A Novel Calmodulin-binding Protein, Belonging to the WD-Repeat Family, Is Localized in Dendrites of a Subset of CNS Neurons

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Abstract. A rat brain synaptosomal protein of 110,000 M_r present in a fraction highly enriched in adenylyl cyclase activity was microsequenced (Castets, F., G. Baillat, S. Mirzoeva, K. Mabrouk, J. Garin, J. d'Alayer, and A. Monneron. 1994. *Biochemistry.* 33:5063–5069). Peptide sequences were used to clone a cDNA encoding a novel, 780–amino acid protein named striatin. Striatin is a member of the WD-repeat family (Neer, E.J., C.J. Schmidt, R. Nambudripad, and T.F. Smith. 1994. *Nature (Lond.).* 371:297–300), the first one known to bind calmodulin (CaM) in the presence of Ca^{++}. Subcellular fractionation shows that striatin is a membrane-associated, Lubrol-soluble protein. As analyzed by Northern blots, in situ hybridization, and immunocytochemistry, striatin is localized in the central nervous system, where it is confined to a subset of neurons, many of which are associated with the motor system. In particular, striatin is conspicuous in the dorsal part of the striatum, as well as in motoneurons. Furthermore, striatin is essentially found in dendrites, but not in axons, and is most abundant in dendritic spines. We propose that striatin interacts, through its WD-repeat domain and in a CaM/Ca^{++}-dependent manner, with one or several members of a surrounding cluster of molecules engaged in a Ca^{++}-signaling pathway specific to excitatory synapses.

SYNAPSES are exquisitely organized subcellular structures (5). In the presynaptic compartment, the storage of neurotransmitters in vesicles, their release upon Ca^{++} influx, and their reuptake involve a host of proteins that include ion channels, enzymes, cytoskeletal elements, regulatory proteins, and transducers (44). In type I synapses, the postsynaptic compartment is characterized by the presence of a postsynaptic density (PSD),[1] a dense, filamentous structure linked to the inner face of the postsynaptic membrane (for review see 20).

We have shown that mammalian brain synapses contain an adenylyl cyclase of unusually high specific activity (31). This has led us to speculate that either synapses contain an as yet unknown type of adenylyl cyclase, or that they are endowed with novel types of transducers or supramolecular complexes particularly efficient in the channeling of stimulatory signals to adenylyl cyclase. To test these possibilities, we have characterized some of the proteins present in synaptosomal preparations enriched in adenylyl cyclase. Among them, a protein of 110,000 M_r yielded, by proteolytic digestion, peptides of unknown sequences. This study reports the cloning of the corresponding cDNA that encodes a novel protein, striatin, shown to directly bind calmodulin in a Ca^{++}-dependent manner. A member of the WD-repeat family of proteins (29), striatin is the first member of this family known to bind calmodulin. Subcellular fractionation studies indicate that striatin is a membrane-associated, Lubrol-soluble protein.

Striatin is shown here to be exclusively expressed in the central nervous system (CNS), principally in the neurons of the motor system: layer V of the sensory motor cortex, brain motor nuclei, especially the dorsal part of the caudate nucleus, and motoneurons, but also in the olfactory tubercle as well as, to a lesser extent, in the hippocampus. This striking distribution goes together with a mostly dendritic subcellular localization, especially dendritic spines, at the exclusion of axons. Striatin is therefore likely to engage in multiple protein interactions in a Ca^{++}-signaling pathway specific to postsynaptic compartments of a defined subset of CNS neurons.

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1. *Abbreviations used in this paper*: CaM, calmodulin; CNS, central nervous system; ORF, open reading frame; PSD, postsynaptic density; UTR, untranslated sequence.

Materials and Methods

Obtaining of Peptides and Microsequencing

Pooled fractions enriched in rat CNS synaptosomal adenylyl cyclase (eluates from forskolin and calmodulin-affinity chiomatographics, referred to as FC fractions in reference 6) were subjected to PAGE (7.5% SDS–polyacrylamide preparative gel). A piece of gel containing 110 (+/− 5)-kD proteins was cut out and digested with 2 μg/ml of endoproteinase Lys-C (Boehringer Mannheim GmBH, Mannheim, Germany) for 18 h at 30°C. The digested material was injected onto a DEAE column linked to a C 18 reversed-phase HPLC column. Eluted peptides were sequenced on a gas-phase sequencer (470; Applied Biosystems, Foster City, CA). All 10 peptides sequenced (KxELTDIAIVLDNFK, KxVIDTSTIVRK, KxWNPK, KMWNLQK, KxxIAEAGAxALAK, KDEPGAWEETF, KTLFGK, KPAAVVLQTK, KNLFSYQEPK, and KVSLTLGMQxK) were not found in data banks.

Amplification of DNA Using Reverse Transcription–PCR

Total rat brain RNA was prepared by the urea-LiCl method (3). mRNAs were purified on oligo(dT)-cellulose spin columns (Clontech, Palo Alto, CA). Rat brain cDNAs were obtained by reverse transcription, using avian myeloblastosis virus reverse transcriptase and random hexanucleotides according to the manufacturer's protocol (Amersham Intl., Little Chalfonet, England). Using the cDNAs as templates, PCR was achieved with degenerate oligonucleotide primers derived from the sequences of five of the peptides obtained from the protein of 110,000 M_r, in the presence of Taq polymerase (Eurobio, Les Ulis, France). A 0.8-kbp DNA fragment was reproducively obtained using the sense primer corresponding to the sequence IVLDNFK: 5′-ACGGATCCAT(A,C,T)GT (A,C,G,T)(CT)T (A,C,G,T)GA(C,T)AA(C,T)TT(C,T)AA-3′, and the antisense primer corresponding to peptide KMWNLQ: 5′-GAGTC-GACTG(A,C,G,T) A(A,G)(A,G)TTCCACAT(T,C)TT-3′. Cycles were as follows: 3 min, 94°C, 2 cycles with 1 min, 58°C, 2 min, 72°C, 1 min, 94°C; 2 cycles with 1 min, 53°C, 2 min, 72°C, 1 min, 94°C; 40 cycles with 1 min, 48°C, 2 min, 72°C, 1 min, 94°C in a thermocycler (Bio Rad Laboratories, Irvine, CA). The 0.8-kb amplified DNA fragment was cloned in plasmid pUC18, yielding pUC18 FC used to generate a probe with which to screen a cDNA library.

Cloning of the cDNA Encoding Striatin

Among three rat brain cDNA libraries analyzed by PCR screening using a cDNA fragment obtained with oligonucleotides internal to the 0.8-kbp insert (5′-CAGTGATGAAGACGAGGATG-3′ and 5′-CCCTACTGAT-GTCCTGCTC-3′), one, constructed in λ ZAP II (randomly primed; a gift of Dr. Marius Sudol, Rockefeller University, New York), was positive. *Escherichia coli* Y 1090 were infected with this library, and filter replicas (2×10^6 phage plaques) were hybridized using the 0.8-kbp insert from pUC18 FC, radiolabeled using a nick-translation kit and [α^{32}P]dCTP (Amersham Intl.). Replicas (Hybond-N+ membranes; Amersham Intl.) were hybridized at 42°C overnight in 5× SSC, 1× Denhardt's, 50% formamide, 20 μg/ml calf thymus DNA, and 0.3% SDS. Filters were washed with 0.1× SSC, 0.1% SDS at 50°C. 14 plaque-purified phage were isolated with three rounds of screening (40). In vivo excision of the plasmids, called pFC, was carried out using helper phage M13 K07. The plasmid constructs were ordered relative to each other by PCR and restriction mapping; the inserts were sequenced and analyzed as described.

DNA Sequencing

Recombinant plasmids were purified by alkaline lysis (4) and sequenced. The inserts were sequenced on both strands by the dideoxy method of Sanger, according to Garoff and Ansorge (14), using T7 DNA polymerase (Pharmacia, Uppsala, Sweden) or Sequenase (United States Biochemical Corp., Cleveland, OH) and [α^{35}S]dATP. T3 and T7 primers (Pharmacia), as well as primers defined by the OLIGO program (37) for the walking procedure, were used. Sequences were analyzed with the UWGCG program on a Silicon Graphics station (Mountain View, CA). The BLAST algorithm (2) was used for homology search on the GenBank data base.

Construction of Plasmids

Construction of the Plasmid Encoding the Full-Length Striatin Sequence. The 0.7-kbp KpnI–HpaI fragment of pFC5, which contains the 5′ part of the coding sequence, was inserted into pFC9 previously deleted from its KpnI–HpaI fragment, yielding pFCC encoding striatin (Fig. 1).

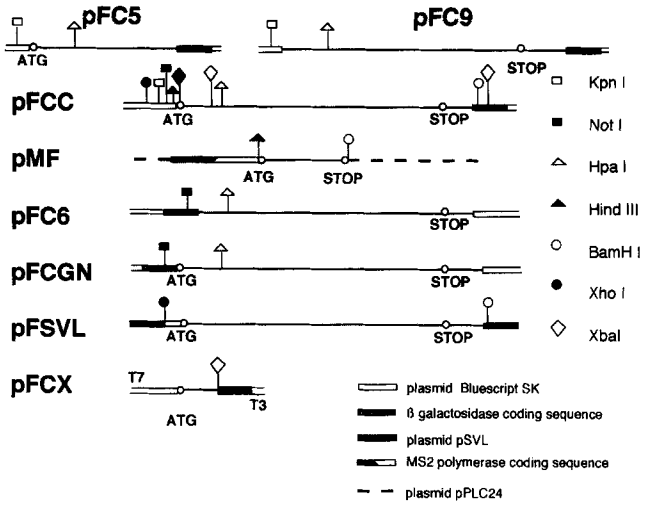

Figure 1. Cloning of striatin cDNA and constructions of expression vectors. (*Solid lines*) Striatin cDNA sequence. (*Boxes*) Flanking plasmid sequences. pFC 5, 6, and 9 are three of the fourteen isolated pBluescript clones containing striatin inserts. *pFCC* is a full-length clone constructed from *pFC5* and *pFC9*, and *pFCGN* is constructed from *pFCC* and *pFC6*. *pFSVL* and *pFDNA* result from the insertion into pSVL and pcDNA 3 of the appropriate *pFCC* fragments. *pFCX* was obtained by digestion of pFCC.

Construction of Prokaryotic Expression Plasmids The pMF plasmid (Fig. 1) was constructed by inserting the first 150 bp of striatin cDNA into the BamHI and HindIII linearized plasmid pPLc 24, in frame with the coding sequence of the NH_2-terminal portion (first 98 residues) of bacteriophage MS 2 polymerase (35). The 150-bp fragment was obtained by PCR using pFCC as template and the following oligonucleotides: 5′-ATGGATC-CCATGGACGAGCAGGCGGG-3′ and 5′-ATAAGCTTAATTAAT-TATGCAGGAAGTGCAAGATCCCC-3′. pMF was used to overproduce a fusion protein containing the first 50 amino acids of striatin, called fusion protein I (Fig. 2 *B*). The pFCGN plasmid was constructed by insertion of the NotI–HpaI fragment of pFCC into pFC 6 previously digested by the same enzymes, in frame with the sequence coding for the first 27 amino acids of the α fragment of β-galactosidase (Fig. 1). This plasmid was used to produce a hybrid protein containing the entire sequence of striatin, the so-called complete fusion protein. pFCX, used to generate a set of riboprobes (see below), was obtained by digesting pFCC with XbaI (Fig. 1).

Construction of Mammalian Expression Vectors. The pFSVL plasmid resulted from the insertion into XhoI–BamHI-digested pSVL (Pharmacia) of the XhoI–BamHI fragment from pFCC (Fig. 1).

DNA and RNA Blot Hybridization

Southern blots of rat genomic DNA digested by EcoRI, HindIII, BamHI, and PstI were carried out as described (40), using a probe obtained by PCR, spanning sequence 1,293-2,316 (which contains an EcoRI site at bp 1955), and labeled by random priming with [α^{32}P]dCTP. mRNA from various rat and mouse tissues was purified either from total RNA using a Clontech kit, or from the tissues or cells (L1210 lymphocyte cell line) using a messenger RNA isolation kit from Stratagene (La Jolla, CA). Approximately 5 μg of each mRNA were loaded on 1% agarose and 6% formaldehyde gels, electrophoresed, and transferred to Hybond membranes by capillary transfer. The blots were hybridized with the appropriate radioactive probes as described (40). The probes consisted of several fragments of striatin cDNA, which together spanned the entire coding sequence. They were prepared either by digestion of pFC 16 plasmid by HincII, yielding a fragment corresponding to bp 668–1,647, or by PCR, yielding DNA fragments corresponding to striatin sequences 1–720 (Fig. 2 *B*,

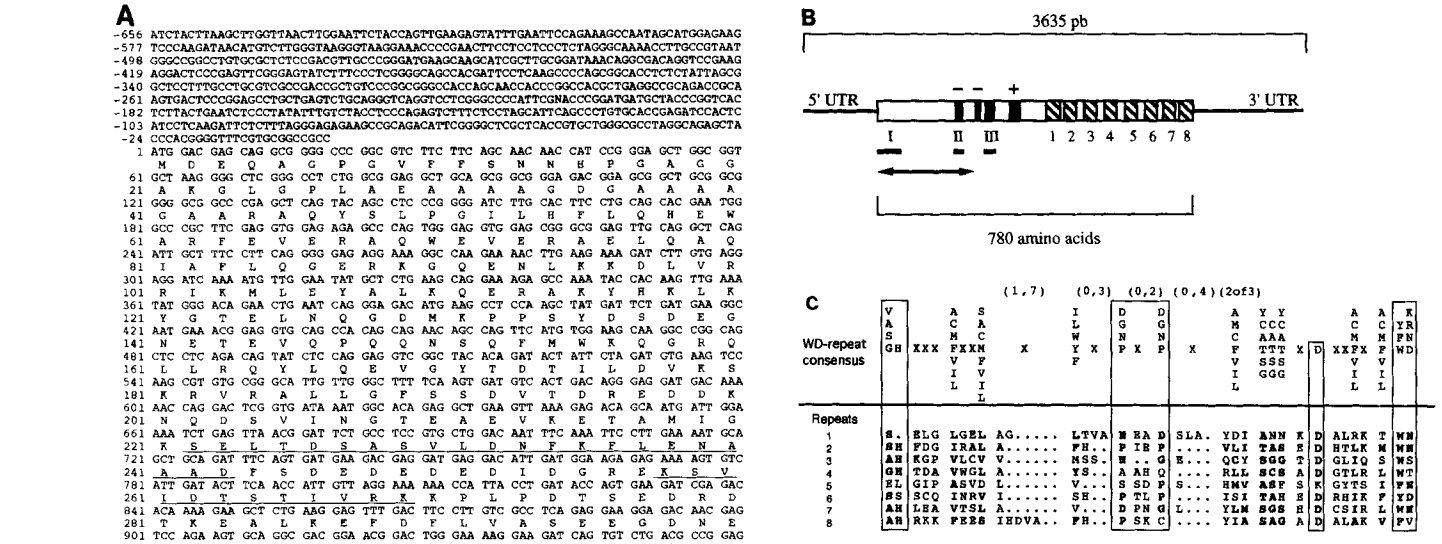

Figure 2. (*A*) Nucleotide and predicted amino acid sequences of the striatin cDNA. Nucleotides are positively numbered from the first base of the putative initiation codon, in the 5′ to 3′ direction; the nucleotides on the 5′ side of residue 1 are indicated by negative numbers. The amino acid sequence, in single-letter code, is shown below the nucleotide sequence. The sequences of the five peptides generated by digestion of the brain 110-kD protein are underlined. (*B*) Schematic representation of striatin ORF and its flanks. The single straight lines depict the 5′ and 3′ UTR regions of the cDNA; the protein coding sequence is shown as a box. The four dark rectangles indicate regions coding for highly charged peptides, two negatively charged (−), and one positively charged (+), and the diagonally striped bars correspond to the WD-repeats, numbered 1–8. Below the model, the horizontal bars numbered I, II, and III indicate the amino acid sequences 1–50, contained in fusion protein I, 191–205, corresponding to peptide II, and 267–287, corresponding to peptide III, respectively. Antibodies have been obtained against these peptides. (*Double-headed arrow*) Nucleotide sequence 1–750 corresponding to probe 1, one of the probes used for Northern blots. (*C*) Comparison of the amino acid sequences of the "WD-repeat" consensus defined by Neer et al. (29) (Fig. 2 *C*, *upper*) and of striatin (*lower*). These sequence data are available from EMBL/GenBank/DDBJ under accession number X99326.

double-headed arrow), 720–1,215, 1,293–2,316, and 1,970–2,685. The fragments were purified using a GeneClean kit (Bio 101, LaJolla, CA). 25 ng of each were radiolabeled (2×10^8 cpm/μg DNA) by random priming using a Stratagene kit in the presence of [α^{32}P]dCTP. An actin probe (M. Buckingham, Institut Pasteur, Paris, France) was radiolabeled in the same way. After the hybridization step, the blots were washed three times (20 min each) at 50°C in buffer L (0.1× SCC, 0.1% SDS). Autoradiograms (Fuji RX films and amplifying screens [Fuji Photo Film Co., Ltd., Tokyo, Japan]) were exposed at −80°C.

In Situ Hybridization

Four adult Wistar rats (200–250 g) were used. The animals were killed by decapitation, and the brains were quickly removed, frozen on powdered dry ice, and stored at −70°C. Frontal and sagittal sections of 10 μm were cut on a cryostat and thaw-mounted on subbed slides. In situ hybridization histochemistry was performed as described (38). Briefly, sections were fixed in formaldehyde, acetylated, dehydrated, and hybridized in a humid chamber at 50°C for 3.5 h with 3–5 ng of ^{35}S-radiolabeled RNA probes complementary to (antisense) and similar to (sense) striatin mRNA. Sections were then washed at 52°C in 50% formamide and 2× SSC and incubated with RNase A (100 μg/ml) in 2× SSC at 37°C for 30 min. Sections were dehydrated, defatted, and apposed to films (X-OMAT AR; Eastman-Kodak Co., Rochester, NY) for 6–10 d. The ^{35}S-radiolabeled RNA probes were obtained as follows: pFC 10, encoding sequence 840–1,192, and pFCX (Fig. 1) were linearized and transcribed using T3 and T7 polymerases (RNA transcription kit from Stratagene) in the presence of 50 μCi [^{35}S]UTPαS (>1,000 Ci/mmol, 10 mCi/ml: Amersham Intl.) in a final volume of 20 μl.

Tissue and Cell Fractionation, Calmodulin-Sepharose Affinity Purification, Western Blots, and Immunoprecipitation

Protein was determined by the method of Schaffner and Weissmann (41). Homogenates of rat whole brain, striatum, hippocampus, hypothalamus, cerebellum, and brain cortex were heated in Laemmli sample buffer; equal amounts of protein were electrophoresed on 7.5% SDS-PAGE gels and blotted as described (31). Other types of PAGE described by Neville

(30), by Penin et al. (32), or including 6 M urea in all buffers were also used. Fractions enriched in synaptosomes were prepared by flotation as described (25). Rat brain, striatum, or synaptosomes were homogenized in ice-cold buffer B (50 mM triethanolamine, pH 7.6, 5 mM $MgCl_2$, 2 mM DTT, 0.3 M sucrose, inhibitors of proteases: 0.1mg/ml trypsin inhibitor, 2 μg/ml leupeptin, aprotinin, and pepstatin, 1 mM Pefabloc [Boehringer Mannheim GmBH], and then centrifuged at 100,000 *g* for 70 min at 4°C. The supernatants were saved, and the pellets were homogenized in the presence of 0.9% Lubrol-PX (Lubrol/protein ratio of 2.5) and centrifuged as described (10). The Lubrol-soluble fractions were incubated for 4 h at 4°C with calmodulin (CaM)–Sepharose (~1 g resin per 10 μg protein) in the presence of 1 mM Ca^{++}, as described (9). The flow-through was saved. After several washes, the CaM-binding proteins were eluted in a 1 mM EDTA- or EGTA-containing buffer and TCA precipitated (9).

PSDs were prepared according to Cohen et al. (7). Fractions were analyzed by Western blots, using preimmune and immune sera directed against fusion protein I and striatin peptides II and III (see below). Either the enhanced chemiluminescence (ECL) (Amersham Intl.) procedure, or the procedure using alkaline phosphatase-coupled anti-rabbit antibodies (Promega, Madison, WI) were applied. In other experiments, immunoprecipitation studies were achieved as described (40), using Pansorbin (Calbiochem-Novabiochem Corp., La Jolla, CA) preincubated with the antibodies described below (12 μl of serum for 50 μl of a 10% Pansorbin suspension). The samples to be studied were incubated with mild agitation at 4°C with the antibody-treated Pansorbin aliquots for 2 h. Adenylyl cyclase activities were determined in the pellets and supernatants (31). In parallel, both fractions were analyzed by SDS-PAGE and Western blots.

Preparations highly enriched in synaptosomal adenylyl cyclase activity, called FC fractions (31), were incubated in the presence of various concentrations of affinity-purified anti-peptide II or III antibodies; adenylyl cyclase activity was measured in basal condition, in the presence of 100 μM forskolin, various concentrations of Ca^{++}, or 1 μM calmodulin +/− 10 μM Ca^{++} or +/− 1 mM EGTA as described (31).

Expression of Striatin in E. coli Cells

pMF was used to direct the synthesis of fusion protein I in *E. coli* SG 4044 cells grown at 41°C. The fusion protein was produced as inclusion bodies that were purified. They were dissolved in LSB sample buffer (4 M urea, 25 mM Tris-HCl, pH 8.0, 10 mM EDTA, 50 mM DTT) and electrophoresed on 15% SDS–polyacrylamide gels. The 18-kD protein band was separated on a preparative SDS–polyacrylamide gel by reverse electrophoresis (24) and TCA precipitated. Scanned Coomassie blue–stained gels containing purified fusion protein I showed that it was at least 90% pure. 3 mg fusion protein I was obtained from 1 liter of bacterial culture.

pFCGN was used to infect *E. coli* cya-TP 610 (18) and TP 610 CaM cells (15), grown on Luria-Bertani broth and selected on Mac-Conkey–maltose medium. Induction of striatin synthesis was achieved by adding 15 μg/ml isopropylthio-β-D-galactoside. The adenylyl cyclase activities of the control and transformed lysed cells were determined. In other experiments, the soluble fraction of the lysate of pFCGN-transformed *E. coli* cells was complemented with 1 mM Ca^{++} and loaded on a CaM–Sepharose column, as described above. The flow-through was saved, and the resin was washed. The EDTA- or EGTA-eluate was collected, TCA precipitated, and analyzed by Western blotting.

Expression of Striatin in COS-1 Cells

COS-1 cells were cultured in DME (GIBCO BRL, Gaithersburg, MD) containing 10% FCS, 4 mM glutamine, and antibiotics; they were transfected either by the DEAE-dextran procedure (40) or by the liposome method (adapted from 36), using pSVL or pFSVL (5 μg per one 10-cm petri dish). Trypsinized COS-1 cells were centrifuged, and the pellets were lysed for 30 min on ice in lysis buffer (50 mM Tris-HCl pH 7.5, 1% Triton X-100, 50 mM NaCl, 25 mM NaF, 1 mM EDTA, and the above cited inhibitors of proteases). The lysates were centrifuged twice (20,000 *g*, 30 min, 4°C), and the supernatants were subjected to immunoprecipitation. Triplicate dishes of COS-1 cells, untransfected and transfected by pFSVL or pSVL, were incubated, 48 h after transfection, in DME without FCS containing 2 μCi/ml of [^{3}H]adenine (24 Ci/mmol; Amersham Intl.), according to the procedure described by Salomon (39). After several washes and a 10-min incubation in the presence of 0.1 mM isobutylmethylxanthine (EGA-Chemie), they were incubated for 10 min in the presence or absence of 10 μM forskolin, with or without 1 μM isoproterenol, or with the Ca^{++} ionophore A 23187 (Sigma Chemical Co., St. Louis, MO). Nucleotides were extracted by adding 2.5% cold PCA to the dishes. Samples were neutralized and [^{3}H]cAMP was measured, using [^{32}P]cAMP for calibration of the chromatographic columns. Untransformed and transformed (48 h) COS cells, grown in triplicate dishes for each condition, were lysed and assayed for protein and adenylyl cyclase activity (basal and forskolin-stimulated activities).

Antibodies

Three rabbits were immunized with the purified fusion protein I (150 μg per injection) according to published procedures. Titers were determined by ELISA, using bacterial soluble lysate containing the complete fusion protein as coating antigen. Titers were of the order of 1/10,000 after 4 mo.

Peptides corresponding to striatin sequences 191–205 (peptide II) and 267–287 (peptide III) (Fig. 2 *B*) were synthesized with an NH_2-terminal added cysteine (P. Fourquet, CIML, CNRS, Université de la Méditerranée, Marseille, France). They were coupled to pig thyroglobulin (Sigma Chemical Co.) by means of sulfo-MBS (Pierce Chemical Co., Rockford, IL) according to described procedures (45). Three rabbits were injected with peptide II, and two rabbits were injected with peptide III. Titers were determined by ELISA, using peptides coupled to ovalbumin by means of 1-ethyl-3-[3-dimethylamino-propyl]carbodiimide hydrochloride (EDC) (Pierce Chemical Co.) as coating antigens. Specific anti-peptide antibodies were prepared by adsorbing sera on Sulfogel (Pierce Chemical Co.) coupled to each peptide (1 mg peptide per ml). Antibodies were eluted from the resin by adding a 10 mM glycine–HCl buffer, pH 3.0; the eluates were extensively dialyzed and concentrated by centrifugation in a Centriprep 30 (Amicon Corp., Danvers, MA).

Immunocytochemistry

15 young male Sprague-Dawley rats (120–140 g) were deeply anesthetized and perfused transcardiacally with 300 ml of a cold 3% paraformaldehyde solution containing 0.08% glutaraldehyde. Coronal Vibratome sections of the striatum were collected in ice-cold PBS containing 50 mM NH_4Cl and cryoprotected before being permeabilized by three cycles of freeze-thawing in liquid nitrogen. The preembedding free-floating technique was used. Briefly, the blocked sections were soaked overnight in ice-cold solutions containing 1–3 μg/ml of affinity-purified antibodies directed against striatin peptides II and III (Fig. 1 *B*). Biotinylated goat anti-rabbit second antibody (Vector Laboratories, Burlingame, CA) was used at a dilution of 1:100 and decorated with avidin-biotin-HRP complex (Vector Laboratories). The sections were postfixed with 2% glutaraldehyde, and the peroxidase complex was revealed with 0.05% 3.3'DAB tetrahydrochloride and 0.033% H_2O_2. Sections were fixed in 1% OsO_4, contrasted in 1% uranyl acetate, dehydrated, and flat-embedded in Durcupan (Fluka AG, Buchs, Switzerland). Ultrathin sections were observed without further staining on an electron microscope (CM 10; Philips Electronic Instruments, Mahwah, NJ). A few sections were processed for light microscopy just after the DAB reaction. Three purified anti-peptide antibodies raised against peptides II and III were used: two gave comparable immunocytochemical reactions, and one gave no reaction and was used as a control. Other controls included (*a*) the omission of the primary antibodies, and (*b*) the use of primary antibodies adsorbed with their peptides added at a 100:1 molar ratio.

Results

cDNA Cloning of Striatin and Analysis of the Predicted, Encoded Protein Sequence

A 110,000-M_r, Lubrol-soluble protein present in an adenylyl cyclase–enriched synaptosomal fraction was obtained by PAGE and digested in the gel. 10 of the obtained peptides were sequenced and found to be original. Sense and antisense degenerate oligonucleotides deduced from five of them were used as primers in reverse transcription–PCR reactions with cDNAs obtained by reverse-transcribed rat brain mRNAs as template. A novel 0.8-kbp DNA fragment was obtained, encoding four of the se-

quenced peptides. The screening of a randomly primed, λ ZAP rat brain cDNA library with this probe yielded 14 overlapping clones, defining a novel 3,635-bp sequence. This sequence contains an open reading frame (ORF) of 2,343 nucleotides (nt) flanked by a 656-nt-long 5′ untranslated sequence (UTR) and a 636-nt-long 3′ UTR (Fig. 2, *A* and *B*). The 3′ untranslated sequence does not contain a polyadenylation signal. The protein encoded by the ORF has been called striatin since, as shown below, it is predominantly localized in the striatum. An ATG codon, preceded by several stop codons, lies within a canonical eukaryotic translation start sequence (22). The predicted protein sequence encodes 780 amino acids. It contains five of the ten peptides originating from the digestion of the 110,000-M_r component. Thus, in the preparative gel, another synaptosomal protein migrates with an apparent relative molecular mass identical to that of striatin. We have sequenced the latter protein, which appears to be a glycosidase (to be reported elsewhere).

A search for protein homologies in the NCBI data bank shows the presence, at the COOH-terminal end, of eight short sequences known as β-repeats or WD units at positions 419–454, 461–491, 514–544, 567–597, 613–643, 662–692, 704–734, and 746–780 (Fig. 2 *B*, numbered from 1 to 8); striatin is thus a member of the WD-repeat family (29). One WD-unit perfectly matches the consensus (repeat 2), and four contain three or fewer mismatches (repeats 4, 6, 7, 8); unit 1 (six mismatches), 3 (4 mismatches), and 5 (8 mismatches) only loosely conform to the consensus (Fig. 2 *C*). Besides, numerous potential phosphorylation sites are scattered over the sequence: 10 for calcium/phospholipid–dependent protein kinase C (serine or threonine 180, 194, 382, 405, 458, 486, 511, 592, 695, and 729) and 18 for casein-kinases. Four highly charged short sequences, one bearing a net positive charge of 9 (331–353), and another bearing a net negative charge of 7 (246–258), are found in the NH_2-terminal region. The region spanning sequence 87–104 is predicted to be an amphipatic α helix having a positively charged side and a hydrophobic side. Some of these structural elements are shown in Fig. 2 *B*. Analysis of the striatin protein sequence does not reveal hydrophobic α helices suggestive of transmembrane segments. No known ATP- or GTP-binding consensus sequences are present. Striatin has a calculated isoelectric point of 5.16.

While our study was completed, the cDNA of a new member of the WD-repeat family, coding for a shorter, 713–amino acid human nuclear protein, SG2NA, of unknown function, was published (28). Its protein sequence is 80% similar to and 66% identical with sequences of a similar length from striatin, in both the NH_2-terminal and the WD-repeat COOH-terminal domains, but not in the central domain. Although Muro et al. (28) report six WD repeats in the SG2NA sequence, we indeed noticed in this sequence two more WD repeats, at positions 353–387 and 679–713. Having now obtained the human striatin sequence (to be published elsewhere), we can state that striatin and SG2NA are different proteins, defining a new subfamily of WD-repeat proteins.

Striatin Is a Detergent-soluble 110,000-M_r Protein

To identify the product of the *striatin* gene, antisera were obtained. Some were raised against a fusion protein expressed in *E. coli* by plasmid pMF (Fig. 1), containing the first 50 amino acids of striatin (fusion protein I; Fig. 2 *B*); others were directed against two charged striatin peptides II (sequence 191–205) and III (sequence 267–287), located in the NH_2-terminal region of the protein (Fig. 2 *B*). Immunoblot studies carried out with each type of anti-striatin antibodies reveal the presence of only one 110,000-M_r protein in rat brain and spinal cord homogenates as well as in synatosomal purified membrane fractions where this protein is enriched (Fig. 3, *A* and *B*, lanes *2* and *1*, respectively). Striatin has a theoretical molecular mass of 86,225. The apparent relative molecular mass of striatin deduced by PAGE is thus higher than predicted by 23,800. When

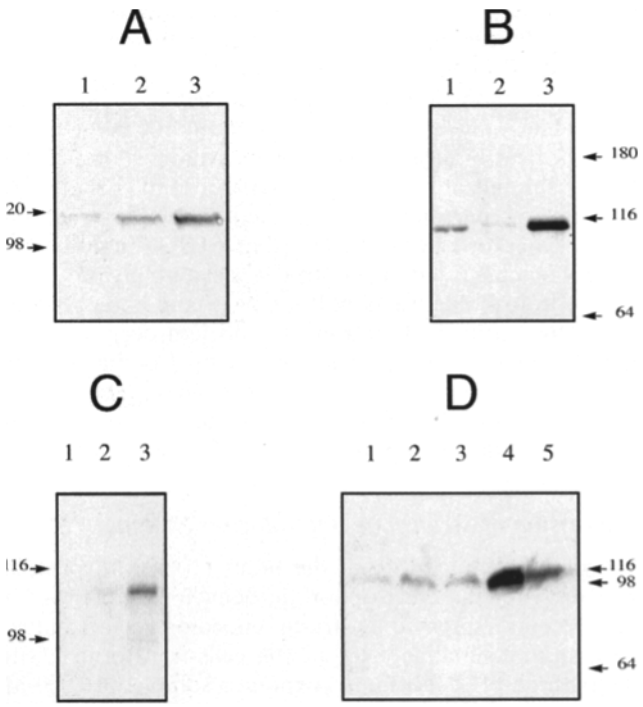

Figure 3. Expression of striatin, as analyzed by Western blots revealed by antibodies directed against fusion protein I (anti-peptide I), using phosphatase-coupled second antibodies (*A*, *B*, and *C*) or the ECL procedure (*D*). (*A*) Soluble fraction of the lysate of *E. coli* cells transformed by plasmid pFCGN (lane *1*; 40 μg/lane), adult rat brain homogenate (lane *2*; 80 μg/lane), and lysate of COS-1 cells transfected with plasmid pFSVL (lane *3*; 70 μg/lane). Striatin migrates at the same position in SDS-PAGE, whatever its origin. (*B*) Adult rat brain striatin binds to CaM–Sepharose in the presence of Ca^{++}. (Lane *1*) Brain Lubrol-soluble proteins before loading on CaM–Sepharose (total protein: 4.5 mg; 48 μg/lane *1*); (lane *2*) proteins in the flow-through (total protein: 2.8 mg; 48 μg/lane *2*); (lane *3*) proteins in the EDTA-eluate (total protein: 150 μg; 18 μg/lane *3*). (*C*) The soluble fraction of the lysate of *E. coli* cells transformed by plasmid pFCGN (total amount: 30 mg protein; 120 μg/lane *2*) was incubated with CaM–Sepharose (3 ml); the flow-through was collected (18 mg protein; 86 μg/lane *1*); and the resin was washed (10 mg protein; not shown). EDTA-eluates were then collected (first 4 ml, 80 μg protein; lane *3*: 40 μg; next 4 ml, 10 μg, not shown). (*D*) Normalized amounts of homogenates of various rat brain regions were analyzed by Western blot. (Lane *1*) Hypothalamus; (lane *2*) hippocampus; (lane *3*) cerebellum; (lane *4*) striatum; (lane *5*) cerebral cortex.

striatin is analyzed on other gel systems, Neville SDS gels (30) or gels containing either 6 M urea or an anionic detergent instead of SDS (32), it again has a 110,000 M_r, confirming its anomalous electrophoretic behavior. Striatin is particulate, only $\sim 1\%$ being found in the 100,000-*g* supernatant of rat brain homogenates or synaptosomes. Upon Lubrol solubilization of brain or synaptosomal pellets, striatin mostly partitions in the Lubrol-soluble fractions. When synaptosomes are resolved in a PSD fraction and a Lubrol-soluble fraction (7), striatin is essentially present in the Lubrol-soluble fraction. Even in striatal homogenates, the richest source of striatin, this protein occurs in very low abundance, not being revealed by Coomassie blue even in overloaded gels. This fact is in agreement with the scarcity of the 110,000-M_r synaptosomal protein from which we obtained peptides.

Expression of Striatin in Heterologous Systems

To confirm the identity of the brain 110,000-M_r protein, we compared its electrophoretic behavior with those of the proteins expressed by striatin-encoding vectors in bacteria and mammalian cells. *E. coli* cells transformed with the plasmid pFCGN (Fig. 1) express a soluble, 110,000-M_r fusion protein (expected M_r: 89,200), migrating at the same position in SDS-PAGE as brain striatin (Fig. 3 *A*, lane *1*). This is in favor of pFCGN being a full-length clone. It also follows that eukaryotic posttranslational modifications are not likely to account for the anomalous behavior of striatin in SDS-PAGE.

Mammalian COS-1 cells were transfected with plasmid pFSVL encoding striatin (Fig. 1). A 110,000-M_r protein, migrating in SDS-PAGE at the same position as brain striatin, can readily be detected by anti-peptide antibodies II and III in Western blots of lysates or immunoprecipitates of the lysed cells (Fig. 3 *A*, lane *3*).

Striatin Directly and Quantitatively Binds Ca^{++}-CaM

Brain striatin quantitatively binds CaM–Sepharose in the presence of Ca^{++} (Fig. 3 *B*, lanes *1* and *2*), but not in its absence. It is eluted from the affinity-resin by EDTA- or EGTA-containing buffers, as shown by Western blots (Fig. 3 *B*, lane *3*).

The bacterially expressed fusion protein comprising the full-length striatin also quantitatively binds CaM–Sepharose in the presence of Ca^{++} (Fig. 3 *C*, lanes *1* and *2*), but not in its absence, and is eluted from the resin by EDTA- or EGTA-containing buffer (Fig. 3 *C*, lane *3*). Since calmodulin is not found in bacteria, and since bacterial proteins do not bind CaM–Sepharose in a Ca^{++}-dependent manner, this observation demonstrates that striatin directly binds Ca^{++}-calmodulin.

Striatin Is Not Directly Involved in cAMP Synthesis

Since striatin was discovered in synaptosomal adenylyl cyclase–enriched fractions, various studies have been performed to establish whether or not this protein is involved in cAMP production, by itself or by interacting with adenylyl cyclase components.

The bacterial full-length fusion protein expressed by plasmid pFCGN is unable to restore a cya phenotype in *E. coli* cells deficient in adenylyl cyclase, whether these cells harbor a plasmid encoding calmodulin or not. The bacterially expressed striatin thus does not encode an adenylyl cyclase able to function in *E. coli*.

Pellets of immunoprecipitated brain striatin do not contain any measurable adenylyl cyclase activity. Brain FC fractions highly enriched in adenylyl cyclase activity, when preincubated with affinity-purified anti–peptide I and II antibodies in large excess, display the same adenylyl cyclase activities as do control fractions (not shown). Likewise, FC fractions complemented with various amounts of bacterially expressed striatin (contained in CaM–Sepharose EDTA eluates, as described) have the same adenylyl cyclase activities as control fractions.

We have compared the cAMP contents of COS-1 cells overexpressing striatin (pFSVL-transfected cells) and of control cells, according to the procedure described by Salomon (39). Various assay conditions were tested: basal conditions (cells unchallenged with adenylyl cyclase effectors), stimulatory conditions (addition of 10 μM forskolin, with or without 1 μM isoproterenol, to the culture medium), and assays in the presence of various concentrations of the Ca^{++} ionophore A 23187. No meaningful differences in the cAMP contents of control and transfected cells could be detected (not shown). We conclude that the overexpressed striatin does not grossly modify the cAMP synthesis machinery of COS cells. In those cells, however, the type(s) of adenylyl cyclase expressed is not known, and it is conceivable that striatin, in case it affects the function of adenylyl cyclase, operates in the context of one particular type of adenylyl cyclase.

Striatin Is Mostly Expressed in the Central Nervous System

RNA analysis by Northern blots using four different probes, which together span the whole striatin coding sequence, show that among all tested rat or mouse tissues, striatin is expressed almost exclusively in the central nervous system (Fig. 4 *A*, lanes *1*, *2*, *3*, and *11*). Four rat transcripts of 8.3, 7.7, 5.5, and 4.7 kb are detected. Their lengths greatly exceed that of the striatin ORF (2.3 kb); however, brain messenger RNAs are known to have unusually long 3′ UTRs (43). The 8.3- and 5.5-kb transcripts are most abundant in brain and cerebellum (Fig. 4, lanes *1* and *2*), the 4.7 transcript is the major species in the spinal cord (Fig. 4, lane *3*). Blots exposed for longer times reveal all four transcripts in testes (not shown). In Northern blots of mouse brain revealed with rat probes, only two transcripts of 8.3 and 5.5 kb are readily detected (Fig. 4 *A*, lane *11*). Only two of the four transcripts encode the full-length protein, as shown in Northern blots hybridized with a probe corresponding to the 5′ region (probe 1; Fig. 2 *B*, *double-headed arrow*).

To know whether the four transcripts originate from one or several homologous genes, rat genomic Southern blots have been probed with a DNA probe revealing the four transcripts. They yield a simple pattern, only one fragment being present in the DNA digested with BamHI or PstI (Fig. 4 *C*, lanes *1* and *4*) and two with EcoRI (Fig. 4 *C*, lane *2*), as expected if the four transcripts originate from one gene.

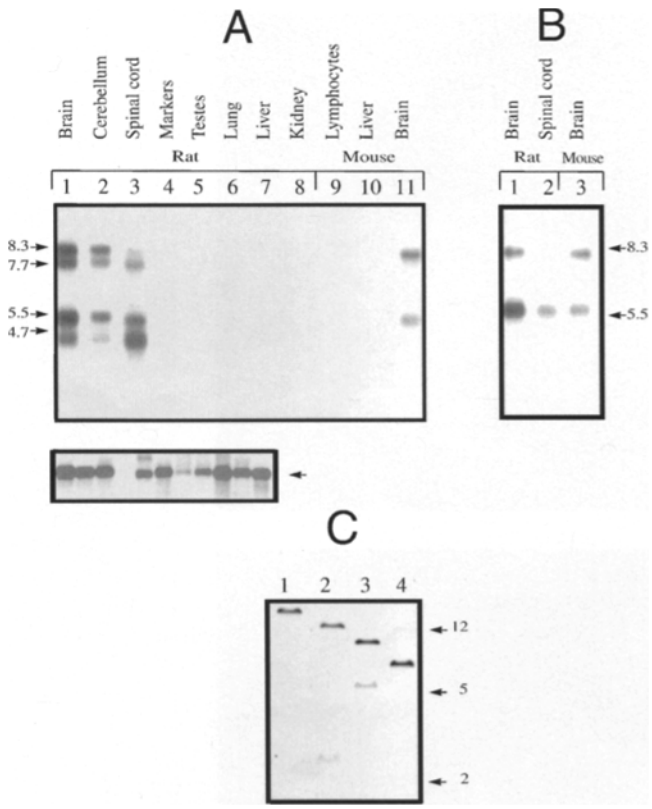

Figure 4. *A* and *B* are Northern blots made with poly(A)$^+$ RNAs from different rat and mouse tissues (5 μg RNA per lane). Striatin is almost only expressed in the CNS. (*A*) A probe spanning striatin sequence 668–1,647 reveals four transcripts in rat CNS (brain, cerebellum, and spinal cord; lanes *1*, *2*, and *3*) and two transcripts in mouse brain (lane *11*). In the encart below, a β-actin probe was applied to the same blot. (*B*) Probe 1 spanning striatin sequence 1–750 reveals only two transcripts in rat and mouse brain (Fig. 4 *B*, lanes *1* and *3*) and one of 5.5 kb in the rat spinal cord. *C* is a Southern blot of rat genomic DNA digested by BamHI (lane *1*), EcoRI (lane *2*), HindIII (lane *3*), and PstI (lane *4*), probed with DNA fragment 1293–2316 (containing an EcoRI site).

The multiplicity of transcripts therefore probably reflects alternative splicing or the existence of several promotors.

In situ hybridization studies using two sets of probes, one spanning the 5′ region of striatin (first 750 bp) and the other spanning the central region, show that striatin is mainly found and intensely expressed in the dorsal striatum and the olfactory tubercle (Fig. 5, *A* and *C*). The nucleus accumbens (ventral striatum) is scarcely labeled. A significant level of striatin mRNA is detected in the cerebral cortex, hippocampus, olfactory bulb, cerebellum (Fig. 5, *A* and *C*) and spinal cord grey matter (not shown). The control, sense, probes give no signal except in the hippocampus and more intensely in the cerebellum, as it is often the case for these two densely populated structures (Fig. 5, *B* and *D*).

Striatin Is Essentially Localized in Striatal Dendritic Spines

The localization of striatin in different regions of the brain, as studied by Western blots, parallels the distribution of its mRNA; the highest amount of immunoreactive material is found in striatal homogenates (Fig. 3 *D*, lane *4*). Striatin is present but less abundant in cortex, hippocampus, and cerebellum (~10 times less per mg protein; Fig. 3, lanes *2*, *3*, and *5*), and scarce in the hypothalamus (Fig. 3, lane *1*).

Immunocytochemical labeling of brain sections using affinity-purified antibodies directed against peptides II and III reveals that striatin is found in the striatum, where the perikarya of most neurons are labeled, as well as many elements of the neuropil (Fig. 6, *A* and *B*). Other structures that build up the motor system, i.e., the pyramidal neurons from layer V in the sensory-motor cortex, brain nuclei such as the red nucleus (to be shown elsewhere) as well as the cerebellum, in the molecular layer, are also labeled (Fig. 6 *D*). In spinal cord sections, striatin is seen in motoneurons, in cell bodies and dendrites (Fig. 6 *E*). Similar results have been obtained with the two sets of antibodies directed against different epitopes of striatin. Sections treated with antibodies preincubated with an excess of the relevant peptide do not show reaction product (Fig. 6 *C*).

At the ultrastructural level in the striatum, the vast majority of neurons are stained. These neurons have a rounded nucleus and all the morphological characteristics of medium spiny, GABAergic neurons. The subcellular structures that contain the highest amount of DAB reaction product are dendritic spines (Fig. 7, *A*, *C*, and *D*); labeled dendritic shafts are sometimes seen (Fig. 7 *B*). In conditions that limit the dispersion of the reaction product away from its origin, the immunolabeling is mostly localized in the vicinity of the postsynaptic density and of the postsynaptic membrane (Fig. 7 *D*). Dendrites are, however, labeled all along their course, whereas axons are unlabeled. In neuronal cell bodies, a discrete labeling occurs in the cytoplasm around the nucleus, but not in the nucleus itself or in the lumen of the rough ER or of the Golgi apparatus (not shown). Sections treated with antibodies preincubated with an excess of the relevant peptide do not show any reaction product (Fig. 7 *E*).

Discussion

Striatin, a novel protein found in the CNS, has several characteristics, suggesting a possible role in postsynaptic signaling. First, it directly binds CaM in the presence of Ca^{++}, as demonstrated by the study of bacterially expressed striatin. Secondly, its sequence contains eight WD-repeats in its COOH-terminal part. The family of WD-repeat proteins is characterized by the presence of a series of four to eight conserved repeating units of 23–41 amino acids, usually beginning with GH and ending with WD, separated by short, variable sequences (29). To our knowledge, striatin is the first known WD-repeat protein that binds Ca^{++}-CaM. Up to now, all known proteins belonging to this family are eukaryotic regulatory proteins, none so far being an enzyme. Some interact with components of the cytoskeleton, like coronin (11), or of vesicles, like Sec1 3p (33), or participate in supramolecular complexes involved in transcription or translation processes. A number of them, among which are the β subunits of heterotrimeric G proteins, play a role in signal transduction. Striatin is not a member of already known WD-repeat subfamilies, since its WD-repeats are not similar to theirs in equivalent positions. In particular, it is not a member of the

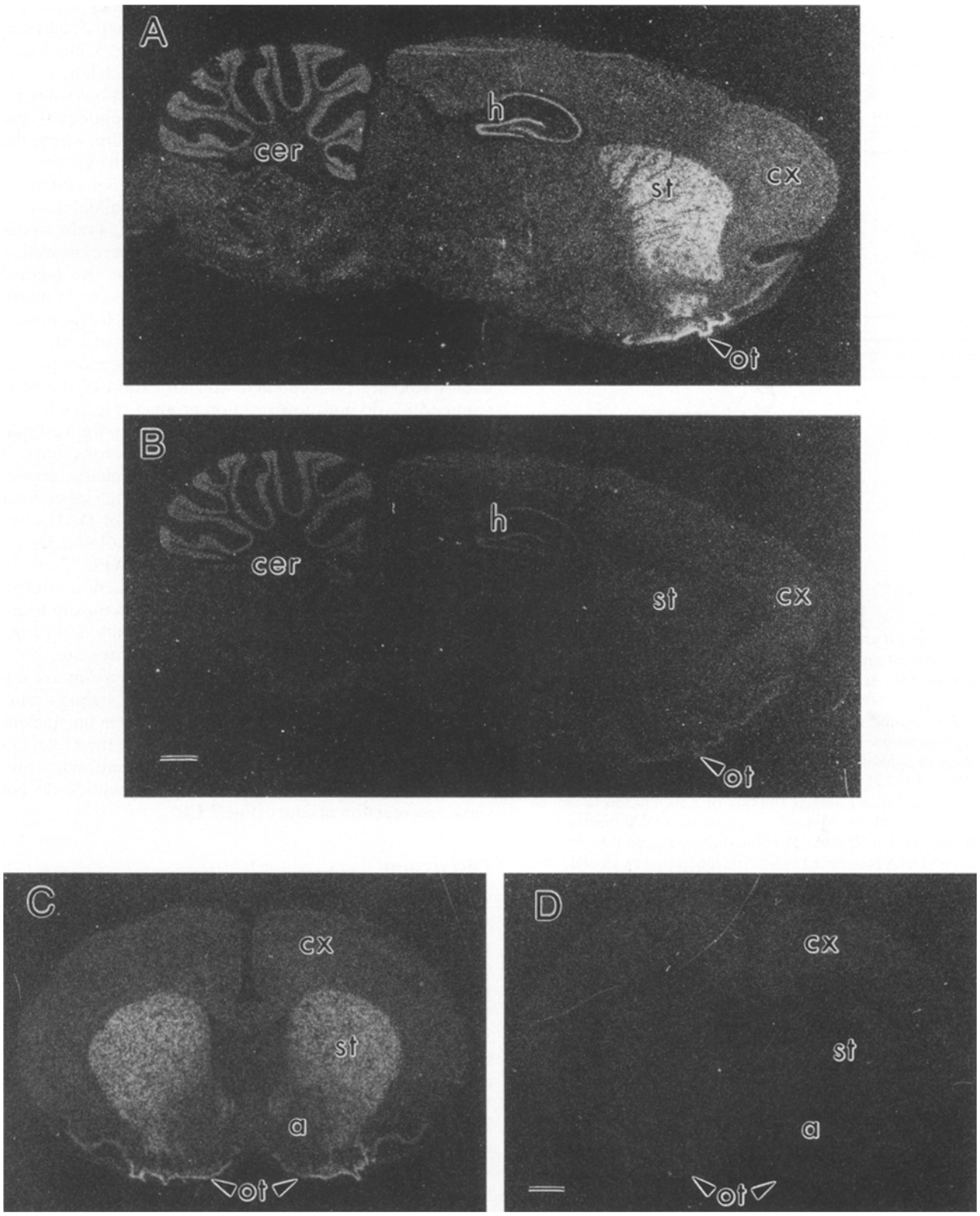

Figure 5. Striatin is predominantly expressed in the striatum. Dark-field autoradiograms of rat brain sagittal (*A* and *B*) and coronal (*C* and *D*) sections. In situ hybridization with the antisense RNA probe (*A* and *C*) shows an intense and specific labeling in the striatum (*st*; *A* and *C*) and the olfactory tubercle (*ot*; *A* and *C*) and a moderate staining of the nucleus accumbens (*a*; *C*) and cortex (*cx*; *A* and *C*), as compared to the sense labeling (*C* and *D*). The positive hybridization signal in the hippocampus (*h*; *A*) detected with the antisense probe is significantly stronger than the weak staining seen with the sense probe (*B*); the signals given by both probes are comparable for the cerebellum (*cer*; *A* and *B*).

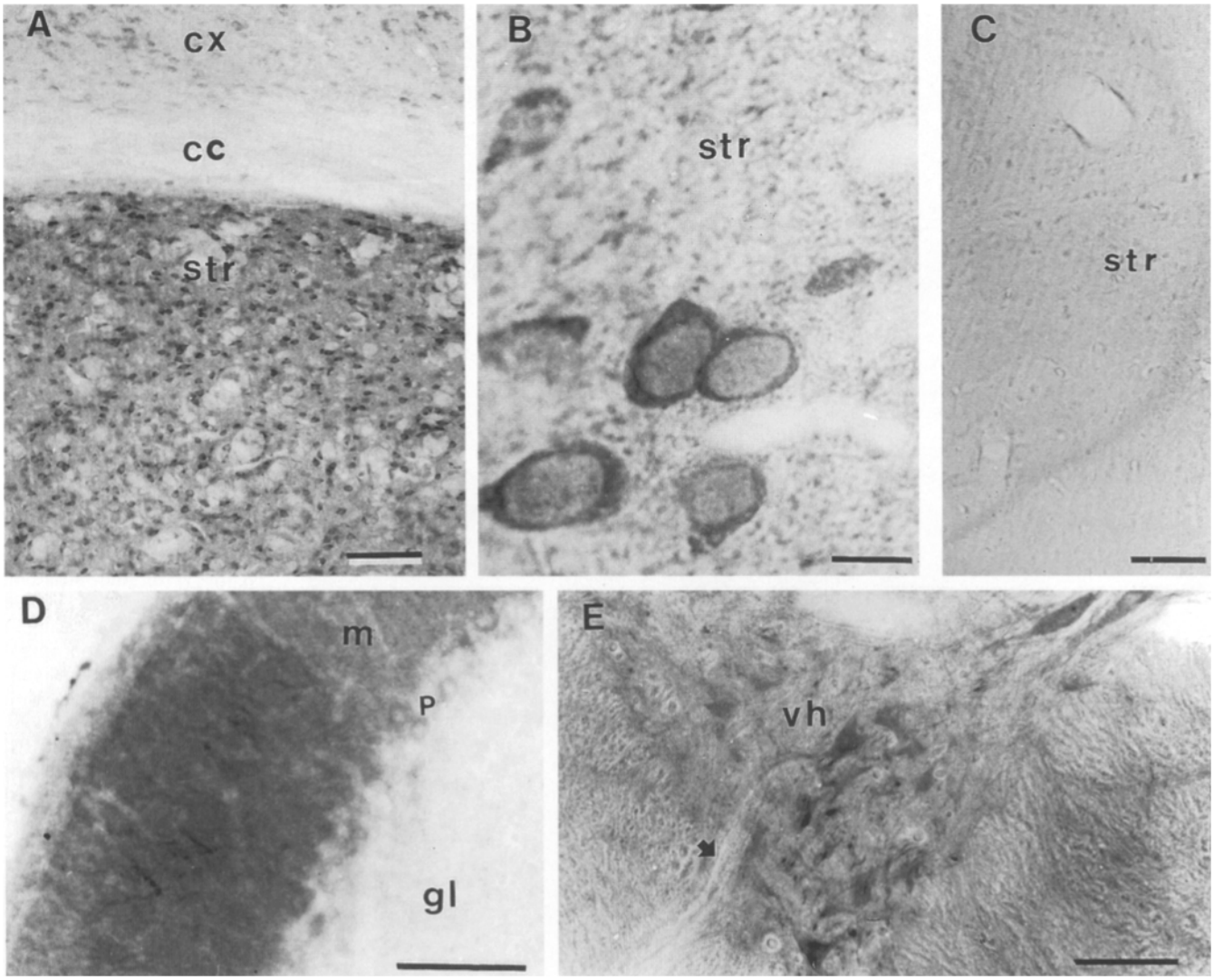

Figure 6. Striatin is present in the CNS motor system. Immunocytochemical localization at the light microscope. Immunolabeling obtained with the affinity-purified anti–peptide III antibody. (*A–C*) Coronal sections of the striatum. (*A*) Low concentration of antibody (1 μg/ml). The striatum (*str*) is more labeled than the cortex (*cx*). No labeling is observed in the corpus callosum (*cc*). (*B*) Higher concentration of antibody (3 μg/ml). The perikarya of most striatal neurons are labeled, but not the nuclei. The neuropil is strongly labeled. (*C*) Adjacent section treated with the antibody preadsorbed with peptide III. (*D*) Coronal section of the cerebellum. The molecular layer (*ml*) is densely stained, whereas no labeling is seen in the granular layer (*gl*). The Purkinje cell bodies (*P*) are lightly labeled. (*E*) Cross-section of the spinal cord, same treatment as in *D*. *vh*, ventral horn. The motoneurons are stained at the level of the cell bodies and dendrites, whereas their axons (*arrows*) are not. Bars, 100 μm (10 μm in *B*).

β subunits of heterotrimeric G proteins. The WD-repeat domains of rat striatin and human SG2NA (29) are, on the contrary, conserved. Although radically differing in their subcellular localizations, and therefore probably in their functions, striatin and SG2NA thus constitute a novel subfamily of WD-repeat proteins.

The peptides whose sequences allowed us to clone striatin originated from a Lubrol-soluble synaptosomal protein thought to be membrane bound (6). The study of brain subcellular fractions with antibodies directed against striatin peptides and a fusion protein confirmed that striatin is almost entirely particulate and that most of it can be solubilized by Lubrol. Striatin is therefore not a protein intrinsic to PSDs, but appears to be associated with membranes. Yet, we found no sequences able to account for hydrophobic α helices or consensus sequences for lipidic, posttranslational modifications, although palmitoylation is theoretically possible. Striatin possesses two polybasic domains, one bearing a net positive charge of 9. They may play a role in the binding of striatin to membranes, since it has been shown that stretches of basic amino acids mediate or at least help to promote protein binding to the membrane (21).

Striatin is largely expressed in structures belonging to the CNS motor system. In situ hybridization and immunocytochemistry show that striatum is the brain structure displaying the highest content of striatin. The caudate nucleus contains significantly more striatin than the nucleus accumbens. In Huntington-affected striata, the neurons that undergo apoptosis are also those present in the caudate nucleus, not those in the nucleus accumbens. Besides

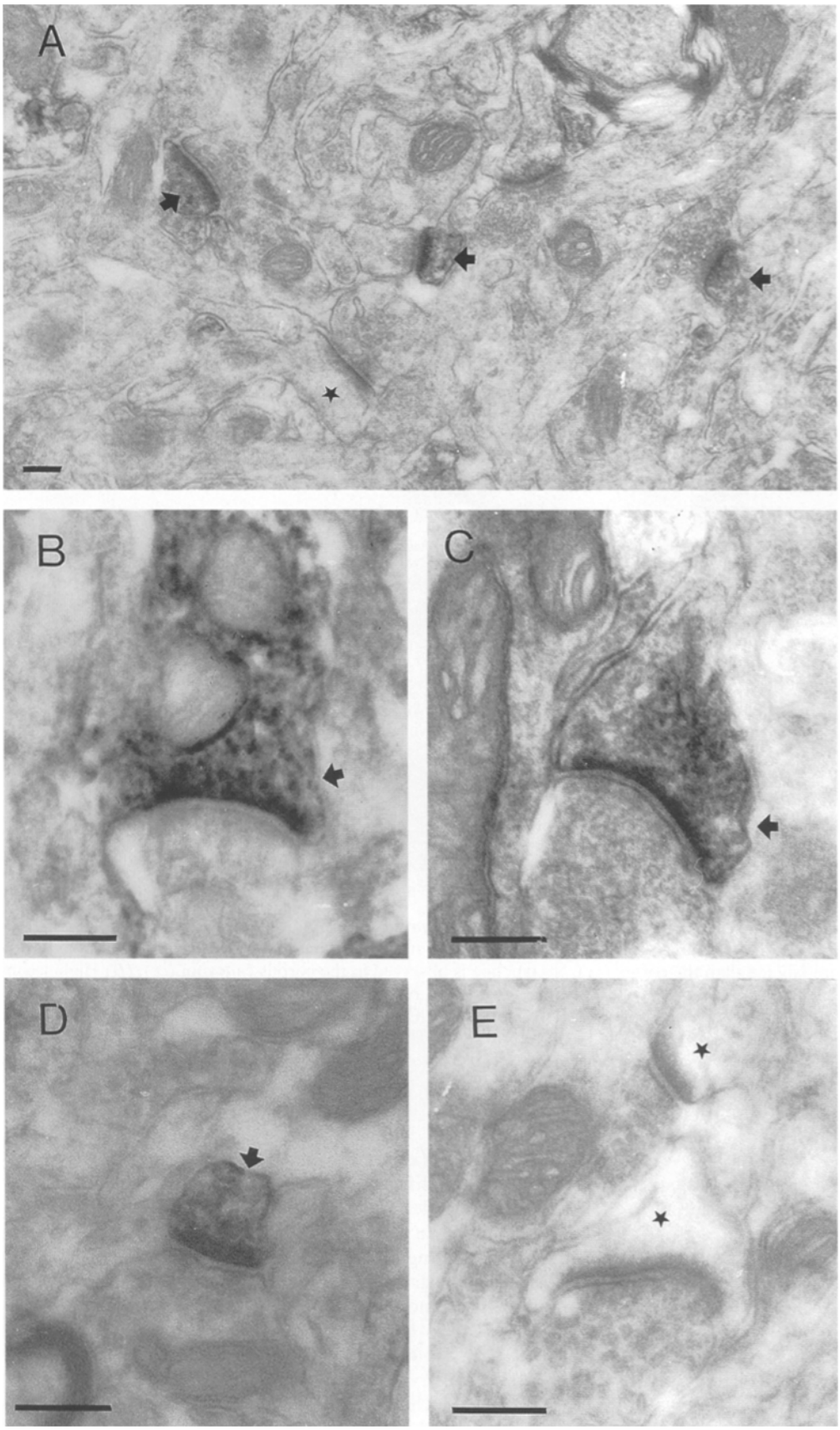

Figure 7. In the striatum, striatin is localized in the postsynaptic compartment of synapses. Electron micrographs. Immunocytochemical localization obtained with an affinity-purified antibody directed against peptide III. (Similar results were obtained with antibody directed against peptide II). (*A*) Low magnification showing immunolabeled dendritic spines (*arrows*) and one unlabeled synapse (*star*). (*B*) Higher magnification showing a labeled structure that is probably a dendritic shaft. In this experiment, both the first antibody (3 μg/ml) and DAB concentrations were intentionally increased to obtain a strong labeling. (*C*) A labeled dendritic spine. (*D*) Lighter labeling obtained at low first antibody (1 μg/ml) and DAB concentrations, suggesting that striatin is localized close to the postsynaptic density (*arrowhead*) and to the postsynaptic membrane in dendritic spines. (*E*) Absence of immunoreaction inside two dendritic spines. In this control, the affinity-purified antibody directed against peptide III was preadsorbed overnight with peptides III and II in a 100-fold molar excess before immunolabeling. Bars, 0.2 μm.

striatum, the brain cortex, cerebellum, spinal cord grey matter, olfactory tubercle, and hippocampus also contain striatin messengers. Immunolabeling studies of brain and spinal cord sections using affinity-purified anti-striatin peptide antibodies confirm the presence of striatin in the motor system, in particular layer V of the sensory-motor cortex, striatum, brain motor nuclei, cerebellum, and spinal cord grey matter (to be described in detail elsewhere). These studies indicate that (*a*) only neurons contain striatin, no glial cells having been shown to contain any. (*b*) In the labeled neurons, two compartments contain striatin: the cell bodies, with the exception of nuclei, and more abundantly, the neuropil. In the cerebellum, the molecular layer is immunolabeled, but not the granular layer. In spinal cord sections, the dendrites of the motoneurons are stained, whereas the axons are unlabeled. Ultrastructural studies show that the vast majority of striatal neurons contain striatin and have the morphological characteristics of medium spiny neurons. Striatin is mostly confined to dendrites, predominantly the dendritic spines, where the reaction product is apposed to the inner face of the synaptic membrane and to the PSDs. Thus, in all neurons examined so far, striatin is present solely in the somato-dendritic compartment, predominantly in the dendritic spines.

The high concentration, in dendritic spines, of components involved in cAMP and Ca^{++} signaling is generally viewed as facilitating the reception, transmission, and integration of signals carried by both cAMP and Ca^{++} (8, 26). Indeed, the postsynaptic compartment contains channels such as voltage-gated ion channels and glutamate (*N*-methyl-D-aspartate [NMDA]) receptor channels, regulatory enzymes, and proteins involved in transduction, such as the abundantly represented calcium/CaM–dependent protein kinase type II, cAMP-dependent protein kinase, CaM, CaM-dependent cAMP phosphodiesterases (23), calcium/phospholipid–dependent protein kinase C (1), calcineurin (17), and adenylyl cyclase (27). Although adenylyl cyclase is expressed in neurons in the same postsynaptic compartment as striatin, so far we have been unable to find a relationship between striatin and the cAMP machinery. Of interest, however, is the fact that striatum is the only one brain structure specifically endowed with adenylyl cyclase type V (16), in association with G_{olf} α (rather than $G_s\alpha$) (12, 19) and Gβ7 subunits (46).

The direct binding of striatin to Ca^{++}-CaM must be of great importance, since dendritic spines are individual calcium compartments (47). The extremely large variations in Ca^{++} concentrations that occur within these structures (34) have numerous biological consequences, such as the local regulation of the ras and MAP kinase pathways (13) or of microtubules, or the mechanisms of Ca^{++} buffering, so important with respect to Ca^{++} cytotoxicity (42). Striatin is thus likely to interact, in a Ca^{++}-dependent manner and through its WD-repeat domain, with one or several members of the surrounding cluster of signaling or cytoskeletal molecules present in dendritic spines. The as yet unknown function of striatin is currently being investigated by the indirect, two-hybrid technique. The special distribution of striatin within the CNS turns it into a marker for a defined subset of neurons, and more particularly, of their dendrites. As such, it might be instrumental to study striatin in the course of neurodegenerative diseases.

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References

1. Akers, R.F., D.M. Lovinger, P.A. Colley, D.J. Linden, and A. Routtenberg. 1986. Translocation of protein kinase C activity may mediate hippocampal long-term potentiation. *Science (Wash. DC).* 231:587–589.
2. Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410.
3. Auffray, C., and F. Rougeon. 1980. Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA. *Eur. J. Biochem.* 107:303–314.
4. Birnboim, H.C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* 7:1513–1523.
5. Burns, M.E., and G.J. Augustine. 1995. Synaptic structure and function: dynamic organization yields architectural precision. *Cell.* 83:187–194.
6. Castets, F., G. Baillat, S. Mirzoeva, K. Mabrouk, J. Garin, J. d'Alayer, and A. Monneron. 1994. A brain synaptosomal adenylyl cyclase of high specific activity is photolabeled with azido-ATP. *Biochemistry.* 33:5063–5069.
7. Cohen, R.S., F. Blomberg, K. Berzins, and P. Siekevitz. 1977. The structure of postsynaptic densities isolated from dog cerebral cortex. I. Overall morphology and protein composition. *J. Cell Biol.* 74:181–203.
8. Cooper, D.M.F., N. Mons, and J.W. Karpen. 1995. Adenylyl cyclases and the interaction between calcium and cAMP signalling. *Nature (Lond.).* 374:421–424.
9. Coussen, F., J. Haiech, J. d'Alayer, and A. Monneron. 1985. Identification of the catalytic subunit of brain adenylate cyclase: a calmodulin-binding protein of 135 kDa. *Proc. Natl. Acad. Sci. USA.* 82:6736–6740.
10. d'Alayer, J., G. Berthillier, and A. Monneron. 1983. Structure of brain adenylate cyclase: proteolysis-dependent modifications. *Biochemistry.* 22: 3984–3953.
11. de Hostos, E.L., B. Bradtke, F. Lottspeich, R. Guggenheim, and G. Gerisch. 1991. Coronin, an actin-binding protein of *Dictyostelium discoideum* localized to cell surface projections, has sequence similarities to G proteins β subunits. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:4097–4104.
12. Drinnan, S.L., B.T. Hope, T.P. Snutch, and S.R. Vincent. 1991. G_{olf} in the basal ganglia. *Mol. Cell. Neurosci.* 2:66–70.
13. Finkbeiner, S., and M.E. Greenberg. 1996. Ca^{++}-dependent routes to Ras: mechanisms for neuronal survival, differentiation and plasticity? *Neuron.* 16:233–236.
14. Garoff, H., and W. Ansorge. 1981. Improvements of DNA sequencing. *Anal. Biochem.* 115:450–457.
15. Glaser, P., D. Ladant, O. Sezer, F. Pichot, A. Ullmann, and A. Danchin. 1988. The calmodulin-sensitive adenylate cyclase of *Bordetella pertussis*: cloning and expression in *Escherichia coli. Mol. Microbiol.* 2:19–30.
16. Glatt, C.E., and S.H. Snyder. 1993. Cloning and expression of an adenylyl cyclase localized to the corpus striatum. *Nature (Lond.).* 361:536–538.
17. Goto, S., Y. Matsukado, E. Miyamoto, and M. Yamada. 1987. Morphological characterization of the rat striatal neurons expressing calcineurin immunoreactivity. *Neuroscience.* 22:189–201.
18. Hedegaard, L., and A. Danchin. 1985. The cya gene region of *Erwina chrysanthemi* B 374: organisation and gene products. *Mol. & Gen. Genet.* 201: 38–42.
19. Hervé, D., M. Lévi-Strauss, I. Marey-Semper, C. Verney, J-P. Tassin, J. Glowinski, and J-A. Girault. 1993. G_{olf} and G_s in rat basal ganglia: possible involvement of G_{olf} in the coupling of dopamine D1 receptor with adenylyl cyclase. *J. Neurosci.* 13:2237–2248.
20. Kennedy, M.B. 1993. The postsynaptic density. *Curr. Opin. Neurobiol.* 3: 732–737.
21. Kim, J., M. Mosior, L.A. Chung, H. Wu, and S. McLaughlin. 1991. Binding of peptides with basic residues to membranes containing acidic phospholipids. *Biophys. J.* 60:135–148.
22. Kozak, M. 1986. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell.* 44: 283–292.
23. Ludvig, N., V. Burmeister, P.C. Jobe, and R.L. Kincaid. 1991. Electron mi-

croscopic immunocytochemical evidence that the calmodulin-dependent cyclic nucleotide phosphodiesterase is localized predominantly at postsynaptic sites in the rat brain. *Neuroscience.* 44:491–500.
24. Mendel-Hartvig, I. 1982. A simple and rapid method for the isolation of peptides from sodium dodecyl sulfate-containing polyacrylamide gels. *Anal. Biochem.* 121:215–217.
25. Monneron, A., and J. d'Alayer. 1980. Effects of cross-linking agents on adenylate cyclase regulation. *FEBS Lett.* 109:75–80.
26. Mons, N., and D.M.F. Cooper. 1995. Adenylate cyclases: critical foci in neuronal signaling. *Trends Neurosci.* 18:536–542.
27. Mons, N., A. Harry, P. Dubourg, R.T. Premont, R. Iyengar, and D.M.F. Cooper. 1995. Immunohistochemical localization of adenylyl cyclase in rat brain indicates a highly selective concentration at synapses. *Proc. Natl. Acad. Sci. USA.* 92:8473–8477.
28. Muro, Y., E.K.L. Chan, G. Landberg, and E.M. Tan. 1995. A cell-cycle nuclear autoantigen containing WD-40 motifs expressed mainly in S and G2 phase cells. *Biochem. Biophys. Res. Commun.* 207:1029–1037.
29. Neer, E.J., C.J. Schmidt, R. Nambudripad, and T.F. Smith. 1994. The ancient regulatory-protein family of WD-repeat proteins. *Nature (Lond.).* 371:297–300.
30. Neville, D.M. 1971. Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. *J. Biol. Chem.* 246:6328–6334.
31. Orlando, C., J. d'Alayer, G. Baillat, F. Castets, O. Jeannequin, J-C. Mazie, and A. Monneron. 1992. A monoclonal antibody directed against the catalytic site of *Bacillus anthracis* adenylyl cyclase identifies a novel mammalian brain catalytic subunit. *Biochemistry.* 31:3215–3222.
32. Penin, F., C. Godinot, and D.C. Gautheron. 1984. Two-dimensional gel electrophoresis of membrane proteins using anionic and cationic detergents. Application to the study of mitochondrial F_0-F_1-ATPase. *Biochim. Biophys. Acta.* 775:239–245.
33. Pryer, N.K., N.R. Salama, R. Schekman, and C.A. Kaiser. 1993. Cytosolic Sec13p complex is required for vesicle formation from the endoplasmic reticulum in vitro. *J. Cell Biol.* 120:865–875.
34. Regher, W.G., and D.W. Tank. 1994. Dendritic calcium dynamics. *Curr. Opin. Neurobiol.* 4:373–382.
35. Remaut, E., P. Stanssens, and W. Fiers. 1981. Plasmid vectors for high-efficiency expression controlled by the p_L promotor of coliphage lambda. *Gene (Amst.).* 15:81–93.
36. Rose, J.K., L. Buonocore, and M.A. Whitt. 1991. A new cationic liposome reagent mediating nearly quantitative transfection of animal cells. *Biotechniques.* 10:520–525.
37. Rychlik, W., and R.E. Rhoads. 1989. A computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and *in vitro* amplification of DNA. *Nucleic. Acids Res.* 17:8543–8551.
38. Salin, P., and M.F. Chesselet. 1993. Expression of GAD (M_r 67,000) and its messenger RNA in basal ganglia and cerebral cortex after ischemic cortical lesions in rats. *Exp. Neurol.* 119:291–301.
39. Salomon, Y. 1991. Cellular responsiveness to hormones and neurotransmitters: conversion to [^{3}H] adenine to [^{3}H] cAMP in cell monolayers, cell suspensions, and tissue slices. *In* Methods in Enzymology. R.A. Johnson and J.D. Corbin, editors. Academic Press, San Diego, CA. 195:22–28.
40. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
41. Schaffner, W., and C. Weissmann. 1973. A rapid, sensitive and specific method for the determination of protein dilute solution. *Anal. Biochem.* 56:502–514.
42. Segal, M. 1995. Dendritic spines for neuroprotection: a hypothesis. *Trends Neurosci.* 18:468–470.
43. Sutcliffe, J.G. 1988. mRNA in the mammalian central nervous system. *Annu. Rev. Neurosci.* 11:157–198.
44. Trimble, W.S., M. Linial, and R.H. Scheller. 1991. Cellular and molecular biology of the presynaptic nerve terminal. *Annu. Rev. Neurosci.* 14:93–122.
45. Van Regenmortel, M.H.V., J-P. Briand, S. Muller, and S. Plaué. 1988. Synthetic Polypeptides As Antigens. Laboratory Techniques in Biochemistry and Molecular Biology. Vol. 19. R.H. Burdon and P.H. Van Knippenberg, editors. Elsevier/North Holland, Amsterdam. 95–130.
46. Watson, J.B., P.M. Coulter II, J.E. Margulies, L. de Lecea, P.E. Danielson, M.G. Erlander, and J.G. Sutcliffe. 1994. G-protein γ7 subunit is selectively expressed in medium-sized neurons and dendrites of the rat neostriatum. *J. Neurosci. Res.* 39:108–116.
47. Yuste, R., and W. Denk. 1995. Dendritic spines as basic functional units of neuronal integration. *Nature (Lond.).* 375:682–684.