. Kainate receptor subunits in the striatum: (A) GluR6/7-immunoreactive elements in the monkey CD. Immunoreactivity is mainly associated with dendrites (DEN) and axon terminals (Te) forming asymmetric synapses (arrowheads) whereas spines (SP) are almost completely devoid of labeling. Non-immunoreactive terminals are indicated with asterisks, (B) GluR6/7-immunoreactive terminal (Te) forming an asymmetric axo-spinous synapse (arrowhead). Asterisks indicate non-immunoreactive terminals. Note the paucity of gold particles on the plasma membrane in the labeled bouton, (C) An anterogradely labeled terminal which displays GluR6/7 immunoreactivity (Te) in the monkey PUT following BDA injections in the M1. The double labelled bouton forms an asymmetric synapse (arrowhead) with an unlabelled dendrite (DEN). An unlabeled terminal is marked in the neuropil (asterisk), (D–E) Post-embedding immunogold localization of GluR6/7 immunoreactivity in the monkey PUT. In immunoreactive terminals, gold particles are associated with vesicular membrane (double arrows in D) or aggregated in the presynaptic grid of asymmetric synapses (E). The postsynaptic immunoreactivity is often associated with the postsynaptic density (double arrows) and plasma membrane of asymmetric synaptic junctions (F). Scale bars: A: 1 μm; B: 0.5 μm (valid for C); D: 0.3 μm (valid for F); E: 0.5 μm.

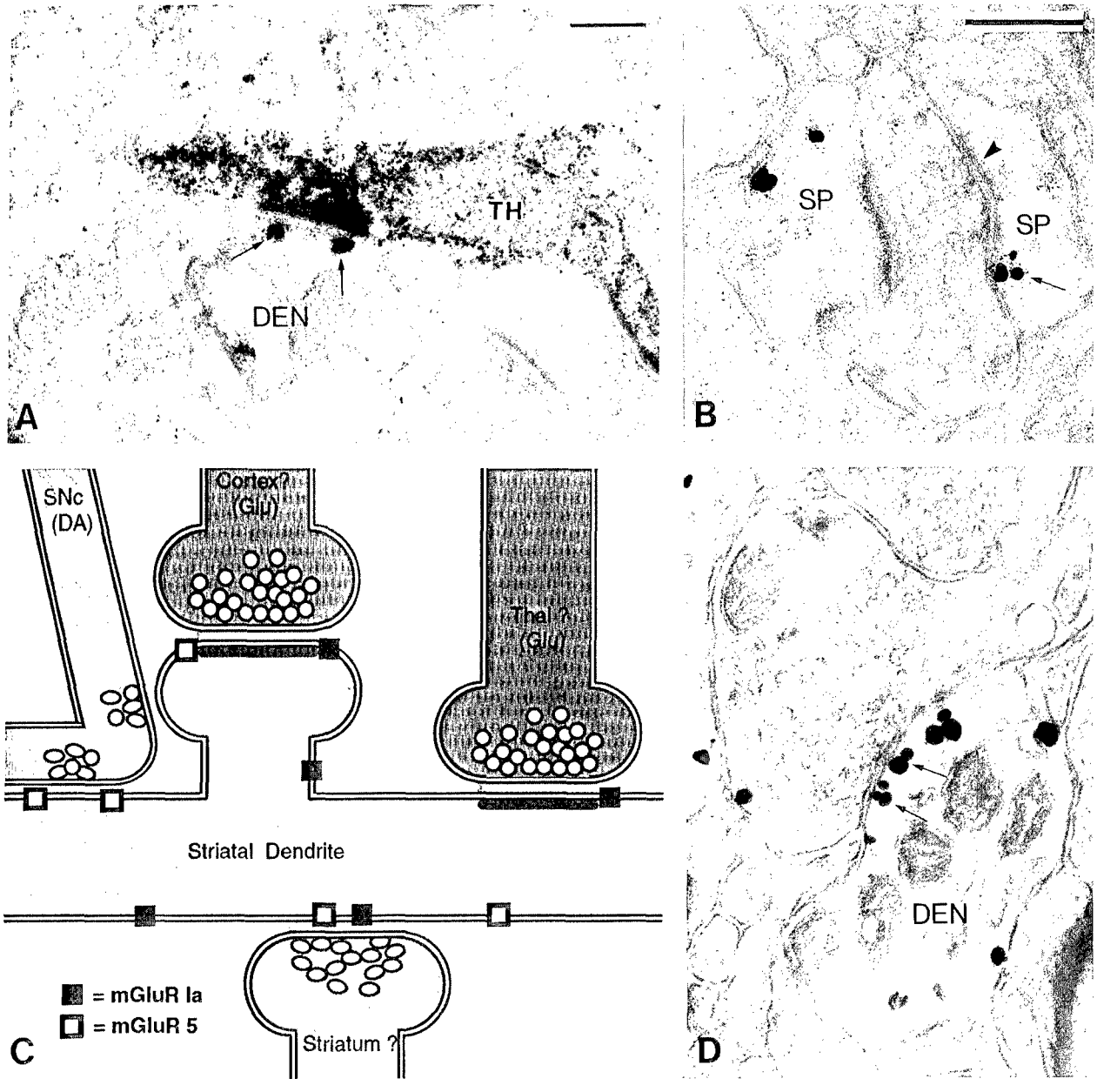

Fig. 3. Group I mGluRs in the monkey striatum: (A) mGluR5 immunoreactivity (arrows) at the edges of a symmetric axo-dendritic synapse established by a TH-positive terminal in the PUT. (B) mGluR1a immunoreactivity (arrow) at the edges of an asymmetric axo-spinous synapse (arrowhead) in the CD. (C) summary diagram of the subsynaptic localization of mGluR1a and mGluR5 immunoreactivities in dendrites and spines of striatal projection neurons in monkeys and (D) mGluR1a immunoreactivity (arrows) in the main body of a symmetric axo-dendritic synapse in the CD. Abbreviations: DA: dopamine; DEN: dendrite; Glu: glutamate; SNc: substantia nigra pars compacta; SP: spines; Thal: thalamus. Scale bars: A: 0.25 μm (valid for D); B: 0.5 μm.

(Fig. 3) (Smith et al., 2000a). Overall, the pattern of group I mGluRs immunoreactivity is the same in the CD and the PUT. Both medium-sized projection neurons and large interneurons display mGluR1a and mGluR5 immunoreactivities. In general, the neuropil staining is much more intense with the mGluR5 than the mGluR1a antiserum. No obvious patch/matrix pattern of distribution of immunoreactive neurons is observed with both antisera (Smith et al., 2000a). At the electron microscope level, the immunoperoxidase reaction product is mostly found in post-synaptic elements including large- and small-sized perikarya with smooth or indented nuclei, dendritic processes of various sizes and dendritic spines. In addition, some axon terminals that form asymmetric axo-spinous synapses display light mGluR1a immunoreactivity, but presynaptic labelling was never encountered in mGluR5-immunostained sections (Smith et al., 2000a). In sections labeled with immunogold, both mGluR1a and mGluR5 immunoreactivities are commonly found at the edges of

asymmetric post-synaptic densities of axo-spinous and axo-dendritic synapses (Fig. 3B,C). In the mGluR5-immunostained sections, aggregates of gold particles are also associated with the main body of symmetric post-synaptic specializations established by terminals that morphologically resemble intrinsic GABAergic boutons (Fig. 3C,D). In sections double labelled for tyrosine hydroxylase (TH) and group I mGluRs, mGluR5 immunoreactivity is occasionally found perisynaptically to symmetric synapses established by TH-containing terminals (Fig. 3A,C). A large number of gold particles are also found extrasynaptic along the membrane of dendrites and spines.

So far, very little is known about the subcellular and subsynaptic localization of group II and group III mGluRs in the primate striatum. Preliminary evidence indicates that group II mGluRs are expressed presynaptically in putative glutamatergic axon terminals and postsynaptically in dendrites and spines (Paquet and Smith, 1997). On the other hand, group III mGluRs (mGluR7a and mGluR4a) are found in both GABAergic and glutamatergic boutons (Paquet and Smith, 1997). On the basis of these anatomical data, it appears that both pre and postsynaptic mGluRs may act at various sites to modulate glutamatergic, dopaminergic and GABAergic synaptic transmission in the primate striatum (Fig. 3). The expression of group II and group III mGluRs in glutamatergic terminals raises the interesting possibility of targeting these receptors to decrease the release of glutamate in Huntington's disease, thereby, protecting striatal projection neurons from excitotoxic cell death.

5.3. Ionotropic GABA-A receptors

Central benzodiazepine receptors associated with GABA-A receptors have been classified into two subtypes, namely BZI and BZII receptors, on the basis of their pharmacology (Johnston, 1996; Mehta and Ticku, 1999). The use of selective radioactive ligands for each receptor subtype allowed to study the distribution of BZ/GABA-A receptors in the human and monkey striatum in both normal and pathological conditions. However, many studies were carried out using ligands that have the same affinity for both BZ receptor subtypes. In the description below, we will refer to either BZI or BZII receptors in cases where specific ligands were used and BZ binding sites in cases where data have been obtained with nonspecific ligands that recognize both receptor subtypes. In brief, BZ binding studies led to the following data: (1) Both receptor subtypes are significantly more abundant in the ventral than dorsal striatum (Young and Kuhar, 1979; Penney and Young, 1982; Walker et al., 1984; Faull and Villiger, 1986, 1988; Waldvogel et al., 1998, 1999), (2) In both the CD and PUT, BZI and BZII binding sites are distributed according to the patch/matrix compartmentation in humans and monkeys (Faull and Villiger, 1986, 1988; Waldvogel et al., 1998, 1999), (3) The density of BZ binding sites is significantly decreased in the striatum of Hutington's patients (Penney and Young, 1982; Walker et al., 1984; Glass et al., 2000), (4) The density of BZ binding sites is decreased in the rostral part of the CD and PUT of MPTP-treated monkeys. This decrease remains unchanged after treatment with D1 or D2 receptor agonists (Calon et al., 1999) and (5) Continuous, but not pulsatile, administration of the D2 agonist, U91356A, in MPTP monkeys leads to a significant decrease of BZ binding sites in the striatum (Calon et al., 1995). PET imaging is another approach that has been used to study the in vivo distribution of BZ binding sites in monkey and human striatum (Brouillet et al., 1990; Moerlein and Perlmutter, 1992; Schmid et al., 1995). Results obtained so far with these methods are largely consistent with in vitro binding data.

The development of antibodies and cDNA probes raised against various GABA-A receptor subunits allowed studies of the detailed cellular localization of these subunits in the human and monkey striatum. Overall, the immunohistochemical labeling for many GABA-A receptor subunits shows a marked heterogeneous distribution, which corresponds to the patch/matrix striatal compartments, throughout the human striatum. In brief, apart from the α1 subunits which are more abundant in the matrix than the patch compartment, all other subunits examined (α2, α3, β2/3, γ2) are expressed in both compartments but significantly more in patches than the matrix in the dorsal striatum (Waldvogel et al., 1999). The α1 and β2/3 subunits display a similar pattern of distribution in the baboon striatum (Waldvogel et al., 1998). It is noteworthy that the pattern of compartmental expression of these receptor subunits, except for the α1 subunit, is different in the ventral striatum where all subunits are preferentially expressed in the matrix compartment (Waldvogel et al., 1999). Co-localization studies using various markers of striatal neurons led to the following conclusions about GABA-A receptor subunit configurations in human striatal cells: (1) The parvalbumin/GABA interneurons are enriched in α1, β2/3 and γ2 subunits whereas the calretinin interneurons express the same subunits but also contain high levels of the α3 subunit, (2) The cholinergic interneurons only express the α3 subunit, (3) The NPY-immunoreactive neurons are completely devoid of GABA-A receptor subunit immunoreactivity and (4) The calbindin-containing projection neurons express a low to moderate level of α2, α3, β2/3 and γ2 subunits immunoreactivity but are devoid of α1 subunit labeling. In monkeys, the α1 subunit is also expressed preferentially in striatal interneurons whereas the β2/3 subunits immunoreactivity is much more homoge-

neously distributed (Waldvogel et al., 1998). Other GABA-A receptor subunits found in the monkey striatum include the α4 and δ subunits which are expressed at high and moderate levels, respectively, in most striatal neurons (Kultas-Ilinsky et al., 1998). In contrast, the β1 and γ1 subunit mRNAs are not detectable in the monkey striatum (Kultas-Ilinsky et al., 1998). These data indicate that the subunit composition of GABA-A receptors displays a considerable degree of regional and cellular heterogeneity in the human and monkey striatum.

So far, the electron microscopic analysis of GABA-A receptor subunits in the primate striatum is limited to immunoperoxidase localization of α1 and β2/3 subunit labeling in baboon and macaque monkeys. In both species, the GABA-A receptor subunits are expressed along the plasma membrane of striatal projection neurons and interneurons. At the subsynaptic level, peroxidase labeling was found to be associated with both symmetric and asymmetric membrane specializations as well as with nonsynaptic sites along the plasma membrane (Waldvogel et al., 1998). Further immunogold studies are essential to characterize the exact synaptic localization of GABA-A receptor subunits in the primate striatum. Recent immunogold data indicate that the α1, β2/3 and γ2 subunit immunoreactivities are expressed postsynaptically in the main bodies of symmetric GABAergic synapses in the rat striatum (Fujiyama et al., 2000).

5.4. Metabotropic GABA-B receptors

The distribution of GABA-B binding sites in the monkey basal ganglia has recently been studied using high affinity radioactive ligands (^{3}HCGP 62349; ^{125}I-CGP 64213) and ^{3}H-GABA. The striatum and the substantia nigra were found to display the highest level of GABA-B binding in the basal ganglia (Ambardekar et al., 1999; Bowery et al., 1999; Calon et al., 2000). In general, the distribution of striatal binding sites was relatively homogeneous throughout the CD, PUT and Acc. No obvious patch/matrix compartmentation of labelling was noticed. The density of striatal GABA-B binding sites is not changed in MPTP-induced parkinsonian monkeys treated with dopaminergic agonists, despite a significant decrease in the substantia nigra and increased binding in the GPi (Calon et al., 2000).

The recent cloning of two GABA-B receptor subtypes (GABA-BR1 and GABA-BR2) and the subsequent development of antibodies and mRNA probes led to a better characterization of the cellular and subcellular localization of GABA-B receptors in rat (Fritschy et al., 1999; Margeta-Mitrovic et al., 1999), monkey (Charara et al., 2000a,b) and human (Makoff, 1999; Billinton et al., 2000) brain. We recently used affinity-purified polyclonal antisera to localize immunocytochemically GABA-BR1 and GABA-BR2 receptor subunits at light and electron microscope level in the monkey basal ganglia (Charara et al., 2000a,b). Overall, the pattern of labelling generated by both antisera is relatively similar throughout the primate basal ganglia, except that the intensity of labelling is generally much higher for GABA-BR1 than GABA-BR2 immunoreactivity. The similarity in distribution for both GABA-B receptor subtypes is consistent with the idea that GABA-BR1 and GABA-BR2 receptors must form heterodimers to be functional (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Kuner et al., 1999; Makoff, 1999).

In the striatum, the staining for both GABA-B receptor subtypes is homogeneous and relatively similar throughout the CD, PUT and Acc. Both medium-sized projection neurons and large interneurons are immunostained. In general, interneurons are more strongly labeled than projection neurons which is in keeping with recent findings showing that the GABA-BR1 immunoreactivity is particularly abundant in a small population of neurons scattered throughout the rat striatum (Margeta-Mitrovic et al., 1999). There is no patchy distribution of immunostaining, indicating that GABA-B receptor immunoreactivity is not differentially expressed in the patch-matrix compartments, which differs from the patchy distribution of some GABA-A receptor subunits in the monkey and human striatum (see above).

At the electron microscope level, the immunoperoxidase product of GABA-BR1 and GABA-BR2 is found in postsynaptic elements including large- and medium-sized cell bodies, dendrites and spines (Charara et al., 2000a,b). Occasionally, GABA-BR1 immunoreactivity is also detected in cell bodies and thin processes of astrocytes. In sections stained with the pre-embedding immunogold method, GABA-BR1 immunoreactivity was found to be localized in the main body of symmetric post-synaptic specializations established by terminal boutons packed with large pleomorphic vesicles or vesicle-filed axonal processes (Fig. 4D–E). Perisynaptic labeling at asymmetric axo-spinous and axo-dendritic synapses is also seen (Fig. 4C,E). Finally, a large number of extrasynaptic gold particles were found in neuronal perikarya, dendrites and spines (Fig. 4E). In addition to post-synaptic elements, GABA-BR1 and GABA-BR2 immunoreactivities were found in many myelinated and unmyelinated axonal segments as well as in cortical- or thalamic-like terminal boutons forming asymmetric axo-spinous and axo-dendritic synapses (Fig. 4A–B, E). In those labelled terminals, the gold particles are found in the presynaptic grid of asymmetric axospinous and axodendritic synapses (Fig. 4B). Another much rarer type of GABA-BR1-immunoreactive terminal forms "en passant" symmetric synapses with immunolabeled dendrites and display the ultra-

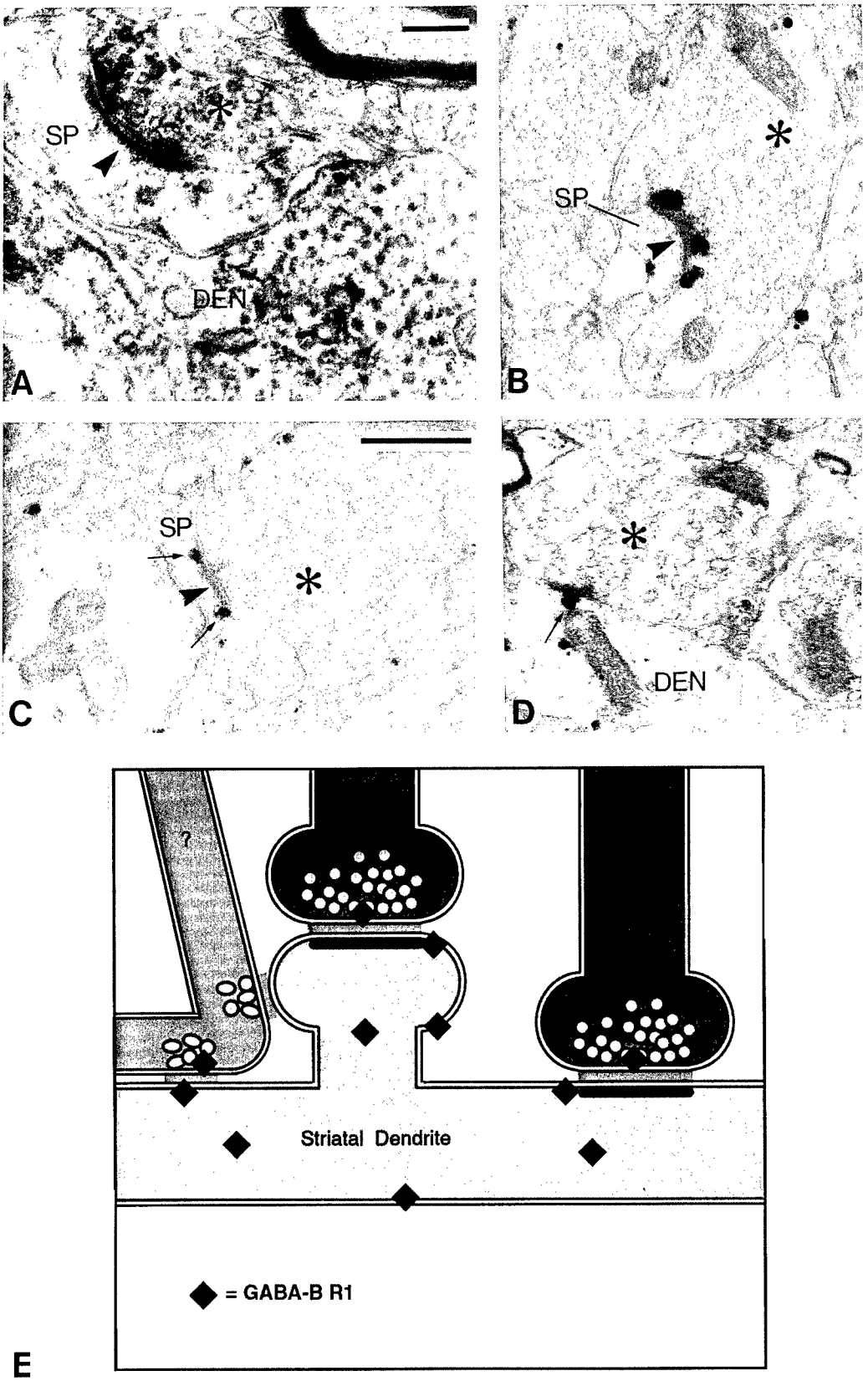

Fig. 4. GABA-BR1 receptor immunoreactivity in the monkey striatum: (A) GABA-BR1-immunoreactive terminal forming an asymmetric axo-spinous synapse (arrowhead) in the monkey PUT. An immunoreactive dendrite (Den) is in the same field, (B) GABA-BR1 (GBR1) immunogold labeling in the presynaptic grid of a putative glutamatergic terminal (asterisk) forming an asymmetric synapse (arrowhead) with a spine (SP) in the PUT, (C) postsynaptic GABA-BR1 labelling (arrows) at the edges of an asymmetric axo-spinous synapse (arrowhead), (D) postsynaptic GABA-BR1 labeling (arrow) at a symmetric axo-dendritic synapse and (E) schematic diagram to summarize the subsynaptic localization of GABA-BR1 immunoreactivity in dendrites and spines of striatal projection neurons. Scale bars: A: 0.25 μm (valid for B); C: 0.5 μm (valid for D).

structural features of either dopaminergic terminals from the substantia nigra or GABA-containing intrinsic striatal boutons (Charara et al., 2000a) (Fig. 4E). Although functions of GABA-B receptors are poorly known in the primate brain, it appears that these receptors are localized to subserve both pre- and postsynaptic control of GABAergic, glutamatergic and, possibly, dopaminergic neurotransmission in the monkey striatum. Functional data in various non-primate species, indeed, suggest that activation of GABA-B receptors modulate glutamatergic and dopaminergic transmission, but the exact localization of receptors which mediate these effects remains controversial (Sawynok and LaBella, 1981; Reinmann, 1983; Wilson and Wilson, 1985; Calabresi et al., 1990; Seabrook et al., 1990; Calabresi et al., 1991; Arias-Montano et al., 1992; Nisenbaum et al., 1992, 1993; Smolders et al., 1995).

6. Glutamate and GABA receptors in the globus pallidus

6.1. Ionotropic glutamate receptors

Both GPe and GPi show a relatively strong binding for various ionotropic glutamate receptor subtypes and are enriched in NMDA, AMPA and kainate receptor subunits in monkeys and humans (Lee and Choi, 1992; Bernard et al., 1996; Tomiyama et al., 1997; Blue et al., 1999). The four AMPA receptor subunits (GluR1–4) are expressed at a moderate to high level in GPe and GPi of rhesus monkeys and humans (Bernard et al., 1996; Paquet and Smith, 1996; Ciliax et al., 1997; Meng et al., 1997; Tomiyama et al., 1997; Betarbet et al., 2000) whereas only low levels of KA1 and KA2 subunits are detectable. On the other hand, the human GPi is devoid of GluR5–7 kainate receptors subunit mRNAs (Bernard et al., 1996). An interesting feature about AMPA receptors in the primate pallidum is the relative lack of GluR1 immunoreactivity in GPi neurons in the squirrel monkey (Paquet and Smith, 1996). This differs from data obtained in rhesus monkeys and humans, using the same antibodies, where all AMPA receptor subunits are strongly expressed in both pallidal segments (Bernard et al., 1996; Ciliax et al., 1997). The functional significance of this potential species difference between old world and new world primates regarding AMPA receptor subunit localization remains to be established.

Neurons in both pallidal segments are enriched in NMDAR1 and NMDAR2D subunits, but also display low level of expression of NMDAR2A,B,C in humans (Kosinski et al., 1998). In GPi, a subpopulation of neurons exhibiting low NMDAR2D mRNA signal can be easily separated from the majority of pallidal neurons which display intense labelling for this subunit (Kosinski et al., 1998). Taking into account that the bulk of glutamatergic afferents to the globus pallidus arises from the STN, it is likely that both AMPA and NMDA receptors with different subunit composition are expressed at subthalamopallidal synapses in primates. Direct evidence in favor of this hypothesis has been obtained in rats (Bernard and Bolam, 1998).

Based on the functional model of basal ganglia circuitry suggesting that the STN is hyperactive in PD, one could hypothesize that this hyperactivity results in a decreased glutamate receptor expression in the pallidum. It was, indeed, recently shown that the GluR1 mRNA expression and protein levels are significantly decreased in GPi and SNr of parkinsonians (Bernard et al., 1996; Betarbet et al., 2000).

6.2. Metabotropic glutamate receptors

Although many studies have addressed the issue of mGluRs localization in the rodent pallidum, data in primates are much rarer and restricted to group I and group II mGluRs. In a recent light microscopic immunoperoxidase study, Phillips et al. (2000) have demonstrated that the neuropil of GPe and GPi displays a moderate to high mGluR2 immunoreactivity in humans with a slightly higher staining intensity in GPe than in GPi. In both pallidal segments, the immunoreactivity appeared to be associated with afferent fibers rather than pallidal neurons per se (Phillips et al., 2000), which suggests the existence of presynaptic group II mGluRs in the human pallidum. These data are strikingly different from those obtained in rodents which revealed low level of mGluR2 mRNAs and immunoreactivity in the rat and mouse globus pallidus (Ohishi et al., 1993; Testa et al., 1994, 1998). Whether this indicates a true species difference between rodents and primates regarding the presynaptic localization of mGluR2 in the pallidal complex or relies upon technical differences in the sensitivity of the different antibodies used in these studies remains to be established. It is worth noting that functional group II mGluRs were found to be expressed on putative STN glutamatergic terminals in the rat SNr (Bradley et al., 2000). If the intense mGluR2 immunolabeling seen in the human pallidum corresponds to presynaptic STN terminals, the group II mGluRs become a very promising target to reduce the release of glutamate from hyperactive subthalamopallidal terminals in PD. To further characterize this issue, electron microscopic studies are essential to determine the exact source of presynaptic mGluR2 labeling in the primate pallidum.

We recently carried out a detailed electron microscopic study of the subsynaptic localization of group I mGluRs (mGluR1a and mGluR5) in GPe and GPi of monkeys (Hanson and Smith, 1999; Smith et al.,

2000a). These data indicate that both receptor subtypes are largely expressed postsynaptically in neuronal elements including dendrites and perikarya. At the subsynaptic level, mGluR1a and mGluR5 immunoreactivities are found at the edges of asymmetric postsynaptic specializations of putative glutamatergic synapses (Fig. 5B–C); a pattern of labelling consistent with that found in the rat cerebellum and hippocampus (Nusser et al., 1994; Lujan et al., 1996; Ottersen and Landsend, 1997). However, a surprising observation was a strong mGluR1a and mGluR5 labelling associated with the core of symmetric synapses established by putative GABAergic striatal terminals (Hanson and Smith, 1999) (Fig. 5A,C). A large proportion of group I mGluR labelling was also found at extrasynaptic sites along the dendrites of GPe and GPi neurons. These observations raise questions about the potential sources of glutamate that activates these receptors and their functional significance at GABAergic synapses (see below).

To our knowledge, the distribution of group III mGluRs has not yet been studied in the primate pal-

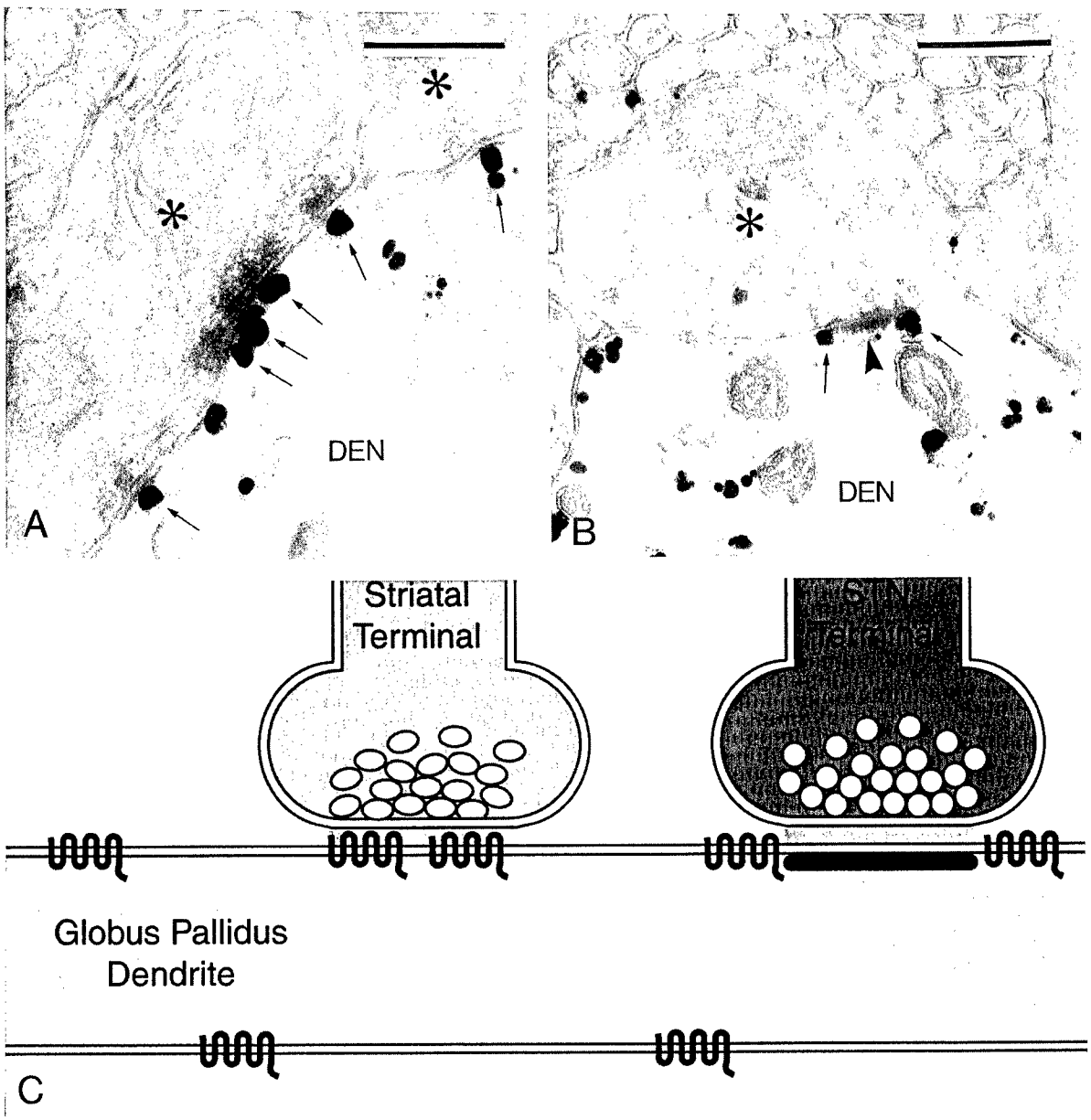

Fig. 5. Group I mGluR immunoreactivity in the monkey pallidum: (A) MGluR1a immunogold labeling (arrows) in the main body of symmetric axo-dendritic synapses established by striatal-like boutons (asterisks) in Gpi, (B) perisynaptic mGluR5 labeling (arrows) at a putative subthalamopallidal asymmetric synapse (arrowhead) and (C) summary diagram to illustrate the subsynaptic localization of group I mGluRs in the monkey pallidum. Note that group I mGluRs are expressed at both symmetric and asymmetric synapses. A substantial proportion of gold labeling was also found extrasynaptically. Scale bars: A: 0.3 μm; B: 0.6 μm.

lidum. However, data in rodents indicate that mGluR4a and mGluR7a,b are expressed pre-synaptically in striatopallidal terminals where they may modulate GABA release from the striatum (Kinoshita et al., 1998; Bradley et al., 1999; Kosinski et al., 1999). The fact that mGluR4a is selectively expressed on striatopallidal, but not striatonigral, terminals makes it an ideal target to reduce GABA release from the overactive striatopallidal projection in PD.

6.3. GABA-A receptors

Overall, the density of BZ binding sites in the primate globus pallidus is much lower than in the striatum and restricted to BZI receptor subtypes (Faull and Villiger, 1988; Waldvogel et al., 1998, 1999). The level of binding is higher in the ventral pallidum than the dorsal pallidum, and more prominent in GPe than in GPi (Faull and Villiger, 1988; Waldvogel et al., 1998, 1999). Differential changes in binding intensity between the two pallidal segments were found in postmortem brains of Huntington's patients. Whereas the GPe shows an increased binding at the very early stage of the disease, the GPi is much more strongly labelled than GPe in advanced grades of Huntington's disease (Glass et al., 2000). These data are consistent with previous findings showing that striato-GPe neurons degenerate before striato-GPi neurons in Huntington's patients (see Vonsatell and DiFiglia, 1998 for a review). The increased GABA-A binding sites might reflect a compensatory mechanism for the decrease in GABA release due to striatal cell death. Changes in pallidal BZ binding sites were also noticed in animal models of PD. MPTP-treated monkeys show an increased level of GABA/BZ binding in the GPi which could be reversed by treatment with the long acting D2 receptor agonist, cabergoline, but not by the D1 receptor agonist, SKF 82958 (Robertson et al., 1990; Calon et al., 1995, 1999). In contrast, the density of GABA/BZ binding sites is decreased in GPe after MPTP treatment (Griffiths et al., 1990; Robertson et al., 1990). This up- and down-regulation of GABA-A receptors in GPi and GPe, respectively, is consistent with the current functional model of basal ganglia circuitry which suggests that the activity of the "so-called" direct striato-GPi pathway is decreased in PD whereas the activity of the "indirect" striato-GPe projection is increased (Albin et al., 1989; DeLong, 1990).

As expected, based on their strong GABAergic innervation from the striatum (Shink and Smith, 1995), pallidal neurons are enriched in various GABA-A receptor subunits in primates and non-primates (Zhang et al., 1991; Fritschy and Möhler, 1995; Charara and Smith, 1998; Kultas-Ilinsky et al., 1998; Waldvogel et al., 1998, 1999). Using double labeling immunofluorescence techniques, Waldvogel et al. (1999) studied the expression of various GABA-A receptor subunits in chemically characterized neurons in the human pallidum. Their main findings are: (1) Pallidal neurons are devoid of $\alpha 2$ subunit immunoreactivity, (2) GABAergic pallidal neurons immunoreactive for parvalbumin and a subpopulation of strongly immunoreactive calretinin (CR)-containing neurons express high levels of $\alpha 1$, $\alpha 3$, $\beta 2/3$ and $\gamma 2$ immunoreactivity and (3) a subpopulation of pallidal neurons which display very intense CR immunoreactivity express $\alpha 1$, $\beta 2/3$ and $\gamma 2$ subunit immunoreactivities. Findings in monkeys are consistent with those data except that the $\alpha 2$ mRNA is expressed at a moderate level in GPe and GPi neurons in non-human primates (Kultas-Ilinsky et al., 1998). The lack of $\alpha 2$ subunit in pallidal neurons seems to be a feature unique to the human pallidum since GP neurons also display $\alpha 2$ immunoreactivity in rats (Fritschy and Möhler, 1995). It is worth noting that monkey pallidal neurons also express moderate to high level of $\alpha 4$ and δ subunit mRNAs, but are devoid of $\gamma 1$ subunit (Kultas-Ilinsky et al., 1998). The $\beta 1$ subunit mRNA is expressed at a low level in GPe but is not detectable in the monkey GPi (Kultas-Ilinsky et al., 1998). The subcellular localization of $\alpha 1$ and $\beta 2/3$ subunit immunoreactivity has been studied at the electron microscope level using pre-embedding immunoperoxidase methods (Charara and Smith, 1998; Waldvogel et al., 1998). Both antibodies resulted in intense labeling of the plasma membrane of GPe and GPi neurons. In some cases, aggregates of peroxidase reaction product were associated with symmetric and asymmetric post-synaptic specializations (Waldvogel et al., 1998), suggesting the existence of GABA-A receptor subunits at both GABAergic and putative glutamatergic synapses. Strong labeling was also found at non-synaptic sites along the plasma membrane. To further characterize the subsynaptic localization of the GABA-A receptor subunits in the monkey pallidum, we started a series of electron microscopic post-embedding immunogold studies of $\alpha 1$, $\beta 2/3$ and $\gamma 2$ subunits immunoreactivity in rhesus monkeys. So far, our data indicate that the $\alpha 1$ and $\beta 2/3$ subunit immunoreactivity is confined to the main body of symmetric synapses (Fig. 6A, Fig. 7), which is consistent with recent data in rodents using the same approach (Somogyi et al., 1996; Fujiyama et al., 1998).

6.4. GABA-B receptors

Low to moderate levels of GABA-B binding sites are expressed in both pallidal segments in normal monkeys (Ambardekar et al., 1999; Bowery et al., 1999). After MPTP treatment, the level of GABA-B receptors is significantly increased in GPi, but no changes are seen in GPe, striatum and SNr (Calon et al., 2000). The increase in GPi is not affected by treatment with D1

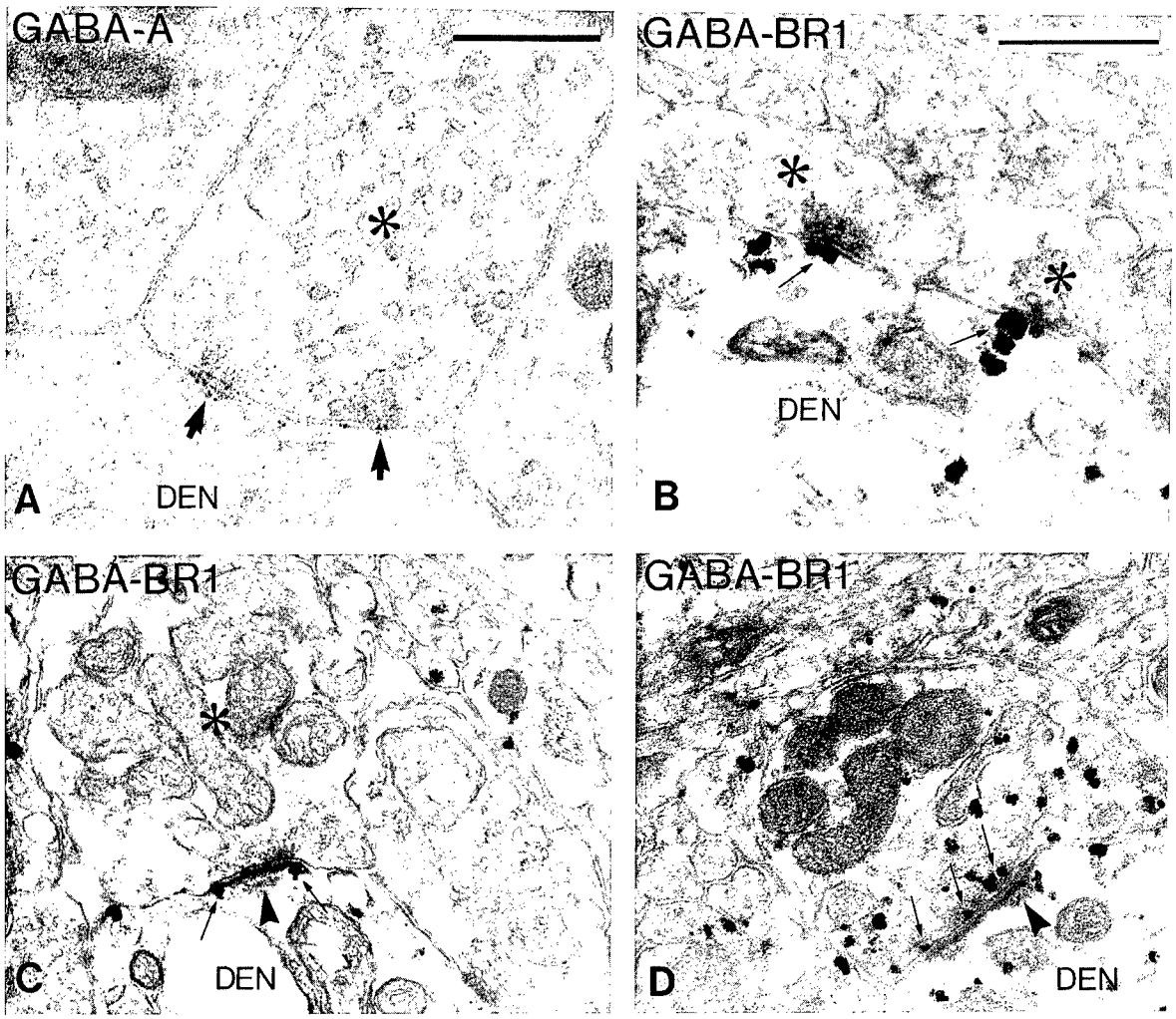

Fig. 6. GABA-A and GABA-B receptors in the monkey pallidum: (A) GABA-A receptor α1 subunit immunoreactivity in the main body of a symmetric synapse (small arrows) established by a striatal-like bouton (asterisk), (B) postsynaptic GABA-BR1 immunoreactivity (small arrows) at symmetric synapses established by putative striatal boutons (asterisks), (C) postsynaptic labelling (arrows) at the edges of an asymmetric synapse estalished by a STN-like bouton in GPi (asterisk) and (D) presynaptic immunogold labeling of a STN-like bouton in GPi. Note that some gold particles are located in the presynaptic grid of the asymmetric synapse (arrows), but the majority of labeling is intracellular. Scale bars: A: 0.3 μm; B: 0.5 μm (valid for C–F).

dopamine receptor agonist, but is partly reversed by cabergoline, a potent D2 dopamine receptor agonist (Calon et al., 2000).

Recent immunohistochemical studies revealed the existence of both GABA-BR1 and GABA-BR2 receptor subunit immunoreactivity in virtually all pallidal neurons in humans (Billinton et al., 2000) and monkeys (Charara et al., 2000a,b). Overall, the pattern of both GABA-B receptor subtypes is the same throughout GPe and GPi except that the intensity of labelling for GABA-BR1 is much stronger than that of GABA-BR2.

At the electron microscope level, GABA-BR1 and GABA-BR2 immunoreactivity is enriched in post-synaptic neuronal elements including perikarya and dendritic shafts of various sizes (Charara et al., 2000a,b). At the subsynaptic level, GABA-BR1 immunoreactivity is commonly found in the main body of symmetric synapses established by striatal-like GABAergic terminals in both GPe and GPi or at the edges of asymmetric post-synaptic specializations of axo-dendritic synapses (Fig. 6B–C, Fig. 7). Extrasynaptic labeling is also detected in neuronal perikarya and dendrites. The most striking feature of GABA-BR1 and GABA-BR2 immunoreactivity in the GPe and GPi is the pre-synaptic labeling of numerous unmyelinated axonal segments and putative STN-like glutamatergic terminals forming asymmetric synapses (Charara et al., 2000a,b) (Fig. 6D, Fig. 7), suggesting that GABA-B act as heteroreceptors to modulate glutamate release in the globus pallidus. Another population of lightly labeled terminals that display the ultrastructural features of striatal boutons and form symmetric axo-dendritic synapses also display GABA-BR1 and GABA-BR2 immunoreactivity in both pallidal segments.

7. Glutamate and GABA receptors in the subthalamic nucleus

7.1. Ionotropic and metabotropic glutamate receptors

Very little is known about ionotropic glutamate receptor localization in the primate STN. Binding studies indicate that NMDA, AMPA and kainate receptors are expressed at a low to moderate level in this brain region in humans (Lee and Choi, 1992; Ball et al., 1994). Immunocytochemical studies revealed the existence of strong neuronal labeling for the AMPA GluR1 subunit in monkeys (Ciliax et al., 1997). Nigrostriatal dopaminergic denervation does not induce significant changes in GluR1 protein expression in STN neurons of MPTP-treated monkeys (Betarbet et al., 2000). Although immunohistochemical studies of other ionotropic glutamate receptor subunits have not been carried out in the primate STN, data in rodents indicate that various types of NMDA, AMPA and kainate receptor subunits are expressed in this brain structure (Petralia and Wenthold, 1992; Standaert et al., 1994; Petralia et al., 1994a,b; Bischoff et al., 1997). At the subsynaptic level, AMPA and NMDA receptor subunits are co-expressed in the core of asymmetric synapses, though some of the AMPA GluR2/3 immunoreactivity is also associated with non-synaptic sites and symmetric synaptic junctions in rats (Clarke and Bolam, 1998). Such information has not yet been gathered in primates.

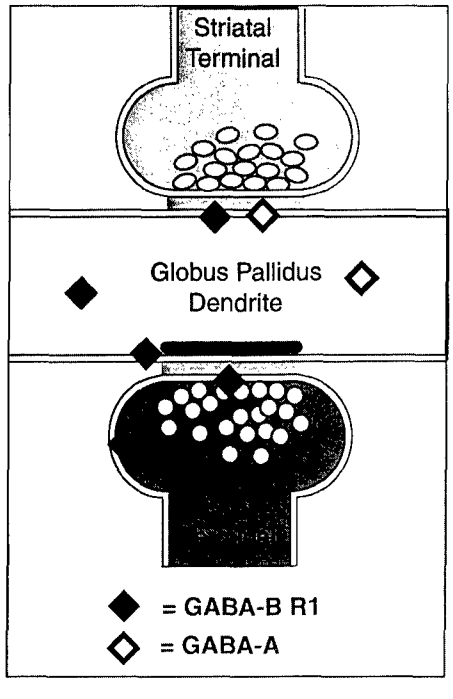

Fig. 7. Summary diagram to illustrate the subsynaptic localization of GABA-A and GABA-BR1 immunoreactivity in the monkey pallidum.

Group I and group II mGluRs are expressed in the primate STN. Immunoreactivity for mGluR2 is far less intense in the STN than other parts of the basal ganglia in humans (Phillips et al., 2000). This is surprising since STN neurons show high levels of mGluR2 mRNA expression in the rat (Testa et al., 1994). Although neuronal perikarya are lightly labeled, the neuropil of the STN displays strong immunoreactivity for both mGluR1a and mGluR5 in monkeys. At the electron microscope level, both group I mGluRs are found almost exclusively in post-synaptic elements. Immunogold particles are commonly found at the edges of symmetric and asymmetric post-synaptic specializations (Fig. 8A,C). Interestingly, labelling is also found at the edges of puncta adherentia between putative GABAergic GPe terminals and STN dendrites (Fig. 8D). In some cases, gold particles are associated with the main body of "en passant type" symmetric synapses established by vesicle-filled axon-like processes or at adherent junctions between putative glial processes and neuronal structures (Fig. 8B,D). Extrasynaptic labeling is frequently found for both receptor subtypes. The group III mGluRs localization has not been studied in primates, but in situ hybridization and immunocytochemical data indicate that the mGluR4 and mGluR7 expression is very low in the rat STN (Ohishi et al., 1995; Bradley et al., 1999; Kosinski et al., 1999).

7.2. Ionotropic and metabotropic GABA receptors

In the monkey, STN neurons express high level of mRNA for the $\alpha 1$, $\alpha 3$, $\beta 2$, $\beta 3$, $\gamma 2$ and δ subunits of the GABA-A receptors, but are devoid of $\alpha 2$, $\alpha 4$, $\beta 1$ and $\gamma 1$ subunits (Kultas-Ilinsky et al., 1998). A recent study showed that the human STN is particularly enriched in the ε subunit (Davies et al., 1997). Although STN neurons also display strong immunoreactivity for various GABA-A receptor subunits in rats and monkeys (Zhang et al., 1991; Wisden et al., 1992; Fritschy and Möhler, 1995; Charara and Smith, 1998), there is some discrepancy between rodents and primates regarding the type of GABA-A receptor subunits expressed in this nucleus. For instance, the $\alpha 2$ subunit immunoreactivity is abundant in rat STN neurons whereas the mRNA encoding this subunit is not detectable in the monkey STN. In contrast, the δ subunit mRNA is abundant in the monkey STN but the immunoreactivity for this protein does not reach a detectable level in rats (Fritschy and Möhler, 1995). Whether these data indicate a real species difference in the subunit composition of GABA-A receptors between rodents and primates remains to be established.

STN neurons display low to moderate immunoreactivity for GABA-BR1 and GABA-BR2 subtypes in monkey and human STN (Billinton et al., 2000; Charara et al., 2000a,b). At the electron microscope

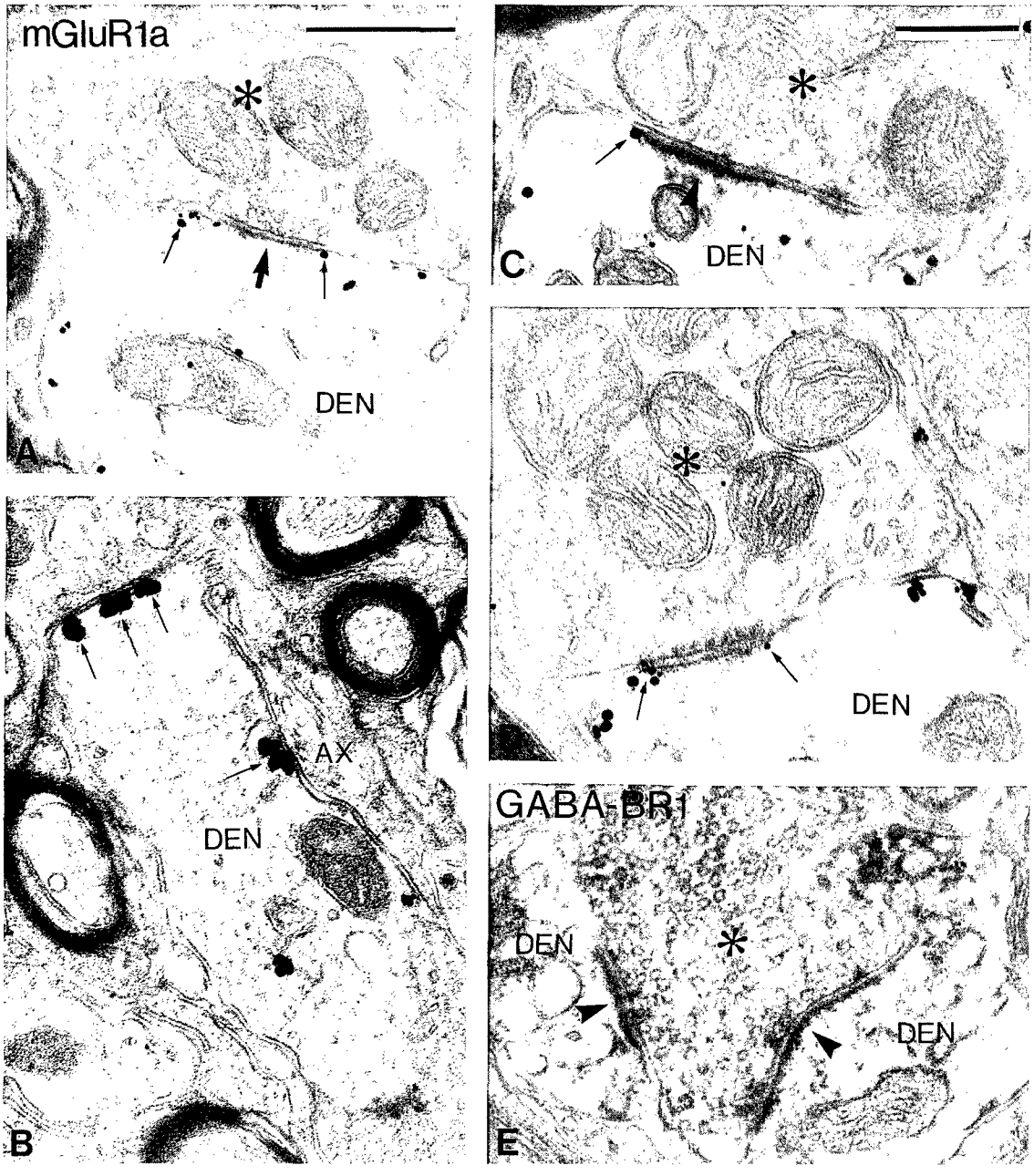

Fig. 8. Group I mGluRs and GABA-B receptor immunoreactivity in the STN: (A) mGluR1a labeling (small arrows) at the edges of a symmetric axo-dendritic synapse (arrows), (B) mGluR1a immunoreactivity at a "en passant" type symmetric synapse established by a vesicle- filled axon-like process (AX). Aggregates of gold particles at extrasynaptic sites along the labeled dendrite are not shown in these micrographs. (C–D) mGluR1a immunoreactivity at the edges of an asymmetric postsynaptic specialization (C) or a puncta adherentia between a putative GABAergic GPe terminal and a dendrite (D) and (E) A GABA-BR1-immunoreactive terminal forming asymmetric synapses (arrowheads) with dendrites (DEN). Scale bars: A: 0.2 μm (valid for B–E).

level, both receptor subtypes are expressed post-synaptically on dendrites of STN neurons and pre-synaptically in putative glutamatergic axon terminals in monkeys (Charara et al. 2000a,b) (Fig. 8E). As was found in other basal ganglia structures, the GABA-BR2 immunoreactivity is far less intense than the GABAR1 immunostaining in the STN (Charara et al., 2000a,b). Together, these data indicate that both GABA-A and GABA-B receptors are likely to mediate postsynaptic inhibition by GPe in STN neurons. In addition, GABA-B receptors may also control the activity of STN neurons by presynaptic inhibition of neurotransmitter release from extrinsic and/or intrinsic glutamatergic terminals.

8. Glutamate and GABA receptors in the substantia nigra

8.1. Ionotropic and metabotropic glutamate receptors in midbrain dopaminergic neurons

Glutamate plays a major role in controlling the firing rate and firing pattern of midbrain dopaminergic neurons in rats (Grace and Bunney, 1984; Smith and Grace, 1992). On the other hand, glutamate may also become excitotoxic to dopaminergic neurons in PD. Although the exact mechanisms underlying this excitotoxic phenomenon still remains to be established, there is increasing evidence that an intracellular rise in calcium via NMDA receptor activation might be involved (Blandini et al., 1996). If such is the case, one would expect neurons in the VTA and dorsal tier of the SNc, which are less sensitive to neurodegeneration in PD, to express a lower level of Ca^{+2}-permeable NMDA receptors than the highly sensitive ventral tier SNc neurons. Recent in situ hybridization data in humans indicate that such is not the case (Counihan et al., 1998). The levels of NMDAR1 and NMDAR2 (a–d) subunit mRNAs are not significantly different between the various midbrain dopaminergic cell groups, except that neurons of the pars lateralis express a slightly higher level of labelling for all NMDA receptor subunits examined (Counihan et al., 1998). These data also show that the NMDAR2D is, by far, the most abundant NMDAR2 subunit expressed in the different subgroups of SNc neurons. On the other hand, data in squirrel monkeys demonstrate that the NMDAR1 subunit mRNA expression is significantly higher in ventral tier SNc neurons than in the dorsal tier of the SNc and VTA (Paquet et al., 1997). Similarly, the AMPA GluR2 subunit is more abundant in ventral than dorsal SNc neurons whereas the GluR1 subunit is homogeneously distributed among midbrain dopaminergic cell groups (Paquet et al., 1997). These mRNA data are consistent with immunohistochemical findings showing that SNc and VTA dopaminergic neurons display moderate to strong immunoreactivity for the NMDAR1 and the AMPA (GluR1, GluR2/3 and GluR4) glutamate receptor subunits in monkeys (Paquet et al., 1997). However, SNc/VTA neurons are almost completely devoid of NMDAR2 A/B immunoreactivity, which is in line with recent rodent data (Standaert et al., 1994; Albers et al., 1999). At the subcellular level, the GluR1, GluR2/3 and NMDAR1 immunoreactivity is mostly associated with postsynaptic elements, though a small number of preterminal axons, axon terminals and glial cell processes are also labelled (Paquet et al., 1997).

Both group I mGluR subtypes (mGluR1a and mGluR5) are expressed in midbrain dopaminergic neurons in monkeys. Analysis of immunogold labelling at the electron microscopic level revealed that both receptor subtypes are mostly expressed postsynaptically: (1) at the edges of asymmetric post-synaptic specializations (Fig. 9A), (2) in the main body of symmetric, putative GABAergic, synapses (Fig. 9B) and (3) extrasynaptically along the neuronal plasma membrane. A major difference between the subcellular distribution of mGluR5 and mGluR1a immunoreactivity is that a large part of mGluR1a labelling is bound to the plasma membrane whereas most mGluR5 immunostaining is intracellular. This differential distribution seems to be a common feature for the two group I mGluR subtypes in both SNr and SNc in rats and monkeys (Hubert and Smith, 1999). The functional significance of this large internalized pool of mGluR5 under basal conditions remains to be established. The dopaminergic neurons do not show any detectable mGluR2 immunoreactivity in the human substantia nigra (Phillips et al., 2000). Although group III mGluR localization has not been studied in the primate SN, recent data indicate that both SNc and SNr neurons do not express detectable levels of mGluR4 and mGluR7 immunoreactivity or mRNAs in rats (Ohishi et al., 1995; Bradley et al., 1999; Kosinski et al., 1999).

8.2. GABA-A and GABA-B receptors in midbrain dopaminergic neurons

Midbrain dopaminergic neurons in the SNc express varying degrees of GABA-A receptor subunits in monkeys. The α1 and α2 subunits are expressed at a low level whereas the α3, α4, β1, β2, β3, γ2 and δ are much more abundant. It is worth noting that only SNc neurons express the β1 subunit mRNA in monkey basal ganglia (Kultas-Ilinsky et al., 1998). As is the case for most basal ganglia structures, SNc neurons do not express a detectable level of γ1 subunit mRNA.

The SNc displays the highest GABA-B receptor binding site densities in the monkey basal ganglia (Ambardekar et al., 1999) and expresses strong and moderate GABA-B R1 and GABA-B R2 immunoreactivity, respectively (Billinton et al., 2000; Charara et al., 2000a,b). MPTP lesion of dopaminergic neurons results in a significant loss of GABA-B binding sites in the monkey SNc, suggesting that GABA-B receptors are largely expressed on SNc neurons (Calon et al., 2000). At the electron microscopic level, immunoreactivity for both GABA-B receptor subtypes is, indeed, largely found postsynaptically in neuronal perikarya and dendrites. Rare pre-terminal axons and terminal boutons forming asymmetric synapses are occasionally labelled (Charara et al., 2000a,b) (Fig. 9C).

8.3. Ionotropic and metabotropic glutamate receptors in SNr GABAergic neurons

The pattern of distribution of ionotropic glutamate

receptors in SNr neurons is similar to that seen in the globus pallidus. Furthermore, as is found in the monkey GPi, the expression of the AMPA receptor subunit, GluR1, is downregulated in SNr neurons of parkinsonian monkeys (Betarbet et al., 2000).

8.4. GABA-A and GABA-B receptors in SNr GABAergic neurons

The pattern of GABA-A/BZ binding sites in the SNr and the expression of GABA-A receptor subunits is

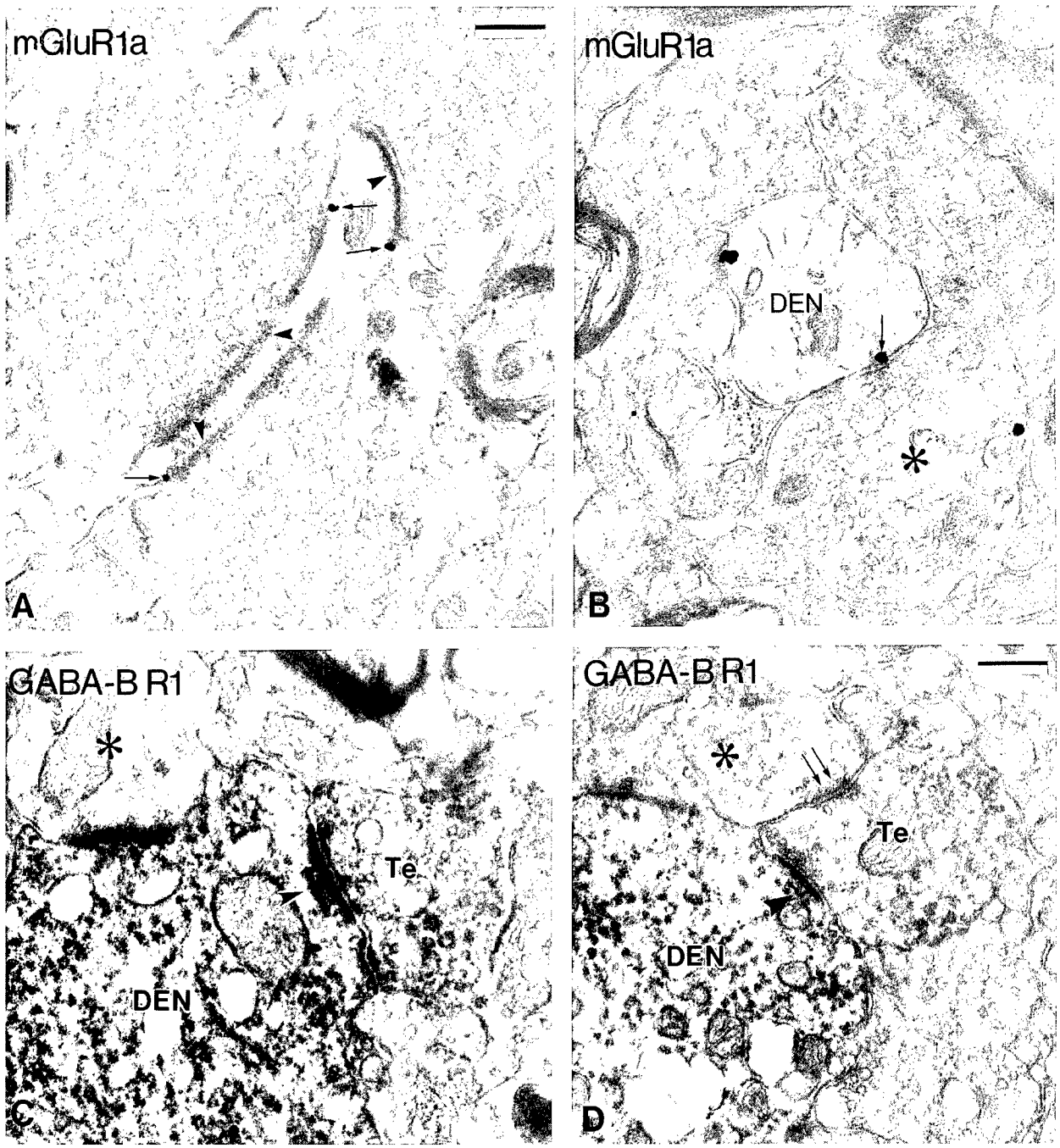

Fig. 9. Group I mGluRs and GABA-B receptors in monkey SNc and SNr: (A) MGluR1a immunogold particles at the edges of asymmetric synapses on a small spine-like process in SNc, (B) MGluR1a labeling in the main body of a symmetric axo-dendritic synapse in SNr, (C) A GABA-BR1-immunoreactive axon terminal forming an asymmetric synapse with a labeled dendrite. The asterisk indicates an unlabeled bouton in contact with the same dendrite in SNc and (D) A GABA-BR1-containing terminal in asymmetric contact with an immunoreactive dendrite in the SNr. The asterisk indicates a nonimmunoreactive terminal in contact with the labeled terminal. Scale bars: A: 0.25 μm (valid for B–C); D: 0.5 μm.

largely similar to that in the globus pallidus (Robertson et al., 1989) (see above), except for the expression of the α4 subunit mRNA which is abundant in GPi but not detectable in the monkey SNr (Kultas-Ilinsky et al., 1998). Following MPTP treatment, a significant increase in GABA-A/BZ binding sites is observed in the monkey SNr (Robertson et al., 1989), which is consistent with the hypothesis that the "direct" striatonigral GABAergic pathway is underactive in PD (DeLong, 1990). Although a detailed electron microscopic analysis of the subsynaptic localization of these receptors has not yet been carried out in primates, recent immunogold data indicate that most GABA-A receptor subunits are expressed in the core of symmetric striatonigral synapses in rats (Fujiyama et al., 1998).

In general, the SNr displays a low level of GABA-B receptor binding sites (Ambardekar et al., 1999) and is lightly labelled with GABA-BR1 and GABA-BR2 receptor antibodies in monkeys (Charara et al., 2000a,b) and humans (Billinton et al., 2000). No change in GABA-B receptor binding is seen in the SNr following MPTP treatment (Calon et al., 2000). The pattern of subcellular distribution of GABA-B receptor subtypes in the monkey SNr resembles that seen in the globus pallidus, i.e. dendrites and many preterminal unmyelinated axons as well as a population of STN-like terminals forming asymmetric synapses display GABA-BR1 and GABA-BR2 immunoreactivity (Charara et al., 2000a,b) (Fig. 9(D)). Another population of striatal-like boutons display immunoreactivity for both receptor subtypes (Charara et al., 2000a,b).

9. Potential sources of activation of metabotropic receptors

One of the main features which characterizes the subsynaptic localization of metabotropic glutamate and GABA receptors is their strong expression at non-synaptic sites. In fact, this pattern of distribution was also found for other types of G-protein-coupled receptors such as dopamine, muscarinic and opiate receptors (Yung et al., 1995; Svingos et al., 1997; Bernard et al., 1998; Muriel et al., 1999). These data suggest that extrasynaptic spillover of neurotransmitter or neuropeptides might be a common mechanism to activate G protein-coupled receptors in the CNS.

One of the most surprising findings presented in this review was the localization of group I mGluRs at putative GABAergic striatal synapses in GPe, GPi and SNr (Hanson and Smith, 1999). This raises questions about the sources of activation and potential functions of these receptors at GABAergic synapses. A first possible source of glutamate might be the spillover of transmitter released from glutamatergic terminals. Extrasynaptic diffusion of glutamate to activate AMPA and NMDA receptors was, indeed, demonstrated in the rat hippocampus and cerebellum (Asztely et al., 1997; Barbour and Hausser, 1997; Kullmann and Asztely, 1998; Dzubay and Jahr, 1999). Since mGluRs display a stronger affinity for glutamate than ionotropic receptors (Conn and Pin, 1997), it is likely that even a small amount of spilled over neurotransmitter is enough to induce mGluRs activation. Another possibility is that glutamate released from astrocytes activates mGluRs located at symmetric synapses and, possibly, those located extrasynaptically. Data obtained over the past few years showing that astrocytes express various ion channels and contain glutamate receptors (Sontheimer et al., 1996; Steinhauser and Gallo, 1996; Verkhratsky and Kettenmann, 1996; Carmignoto et al., 1998; Porter and McCarthy, 1997), have shifted the traditional concept of astrocytes as simple structural support for neurons to a view in which glial cells play a more active role in information processing and neuronal communication in the central nervous system (Parpura et al., 1994; Antanitus, 1998). It is well established that neuronal stimulation induces waves of elevated intracellular calcium which propagate between glial cells and lead to glutamate release (Parpura et al., 1994; Araque et al., 1999). A third possibility is that striatal terminals, under certain circumstances, release excitatory amino acids. Although this is not consistent with the current view of neurotransmission at striatofugal synapses, indirect evidence suggests that striatal neurons may co-express, and possibly co-release, GABA and glutamate as neurotransmitters. First, striatopallidal neurons possess a high-affinity uptake system for glutamate and aspartate (White et al., 1994). Second, excitatory post-synaptic currents sensitive to the glutamate antagonist CNQX are found in cultures consisting only of dissociated striatal neurons (Dubinsky, 1989). Third, in vivo stimulation of the CD produces a combination of excitatory (EPSPs) and inhibitory (IPSPs) post-synaptic potentials in the rat globus pallidus (Levine et al., 1974; Kita and Kitai, 1991). Although these excitatory effects can be attributed to activation of axons extrinsic to the striatum or multisynaptic pathways, they might also originate from intrinsic striatal neurons. Even if co-release of fast neurotransmitters such as glutamate and GABA is clearly not a common feature in the CNS, the synaptic co-release of GABA and glycine was shown in the spinal cord (Jonas et al., 1998). Furthermore, Jo and Schlichter (1999) recently showed that the fast excitatory neurotransmitter ATP is co-released with the inhibitory neurotransmitter GABA at individual synapses in cultured spinal neurons. If glutamate, indeed, activates mGluRs at GABAergic synapses, it is likely that the post-synaptic mGluR responses regulate GABA currents in pallidal neurons either by changing membrane excitability through modulation of calcium and potassium channels (Conn and Pin, 1997) or via direct physical interactions with GABA-A or GABA-B

receptors as was recently shown in vitro for dopamine D5 and GABA-A receptors (Liu et al., 2000). Modulatory effects of GABAergic transmission by mGluRs were shown in various brain regions in the rat including the spinal cord, the nucleus of the solitary tract, the SNc and the hippocampus (Glaum and Miller, 1993, 1994; Bonci et al., 1997; Morishita et al., 1998).

Another surprising observation made in our studies was the localization of pre- and postsynaptic GABA-BR1 and GABA-BR2 receptors immunoreactivity associated with putative glutamatergic terminals. That axo-axonic synapses are very rare in the basal ganglia rule out the hypothesis of direct synaptic release of GABA to activate these receptors. Another possibility would be that, once released, GABA diffuses out of the synaptic cleft and activates extra- and presynaptic GABA-B heteroreceptors (Attwell et al., 1993). Evidence for such a paracrine mode of GABA-B receptor activation was, indeed, demonstrated in the rat hippocampus (Isaacson et al., 1993). The efficacy of such a non-specific mode of transmission largely depends on the extent to which GABA can diffuse and the affinity of GABA-B receptors for its transmitter. Although such information is still lacking for basal ganglia structures, it is worth noting that presynaptic GABA-B receptors were found to have a much higher affinity for GABA than GABA-A receptors in the rat hippocampus (Yoon and Rothman, 1991).

10. Metabotropic glutamate and GABA-B receptors: novel therapeutic targets for Parkinson's disease

An imbalance of activity between the direct and indirect striatofugal pathways in favor of the indirect pathway is thought to underlie most symptoms of PD (DeLong, 1990). The increased activity of the glutamatergic subthalamopallidal and, possibly, corticostriatal projections in animal models of PD led various groups to test the potential therapeutic benefits of ionotropic glutamate receptor antagonists in alleviating parkinsonian symptoms (see Starr, 1995; Blandini et al., 1996 for reviews). Systemic administration of NMDA and non-NMDA antagonists with subthreshold doses of L-DOPA or D2 dopamine receptor agonist has proven to ameliorate symptoms in primate models of PD (Starr, 1995; Blandini et al., 1996). Data reported in this review strongly suggest that interactions with metabotropic glutamate and GABA-B receptors may also have beneficial effects in PD. Drugs interacting with these receptors are expected to influence the induction and progression of the symptoms of the disease without hampering the efficiency of fast glutamatergic and GABAergic synaptic transmission, thereby, reducing unwanted side effects commonly seen with drugs that target ionotropic receptors (Starr, 1995).

The group I mGluRs located perisynaptically at STN synapses in GPe and GPi should be considered as a potential target in PD because the perisynaptic mGluR1a and mGluR5 are likely to be activated by excessive amounts of glutamate released during hyperactivity of subthalamopallidal synapses in parkinsonians. Group I mGluR activation might, then, lead to increased activity of basal ganglia output neurons through various mechanisms including potentiation of ionotropic glutamatergic transmission, reduction of K^+ conductances etc. (see Conn and Pin, 1997 for details). Group I mGluR antagonists should, therefore, reduce the over-excitatory drive generated by the STN in pallidal neurons.

Based on rodent data, activation of the group III mGluRs, mGluR4, seems to be an ideal strategy to alleviate symptoms of PD. It is well established that activation of presynaptic group III mGluRs reduces neurotransmitter release in the hippocampus (Conn and Pin, 1997). If such is also the case in GP, activation of these receptors in parkinsonians should reduce the activity of the overactive indirect pathway by reducing GABA release at striatopallidal synapses, thereby inhibiting subthalamopallidal neurons which, in turn, relieve basal ganglia output neurons in GPi and SNr from their tonic excitatory drive. The final outcome of such therapy should be an increased activity of thalamocortical neurons and facilitation of motor behaviors.

Another mGluR subtype of interest for PD therapy is mGluR2, which was found to be expressed on subthalamonigral terminals in rats (Bradley et al., 2000). Furthermore, activation of these receptors in brain slices reduces glutamatergic transmission at subthalamonigral synapses (Bradley et al., 2000) and systemic administration of group II agonist reverses haloperidol-induced catalepsy (Bradley et al., 2000).

So far, only a few specific agents for group II (LY354740 and LY379268) and group I (MPEP) mGluRs were found to produce central pharmacological actions when administered systemically in animals (Helton et al., 1998; Moghaddam and Adams, 1998; Bordi and Ugolini, 1999; Schoepp et al., 1999). However, the potential therapeutic benefit of such agents will likely drive the development of additional compounds that could be administered systemically for novel medical purposes.

The expression of GABA-B receptors in subthalamopallidal and subthalamonigral terminals (Charara et al., 2000a,b) suggests that activation of these pre-synaptic heteroreceptors might attenuate the overflow of glutamate released by STN neurons in PD. In support of this hypothesis, application of baclofen was found to reduce the evoked synaptic currents mediated by glutamate in the rat SNr in vitro (Shen and Johnson, 1997). The current use of GABA-B agonists in therapeutics is mostly restricted to baclofen in the treatment of spastic-

ity (Porter, 1997). In fact, the beneficial antispastic effect of baclofen is believed to derive from the suppression of excitatory neurotransmitter release to motoneurons in the spinal cord (Fox et al., 1978; Davies, 1981; Bonanno et al., 1998). Future behavioural studies of baclofen in animal models should help ascertain the potential therapeutic efficacy of this drug for PD and understand better the functions of GABA-B in modulating glutamatergic neurotransmission in the basal ganglia circuitry. Interestingly, baclofen was found to reduce haloperidol-induced dyskinesias without causing gross motor depression in squirrel monkeys (Neale et al., 1984).

11. Concluding remarks

The data reviewed in this paper highlight the complexity of GABAergic and glutamatergic synaptic transmission in the primate basal ganglia. The rather unusual pattern of subsynaptic localization of mGluRs and GABA-B receptors in various basal ganglia structures suggests that activation of these receptors may mediate complex presynaptic heteroreceptor functions and/or induce postsynaptic receptor interactions with other neurotransmitter receptor subtypes. These observations, combined with recent evidence for the extrasynaptic diffusion of GABA and glutamate in the CNS, clearly indicate that the activation of metabotropic receptors is likely to be far more complex than the current concept of synaptic receptor activation largely based on ionotropic receptor studies. A better understanding of GABAergic and glutamatergic transmission in normal and pathological basal ganglia functions surely relies upon further analyses of the anatomical localization, physiological effects and pharmacological properties of mGluR and GABA-B receptor subtypes in the primate basal ganglia.

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LETTER TO NEUROSCIENCE

PRE-SYNAPTIC KAINATE RECEPTORS IN GABAERGIC AND GLUTAMATERGIC AXON TERMINALS IN THE MONKEY GLOBUS PALLIDUS

R. KANE-JACKSON AND Y. SMITH*

Division of Neuroscience, Yerkes National Primate Research Center and Department of Neurology, Emory University, 954, Gatewood Road Northeast, Atlanta, GA 30322, USA

Abstract—Although the localization and role of kainate receptors in the CNS remain poorly known, complex, and rather unusual, pre-synaptic auto- and heteroreceptor functions have been disclosed in various brain regions. Basal ganglia nuclei, including the globus pallidus, are enriched in GluR6/7 immunoreactivity. Using electron microscopic immunocytochemistry for GluR6/7 combined with post-embedding immunogold labeling for GABA, we demonstrate that GluR6/7 immunoreactivity is enriched in a large subpopulation of small unmyelinated, presumably pre-terminal, axons as well as GABAergic and putative glutamatergic axon terminals in the internal and external segments of the globus pallidus in monkey. Our findings suggest that kainate receptors are located to subserve pre-synaptic modulation of inhibitory and excitatory transmission in the primate globus pallidus.

Key words: pallidum, striatopallidal, subthalamic nucleus, striatum, heteroreceptor, autoreceptor.

Although kainic acid has long been known as a major neurotoxin, the localization and functions of kainate receptors (KARs) remain poorly understood. The lack of pharmacological agents that could selectively modulate KARs has hampered considerably the advancement of knowledge about these receptors. The recent introduction of specific AMPA receptor antagonists (GYKI compounds) represents a major step forward in understanding KARs function. Data obtained so far, largely from the hippocampus and cerebellum, have revealed that KARs form a rather unique population of ionotropic glutamate receptors that mediate complex pre- and post-synaptic effects on glutamate and GABA neurotransmission (see Lerma et al., 1997, 2001; Chittajallu et al., 1999; Kamiya, 2002 for reviews).

Apart from two recent studies in the striatum (Chergui et al., 2000; Casassus and Mulle, 2002), the knowledge of KARs functions in the basal ganglia circuitry remains elusive. We recently showed that KARs are expressed pre- and post-synaptically in the monkey striatum (Charara et al., 1999; Kieval et al., 2001). Pre-synaptic receptors are confined to glutamatergic afferents while the post-synaptic labeling is mainly expressed in dendrites of projection neurons and interneurons. These observations indicate that KARs may pre- and post-synaptically modulate glutamatergic transmission in the monkey striatum. Knowing that KARs act as heteroreceptors at GABAergic synapses in various brain regions (Lerma et al., 2001) another potential outcome of our striatal data could be that KARs, synthesized in striatal projection neurons, may be transported along striatofugal axons to the globus pallidus and substantia nigra where they may act as heteroreceptors to modulate GABA release. To test this hypothesis, we performed pre- and post-embedding immunolabelling for GluR6/7 KAR subunits and GABA, respectively, in the internal (GPi) and external (GPe) segments of the globus pallidus in rhesus monkeys.

EXPERIMENTAL PROCEDURES

Three adult rhesus monkeys were used in this study. After perfusion with 4% paraformaldehyde and 0.1% glutaraldehyde, the brains were cut in 60 μm-thick sections with a vibratome and processed for immunocytochemistry using a highly specific commercially available polyclonal GluR6/7 antiserum (Upstate Biotech, Lake Placid, NY; dilution 1.5 μg/ml) raised against a synthetic peptide corresponding to the intracellular carboxyl terminus of the GluR6 subunit (TFNDRRLPGKETMA). The specificity of this antibody was determined by immunoblots of cell membranes from transfected human embryonic kidney cells (HEK 293 cells; Petralia et al., 1994; Wenthold et al., 1994) and monkey brain tissue (Kieval et al., 2001). In both cases, the antibody labeled a single band, which corresponds to the molecular weight of the GluR6 subunit (Kieval et al., 2001). Preabsorption of the antibody with the synthetic antigenic peptide (10 μg/ml for 1 h) abolished completely immunoreactivity on immunoblots and fixed monkey brain tissue (Kieval et al., 2001). However, as a result of the sequence homology at the carboxyl terminus between the GluR6 and the GluR7 subunits, the antibodies also recognize the GluR7 subunit to some degree; hence the term GluR6/7 for this antiserum. To confirm that the sequence of amino acids of the synthetic peptides used to produce these antibodies are not found in other known proteins, we performed a search for amino acids sequence alignment in the BLAST database (Altschul et al., 1997), and found that there was no significant homology with any proteins other than the GluR6/7 subunits. This search also revealed that the amino acids sequence used is found in the

*Corresponding author. Tel: +1-404-727-7519; fax: +1-404-727-3278.
E-mail address: yolands@rmy.emory.edu (Y. Smith).

Abbreviations: ANOVA, analysis of variance; DAB, 3,3′ diaminobenzidine tetrahydrochloride; GPe, globus pallidus, external segment; GPi, globus pallidus, internal segment; KARs, kainate receptors; STN, subthalamic nucleus.

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Fig. 1. Light micrograph showing GluR6/7 immunoreactive elements in GPe and putamen (Put). Note the dense neuropil and cellular staining in both regions. The arrow indicates an immunoreactive neuronal perikaryon in GPe. Scale bar=100 μm.

GluR6/7 subunit of both rats and humans, suggesting that these antibodies should recognize their corresponding antigenic sites in both primates and nonprimates.

The anesthesia and euthanasia procedures were performed according to the National Institutes of Health guidelines and have been approved by the Institutional Animal Care and Use committee at Emory University. All efforts were made to minimize the number of animals used and their suffering. The GluR6/7 immunoreactivity was localized with the avidin biotin peroxidase method (Hsu et al., 1981) using 3,3′diaminobenzidine (DAB) as chromogen (see details in Charara et al., 1999; Kieval et al., 2001). After processing for electron microscopy (osmium fixation, and dehydration) and embedding in resin (Durcupan, ACM; Fluka), blocks of tissue from GPe and GPi were cut in ultrathin sections and examined with a Zeiss EM10C electron microscope. A series of sections were processed for postembedding immunogold localization of GABA prior to electron microscope analysis. The specificity of the GABA antiserum (Sigma Chemical Co, St. Louis, MO, USA) and the postembedding immunogold approach used in this study have been previously described (Kieval et al., 2001; Dallvechia-Adams et al., 2002).

To estimate the relative abundance of GluR6/7-immunoreactive elements in GPe and GPi, a series of 50 electron micrographs were taken at 25,000× from two blocks randomly chosen in both pallidal segments of three animals. These micrographs covered a total surface of approximately 2120 μm^2 of pallidal tissue per animal. All micrographs were taken from tissue on the surface of the blocks where the intensity of labeling was optimal. In each micrograph, immunoreactive elements were categorized as unmyelinated axons, axon terminals, dendrites or glia based on ultrastructural features (Peters et al., 1991). The mean percentage of labeled elements in each category was then calculated and a two-way analysis of variance (ANOVA) was performed to compare the relative abundance of GluR6/7-immunoreactive elements between GPe and GPi.

To determine whether the GluR6/7-containing axon terminals displayed GABA immunoreactivity, the density of gold particles associated with GluR6/7-positive axon terminals that formed symmetric synapses was compared with that of unlabeled or GluR6/7-immunoreactive axon terminals forming asymmetric synapses. The quantitative measurements were carried out as follows: ultrathin sections were scanned and each randomly encountered GluR6/7-containing terminal was digitally recorded at 31,500× at the electron microscope. The images were imported into Adobe Photoshop on an IBM computer. The density of gold particles was then determined by measuring the cross-sectional area of individual axon terminals with the NIH Image software. A one-way ANOVA was conducted to test for the difference in GABA labeling between GluR6/7-containing axon terminals forming symmetric synapses and GluR6/7-positive or unlabeled axon terminals forming asymmetric synapses.

RESULTS

At the light microscopic level, neuronal perikarya and dendrites in both pallidal segments displayed moderate GluR6/7 immunoreactivity (Fig. 1). Fine neuropil elements were found throughout the whole extent of GPe and GPi. There was no obvious difference in the pattern of distribution or intensity of GluR6/7 immunolabeling between the two pallidal segments (Fig. 1).

At the electron microscopic level, pre- and post-synaptic elements displayed GluR6/7 immunoreactivity. Dendrites and small unmyelinated axons were the most commonly encountered immunoreactive elements followed by axon terminals and glia (Figs. 2, 3). Both large and small sized dendrites were strongly labeled (Fig. 2), suggesting that KARs are expressed on proximal and distal dendrites. An immunogold analysis is in progress to determine the pattern of subsynaptic localization of GluR6/7 immunoreactivity in relation to specific afferent inputs to individual pallidal neurons. Two populations of immunoreactive axon terminals were found in the pallidum: A first group (N=56; 64.3%) that comprised boutons packed with large pleomorphic vesicles and a few mitochondria formed symmetric axo-dendritic synapses (Fig. 2A, C) while the second population included boutons that contained many round electron-lucent vesicles (N=9; 10.5%), a few mitochondria and formed asymmetric synapses (Fig. 2B). The remainder of labeled boutons (N=22; 25.2%) could not be categorized in any of these groups because the synaptic specializations were not seen in the plane of section. Although the relative density of immunoreactive axons and terminals was slightly higher in GPi than GPe (Fig. 3), this difference was not statistically significant (P=0.127).

To further characterize the phenotype of the labeled axon terminals, post-embedding immunogold staining for GABA was performed. In these double-immunostained sections, all GluR6/7-positive axon terminals that formed symmetric synapses were overlaid with a high density of gold particles whereas the GluR6/7-imunoreactive boutons forming asymmetric synapses expressed a level of GABA immunoreactivity not significantly different from background (Figs. 2C, 4). Quantitative analysis showed that the density of immunogold labeling associated with GluR6/7-containing boutons forming symmetric or asymmetric synapses was not significantly different (P=0.11) from that overlying GluR6/7-negative axon terminals forming symmetric or asymmetric synapses, respectively (Fig. 4), which indicate that the DAB deposit in GluR6/7-positive axon terminals had no effect on the intensity of GABA immunogold labeling.

DISCUSSION

Data presented in this study provide the first evidence for pre-synaptic KARs in the monkey globus pallidus. Small

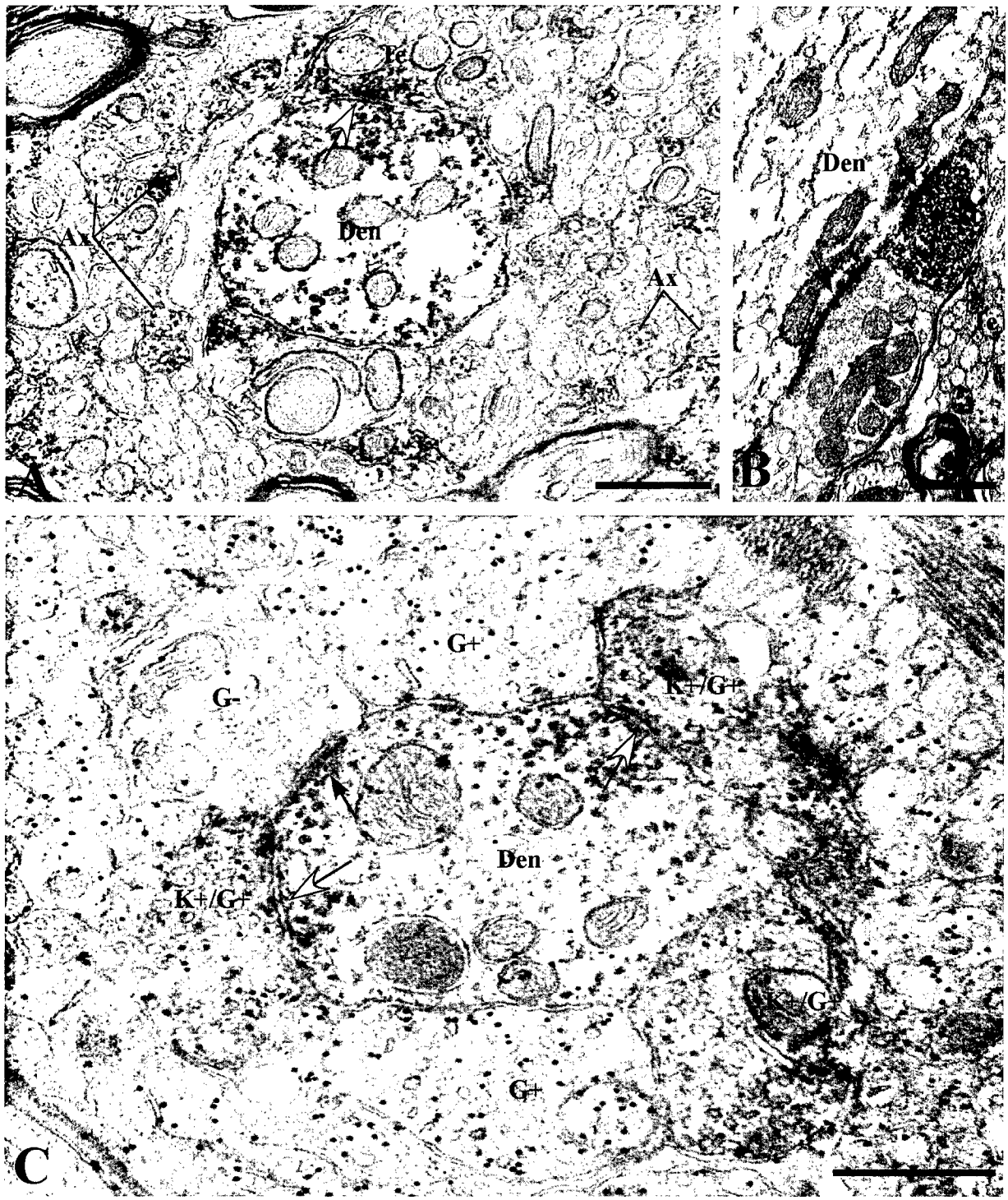

Fig. 2. GluR6/7 immunoreactivity in the monkey pallidum. (A) A multitude of GluR6/7-immunoreactive unmyelinated axonal processes (Ax) in the vicinity of an immunoreactive dendrite (Den) that receives a symmetric synaptic input (open arrow) from a GluR6/7-positive terminal (Te). (B) A Te forms an asymmetric axo-dendritic synapse (arrow). Note the non-immunoreactive bouton (asterisk) forming an asymmetric synapse on the same Den. (C) Shows three GluR6/7-positive axon terminals enriched in GABA immunoreactivity (K+/G+). Two of these boutons form symmetric axo-dendritic synapses (open arrows). The density of gold particles over these boutons is comparable to that associated with other GABA-positive axon terminals (G+) apposed to the same Den. Note the lower density of gold particles associated with a putative glutamatergic terminal (G−) that forms an asymmetric synapse (arrow) on the same Den. Scale bars=0.25 μm.

unmyelinated axons, which presumably represent pre-terminal axonal segments, as well as GABAergic and putative glutamatergic boutons express strong GluR6/7 immunoreactivity in GPe and GPi. Based on previous ultrastructural

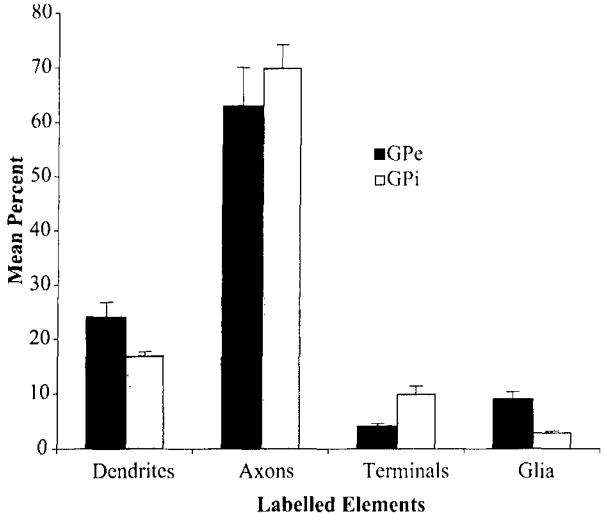

Fig. 3. Histogram showing the relative abundance of various GluR6/7-immunoreactive elements in GPe and GPi. Data are expressed as mean percent (±S.D.) of immunoreactive elements in GPe and GPi of three monkeys. A total surface of approximately 5400 μm^2 of tissue was examined from six blocks in GPe and GPi. The relative abundance of immunoreactive elements in GPe and GPi was not statistically significant.

studies (Smith et al., 1998), it is reasonable to suggest that the KARs/GABA-positive boutons largely arise from the striatum whereas most of the KARs/GABA-negative axon terminals originate from the subthalamic nucleus (STN). However, other minor sources of putative glutamatergic axon terminals forming asymmetric synapses in the globus pallidus, such as the thalamus and pedunculopontine nucleus, should also be considered. Therefore, our findings indicate that pre-synaptic KARs are located to subserve modulation of both GABAergic and glutamatergic transmission in the monkey pallidum.

Although KARs functions have long remained poorly understood, the existence of pre-synaptic kainate auto- or heteroreceptors is now well established in various brain regions (see Lerma et al., 2001 and Kamiya, 2002 for reviews). However, the exact mechanisms underlying their pre-synaptic effects are complex, unusual and, in many cases, poorly characterized (Lerma et al., 1997, 2001; Kamiya, 2002). For instance, one of the main features of pre-synaptic kainate autoreceptors in the hippocampus and cerebellum is that their stimulation can modulate glutamate release bi-directionally; weak activation enhances glutamate release while strong activation leads to inhibition of neurotransmitter release (Schmitz et al., 2000; Delaney and Jahr, 2002; Kamiya, 2002). This activity-dependent process might be critical in setting up differential pre-synaptic KARs functions in the monkey pallidum under normal versus pathological conditions. For instance, the fact that STN becomes hyperactive in Parkinson's disease (Wichmann and DeLong, 1996) suggests that pre-synaptic KARs may have inhibitory effects on glutamate release in parkinsonians, whereas it might facilitate transmission along the subthalamofugal pathways under normal conditions. Electrophysiological studies in brain slices are in progress to further characterize this issue.

Multifarious pre-synaptic effects of KARs activation on GABA release have also been clearly demonstrated in the hippocampus and other brain regions (see Lerma et al., 1997, 2001 for reviews). (1) Suppression of GABAergic transmission that involves metabotropic processes sensitive to pertussis toxin (Rodriguez-Moreno and Lerma, 1998): These results are interesting, but rather surprising, because KAR subunits do not have the structural functional domains to interact with G proteins and second messenger pathways. The exact mechanism(s) that underlies this G protein-mediated interaction remains to be established. (2) Increase in GABA release from cultured

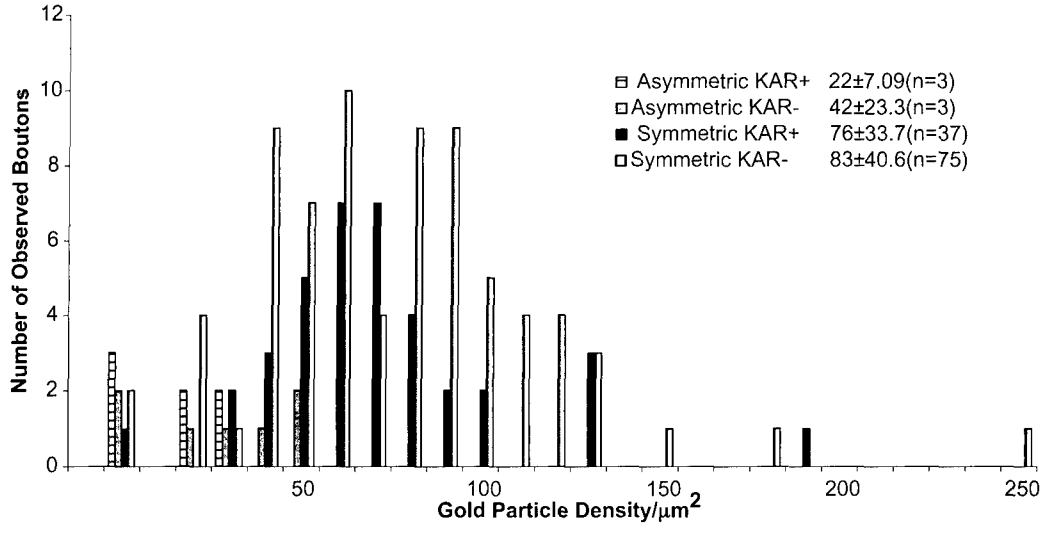

Fig. 4. Relative density of immunogold particles for GABA associated with GluR6/7-positive and GluR6/7-negative axon terminals forming symmetric or asymmetric synapses in GPe and GPi. The total number of axon terminals examined and the mean (±S.D.) density of gold particles over each population of boutons are given in parentheses. The density of gold particles over axon terminals forming symmetric synapses was significantly higher than that associated with axon terminals forming asymmetric synapses ($P<0.01$).

hypothalamic neurons (Liu et al., 1999) and (3) potentiation of GABAergic synapses in the CA1 region of the hippocampus through an unresolved mechanism that does not involve metabotropic intracellular pathways (Jiang et al., 2001). Whether pre-synaptic KARs in striatofugal GABAergic axon terminals in GPe and GPi mediate their effects through any of those mechanisms surely deserve further consideration. Knowing that a proper balance of GABAergic transmission to GPe and GPi is essential for normal basal ganglia functions (Wichmann and DeLong, 1996), a deeper understanding of the roles of KARs in the two pallidal segments may also prove useful for the development of therapeutic strategies aimed at targeting pre-synaptic KARs in basal ganglia diseases.

In brief, anatomical data presented in this and previous studies (Charara et al., 1999; Kieval et al., 2001) strongly suggest that KARs are located to subserve pre- and post-synaptic functions in the primate striatopallidal complex. The recent evidence for mutation of the GluR6 gene in a subset of patients with Huntington's disease (Rubinsztein et al., 1997; MacDonald et al., 1999) emphasizes the potential importance of these receptors in the normal and pathological functioning of the basal ganglia circuitry. Electrophysiological experiments are currently in progress to determine the detailed mechanisms by which pre-synaptic KARs control inhibitory and excitatory synaptic transmission in the globus pallidus.

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Subcellular and Subsynaptic Localization of Presynaptic and Postsynaptic Kainate Receptor Subunits in the Monkey Striatum

Jeremy Z. Kieval, G. W. Hubert, Ali Charara, Jean-François Paré, and Yoland Smith

Division of Neuroscience, Yerkes Regional Primate Research Center, and Department of Neurology, Emory University, Atlanta, Georgia 30322

The localization and functions of kainate receptors (KARs) in the CNS are still poorly known. In the striatum, GluR6/7 and KA2 immunoreactivity is expressed presynaptically in a subpopulation of glutamatergic terminals and postsynaptically in dendrites and spines. The goal of this study was to further characterize the subcellular and subsynaptic localization of kainate receptor subunits in the monkey striatum. Immunoperoxidase data reveal that the relative abundance of GluR6/7- and KA2-immunoreactive terminals is homogeneous throughout the striatum irrespective of the differential degree of striatal degeneration in Huntington's disease. Pre-embedding and post-embedding immunogold data indicate that >70% of the presynaptic or postsynaptic GluR6/7 and KA2 labeling is expressed intracellularly. In material stained with the post-embedding immunogold method, approximately one-third of plasma membrane-bound gold particles labeling in axon terminals and spines is associated with asymmetric synapses, thereby representing synaptic kainate receptor subunits. On the other hand, >60% of the plasma-membrane bound labeling is extrasynaptic. Both GluR6/7 and KA2 labeling in glutamatergic terminals often occurs in clusters of gold particles along the membrane of large vesicular organelles located at various distances from the presynaptic grid. Anterograde labeling from the primary motor cortex or the centromedian thalamic nucleus indicate that both corticostriatal and thalamostriatal terminals express presynaptic GluR6/7 and KA2 immunoreactivity in the postcommissural putamen. In conclusion, these data demonstrate that kainate receptors in the striatum display a pattern of subcellular distribution different from other ionotropic glutamate receptor subtypes, but consistent with their metabotropic-like functions recently shown in the hippocampus.

Key words: Huntington's disease; excitotoxicity; presynaptic receptor; corticostriatal pathway; thalamostriatal pathway; post-embedding immunogold

Glutamate is the major excitatory neurotransmitter in the CNS. Its activity is mediated by three groups of ionotropic receptors: NMDA, AMPA, and kainate receptors (KARs). Kainate receptors are comprised of five subunits, GluR5, 6, 7, and KA1–2 (Hollmann and Heinemann, 1994). Until a few years ago, the inability to pharmacologically differentiate AMPA from KA receptors had limited the understanding of a distinct functional role of KARs in the CNS. However, the use of novel benzodiazepine compounds (GYKI compounds), which act as selective antagonists of AMPA receptors (Paternain et al., 1995), has provided a means of demonstrating a distinct role of KARs in modulating synaptic transmission. Interestingly, several *in vitro* electrophysiological and pharmacological studies have shown that KARS mediate presynaptic effects on GABAergic and glutamatergic neurotransmission in various brain regions (Clarke et al., 1997; Lerma et al., 1997; Rodriguez-Moreno et al., 1997; Mulle et al., 1998; Rodriguez-Moreno and Lerma, 1998; Chittajallu et al., 1999; Liu et al., 1999; Min et al., 1999; Perkinton and Sihra, 1999; Chergui et al., 2000; Contractor et al., 2000; Frerking and Nicoll, 2000; Kamiya and Ozawa, 2000). Furthermore, the excitotoxic effects of KAR agonists were found to be greatly reduced in the CA3 region of the rat hippocampus after mossy fiber denervation (Debonnel et al., 1989). Similar results of decreased susceptibility to kainate-induced seizures and cell death have recently been shown in mutant mice knock-outs of the GluR6 gene (Mulle et al., 1998), providing evidence that these excitotoxic effects are produced through the activation of presynaptic KARs.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by a massive death of striatal projection neurons. In >60% of HD patients, increasing length of CAG repeats correlates highly with a decrease in the age of onset of the disease or the extent of striatal degeneration (Persichetti et al., 1994; Aronin et al., 1995; Penney et al., 1997). However, recent findings have reported that the variance in the age of onset of HD could also be attributed to mutations in the gene encoding the GluR6 KAR subunit (Rubinsztein et al., 1997; MacDonald et al., 1999). Injections of kainic acid into the striatum have, indeed, been known to cause cell death in striatal projection neurons, but to have no such effect on axons crossing or terminating in the area (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). The fact that these neurotoxic effects of kainate in the striatum are attenuated after decortication (McGeer et al., 1978; Biziere and Coyle, 1979), implies that these effects are mediated via cortical terminal glutamate release. In line with these observations, recent findings from our laboratory have demonstrated the presence of KAR immunoreactivity on glutamatergic nerve terminals in the monkey striatum (Charara et al., 1999). To further extend these data and better understand the functions of kainate receptors in the primate striatum, the aim of the present study is to elucidate the

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Correspondence should be addressed to Yoland Smith, Yerkes Regional Primate Research Center, 954 Gatewood Road Northeast, Emory University, Atlanta, GA 30322. E-mail: yolands@rmy.emory.edu.

subcellular and subsynaptic localization of the GluR6/7 and KA2 KAR subunits in the monkey striatum.

The findings presented in this study have been published in abstract form (Kieval et al., 2000).

MATERIALS AND METHODS

Animals and preparation of tissue

Four male adult rhesus monkeys and two male adult squirrel monkeys were used in the present study. The two squirrel monkeys were used for tracing studies (see below), whereas the four rhesus monkeys were processed for KAR immunocytochemistry. After deep anesthesia with an overdose of pentobarbital, rhesus monkeys were perfusion-fixed with 500 ml of cold oxygenated Ringer's solution followed by 2 l of fixative containing 4% paraformaldehyde and 0.1–0.75% glutaraldehyde in phosphate buffer (PB; 0.1 M, pH 7.4). The anesthesia and perfusion of the animals were performed in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* (1996) and the Emory University Animal Care and Use Committee. The brains were then cut in 60-μm-thick sections with a vibrating microtome and processed for the immunohistochemical localization of GluR6/7 and KA2 at the electron microscopic level. A series of sections were cut at 100 μm and processed for the freeze substitution technique and post-embedding immunogold localization of GluR6/7 and KA2 receptor subunits as described below.

Kainate receptor antisera

Commercially available affinity-purified polyclonal antisera generated against synthetic peptides corresponding to the C terminus of GluR6 (TFNDRRLPGKETMA) (Upstate Biotechnology, Lake Placid, NY) and KA2 (GPTGPRELTEHE) (Upstate Biotechnology) were used in this study. The specificity of the anti-GluR6 and anti-KA2 antibodies was determined by immunoblots of cell membranes from transfected human embryonic kidney cells (HEK 293 cells) (Petralia et al., 1994; Wenthold et al., 1994). Immunoblot analyses showed that these antibodies label a single band that corresponds to the molecular weight of their respective receptor subunit. However, as a result of the sequence homology at the C terminus between the GluR6 and the GluR7 subunits, the antibody to GluR6 also recognizes the GluR7 subunit to some degree; hence, the term GluR6/7 for this antiserum. To confirm that the sequence of amino acids of the synthetic peptides used to produce these antibodies are not found in other known proteins, we performed a search for amino acids sequence alignment in the basic local alignment search tool (BLAST) database (Altschul et al., 1997), and we found that there was no significant cross-reactivity with any proteins other than the GluR6/7 and KA2 kainate receptor subunits. This search also revealed that the amino acids sequences used are found in GluR6/7 and KA2 subunits of both rats and humans, suggesting that these antibodies should recognize their corresponding antigenic sites in both primates and nonprimates.

The specificity of the two antisera was further confirmed in the present study by the complete lack of labeling in sections of monkey striatum incubated in solutions from which the antisera were replaced by either nonimmune rabbit serum or antiserum that has been preadsorbed with 10 μg/ml homologous peptides for 1 hr at room temperature (Fig. 1*B,D*). Immunoblotting was also performed to test the specificity of the GluR6/7 antiserum on monkey striatal tissue (Fig. 2). The Western blot procedure was performed as follows: samples of protein were subjected to SDS-PAGE and transferred by electroblotting onto polyvinylidene fluoride membranes (Invitrogen, Carlsbad, CA). The blots were blocked with 5% nonfat dry milk, 0.% Tween 20 in Tris-buffered saline (TBS) (20 mM Tris-HCl plus 137 mM NaCl, pH 7.4) at room temperature for 1 hr, and then incubated overnight at 4°C with antibodies raised against the C terminus of the GluR6/7 subunit (0.5 μg/ml; Upstate Biotechnology) in blocking buffer. The blots were then rinsed for 20 min in blocking buffer and incubated for 1 hr in horseradish peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad, Hercules, CA), diluted 1:10,000 in blocking buffer. After several washes in TBS, the immunoreactive proteins were visualized with enhanced chemiluminescence (Amersham Pharmacia Biotech, Buckinghamshire, UK). For preadsorption experiments, antibodies were preadsorbed with 10 μg/ml homologous peptide for 1 hr at room temperature.

Electron microscope KAR immunocytochemistry

Pre-embedding immunoperoxidase. Sections prepared for pre-embedding immunoperoxidase were pretreated with sodium borohydride (1% in PBS; 0.01 M; pH 7.4) and then cryoprotected in a solution of 25% sucrose and 10% glycerol before being frozen at −80°C for 20 min. They were then thawed and returned to a graded series of cryoprotectant and PBS. Afterward, sections were preincubated in 10% normal goat serum (NGS) in PBS for 1 hr, followed by incubation for 48 hr at 4°C in rabbit polyclonal antisera (GluR6/7, 7.5 μg/ml; KA2, 0.55 μg/ml) diluted in PBS supplemented with 1% NGS. After three 10 min washes in PBS, the sections were incubated in biotinylated goat anti-rabbit IgG (1:200; Vector Laboratories, Burlingame, CA) for 90 min at room temperature, which was followed by three 10 min washes in PBS. Incubation in the avidin–biotin–peroxidase complex (ABC; 1:100; Vector Laboratories) (Hsu et al., 1981) subsequently followed for a period of 90 min. After two 10 min washes in PBS and one 10 min wash in TRIS buffer (0.05 M, pH

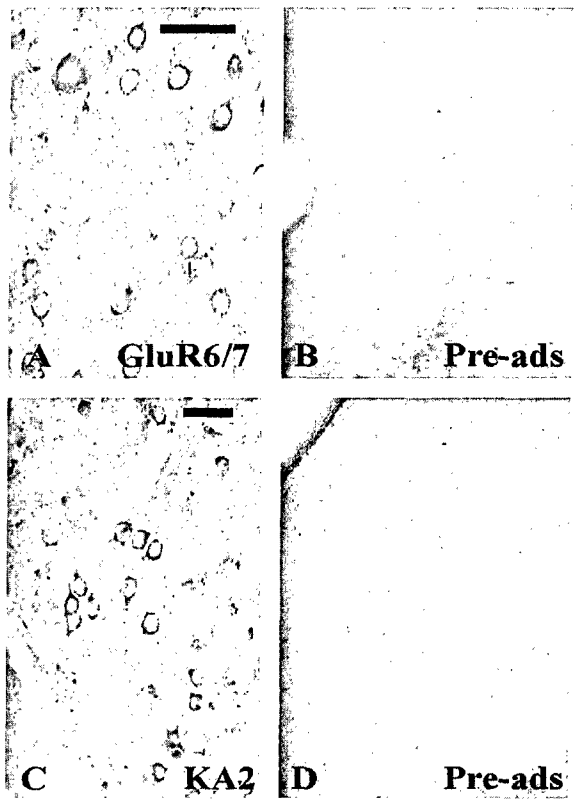


Figure 1. Controls for the specificity of GluR6/7 (*A*, *B*) and KA2 (*C*, *D*) antisera on monkey striatal tissue. In *B* and *D*, the antisera were preadsorbed with 10 μg/ml of homologous peptides for 1 hr before incubation. Scale bars: *A*, 50 μm (valid for *B*); *C*, 50 μm (valid for *D*).

Figure 2. Western blot analysis demonstrating the specificity of the GluR6/7 antiserum. The antibodies detected a single band that corresponds to the approximate molecular weights predicted for GluR6 and GluR7 subunits (~118 kDa). Immunoreactivity is completely abolished when antibodies are preadsorbed with the synthetic GluR6/7 peptide 1 hr before immunoblotting. Molecular weight standards are indicated on the left (10^3 molecular weight).

7.6), the immunostaining was revealed by incubation for 10 min in a solution containing 0.025% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO), 0.01 M imidazole (Fisher Scientific, Atlanta, GA), and 0.006% hydrogen peroxide (H_2O_2). The reaction was stopped by repeated washes in PBS.

Pre-embedding immunogold. Sections processed for pre-embedding immunogold were prepared as described in previous studies (Hanson and Smith, 1999). In brief, sections were pretreated with sodium borohydride, cryoprotected, and frozen at −80°C in the same manner as those processed for immunoperoxidase. They were then preincubated in 10% NGS in PBS containing 0.5% bovine serum albumin, 0.05% Tween 20, and 0.1% gelatin (PBS–BSA) for 1 hr. This was followed by an overnight incubation at room temperature in the primary antibody solution diluted as described above. After three 10 min washes in PBS–BSA, the sections were incubated in goat anti-rabbit IgG conjugated to 1.4 nm colloidal gold particles (1:100 in PBS–BSA; Nanogold; Nanoprobes, Stony Brook, NY) for 2 hr at room temperature. After a 5 min wash in PBS–BSA and two 5 min washes in PBS, the sections were post-fixed in 1% glutaraldehyde in PBS for 10 min at room temperature. After washing in PB (0.1 M, pH 7.4) for 5 min, the gold labeling was intensified by using a silver enhancement kit (HQ silver; Nanoprobes) for 5–10 min at room temperature in the dark and stopped by several washes in distilled water.

Anterograde labeling of corticostriatal and thalamostriatal afferents

Two squirrel monkeys received bilateral iontophoretic injections of biotinylated dextran amine (BDA) either in the primary motor cortex or the centromedian (CM) intralaminar thalamic nucleus. After being intubated and anesthetized with isoflurane, the animals were fixed in a stereotaxic frame, and intracerebral injections of BDA were performed according to procedures described in details in many of our previous studies (Shink et al., 1996; Sidibé and Smith, 1996; Sidibé et al., 1997). In brief, the BDA was injected for 20 min through glass micropipettes with a tip diameter ranging from 20 to 50 μm using a 6 μA positive current delivered at a 7 sec ON/7 sec OFF cycle. Two injection sites along a single penetration were made in CM, whereas a total of 10 injection sites along eight penetrations were made in the cerebral cortex. The depth of anesthesia was monitored throughout the surgery by measuring hearth rate, blood oxygen level, and toe pinch reflex. After surgery, animals received systemic injections of analgesic for 48 hr. The surgical and anesthesia procedures used in these experiments are consistent with those of the National Institute of Health and approved by the Institutional Animal Care and Use Committee of Emory University. After 7–10 d survival, the animals were deeply anesthetized with sodium pentobarbital and perfused transcardially as described above for rhesus monkeys, except that the volumes of Ringer's and fixative were reduced to 350 ml and 1 l, respectively.

BDA histochemistry combined with KAR immunocytochemistry

The BDA was revealed with the ABC method as described in previous studies. In brief, after sodium borohydride treatment, cryoprotection, and −80°C freezing, sections were washed in PBS and incubated overnight at room temperature in standard ABC complex (1:100 dilution). The peroxidase bound to the BDA was then localized with DAB as described above for KAR immunoperoxidase localization. Once the BDA has been revealed, striatal sections that contained large amount of anterogradely labeled fibers were processed for the pre-embedding immunogold localization of GluR6/7 and KA2 as described above. Double-labeled sections were then prepared for electron microscopy.

As controls, a series of sections were processed to reveal BDA followed by incubation in solution containing nonimmune rabbit serum rather than KAR antisera.

Preparation for electron microscopy. All sections prepared for electron microscopy were washed in PB (0.1 M, pH 7.4) before being post-fixed in osmium tetroxide (1% solution in PB) for 10–20 min. They were then washed five times (5 min each) in PB and dehydrated in a graded series of alcohol and propylene oxide. Uranyl acetate was added to the 70% ethanol to improve the contrast in the electron microscope. The sections were then embedded in resin (Durcupan, ACM; Fluka, Buchs, Switzerland) on microscope slides and put in the oven for 48 hr at 60°C. After examination in the light microscope, areas of interest in the striatum were cut out from the slides and glued on top of resin blocks with cyanoacrylate glue. Ultrathin 60-nm-thick sections were cut on a Leica (Nussloch, Germany) UCT ultramicrotome and collected on pioloform-coated, single slot copper or gold grids. Some sections were stained with lead citrate (Reynolds, 1963) and examined with a Zeiss EM10C electron microscope.

High-pressure freezing, freeze substitution, and post-embedding immunogold technique for KAR localization. Tissue from the putamen and the head of the caudate nucleus from three rhesus monkeys were used in this study. After cryoprotection, small areas of 2 mm in diameter were taken from 100-μm-thick striatal sections and placed between two aluminum planchettes that were then instantly frozen in liquid nitrogen using a high-pressure freezer (Balzers HPM 010). Sections were stored in liquid nitrogen until transfer into a freeze substitution apparatus (Bal-Tec FSU 010) whereby the temperature was increased from −90 to −45°C in four major steps over a period of 30 hr. The specimens were freeze-substituted in 0.5% uranyl acetate dissolved in methanol and then embedded in Lowicryl HM-20 (−45°C) in a low temperature polymerization unit (Bal-Tec LTPU 010) for 48 hr. Ultrathin 80-nm-thick sections were cut on a Leica UCT ultramicrotome and collected on pioloform-coated, 400 mesh gold grids.

Sections of freeze-substituted material were first treated in a saturated solution of sodium hydroxide in 100% ethanol (<1 sec.). After being washed in Tris-buffered saline containing 0.01% Triton X-100 (TBS-T), they were incubated for 10 min on drops of 0.1% sodium borohydride and 0.05 M glycine diluted in TBS-T. They were then washed in TBS-T and preincubated for 30 min in TBS-T containing 10% normal serum and 2% human serum albumin (HSA) before being incubated overnight at room temperature with the GluR6/7 (15 μg/ml) and KA2 (5.5 μg/ml) antisera diluted in a solution of TBS-T containing 1% normal serum and 2% HSA. After many washes in TBS-T, the grids were incubated for 90 min in the 10 nm gold-conjugated secondary antibodies (1:180; BBInternational) diluted in TBS-T containing 1% normal serum and 2% HSA. Grids were washed in ultrapure water and contrasted in a 1% aqueous solution of uranyl acetate for 90 min. The grids were then stained with lead citrate (Reynolds, 1963) before observation.

Control sections were incubated in solutions from which the primary antisera were replaced by 1% nonimmune rabbit serum, whereas the rest of the protocol remained the same as described above.

Analysis of material

Immunoperoxidase data. To estimate the relative abundance of GluR6/7- and KA2-immunoreactive elements in different striatal regions, a series of 50 electron micrographs were taken at 16,500× from randomly chosen areas of the tail and body of the caudate nucleus, the putamen, and the nucleus accumbens in three monkeys. These micrographs covered a total surface of 7148 μm^2 of striatal tissue in each animal. All micrographs were taken from tissue on the surface of the blocks where the intensity of labeling was optimal. In each micrograph, immunoreactive elements were categorized as unmyelinated axons, terminals, spines, or small and large dendrites based on the following ultrastructural features. The unmyelinated axons were distinguished by their small diameter (0.1–0.2 μm), regular contours, lack of synaptic inputs, and presence of microtubules, whereas axon terminals contained synaptic vesicles and did not receive synaptic inputs. The heads of dendritic spines were recognized by their electron lucent and bulbous appearance, lack of mitochondria, and absence of microtubules. They also frequently received asymmetric synaptic inputs. Finally, most dendrites were easily distinguishable from other neuronal elements by their large size and enrichment in mitochondria and microtubules. Elements that could not be categorized according to these ultrastructural features were not considered in the analysis. The mean percentage of labeled elements in each category was then calculated, and χ^2 tests were performed to compare the relative abundance of immunoreactive terminals in the different striatal regions.

Pre-embedding immunogold data. In tissue immunostained with the pre-embedding immunogold technique, the proportion of gold particle labeling associated with the plasma membrane and intracellular compartments was calculated from a series of 200 electron micrographs taken at 25,000× in the putamen and the head of the caudate nucleus in three monkeys. These micrographs covered a total surface of 2843 μm^2 of striatal tissue. To avoid false-positive data generated by light background staining, an element had to contain, at least, three gold particles to be considered immunoreactive. The total number of gold particles in each immunoreactive element encountered in these micrographs was then calculated and categorized as intracellular or bound to the plasma membrane. Taking into consideration the size of primary and secondary antibodies, the maximum distance between the 1.4 nm gold particle and the epitope would be ~17 nm (Blackstad et al., 1990). Based on this

criterion, presynaptic or postsynaptic gold particle labeling was categorized as plasma membrane-bound if it was found inside an area not further than 16 nm plus radius of gold particles from the presynaptic or postsynaptic plasma membranes, respectively. All other gold particles were categorized as "intracellular". To avoid problems in categorizing the membrane-bound gold particles, only those elements that displayed good ultrastructural preservation with well preserved plasma membrane were considered.

Post-embedding immunogold data. This method was used to characterize the subsynaptic localization of GluR6/7 and KA2 labeling. The advantages of this approach over the pre-embedding immunogold technique to label synaptic receptors have been discussed in details in previous studies (Lujan et al., 1996; Ottersen and Landsend, 1997). In brief, the fact that the entire cut length of the plasma membrane is uniformly exposed to the antibodies increases the accessibility to the antigenic sites, which provides a condition for quantitative analysis of synaptic receptor localization. Quantitative measurements were made from a series of 100 electron micrographs taken at 31,500× from putamen and caudate tissue immunostained with GluR6/7 and KA2 by the post-embedding immunogold method. Because the quality of ultrastructural preservation of post-embedding immunostained tissue on the same grid is variable, we chose immunostained areas where the preservation was optimal for this analysis. To verify whether the pattern of distribution of immunogold labeling was the same as that found with the pre-embedding immunogold technique, we counted the total number of gold particles in a series of labeled terminals and spines and determined the proportion that were bound to the plasma membrane or intracellular. Furthermore, we categorized the plasma membrane-bound gold particles as "synaptic" or "extrasynaptic" based on their respective localization to synapses. A gold particle was categorized synaptic if it was located inside an area not further than 21 nm (antibody bridges, 16 nm; radius of 10 nm gold particles, 5 nm) from the presynaptic or postsynaptic plasma membrane (Matsubara et al., 1996; Valtschanoff and Weinberg, 2001; Nyiri et al., 2001). All other plasma membrane-bound gold particles were put in the extrasynaptic category. We used an arbitrary criterion that an element had to contain at least three gold particles or more to be considered immunoreactive.

To verify whether the distribution of presynaptic labeling displayed any relationship with the synaptic active zones, a series of immunostained terminals for GluR6/7 or KA2 were randomly selected for measurement of the shortest distance of individual gold particles from the presynaptic plasma membrane. To do so, we measured the distance that separated any gold particles bound to vesicular organelles from the closest part of the presynaptic membrane.

Anterograde labeling and pre-embedding immunogold labeling. Five blocks of striatal tissue (three for CM labeling, two for M1 labeling) immunostained for GluR6/7 or KA2 and BDA were chosen for this analysis. In the electron microscope, sections from the surface of the blocks were scanned for the presence of BDA-labeled terminals. Once such boutons were found, they were photographed at low (12,500×) and high (31, 500×) magnification and categorized as double labeled if they contained three gold particles or more. To avoid false-negative data because of the poor penetration of the gold-conjugated secondary antibodies in tissue, only those BDA-containing terminals that were found in the vicinity of other non-BDA-labeled KAR-immunoreactive boutons were considered in this analysis.

RESULTS

Tests for antibody specificity

As previously shown (Charara et al., 1999), both GluR6/7 and KA2 immunoreactivities were found in numerous neuronal perikarya that displayed morphological features of both medium-sized projection neurons and large interneurons throughout the monkey striatum (Fig. 1*A,C*). After preadsorption of either antisera with homologous peptides, the striatum was completely devoid of immunostaining (Fig. 1*B,D*). Similar results were obtained after omission of the two primary antisera from the incubation solutions. Furthermore, a single band of labeling that corresponds to the molecular weight of GluR6 and GluR7 (Wenthold et al., 1994) was found in Western blots analysis of monkey striatum (Fig. 2). This band was completely abolished after preadsorption of the antiserum with homologous peptides (Fig. 2).

Relative distribution of GluR6/7 and KA2-immunoreactive elements in the striatum

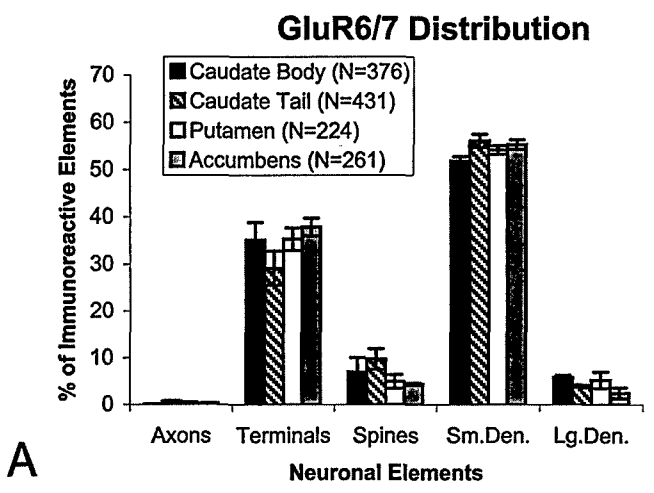
Tissue from three rhesus monkeys was processed for immunoperoxidase to characterize the general distribution of GluR6/7 and KA2 immunoreactivity in different striatal regions. The goal of this first series of experiments was to determine whether the relative abundance of kainate receptor subunit-immunoreactive glutamatergic terminals correlate with the sensitivity of the different striatal regions to neurodegeneration in HD (Vonsattel and DiFiglia, 1998). To do so, we characterized the nature of labeled elements in striatal areas known to be more or less sensitive to neurodegeneration in HD. The tail and body of the caudate nucleus as well as the dorsal putamen were chosen as sensitive areas, whereas the nucleus accumbens served as the least sensitive region (Vonsatell and DiFiglia, 1998). In all striatal regions, the pattern of distribution and intensity of immunoreactivity for the two antibodies was consistent with previous data from our laboratory (Charara et al., 1999). KAR immunoreactivity was expressed presynaptically in small unmyelinated axons and axon terminals forming asymmetric axospinous and axodendritic synapses. The postsynaptic labeling was found predominantly in small dendrites, whereas labeled spines were much less abundant (Fig. 3). Both medium-sized and large neuronal perikarya also displayed light patchy immunoreactivity (data not shown; Charara et al., 1999). Overall, 20–40% of GluR6/7-containing striatal elements were axon terminals, whereas the proportion of KA2-immunoreactive boutons ranged from 10 to 15% (Fig. 3). Although there was a slight variation in the relative abundance of labeled terminals among the four striatal regions examined, these changes were not significantly different, which indicates that presynaptic KARs are homogeneously distributed throughout the striatum irrespective of the degree of sensitivity to degeneration in HD.

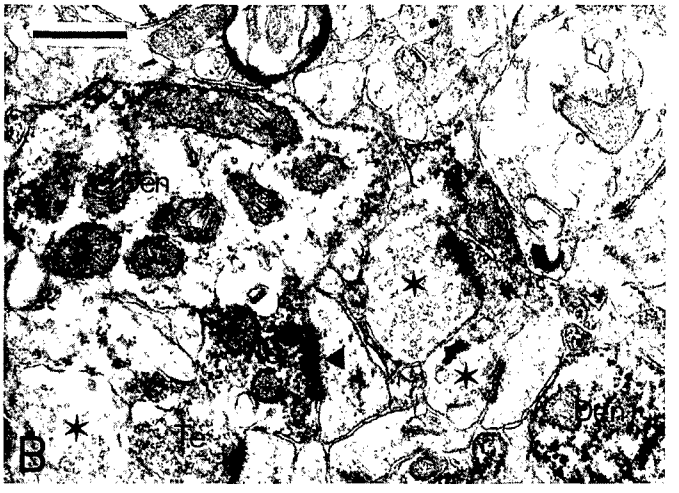
Subcellular localization of kainate receptor subunit immunoreactivity

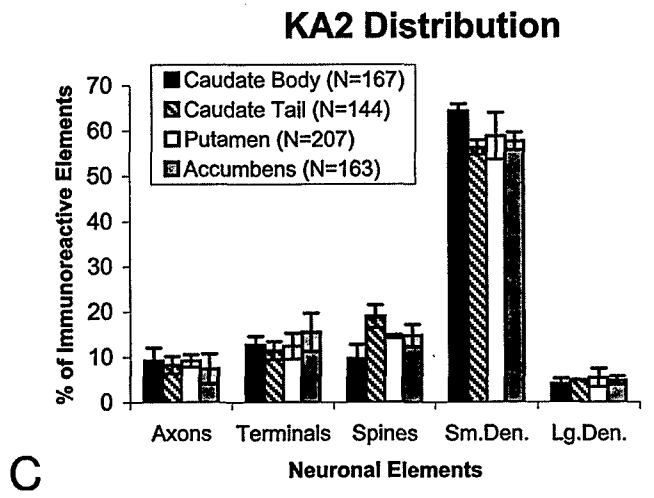
Pre-embedding immunogold

Because of the poor spatial resolution of the immunoperoxidase deposit, this approach is not suitable to analyze the subcellular and subsynaptic localization of receptors. To overcome this problem we used pre-embedding and post-embedding immunogold procedures that allow to determine more precisely the exact localization site of receptors.

Overall, the pattern of pre-embedding immunogold labeling was consistent with that of the immunoperoxidase data, i.e., gold particles were mostly found in dendrites and axon terminals, although light immunoreactivity was occasionally seen in dendritic spines (Figs. 4, 5). In both large and small neuronal perikarya, gold particles were primarily expressed intracellularly in vesicular and tubular intracellular organelles that likely correspond to parts of endoplasmic reticulum and Golgi apparatus, whereas the plasma membrane was almost completely devoid of immunoreactivity (Fig. 4). To study the subcellular localization of KARs, randomly selected fields of immunoreactive elements were photographed in the putamen and the caudate nucleus of three monkeys. The gold particles were then counted and categorized as intracellular or plasma membrane-bound according to criteria described in Materials and Methods. One of the striking feature that characterized the localization of KAR subunit immunoreactivity was that >70% of GluR6/7 and KA2 labeling was expressed intracellularly in all labeled elements (Figs. 4, 5, Table 1). In terminals, most gold particles were homogeneously distrib-







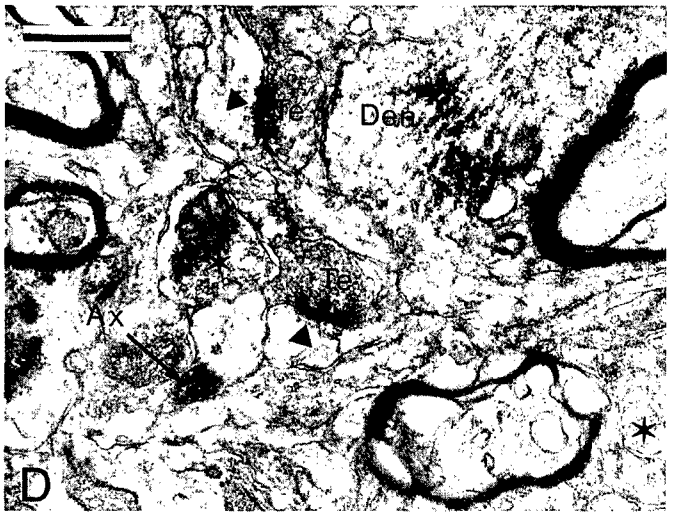


Figure 3. Relative distribution of GluR6/7 and KA2 immunoreactivity in different striatal regions. *A*, Histogram showing the relative proportion of neuronal elements immunoreactive for GluR6/7 in different striatal regions. *B*, GluR6/7-immunoreactive elements in the body of the caudate nucleus. Note the presence of labeled terminals (*Te*) forming asymmetric synapses (*arrowheads*) and immunoreactive dendrites (*Den*). The *asterisks* indicate unlabeled boutons. *C*, Histogram showing the relative distribution of neuronal elements immunoreactive for KA2 in different striatal regions. *D*, KA2-containing elements in the body of the caudate nucleus. Two immunoreactive terminals (*Te*), a labeled dendrite (*Den*), and an unmyelinated axon (*Ax*) are shown. The *asterisks* indicate unlabeled boutons. Statistical analysis revealed no significant difference in the relative abundance of GluR6/7 or KA2-containing elements among the different striatal regions (χ^2; $p < 0.001$). Scale bars, 0.5 μm.

uted over synaptic vesicles, although dense aggregates of three or more particles were occasionally seen (Fig. 5). Because of the large size of silver-intensified gold particles, the exact localization site of presynaptic labeling was difficult to ascertain. Similarly, the ultrastructural preservation of intracellular organelles in dendrites and spines was not good enough to determine the exact binding site of intracellular postsynaptic gold labeling (Fig. 5).

Post-embedding immunogold

Because of the large size of silver-intensified gold particles and poor penetration of gold-conjugated antibodies through thick sections, the pre-embedding immunogold technique provides limited information on the quantitative distribution of subsynaptic antigenic sites. To gain a higher level of spatial resolution and quantitative estimates of the relative distribution of GluR6/7 and KA2 receptor subunit immunoreactivity at the synaptic level, the freeze-substitution post-embedding immunogold technique was performed on striatal tissue. Overall, the distribution of labeling was consistent with results obtained with the pre-embedding immunogold and immunoperoxidase methods (Fig. 6), i.e., gold particles were seen over axon terminals forming asymmetric synapses, unmyelinated axons, as well as postsynaptic dendrites and spines. In contrast, terminals forming symmetric synapses were completely devoid of labeling. To further ascertain that most of the GluR6/7 and KA2 immunoreactivity was expressed intracellularly, as revealed by the pre-embedding immmunogold technique (Table 1), we quantified the density of post-embedding immunogold labeling associated with intracellular elements versus the plasma membrane in a series of spines and axon terminals with good ultrastructural preservation in GluR6/7- and KA2-immunostained material (Table 1). These data revealed that more than two-thirds of both presynaptic and postsynaptic GluR6/7 or KA2 labeling is expressed intracellularly in the monkey striatum (Table 1). However, a main difference between data obtained with the pre-embedding and post-embedding methods relates to the density of synaptic labeling for GluR6/7 and KA2. Although very few gold particles were associated with synapses in material prepared with the pre-embedding immunogold technique (Figs. 4, 5), 30–40% of post-embedding immunogold label-

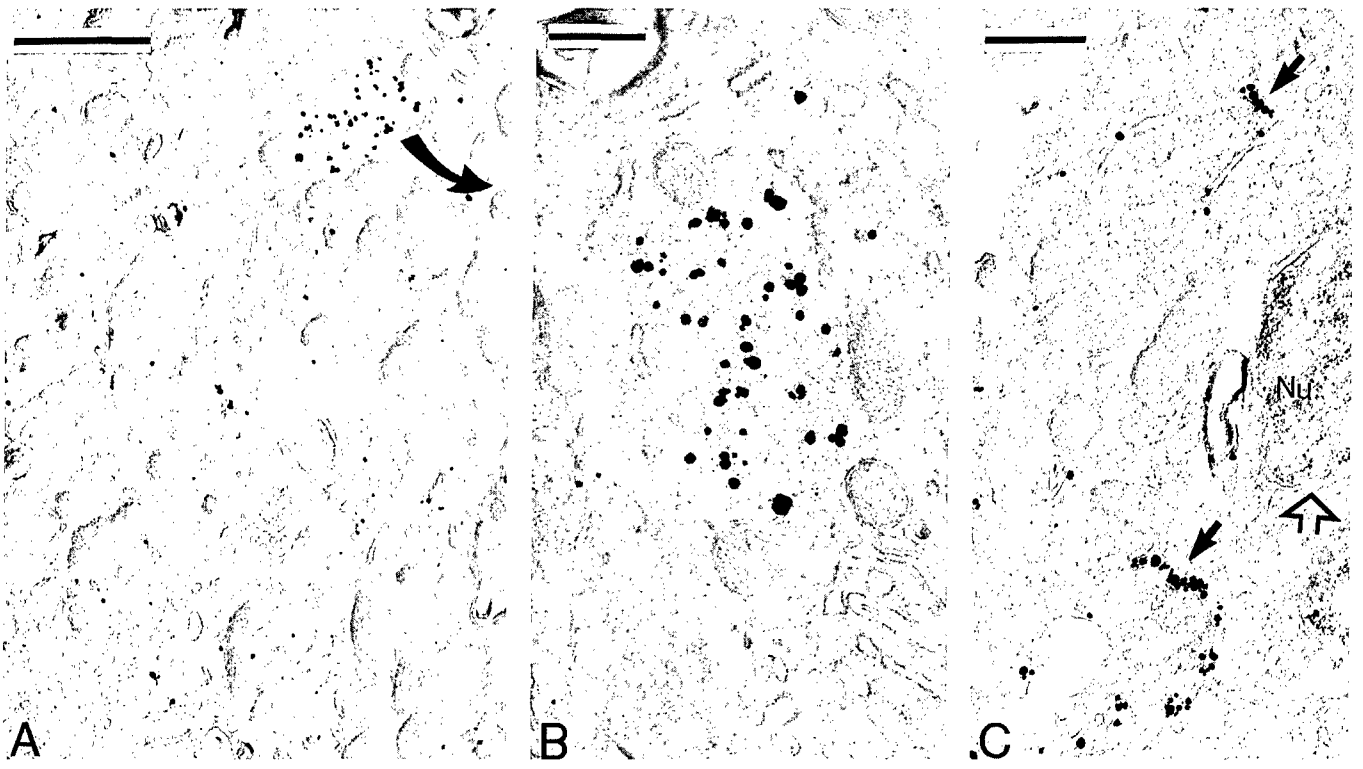


Figure 4. GluR6/7 and KA2 immunoreactivity in neuronal perikarya. *A*, A GluR6/7-containing neuronal perikaryon in the putamen. *B*, High magnification of gold particles labeling associated with the endoplasmic reticulum (*ER*) in this cell body. *C*, KA2 immunoreactivity in Golgi or ER-like tubular organelles in a neuronal perikaryon (*arrows*) of an interneuron (invaginated nuclear membrane; *open arrow*). *Nu*, Nucleus. Scale bars: *A*, 1.0 μm; *B*, 0.25 μm; *C*, 0.5 μm.

Table 1. Relative distribution of intracellular versus plasma membrane-bound immunogold particle labeling for GluR6/7 and KA2 as revealed by pre-embedding and post-embedding immunogold method

KAR subunits/ neuronal elements	% Intracellular/plasma membrane-bound		
	Spines	Terminals	Dendrites
GluR6/7			
Pre-embedding	71/29 (n = 237)	74/26 (n = 526)	78/22 (758)
Post-embedding	76/24 (n = 99)	81/19 (n = 168)	—
KA2			
Pre-embedding	76/24 (n = 144)	77/23 (n = 97)	75/25 (n = 492)
Post-embedding	75/25 (n = 93)	73/27 (n = 182)	—

Measurements were made from: (1) pre-embedding material, GluR6/7: 58 spines, 88 terminals, 86 dendrites; KA2: 54 spines, 22 terminals, 82 dendrites. (2) Post-embedding material, GluR6/7: 15 spines, 20 terminals; KA2: 15 spines, 20 terminals. Dendrites were not analyzed in the postembedding material. N = Total number of gold particles.

ing in spines and 20–30% of labeling in terminals was considered synaptic (Table 2). This difference in synaptic labeling between the pre-embedding and post-embedding immunogold methods has been previously described for other types of glutamate and GABA-A receptors (Baude et al., 1993; Nusser et al., 1995a,b). The poor penetration of gold-conjugated secondary antibodies through thick sections likely represents the main limiting factor of the pre-embedding immunogold method to label synaptic receptors.

As mentioned above, subsets of axon terminals forming asymmetric synapses display strong immunoreactivity for KAR subunits which, for the most part, was found to be expressed intracellularly over synaptic vesicles. The post-embedding immunogold method allowed to better characterize this presynaptic labeling and revealed that the majority of gold particles in terminal boutons were often clustered along the membrane of large vesicular organelles that were randomly distributed in the terminal (Fig. 6*D,F*). In addition, clusters of gold particles were occasionally seen in the presynaptic grid of asymmetric synapses (Fig. 6*B,E*).

To verify the relationships between presynaptic KARs and the synaptic release sites of glutamate, we measured the shortest distance between GluR6/7 and KA2 presynaptic immunogold particles bound to vesicular organelles and the synaptic active zone in 15 immunoreactive axon terminals immunostained with each antibodies (Fig. 7). These measurements indicate that both KAR subtypes display a wide range of distribution in relation to asymmetric synapses; some gold particles being found right at the active zones (Figs. 6*B,E*, 7) others being located as far as 1.0 μm away from the synaptic junctions (Figs. 6*D,F*, 7). Control grids were devoid of labeling except for a few scattered gold particles.

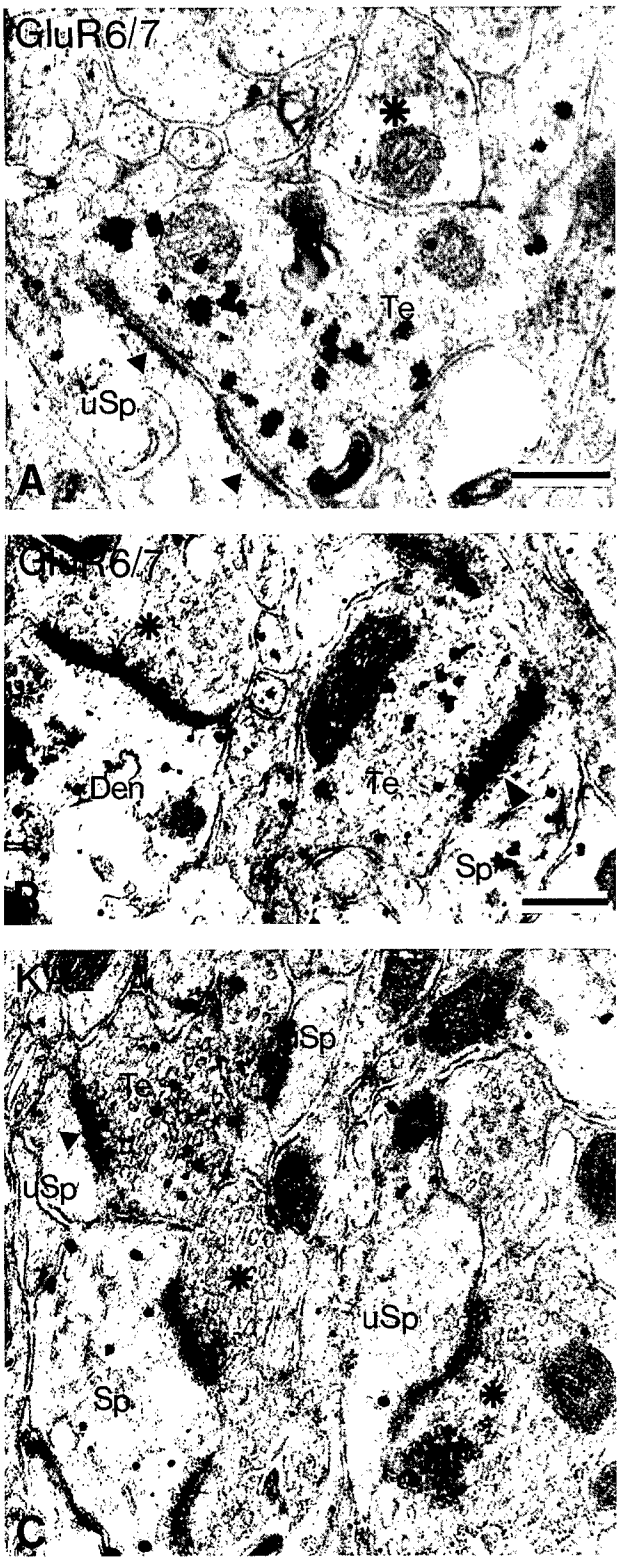


Figure 5. GluR6/7-immunoreactive (*A, B*) and KA2-immunoreactive (*C*) elements in the striatum as revealed with the silver-intensified preembedding immunogold method. *A, B*, GluR6/7-containing axon terminals (*Te*) forming asymmetric axospinous synapses (*arrowheads*) in the caudate nucleus. Nonimmunoreactive terminals (*) and unlabeled spines (*uSp*) are shown in the same region. Labeled dendrites (*Den*) and spines (*Sp*) are indicated. *C*, A KA2-immunoreactive terminal (*Te*) in the putamen. Nonimmunoreactive boutons (*) and spines (*uSp*) are shown in the same field. Note that most gold particles in axon terminals, dendrites (*Den*), and spines are located intracellularly. Scale bars: *A*, 0.25 μm; *B*, 0.25 μm (valid for *C*).

Sources of immunoreactive terminals

Because both cortical and thalamic afferents provide glutamatergic terminals forming asymmetric synapses in the monkey striatum (Sadikot et al., 1992; Smith et al., 1994), we tested whether KAR subunits were associated with both glutamatergic inputs. Injections of BDA were made in the centromedian nucleus of the thalamus or the primary motor cortex in two squirrel monkeys. The thalamic injection sites were confined to CM with slight contamination of the overlying mediodorsal nucleus and the subparafascicular nucleus, whereas the cortical injections mostly involved the leg and trunk areas of M1 (Fig. 8). As expected based on previous studies (Sadikot et al., 1992; Smith et al., 1994), both injections produced large amount of anterograde labeling in fibers and axon terminals in the postcommissural putamen. In sections double-labeled for GluR6/7 or KA2 and BDA, 30–60% of anterogradely labeled terminals (DAB-stained) displayed KAR subunit immunoreactivity (Table 3). The double-labeled boutons were easily distinguishable from unlabeled terminals in the same field by the coexpression of dense amorphous DAB reaction product (BDA labeling) overlaid by three gold particles or more (KAR labeling) (Fig. 9). Although the relative abundance of KA2-containing terminals was quite similar after thalamic and cortical injections, the proportion of GluR6/7-containing thalamostriatal boutons was higher than corticostriatal terminals (Table 3).

DISCUSSION

This study provides the first detailed analysis of the subcellular and subsynaptic localization of kainate receptor subunits in the CNS. Four major conclusions can be drawn from our data: (1) kainate receptor-containing glutamatergic terminals are homogeneously distributed throughout the monkey striatum irrespective of the differentital sensitivity of striatal regions to Huntington's disease, (2) presynaptic and postsynaptic kainate receptor subunits are primarily expressed intracellularly in cell bodies, dendrites, spines and axon terminals throughout the striatum, (3) the majority of presynaptic and postsynaptic plasma membrane-bound immunogold labeling for GluR6/7 and KA2 is found extrasynaptically, although approximately one-third is also associated with asymmetric synapses, and (4) both thalamic and cortical terminals in the sensorimotor striatum express presynaptic KAR subunits. These results will now be discussed in light of previous functional studies of kainate receptors in the CNS and their potential implication in the progressive death of striatal neurons in HD.

Presynaptic kainate receptors are homogeneously distributed in the striatum

The immunoperoxidase data presented in this study extend previous findings of our laboratory showing that presynaptic kainate receptors are expressed in a subpopulation of putative glutamatergic boutons in the monkey striatum (Charara et al., 1999). In this study we tested the hypothesis that the relative abundance of KAR-containing terminals was greater in areas that are more sensitive to neurodegeneration in HD. It has, indeed, been shown that variation of the GluR6 subunit genotype is correlated with the age on onset of HD that cannot be accounted for by the number of CAG repeats (Rubinsztein et al., 1997; MacDonald et al., 1999). These observations, combined with the fact that some striatal regions are more sensitive than others to neurodegeneration, led us to consider the possibility that this particular pattern of neuronal death might be

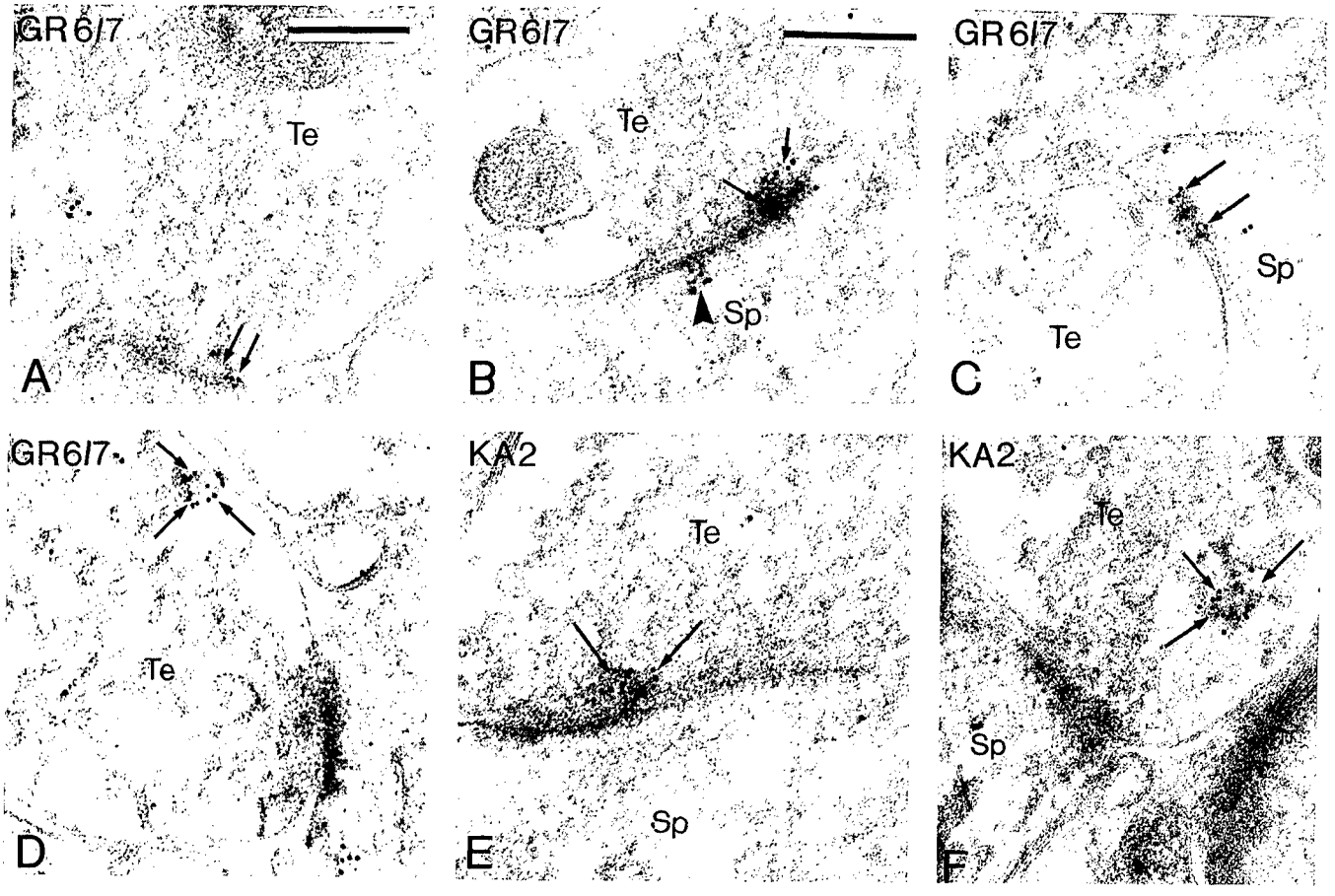


Figure 6. Post-embedding immunogold localization of GluR6/7 (*A–D*) and KA2 (*E*, *F*) immunoreactivity in the striatum. *A*, *C*, GluR6/7 labeling (*double arrows*) in the active zone of asymmetric axospinous synapses. *B*, Presynaptic and postsynaptic GluR6/7 labeling at an asymmetric axospinous synapse. *D*, Presynaptic GluR6/7 immunoreactivity along the surface of a large vesicular structures (*arrows*) in a terminal forming an asymmetric synapse with a spine. Note that gold particles in the axon terminal are aggregated at the presynaptic grid (*arrows*), whereas the spine labeling is associated with the postsynaptic density of the asymmetric postsynaptic specialization (*arrowhead*). *E*, Dense KA2 labeling (*arrows*) in the presynaptic grid of an asymmetric axospinous synapse. *F*, KA2 labeling (*arrows*) along the surface of a large vesicular organelle in an axon terminal apposed to a spine (*Sp*). Scale bars: *A*, 0.25 μm (valid for *C*, *D*, *F*); *B*, 0.25 μm (valid for *E*).

Table 2. Proportion of synaptic versus extrasynaptic immunogold labeling for kainate receptor subunits at asymmetric synapses in the striatum

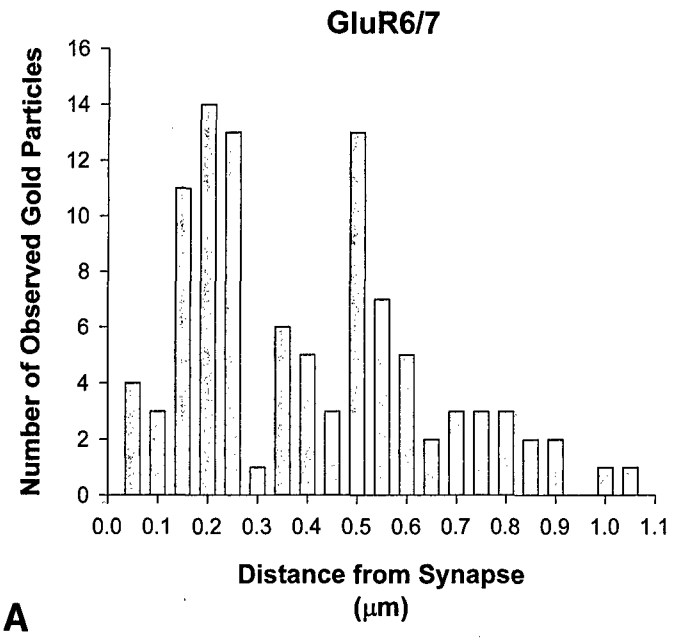
KAR subunits/ neuronal elements	% Synaptic/extrasynaptic gold labeling	
	Spines	Terminals
GluR6/7	38/62 (n = 345)	22/78 (n = 974)
KA2	43/57 (n = 112)	29/71 (n = 345)

Measurements were made from: GluR6/7, 56 spines; 77 terminals; KA2, 33 spines, 31 terminals. N = Total number of gold particles.

attributable to a differential distribution of KAR-containing terminals throughout the striatum. We, therefore, hypothesized that areas like the tail of the caudate nucleus, which degenerates first in HD, contains more KAR-positive terminals than the nucleus accumbens, which remains intact in >50% of HD patients (Vonsatell and DiFiglia, 1998). Our data revealed that such is not the case. We did not find any significant difference in the relative abundance of KAR-positive terminals between the tail of the caudate nucleus and other striatal regions. Of course, these data do not rule out the possibility that the malfunctioning of kainate receptors might underlie the pattern of striatal neurodegeneration in HD. However, if such is the case, this is unlikely to rely on a simple difference in the relative abundance of presynaptic kainate receptors in the different striatal regions. Variation in the GluR6 genotype might rather lead to changes in pharmacological and physiological properties of particular subsets of kainate receptors expressed presynaptically in specific striatal regions.

Subcellular localization of kainate receptor subunits

An interesting feature that characterized the subcellular localization of both GluR6/7 and KA2 receptor subunits is their strong intracellular expression. Our data demonstrate that >70% of both presynaptic and postsynaptic KAR subunit immunoreactivity is expressed intracellularly and that almost two-thirds of the plasma membrane-bound KARS are extrasynaptic. This pattern of distribution resembles that of G-protein-coupled metabotropic receptors which, for the most part, are expressed intracellularly or at nonsynapic sites along plasma membrane (Pasquini et al., 1992; Wang et al., 1997; Bernard et al., 1999; Hanson and Smith, 1999; Smith et al., 2000, 2001). Interestingly, both the presynaptic and postsynaptic effects of kainate receptors are consistent with those of metabotropic glutamate receptor functions (for review, see Conn and Pin, 1997; Anwyl, 1999; Cartmell and Schoepp, 2000). For instance, kainate receptors mediate slow postsynaptic cur-



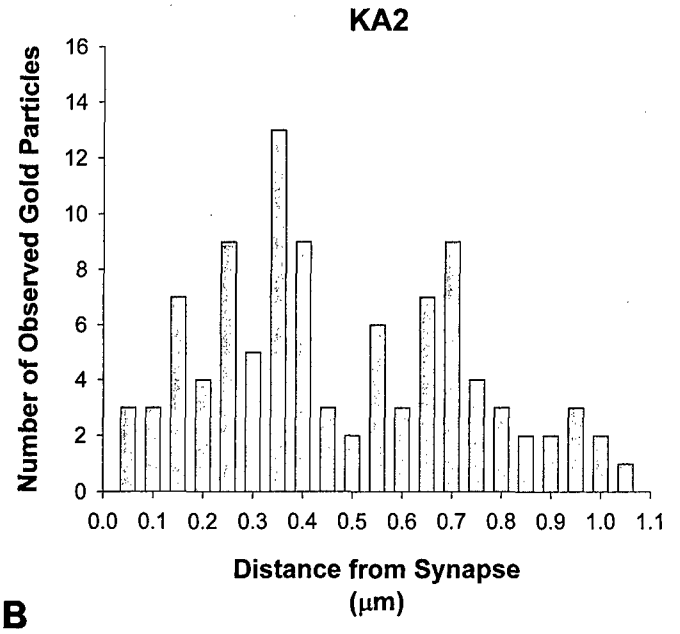


Figure 7. Histograms showing the distribution of gold particle labeling for GluR6/7 (*A*) and KA2 (*B*) in relation to the presynaptic grids of asymmetric synapses in the putamen. The mean (±SD) shortest distance of gold particles from the active zones is relatively similar for both KAR subunits antibodies (0.40 ± 0.20 μm for GluR6/7; 0.47 ± 0.25 μm for KA2). Fifteen terminals immunoreactive for GluR6/7 or KA2 were examined.

rents that could be elicited only after high-frequency stimulation of hippocampal mossy fiber pathway in CA3 pyramidal neurons in rats (Castillo et al., 1997; Vignes and Collingridge, 1997; Rodriguez-Moreno and Lerma, 1998). Furthermore, it has been shown that the presynaptic control of GABA release by kainate receptors in the hippocampus is mediated by a metabotropic process that is sensitive to Pertussis toxin and independent of ion channel current (Rodriguez-Moreno and Lerma, 1998). These functional data combined with results of our study clearly indicate that both the localization and functions of kainate receptors are

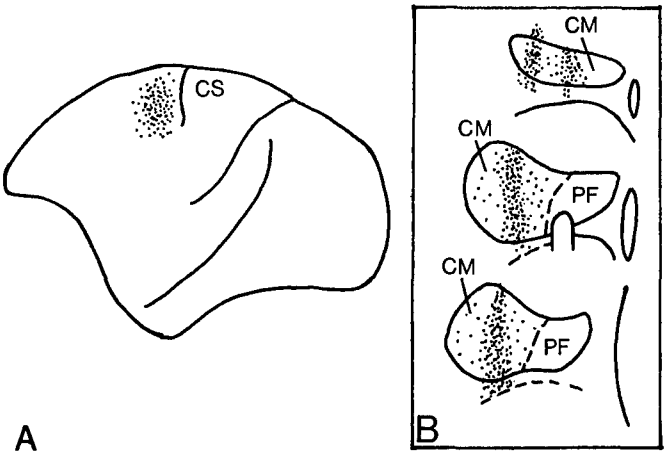


Figure 8. BDA injection sites in the primary motor cortex (*A*) and the centromedian nucleus (*B*) in squirrel monkeys. *CM,* Centromedian nucleus; *CS,* central sulcus; *FR,* fasciculus retroflexus; *PF,* parafascicular nucleus.

Table 3. Kainate receptor subunit immunoreactivity in anterogradely labeled thalamic and cortical terminals in the putamen

	Kainate receptor subunit immunoreactivity	
Sources of terminals	GluR6/7 (%)	KA2 (%)
CM	60 (N = 55)	40 (N = 42)
M1	28 (N = 58)	43 (N = 51)

N = Total number of anterogradely labeled terminals examined.

strikingly different from those of conventional ionotropic glutamate receptors that are largely confined to the main bodies of synaptic active zones (Bernard et al., 1997; Ottersen and Landsend, 1997) and mediate fast synaptic transmission.

It is noteworthy that a substantial level of intracellular AMPA glutamate receptor subunits immunoreactivity has recently been found in dendrites of dorsal cochlear neurons in rats (Rubio and Wenthold, 1999). Interestingly, the receptor subunit labeling was often found in clusters of 2–12 gold particles associated with vesicular structures that strikingly resemble the large organelles immunoreactive for KAR subunits observed in the present study (Rubio and Wenthold, 1999). Although Rubio and Wenthold (1999) showed that some of these structures displayed immunoreactivity for specific markers of endoplasmic reticulum, the majority of labeling was found over elements that were not immunoreactive for ER markers. Future studies are essential to better characterize the exact nature of these glutamate receptor packaging organelles. Presynaptic delta opioid receptors are also expressed on the membrane of large vesicles in primary afferents to the rat spinal cord (Zhang et al., 1998). Whether these vesicles represent an early stage of endosomes and/or a different type of dense-core vesicles remains to be established.

A substantial proportion of presynaptic and postsynaptic gold labeling was also associated with asymmetric synapses, suggesting that kainate receptors may mediate fast excitatory transmission at some glutamatergic synapses in the monkey striatum. However, recent electrophysiological studies could not demonstrate any synaptic activation of kainate receptors after stimulation of striatal glutamatergic afferents (Chergui et al., 2000). At present, there is no clear explanation for this apparent discrepancy between anatomical and functional data. However, these two sets of

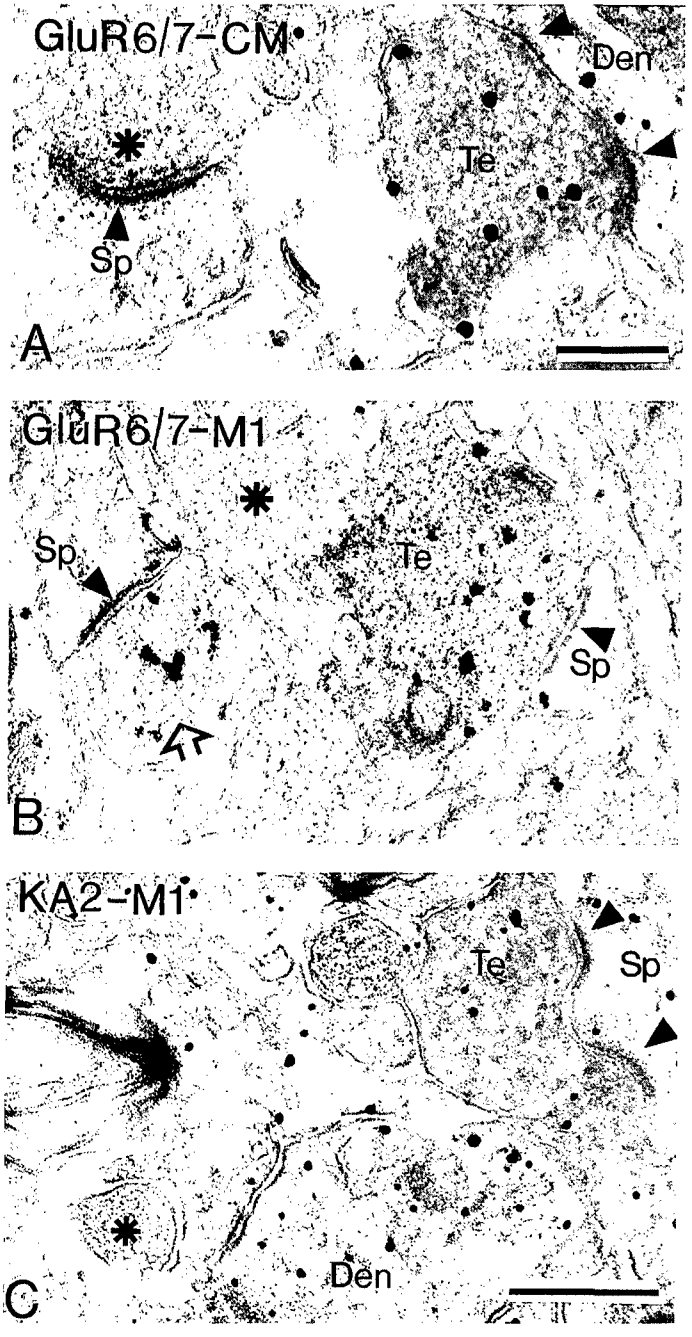


Figure 9. GluR6/7-immunoreactive (*A*, *B*) and KA2-immunoreactive (*C*) terminals (*Te*) labeled anterogradely from the centromedian thalamic nucleus (*CM*) or the primary motor cortex (*M1*) in the putamen. Nonimmunoreactive terminals are indicated by *asterisks*. *Arrowheads* point to asymmetric synapses. The *open arrow* indicates an unlabeled bouton that displays GluR6/7 immunoreactivity. Scale bars: *A*, 0.25 μm (valid for *B*); *C*, 0.5 μm.

findings, combined with those obtained in other brain regions (see below), indicate that the distribution and role of kainate receptors in the CNS are different and likely to be much more complex that those of conventional ionotropic glutamate receptors.

Potential functions of presynaptic striatal kainate receptors

The roles of kainate receptors in the striatum are poorly known. However, the toxic effects of kainic acid for striatal projection neurons have long been established although the exact mechanism by which kainic acid induces striatal cell death is still unknown. In this regard, it is noteworthy that the excitotoxic effects of kainic acid for striatal neurons are abolished after decortication (Biziere and Coyle, 1979), which indicates that the glutamatergic corticostriatal projection is somehow involved in mediating this effect. In addition, the fact that striatal cultures become sensitive to kainic acid only when cocultured with cortical neurons is further evidence for the involvement of corticostriatal afferents (Campochiaro and Coyle, 1978; Panula, 1980). In line with these early observations, our data demonstrate that kainate receptors are, indeed, associated presynaptically and postsynaptically with corticostriatal terminals in monkeys. Classically, postsynaptic AMPA and kainate receptors are ligand-gated ion channels permeable to cations, such that activation of these receptors leads to increased Na^+ and Ca^{2+} conductances, and thus neuronal membrane depolarization. If the presynaptic kainate receptors are similar to their postsynaptic counterparts, depolarization of the nerve terminal plasma membrane could conceivably facilitate the opening of Ca^{2+} channels linked to glutamate release on arrival of an action potential, and thus potentiate the release of glutamate. Interestingly, recent evidence indicates that activation of GluR6-containing presynaptic kainate receptors facilitates glutamate exocytosis from cerebral cortex nerve terminals in a synaptosome preparation (Perkinton and Sihra, 1999). Similarly, kainate acts at presynaptic receptors to increase GABA release from hypothalamic neurons (Liu et al., 1999). However, kainate was found to downregulate GABAergic transmission in the rat hippocampus (Rodriguez-Moreno et al., 1997). These findings suggest that the effects of presynaptic kainate receptors are strongly dependent on the neuronal type in which they are expressed. It is likely that the mechanisms of action of kainate receptors are also complex and different from one neuronal population to another. For instance, it seems that the presynaptic inhibition of GABA release in the hippocampus is independent of ion channel activity but rather involves the activation of phospholipase C and protein kinase C (PKC) through a Pertussis toxin-sensitive G-protein (Rodriguez-Moreno and Lerma, 1998). One could speculate that the facilitatory effects observed in the cereberal cortex and hypothalamus are mediated via increased calcium conductances in the membrane of the nerve terminals and/or activation of a pool of PKC that facilitates neurotransmitter release. Whether any of these effects are suitable to explain the functions of kainate receptors in the striatum remains to be established.

At first glance, it appears reasonable to believe that presynaptic kainate receptors facilitate glutamate release in the striatum, which might explain why the excitotoxic effects of kainic acid are dependent on the presence of cortical afferents. One could also speculate that the malfunctioning of such receptors in some HD patients might lead to an increased release of glutamate and excitotoxic cell death. However, a recent *in vitro* slice preparation study showed that kainate receptors could not be activated neither by a single or repetitive stimulation of glutamatergic afferents in the rat striatum (Chergui et al., 2000). The authors rather demonstrated that kainate receptors depress GABAergic synaptic transmission indirectly via release of adenosine acting on A2a receptors (Chergui et al. 2000). These data are surprising and difficult to reconcile with our electron microscopic findings showing the abundance of presynaptic kainate receptors in both cortical and thalamic glutamatergic afferents (Charara et al., 1999). However, the physiological conditions under which these kainate receptors are activated remains to be determined. If, under the

experimental conditions used by Chergui et al. (2000), kainate receptors remained in intracellular compartments rather than on the plasma membrane, their lack of responses to stimulation of glutamatergic afferents is not surprising. Future studies are essential to elucidate this issue.

Concluding remarks

In conclusion, it appears that kainate receptors underlie novel modulatory functions of synaptic transmission. Their extrasynaptic localization combined with metabotropic-like effects shown in the hippocampus suggest that these receptors probably mediate slow modulatory effects rather than fast synaptic transmission. The evidence that the gene encoding the GluR6 subunit might be affected in a subset of HD patients highlights the importance of these receptors in striatal functions in normal and pathological conditions. The use of selective antagonists for kainate and AMPA receptors in normal animals, knock-out mice, and animal model of neurodegenerative diseases will certainly prove useful to clearly assess the role of kainate receptors in the striatum.

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The thalamostriatal system: a highly specific network of the basal ganglia circuitry

Yoland Smith, Dinesh V. Raju, Jean-Francois Pare and Mamadou Sidibe

Yerkes National Primate Research Center and Department of Neurology, Emory University, Atlanta, GA 30322, USA

Although the existence of thalamostriatal projections has long been known, the role(s) of this system in the basal ganglia circuitry remains poorly characterized. The intralaminar and ventral motor nuclei are the main sources of thalamic inputs to the striatum. This review emphasizes the high degree of anatomical and functional specificity of basal ganglia–thalamostriatal projections and discusses various aspects of the synaptic connectivity and neurochemical features that differentiate this glutamate system from the corticostriatal network. It also discusses the importance of thalamostriatal projections from the caudal intralaminar nuclei in the process of attentional orientation. A major task of future studies is to characterize the role(s) of corticostriatal and thalamostriatal pathways in regulating basal ganglia activity in normal and pathological conditions.

Existence of the thalamostriatal system was first suggested by Vogt and Vogt [1]. Confirmation of this suggestion and the starting point for further extensive analyses of this system came 15 years later in an extensive cell degeneration study by Powell and Cowan [2] that showed profuse striatal projections from the whole intralaminar nuclear complex in primates. Since then, anatomical and functional organization of this projection and its potential role in regulating neurotransmitter homeostasis in the basal ganglia under normal and pathological conditions has been examined. This review summarizes the key chemoanatomical and functional characteristics of the thalamostriatal system and proposes a series of functional basal ganglia–thalamostriatal loops through which segregated information can be processed and integrated at different levels of the basal ganglia circuitry. Readers are referred to previous reviews for additional information and broader coverage of early literature on the thalamostriatal projections [3–7].

Source(s), topography and intrastriatal organization of thalamostriatal projections

Intralaminar thalamostriatal projections

The main source(s) of thalamostriatal projections are intralaminar thalamic nuclei, but substantial inputs from midline and specific relay nuclei have also been described in rats [7–11], cats [12,13] and monkeys [14–18] (**Figure 1**). The rostral intralaminar complex comprises the central medial, paracentral and central lateral nuclei, whereas the caudal intralaminar group includes the centromedian (CM)–parafascicular (PF) nuclear complex in primates. Although the CM is not clearly delineated in rodents, the lateral part of the PF is considered the homolog of the primate CM, whereas the medial PF displays strong similarities with the PF proper in monkeys [3,7]. The topography of thalamostriatal projections from these nuclei is depicted in **Figure 1**.

In monkeys, organization of thalamostriatal projections from the caudal intralaminar nuclei has been studied in detail, but little is known about the topography of the rostral intralaminostriatal pathways. Based on their striatal targets and sources of basal ganglia inputs, the CM–PF complex is divided into five major compartments in primates: (i) the rostral third of PF projects predominantly to the nucleus accumbens; (ii) the caudal two-thirds of PF innervate the caudate nucleus; (iii) the dorsolateral extension of PF (PFdl) projects to the anterior putamen; (iv) the medial two-thirds of CM (CMm) project to the post-commissural putamen; and (v) the lateral third of CM (CMl) projects to the primary motor cortex (**Figure 1**). Through these highly topographic and specific projections, the CM–PF complex influences widespread striatal regions involved in processing functionally segregated information (**Figure 1**). The rostral PF is mainly related to the limbic striatum, the rest of the PF and PFdl are preferentially connected with associative striatal territories, and the CMm is the main source of inputs to sensorimotor striatal regions [17,19,20] (**Figure 1**).

Non-intralaminar thalamostriatal projections

Thalamostriatal projections also arise from midline and specific thalamic nuclei (**Figure 1**). In rats, the midline nuclear group comprises the paraventricular, parataenial, interanteromedial, intermediodorsal, rhomboid and reuniens nuclei. Projections from these nuclei are mainly confined to the ventral striatum but they also provide significant inputs to dorsal striatal regions [7,8] (**Figure 1**).

Another major source of thalamic inputs to the striatum are ventral motor thalamic nuclei [9,12,15,18]. Complex organization of these projections and tight relationships between functionally related ventral motor thalamostriatal and corticostriatal projections from motor,

Corresponding author: Yoland Smith (yolands@rmy.emory.edu).
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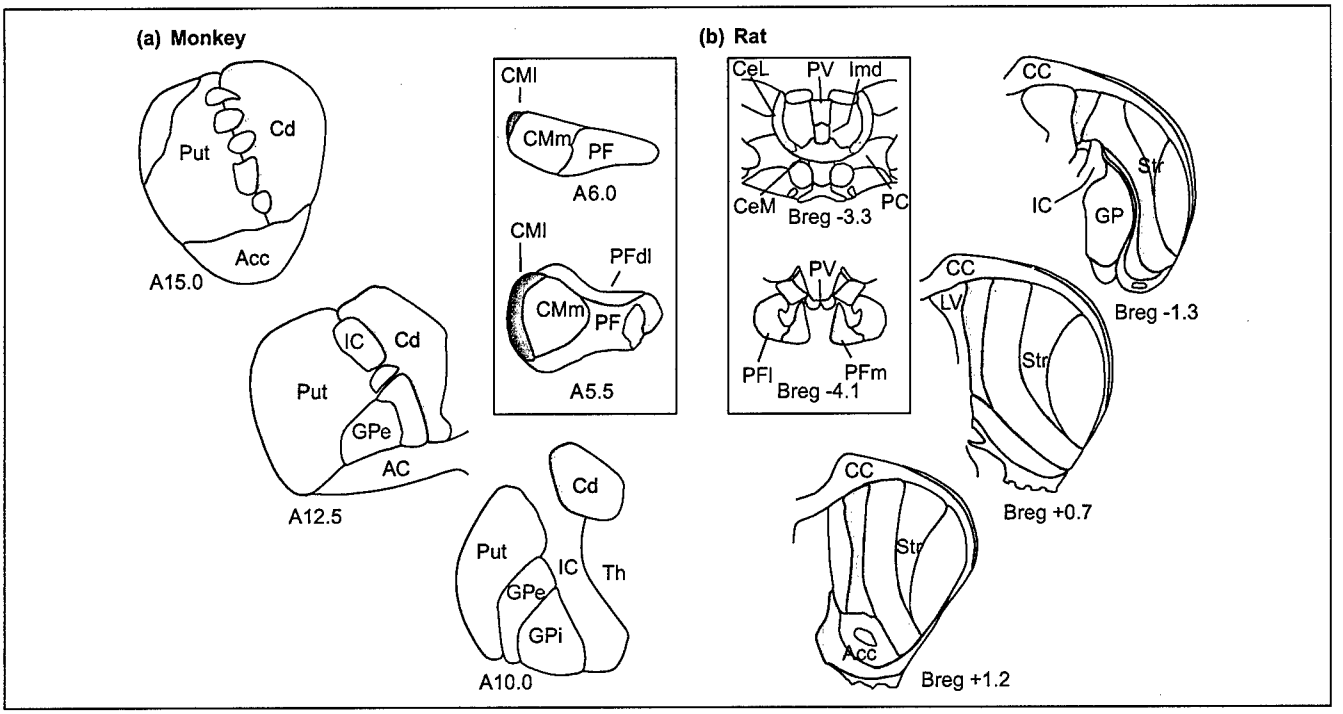

Figure 1. Rostrocaudal striatum (Str), showing the pattern of distribution of thalamic inputs from caudal intralaminar thalamic nuclei in monkey **(a)** and from rostral intralaminar, caudal intralaminar and midline thalamic nuclei in rat **(b)**. Striatal territories receive inputs from the corresponding color-coded thalamic nuclei (middle boxes). The lateral centromedian (CMl) nucleus in monkey projects to the primary motor cortex but not to the striatum. In rats, projections from the central medial (CeM) and medial parafascicular (PFm) nuclei terminate along the rostrocaudal extent of the medial third of the caudate (Cd)–putamen (Put) complex, whereas inputs from the lateral parafascicular (PFl) nucleus innervate profusely the lateral sector of the dorsal striatum. Projections from central lateral (CeL) and paracentral (PC) nuclei occupy the central core of the dorsal striatum. Projections from the parataenial nucleus (not shown) provide a substantial innervation of the central portion of the rostral part of the nucleus accumbens (Acc). Inputs from intermediodorsal (Imd) nucleus are concentrated in the core of the nucleus accumbens rostrally, and caudally they invade the ventromedial part of the caudate–putamen complex and a striatal region along the dorsal border of the globus pallidus (GP). Anteroposterior stereotaxic coordinates that these drawings correspond to are indicated below each section. Additional abbreviations: A, anterior; AC, anterior commissure; Breg, bregma; CC, corpus callosum; CMm, medial centromedian nucleus; GPe, globus pallidus, external segment; GPi, globus pallidus, internal segment; IC, internal capsule; LV, lateral ventricle; PF, parafascicular nucleus; PFdl, dorsolateral parafascicular nucleus; PV, paraventricular nucleus; Th, thalamus. Rat data are reproduced, with permission, from Ref. [8] © (1990) Wiley-Liss, Inc., a subsidiary of John Wiley & Sons.

premotor and supplementary motor areas at the level of the striatum have been demonstrated in monkeys [4,15,21]. Interconnected regions of the ventral motor thalamic nuclei and motor cortices send convergent inputs to the sensorimotor striatum, suggesting functional interactions between corticostriatal and thalamostriatal projections in motor behaviors [4,21].

Several other thalamic nuclei have been recognized as potential sources of thalamostriatal projections in rats, cats and monkeys [3,6,12,18,22], but detail on the topography and intrastriatal arborization of these projections is scarce and will not be discussed further in this review.

Thalamostriatal versus thalamocortical systems: segregated or collateralized axonal sources

Overall, retrograde double labeling studies agree that a substantial proportion of neurons in the rostral intralaminar nuclear group and some specific thalamic nuclei (the ventral anterior and ventral lateral complex, and the mediodorsal nucleus) provide axon collaterals to both the striatum and the cerebral cortex, whereas thalamostriatal and thalamocortical neurons are largely segregated in the caudal intralaminar CM–PF nuclear complex [6,23,24]. For instance, CM projections to the primary motor cortex in monkeys arise from a restricted neuronal population confined to CMl, whereas neurons in CMm project to the post-commissural putamen (Figure 1). However, single-cell-filling injection data suggest that most, if not all, PF neurons that project to the caudate–putamen complex send sparse collaterals to the cerebral cortex in rats [25]. This pattern is opposite from that displayed by projections from the central lateral nucleus, which provide scarce, loosely organized long varicose processes in the striatum but form dense patches of terminals in the rat cortex [11,26].

Differential synaptic organization of thalamostriatal versus corticostriatal projections

Differential pattern of synaptic innervation of striatal neurons

Both medium spiny projection neurons and aspiny interneurons are targeted by thalamostriatal and corticostriatal projections, but the pattern of distribution and density of innervation vary depending on the chemical phenotype and projection sites of the postsynaptic striatal neurons. In contrast to cortical inputs, which terminate predominantly on the heads of dendritic spines [27], the vast majority of thalamic terminals from caudal intralaminar nuclei form asymmetric synapses on dendritic shafts of medium-sized projection neurons in rats and monkeys [17,27–29] (Figure 2; Table 1). However, studies in rats implanted with striatal grafts demonstrate that inputs from rostral intralaminar nuclei target

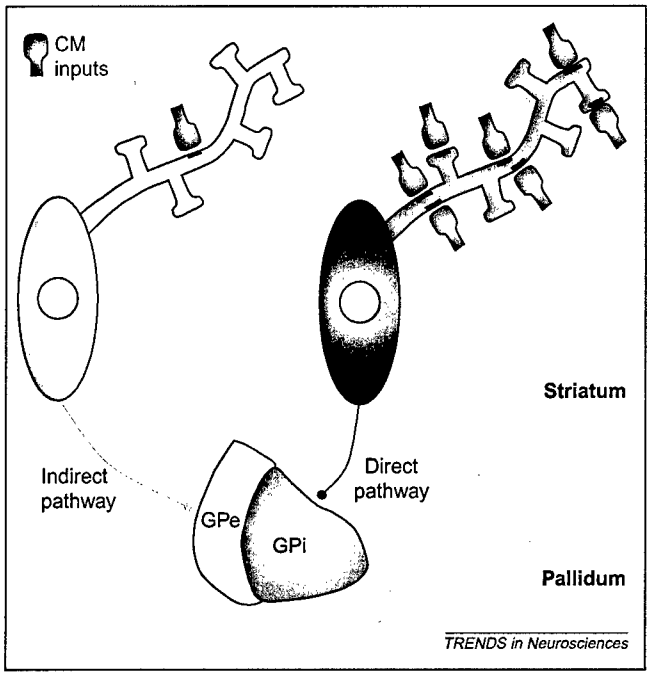

Figure 2. Different degrees of innervation of striatal neurons projecting to the external globus pallidus (GPe; 'indirect pathway', yellow) versus striatal neurons projecting to the internal globus pallidus (GPi; 'direct pathway', blue) by inputs from the centromedian nucleus (CM; red terminals) in the monkey sensorimotor striatum [29]. Centromedian nucleus inputs form asymmetric synapses preferentially on dendrites of 'direct' striatofugal neurons projecting to the internal globus pallidus.

preferentially dendritic spines in the host neostriatum [30], suggesting a certain degree of variability in the postsynaptic targets of thalamostriatal projections depending on their origin. Additional studies in normal striatum must be performed to confirm these observations.

Differential innervation of direct versus indirect striatofugal neurons

Another feature of intralaminar thalamic inputs to striatofugal neurons is the different degree of innervation of so-called 'direct versus indirect' striatal output neurons by CM (**Figure 2**). Projections from CM form synapses more frequently with striatofugal neurons that project to the internal versus the external globus pallidus in monkeys, which suggests that CM modulates preferentially the direct output pathway of the sensorimotor striatum in primates [29] (**Figure 2**; **Table 1**). However, it is noteworthy that PF lesions prevent upregulation of enkephalin mRNA (a marker of indirect- pathway neurons), but do not affect downregulation of substance P mRNA (a marker of direct-pathway neurons), in the striatum of 6-hydroxydopamine-treated rats [31,32]. Although additional studies must be performed in parkinsonian monkeys to correlate these two sets of data, they pave the way for complex, direct or indirect, modulatory functions of thalamic inputs to the two populations of striatofugal neurons in both normal and pathological conditions.

Interestingly, sensorimotor corticostriatal afferents also have different influences on the two segregated populations of striatofugal neurons. More than 75% of striatofugal neurons that express c-Fos following microstimulation of physiologically corresponding sites in the primary motor and somatosensory cortex give rise to the 'indirect' pathway [33–35]. Whether the differential functional effects are mediated by a preferential synaptic innervation of indirect striatofugal neurons by primary motor cortical inputs is not clear. There is evidence that this might be the case in monkeys but not in rats [36,37].

Differential synaptic interactions with dopaminergic afferents

The importance of dopamine–glutamate interactions in the striatum is unequivocal. An improper balance of activity between these two neurotransmitters is a key feature that underlies some of the behavioral changes in Parkinson's disease, schizophrenia and addiction to psychomotor stimulants [38–40]. The convergence of corticostriatal and nigrostriatal afferents on the head and neck of dendritic spines, respectively, is a well-established anatomical substrate that underlies some of the functional relationships between cortical and nigral inputs to the striatum [27]. By contrast, thalamic inputs from CM and dopaminergic terminals are not in close proximity on the same postsynaptic targets in the monkey striatum [28] (**Table 1**). Together, these anatomical arrangements suggest that dopaminergic afferents are located to subserve more specific modulation of afferent cortical inputs than afferent thalamic inputs in the sensorimotor territory of the primate striatum [28]. However, this does not rule out the possibility that dopaminergic and thalamic inputs could terminate at distant locations on the same neurons. As already

Table 1. Differences between thalamostriatal projections from caudal intralaminar nuclei and corticostriatal projections[a]

Caudal intralaminar thalamostriatal projections	Corticostriatal projections	Refs
Target mainly dendrites of striatal output neurons	Target mainly spines of striatal output neurons	[17,27–29,36]
Do not display any synaptic relationships with dopaminergic afferents on striatal output neurons	Frequently converge with dopaminergic inputs onto dendritic spines	[27,28]
Form synapses preferentially with 'direct' striatofugal neurons	Cortical stimulation induces c-Fos expression preferentially in 'indirect' striatofugal neurons	[29,33,35]
Provide strong synaptic inputs to the proximal dendrites and perikarya of cholinergic interneurons	Provide scarce inputs to distal dendrites of cholinergic interneurons	[41–43]
Facilitate striatal ACh release through NMDA receptor activation	Facilitate striatal ACh release through non-tonic activation of AMPA receptors	[44]
Are parvalbumin and calretinin immunoreactive	Do not display Ca^{2+}-binding-protein immunoreactivity	[46]
Almost 50% display mGluR1a immunoreactivity	Less than 5% display mGluR1a immunoreactivity	[51]
Mainly use VGluT2	Mainly use VGluT1	[31,58]

[a]Abbreviations: mGluR1a, metabotropic glutamate receptor 1a; VGluT, vesicular glutamate transporter.

discussed, there is good evidence that excitatory influences provided by intralaminar thalamic inputs are a major drive for the regulatory changes in neurochemical phenotype of striatal neurons that are induced by dopamine-mediated nigrostriatal modulation under normal and pathological conditions [31,32].

Differential synaptic innervation of interneurons

Differential thalamic and cortical innervation has also been found at the level of striatal cholinergic versus parvalbumin-containing GABAergic interneurons. Cholinergic striatal interneurons receive substantial inputs from CM–PF but are almost devoid of cortical afferents, except for light innervation of distal dendrites [41–43] (Table 1). These electron microscopic data are consistent with microdialysis studies showing a more powerful and long-lasting effect of PF stimulation than cortical stimulation on NMDA-mediated ACh release in the striatum [44]. By contrast, parvalbumin-containing GABAergic interneurons are massively innervated by both cortical and CM inputs in monkeys [45,46]. However, they receive scarce thalamic innervation from PF in rats [47]. These electron microscopic data are in line with the significant increase in c-Fos expression in parvalbumin-containing interneurons, but not in choline acetyltransferase (ChAT)-immunoreactive neurons, following motor cortical stimulation in rats and monkeys [33,35]. The calretinin-containing interneurons are devoid of CM inputs [46] and only ~10% of them show c-Fos expression following cortical activation in monkeys [35], suggesting that the extrinsic glutamatergic cortical and thalamic inputs might not be the primary drive of these striatal interneurons in primates. Interneurons containing somatostatin, nitric oxide and neuropeptide Y receive direct inputs from CM in monkeys [46] and respond to both cortical and thalamic activity changes in primates and rodents [33,35]. Surprisingly, some authors have been unsuccessful in demonstrating direct PF inputs to neuropeptide-Y-containing neurons in rats [48].

Neurotransmitters, neuromodulators and receptors associated with the thalamostriatal system

Glutamate is the main neurotransmitter used by the thalamostriatal projection. Ca^{2+}-binding proteins coexist with glutamate along the thalamostriatal projection from CM in monkeys [46]. NMDA, AMPA, kainate and group I metabotropic glutamate receptors are located to subserve postsynaptic effects and/or modulation of neurotransmitter release at thalamostriatal synapses (**Figure 3**). High-resolution electron microscopic studies in rats provide ultrastructural evidence for AMPA and kainate receptor subunit immunoreactivity in the main body of asymmetric axospinous and axodendritic synapses [49,50]; an indication that both thalamostriatal and corticostriatal inputs are endowed with postsynaptic AMPA and kainate receptors (**Figure 3**). In monkeys, both group I metabotropic glutamate receptors (mGluR1a and mGluR5) are located at the edges of corticostriatal and thalamostriatal inputs [51]. However, mGluR1a, but much less frequently mGluR5, is expressed presynaptically in CM afferents to the sensorimotor putamen [51]. By contrast, most corticostriatal terminals from the primary motor cortex do not display significant mGluR1a immunoreactivity, suggesting that mGluR1a has more powerful

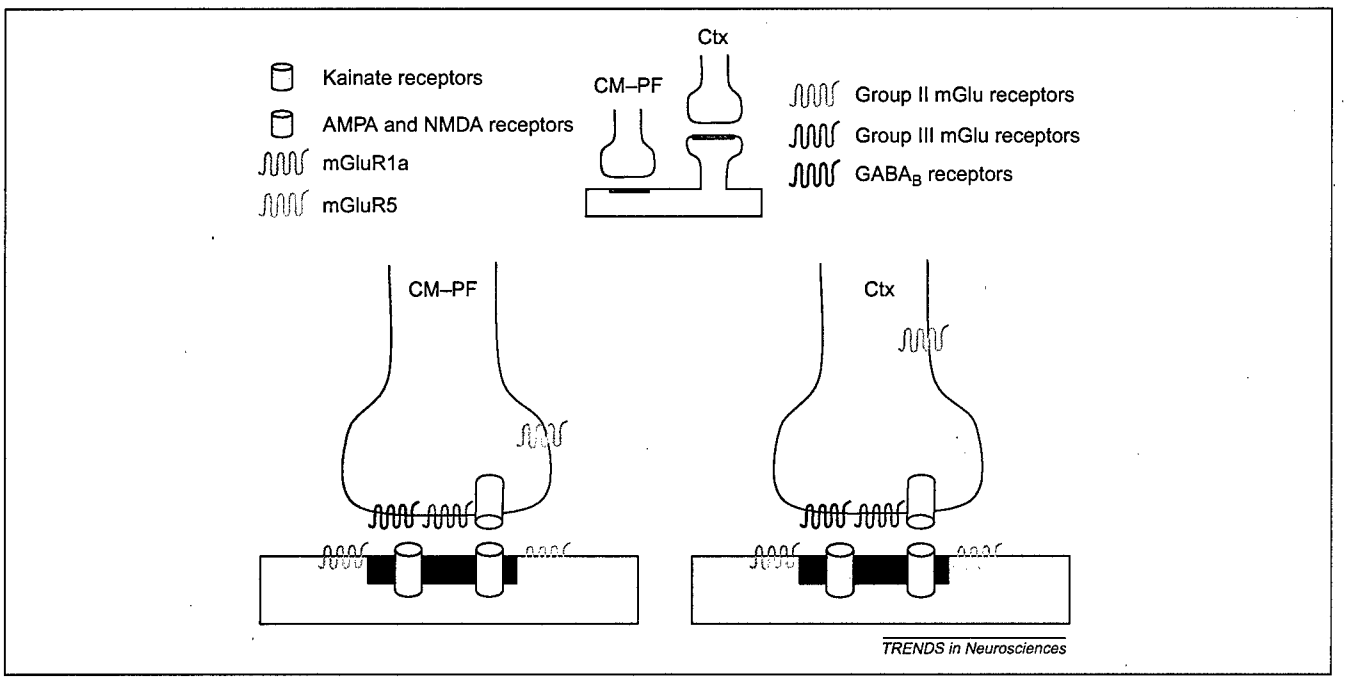

Figure 3. Localization of various subtypes of glutamate and $GABA_B$ receptors at striatal synapses of neurons from the cerebral cortex (Ctx, yellow terminal) and from the thalamic centromedian–parafascicular complex (CM–PF, green terminal), as revealed by electron microscopic immunoperoxidase and immunogold techniques combined with tract-tracing methods [49–53,57]. Note that extrasynaptic and intracellular pools of receptors are not illustrated. Both inputs are endowed with the same combination of glutamate and $GABA_B$ receptors, except for presynaptic metabotropic glutamate (mGlu) receptor 1a (mGluR1a), which is found predominantly on thalamostriatal afferents. Group II metabotropic glutamate receptors are expressed on pre-terminal axonal segments of cortical afferents but their localization on thalamostriatal inputs has not yet been shown.

control of glutamate release at thalamostriatal than corticostriatal synapses in primates [51] (**Figure 3**; **Table 1**). Group III metabotropic glutamate receptors (mGluR4 and mGluR7) are expressed in putative cortical or thalamic terminals forming axospinous or axodendritic synapses, respectively, in the rat striatum [52,53] (**Figure 3**). Although there is solid evidence for group II (mGluR2 and mGluR3) metabotropic glutamate receptor expression on the pre-terminal axonal segments of corticostriatal afferents, their localization on thalamostriatal projections remains to be established [54] (**Figure 3**). Group II and group III metabotropic glutamate receptor activation reduces glutamate-mediated transmission at corticostriatal synapses in slices of rat striatum [55,56] but its effect on the thalamostriatal projection is unknown. Both thalamostriatal and corticostriatal inputs express presynaptic GluR6/7 kainate receptor subunits and $GABA_{B1}$ receptor immunoreactivity in the monkey striatum [50,57] (**Figure 3**). Interestingly, the two main vesicular glutamate transporters, VGluT1 and VGluT2, are differentially expressed in corticostriatal and thalamostriatal terminals. VGluT1 is mainly found in cortical boutons, whereas VGluT2 is preferentially associated with thalamic afferents [31,58] (**Table 1**).

Functional basal ganglia–thalamostriatal loops

Ventral motor nuclei and the CM–PF complex are the main thalamic targets of GPi efferents [6,19]. The majority of GPi cells that project to the ventrolateral nucleus send axon collaterals to the CM–PF complex [6]. In turn, both the ventral motor and CM–PF nuclear groups send highly topographic and specific inputs to different functional territories of the striatum [15,17,20,29] (**Figure 1**). In monkeys, functionally segregated outputs from GPi largely innervate different regions of CM–PF [19]. Together, these anatomical data provide a substrate for the formation of functionally segregated basal ganglia–thalamostriatal loops in primates (**Figure 4**).

Recent evidence suggests the existence of functional basal ganglia–thalamostriatal loops that involve the ventral motor thalamic nuclei in primates. Although the ventral anterior and ventral lateral complex is commonly seen as the main source of basal ganglia outflow to the cerebral cortex, it also provides substantial and highly topographic projections to the striatum [4,6,9,15,21]. Therefore, the ventral motor thalamic nuclei should not be seen solely as a relay for basal ganglia outflow to the cerebral cortex but, rather, as a group of thalamic nuclei that also provide a significant positive feedback to the sensorimotor striatum (**Figure 4**).

Non-striatal basal ganglia targets of intralaminar thalamic projections

In addition to the striatum, CM–PF projections innervate other basal ganglia nuclei, including the subthalamic nucleus (STN), the globus pallidus and the substantia nigra [10,11,25,26,59]. In monkeys, the thalamosubthalamic projection is functionally organized so that sensorimotor neurons in CMm terminate preferentially in the 'motor-related' dorsolateral part of the STN, whereas limbic and associative neurons in PF project almost exclusively to the medial 'limbic-related' region of

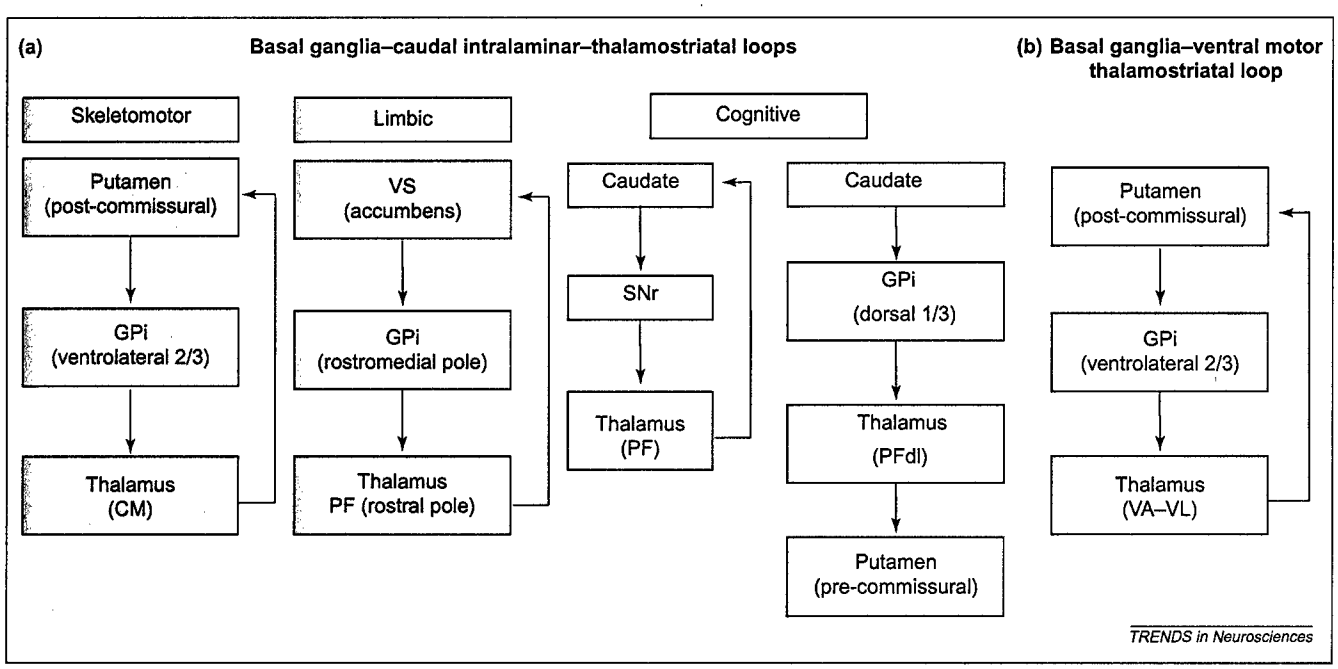

Figure 4. Segregated basal ganglia–thalamostriatal loops through the caudal intralaminar and ventral motor thalamic nuclei in monkeys. Black arrows indicate GABAergic projections; red arrows indicate glutamatergic pathways. **(a)** The skeletomotor internal globus pallidus (the ventrolateral two-thirds of the GPi) projects exclusively to the centromedian nucleus of the thalamus (CM), whereas the limbic GPi (its rostromedial pole) and associative GPi (its dorsal third) innervate the rostral pole of the parafascicular nucleus (PF) and the dorsolateral PF (PFdl), respectively. In turn, CM and PF neurons project back to the corresponding functional territories of the dorsal and ventral striatum (VS). The CM–PF complex is part of segregated functional loops with the striatopallidal complex. The substantia nigra pars reticulata (SNr) innervates PF neurons that project to the caudate nucleus [5,20], which provides an additional 'associative' circuit between basal ganglia and thalamostriatal neurons. **(b)** A basal ganglia–ventral motor thalamostriatal loop that involves specific regions of the ventral anterior and ventral lateral nuclear complex (VA–VL), the post-commissural putamen and the ventrolateral two-thirds of the GPi has been described in monkeys [21].

the STN [23]. A similar topographical organization was also found for PF–STN projections in rats [60]. Although thalamostriatal and thalamosubthalamic neurons were originally thought to be largely segregated [61], a single-cell-filling study showed that some PF neurons that project to the striatum send axon collaterals to the STN in rats [25]. The thalamosubthalamic input is excitatory and tonically drives activity of STN neurons [62]. This excitatory thalamic drive might be crucial in mediating some of the pathophysiological effects of Parkinson's disease in humans [62].

The thalamopallidal projection arises predominantly from axon collaterals of thalamostriatal axons from the PF, although rostral intralaminar nuclei also provide significant pallidal inputs [11,25]. This projection is highly topographic and supplies functionally segregated information to the associative, sensorimotor and limbic parts of the rat globus pallidus [11,23].

Role of the thalamostriatal projections from CM–PF in attention

The exact role of the thalamostriatal pathway remains poorly understood. Kimura and colleagues [63–65] recently proposed that CM and PF supply striatal neurons with information that have attentional values, thus acting as detectors of behaviorally significant events occurring on the contralateral side. These observations are consistent with a positron emission tomographic study in humans showing activation of the CM–PF complex when participants switch from a relaxed awake state to an attention-demanding reaction-time task [66]. Two main functional characteristics of CM–PF neurons were disclosed in monkeys [63]. First, CM–PF neurons have multimodal properties – that is, they respond to a large variety of sensory stimuli (auditory, visual and somatosensory) presented either in or outside sensorimotor conditioning tasks. Second, CM–PF neurons are temporally tuned – that is, they can generate in a timely manner discrete and coherent responses to a wide variety of sensory stimuli. On the basis of their latency and pattern of responses to sensory stimuli, CM–PF neurons have been categorized into two main populations, namely those that display short-latency facilitatory responses (SLF neurons) and those that display long-latency facilitatory responses (LLF neurons) to sensory events [63]. These two populations are largely segregated in the CM–PF complex, SLF neurons being mainly found in PF whereas LLF are particularly abundant in CM. Responses of both types of neurons are not associated with reward. This contrasts them with the tonically active neurons (TANs) – putative striatal cholinergic interneurons that are a major striatal target of SLF and LLF neurons – which under the same experimental conditions respond preferentially to rewarding stimuli [63]. However, CM–PF inputs are required for the sensory responses of TANs that are acquired through sensorimotor learning. Inactivation of CM–PF decreases the characteristic pause and subsequent rebound facilitation, but does not affect the early short-latency facilitation, of TANs in response to sensorimotor conditioning [63,67]. Considering the importance of the dopamine system in modulating striatal activity through TANs [67], one might suggest that the behaviorally sensory events transmitted along the thalamostriatal projections from CM–PF, in coordination with the motivational value of the dopamine inputs, provide a strong basis for proper selection of actions through the basal ganglia thalamocortical–striatal circuitry [63].

Information flowing along the thalamostriatal pathway from CM–PF and its functional relevance for basal ganglia functions are likely to differ from the signals provided to the striatum by thalamostriatal projections from the ventral motor nuclear group [4,21] (**Figure 4**). Although the functions of the ventral thalamostriatal projection remain unclear, it is possible that this system might be a positive reinforcer of striatal neurons involved in performing a selected behavior [21].

CM–PF degeneration in basal ganglia diseases

The tight association between the caudal intralaminar nuclei and the basal ganglia is further exemplified by the neuropathological evidence for >50% neuronal loss in the CM–PF complex of patients with Huntington's and Parkinson's diseases [68–70]. In parkinsonians, parvalbumin-containing neurons seem to be differentially affected in the CM and PF: 50% of parvalbumin-containing neurons are affected in PF whereas 70% loss of parvalbumin-negative neurons has been found in CM [70]. Significant neuronal loss in CM–PF has also been reported in progressive supranuclear palsy (PSP) [69]. In Parkinson's disease, Lewy bodies are found in CM–PF, and in PSP, abundant intracellular neurofibrillary and glial tangles concentrate in this complex. Other thalamic nuclei do not appear to be affected by these degenerative processes, emphasizing the high specificity of relationships between basal ganglia and caudal intralaminar nuclei in both normal and pathological conditions in primates.

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CHAPTER 165

Anatomy and Synaptic Connectivity of the Basal Ganglia

YOLAND SMITH ■ ALI CHARARA

OVERALL ORGANIZATION OF THE BASAL GANGLIA

The basal ganglia are a group of subcortical nuclei of the mammalian brain that are intimately involved in motor control but also play complex roles in mediating cognitive and limbic functions. The basal ganglia traditionally include the striatum, which is composed of the caudate nucleus, the putamen, and the nucleus accumbens; the external globus pallidus (GPe; globus pallidus in nonprimates); the internal globus pallidus (GPi; entopeduncular nucleus in nonprimates); the substantia nigra, which includes the dopaminergic neurons in the substantia nigra pars compacta (SNc) and the γ-aminobutyric acid (GABAergic) neurons in the substantia nigra pars reticulata (SNr); and the subthalamic nucleus.

They are a complex and highly interconnected group of nuclei that have been the subject of intensive studies over many decades because of their clear involvement in neurological disorders that manifest as abnormal motor activities. The striatum and, to a lesser extent, the subthalamic nucleus are the main entrances of extrinsic information to the basal ganglia circuitry. The striatal architecture is divided into two compartments called *patches* (or striosomes) and *matrix*, which are characterized by differential expression of various neurotransmitters, receptors, and input-output connections.[1] The cerebral cortex and the intralaminar thalamic nuclei are the two major sources of excitatory glutamatergic afferents to the striatum and subthalamic nucleus. Dopaminergic inputs from the SNc and the ventral tegmental area, as well as serotonin inputs from the dorsal raphe, tightly interact with glutamatergic afferents to modulate striatal neuronal activity. After integration at the striatal level, the information is conveyed by medium-sized spiny projection neurons to the basal ganglia output nuclei (i.e., GPi and SNr) that forward basal ganglia outflow to frontal areas of the cerebral cortex through the ventrolateral thalamus or various brainstem structures (e.g., superior colliculus, lateral habenular nucleus, pedunculopontine nucleus, parvicellular reticular formation). Part of the information flowing through the GPi returns to the striatum via connections with thalamostriatal neurons in the caudal intralaminar nuclei (Fig. 165–1).

Major aspects of the chemical anatomy and synaptic connectivity of the basal ganglia are reviewed in this chapter, and findings that have introduced novel concepts of basal ganglia organization are highlighted. A detailed account of the earlier literature is provided in several reviews published during the 1990s.[1–13]

AFFERENTS TO THE STRIATUM

Functional Organization of the Corticostriatal Projection

The entire cortical mantle provides a highly topographic input to the striatum. In primates, the somatosensory, motor, and premotor cortices project somatotopically to the postcommissural region of the putamen and the associative cortical areas project to the caudate nucleus and the precommissural putamen. The limbic cortices, the amygdala and the hippocampus, terminate preferentially in the ventral striatum, which includes the nucleus accumbens and the olfactory tubercle.[6, 7, 10, 12, 13]

Processing and integrating functionally related information within these striatal territories is probably very complex and governed by convergence and segregation of cortical inputs. For instance, projections from prefrontal oculomotor areas interconnected by corticocortical projections (i.e., frontal eye field and supplementary eye field) tightly overlap within the monkey striatum.[14] Conversely, Selemon and Goldman-Rakic[15] demonstrated that projections from connection-linked associative cortical areas in the frontal, parietal, and temporal lobes are completely segregated or interdigitated within a zone of overlap in the monkey striatum. The results of this study also introduced a novel concept of corticostriatal projection organization related to which associative cortices project to longitudinally extensive domains aligned along the mediolateral axis of the caudate nucleus in primates.[15] Complex patterns

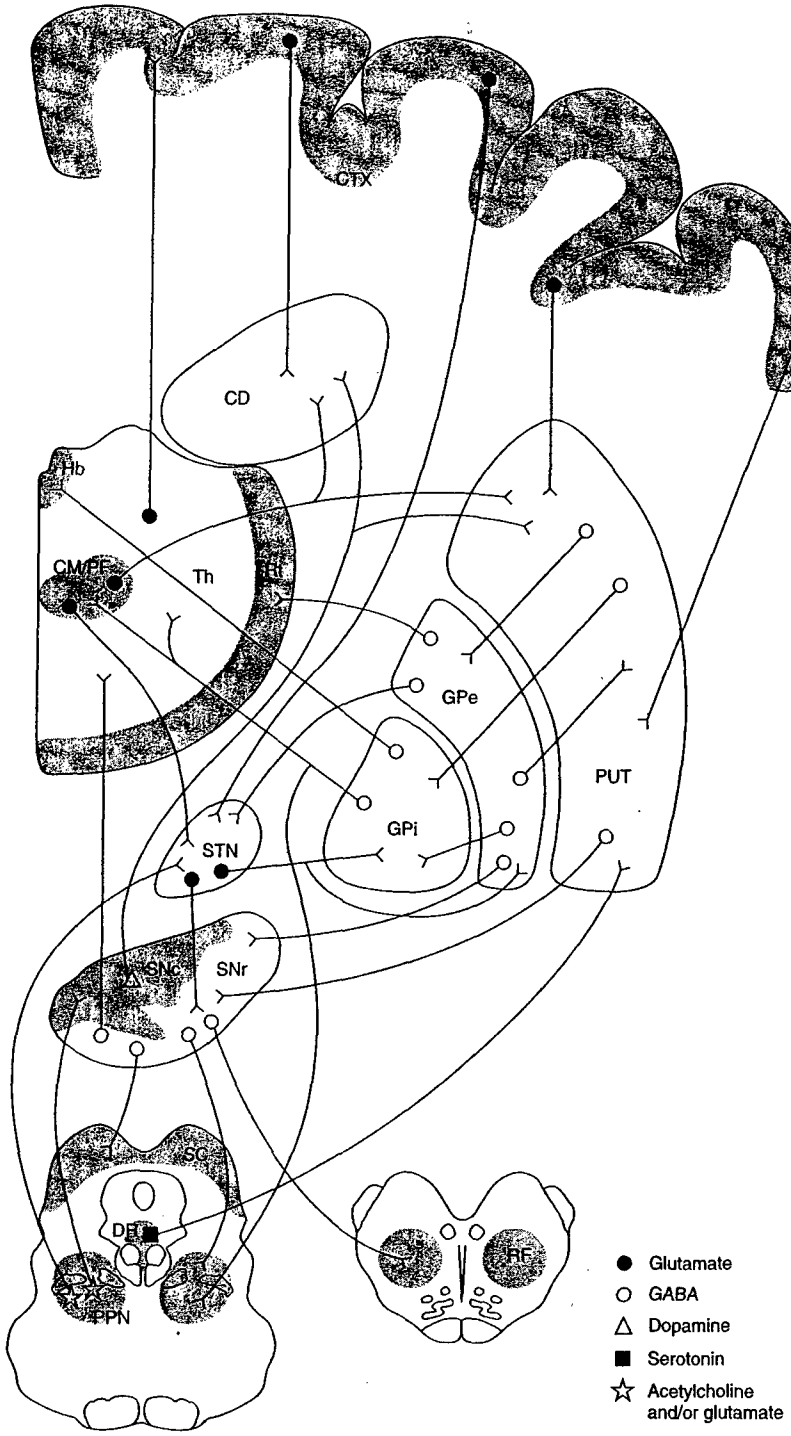

FIGURE 165–1. In the simplified schematic diagram of basal ganglia connectivity in primates, some connections have been omitted. The symbols used to label neuronal cell bodies indicate the main neurotransmitter used by these neurons. CD, caudate nucleus; CTX, cerebral cortex; CM/PF, center median/parafascicular nuclei; DR, dorsal raphe; GPe, globus pallidus, external segment; GPi, globus pallidus, internal segment; Hb, habenula; PUT, putamen; Rt, reticular nucleus; RF, reticular formation; SC, superior colliculus; SNc, substantia nigra, pars compacta; SNr, substantia nigra, pars reticulata; STN, subthalamic nucleus; Th, thalamus; PPN, pedunculopontine nucleus.

of intrastriatal organization were also found for projections from sensorimotor cortical areas.[16, 17] For example, regions representing homologous body parts in different somatosensory cortical regions and primary motor cortex send projections that converge within the ipsilateral putamen, whereas contralateral projections from the primary motor cortex, except those from the face area, interdigitate with ipsilateral primary motor cortex and somatosensory cortical projection sites in squirrel monkeys.[17]

The striatum is composed of two main populations of neurons: the medium-sized GABAergic projection neurons, which have their dendrites densely covered with spines and account for more than 90% of the total neuronal population of the striatum, and the aspiny neurons, which are much less abundant and are generally considered to be interneurons.[3, 7, 10, 18] Dendritic spines of projection neurons are by far the main targets of corticostriatal afferents. Convergence of cortical and dopamine inputs at the level of individual spines was

found to be a major feature of the synaptic connectivity of striatofugal neurons in rats and monkeys.[3, 12, 13] GABAergic interneurons also receive significant cortical inputs,[19] whereas cholinergic interneurons are almost completely devoid of cortical afferents except for sparse inputs on their distal dendrites.[20–21a] The projection neurons have extensive overlapping dendritic trees and emit axon collaterals that form symmetrical synapses with dendrites and spines of neighboring neurons in the striatum.[3, 7, 22] Although these intrastriatal connections have long been considered the main substrate for the mutual GABAergic inhibition between striatofugal neurons, electrophysiologic studies show that the inhibition among spiny neurons is rather weak.[23, 23a] This connection is powerful in terms of modulating the timing of action potentials generated by nearby spiny neurons.[23b] On the other hand, the feed-forward pathway through GABAergic interneurons is probably a better candidate for generating these inhibitory influences.[7, 8, 18, 24]

Three types of cortical neurons project to the striatum in rats.[7, 10] The most common type includes large cortical neurons located in deep layer V. These cells have extensive intracortical axon arborizations and emit fine collaterals, with only a few terminals in the ipsilateral striatum, which indicates that these cortical neurons innervate a limited population of striatal cells according to strict topographic organization. A less common type of pyramidal tract cell that contributes to the corticostriatal projection is medium sized and located in superficial layer V. These neurons have limited intracortical arborization but terminate profusely in the ipsilateral striatum. A third type of corticostriatal neuron is located in superficial layer V and the deep part of layer III. These neurons give rise to an extensive axonal arbor in the region of the parent perikaryon and form diffuse plexuses of axon terminals that occupy a large volume of the ipsilateral and contralateral striata. The region of the striatum innervated by these axons can be as large as 1 mm across, but within these regions, the density of axonal arborization is very sparse, leaving large areas not innervated. This pattern of arborization implies that individual cortical fibers cross the dendritic fields of many striatal neurons but form few synapses with any given cell.[8, 10] Conversely, striatal neurons can be expected to receive inputs from a large number of cortical fibers but not to receive many synapses from any one of them. The functional implications of such a pattern of organization are twofold. It suggests that striatal neurons may increase their firing rate only if there is activation of convergent input from many different cortical neurons and that nearby striatal neurons with totally overlapping dendritic volumes have few presynaptic cortical axons in common.[8, 10] These anatomic findings strongly support a high degree of specificity of the corticostriatal projection and explain the absence of redundancy in the responses of neurons near each other in the striatum.[7, 8]

Functional Organization of the Thalamostriatal Projection

In addition to the cerebral cortex, the intralaminar thalamic nuclei are a major source of excitatory afferents to the striatum. However, the influence of thalamic inputs on the activity of striatal neurons has received much less attention than corticostriatal projections. Anterograde tracing studies in rats and monkeys indicate that the thalamostriatal projection is massive, topographically organized, and highly specific.[6, 13, 25] In primates, the caudal intralaminar nuclear group (i.e., centromedian and parafascicular nuclei) provides massive inputs that largely terminate in different functional territories in the striatum.[6, 26, 27] Centromedian and parafascicular inputs terminate preferentially in the matrix compartment of the dorsal and ventral striatum.[26] The centromedian nucleus projects massively to the postcommissural sensorimotor part of the putamen, whereas the parafascicular nucleus innervates predominantly the caudate nucleus and, to a lesser extent, the ventral striatum.[26, 27] The striatal input from the parafascicular nucleus terminates in a patchlike manner that preferentially invades the matrix compartment in the caudate nucleus and the nucleus accumbens. The precommissural putamen receives inputs from the so-called dorsolateral parafascicular nucleus, a group of fusiform neurons that extends mediolaterally along the dorsal border of the centromedian.[28] In rats, thalamic inputs to the ventral striatum arise predominantly from midline and rostral intralaminar nuclei.[25] Specific relay nuclei also project to the striatum, although to a much lesser extent than intralaminar nuclei.[6, 13] As is the case for the motor and somatosensory cortical afferents, the centromedian nucleus input terminates in a bandlike fashion.[26, 27] Whether the thalamostriatal projection is somatotopic and overlaps with corticostriatal afferents is unknown.

The medium spiny neurons are the main targets of thalamic afferents, but in contrast to cortical terminals, which mostly terminate on the head of dendritic spines,[3] centromedian and parafascicular inputs preferentially innervate the dendritic shafts of striatal neurons.[13, 26, 27, 29] However, afferents from rostral intralaminar nuclei terminate almost exclusively on dendritic spines in rats.[30] In contrast to cortical and dopaminergic inputs, which often converge on common postsynaptic targets, centromedian and dopaminergic terminals largely innervate different striatal elements,[31] which suggests that dopaminergic afferents are located to subserve a more specific modulation of afferent cortical input than afferent thalamic input in the striatum. Striatal interneurons immunoreactive for choline acetyltransferase, parvalbumin, and somatostatin, but not those containing calretinin, receive inputs from the centromedian nucleus in monkeys.[32, 33] Centromedian inputs also display a high degree of specificity in their pattern of synaptic innervation of striatal projection neurons.

Together, these anatomic findings indicate that the thalamostriatal projections are more massive and much better organized than previously thought.[6, 13, 25–28, 31, 32] A major task for the coming years will be to elucidate the sources and better characterize the types of information transmitted by the different populations of thalamostriatal neurons. This will help to better understand the mechanisms by which thalamic and cortical

inputs interact to control the activity of striatofugal neurons.

Some thalamic relay nuclei also project to the striatum in a highly specific and organized fashion.[34] Data demonstrate the convergence of interconnected cortical and ventral thalamic areas to specific regions of the sensorimotor striatum in monkeys.[35]

γ-Aminobutyric Acid and Glutamate Receptors in the Striatum

Although glutamate is the transmitter of cortical and thalamic afferents, highly specific actions on particular postsynaptic targets may be achieved through the different types of glutamate receptors. There are two categories of glutamate receptors: the ionotropic receptors, which mediate fast- and short-lasting excitatory effects, and the metabotropic receptors, which mediate slow- and long-lasting modulatory effects of glutamatergic transmission. Ionotropic glutamate receptors include *N*-methyl-D-aspartate (NMDA; NR1, NR2A-D), α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA; GluR1 through GluR4), and kainate (GluR5 through GluR7, KA1, KA2) subtypes. Metabotropic receptors include eight subtypes pooled into three major groups: group I (mGluR1, mGluR5), group II (mGluR2, mGluR3), and group III (mGluR4, mGluR6, mGluR7, mGluR8). Study results indicate that various presynaptic and postsynaptic ionotropic and metabotropic glutamate receptors are involved in mediating and modulating excitatory effects in the striatum (Fig. 165–2).

Striatal projection neurons and interneurons express several NMDA, AMPA, and kainate receptor subunits.[36–40] AMPA and NMDA receptor subunits are found exclusively postsynaptically in the core of asymmetrical axodendritic and axospinous synapses in the rat striatum.[38, 41] Moreover, studies in the monkey striatum revealed that kainate receptors are found postsynaptically and presynaptically in cortical-like terminals making asymmetrical synapses.[42, 42a] In addition to ionotropic receptors, striatal neurons are enriched in metabotropic glutamate receptors. Group I and some of group III (mGluR4 and mGluR7) metabotropic glutamate receptors are expressed at a moderate to high level by most striatal neurons.[43, 44] Whereas strong immunoreactivity for group III mGluRs is found in glutamatergic corticostriatal terminals,[45, 46, 46a] group I mGluRs are found postsynaptically, perisynaptic to asymmetrical axospinous synapses, in the core of GABAergic synapses, and perisynaptic to dopaminergic synapses.[47, 48, 48a] The mGluR2 receptor is confined to striatal cholinergic interneurons, whereas mGluR3 is mostly expressed by glial cells.[43, 49, 50] A large population of corticostriatal terminals display mGluR2 and mGluR3 immunoreactivity.[51] These observations indicate that corticostriatal terminals are associated with presynaptic and postsynaptic glutamate receptors (see Fig. 165–2).

In addition to glutamatergic afferents, the striatum receives GABA-containing inputs from GABAergic interneurons, local collateral axons of medium-sized projection neurons, and from GPe cells. The inhibitory actions of GABA are mediated through two major groups of receptors: the ionotropic $GABA_A$ receptors, which are involved in fast synaptic inhibition and include 14 subunits (α_1 through α_6, β_1 through β_3, γ_1 through γ_3, δ, ϵ),[52, 53] and the metabotropic $GABA_B$ receptors, which mediate slow- and long-lasting inhibi-

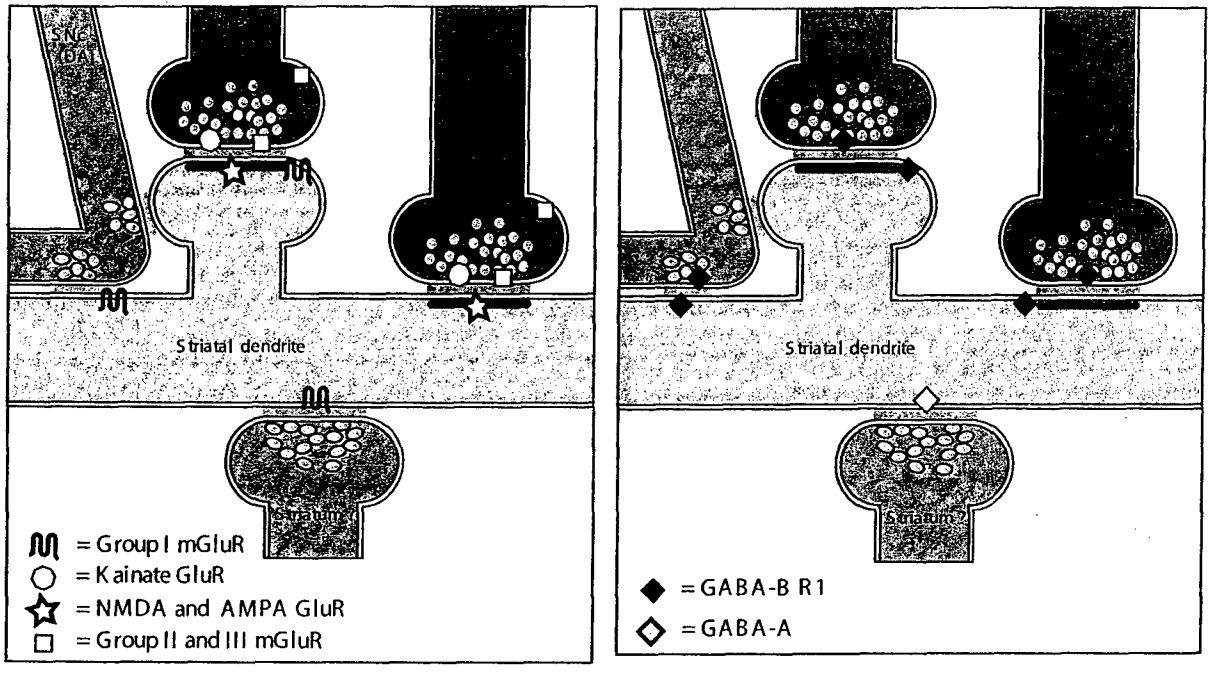

FIGURE 165–2. Schematic drawings of striatal dendrites show the pattern of subsynaptic distribution of glutamate and γ-aminobutyric acid (GABA) receptors in relation to the main striatal afferents in monkeys.

tion and include $GABA_B$-R1 and $GABA_B$-R2 subunits.[54-56] As expected, striatal neurons are enriched in $GABA_A$ and $GABA_B$ receptors.[53, 56-60] Whereas $GABA_B$ receptors are homogeneously distributed and found in medium-sized projection neurons and interneurons,[59] the $GABA_A$ receptor subunits display different patterns of distribution: the β_2 and β_3 subunits are confined to cholinergic neurons, whereas the α_1 subunit is abundant in the matrix compartment, where it is expressed in medium-sized aspiny neurons.[60] $GABA_A$ receptors are localized exclusively postsynaptically in the main bodies of symmetrical synapses.[60, 61] $GABA_B$ receptors are found postsynaptically in the main body of many symmetrical synapses and presynaptically in terminals making asymmetrical synapses (see Fig. 165–2).[48, 48a, 59] A small population of axons forming en passant type of symmetrical synapses also express $GABA_B$ receptors.[59] These data suggest that GABA can act postsynaptically through $GABA_A$ and $GABA_B$ receptors to modulate GABAergic transmission or presynaptically through $GABA_B$ receptors to modulate the activity of glutamatergic afferents in the striatum.

Other Afferents to the Striatum

The striatum is the target of many other afferents that are not discussed in detail in this chapter because of space limitations. These afferents include the massive projections from midbrain dopaminergic neurons in the SNc, ventral tegmental area, and retrorubral area[62, 63] as well as subthalamostriatal projections. In monkeys, subthalamic neurons that project to the caudate nucleus and putamen arise from two distinct neuronal populations; those that innervate the putamen are located in the sensorimotor-related dorsolateral two thirds of the subthalamic nucleus, and subthalamocaudate neurons are found ventromedially in the associative territory.[34, 64] Other minor inputs to the striatum arise from the tuberomamillary nucleus,[65] the brainstem pedunculopontine nucleus,[34, 66] the locus ceruleus,[34] the spinal nucleus of the trigeminal nerve,[67] the peripeduncular nucleus,[68] and the substantia innominata.[69]

DIRECT AND INDIRECT STRIATOFUGAL PROJECTIONS

The cortical inputs, together with the many other intrinsic and extrinsic afferents, are integrated by medium-sized projection neurons in the striatum. After processing at the striatal level, the cortical information is conveyed to the output nuclei of the basal ganglia (i.e., GPi and SNr) through two routes, the so-called direct and indirect striatofugal pathways.[10, 70-72] The *direct pathway* arises from a subpopulation of spiny neurons that project directly to the GPi or SNr, whereas the *indirect pathway* arises from a separate population of spiny neurons that project to the GPe. The GPe conveys the information to the subthalamic nucleus, which relays it to the output nuclei of the basal ganglia. The subpopulations of striatal output neurons that give rise to the direct and indirect pathways are further distinguished by their expression of neuropeptides and dopamine receptor subtypes. Although all striatal spiny neurons use GABA as their main transmitter, the subpopulation that gives rise to the direct pathway is characterized by the presence of the neuropeptides substance P and dynorphin and by the preferential expression of the D_1 subtype of dopamine receptors. The subpopulation that gives rise to the indirect pathway expresses preferentially enkephalin and the D_2 subtype of dopamine receptors.[10] Imbalance in the activity of these two pathways underlies some of the motor deficits in Parkinson's disease.[10, 70, 72]

The model of direct and indirect pathways as originally introduced was by necessity a simplification and included only the major projections of subnuclei of the basal ganglia. Since its introduction, evolving knowledge about the anatomic and synaptic organization of the basal ganglia has led scientists to reconsider and update some aspects of the model. One of the most important new findings regarding the anatomic organization of the basal ganglia is the demonstration of multiple indirect pathways of information that flow through the basal ganglia. In addition to the classic indirect pathway through the GPe and the subthalamic nucleus, the GPe gives rise to GABAergic projections that terminate in basal ganglia output structures (i.e., GPi and SNr) and the reticular nucleus of the thalamus.[6, 12, 13] A projection from the GPe to the striatum, which in rats preferentially targets subpopulations of striatal interneurons,[73] has also been described.[6, 12, 13] Although the exact functions of these connections remain unknown, the circuitry of the basal ganglia as outlined in the original model of direct and indirect pathways is likely to be more complex than previously thought.[12]

Molecular and anatomic data challenge the organization of direct and indirect pathways. Reverse transcriptase polymerase chain reaction techniques show a higher level of colocalization of D_1 and D_2 receptors than that revealed by in situ hybridization methods in striatal neurons.[10] However, the relative abundance of the two receptor subtypes in direct and indirect striatofugal neurons is strikingly different, which is consistent with in situ hybridization data. Indirect striatofugal neurons that contain enkephalin express high levels of D_2 mRNA and low levels of D_1 mRNA, whereas direct striatofugal neurons that contain substance P express high levels of D_1 mRNA and low levels of D_2 mRNA. The only striatal projection neurons that coexpress high levels of D_1 and D_2 receptor subtypes are a small population of projection neurons that contain enkephalin and substance P. Another set of data that has led to the reconsideration of some aspects of the model was obtained in intracellular staining studies. These data showed that the segregation of striatofugal neurons into striatopallidal and striatonigral neurons is not as clear-cut as originally suggested based on differential peptide expression and retrograde double-labeling studies.[10] It appears that striatal projection neurons innervate the pallidal segments and the substantia nigra in rats and monkeys to some extent.[10] Kawaguchi and

coworkers[22] divided striatofugal neurons into two major types based on their pattern of axonal arborization. The first type, referred to as *indirect* striatofugal neurons, has axons that arborize profusely and exclusively in the globus pallidus. The second type of neurons, referred to as *direct* striatofugal neurons, projects massively to the entopeduncular nucleus or the substantia nigra, or both, but also sends thin axon collaterals to the globus pallidus.[10] Although this does not rule out the concept of segregation of striatofugal neurons, these findings must be kept in mind while considering the functional significance of the direct and indirect striatofugal pathways.

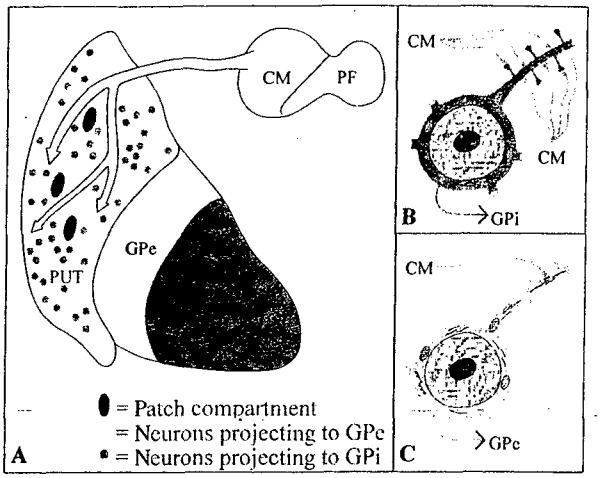

FIGURE 165–4. Compartmental *(A)* and synaptic *(B* and *C)* relationships between striatopallidal neurons and thalamic afferents from the centromedian (CM) nucleus in squirrel monkeys. These data were obtained after simultaneous injections of anterograde tracers in the CM nucleus and retrograde tracers in either segment of the globus pallidus. The thalamic inputs project mainly to the matrix striatal compartment *(A)* that contains neurons projecting to the external globus pallidus (GPe) *(light gray circles)* or internal globus pallidus (GPi) *(dark gray circles).* The large ovoid black areas represent the patch compartment, which does not receive input from the CM. The thalamic terminals form asymmetrical synapses, frequently with striato-GPi neurons *(B)* but rarely with striato-GPe cells *(C).* (Adapted from Sidibé M, Smith Y: Differential synaptic innervation of striatofugal neurons projecting to the internal or external segments of the globus pallidus by thalamic afferents in the squirrel monkey. J Comp Neurol 365:445–465, 1996.)

Glutamate and γ-Aminobutyric Acid Receptors in the Globus Pallidus

GABA and glutamate are the two main transmitters that mediate activity along the direct and indirect pathways of the basal ganglia. Pallidal neurons receive massive axodendritic GABAergic inputs from the striatum and strong somatic innervation from local collaterals of GPe neurons. The glutamatergic terminals from the subthalamic nucleus, which account for less than 10% of the total population of boutons in contact with pallidal neurons, are homogeneously distributed among GABAergic terminals on neuronal cell bodies and dendrites (Fig. 165–3). The effects of GABA and glutamate on pallidal neurons depend on the subtype and subunit composition of the receptors expressed by the postsynaptic neurons and on their spatial relationships to glutamate and GABA release sites. In this respect, pallidal neurons express various NMDA and AMPA receptor subunits in the core of asymmetrical glutamatergic synapses in the rat pallidum.[41, 74] Pallidal neurons are also enriched in group I (mGluR1 and mGluR5) metabotropic glutamate receptors.[43] Surprisingly, both subtypes of group I mGluRs were found postsynaptically in the core of striatopallidal GABAergic synapses and perisynaptically at subthalamopallidal glutamatergic synapses in monkeys (Fig. 165–5).[47-48a, 75] However, pallidal neurons express low levels of group II mGluRs that abound in glial cells.[43, 49, 50] The group III mGluRs (mGluR4a and mGluR7a, b) are mostly expressed presynaptically in striatopallidal GABAergic terminals, where they may act as heteroreceptors to modulate GABA release from striatal terminals (see Fig. 165–5).[45-46a]

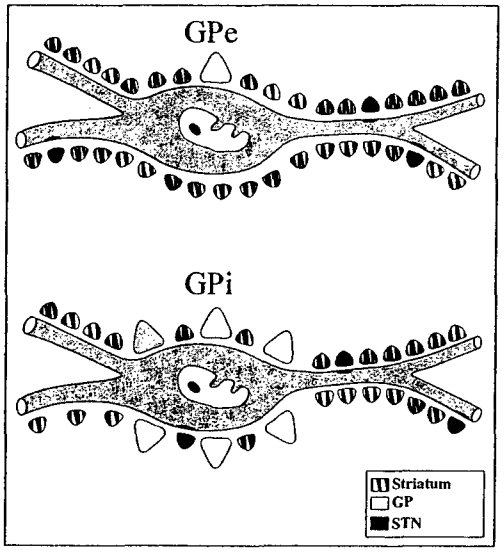

FIGURE 165–3. The pattern of innervation of neurons shown in both segments of the globus pallidus is based on data obtained in squirrel monkeys using anterograde tracing techniques and postembedding immunogold labeling for γ-aminobutyric acid (GABA) or glutamate. The relative size and proportion of each category of terminal are represented. The major difference between innervation of neurons of the two segments of the globus pallidus is that internal globus pallidus (GPi) neurons receive strong somatic inputs from the external globus pallidus (GPe), whereas striatal and subthalamic terminals are evenly distributed on GPe and GPi neurons. (Adapted from Smith Y, Bevan MD, Shink E, et al: Microcircuitry of the direct and indirect pathways of the basal ganglia. Neuroscience 86:353–387, 1998.)

As expected, pallidal neurons express moderate to high levels of $GABA_A$ and $GABA_B$ receptors.[53, 58-60] The $GABA_A$ receptor subunits are mostly found postsynaptically in the core of symmetrical striatopallidal GABAergic synapses,[48a, 60, 76, 77] whereas the $GABA_B$ receptors are present on the postsynaptic membrane of striatopallidal synapses, perisynaptic to asymmetrical synapses, and presynaptic in subthalamic glutamatergic terminals (see Fig. 165–5).[48, 48a, 59] These data

indicate that $GABA_B$ receptors may act at various sites to modulate GABAergic and glutamatergic neurotransmission in the pallidal complex.

Differential Innervation of Direct and Indirect Striatofugal Neurons and Interneurons

Although all medium-sized spiny striatofugal neurons display a similar pattern of synaptic innervation, evidence indicates that some extrinsic afferents preferentially target direct or indirect striatal projection neurons (see Fig. 165–4). For instance, thalamic inputs from the centromedian nucleus form synapses much more frequently with direct than indirect striatofugal neurons in squirrel monkeys (see Fig. 165–4).[27] However, sensorimotor cortical inputs influence preferentially striato-GPe neurons in rats[78] and monkeys.[79] After microstimulation of physiologically corresponding sites in the primary motor cortex and somatosensory cortical region, 75% of the striatofugal neurons that displayed FOS immunoreactivity (i.e., neurons that changed their activity) were enkephalin immunoreactive, indicating that they give rise to the indirect pathways. Whether those functional effects were mediated by a differential density of sensorimotor cortical terminals in contact with striato-GPe and striato-GPi neurons remains to be established. Such does not seem to be the case in rats, however, because inputs from the motor cortex form synapses more frequently with neurons of the direct pathway than those of the indirect pathways.[80]

This differential synaptic innervation was also found at the level of striatal interneurons. For example, cholinergic interneurons receive massive inputs from thalamic intralaminar nuclei but are much less innervated by cortical afferents.[20–21a, 81] However, calretinin-immunoreactive interneurons appear to be devoid of centromedian inputs, whereas parvalbumin- and somatostatin-containing neurons receive centromedian and cortical inputs in monkeys.[81] In rats, parvalbumin-containing neurons are preferentially innervated by cortical afferents.[33]

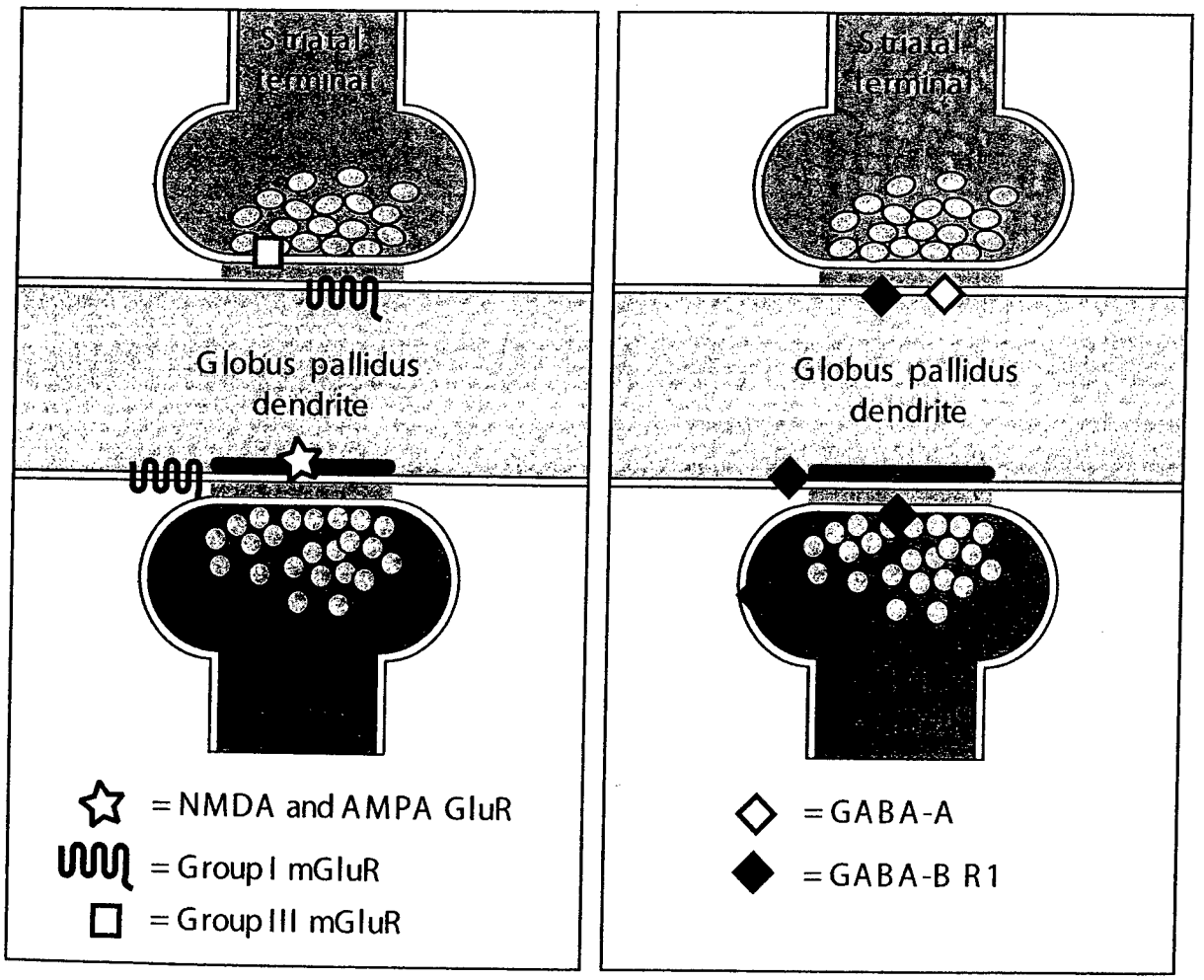

FIGURE 165–5. Schematic drawings of pallidal dendrites show the pattern of subsynaptic distribution of glutamate and γ-aminobutyric acid (GABA) receptors in relation to striatal and subthalamic afferents in monkeys.

CORTICOSUBTHALAMIC AND THALAMOSUBTHALAMIC PROJECTIONS: ADDITIONAL ENTRANCES TO THE BASAL GANGLIA CIRCUITRY

As is the case for the striatum, the subthalamic nucleus also receives excitatory glutamatergic projections from the cerebral cortex. In primates, anatomic evidence indicates that the corticosubthalamic projection is exclusively ipsilateral and arises mainly from the primary motor cortex (area 4), with a minor contribution of prefrontal and premotor cortices. Somatosensory and visual cortical areas do not project to the subthalamic nucleus, but they substantially innervate the striatum. Attempts to determine the exact origin of corticosubthalamic projections in the cat and monkey by retrograde transport have been inconclusive. In rats, however, the corticosubthalamic projection originates mainly from layer V neurons that, in some cases, send axons collateral to the striatum. In rats and monkeys, the corticosubthalamic projection is topographically organized so that afferents from the primary motor cortex are confined to the dorsolateral part of the subthalamic nucleus, whereas the premotor (areas 8, 9, and 6), supplementary motor, and presupplementary motor areas, as well as adjacent frontal cortical regions, innervate preferentially the medial third of the nucleus. Inputs from the prefrontal-limbic cortices are confined to the medial-most tip of subthalamic nucleus.[6, 12, 13] By virtue of its cortical inputs, the dorsolateral sector of the subthalamic nucleus is involved in the control of motor behaviors, whereas the ventromedial sector processes oculomotor, associative, and limbic information.[12, 13]

Like cortical afferents to the striatum, the corticosubthalamic projection from the primary motor cortex is somatotopically organized; the face area projects laterally, the arm area centrally, and the leg area medially. The arrangement of somatotopic representations from the supplementary motor area to the medial subthalamic nucleus is reversed from the ordering of the primary motor cortex to the lateral subthalamic nucleus in macaque monkeys.[82] The cerebral cortex imposes a specific functional segregation on the striatum and at the level of the subthalamic nucleus.[83] However, subthalamic nucleus neurons have long dendrites that may cross boundaries of functional territories imposed by cortical projections in rats.[84] This anatomic arrangement opens the possibility for some functionally segregated information at the level of the cerebral cortex to converge on individual subthalamic nucleus neurons in rodents.

As described for the striatum, another source of excitatory inputs to the subthalamic nucleus arises from the centromedian-parafascicular nuclear complex. No other thalamic nuclei are known to innervate the subthalamic nucleus. The thalamosubthalamic projection arborizes ipsilaterally in discrete portions of the subthalamic nucleus. This input respects the functional organization of the subthalamic nucleus (i.e., sensorimotor neurons in the centromedian terminate preferentially in the dorsolateral part of the nucleus), whereas limbic- and associative-related neurons in the parafascicular nucleus project almost exclusively to the medial subthalamic nucleus. In rats, the thalamosubthalamic projection is excitatory and tonically drives the activity of subthalamic nucleus neurons. Although some parafascicular neurons that project to the striatum send axon collaterals to the subthalamic nucleus, the thalamosubthalamic and thalamostriatal projections largely arise from segregated populations of parafascicular neurons in rats.[85]

Even if cortical and thalamic inputs are relatively sparse and terminate on the distal dendrites and spines of subthalamic nucleus neurons, electrophysiologic experiments show that activation of these inputs results in very strong, short-latency, monosynaptic excitatory postsynaptic potentials in subthalamic nucleus neurons. The information flowing through the subthalamic nucleus reaches basal ganglia output structures much faster than information transmitted along the striatofugal pathways.[86–88a] These anatomic and electrophysiologic data suggest that the subthalamic nucleus is another main entrance of information to the basal ganglia circuitry.

γ-Aminobutyric Acid and Glutamate Receptors in the Subthalamic Nucleus

Subthalamic nucleus neurons display strong immunoreactivity for various $GABA_A$ receptor subunits,[48a, 77] which is consistent with electrophysiologic studies showing that the pallidal inhibition of subthalamic nucleus neurons is largely mediated by $GABA_A$ receptor activation. Subpopulations of subthalamic nucleus neurons display moderate immunoreactivity for $GABA_B$ receptors.[59] At the electron microscopic level, $GABA_B$ receptors are expressed postsynaptically on dendrites of subthalamic nucleus neurons and presynaptically in putative glutamatergic axon terminals.[59] Together, these data indicate that $GABA_A$ and $GABA_B$ receptors probably mediate postsynaptic inhibition from the GPe in subthalamic nucleus neurons. $GABA_B$ receptors also may control the activity of subthalamic nucleus neurons by presynaptic inhibition of neurotransmitter release from extrinsic or intrinsic, or both, glutamatergic terminals.

Subthalamic nucleus neurons express a high level of immunoreactivity for various NMDA and AMPA glutamatergic receptor subunits. Ultrastructural analysis reveals that both types of ionotropic glutamate receptors are expressed preferentially in the postsynaptic membrane of putative glutamatergic synapses, although AMPA receptor subunit immunoreactivity is also found at symmetrical GABAergic synapses in rats.[74] The synaptic localization of mGluRs has not been studied in great detail in the subthalamic nucleus, but preliminary data indicate that group I mGluRs are found postsynaptically at the edges of both asymmetrical glutamatergic synapses or symmetrical GABAergic synapses.[48a, 89] A particular feature that characterizes subthalamic nucleus neurons is their strong expression of group II (mGluR2) mGluR mRNAs relative to other

populations of basal ganglia neurons.[43, 50] Consistent with this, group II mGluR agonists reduce subthalamic nucleus–mediated excitatory postsynaptic potentials in rat SNr neurons.[89a] These effects are probably mediated by activation of presynaptic mGluR2 receptors expressed on subthalamic nucleus axons and terminals in the SNr. Very low levels of group III mGluR mRNAs are found in subthalamic nucleus neurons.[43, 44]

THE MOTOR THALAMUS: A MAJOR TARGET OF BASAL GANGLIA AND CEREBELLAR OUTFLOW

The GPi and the SNr are the two output structures of the basal ganglia. They convey basal ganglia outflow to motor, cognitive, and intralaminar thalamic nuclei and to various brainstem structures (see Fig. 165–1). In this section, we discuss the organization of basal ganglia projections from GPi and SNr to the thalamus and compare the pattern of distribution of basal ganglia inputs with that of cerebellar afferents.

Nomenclature of Motor Thalamic Nuclei

The motor thalamus is composed of the ventral anterior and ventral lateral nuclear groups. Various nomenclatures have been introduced for the terminology of the subdivisions of motor thalamic nuclei in primates (Table 165–1). To facilitate the comparison of data obtained in different studies, it is important to understand the correspondence between these terminologies. Table 165–1 compares the nomenclature for the ventral anterior and ventral lateral subdivisions introduced by various groups to define motor thalamic nuclei in Old World[90–93] and New World[94] monkeys. Further details on the nomenclature of thalamic nuclei in humans and nonhuman primates are provided in an extensive review by Percheron and colleagues.[95]

Basal Ganglia and Cerebellar Inputs to Motor Thalamic Nuclei

Although the GPi and SNr project to the ventral anterior and ventral lateral nuclei, the nigral and pallidal afferents largely terminate in different subdivisions of this nuclear group,[96, 97] which were originally thought to be completely separate from cerebellar projection sites in the primate thalamus.[91, 96] However, investigations using multiple-labeling techniques have demonstrated that, even if cerebellar and pallidal projections mainly innervate different thalamic nuclei, a substantial level of convergence exists between these two major thalamic afferents.[98–101] These observations led to reconsideration of many aspects of basal ganglia thalamocortical relationships, taking into account that basal ganglia information is conveyed to premotor and supplementary motor cortical areas and the primary motor cortex. Conversely, the cerebellar outflow, which was thought to be conveyed exclusively to primary motor cortex, also reaches premotor and supplementary motor cortical regions.[98–102]

Another important concept that has been emphasized in the past few years is that cerebellar and basal ganglia thalamic projections terminate in motor thalamic territories and reach major associative and limbic regions of the primate thalamus.[102] Although the nonmotor functions of basal ganglia and cerebellum have long been known, a series of anatomic data demonstrated the existence of various connections through which basal ganglia and cerebellar information can reach various cortical areas in the frontal, parietal, and temporal lobes known to be involved in cognitive functions.[102] The use of trans-synaptic, retrograde virus transport after injections in various cortical areas led Strick and associates[102] to propose that nigral, pallidal, and cerebellar outputs to the cerebral cortex flow through various channels that arise from segregated regions of basal ganglia output structures and deep cerebellar nuclei (Fig. 165–6).

NIGROTHALAMIC PROJECTION

The ventral anterior and mediodorsal thalamic nuclei are the main targets of nigrothalamic projections in primates.[6, 97] In an elegant anatomic study combining anterograde and retrograde labeling methods, Ilinsky and coworkers[97] came to some conclusions regarding the nigrothalamocortical projections in monkeys. First, inputs from the medial part of the SNr terminate mostly in the medial magnocellular divisions of the

TABLE 165–1 ■ **Nomenclature of Subdivisions of the Ventral Anterior and Ventral Lateral Nucleus Nuclear Complex in New World and Old World Monkeys**

Olszewski[90]	VLo	VPLo	Area X	VLc (and VLps)	VLm	VApc	VAmc
Jones[91]	VLa	VLp	VLp	VLp	VMp	VA	VA
Ilinksy and Kultas-Ilinsky[92]	VAdc	VL	VL	VLd	VM	VApc	VAmc
Paxinos et al[93]	VAL (Vo)	VLL	VLM	VAL	VAM	VAL (Vo)	VAM
Stepniewska et al[94]	VLa	VLp	VLx	VLd	VM	VApc	VAmc

VA, ventral anterior nucleus; VAdc, ventral anterior nucleus, densocellular part; VAL, ventral anterior nucleus, lateral part; VAM, ventral anterior nucleus, medial part; VAmc, ventral anterior nucleus, magnocellular part; VApc, ventral anterior nucleus, parvocellular part; VL, ventral lateral nucleus; VLa, ventral lateral nucleus, anterior division; VLc, ventral lateral nucleus, pars caudalis; VLd, ventral lateral nucleus, dorsal division; VLL, ventral lateral nucleus, lateral part; VLM, ventral lateral nucleus, medial part; VLo, ventral lateral nucleus, pars oralis; VLp, ventral lateral nucleus, principal division; VLps, ventral lateral nucleus, pars posterma; VLx, ventral lateral nucleus, medial division; VM, ventral medial nucleus; VMp, ventral medial nucleus, principal division; Vo, ventralis oralis nucleus; VPLo, ventral posterior lateral nucleus, pars oralis.

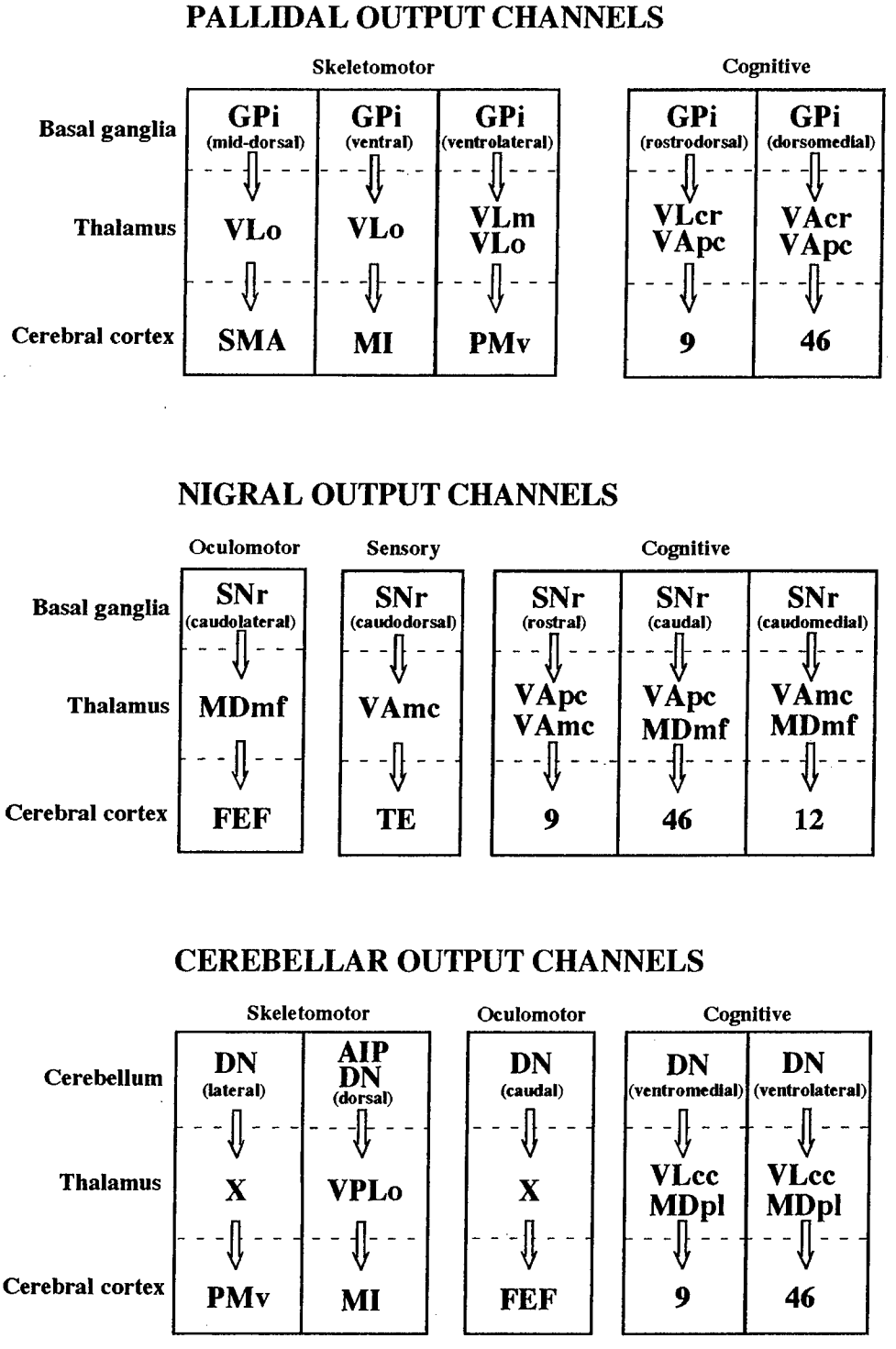

FIGURE 165–6. Motor and nonmotor connections between basal ganglia output structures or deep cerebellar nuclei and various functional regions of the monkey cerebral cortex. The internal globus pallidus (GPi), substantia nigra pars reticulata (SNr), and dentate cerebellar nucleus (DN) project to different subdivisions of the ventral anterior and ventral lateral (VA/VL) nuclei and the mediodorsal (MD) nucleus, which reach functionally segregated cortical areas involved in motor, cognitive, and sensory functions. The nomenclature of thalamic nuclei used in this diagram is that of Olszewski[90] (see Table 165–1). AIP, accessory interpositus nucleus; FEF, frontal eye field; MDmf, mediodorsal nucleus pars multiformis; MDpl, mediodorsal nucleus pars lateralis; TE, area of the inferotemporal cortex. (Adapted from Middleton FA, Strick PL: Basal ganglia and cerebellar loops: Motor and cognitive circuits. Brain Res Rev 31:236–250, 2000.)

ventral anterior nucleus (VAmc) and the mediodorsal nucleus (MDmc), which innervate anterior regions of the frontal lobe, including the principal sulcus (i.e., Walker's area 46) and the orbital cortex (i.e., Walker's area 11). Second, neurons in the lateral part of the SNr project preferentially to the lateral posterior region of the VAmc and to different parts of the mediodorsal nucleus mostly related to posterior regions of the frontal lobe, including the frontal eye field and areas of the premotor cortex. On the basis of retrograde transsynaptic viral tracing studies, Strick and associates[102] extended these findings and proposed that the nigral outputs to the thalamus flow along five separate channels that target various cortical areas involved in cognitive, sensory, and oculomotor functions (see Fig. 165–6). Another thalamic target of SNr neurons is the caudal intralaminar parafascicular nucleus.[94, 103] The organization of this projection is discussed in a separate section.

PALLIDOTHALAMIC PROJECTION

The main thalamic targets of GPi neurons are the ventral anterior and ventral lateral nuclei and caudal intralaminar thalamic nuclei.[6, 104] Efferents from the sensorimotor GPi remain largely segregated from the associative and limbic projections at the level of the thalamus, whereas, they partly overlap in the tegmental pedunculopontine nucleus (PPN).[104, 105] The limbic and associative pallidal projections innervate common nuclei in the thalamus and PPN.[104, 105] In squirrel monkeys, the sensorimotor GPi outputs are directed toward the posterior ventral lateral nucleus (VLp), whereas the associative and limbic GPi preferentially innervate the parvocellular ventral anterior nucleus (VApc) and the dorsal ventral lateral nucleus (VLd). The ventromedial nucleus receives inputs from the limbic GPi only.[104] These findings reveal that some associative and limbic cortical information, which is largely processed in segregated corticostriatopallidal channels, converge to common thalamic nuclei in monkeys.[104] After processing at the thalamic level, the basal ganglia influences are conveyed to the cerebral cortex through the ventral anterior and ventral lateral nuclei. Retrograde transneuronal virus studies showed that different populations of GPi neurons project to thalamocortical neurons directed toward the supplementary motor area, primary motor cortex, and premotor area,[102] each of which is involved in the control of various aspects of skeletomotor activity (see Fig. 165–6). The cognitive information from the dorsal part of GPi is transmitted to prefrontal cortical areas 9 and 46 and is involved in planning and spatial working memory through the VApc (see Fig. 165–6).[102] About 10% to 20% of pallidothalamic neurons in the monkey GPi project to the contralateral ventral anterior and ventral lateral nuclei.[106]

CEREBELLOTHALAMIC PROJECTION

Cerebellar afferents are partly segregated from nigral and pallidal projections in the primate thalamus. Although the cerebellum is commonly seen as a brain region involved in skeletomotor control, the importance of this structure in cognitive functions is well established.[101,107] In support of such nonmotor cerebellar functions, anatomic studies indicate that the dentate nucleus gives rise to at least two different channels of cognitive cerebellar-thalamic-cortical information in monkeys (see Fig. 165–6). These two circuits, which largely arise from the ventral part of the dentate nucleus, reach cortical areas 9 and 46 through relays in specific parts of the ventrolateral and mediodorsal nuclei (see Fig. 165–6). The skeletomotor-related outflow reaches premotor and primary motor cortical areas through relays in area X and VPLo, respectively. Cerebellar information related to eye movement arises from the caudal part of the dentate nucleus and reaches the frontal eye field cortical area through area X (see Fig. 165–6). Although cerebellar projections to the intralaminar thalamic nuclei have been described in nonprimates,[91, 108, 109] the existence of such connections still remains to be established in monkeys.

Basal Ganglia Inputs to Intralaminar Thalamic Nuclei

Most pallidal neurons that project to thalamic relay nuclei send axon collaterals to the caudal intralaminar nuclei, where they follow a highly specific pattern of distribution.[6, 104] Pallidal axons arising from the sensorimotor GPi terminate exclusively in the centromedian nucleus, where they form synapses with thalamostriatal neurons projecting back to the sensorimotor territory of the striatum (Fig. 165–7).[103, 104] In contrast, associative inputs from the caudate-receiving territory of the GPi terminate massively in a dorsolateral extension of the parafascicular nucleus (PFdL), which, surprisingly, does not project back to the caudate nucleus but rather innervates preferentially the precommissural region of the putamen (see Fig. 165–7).[103] The limbic GPi selectively innervates the rostrodorsal part of the parafascicular nucleus, which projects back to the nucleus accumbens. SNr projections are confined to the parafascicular nucleus, where they largely overlap with thalamostriatal neurons projecting to the caudate nucleus.[103] It appears that the centromedian-parafascicular complex is part of closed and open functional loops with the striatopallidal complex (see Fig. 165–7).

DESCENDING PALLIDAL AND NIGRAL PROJECTIONS TO THE TEGMENTAL PEDUNCULOPONTINE NUCLEUS

In monkeys, more than 80% of GPi neurons that project to the PPN send axon collaterals to the ventral thalamus.[6] In contrast to the ventral lateral nucleus, which largely conveys basal ganglia information to the cerebral cortex, the PPN gives rise to descending projections to the pons, medulla, and spinal cord as well as prominent ascending projections to the different structures of the basal ganglia, thalamus, and basal forebrain.[109–112] The pallidotegmental projection may be a

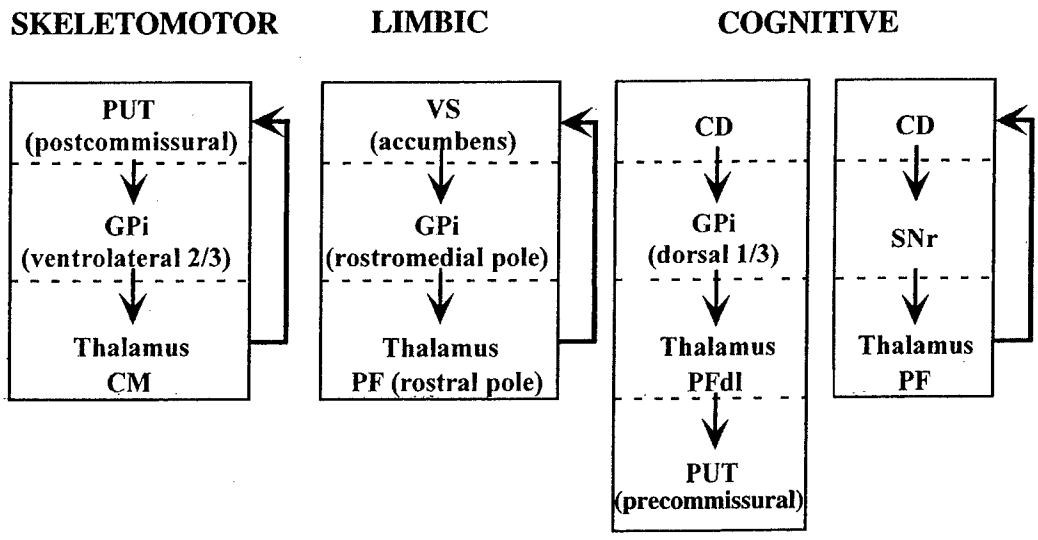

FIGURE 165–7. The functional interactions between the basal ganglia and thalamostriatal neurons in monkeys are shown schematically. These data were obtained after simultaneous injections of retrograde tracers in different functional territories of the striatum and anterograde tracers in the corresponding functional regions of internal globus pallidus (GPi) or substantia nigra pars reticulata (SNr) in squirrel monkeys. Notice that the caudal intralaminar thalamic nuclei (centromedian-parafascicular complex) and the basal ganglia are interconnected by closed and open functional loops. (Data from Sidibé M, Pare J-F, Smith Y: Nigral and pallidal inputs to functionally segregated thalamostriatal neurons in the centromedian/parafascicular intralaminar nuclear complex in monkey. J Comp Neurol 447:286–299, 2002; and Sidibé M, Bevan MD, Bolam JP, et al: Efferent connections of the internal globus pallidus in the squirrel monkey: I. Topography and synaptic organization of the pallidothalamic projection. J Comp Neurol 382:323–347, 1997.)

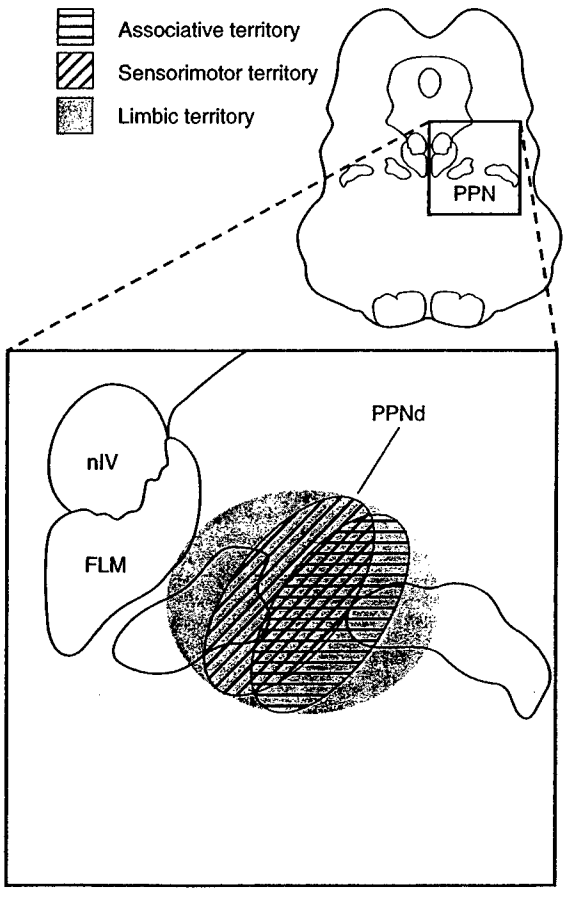

FIGURE 165–8. The location of anterogradely labeled fibers in the tegmental pedunculopontine nucleus (PPN) are shown schematically after injections of anterograde tracers in the associative, sensorimotor, and limbic territories of the internal globus pallidus (GPi) in squirrel monkeys. Notice that projections from the different functional territories of the GPi largely overlap in the pars diffusa of the PPN (PPNd). nIV, trochlear nucleus; FLM, medial longitudinal fasciculus; Sp, superior cerebellar peduncle. (Adapted from Shink E, Sidibé M, Smith Y: Efferent connections of the internal globus pallidus in the squirrel monkey: II. Topography and synaptic organization of the pallidal efferents to the pedunculopontine nucleus. J Comp Neurol 382:348–363, 1997.)

route by which information can escape from the basal ganglia–thalamocortical circuitry and reach lower motor and autonomic centers. Another possibility is that the PPN acts as an interface between different functional territories of the GPi and sends back the integrated information to the basal ganglia circuitry mainly through its massive projection to the dopaminergic neurons of the SNc.[6, 13] We investigated the pattern of distribution of functionally segregated pallidofugal information in the PPN of squirrel monkeys.[105] The results of this study are summarized in Figure 165–8. Injections of anterograde tracers in different functional territories of the GPi lead to anterograde labeling that largely converges to common regions of the so-called pars diffusa of the PPN (PPNd). The fields of fibers that arise from the associative and limbic territories of the GPi are more widely spread than the afferents from the sensorimotor territory of the GPi. Another major finding of this study is that pallidal fibers largely avoid cholinergic neurons in the pars compacta of the PPN. These anatomic data suggest that the noncholinergic neurons of the PPNd are potential targets for the integration of information arising from different functional territories of the GPi in primates. The PPNd is in a position to act as an interface for motivational, cognitive, and motor information transmitted along the pallidotegmental projection in primates (Fig. 165–8).

The SNr also provides substantial inputs to cholinergic and noncholinergic neurons of the PPN in rats.[113, 114] Although the existence of this projection has been shown in monkeys by means of retrograde labeling studies,[6] the exact targets of nigrotegmental projections remain to be established.

CONCLUSIONS

Our knowledge of the basal ganglia anatomy has increased tremendously over the past 10 years, mainly because of the introduction of highly sophisticated and sensitive tract-tracing and immunocytochemical methods suitable to light and electron microscopic analysis. Anatomic data led some to reconsider certain aspects of the functional circuitry of the basal ganglia. For instance, the thalamostriatal projection, which is largely neglected in functional models of basal ganglia connectivity, deserves attention. This projection is massive and follows a highly specific pattern of functional connectivity with the striatopallidal complex. The fact that thalamic inputs are directed preferentially toward specific populations of striatal projection neurons and interneurons strongly indicates that these inputs may play a major role in the basal ganglia circuitry.

An important concern has been raised during the past few years about the validity of the direct and indirect pathways of the basal ganglia. The evidence that subpopulations of striatofugal neurons express D_1 and D_2 dopamine receptors, combined with the fact that striatofugal neurons are more collateralized than previously thought, has challenged the concept of segregation of striatal projection neurons. Despite these anatomic refinements of the basal ganglia circuitry, it is clear that the functional concept of direct and indirect pathways still remains the basic working model for understanding changes in the basal ganglia circuitry in pathologic conditions and for developing more accurate surgical and pharmacologic therapies for basal ganglia diseases.

Another critical aspect of the basal ganglia circuitry, which should receive more attention, is the relative importance of the subthalamic nucleus and the striatum as major entrances of cortical information to the basal ganglia. Although the striatum receives a much more massive input from the cerebral cortex and thalamus than the subthalamic nucleus, the fact that the information flowing through the corticosubthalamic or thalamosubthalamic projections reaches the output structures of the basal ganglia (i.e., GPi and SNr) before the information traveling through the striatum deserves consideration. The long-held belief that the basal ganglia and cerebellum were solely involved in motor behaviors should be abandoned in light of various behavioral and clinical studies showing the clear implication of these brain regions in cognitive functions. The anatomic data presented in this chapter demonstrate that basal ganglia and cerebellar outflow have access to both motor-related cortical areas and invade large regions of the frontal, temporal, and parietal lobes devoted to various aspects of cognitive behaviors.

The large amount of neurotransmitters and neuropeptides involved in mediating synaptic communication between structures of the basal ganglia makes the chemical pedigree of basal ganglia structures extremely complex. Data showing that G protein–coupled glutamate and GABA receptors are largely expressed extrasynaptically or at synapses unrelated to the release site of their stimulating neurotransmitter further enhance this complexity and raise exciting issues about the functions and mechanisms of activation of metabotropic receptors in the basal ganglia.

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LOCALIZATION AND FUNCTIONS OF KAINATE RECEPTORS IN THE RAT GLOBUS PALLIDUS

Xiao-Tao Jin and Yoland Smith*

1. INTRODUCTION

Kainate receptors (KARs) are a subtype of ionotropic glutamate receptors composed five subunits (GluR5, GluR6, GluR7, KA1 and KA2) (Hollmann and Heinemann, [redacted]). Recent studies have demonstrated that activation of KARs mediates a large variety of pre- and post-synaptic effects on neurotransmission. For instance, activation of presynaptic KARs modulates glutamatergic and GABAergic synaptic transmission, while postsynaptic activation induces a small and slow component of glutamatergic response at various synapses in the CNS (for review see Lerma, 2003; Huettner, 2003).

The globus pallidus (GP) plays a central integrative role in the basal ganglia circuitry (Plenz and [redacted], 1999; Bevan et al., 2002). The GP receives glutamatergic inputs from the subthalamic nucleus (STN) and sends GABAergic outputs back to the STN and other basal ganglia nuclei. The hyperactivity of the subthalamofugal glutamatergic projection and changes in the firing pattern of STN and GP neurons are critical in mediating abnormal activity of the basal ganglia circuitry under pathological conditions (Plenz and Kitai, 1999; Bevan et al., 2002).

We have shown that KARs are expressed pre- and post-synaptically in the monkey striatum ([redacted]., 1999; Kieval et al., 2001). Although recent functional studies demonstrated that activation of these receptors by exogenous application of KA modulates glutamatergic transmission in the striatum, none of these studies addressed the role of synaptically activated KARs in the basal ganglia (Casassus and Mulle, 2002; Crowder and Weiner, 2002). Recently, we have demonstrated that KARs immunoreactivity is also expressed in dendrites and glutamatergic axon terminals in the monkey GP (Kane-Jackson and Smith, 2003). These findings pave the way for pre- and post-synaptic KARs- mediated effects in this brain region. To further address this issue, we employed electron microscopic immunocytochemistry and whole cell recording techniques to determine the localization and functions of KARs in rat GP. Consistent with our previous findings in monkeys, we found that GluR6/7 immunoreactivity is

* X-T. Jin and Y. Smith, Yerkes National Primate Research Center and Department of Neurology, Emory University, Atlanta, GA, USA.

expressed in dendrites and glutamatergic terminals in the rat GP. Furthermore, our electrophysiological data provide evidence for KAR-mediated postsynaptic effects and presynaptic regulation of glutamatergic transmission in the rat GP.

2. MATERIALS AND METHODS

2.1 Electron Microscopic Immunocytochemistry

Two adult and two 17-d old Sprague Dawley rats were used in the present study. After deep anesthesia with an overdose of pentobarbital, rats were perfusion-fixed with 500 ml of cold oxygenated Ringer's solution followed by 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer (PB) (0.1 M, pH 7.4). The brains were then cut in 60-μm-thick sections with a vibrating microtome and processed for the immunohistochemical localization of GluR6/7 at the electron microscopic level.

Commercially available affinity-purified polyclonal GluR6/7 antiserum (Upstate Biotech, Lake Placid, NY, dilution 1.5 μg/ml) raised against a synthetic peptide corresponding to the C terminal of GluR6 subunit (TFNDRLPGKETMA) were used in this study. Details of the immunostaining protocols for light and electron microscopy are found in previous studies ([redacted] 1999; Kieval et al., 2001). Sections processed for immunoperoxidase were stained with diaminobenzidine (DAB) using the avidin-biotin-peroxidase method (ABC, Vector Labs, Burlingame, CA, USA). After immunostaining, sections were processed for electron microscopy, and ultrathin sections of GP region were prepared.

Ultrathin sections collected from immunostained GP were scanned in the electron microscope for the presence of immunoreactive elements. Labeled structures were photographed and characterized on the basis of ultrastructural features described in Peters et al (1990). The total number of labeled elements from a series of ultrathin sections collected from blocks of GP tissue of 4 rats were tabulated and expressed as relative percentages of immunoreactive elements (see Charara et al., 1999; Kieval et al., 2001 for detail).

2.2 Whole-Cell Patch Clamp Recording

Whole-cell patch clamp recordings were performed on slices (300 μm) from 13 to 17-d old Sprague Dawley rats (Charles River Laboratories, Wilmington, MA). Parasagittal slices were made on a Vibratome 3000 (The Vibratome Company, St. Louis, MO) in ice-cold oxygenated sucrose buffer. Slices were stored at room temperature in a chamber containing artificial cerebrospinal fluid (ACSF) (in mM): 124 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1.0 NaH_2PO_4 and 2.0 $CaCl_2$, 20 glucose, 26 $NaHCO_3$ at pH 7.3-7.4 with 95% O_2, 5% CO_2 bubbling through it. The osmolarity of the ACSF was ~ 310 mOsm. During the recording, slices were maintained fully submerged in the recording chamber and perfused with oxygenated ACSF. GP neurons were visualized by IR-differential interference contrast microscopy (BX51WI) using a 40X water immersion objective (Olympus, Pittsburgh, PA). Whole-cell recordings were made with glass pipettes (3-4 mΩ), that contained in (mM): 140 CsCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES, 2 Mg_2ATP, 0.2 GTP, and 0.5% biocytin, pH 7.4 (300-310 mOsm). Cells were

characterized as GP neurons if they fulfilled the electrophysiological criteria established in previous studies (Kita and Kitai, 1991; Nambu and Llinas, 1994; Cooper and Stanford, 2000). Exogenous KA-induced currents were isolated pharmacologically by including 100 μM GYKI 52466, an AMPA receptor antagonist, 50 μM D-AP5, a NMDA receptor antagonist, 50 μM bicuculline, a GABA receptor antagonist and 0.5-1μM tetrodotoxin (TTX), a Na^+ channel blocker. To record excitatory postsynaptic currents (EPSCs) in GP, a bipolar tungsten stimulation electrode (FHC, Bowdoinham, ME) was placed in the internal capsule. EPSCs were evoked with single pulses that ranged from 3 to 10 V delivered once every 15-20 sec. The paired-pulse facilitation (PPF) of evoked EPSCs was performed as follows: two stimuli of internal capsule were paired with an interstimulus interval of 40-50 ms. The ratio of peak 2/peak1 was calculated.

All drugs were purchased from Toris Cookson (Ellisville MO). Signals were filtered at 5 kHz and digitized with a Digidata 1200 analog-to-digital converter (Axon Instruments, Foster City, CA). Data were analyzed off-line using pClamp 6 (Axon Instruments). All values were expressed as means ± SEM. Statistical significance was assessed by Student's *t* tests.

3. RESULTS

3.1. Pre and Postsynaptic Localization of GluR6/7 in The GP.

In a first set of experiment, we used commercially available affinity-purified polyclonal antibody to immunohistochemically localize GluR6/7 at the electron microscope level. Consistent with our primate data (Kane-Jackson and Smith, 2003), proximal and distal dendrites were the most common postsynaptic immunoreactive elements encountered in the rat GP. In addition to postsynaptic elements, terminals forming symmetric or asymmetric synapses and small preterminal unmyelinated axons comprised most of the presynaptic labeling (Fig. 1A, B). Although the exact source of the small unmyelinated axons could not be definitively determined, the presence of GluR6/7 on terminals forming asymmetric synapses indicates that GluR6/7 is expressed in presynaptic glutamatergic boutons.

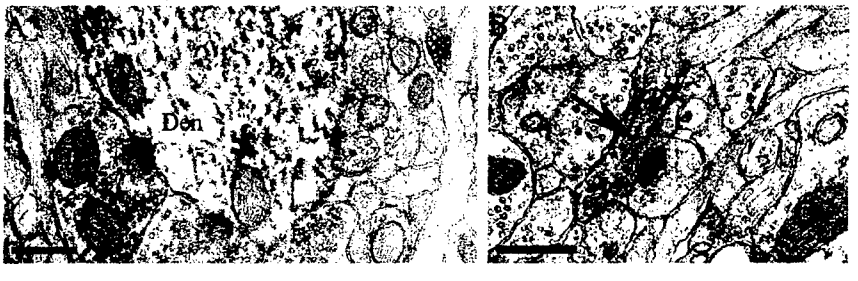

Figure 1. (A) GluR6/7-labeled dendrite (den), an axon terminal (TE) forming an asymmetric synapse. (B) GluR6/7-labeled unmyelinated axon (arrow). Scale bars, 0.5 μm.

3.2. Functional Kainate Receptors Are Present on Postsynaptic GP Neurons

Our electron microscopic immunocytochemistry data demonstrate that GluR6/7 immunoreactivity is enriched in postsynaptic elements in the rat GP (Fig. 1A). To test whether these KAR subunits form functional receptors, we measured the amplitude of inward currents induced by bath application of kainate using whole-cell patch-clamp recording techniques. As described previously by others (Kita and Kitai, 1991; Nambu and Llinas, 1994; Cooper and Stanford, 2000) and in a recent study from our laboratory (Poisik et al., 2003), we recorded heterogeneous population of neurons that can be classified into three types on the basis of difference in spike frequency adaptation, time-dependent inward rectification, cell's input resistance, and rebound spiking. Since we observed no significant differences in responses to kainate between these different populations of neurons, we combined the results.

GYKI 52466 is a specific AMPA receptor antagonist, which at a concentration of 100 μM, blocks AMPA receptors-mediated currents without significant effects on KARs (Paternain et al., 1995). Bath application of KA (3 μM) induced inward currents in presence of 50 μM D-AP5, 50 μM BIC, 0.5 μM tetrodotoxin (TTX) and 100 μM GYKI 52466. The inward currents evoked by KA were not significantly different [redacted] or presence of 100 μM GYKI 52466 (69 ± 4.5 and 59 ± 1.8 pA, respectively. $n = 5$, $p = 0.08$, *t*-test) (Fig. 1A, B). However, it was significantly inhibited by 50 μM CNQX, an AMPA/KAR antagonist (4.4 ± 2.2 pA. $n = 5$, $p < 0.001$) (Fig. 2A, B).

To confirm that 100 μM GYKI 52466 is a selective antagonist for AMPA receptors in the slice of rat GP, we examined the effect of 100 μM GYKI 52466 on AMPA-induced inward currents. Bath application of AMPA (4 μM) induced inward currents that were significantly blocked by pretreatment of 100 μM GYKI 52466 (data not shown). Therefore, 3 μM of KA likely activates KARs and not AMPARs in the presence of 100 μM GYKI 52466. This is consistent with a previous study demonstrating that bath application of KA (1-3 μM) does not induce significant inward currents in nucleus accumbens neurons of GluR6$^{-/-}$ mice (Casassus and Mulle, 2002).

While exogenous application of kainate induces inward whole-cell membrane currents, it is unclear whether synaptically released glutamate can activate these postsynaptic KA receptors. To test this hypothesis, we stimulated the internal capsule medial and ventral to the GP and recorded evoked excitatory post synaptic currents (EPSCs) in presence of 50 μM D-AP5 and 50 μM BIC. Superfusion of slices with 100 μM GYKI 52466 reduced, but did not completely block, the EPSCs ($n = 6$) (Fig. 2C, D). On the other hand, GYKI 52466 plus 50 μM CNQX always produced almost complete inhibition of EPSCs ($n = 6$) (Fig. 2C, D). The remaining EPSC (the amplitude of residual EPSC) after application of GYKI 52466 was 18 ± 4% of control ($n = 6$) (Fig. 2D). The decay time for residual EPSCs was significantly longer than that condition in the absence of GYKI 52466 application.

3.3. Kainate Receptor Activation Reduces Evoked EPSCs

Previous studies from others have demonstrated that a low dose of KA (0.3-1 μM) modulates excitatory synaptic transmission in the striatum (Cassassus and Mulle, 2002; Crowder and Weiner, 2002). We therefore tested KA (0.1-1 μM) on glutamatergic

synaptic transmission in GP. EPSCs were evoked every 15 s in GP neurons in presence of 50 μM D-AP5 and 50 μM BIC. Bath application of KA reversibly decreased EPSC amplitude as shown in figure 3A. On average, 1μM of KA reduced EPSC amplitude to 43.5 ± 3.7% ($p<0.001$, n = 7) (Fig. 3B). We have demonstrated that 1μM KA is unlikely to active AMPA receptors under our experimental conditions. This indicates that 1μM KA- induced EPSC depression is unlikely to be due to activation of AMPA receptors.

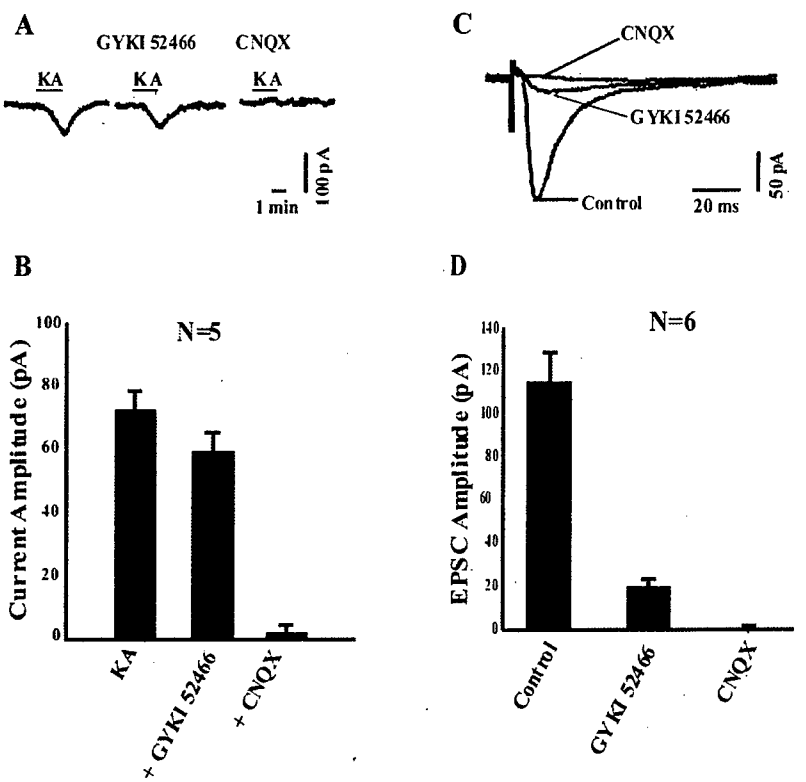

Figure 2. Functional expression of kainate receptors in rat GP. Inward currents evoked by 3 μM kainate in a GP neuron (A) and mean amplitude of inward currents evoked by 3 μM KA (mean ± SEM) (B). Currents were evoked by KA alone, in presence of GYKI 52466 (100 μM) or CNQX (50 μM). Synaptically evoked EPSCs recorded from a GP neuron (C) and mean amplitude of EPSC recorded in control condition and following bath application of GYKI 52466 or CNQX (mean ± SEM) (D).

If this is true, 1 μM KA should also reduce NMDA receptor-mediated EPSCs. Indeed, we found that application of 1 μM KA depressed NMDA receptor-mediated EPSCs to a

similar degree as it did for AMPA receptor-mediated EPSCs (46.4 ± 2.9%, p<0.001, n=13) (Fig. 3C, D). However, when slices were pre-treated with AMPA/KA receptor

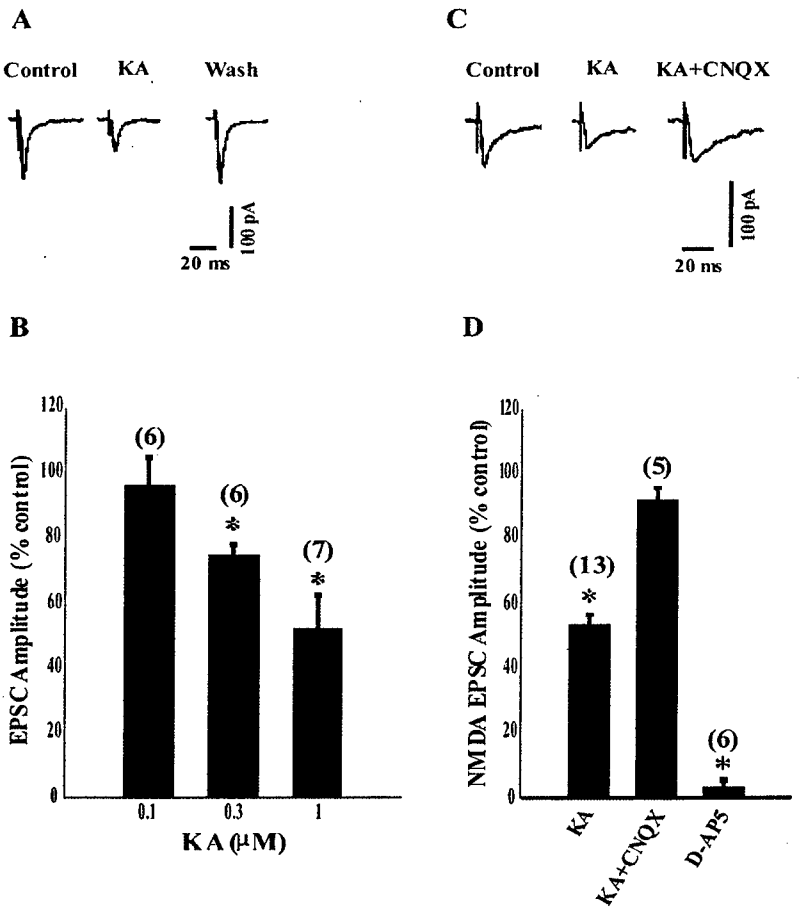

Figure 3. KAR activation inhibits glutamatergic synaptic transmission in rat GP. Application of KA (1 μM) depressed the evoked EPSCs recorded from a GP neuron (A). Summary bar graph showing the effect of bath application of KA (0.1-1μM) on EPSC amplitude as percent of control (mean ± SEM) (B). NMDA-mediated EPSCs were reduced by KA (1 μM) and this inhibition was blocked by CNQX (C). Bar graph summarizes mean amplitude of NMDA-EPSC in presence KA alone, KA plus CNQX and D-AP5 alone as percent of control ± SEM. All recordings were done in presence of GYKI 52466, and bicuculline. Numbers in parentheses indicate the number of cells tested under each condition; significant difference from control: * $P < 0.001$.

antagonist CNQX, the effects of KA on the NMDAR-mediated EPSCs were abolished (8.4 ±3.4%, p=0.07, n=5) (Fig. 3C, D). These results suggest that activation of KARs, but not AMPARs, are responsible for KAR activation-induced EPSC depression.

3.4. Kainate Receptor Activation Reduces Evoked EPSCs via a Presynaptic Mechanism

To test whether KA-induced EPSC depression is modulated via a presynaptic mechanism, we studied the effect of KA on paired-pulse facilitation (PPF) ratio of

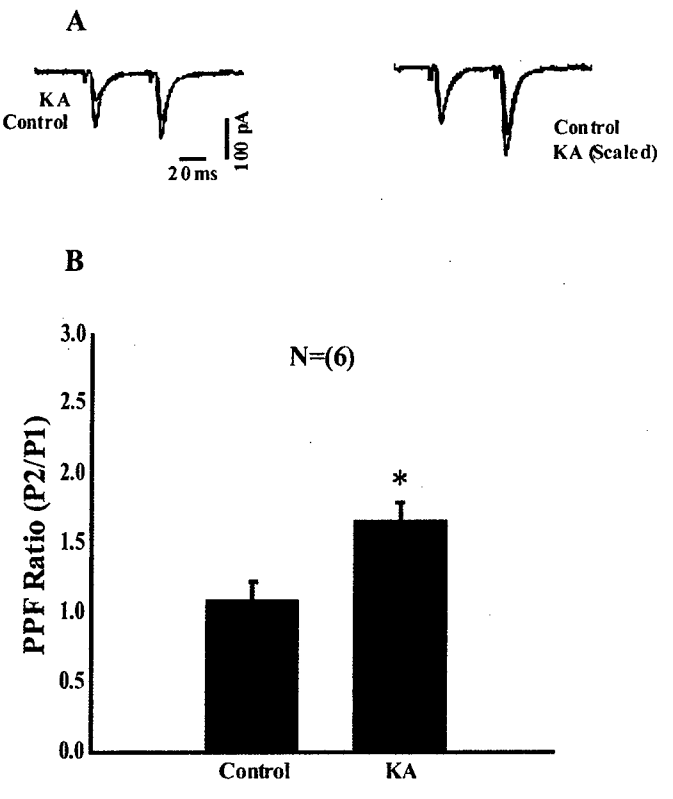

Figure 4. KAR activation increases paired-pulse facilitation (PPF) ratio at glutamatergic synapses in the GP. Effect of KA on paired EPSCs recorded from a GP neuron (A, left traces). The paired-pulse response to KA after scaling to the first EPSC (A, right traces). Bar graph summarizing the PPF ratio of EPSCs, expressed as mean ratio of P2/P1 ± SEM, in the absence or presence of KA. Significant difference from control: * $P < 0.001$.

evoked EPSCs. To record paired-EPSCs, two stimuli of internal capsule were paired with an interstimulus interval of 40-50 ms. As shown in figure 4, the peak of the second EPSC

is usually larger than the first one. We then calculated [redacted] ratio of *peak2/peak1* in the presence or absence of KA (1 μM). The ratio of *peak2/peak1* was significantly increased in presence of KA ($p<0.01$, $n = 6$) (Fig. 4), indicating a presynaptic effect. We also found that at 1μM concentration, bath application of KA did not significantly change [redacted] cell's input resistance (data not shown). Therefore, it seems unlikely that KARs-induced depression of glutamatergic synaptic transmission was due to change in passive membrane properties.

4. DISCUSSION

The data presented in this study provide the first evidence for postsynaptic activation of KARs by exogenous applied KA and synaptic released glutamate in the rat globus pallidus. In line with EM results, we demonstrated that activation of KARs reduces glutamatergic synaptic transmission in GP neurons and that this effect is mediated by a presynaptic mechanism.

Under the condition of blockade of AMPA, NMDA, and $GABA_A$ receptors with their selective antagonists, bath application of 3 μM KA induced an inward current in all recorded GP neurons. Furthermore, these KA-induced currents were completely blocked by CNQX, an AMPA/KA receptor antagonist. The fact that these currents were resistant to AMPA receptor antagonist, but blocked by AMPA/KA receptor antagonist, demonstrates that they were mediated through activation of KARs. These results are consistent with previously published data showing that KA (1-3 μM) does not induce AMPA-mediated inward currents in nucleus accumbens neuron of GluR6-deficient mice (Casassus and Mulle, 2002).

Despite the fact that the amplitude of KAR-mediated EPSCs is much smaller than that of AMPARs-induced EPSC (for review see Lerma, 2003; Huettner, 2003), we recorded small EPSCs evoked by individual stimuli in internal capsule in the presence of AMPA and NMDA receptor antagonists. Consistent with previous reports, we found that this EPSC was resistant to GYKI 52466, but completely blocked by CNQX. Furthermore, the decay time for residual EPSCs were significantly longer than that of AMPAR-mediated EPSCs (Castillo et al., 1997; Kidd and Isaac, 1999; Li and Rogawski, 1998; Li et al., 1999). These results suggest that KARs in rat GP could be activated by synaptically released glutamate.

In line with several studies conducted in hippocampus (Contractor et al., 2000; Kamiya and Ozawa 1998, 2000; Schmitz et al., 2000; Frerking et al., 2001) and nucleus accumbens core region (Crowder and Weiner, 2002; Casassus and Mulle, 2002), our data demonstrate that bath application of KA reduces the amplitude of EPSCs recorded in GP neurons. This effect is likely modulated by KARs because the concentration of KA (1μM) used in our experiments did not activate AMPA receptors (Casassus and Mulle, 2002). In addition, 1 μM KA also inhibited NMDAR-mediated EPSCs to a similar degree as it did for AMPAR-mediated EPSCs, and this inhibition was insensitive to GYKI 52466, but completely blocked by CNQX.

We next sought to determine the action site of KA on glutamatergic synaptic transmission in rat GP. It has been described in several brain regions that the inhibition of synaptic transmission by KA is modulated, at least in part, by activation of presynaptic

KARs (Contractor et al., 2000; Kamiya and Ozawa 1998, 2000; Schmitz et al., 2000; Frerking et al., 2001; Crowder and Weiner, 2002). Our results, indeed, showed that KA inhibition of EPSCs was associated with a significant increase in PPF ratio. Increase in PPF suggests a presynaptic decrease in the probability of neurotransmitter release (Manabe et al., 1993). In line with this result, our electron microscope immunohistochemical data have demonstrated that GluR6/7 immunoreactivity is expressed in putative glutamatergic axon terminals in the rat GP (Fig. 1). In addition to presynaptic modulation, it has been discussed that kainate receptor activation in the postsynaptic neuron reduces the input resistance, which could shunt IPSCs or EPSCs (Contractor et al., 2000; Frerking et al., 1999). However, we did not record a significant amount of inward current following application of 1 μM KA. Thus, we conclude that the reduction of EPSCs induced by KA was modulated by a presynaptic mechanism that did not associate with a significant change in input resistance of GP neurons.

It is known that the STN is by far the main source of glutamatergic inputs to the GP. Thus, KARs-modulated inhibition of EPSCs in rat GP is likely due to activation of KARs in subthalamo-pallidal terminals. However, because a small number of brainstem and thalamic glutamatergic fibers also innervate the GP (Kincaid et al., 1991; Naito and Kita, 1994; Mouroux et al., 1997), we cannot rule out the possibility that activation of KARs at these terminals may also account for the pre-synaptic effect demonstrated in our study.

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3

Glutamatergic Pathways

Their Relevance for Psychiatric Diseases

Yoland Smith

1. INTRODUCTION

Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system (CNS). Its effects are mediated through a large variety of ionotropic and metabotropic receptors abundantly expressed along the whole extent of the neuraxis. Abnormal regulation of glutamatergic transmission is, therefore, a key factor that underlies the appearance and progression of many neurodegenerative and psychiatric diseases. Unfortunately, the success of therapeutic strategies aimed at modulating glutamatergic transmission has been variable owing to the widespread distribution of glutamate receptors throughout the brain and the importance of glutamate in normal brain functioning. Although the importance of glutamatergic transmission in the modulation of neuronal activity involved in processing limbic and cognitive information has long been established, the complexity of the neuronal pathways involved combined with the multifarious effects glutamate could mediate via pre- and postsynaptic interactions with various receptor subtypes, have led to important controversies regarding the exact role glutamate plays in psychiatric diseases. However, substantial progress has been made over the past 10 yr in dissecting out the anatomy, physiology, and pharmacology of various neuronal pathways whereby glutamate could functionally modulate integrative processing of complex cognitive information. This chapter briefly summarizes some of these observations and considers their implications in our understanding of the anatomo-patho-physiology of psychiatric diseases, particularly schizophrenia, for which various hypotheses based on abnormal glutamatergic/dopaminergic transmission have been put forward to explain the neurochemical dysfunction of this disease *(1–15)*.

This review does not intend to cover the whole literature on the potential implications of glutamatergic pathways in psychiatric diseases, but will rather focus on recent developments regarding the anatomy and the potential mechanisms whereby glutamatergic pathways may interact to modulate neuronal integration in cortical and subcortical brain regions known to be affected in psychiatric diseases (Fig. 1).

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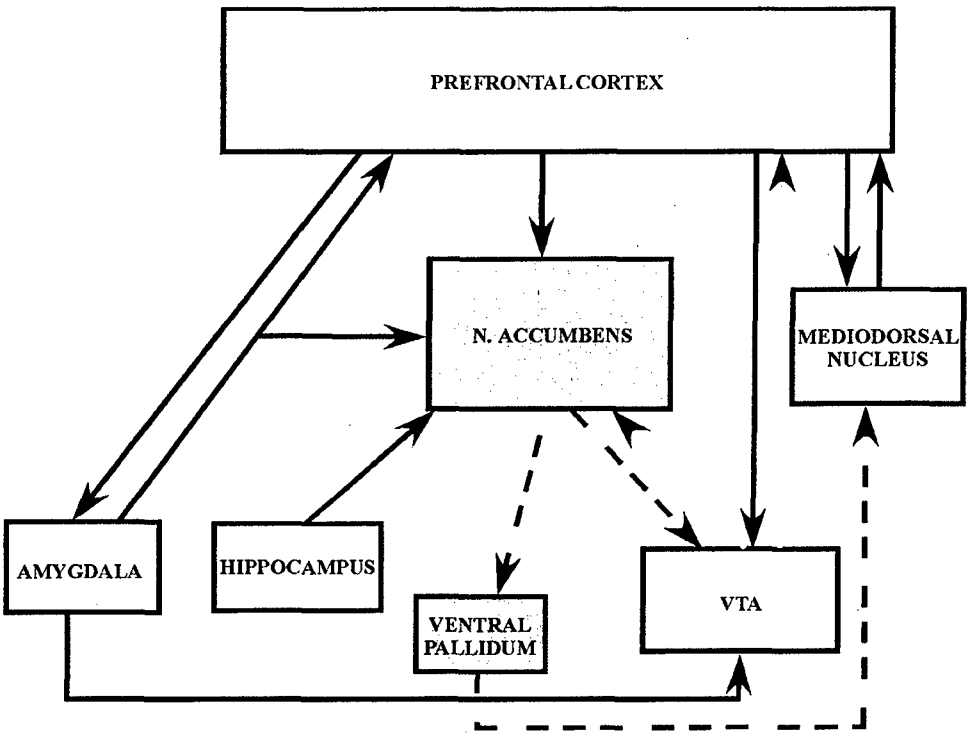

Fig. 1. Summary diagram and the main subcortical glutamatergic circuitry (black arrows) involved in the processing of limbic and cognitive information related to psychiatric diseases. These pathways play important roles in regulating dopaminergic outflow from the ventral tegmental area (light gray arrows) and γ-aminobutyric acid output from the nucleus accumbens (dashed arrows). Note that many connections have been purposefully omitted from this diagram.

2. DOPAMINERGIC/GLUTAMATERGIC HYPOTHESES OF PSYCHIATRIC DISORDERS

It has long been thought that schizophrenia and other psychiatric disorders were mediated by direct alterations of dopamine neuronal activity. This long-term belief was based on two main observations:

1. Drugs that increase dopamine levels in the brain create conditions that resemble those of schizophrenic psychosis in normals and exacerbate the psychosis problems in schizophrenic patients.
2. Drugs currently used to treat schizophrenics block dopamine receptors.

Although abnormal dopaminergic transmission remains a key component of the changes in neural activity that underlie psychiatric disorders *(14)*, it appears that the main abnormality of dopamine transmission in schizophrenics is largely mediated by changes in extrinsic regulatory influences of dopamine release either at the level of the ventral tegmental area or in the prefrontal cortex and nucleus accumbens *(1,3,5,12,14)*.

There are now various sets of data suggesting that forebrain dopamine systems may not be the primary site of neuropathology that is schizophrenia. Numerous studies have found structural and metabolic abnormalities in anterior temporal lobe and prefrontal

cortices *(8,14,16)*. Therefore, it seems that dopamine transmission is not affected in schizophrenia owing to a major defect in midbrain dopamine cell functions but rather results from an abnormal modulation by glutamatergic influences from limbic and prefrontal cortical regions (*1,3,5,12* and Chapter 7). In addition to the cerebral cortex, other forebrain structures and pathways that use glutamate as a neurotransmitter have been considered potential targets of schizophrenia and other psychiatric diseases. These include the amygdala, hippocampal formation, and mediodorsal thalamic nucleus *(6,14)*. Furthermore, the functional interactions between cortical and subcortical glutamatergic pathways at the level of the nucleus accumbens have received considerable attention over the past decades in regard to their potential involvement in neuropsychiatric diseases *(5)*. In this chapter, I will give an overview of the main features that characterize the anatomical and functional organization of these glutamatergic pathways (Fig. 1) and discuss recent findings that suggest their involvement in cognitive, emotional, and limbic-related behaviors.

3. CHANGES IN THALAMOCORTICAL AND INTRINSIC CORTICO-CORTICAL GLUTAMATERGIC CONNECTIONS IN PSYCHIATRIC DISEASES

The prefrontal cortex plays a major role in cognitive, limbic, and memory functions. Abnormalities in information processing or neurological damage to the prefrontal cortex may lead to a myriad of symptoms ranging from changes in personality traits to working memory deficits, and psychiatric diseases *(6,14)*. The anatomical organization of the prefrontal cortex in primates is very complex and comprises a multitude of functional areas characterized by differential patterns of connectivity and electrophysiological properties *(17–19)*. The activity of the prefrontal cortex is under the control of various afferent inputs that use glutamate as neurotransmitter. One of the main sources of thalamic afferents to the prefrontal cortex is the mediodorsal nucleus (MD), although projections from high-order, intralaminar and midline thalamic nuclei have also been reported *(20,21)*. The MD comprises various subdivisions and it appears that each of these subnuclei contribute to the innervation of different prefrontal cortical regions. For instance, the ventral part of the magnocellular MD (MDmc) projects to lateral regions of the ventral and medial prefrontal cortex including Walker's areas 11 and 12, whereas the dorsal part of MDmc is mainly connected with ventromedial regions of the prefrontal cortex (areas 13 and 14). In contrast, the lateral parvicellular MD (MDpc) innervates preferentially dorsolateral and dorsomedial prefrontal areas (Walker's 46, 9, and 8B); the multiform MD (MDmf) is mainly connected with area 8A, whereas area 10 has connections with the anterior part of MD. Thalamic inputs from MD are invariably confined to layer IV and adjacent deep layer III *(22)*. Interestingly, both the number of neurons and volume of MD are reduced in the brains of schizophrenic patients *(23,24)*. In line with these observations, other studies have reported fewer putative thalamic axon terminals and fewer dendritic spines on cortical pyramidal neurons in the prefrontal cortex of schizophrenics *(25,26)*. Although the exact functional implication of decreased thalamic influences on the prefrontal cortex in schizophrenia remains to be established, it has been suggested that they may lead to abnormalities in the inhibitory γ-aminobutyric acid (GABA)ergic microcircuitry of the primate prefrontal cortex *(27,28)*. However, acute lesion of the MD does not result in any significant changes in the expression of glutamic acid decarboxylase

67 (GAD67) mRNA in the prefrontal cortex of rats suggesting that the cortical abnormalities in GABAergic transmission observed in schizophrenia may be mediated by more complex changes in cortical microcircuitry than a mere decreased activity of thalamocortical glutamatergic inputs *(29)*.

The prefrontal cortex is also endowed with extensive glutamatergic corticocortical connections that may be affected in schizophrenia *(6)*. Although some of these connections involve posterior and temporal association areas, profuse horizontal axonal projections from layers II and III of dorsolateral prefrontal areas 9 and 46 to neighboring cortical areas have been described. These local projections are organized in a cluster-like manner that forms a series of elongated stripes within the same areas of the dorsolateral prefrontal cortex. Furthermore, these connections are reciprocal, suggesting that they form distinct interconnected functional modules that could play an important role in the integration and processing of prefrontal cortical information relating to working memory, one of the most fundamental cognitive process affected in schizophrenia. Although there is no direct evidence that these connections are specifically affected in psychiatric diseases, the fact that the size of layer III neuronal perikarya is reduced, combined with the evidence for a decrease in the density of dendritic spines on layer III pyramidal neurons in the prefrontal cortex of schizophrenic patients, are strong evidence in favor of abnormalities in the intrinsic glutamatergic microcircuitry in schizophrenia *(6)*.

4. GLUTAMATERGIC INPUTS TO MIDBRAIN DOPAMINERGIC NEURONS: KEY FACTORS IN CHANGES OF DOPAMINERGIC TRANSMISSION IN PSYCHIATRIC DISORDERS

Extrinsic glutamatergic inputs play a critical role in controlling the firing rate and firing pattern of midbrain dopaminergic neurons in the ventral tegmental area (VTA) *(3)*. Local application of glutamate or stimulation of glutamatergic afferents from the prefrontal cortex or the subthalamic nucleus results in an increased burst firing in midbrain dopaminergic neurons, thereby increased phasic dopamine release in the nucleus accumbens *(3,30,31)*. Midbrain dopaminergic neurons, in particular those in the VTA, receive massive glutamatergic inputs in primates *(32)*. Almost 70% of the total synaptic innervation of VTA dopaminergic neurons arises from glutamatergic boutons in monkeys *(32)*. The prefrontal cortex, subthalamic nucleus, and the brainstem pedunculopontine tegmental nucleus are likely to be the main sources of this innervation *(3,33–35)*.

The VTA is made up of largely segregated populations of dopaminergic and non-dopaminergic projection neurons that project to various cortical and subcortical brain structures, including the nucleus accumbens and the prefrontal cortex. Interestingly, glutamatergic inputs from the prefrontal cortex display a high degree of synaptic specificity in the rat VTA, targeting selectively GABA-containing mesoaccumbens neurons and dopamine-containing mesocortical cells *(36)*. These anatomical data provide a basic substrate for highly specific mechanisms through which prefrontal inputs may control the activity of ascending dopaminergic and GABAergic outflow from the VTA. It is noteworthy that the prefrontal cortex may also control the burst firing of midbrain dopaminergic neurons via its projections to the nucleus accumbens, which, in turn, sends GABAergic inputs to the VTA either directly or indirectly through disinhibition of the ventral pallidum *(3,37)*.

Other sources of glutamatergic projections that mediate changes in firing pattern of VTA neurons include the ventral hippocampus, entorhinal cortex, and amygdala, most likely via polysynaptic pathways that involve projections to the ventral striatum. However, it is important to note that the extended amygdala *(38)*, including the central nucleus and the bed nucleus of stria terminalis provides direct topographic inputs to midbrain dopaminergic neurons *(39,40)*. These represent additional routes through which glutamate could exert direct control on midbrain dopaminergic neuron activity.

5. THE NUCLEUS ACCUMBENS: A CRITICAL SITE FOR PREFRONTAL CORTICAL GLUTAMATERGIC MODULATION OF TONIC DOPAMINE RELEASE

Another way through which prefrontal glutamatergic outputs regulate subcortical dopaminergic transmission is via projections to the striatum (*see* Section 7). Corticostriatal glutamatergic afferents utilize multiple pathways to regulate striatal dopamine release and levels of extracellular dopamine *(3)*. In vitro and in vivo studies have proposed various pre- and postsynaptic mechanisms that involve both ionotropic and metabotroic glutamate receptors, as well as indirect multisynaptic pathways that could mediate these effects *(3)*. Grace and his colleagues have proposed that the glutamatergic modulation of intrastriatal dopamine release is mainly responsible for the maintenance of tonic dopamine levels in the striatum, whereas glutamatergic inputs to midbrain dopaminergic neurons regulate phasic dopamine release *(3)*. Although the prefrontal cortex is a key component for the control of intrastriatal dopamine levels, other glutamatergic inputs from the amygdala and hippocampus also appear to be involved through complex interactions functional interactions at the level of the nucleus accumbens (*see* Section 7).

6. STRESS-INDUCED DISRUPTION OF GLUTAMATERGIC TRANSMISSION FROM THE PREFRONTAL CORTEX AND ITS IMPACT FOR PSYCHIATRIC DISORDERS

Because of its functional importance in regulating dopaminergic transmission at cortical and subcortical levels, abnormal activity of prefrontal glutamatergic influences on the nucleus accumbens and the VTA may play a critical role in various psychiatric diseases *(12)*. The role of stress in the induction, maintenance, and relapse of psychiatric dysfunctions is well established and there is good evidence that changes in glutamatergic transmission in the prefrontal cortex and, possibly the hippocampus, may be responsible for the dopamine-mediated behavioral abnormalities seen in psychiatric diseases *(12)*. Stress induces two temporally different glutamate-mediated events in the prefrontal cortex. The first is an initial acute response characterized by an increase of fast glutamatergic synaptic transmission. This first event, which underlies immediate responses to stress, is likely to be induced by increased transmission of thalamocortical sensory inputs to prefrontal and limbic cortical areas *(12)*. This acute response is followed by long-lasting increases of glutamate and monoamine releases in prefrontal, limbic, and hippocampal cortices. Long-lasting changes in gene expression and protein synthesis also characterize this second event.

The prefrontal cortex also plays an important role in regulating the hypothalamo–pituitary axis (HPA) and glucocorticoid secretion during stress. It appears that the rather slow increased glutamate release in the hippocampus following stress might be mediated through HPA-regulated mechanisms, whereas the fast changes in glutamatergic transmission that

occur in the prefrontal cortex might be independent of the HPA axis and, rather, involve increased synaptic release of glutamate from intracortical or extrinsic afferents *(12)*.

7. FUNCTIONAL INTERACTIONS BETWEEN GLUTAMATERGIC INPUTS FROM THE AMYGDALA, HIPPOCAMPUS, AND PREFRONTAL CORTEX TO THE NUCLEUS ACCUMBENS

The nucleus accumbens is thought to be a key structure in the neuronal circuitry that underlies the neurobiological bases of psychiatric disorders, most particularly schizophrenia. The convergence of glutamatergic inputs from the amygdala, the prefrontal cortex, and the hippocampus, three brain regions that are affected in schizophrenic patients, combined with the dopaminergic inputs from the VTA, set the stage for multifarious and complex functional interactions that underlie the processing and integration of cognitive and limbic-related information flowing through this brain region. The anatomy and electrophysiology of these projections have been studied in great detail, which led to various hypotheses regarding the mechanisms by which these glutamatergic and dopaminergic projections interact to mediate their functional effects on behavior *(3,5)*. This section briefly summarizes some of the main anatomical features that characterize the organization and synaptic connectivity of these pathways, and discusses recent electrophysiological observations that support an important role for amygdala and hippocampal inputs to gate information flow from the prefrontal cortex to the nucleus accumbens *(5)*.

7.1. The Corticostriatal Projection

Various areas of the prefrontal and cingulate cortices provide substantial inputs to the monkey nucleus accumbens *(43–45)*. Price and his colleagues *(46)* defined the organization of prefronto-cortical projections to the striatum according to two major prefrontal networks involved in the integration and processing of functionally different information. These two networks are characterized by different corticocortical connections and distinct connections with subcortical brain regions including the thalamus, hypothalamus, and amygdala. The *"orbital network"* is thought to be involved predominantly in the processing of sensory information relating to food and feeding, whereas the *"medial network"* is more closely related to visceromotor or emotional motor functions *(45,46)*. The two pathways are tightly connected with various cortical and subcortical limbic structures including the amygdala, entorhinal cortex, and hippocampus, through which they may play important roles in controlling mood and guiding behaviors *(46)*. The two networks are differentially connected with the dorsal and ventral striatum. The ventromedial striatum, which includes the ventral putamen, medial caudate nucleus, and nucleus accumbens, receives its main input from the medial cortical network. Projections from caudomedial areas 32, 25, and 14r innervate mainly the medial edge of the caudate nucleus, the nucleus accumbens, and the ventromedial putamen, whereas projections from cortical areas 10o, 10m, and 11m remain restricted to the medial edge of the caudate nucleus *(46)*. Projections from areas 12o, 13a, and Iai terminate in the lateral accumbens and ventral putamen. On the other hand, projections from the "orbital network" are mainly directed toward the central part of the rostral striatum, which includes the central and lateral parts of the caudate nucleus and the ventromedial putamen *(46)*.

In addition to the prefrontal cortex, the nucleus accumbens also receives cortical inputs from limbic- and associative-related areas of the temporal lobe including the

entorhinal and perirhinal cortices, as well as the rostral portion of the superior temporal gyrus *(41,47–49)*. The cingulate cortex (areas 25, 24a–c, 24 a′–c′) is another major source of topographic cortical inputs to the monkey ventral striatum. The medial ventral striatum is mainly innervated by parts of the anterior cingulate cortex (areas 25, 24a,b) whereas the shell region of the accumbens receives fibers from areas 25, 24a,b and 24 a′,b′. Projections to the core of the accumbens arise primarily from areas 25, 24a,b and the medial part of area 24c, whereas the lateral part of the ventral striatum is mainly targeted by fibers coming from areas 24b,b′ and 23b and medial 24c *(43)*.

The organization of prefrontal corticostriatal projections to the core or shell of the nucleus accumbens has been studied in great detail in rodents by means of retrograde and anterograde tracing methods. The main prefrontal cortical inputs to the medial and lateral shell of the rat accumbens arise from the dorsal peduncular and infralimbic cortices, whereas the dorsal and ventral prelimbic and anterior cingulate cortices innervate preferentially the core. In addition, the lateral shell also receives strong cortical inputs from the agranular insular, perirhinal, rostral piriform, and lateral entorhinal cortices. On the other hand, additional cortical inputs to the medial shell arise from the caudal piriform cortex as well as the lateral and medial parts of the entorhinal cortex, whereas the core is preferentially targeted by inputs from the agranular insular and perirhinal cortices *(50,51)*.

Cortical inputs to the accumbens target preferentially the spines of striatal output neurons. Direct synaptic convergence of prefrontal inputs with dopaminergic terminals and hippocampal afferents have been demonstrated *(52–54)*, which provide a solid anatomical substrate for the gating properties of hippocampal projection on prefrontal cortical inputs in the rat accumbens *(5)*.

7.2. *The Amygdalostrial Projection*

In primates, the amygdalostriatal projection arises preferentially from various components of the basal and accessory basal nuclear complexes *(48,55–57)*. The main striatal target of amydala projections is the ventromedial striatum. Very few, if any, amygdala inputs are sent to the central striatum. The basal and accessory basal inputs innervate both the shell and core of accumbens, except for a restricted region in the dorsomedial shell that receives few basal nucleus inputs. The projection is topographically organized so that parvicellular basal inputs terminate in ventral shell and core, whereas magnocellular inputs target ventral shell and ventromedial putamen *(48)*. The intermediate division of the basal nucleus projects broadly across the whole ventromedial striatum except the dorsomedial part of the shell. The shell also receives specific inputs from the medial part of the central nucleus and periamygdaloid cortex and additional inputs from the medial nucleus *(48,57)*.

In the rat *(51,58–60)*, the amygdalostriatal projection is much more extensive than in monkeys and involves the whole extent of the ventral and dorsal striatum except for the rostrodorsolateral part of the caudate–putamen complex. This projection is highly topographic: the rostral basolateral nucleus projects preferentially to rostral and caudolateral portions of the accumbens and large portions of the dorsal striatum, whereas the caudal basolateral nucleus projects to the rostromedial caudate–putamen complex and caudomedial portion of the nucleus accumbens.

Amygdala terminals form asymmetric synapses mainly with spines and distal dendrites of projection neurons. At the light microscopic level there is a certain degree of overlap of axons from amygdala, hippocampus, prefrontal cortex, and thalamus in

nucleus accumbens *(61,62)* and some studies suggest functional convergence of these inputs onto individual neurons *(63)*. Electron microscopic studies demonstrated synaptic convergence of amygdala inputs with dopamine terminals *(53)* and hippocampal (ventral subicular) afferents onto single striatal neurons *(64)*. These convergent inputs may possibly mediate some of the complex functional interactions disclosed between these various glutamatergic afferents to control accumbens neuronal activity.

7.3. The Hippocampostriatal Projection

In monkeys, the subiculum is the main source of hippocampal inputs to the nucleus accumbens, but additional minor inputs come from parasubiculum, prosubiculum, and CA1 and CA3 regions *(48)*. These projections, which travel through the fornix and arise predominantly from the rostral hippocampus, terminate most densely in medial and ventral portions of accumbens. There is overlap of subicular and amygdala inputs to the medial division of the nucleus accumbens, suggesting potential interactions between these two pathways to modulate information processing in the primate accumbens *(48)*.

In rats and cats, the subiculum, CA1 region, and parahippocampal cortex provide massive heterogeneous projections to the ventral striatum *(65,66)*. The ventral subiculum projects mainly to the caudomedial part of the nucleus accumbens, whereas the dorsal and septal subiculum innervate preferentially its lateral and rostral components. Hippocampal inputs converge with dopaminergic, prefrontal, and amygdala afferents at the single-cell level in the rat accumbens *(33,54,67)*.

7.4. Functional Gating of Prefrontal Cortical Inputs by Hippocampal and Amygdala Afferents to the Nucleus Accumbens

Grace and his colleagues have published a series of elegant studies over the past 5 yr that provide a solid support for tight functional interactions between cortical, amygdala, and hippocampal glutamatergic inputs within the rat nucleus accumbens *(3,5)*. In vivo, accumbens neurons exhibit a bistable steady-state membrane potential alternating from a hyperpolarized nonfiring state to a depolarized state during which neurons can fire action potentials. Inputs from the hippocampal subiculum are responsible for generating the bistable state in these neurons. If fimbria/fornix is transected, none of striatal neurons exhibit the bistable membrane potential *(5,68,69)*. Prefrontal cortical stimulation induces only brief excitatory responses that, by themselves, are unlikely to result in action potentials in accumbens neurons. However, if hippocampal inputs are stimulated first, subsequent stimulation of prefrontal cortical afferents generate action potentials in accumbens neurons *(5,68)*. Activation of the subicular inputs cause the cells to shift to a depolarized state under which conditions prefrontal inputs can generate spike discharges. The hippocampal input, therefore, appears to act as a gate for prefrontal cortical influences to accumbens neurons *(5)*. Once this gate is opened, it allows prefrontal cortical inputs to get through and activates striatal neurons. This interaction is modulated by drugs that affect dopamine transmission because such compounds have an effect on the bistable state frequency of striatal neurons. For instance, systemic injection of D1 and D2 agonists decrease the frequency at which the membrane potential of striatal neurons exhibit depolarized states. Because the depolarized state is necessary for the gating of prefrontal cortical inputs by hippocampal afferents, the effects of prefrontal cortical inputs on striatal neurons are attenuated under these conditions *(5,70)*.

Amygdala inputs also appear to gate prefrontal cortical excitatory afferents to accumbens neurons. Stimulation of amygdala induces a brief depolarization of striatal neurons. If a

stimulus from amygdala is delivered before stimulation of the prefrontal cortex, there is facilitation of prefrontal cortical inputs to induce action potential in striatal neurons. This potentiation depends on the delay between the two stimuli. The amygdala has to be activated 7–30 ms before prefrontal cortical stimulation to mediate the potentiating effects. Inputs from both the amygdala and hippocampus are, therefore, capable of gating prefrontal cortical throughput to the accumbens, but in the case of amygdala, the response is brief and likely represents a phenomenon-related event *(5)*. It is important to note that there are reciprocal functional relationships between the amygdala and the prefrontal cortex; that is, prefrontal cortical stimulation influences neuronal activity in the amygdala and vice versa. Interestingly, cortical stimulation exerts inhibitory influences on the amygdala. This inhibitory effect appears to be mediated through various mechanisms that recruit amygdala GABAergic interneurons including a chloride-mediated hyperpolarization, persistent decrease in neuronal inputs resistance, and shunting of postsynaptic potentials *(71)*. Dopamine appears to be an important modulator of this functional interplay between the prefrontal cortex and amygdala *(71)*.

Although the exact functions of these gates are not clearly defined, Grace and colleagues have hypothesized that the hippocampal subiculum inputs may gate context-related events, whereas the amygdala may be involved in modulating prefrontal cortical stimuli related to emotion or affective states. Given the fact that schizophrenics show deficits in tasks that contain context-related information, one may hypothesize that a primary pathology of these brains relies upon the malfunctioning of the hippocampal gate of prefrontal cortical information at the level of the nucleus accumbens *(3,5)*.

8. CONCLUDING REMARKS

The importance of glutamatergic transmission in psychiatric diseases is now well established. Although much emphasis has been devoted to the prefrontal cortex, data presented in this review highlight the importance of other glutamatergic pathways from the amygdala and hippocampus. The complex functional interactions between these glutamatergic afferents to the nucleus accumbens, combined with the direct and indirect modulation these glutamatergic brain structures may exert on the activity of midbrain dopaminergic neurons, emphasize the importance of a tight balance of activity between glutamatergic and dopaminergic transmission to the prefrontal cortex and the nucleus accumbens for normal integration and processing of cognitive and limbic information. A shift in that balance leading to an increase release of dopamine at cortical and subcortical levels may be a critical factor that underlies the appearance, maintenance, and relapse of psychiatric diseases in humans.

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Localization and function of pre and postsynaptic kainate receptors in the rat globus pallidus.

Xiao-Tao Jin, Jean-Francois Paré, Dinesh V, Raju and Yoland Smith*

Division of Neuroscience, Yerkes National Primate Research Center and Department of Neurology, Emory University, 954, Gatewood Road NE, Atlanta, GA 30322, USA

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* To whom all correspondence should be addressed at:

Dr. Yoland Smith
Division of Neuroscience, Yerkes National Primate Research Center
and Department of Neurology, Emory University, 954, Gatewood Road
Northeast, Atlanta, GA 30322, USA

Telephone: + 1-404-727-7519

Fax: + 1-404-727-3278

Email: yolands@rmy.emory.edu.

Abstract

Kainate receptors are widely expressed the BC widely. In this study, we used electron microscopic immunocytochemistry and whole-cell recording techniques to examine the localization and function of kainate receptors (KARs) in the rat GP. Dendrites were the most common immunoreactive elements, while terminals forming symmetric or asymmetric synapses and unmyelinated axons comprised most of the presynaptic labeling. To determine whether synaptically released glutamate activates KARs, we recorded excitatory post synaptic currents (EPSCs) in GP following single pulse stimulation of the internal capsule. GYKI 52466 (100μM), an AMPA receptor antagonist, reduced, but did not completely block evoked EPSCs. The remaining EPSC component was mediated through activation of KARs because it was abolished by CNQX, an AMPA/KAR antagonist. The rise time (10-90%) and decay time constant (τ) for those EPSCs were longer than those of AMPA-mediated EPSCs recorded before GYKI 52466 application. KAR activation inhibited EPSCs. This inhibition was associated with a significant increase in paired-pulse facilitation ratio suggesting a pre-synaptic action of KAR. KAR inhibition of EPSCs was blocked by G protein inhibitor, N-ethylmaleimide (NEM), or PKC inhibitor calphostin C. Our results demonstrate that KAR activation has dual effects on glutamatergic transmission in the rat GP: (1) it mediates small amplitude EPSCs and (2) it reduces glutamatergic synaptic transmission through a presynaptic G-protein coupled, PKC-dependent, metabotropic mechanism. These findings provide evidence for the multifarious functions of KARs in regulating synaptic transmission, and open up the possibility for the development of pharmacotherapies to reduce the hyperactive subthalamofugal projection in Parkinson's disease.

Introduction

Fast excitatory synaptic transmission in the CNS is mediated predominantly by ionotropic glutamate receptors that can be divided into three classes: α-amino-3-hydroxyl-5-methyl-4-isoxazole propionic acid (AMPA), N-methyl-D-aspartate (NMDA), and kainate receptors (KARs) (Hollmann and Heinemann, 1994). The family of KARs is composed of five different genes that code for the subunits GluR5, GluR6, GluR7, KA1 and KA2 subunits (for reviews, see Hollmann & Heinemann, 1994; Bettler & Mulle, 1995). KAR activation mediates a large variety of pre- and post-synaptic effects on neurotransmission (for review, Frerking & Nicoll, 2000; Lerma, 2003; Huettner, 2003). For instance, activation of presynaptic KARs suppresses glutamatergic and GABAergic transmission in different brain regions (Lerma, 2003; Huettner, 2003). While postsynaptic KAR activation mediates a small and slow component of glutamatergic response at various synapses in the CNS (Cossart et al., 1998; Frerking et al., 1998; Bureau et al., 2000; Li & Rogawski, 1998; Li et al., 1999).

The globus pallidus (GP) (external globus pallidus, GPe, in primate) plays a central integrative role in the basal ganglia circuitry (Plenz & Kital, 1999; Bevan et al., 2002). The GP receives major glutamatergic inputs from subthalamic nucleus (STN) and sends GABAergic outputs back to the STN and other basal ganglia nuclei. The hyperactivity of the subthalamofugal glutamatergic projection and changes in the firing pattern of STN and GP neurons are critical in mediating abnormal activity of the basal ganglia circuitry under pathological conditions (Plenz & Kitai, 1999; Bevan et al., 2002).

We have shown that KARs are expressed pre- and post-synaptically in the monkey striatum (Charara et al., 1999; Kieval et al., 2001). Although recent functional studies demonstrated that activation of KAR by exogenous application of KA modulates neurotransmission in the striatum and substantia nigra pars compacta (SNc), none of these studies addressed the role of synaptically activated KARs in the basal ganglia (Casassus & Mulle, 2002; Crowder & Weiner, 2002; Nakamura et al., 2003). Recent findings from our laboratory have demonstrated strong GluR6/7 immunoreactivity in dendrites, axons and glutamatergic axon terminals in the monkey pallidum (Kane-Jackson & Smith, 2003). These findings

paved the way for pre- and post-synaptic KARs-mediated effects in the GP. To further address this issue, we employed electron microscopic immunocytochemistry and whole cell patch-clamp recording techniques to determine the localization and functions of KARs in the rat GP. Our data provide evidence for KAR-mediated postsynaptic currents and G-protein-mediated presynaptic regulation of glutamatergic transmission in rat GP.

Findings of this study have been presented on abstract forms (Jin & Smith, 2003; 2004).

Materials and methods

I. Immunocytochemistry

Animals and preparation of tissue

Two adult and two 17-d old Sprague Dawley rats were used for this part of the study deep anesthesia with an overdose of pentobarbital, rats were perfusion-fixed with 50 ml of cold oxygenated Ringer's solution followed by 350 ml of fixative solution containing 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer (PB) (0.1 M, pH 7.4). The anesthesia and perfusion of the animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (1996) and the Emory University Animal Care and Use Committee. The brains were then cut into 60μm-thick sections with a vibrating microtome and processed for the immunohistochemical localization of GluR6/7 at the electron microscopic level.

Pre-embedding immunoperoxidase

Sections prepared for pre-embedding immunoperoxidase were pretreated with sodium borohydride (1% in PBS; 0.01 M; pH 7.4) and then cryoprotected in a solution of 25% sucrose and 10% glycerol before being frozen at -80°C for 20 min. They were then thawed and returned to a graded series of cryoprotectant and PBS. Afterwards, sections were preincubated in 10% normal goat serum (NGS) in PBS for one hour, followed by incubation for 48 hr at 4°C in the rabbit polyclonal GluR6/7 antiserum (7.5 μg/ml) (Upstate Biotechnology, Lake Placid, NY) diluted in PBS supplemented with 1% NGS. The

preparation and specificity test for this antiserum were discussed in detail elsewhere (Petralia et al., 1994; Kieval et al., 2001). After three 10 min washes in PBS, the sections were incubated in biotinylated goat anti-rabbit IgG (1:200; Vector Laboratories, Burlingame, CA) for 90 min at room temperature, which was followed by three 10 min washes in PBS. Incubation in the avidin-biotin-peroxidase complex (ABC; 1:100; Vector Laboratories) (Hsu et al., 1981) subsequently followed for a period of 90 min. After two 10 min washes in PBS and one 10 min wash in TRIS buffer (0.05 M, pH 7.6), the immunostaining was revealed by incubation for 10 min in a solution containing 0.025% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO), 0.01 M imidazole (Fisher Scientific, Atlanta, GA), and 0.006% hydrogen peroxide (H_2O_2). The reaction was stopped by repeated washes in PBS.

Preparation for electron microscopy

All sections prepared for electron microscopy were washed in PB (0.1M, pH 7.4) before being post-fixed in osmium tetroxide (1% solution in PB) for 20 min. They were then washed five times (5 min each) in PB and dehydrated in a graded series of alcohol and propylene oxide. Uranyl acetate (1%) was added to the 70% ethanol to improve the contrast in the electron microscope. The sections were then embedded in resin (Durcupan, ACM; Fluka, Buchs, Switzerland) on microscope slides and put in the oven for 48 hr at 60°C. After examination in the light microscope, areas of interest in the GP were cut out from the tissue. Ultrathin 60 nm-thick sections were cut on a Leica (Nussloch, Germany) UCT ultramicrotome and collected on pioloform-coated, single slot copper grids. Sections were then stained with lead citrate (Reynolds, 1963) and examined with a Zeiss EM10C electron microscopy.

Analysis of immunoperoxidase data

Ultrathin sections collected from 6 blocks of tissue (3 blocks from young, 3 blocks from adult) taken from the immunostained GP in the rats used for this study were scanned in the electron microscope for the presence of immunoreactive elements. A total of 130 micrographs were taken from young GP tissue (total surface, 1395μm^2) while 141 micrographs (total surface, 1513 μm^2) were taken from adult at

25000x. Labeled structures were categorized on the basis of ultrastructural features described in Peters et al (1991). The relative density of labeled elements in each category was expressed as the percentage of total labeled elements in adult and young rat GP. The difference in the distribution of GluR6/7 immunoreactivity between young and adult rats was analyzed statistically using Chi-Square analysis.

II. Electrophysiology

Slice preparation

All electrophysiological experiments were performed on slices from 13 to 17-d old Sprague Dawley rats (Charles River Laboratories, Wilmington, MA). After decapitation, brains were removed and quickly submerged in the ice-cold oxygenated sucrose buffer containing (in mM): 223.4 sucrose, 20 glucose, 47.3 $NaHCO_3$, 3 KCI, 1.9 $MgSO_4$, 1.2 KH_2PO_4, and 2 $CaCl_2$ (Poisik et al., 2003; 2004). Parasagittal slices (300μm in thickness) were made on a Vibratome 3000 (The Vibratome Company, St. Louis, MO) in ice-cold oxygenated sucrose buffer. Slices were stored at room temperature in a chamber containing artificial cerebrospinal fluid (ACSF) (in mM): 124 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1.0 NaH_2PO_4 and 2.0 $CaCl_2$, 20 glucose, 26 $NaHCO_3$ at pH 7.3-7.4 with 95% O_2, 5% CO_2 bubbling through it. The osmolarity of the ACSF was ~ 310 mOsm.

Whole-cell patch-clamp recordings

Whole-cell path-clamp recordings were performed as described previously (Marino et al., 2001; Poisik et al., 2003; 2004). During the recording, the slice was maintained fully submerged in the recording chamber and perfused with oxygenated ACSF (~3ml/min). GP neurons were visualized by IR-differential interference contrast microscopy (BX51Wl) using a 40X water immersion objective (Olympus, Pittsburgh, PA). Whole cell patch electrodes were pulled from borosilicate glass on a vertical patch pipette puller (Narishige, Tokyo, Japan) to have resistance in the range of 3-5 MΩ when filled with an intracellular patch solution. All experiments were performed at room temperature. Tight-seal (>1GΩ) whole-cell recording was obtained from the cell body of GP neurons. Series resistance was regularly monitored during recording, and cells were rejected if it changed by > 20%. Cells were

categorized as GP neurons if they fulfilled the electrophysiological criteria established in previous studies (Kita & Kitai, 1991; Nambu & Llinas, 1994; Cooper & Stanford, 2000). As described in these studies, two types of GP neurons (type A and B) were recorded in our slice preparation but since no difference was found in kainate-mediated effects between these two populations, results were combined. Recorded neurons were filled with biocytin to assess their exact localization in GP.

For whole- cell voltage- clamp experiments, patch pipettes were filled with the internal solution containing (in mM): 140 CsCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES, 2 Mg_2ATP, 0.2 GTP, and 0.5% biocytin, pH 7.4 (300-310 mOsm). In some experiments, 5mM N-(2,6-Dimethylphenylcarbamoylmethyl) triethylammonium bromide (QX314) was added in the pipette solution. Neurons were voltage-clamped at a holding potential of -70 mV and whole-cell membrane currents were recorded with a Patch-Clamp PC-501A (Warner Instruments, Hamden, CT). For whole-cell current- clamp recordings, the internal solution contained (in mM): 120 potassium gluconate, 20 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES, 2 Mg_2ATP, 0.2 GTP, and 0.5% biocytin, pH 7.4 (300-310 mOsm). Exogenous KA-induced currents or membrane potentials were isolated pharmacologically by including 100 μM GYKI 52466, an AMPA receptor antagonist, 1 μM NBQX, an AMPA/KA receptor antagonist, 50 μM CNQX, an AMPA/KA receptor antagonist, 50 μM D-AP5, a NMDA receptor antagonist, 50 μM bicuculline, a $GABA_A$ receptor antagonist and 1μM tetrodotoxin (TTX). N-ethylmaleimide (NEM) (200 μM), a pertussis toxin-sensitive G-proteins inhibitor, Calphostin C (2 μM), a selective protein kinase C (PKC) inhibitor, staurosporine (0.5 μM), a broad spectrum inhibitor for protein kinases, and (H-89) (0.5 μM), a selective protein kinase A inhibitor (PKA). AMPA receptor-mediated excitatory postsynaptic currents (EPSCs) were isolated pharmacologically in presence of D-AP5 and bicuculline, while NMDA receptor-mediated EPSCs were isolated in presence of GYKI 52466 and bicuculline. The holding potential for AMPA and NMDA receptor-mediated EPSCs was -70 mV and -40 mV, respectively. Three EPSCs were averaged and their peak amplitude was measured before and during KA application. In some experiments, the input resistance of GP neurons was determined

during the KA application, which indicated by changing the holding current during a -10mV step from the command voltage of -70 mV. Kainate, AMPA, D-AP5, CNQX, NBQX, GYKI 52466, TTX and staurosporine were purchased from Tocris Cookson (Ellisville MO). Calphostin C and H-89 were purchased from Sigma (St Louis MO). All compounds were made in a 1000x and diluted into the ACSF immediately before use. Compounds were aliquoted and stored at -20°C.

Electrical stimulation

Bipolar tungsten stimulating electrodes (FHC, Bowdoinham, ME) were placed in the internal capsule medial to the GP. Evoked excitatory postsynaptic currents (EPSCs) in GP neurons were recorded by stimulation of internal capsule with single pulses that ranged from 3 to 10 V, 150-200 μsec, delivered once every 20 sec. The paired-pulse facilitation (PPF) of evoked EPSCs was performed as follows: two stimuli of the internal capsule were paired with an interstimulus interval of 40-50 ms. The ratio of peak 2/peak1 was calculated. The rise time of synaptic currents was measured from 10 to 90% of peak amplitude. The decay kinetics was calculated by fitting a single exponential to the decay from ~10% below the peak.

Biocytin histochemistry

Biocytin-filled neurons were visualized using either Rhodamine (TRITC)-conjugated streptavidin or avidin-biotin-peroxidase (ABC). Following overnight fixation in 10% neutral formalin, the slices processed with TRITC were treated for 20 min in 1% sodium borohydride and rinsed in PBS for 10 min. They were then placed in a pre-incubation solution containing 5% normal donkey serum, 1% BSA, 0.3% Triton and PBS for 1 hour. After 3 rinses in PBS, the slices were incubated in a primary solution containing 1% NDS, 1% BSA, 0.3% Triton X-100 and 1% streptavidin coupled with Rhodamine (Jackson ImmunoResearch) overnight. Slices were then rinsed 3 times in PBS and mounted on slide using the VectaShield mounting media (Vector Laboratories).

The slices processed ABC method and DAB staining were rinsed in PBS for 30 min, incubated in PBS containing 1% Sodium borohydride for 20 min. They were then incubated in an ABC solution overnight

at room temperature. After two 10 min washes in PBS and one 10 min wash in TRIS buffer (0.05 M, pH 7.6), the immunostaining was revealed by incubation for 10 min in a solution containing 0.025% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO), 0.01 M imidazole (Fisher Scientific, Atlanta, GA), and 0.006% hydrogen peroxide (H_2O_2). The reaction was stopped by repeated washes in PBS. The biocytin-labeled neurons were then viewed with a Leica DMRB microscope, equipped with Leica digital Imaging system software.

Data analysis

All signals were filtered at 5 kHz and digitized with a Digidata 1200 analog-to-digital converter (Axon Instruments, Foster City, CA). Data were analyzed off-line using pClamp 6 (Axon Instruments). All values were expressed as means ± SEM. Statistical significance was assessed by Student's *t*-test.

Results

Localization of GluR6/7 in the GP

Previous studies from our laboratory have shown pre and postsynaptic GluR6/7 KAR subunits expression in the adult monkey striatum and globus pallidus (Kieval et al., 2001; Kane-Jackson & Smith, 2003). To make sure the pattern of KARs distribution found in monkeys was valid in rats, we studied the localization of GluR6/7 immunoreactivity in the GP of adult rats at the electron microscopic level. Consistent with our primate data (Kane-Jackson & Smith, 2003), proximal and distal dendrites were the most common postsynaptic immunoreactive elements encountered in the rat GP (Fig. 1A). In addition, terminals forming symmetric or asymmetric synapses and small preterminal unmyelinated axons comprised most of the presynaptic labeling (Fig. 1B and C). Immunoreactive terminals often found expression with immunoreactive dendrites, as shown in Fig. 1D. Since our whole cell patch clamp recording studies were performed in slices of young rats, we compared the pattern of GluR6/7 immunoreactivity in the GP of adult rats with that found in P13-P17 rats. As displayed in figure 1F, no significant difference in the relative abundance of GluR6/7-labeled elements was found between the two

age group (χ^2; P = 0.63) (Fig. 1F). These findings provide a substrate for pre- and post-synaptic KAR-mediated effects in the rat GP. The following electrophysiological and pharmacological studies examine the functional roles of KARs in rat GP.

Functional postsynaptic KARs in the GP

To test whether functional KARs are expressed on GP neurons, we measured the amplitude of inward currents induced by bath application of kainate (KA) using whole-cell patch-clamp recording techniques. As described in previous studies (Kita & Kitai, 1991; Nambu & Llinas, 1994; Cooper & Stanford, 2000; Poisik et al., 2003), the GP contains a heterogeneous population of neurons that can be classified into two main sub-types on the basis of differences in spike frequency adaptation, time-dependent inward rectification, cell's input resistance, and rebound spiking. Type A or II neurons possess two main cardinal features: (1) a sag in membrane potential during a hyperpolarizing current injection in current clamp that corresponds to a time- and voltage-dependant inward current (I_h) and (2) a high input resistance and spontaneous activity at rest (Fig. 2A). A less frequent phenotype categorized as type B or I neurons, were identified by the absence of sag and a lower input resistance than type II neurons. Those two types of neurons likely represent the most common type of recorded neurons in our study. Since we did not observe any significant difference in responses to kainate application in the pool of neurons examined in our study, we did not attempt of charactering the effect of KA on type I or type II neurons.

When applied at a concentration of 100 μM, GYKI 52466, selectively blocks AMPA receptor-mediated currents with minor effect on KARs (Paternain et al., 1995). Bath application of KA (0.1-10 μM) induced concentration-dependent inward currents in presence of 50 μM D-AP5, 50 μM BIC, 1 μM tetrodotoxin (TTX) and 100 μM GYKI 52466 (n = 6, Fig. 2B and C). In these experiments, each concentration of KA was applied for two min and was washed for at least four min before testing the next concentration. At 3 μM, KA induced significant inward currents (Fig. 2C). To test if this concentration of KA generates AMPA-mediated currents in GP neurons, we recorded inward currents

induced by 3 μM of KA in absence or presence of 100 μM GYKI 52466 (Fig. 2D). In each condition, we applied KA for 2 min and waited for 4 min before perfused KA again. We found no significant difference in the amplitude of KA-evoked currents recorded in the presence or absence of GYKI 52466 (69 ± 4.5 before and 59 ± 1.8 pA during GYKI 52466, respectively. n = 5, p = 0.08) (Fig. 2E). In both instances, currents were blocked by 50 μM CNQX, an AMPA/KAR antagonist (4.4 ± 2.2 pA, p<0.001) (Fig. 2D and E). NBQX is an AMPA/KAR antagonist which has also been used to isolate KAR-mediated response from AMPA receptors. At 1 μM, NBQX maximally blocks AMPAR-mediated currents without any effect on KAR-mediated currents (Bureau et al., 1999; Crowder & Weiner, 2002). As expected, we found no significant difference in the amplitude of currents induced by 3 μM KA in absence or presence of 1μM NBQX (63 ± 4.3 and 62.8 ± pA, respectively. n = 5, p = 0.9) (Fig. 2E). These results indicate that a low dose KA (1-3 μM) selectively activates KARs, without any significant effect on AMPA receptors.

To ensure that inward currents induced by 3 μM KA depolarize GP neurons, we performed whole-cell current clamp recordings and tested the effect of 3μM KA on the membrane potential of GP neurons in presence of 50 μM D-AP5, 50 μM BIC and 1 μM tetrodotoxin (TTX). KA depolarized GP neurons when slices were pretreated with 100 μM GYKI 52466 (13.8 ± 1.5 mV) and this depolarization was completely blocked by 50 μM CNQX (0.5 ± 0.9 mV. n = 6, P < 0.001) (Fig. 2F). Similar to what was observed in our voltage clamp studies, 1 μM NBQX had no significant effect on the kainate-induced depolarizations (12.7 ± 1.3 mV. n =10) (Fig. 2F).

To confirm that 100 μM GYKI 52466 and 1μM NBQX are selective antagonists for AMPA receptors in the rat GP, we examined the effect of these antagonists on AMPA-induced inward currents. Application of 5 μM AMPA, which is sufficient to elicit 50% of maximal activation at AMPA receptors (Wong et al., 1994), did not induce any KAR-mediated currents (Wilding & Huettner, 1997). Consistent with studies from cultured neurons and brain slice preparations (Paternain et al., 1995; Bureau et al.,

1999; Crowder & Weiner, 2002), bath application of AMPA (4μM) induced inward currents that were completely blocked by pretreatment with 100 μM GYKI 52466 or 1 μM NBQX (data not shown).

Synaptic release of glutamate activates postsynaptic kainate receptors in the rat GP

Although exogenous application of KA induced whole-cell membrane currents and depolarized the membrane of GP neurons, it is unclear whether synaptically released glutamate can have the same effect in the GP. To address this issue, we stimulated the internal capsule medial and ventral to the GP and recorded evoked EPSCs in presence of 50 μM D-AP5 and 50 μM BIC. Superfusion of slices with 100 μM GYKI 52466 reduced, but did not completely block, the EPSCs (n = 6) (Fig. 3A). On the other hand, 50 μM CNQX always produced an almost complete inhibition of EPSCs recorded from the same cells (Fig. 3A and D). The amplitude of residual EPSC after application of GYKI 52466 was 18 ± 4% of control (n = 6) (Fig. 3D). The decay of the GYKI 52466-resistant component (τ = 35 ± 6.4 ms) was slower than that for the GYKI 52466-sensitive component (τ = 12.7 ± 2 ms) (n = 7, $p < 0.01$) (Fig. 3E). The rise time (10-90%) of GYKI 52466-resistant EPSC (7.7 ± 0.7 ms) was also slower than for the GYKI 52466-sensitive component in the same cells (4.6 ± 0.4 ms, $p < 0.001$) (Fig. 3E).

To confirm that the residual EPSCs were mediated by KAR activation, we applied SYM 2081, a selective KAR agonist that produces potent receptor desensitization (Jones et al., 1997; Wilding & Huettner, 1997; Castillo et al., 1997). SYM 2081 (5 μM) significantly reduced or abolished the residual EPSCs in presence of GYKI 52466 (23 ± 5.9% before and 3.16 ± 0.8% after. n = 7, $p<0.001$) (Fig. 3F and I), but had no significant effect on the large and fast component of EPSCs recorded in absence of GYKI 52466 (89 ± 5%. n = 7, p = 0.6) (Fig. 3G and I). Cyclothiazide (10 μM), which potentiates AMPAR-mediated currents, but not KAR-mediated currents (Partin et al., 1993; Castillo et al., 1997), had no effect on GYKI 52466-resistant EPSCs (19 ± 2% before and 21 ± 1.3% after. n = 8, p = 0.43) (Fig. 3H and I). These data indicate that the small and slow residual EPSCs recorded in GP neurons following AMPA receptor blockade are mediated by KARs.

Kainate receptor activation reduces evoked EPSCs

Previous studies have demonstrated that a low dose of KA (0.3-1 μM) modulates excitatory synaptic transmission in the striatum (Cassassus & Mulle, 2002; Crowder & Weiner, 2002). Our electron microscopic data demonstrate the expression of pre-synaptic GluR6/7 immunoreactivity in putative glutamatergic terminals in the rat and monkey pallidum (Charara et al., 1999; Kieval et al., 2001; Kane-Jackson & Smith, 2003; Jin & Smith, 2004). We, therefore, tested whether 0.3 to 1μM KA could modulate glutamatergic synaptic transmission in slice of rat GP. Non-NMDA-mediated excitatory post synaptic currents were evoked every 20 seconds in GP neurons in presence of 50 μM D-AP5 and 50 μM BIC. Bath application of KA (0.3-1 μM) reversibly decreased non-NMDA-EPSC amplitude in all cells tested (Fig. 4A and B). On average, the KA-induced inhibition of EPSC amplitude was 25.25 ± 2.5% (n = 6, $p < 0.001$) and 43.5 ± 3.7% (n = 7, $p < 0.001$) of control when perfused with 0.3 and 1 μM, respectively (Fig. 4B). We did not detect any effect of KA on EPSC amplitude when KA concentrations below 0.3 μM (0.025-0.1 μM) were used (Fig. 4B).

Since kainate is also an agonist of AMPA receptors, we had to confirm that 1 μM KA did not cause EPSC depression via activation of AMPA receptors. To do so, we examined the effect of KA on the amplitude of NMDA-mediated EPSC in the presence of GYKI 52466 (100 μM) and BIC (50 μM). Under such conditions, bath application of KA depressed NMDA-mediated EPSCs to the same extent as non-NMDA EPSCs (46.4 ± 2.9%, n = 13, $p<0.001$) (Fig. 4C and D). Pre-treatment of the slice with the AMPA/KA receptor antagonist, CNQX (50 μM), abolished the effect of KA on NMDA- mediated EPSCs. At the end of recordings, slices were treated with 50 μM D-AP5 to confirm that the EPSCs were mediated through NMDA receptors (n = 6) (Fig. 4D). These results demonstrate that activation of KARs, but not AMPARs, is responsible for KA-induced EPSC depression.

KAR activation reduces evoked EPSCs via a presynaptic mechanism

We have shown GluR6/7 immunoreactivity in glutamatergic axon terminals in young and adult of GP (Fig. 1). Thus, activation of presynaptic KARs may be responsible for KA-mediated depression of evoked EPSCs in GP neurons. To test this hypothesis, we conducted two sets of experiments. First, we studied the effect of KA on paired-pulse facilitation (PPF) of evoked EPSCs. To record paired EPSCs, two stimuli of the internal capsule were paired with an interstimulus interval of 40 ms. We then calculated ratio of *peak2/peak*1 in the presence or absence of KA (1 μM). The ratio of *peak2/peak*1 was significantly increased in the presence of KA compared to control (1.63 ± 0.1 and 1.13 ± 0.1, respectively. $n = 6$, $p < 0.01$) (Fig. 5A and B), indicating a presynaptic effect. To test whether 1 μM KA-induced currents (Fig. 2B) change the input resistance that could contribute to part of the EPSCs depression, we performed a second set of experiments to measure the input resistance and EPSC amplitude in seven GP cells during 1 μM KA application. We first determined the input resistance by measuring the holding current during a –10 mV step from the command voltage of – 70 mV. The input resistance was 84.5 ± 3% of control during KA application, a reduction that was not found to be statistically significant ($p = 0.4$) (Fig. 5C, top trace and D). In contrast, evoked EPSCs were profoundly inhibited under the same experimental conditions (56 ± 5.6% of control, $p < 0.001$) (Fig. 5C, bottom trace and D). Therefore, it seems unlikely that changes in passive membrane properties contribute significantly to KARs-induced depression of synaptic transmission in GP neurons.

Suppression of evoked EPSCs by kainate application require activation of NEM toxin-sensitive G-protein and PKC

We have shown that KA reduces glutamate release without changing a detectable depolarization. In the hippocampus, the mechanism required for this type of presynaptic KAR-mediated effects involves a G-protein-dependent signal transduction pathway that requires PKC (Rodríguez-Moreno and Lerma 1998; Rodríguez-Moreno et al., 2000). We, therefore, examined whether the KA-mediated inhibition of glutamatergic synaptic transmission in the GP, involves was due a G-protein-coupled transduction

cascade. First, we tested the effects of the G-protein inhibitor NEM (200 μM) on the KA-mediated inhibition of EPSC. As reported in other brain regions (Frerking et al., 2001; Kubota et al., 2003) bath application of NEM increased synaptic transmission. The amplitude of EPSC was 121% ± 4.5 of control in presence of NEM ($n = 7$, $p < 0.001$) (Fig. 6A and B). After 15 min of NEM perfusion, KA had no effect on EPSC amplitude. The EPSC amplitude in the presence of KA together with NEM was 122.6% ± 4.4 of control, which was not significantly different from the amplitude of EPSCs recorded with NEM alone (121% ± 4.5; $p = 0.87$, $n = 7$) (Fig. 6B). These data demonstrate that the inhibitory effect of KA on EPSC amplitude is modulated through G proteins.

Since G-protein-coupled receptor systems have been hypothesized to indirectly modulate the presynaptic actions of KA, including $GABA_B$, adenosine receptors, and mGluRs (Chergui et al., 2000; Frerking et al., 1999; Nakamura et al., 2003; Schmitz et al., 2001), we examined whether the KA-modulated inhibition of glutamatergic synaptic transmission observed in the GP, was due to the secondary activation of these other receptor systems. We pretreated slices with an mGluR antagonist cocktail (1 mM MCPG and 100 μM CPPG), a $GABA_B$ receptor antagonist (20 μM SCH 50911), a dopamine receptor cocktail (10 μM Sulpiride and 10 μM SCH 23390) or a nonselective adenosine receptor antagonist (10 μM CGS 15943) for 10 min prior to bath application of KA. Pretreatment with any of these drugs had no significant effect on KAR mediated-depression of evoked EPSCs (Fig. 6C).

To address whether or not protein kinase activation downstream of G-protein activity was required for KARs-induced presynaptic inhibition in GP neurons, we studied the effects of KA on EPSC amplitude after incubation slices with staurosporine (0.5 μM, 2-4 hr), a broad spectrum inhibitor for protein kinases. Staurosporine significantly reduced the action of KA on EPSC amplitude (92 % ± 5.6 of control, $n = 5$, $p = 0.2$) (Fig. 7C). To further examine which kinase was involved in this action, we incubated slices with two selective inhibitors: As elective protein kinase inhibitor A (H-89, 0.5 μM, 2-4 hr) and a specific PKC inhibitor (calphostin C, 1 μM, 2-4 hr). We found that bath application of KA significantly reduced EPSC amplitude when slices were treated with H-89 (0.5 μM, 2-4 hr) (47.4% ± 3.2

of control, $p < 0.001$, $n = 6$) (Fig. 7B and C), but had no significant effect on EPSC amplitude when slices were incubated with Calphostin C (1 μM, 2-4 hr) (88% ± 4.8, $p = 0.07$, $n = 5$) (Fig. 7A and C). These results demonstrated that activation of PKC, but not PKA, is involved in KA-induced inhibition of glutamate release in rat GP.

Discussion

Findings presented in this study provide the first evidence that synaptically released glutamate can activate postsynaptic KARs in the rat GP. In addition, we demonstrate that KARs activation depresses glutamatergic synaptic transmission through a G-protein mediated presynaptic mechanism. In the following account, these observations will be discussed in light of the current knowledge of KAR functions in other brain regions. The potential role of KAR in the functional circuitry of the basal ganglia under normal and pathological conditions will also be examined.

Post-synaptic KARs function

Under conditions that block AMPA, NMDA, and $GABA_A$ receptors, bath application of 3 μM KA induced an inward current in all GP neurons examined in this study. These currents were completely blocked by CNQX, an AMPA/KA receptor antagonist. The fact that these currents were resistant to AMPA receptor antagonist, but blocked by AMPA/KA receptor antagonist, demonstrates that they were mediated through activation of KARs.

KAR-mediated EPSCs have been demonstrated at various synapses in the hippocampal formation and other brain regions (Frerking et al., 1998; Cossart et al., 1998; Kidd & Isaac, 1999). In hippocampal interneurons, spinal cord, amygdala and thalamus, single stimuli can elicit KAR-mediated EPSCs (Frerking et al., 1998; Li et al 1999; Li et al., 1998; Kidd & Isaac, 1999), while in other neuronal populations such as the hippocampal pyramidal cells, a paired-pulse stimulation can evoke KAR-mediated EPSCs (Ito et al., 2004). These studies demonstrated that synaptic responses mediated by

kainate receptor share two features: the synaptic current is small and has significantly slower deactivation kinetics than those mediated by AMPA receptors (for review see Lerma, 2003; Huettner, 2003). It was hypothesized that slow kinetics of KAR mediated-EPSCs might be due to intrinsic properties of these postsynaptic receptors (for review see Frerking & Nicoll, 2000; Lerma, 2003). For example, binding of GluR6 receptor to SAP90 (synapse-associated protein 90) leads to kainate receptor clustering which, in turn, reduces the extent of desensitization of these receptors (Garcia et al., 1998). Reducing the KARs desensitization could potentially prolong the decay of the EPSC (Frerking & Nicoll, 2000). In the present study, we recorded small amplitude EPSCs evoked by individual stimuli in the internal capsule in the presence of AMPA and NMDA receptor antagonists in slices of rat GP. Various evidence indicate that these EPSCs are mediated by KARs. First, they were resistant to GYKI 52466, but completely blocked by CNQX, as demonstrated for KA-mediated EPSCs in other brain regions (Frerking et al., 1998; Li et al 1999; Li et al., 1998; Kidd & Isaac, 1999). Second, the activation and deactivation kinetics of these EPSCs were significantly slower than those of AMPA-mediated EPSCs. Third, the GYKI 52466-resistant, but not GYKI 52466-sensitive, EPSC was significantly reduced by SYM 2081 (Li et al., 1999; Castillo et al., 1997), a selective kainate receptor agonist that causes potent receptor desensitization (Jones et al., 1997; Wilding & Huettner, 1997). On the other hand, cyclothiazide, which potentiates currents mediated by AMPA receptors, but not by kainate receptors (Partin et al., 1993), did not have any significant effect on the EPSC amplitude recorded in the presence of GYKI 52466 (Li et al., 1999; Castillo et al., 1997). Together, these data provide solid evidence for the expression of functional post-synaptic KARs in the rat GP. Furthermore, we demonstrated that these receptors can be activated by synaptically released glutamate and that they contribute to glutamatergic EPSCs evoked by single stimuli to GP neurons.

Pre-synaptic KARs modulation of glutamatergic transmission

In line with various studies performed in the hippocampus (Chittajallu et al., 1996; Frerking et al., 2000; Contractor et al., 2000; Kamiya & Ozawa 2000; Schmitz et al., 2000) and striatum (Crowder and Weiner, 2002; Casassus and Mulle, 2002), our data demonstrate that bath application of KA reduces the amplitude of EPSCs recorded from GP neurons. This effect is likely modulated by KARs because the concentration of KA (1μM) used in our experiments does not activate AMPA receptors (Casassus & Mulle, 2002). The fact that 1 μM KA inhibited EPSCs mediated by NMDA receptors to the same degree as those mediated by AMPARs, and that this effect was blocked by AMPA/KA antagonist provide strong evidence that this inhibitory effect on glutamatergic transmission is modulated through KAR activation.

We next sought to determine the action site of KA on glutamatergic synaptic transmission. It has been reported in several brain regions that the inhibition of synaptic transmission by KA is modulated, at least in part, by activation of presynaptic KARs (Contractor et al., 2000; Kamiya & Ozawa 1998, 2000; Schmitz et al., 2000; Frerking et al., 2001; Crowder and Weiner, 2001). Our result, indeed, showed that KA inhibition of EPSCs was associated with a significant increase in PPF ratio, a physiological effect that suggests a presynaptic decrease in the probability of neurotransmitter release (Manabe et al., 1993). In line with this result, our electron microscopic immunocytochemical data have demonstrated that GluR6/7 immunoreactivity is expressed in a subset of putative glutamatergic axon terminals in the GP. Furthermore, we demonstrated that 1 μM KA did not significantly change the input resistance of GP neurons, which provide further support that the reduction of EPSCs induced by KA is modulated by a presynaptic mechanism. However, 1 μM KA could induce dendritic conductance changes that are unlikely to be monitored by somatic recordings. Therefore, we cannot rule out that such dendritic effects may account for some of the KA-induced inhibitory effect on EPSCs.

In addition to the inhibitory effect of KA on glutamatergic transmission in CA3 to CA1, mossy fibre-CA3 synapses and striatum (Contractor et al., 2000; Kamiya & Ozawa, 1998, 2000; Schmitz et al., 2000; Frerking et al., 2001; Crowder and Weiner, 2001), KAR activation can also modulate glutamate release

in a bidirectional manner at hippocampal mossy fiber synapses (Schmitz et al., 2001) and parallel fiber synapses in the cerebellar cortex (Delaney & Jahr, 2002). For instance, under the conditions of AMPA and $GABA_A$ receptors blockade with their antagonists, a short train (3 pulses, 200 Hz) of stimulation to A/C fibers facilitates NMDAR-mediated EPSCs, while a long train (10 pulses, 200 Hz) depresses it (Schmitz et al., 2001). Consistent with these data, bath application of low doses of KA (<300 nM) results in facilitation of glutamate release, whereas high doses of KA (500-5000 nM) induce synaptic depression (Schmitz et al; 2001). Several hypotheses have been proposed to explain these dual effects of KAR activation on glutamatergic transmission. Both presynaptic ionotropic and metabotropic actions of KARs on glutamatergic transmission have been demonstrated in the hippocampus. In the present study, EPSCs evoked by single stimuli of the internal capsule were always reduced by bath application of KA (300-1000 nM). We did not find any effect of KA on EPSC amplitude when very low doses (25-100 nM) of KA was applied suggesting KAR activation does not modulate glutamate release in a bidirectional manner in the rat GP.

It is known that the STN is, by far, the main source of glutamatergic inputs to the GP. We, therefore, believe that EPSCs evoked in GP neurons are generated by STN projections. However, because a small number of brainstem and thalamic glutamatergic fibers also innervate the GP (Kincaid et al., 1991; Naito & Kita, 1994; Mouroux et al., 1997), we cannot rule out the possibility that KARs may modulate synaptic transmission at these synapses. It is possible that the sensitivity of KARs to glutamate at these terminals may be different from that in STN boutons.

Mechanism for KARs-modulated presynaptic effect

Several models have been proposed to describe the possible mechanism (s) underlying the pre-synaptic modulation of synaptic transmission by KA (for reviews see Frerking & Nicoll, 2000; Huettner, 2003; Lerma, 2003). Various studies have indicated that a low concentration of KA could directly depolarize presynaptic terminals which, in turn, facilitate glutamate release (Kamiya & Ozawa, 2000; Schmitz et

al., 2000; 2001b); while others have suggested that the presynaptic effects of KA could be mediated by the indirect activation of other receptor systems (Frerking et al., 1999; Chergui et al., 2000; Nakamura et al., 2003). For instance, KA could act at somatodendritic KARs on local neurons to cause the release of neurotransmitters, such as adenosine or GABA which, in turn, could, act heterosynaptically on neighboring boutons to modulate release of neurotransmitter. In fact, activation of adenosine and $GABA_B$ receptors have been shown to modulate the presynaptic effect of KA at inhibitory synapses in the hippocampus, striatum and substantia nigra pars compacta (Frerking et al., 1999; Chergui et al., 2000; Nakamura et al., 2003). Finally, recent studies also indicated that KAR activation could modulate GABAergic and glutamatergic transmission in the hippocampus via a direct, presynaptic metabotropic action (Rodriguez-Moreno & Lerma, 1998; Rodriguez-Moreno et al., 2000; Frerking et al., 2001). In these studies, the inhibitory effects of pre-synaptic KAR activation on IPSCs and EPSCs were abolished by G-protein inhibitors *N*-ethylmaleimide and pertussis toxin (Frerking et al., 2001; Rodriguez-Moreno & Lerma, 1998), but not by KA-mediated depolarization (Rodriguez-Moreno & Lerma, 1998) or activation of other receptor systems (Frerking et al., 2001; Crowder & Weiner, 2002). Furthermore, PKC activation downstream of G-protein activity is essential for the actions of KARs at GABAergic synapses (Rodriguez-Moreno & Lerma, 1998; Rodriguez-Moreno et al., 2000). Interestingly, more recent studies also demonstrated that KAR-induced postsynaptic response also involve G-protein and PKC activation (Melyan et al., 2002; Melyan et al., 2004; Fisahn et al 2005). In the present study, we demonstrate that KAR-induced depression of glutamatergic synaptic transmission in the globus pallidus requires G-protein and PKC activation but does not rely on the secondary activation of GABAB, mGluRs, adenosine and dopamine receptors. Therefore, our results demonstrate a metabotropic regulation of glutamatergic transmission through activation of KARs in the rat GP.

Functional implications

The present study provides direct evidence that KARs activation contributes a small component to glutamatergic excitatory postsynaptic effects in GP neurons. The KAR-mediated presynaptic inhibition of evoked EPSC also suggests that KARs can function as autoreceptors to modulate glutamatergic transmission at the subthalamo-pallidal synapses. Knowing that increased activity of STN neurons is one of the cardinal features of Parkinson's disease pathophysiology (Delong, 1990; Wichmann & Vitek, 2003), one may hypothesize that KAR activation could have beneficial effects in Parkinson's disease by reduction of the overactive outflow from the STN. In fact, glutamate antagonists that target AMPA, NMDA and metabotropic glutamate receptors have proven to be successful in alleviating parkinsonian symptoms in both rat and monkey models of PD (Greenamyre et al., 1994; Klockgether et al., 1991; Brotchie et al., 1991; Breysse et al., 2002). However, these drugs suffer of important short-comings including potential long-term side effects due to the widespread distribution and the need of AMPA and NMDA receptor activation for normal brain functioning (Starr, 1995). One the other hand, although mGluR5 antagonist is very potent in reducing abnormal behavior in rat model of PD (Ossowska et al., 2001), its effect is very short-lasting, which limits considerably its useful therapeutic purpose in humans. Therefore, KARs provide additional targets whereby glutamate-related drugs might be used as potential therapeutic agents for PD and other movement disorders.

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Abbreviations

ABC, avidin-biotin-peroxidase complex; ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole propionic acid; BIC, bicuculline; CNQX, 6-cynao-7-nitroquinoxaline-2,

3-dione; DAB, 3,3-diaminobenzidine tetrahydrochloride; D-AP5, D-(-)-2-Amino-5-phosphonopentanoic acid; EPSCs, excitatory post synaptic currents; GP, globus pallidus; GYKI 52466, 4-(8-Methyl-9H-1,3-dioxolo[4,5 h]{2,3}benzodiazepine-5-yl)-benzenamine hydrochloride; KARs, kainate receptors; NGS, normal goat serum; NMDA, N-methyl-D-aspartate; PD, Parkinson's disease; PBS, phosphate-buffered saline; PPF, paired-pulse facilitation; QX314, N-(2,6-Dimethylphenylcarbamoylmethyl) triethylammonium bromide; STN, subthalamic nucleus; TTX, tetrodotoxin.

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Figure legends

FIG. 1. Pre- and post synaptic expression of GluR6/7 immunoreactivity in adult and young (P14-17) rat globus pallidus. (A) GluR6/7-containing dendrite (*Den*) contacted by unlabeled terminals. A labeled axon (*Ax*) is also indicated. (B, C) GluR6/7-labeled terminals (*Te*) forming symmetric (B) or asymmetric axon-dendritic synapse (C). (D) A GluR6/7-labeled dendrite (*Den*) contacted by a lightly immunoreactive axon terminal (*Te*) forming an asymmetric synapse. (E) A GluR6/7-labeled unmyelinated axon (Ax). A, B, C are taken from adult; D, E are from young rats. (F). Histogram showing the percent of GluR6/7-immunoreactive elements in the GP. A total of 184 labeled elements were analyzed in the young GP, whereas 214 were examined in adult GP. Chi-Square analysis revealed no significant difference in the relative abundance of GluR6/7-containing elements in adult and young rat GP (χ^2; $P = 0.63$). Scale bars: 0.5 μm.

FIG. 2. Functional kainate receptors (KARs) are expressed on globus pallidus (GP) neurons. (A) Records of membrane potential of a type II GP neuron (left trace) filled with biocytin after recording (right micrograph). (B) KA-induced currents were recorded in the presence of GYKI 52466. (C) Dose-response curve shows the average amplitude (pA ± SEM) of KA-evoked currents in the presence of GYKI 52466. (D) KA-evoked currents recorded in absence of AMPA antagonist (left trace), in the presence of GYKI 52466 (middle trace) or CNQX (right trace). (E) Bar graph summarizing the average amplitude of KA-evoked currents in presence of GYKI 52466, CNQX and NBQX. Significant difference between KA+ GYKI 52466 and KA + CNQX: * P <0.001. NS, Non-significant difference between KA and KA+GYKI 52466 or KA+NBQX: P = 0.08 and 0.09, respectively. (F) KA-induced depolarization (mV ± SEM) in the presence of GYKI 52466, CNQX and NBQX. All recordings (B, C, D and F) were done in the presence of 50 μM D-AP5, 50 μM bicuculline (BIC) and 1 μM tetrodotoxin (TTX). The "n" indicates the number of cells tested under each condition.

FIG. 3. Stimulation of the internal capsule induces GYKI 52466-resistant, CNQX-sensitive EPSCs in GP neurons. (A) EPSCs recorded in control condition, or in the presence of GYKI 52466 and CNQX. (B) The GYKI 52466-resistant component was scaled to the peak of the AMPA-mediated component. (C) EPSC amplitude versus time for the traces shown in A. (D) Bar graph shows the average amplitude (% of control ± SEM) of EPSC recorded in control condition or in the presence of GYKI 52466 and CNQX. (E) Time constant (decay) and rise time (10-90%) for EPSC in control condition or in the presence of GYKI 52466. Significant difference of rise time or decay between control and GYKI 52466: * $P < 0.001$ and ** $P < 0.01$, respectively. (F) EPSCs recorded in control condition or in presence of GYKI 52466, GYKI 52466 plus SYM 2081 and partial recovery in GYKI 52466. (G) EPSCs in control condition or in presence of SYM 2081. (H) GYKI 52466-insensitive EPSCs were not affected by Cyclothiazide. (I) Bar graph shows the average amplitude of EPSC (% of control ± SEM) in the presence of GYKI 52466, GYKI 52466 together with SYM 2081, SYM 2081, and GYKI 52466 plus Cyclothiazide. All recordings were done in the presence of 50 μM D-AP5 and 50 μM bicuculline. Significant difference of EPSC (% of control) between GYKI 52466 and GYKI 52466+SYM 2081: * $P < 0.001$, NS, non-significant difference between GYKI 52466 and GYKI 52466+Cyclothiazide, and between control and SYM 2081: $P = 0.43$ and $P = 0.6$, respectively.

FIG. 4. KAR activation depresses glutamatergic synaptic transmission in GP. (A) Time course of KAR-induced reduction of non-NMDA mediated EPSC amplitude (pA) in presence of 50 μM bicuculline and 50 μM D-AP5. Three EPSCs are averaged in each trace at the time indicated by corresponding letters in the graph. (B) Summary bar graph showing the effect of various concentrations of KA on the amplitude of non-NMDA EPSC as percent of control ± SEM. (C) Time course of KAR-induced inhibition of NMDA-mediated EPSC in presence of KA and KA plus CNQX. Three EPSCs are averaged in each trace at the time indicated by corresponding letters in the graph. (D) Bar graph shows

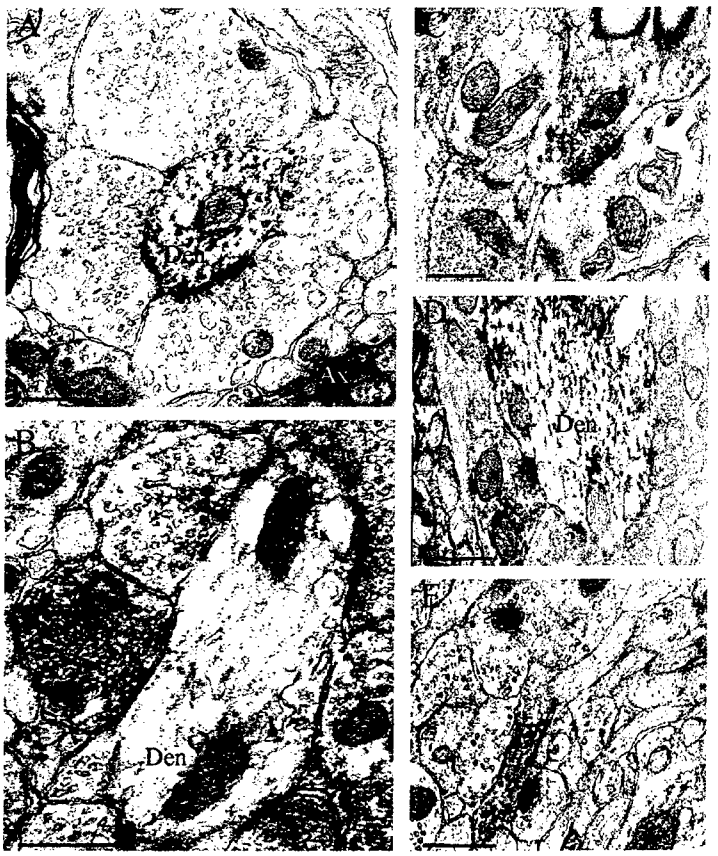
the effect of KA on NMDA-EPSC in each condition. Recordings were done in presence of 100 μM GYKI 52466 and 50μM bicuculline (BIC). Significant difference from control: * $P < 0.001$. NS, not significant.

FIG. 5. KAR activation increases paired-pulse facilitation at glutamatergic synapses in the GP. (A) Paired non-NMDA EPSCs were recorded in control or presence of KA (top traces) and after scaling to the peak of the first EPSC (bottom traces). Time course showing the increase in paired-pulse facilitation ratio (PPFR) of EPSCs in response to KA application (right graph). (B) Bar graph summarizing the PPFR expressed as a mean ratio of P2/P1 ± SEM, in the absence or presence of KA. (C) The effect of KA on both the holding current during a – 10 mV step (top trace) and on the evoked EPSC (bottom trace). (D) Bar graph shows the mean input resistance and EPSC amplitude expressed as % of control ± SEM in the presence of KA. NS, Non-significant difference from control, $P = 0.4$, * significant difference from control, $P < 0.001$.

FIG. 6. Kainate receptor induced inhibition of EPSC involves a NEM-sensitive G protein. (A) The time course of effect of KA (1 μM) on non-NMDA mediated EPSC amplitude (pA) in presence of NEM (200 μM). Three EPSCs are averaged in each trace at the time indicated by corresponding letters in the graph. (B) Summary bar graph showing the effect of KA on the amplitude of non-NMDA EPSC as percent of control ± SEM. (C) Bar graph summarizing the effect of 1 μM KA on non-NMDA EPSC, expressed as percent of control ± SEM, in the presence of mGluR antagonists (1mM MCPG and 100 μM CPPG), the GABAB antagonist (20μM SCH 50911), the adenosine receptor antagonist (10μM CGS 15943), or dopamine receptor antagonists (10μM SCH 23390 and 10 μM sulpiride). NS, not significant.

FIG. 7. Kainate receptor-induced inhibition of EPSC involves activation of protein kinase C, but not protein kinase A. (A) The time course of effect of KA (1 μM) on non-NMDA mediated EPSC amplitude

(pA) in presence of Calphostin (1 μM). Three EPSCs are averaged in each trace at the time indicated by corresponding letters in the graph. (B) EPSCs were recorded in presence of the PKA antagonist H-89 (0.5 μM) or H-89 together with KA (1 μM). (C) Bar graph summarizing the effect of KA on non-NMDA EPSC, expressed as percent of control ± SEM, in the presence of Staurosporine (0.5 μM), Calphostin C and H-89. For comparison, the effect of 1 μM KA in control conditions from Figure 4B is shown. * significant difference from control, $P < 0.001$. NS, NS, not significant.


A
Den
Ax
B
Den
Ax
C
Den
D
E
F
Young (n=2)
Adult (n=2)
% of Immunoreactive Elements
70
60
50
40
30
20
10
0
Den
Axons
Glia
Symmetric
Asymmetric

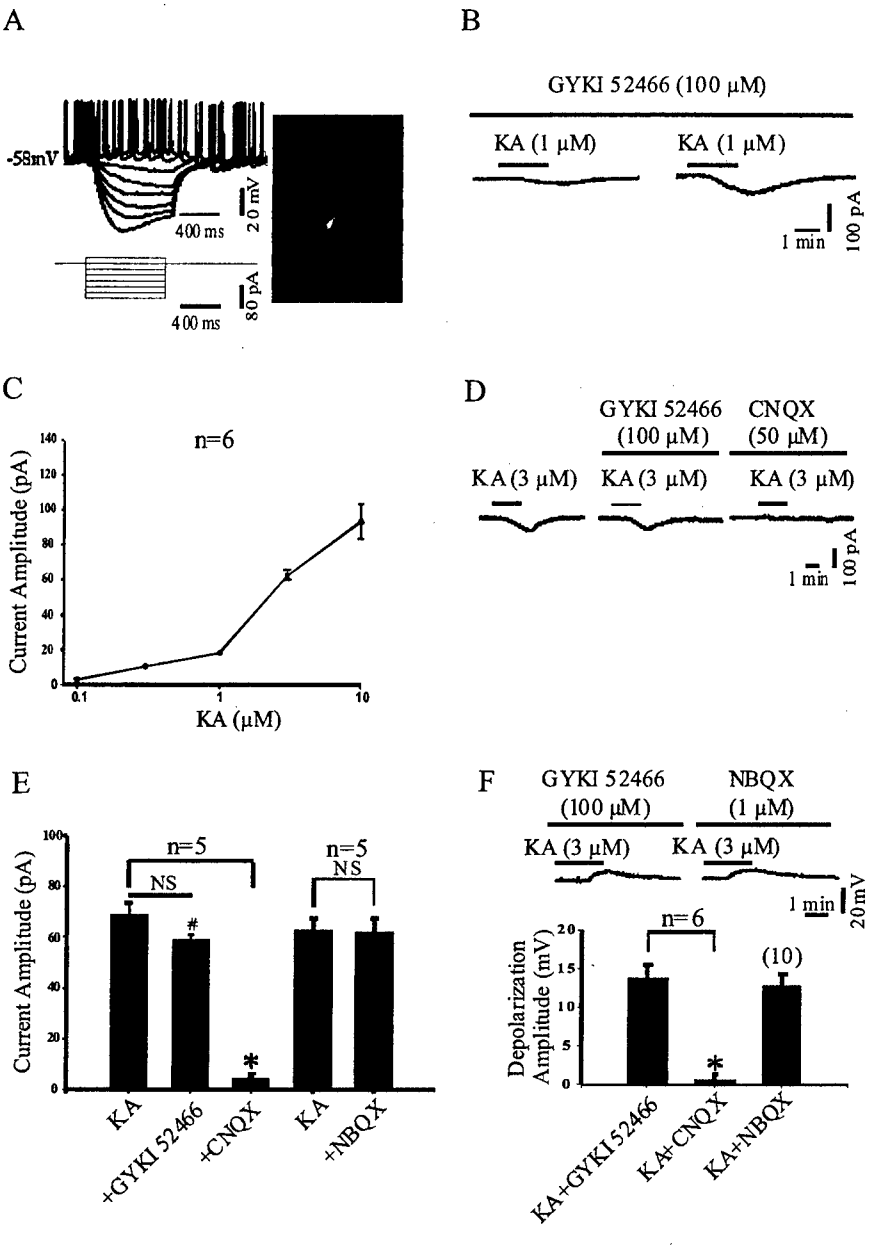

A
-58mV
400 ms
20 mV
80 pA
400 ms
B
GYKI 52466 (100 μM)
KA (1 μM)
KA (1 μM)
1 min
100 pA
C
n=6
Current Amplitude (pA)
KA (μM)
D
GYKI 52466 (100 μM)
CNQX (50 μM)
KA (3 μM)
KA (3 μM)
KA (3 μM)
1 min
100 pA
E
n=5
NS
n=5
NS
Current Amplitude (pA)
KA
+GYKI 52466
+CNQX
KA
+NBQX
F
GYKI 52466 (100 μM)
NBQX (1 μM)
KA (3 μM)
KA (3 μM)
1 min
20mV
n=6
(10)
Depolarization Amplitude (mV)
KA+GYKI 52466
KA+CNQX
KA+NBQX

Figure 2

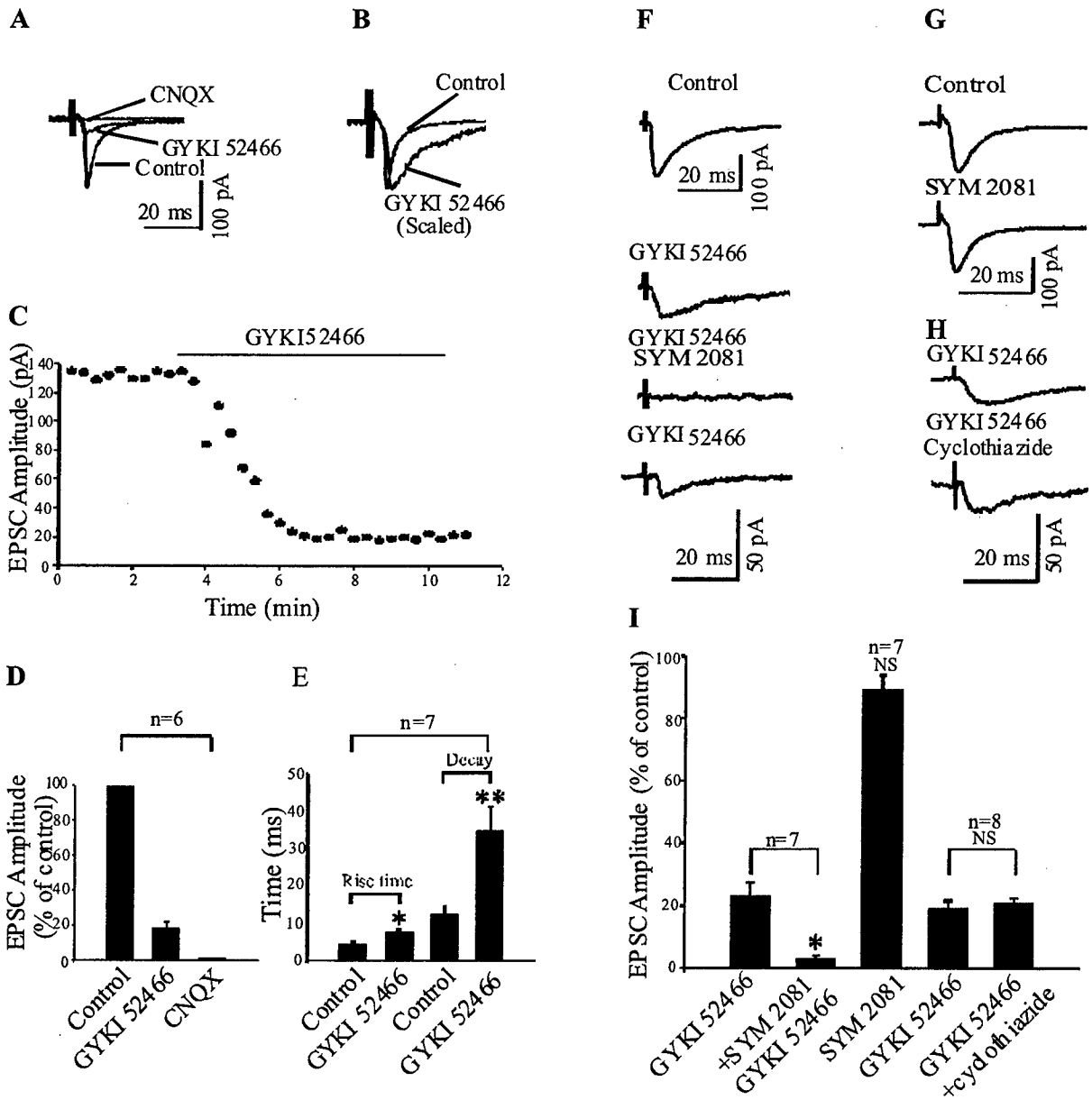

A
CNQX
GYKI 52466
Control
20 ms
100 pA
B
Control
GYKI 52466
(Scaled)
C
GYKI52466
EPSC Amplitude (pA)
Time (min)
D
n=6
EPSC Amplitude (% of control)
Control
GYKI 52466
CNQX
E
n=7
Decay
Rise time
Time (ms)
Control
GYKI 52466
Control
GYKI 52466
F
Control
20 ms
100 pA
GYKI 52466
GYKI 52466
SYM 2081
GYKI 52466
20 ms
50 pA
G
Control
SYM 2081
20 ms
100 pA
H
GYKI 52466
GYKI 52466
Cyclothiazide
20 ms
50 pA
I
EPSC Amplitude (% of control)
n=7
NS
n=7
n=8
NS
GYKI 52466
+SYM 2081
GYKI 52466
SYM 2081
GYKI 52466
GYKI 52466
+cyclothiazide

Figure 3

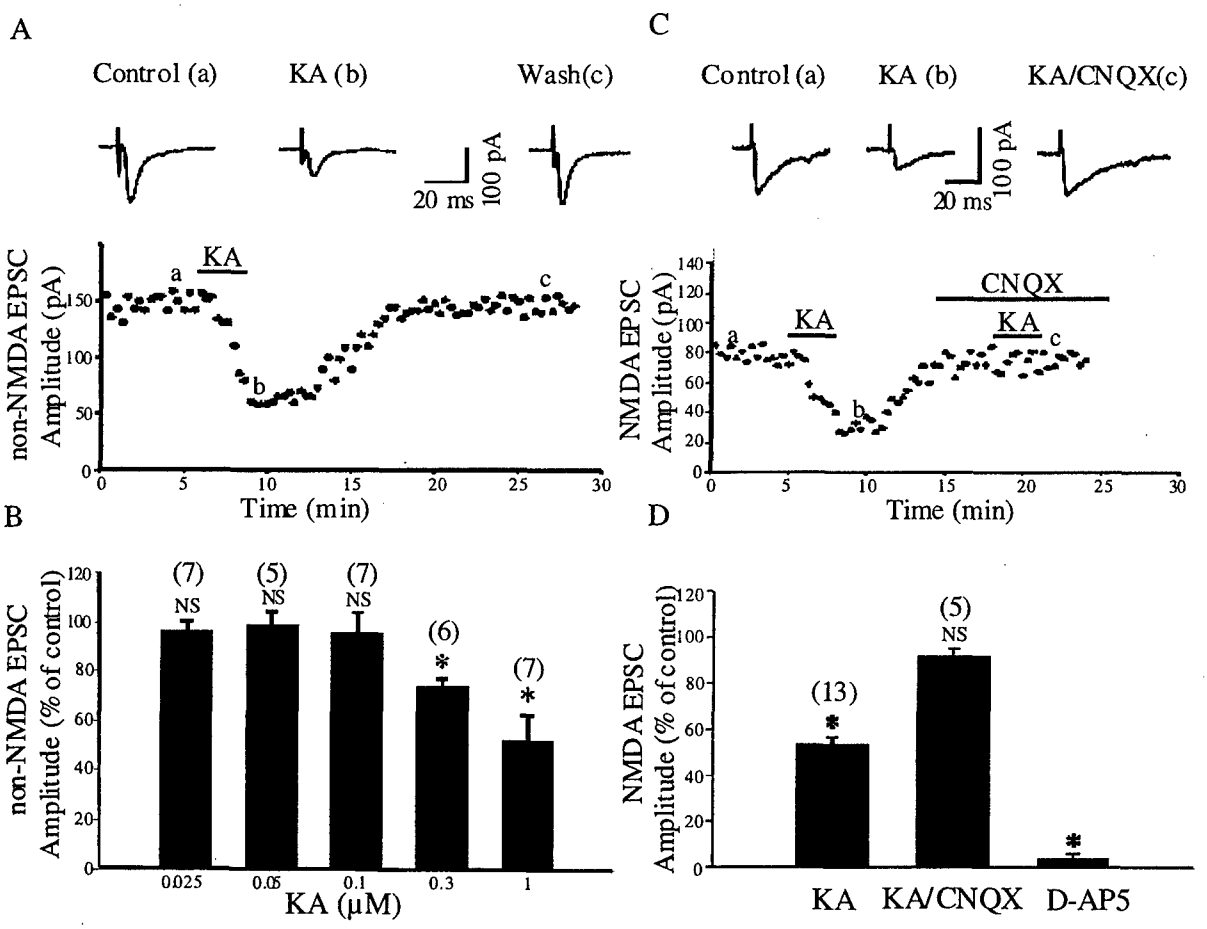

A
Control (a)
KA (b)
Wash(c)
20 ms
100 pA
non-NMDA EPSC Amplitude (pA)
KA
a
b
c
Time (min)
C
Control (a)
KA (b)
KA/CNQX(c)
20 ms
100 pA
NMDA EPSC Amplitude (pA)
CNQX
KA
KA
a
b
c
Time (min)
B
non-NMDA EPSC Amplitude (% of control)
(7)
NS
(5)
NS
(7)
NS
(6)
*
(7)
*
0.025
0.05
0.1
0.3
1
KA (μM)
D
NMDA EPSC Amplitude (% of control)
(13)
*
(5)
NS
*
KA
KA/CNQX
D-AP5

Figure 4

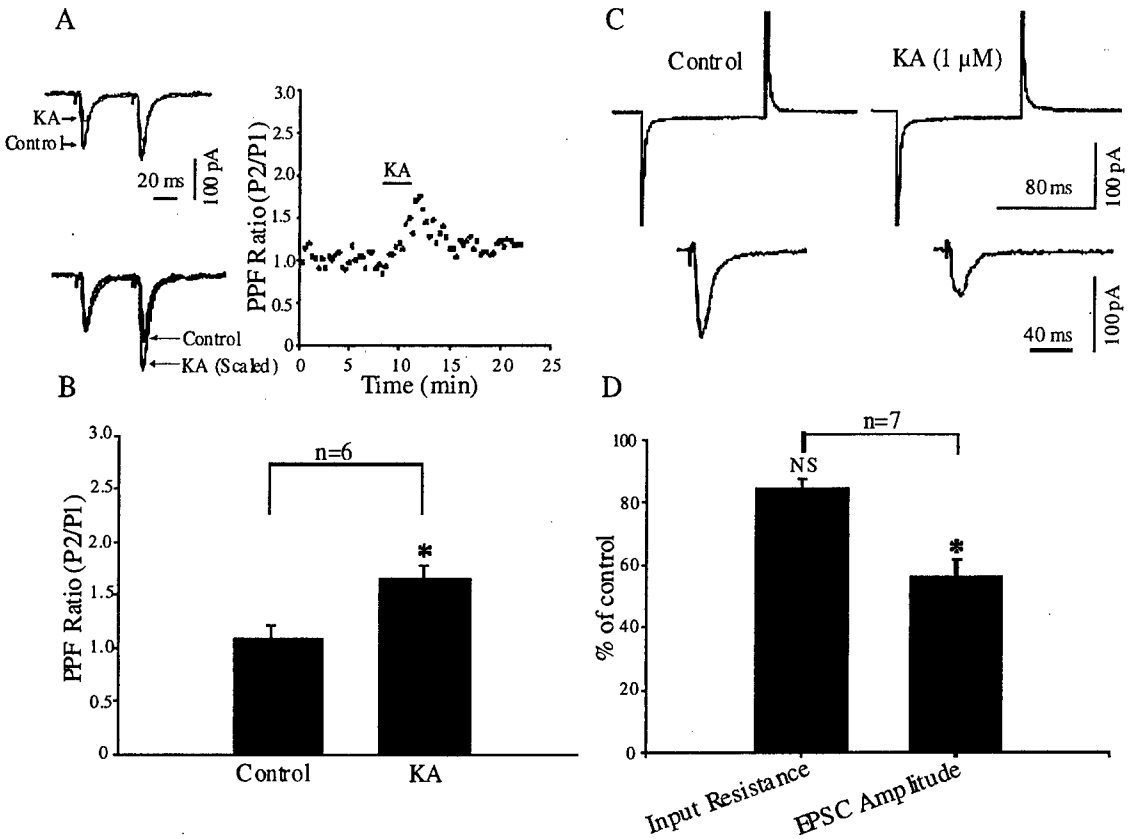

A
KA
Control
20 ms
100 pA
PPF Ratio (P2/P1)
KA
Control
KA (Scaled)
Time (min)
B
n=6
PPF Ratio (P2/P1)
Control
KA
C
Control
KA (1 μM)
80 ms
100 pA
40 ms
100pA
D
n=7
NS
% of control
Input Resistance
EPSC Amplitude

Figure 5

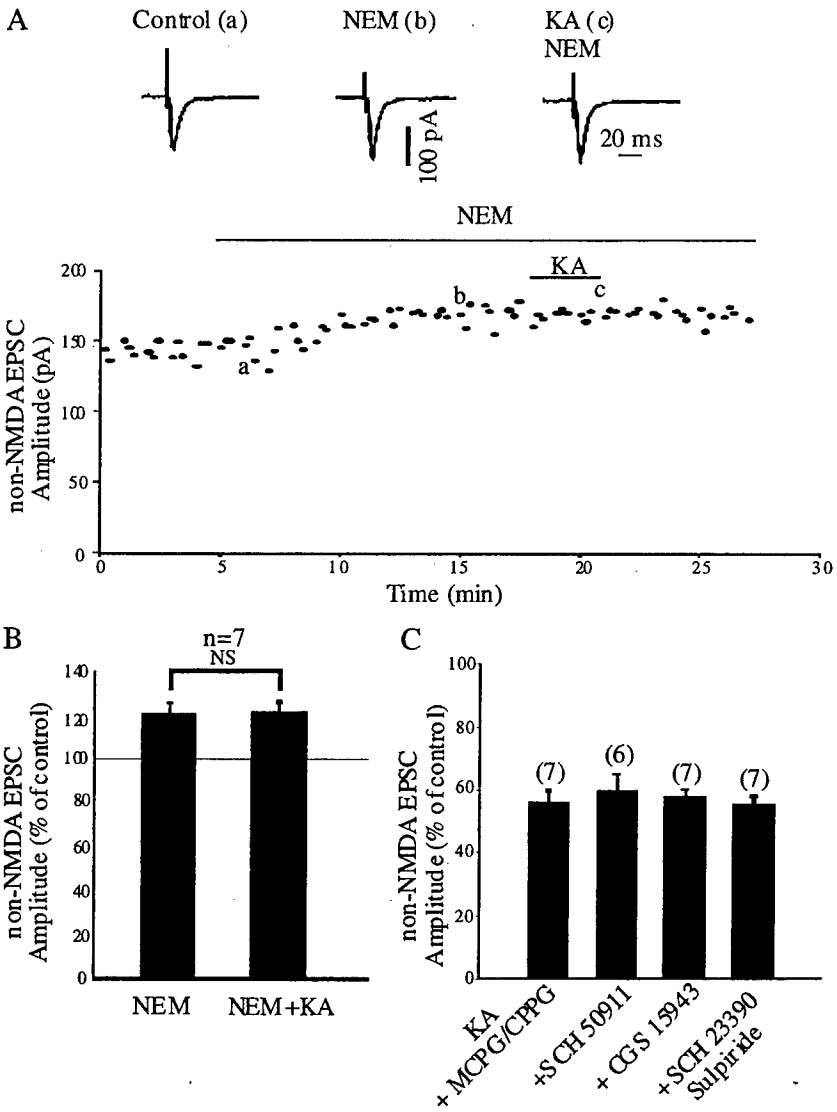

A
Control (a)
NEM (b)
KA (c)
NEM
100 pA
20 ms
NEM
KA
a
b
c
non-NMDA EPSC Amplitude (pA)
Time (min)
B
n=7
NS
non-NMDA EPSC Amplitude (% of control)
NEM
NEM+KA
C
non-NMDA EPSC Amplitude (% of control)
(7)
(6)
(7)
(7)
KA
+MCPG/CPPG
+SCH 50911
+CGS 15943
+SCH 23390
Sulpiride

Figure 6

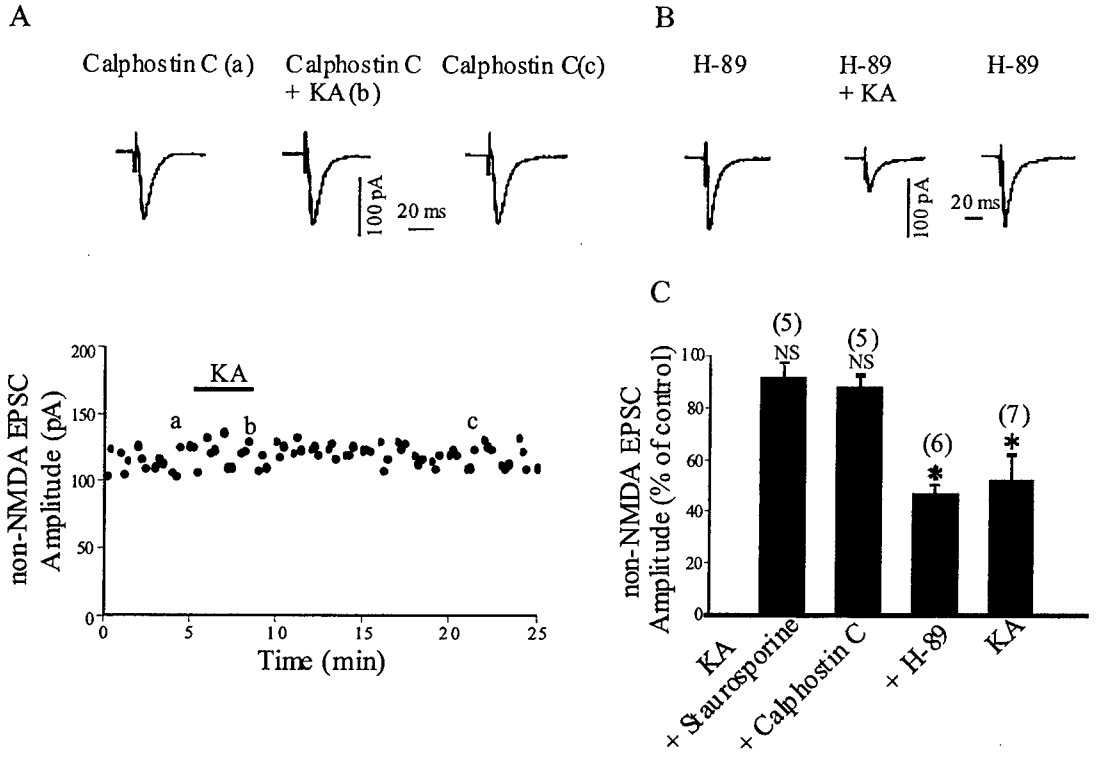

A
Calphostin C (a)
Calphostin C + KA (b)
Calphostin C(c)
100 pA
20 ms
KA
a
b
c
non-NMDA EPSC Amplitude (pA)
Time (min)
B
H-89
H-89 + KA
H-89
100 pA
20 ms
C
non-NMDA EPSC Amplitude (% of control)
(5) NS
(5) NS
(6) *
(7) *
KA
+ Staurosporine
+ Calphostin C
+ H-89
KA

Figure 7