The Journal of Neuroscience, January 1989, 9(1): 339–346

# Inositol 1,4,5-Trisphosphate Receptor Binding: Autoradiographic Localization in Rat Brain

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**Inositol 1,4,5-trisphosphate is a second messenger generated by stimulation of the phosphoinositide cycle, thought to release calcium from intracellular stores. We have mapped the distribution of $^3$H-inositol 1,4,5-trisphosphate receptor binding sites in rat brain by autoradiographic techniques. The cerebellum contains the highest level of inositol 1,4,5-trisphosphate binding sites in brain, which appear to be selectively localized to Purkinje cells. Moderate levels of binding sites are present in the hippocampus, cerebral cortex, caudate, and substantia nigra. Lesion studies indicate that binding in the hippocampus is restricted to intrinsic neuronal elements and in the nigra is found on terminals of the striatonigral projection. Overall, the autoradiographic distribution of inositol 1,4,5-trisphosphate receptors resembles the distribution of phorbol ester binding sites associated with protein kinase C. However, the inositol 1,4,5-trisphosphate receptor has a more restricted distribution since it is not detectable in the spinal cord or olfactory bulb, regions with substantial levels of protein kinase C.**

The phosphoinositide (PI) cycle appears to be a major second-messenger system for a wide range of neurotransmitters, hormones, and growth factors. In the PI cycle, phosphatidylinositol 4,5-bisphosphate is hydrolyzed in response to receptor stimulation to generate inositol 1,4,5-trisphosphate ($IP_3$) and diacylglycerol (Berridge and Irvine, 1984). Diacylglycerol stimulates protein kinase C (PKC), while $IP_3$ acts at membrane sites within the cell to release calcium from intracellular stores (Williamson et al., 1985; Nishizuka, 1986). Putative $IP_3$ receptors have been identified by the binding of radiolabeled $IP_3$ to membrane preparations of peripheral tissues (Baukal et al., 1985; Spät et al., 1986a, b; Guillemette et al., 1987) and the brain (Willcocks et al., 1987; Worley et al., 1987a, b; Supattapone et al., 1988a). In initial studies, we showed biochemically and autoradiographically that $IP_3$ receptor binding sites are distributed heterogeneously in the brain, closely resembling the localization of PKC in some areas but with differences in other regions (Worley et al., 1987a). In the present study, we have employed autoradiography to localize $IP_3$ receptors in brain in greater detail, comparing their distribution with that of PKC and adenylate cyclase, labeled by $^3$H-phorbol 12,13-dibutyrate ($^3$H-PDBu) and $^3$H-forskolin, respectively. We have also characterized the ontogeny of $IP_3$ receptor binding in brain and performed lesion studies to identify neuronal elements enriched in $IP_3$ receptors.

Received Mar. 28, 1988; revised May 23, 1988; accepted May 26, 1988.

This work was supported by USPHS Grants MH-18501 and DA-00266, Physician Scientist Award AG-00256 (to P.F.W.), Research Scientist Award DA-00074 (to S.H.S.). J.M.B. is a Lucille P. Markey and A.P. Sloan Fellow, and this work was supported in part by a grant from the Lucille P. Markey Charitable Trust and the Blades Center for Research in Alcoholism. We wish to thank V.S. Wilson for excellent technical assistance, Nancy Bruce and Dawn C. Dodson for excellent secretarial assistance, and M.L. Oster-Granite for helpful discussions.

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## Materials and Methods

*$^3$H-$IP_3$ autoradiography.* $^3$H-$IP_3$ autoradiography was carried out as described (Worley et al., 1987a). Male Sprague-Dawley rats were anesthetized with pentobarbital and perfused via the left cardiac ventricle with buffer containing 50 mM sodium phosphate, pH 7.5, and 100 mM NaCl, followed by 50 mM sodium phosphate, pH 7.5, in 0.3 M sucrose. Brains were removed and frozen in brain paste on microtome chucks. Cryostat sections (10 μM) were thaw-mounted on subbed slides and stored at −20°C until use. $IP_3$ binding sites were labeled by incubating brain sections for 10 min at 4°C in Buffer A (20 mM NaCl, 20 mM Tris-HCl, pH 7.7, 100 mM KCl, 1 mM EDTA, 1 mg/ml BSA) containing 50 nM $^3$H-$IP_3$ (NEN/DuPont, Boston; 3.6 Ci/mmol), then washed twice for 2 min in the same buffer. Preliminary experiments verified that specific $IP_3$ binding reaches a plateau under these incubation conditions. Nonspecific labeling was determined in the presence of 5 μM unlabeled $IP_3$ and represents less than 5% of total $^3$H-$IP_3$ binding in all areas. Autoradiograms were prepared by apposing labeled sections to Ultrofilm for 4–8 weeks. Autoradiograms were analyzed using a computerized microdensitometer (Loats Associates, Westminster, MD), and optical densities were converted to equivalent disintegrations/min (DPM)/mg of methacrylate (autoradiographic $^3$H-microscales, Amersham, Chicago), as described by Unnerstall et al. (1982). $^3$H-PDBu and $^3$H-forskolin autoradiography were performed as described (Worley et al., 1986a, b).

*Lesion studies.* Quinolinic acid (Sigma, St. Louis) was used to lesion the hippocampal formation and caudate nucleus (Schwarcz et al., 1983), as described (Worley et al., 1986a). The lesions were characterized for extent and selectivity by histologic examination of the brain sections following toluidine blue or acetylcholinesterase stains.

Mice were obtained from Jackson Laboratories (Bar Harbor, ME). The mutants used were *lurcher* and *reeler* (Mullen and Herrup, 1979; Rosenberg, 1986). Cerebellar sections were processed for autoradiography as described above.

## Results

### *Regional distribution of $IP_3$ binding sites*

The techniques employed in this study for autoradiographic analysis of $^3$H-$IP_3$ binding are the same as those used in our earlier report (Worley et al., 1987a). To ensure that $^3$H-$IP_3$ binds selectively to $IP_3$ receptors, we showed that the relative potencies of various inositol phosphates in competing for binding sites in brain sections is the same as in homogenate binding studies. Moreover, we conducted studies to rule out the possibility that $^3$H-$IP_3$ was binding to inositol phosphatases or $IP_3$ kinase (Worley et al., 1987b).

In confirmation of our earlier findings, $IP_3$-associated grains

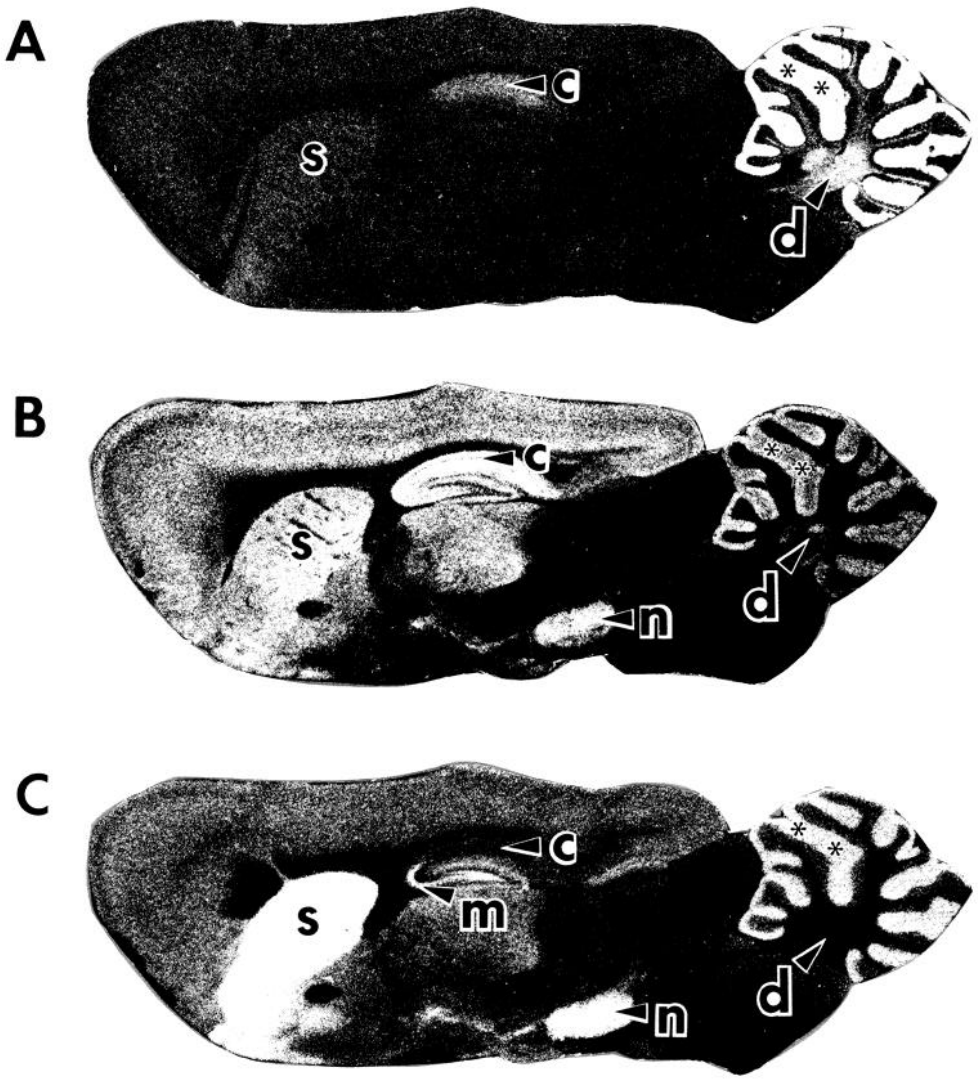


*Figure 1.* Autoradiographic localizations of (*A*) $^3$H-$IP_3$, (*B*) $^3$H-PDBu, and (*C*) $^3$H-forskolin in adult rat brain. Adjacent sagittal brain sections from adult rat were processed for autoradiography to illustrate differential localization of these ligands as discussed in text. Although the density of staining in the cerebellar molecular layer appears similar for all 3 ligands, the number of binding sites for $^3$H-forskolin is approximately 10-fold lower than for either $^3$H-$IP_3$ or $^3$H-PDBu, which are comparable (Worley et al., 1986b, 1987a). Structures are labeled as follows: *d,* deep cerebellar nuclei; *n,* substantia nigra; *s,* striatum; *c,* CA1 region of the hippocampus; *m,* mossy fiber layer; *, molecular layer of cerebellum.

are most dense in the cerebellar molecular layer (Figs. 1 and 2). This localization is in striking contrast to the underlying brain stem, which is devoid of autoradiographic grains. Moderate levels of grain density are apparent in the deep nuclei of the cerebellum and certain white matter regions extending into the cerebellar folia.

PKC, labeled with a tritiated phorbol ester, $^3$H-PDBu, is also enriched in the molecular layer of the cerebellum and the deep nuclei. By contrast, $^3$H-forskolin, which labels the $G_s$-adenylate cyclase complex (Seamon et al., 1984), shows pronounced density in the molecular layer of the cerebellum without labeling the deep nuclei (Worley et al., 1986b).

The second highest density of $IP_3$ receptors occurs in the hippocampus, resembling levels in the cerebellar deep nuclei. Within the hippocampus, binding is most concentrated in the CA1 region; much less binding occurs in CA3 and CA4, though a moderate level of binding is apparent in the dentate gyrus. In contrast to the closely similar localization of $IP_3$ receptors and PKC in the cerebellum, they differ in their localizations in the hippocampus, as PKC is present in high levels in all regions of the hippocampus. As observed previously (Worley et al., 1986b), $^3$H-forskolin binding differs strikingly from that of $IP_3$ in the hippocampus, with highest densities in the molecular layer of the dentate gyrus and the mossy fiber projection to CA3.

Levels of $IP_3$ receptors in the caudate are somewhat lower than those in the hippocampus, with still lower, barely detectable levels in the adjacent globus pallidus. These relative distributions are similar to those of $^3$H-PDBu. However, levels of $^3$H-PDBu binding in the caudate are similar to those in the cerebellum, while $IP_3$ receptor binding is much greater in the cerebellum than the caudate. Relative to the cerebellum, $^3$H-forskolin densities in the caudate are the highest of the 3 ligands examined. For $^3$H-forskolin and $^3$H-PDBu, grain densities are readily visualized in the descending striatonigral pathway and the substantia nigra.

In the cerebral cortex, $IP_3$ binding is most concentrated in the superficial layers. In the more anterior cortex, discrete densities of $IP_3$ binding sites are apparent in the anterior olfactory nucleus. $IP_3$ receptors are not detected in the adjacent olfactory bulb, contrasting with the high density of PKC in the external plexiform layer of the olfactory bulb reported previously (Worley et al., 1986a).

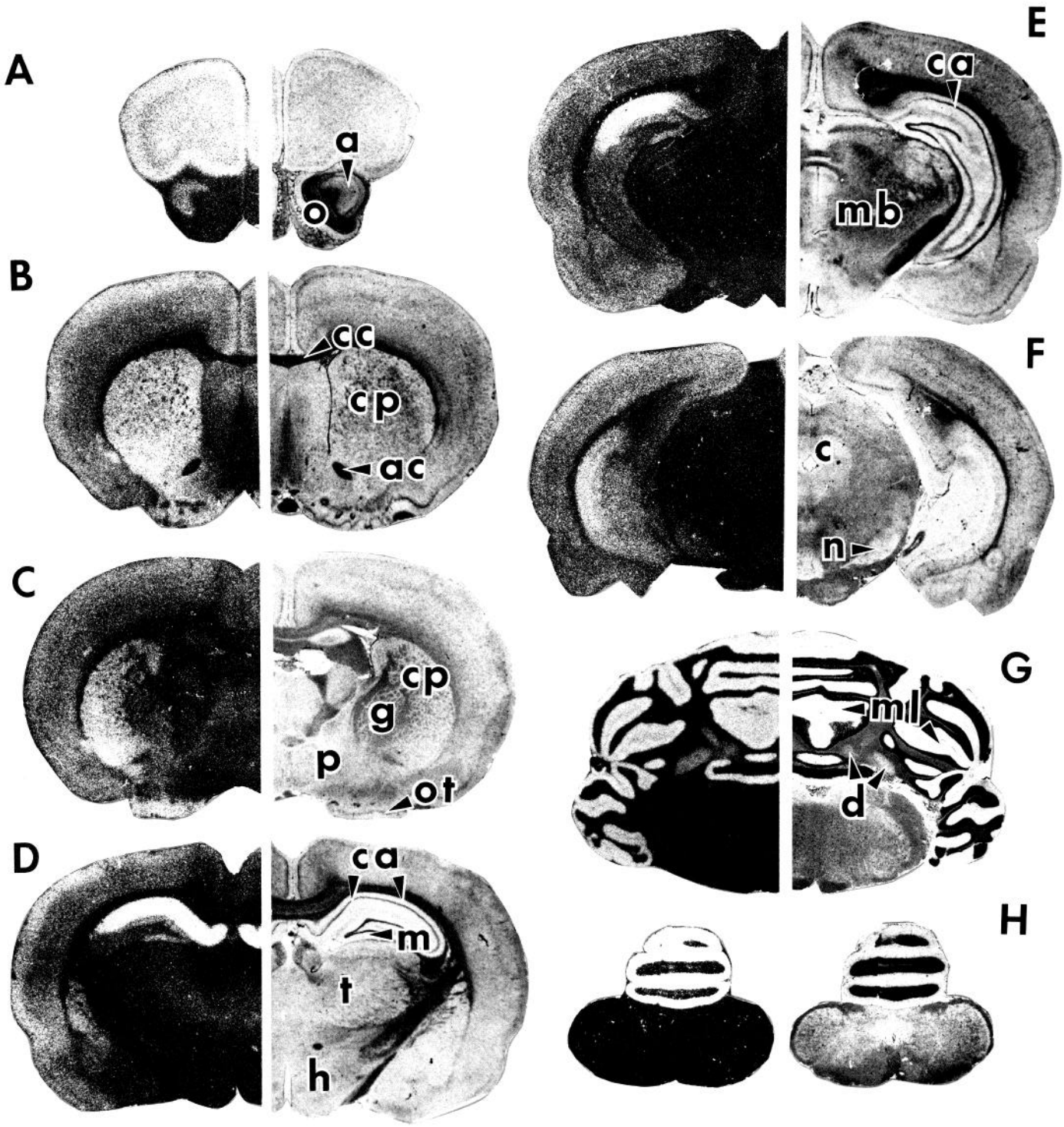


*Figure 2.* Autoradiographic localization of $^3$H-IP$_3$ receptor binding sites in rat brain. Coronal sections of adult rat brain at 8 levels were processed for $^3$H-IP$_3$ autoradiography (*left* half of each composite) or standard histology with toluidine blue (*right* half of each composite). *A,* Heavy labeling with $^3$H-IP$_3$ is apparent in the anterior olfactory nucleus (*a*) but not in the olfactory bulb (*o*). *B,* Superficial layers of the neocortex are more intensely labeled than deep layers. The caudate-putamen (*cp*) is also labeled, while the corpus callosum (*cc*) and anterior commissure (*ac*) are devoid of staining. *C,* Caudate-putamen (*cp*) and olfactory tubercle (*ot*) are more heavily labeled than the globus pallidus (*g*) or the preoptic area (*p*). *D* and *E,* The CA1 region of the hippocampus (*ca*) shows marked selective labeling, with lower levels in molecular layer of the dentate (*m*). $^3$H-IP$_3$ labeling of the thalamus is low but detectable, while labeling of the hypothalamus (*h*) and midbrain (*mb*) are negligible. *F,* Although most of the brain stem, including the central gray (*c*) is devoid of labeling, significant binding is apparent in the substantia nigra (*n*). *G* and *H,* The cerebellum contains intense labeling restricted to the molecular layer and deep cerebellar nuclei, while the brain stem has none.

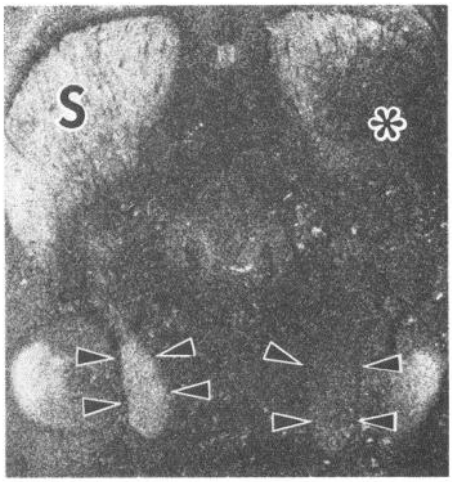


*Figure 3.* Effect of striatal lesion on $^3$H-$IP_3$ binding. Quinolinic acid (200 nmol) was injected in the striatum unilaterally 4 d prior to sacrifice. In a horizontal section through the striatum (*s*) and substantia nigra (*arrowheads*), processed for $^3$H-$IP_3$ binding, $^3$H-$IP_3$ binding sites are decreased in the injected striatum (*asterisk*) and the ipsilateral nigra.

### *$IP_3$ receptor distribution in mutant mice and in lesioned rats*

$IP_3$ binding is present in both the caudate and the zona reticulata of the substantia nigra (Fig. 3). To ascertain if $IP_3$ receptors, like forskolin binding sites and PKC, are contained in the descending striatonigral pathway, we evaluated the effect of unilateral quinolinic acid lesions of the caudate on $^3$H-$IP_3$ binding. Animals were sacrificed 4 d after injection to minimize possible transsynaptic effects on binding. These caudate lesions induce a loss of $IP_3$ receptor-associated silver grains in the ipsilateral nigra, indicating that $IP_3$ receptors are contained in the descending striatonigral pathway.

Previously, we showed that quinolinic acid lesions of the hippocampus, which destroy intrinsic neuronal elements, deplete a major portion of $^3$H-PDBu binding (Worley et al., 1986a). Similar lesions, evaluated 4 d postinjection, elicit a total depletion of $IP_3$ binding in the hippocampus (Fig. 4). The selectivity of this quinolinic acid lesion is ensured by the preservation of acetylcholinesterase staining in these sections, as acetylcholinesterase is contained in the terminals of the septohippocampal afferent projection (Lewis et al., 1967). Accordingly, in the hippocampus, $IP_3$ receptors appear to be localized to intrinsic neurons.

To establish the localization of $IP_3$ receptors in the cerebellum, we utilized strains of mutant mice. The *lurcher* mouse (lc/+) displays a total loss of Purkinje cells with no other major neuronal cell loss at the age studied (Rosenberg, 1986). $IP_3$ receptors are totally absent in cerebellar sections from *lurcher* mice, while a normal pattern is apparent in unaffected littermates (+/+) (Fig. 5). By contrast, in *lurcher* mice, $^3$H-forskolin binding is retained in the cerebellum, although at reduced levels. The lower levels of $^3$H-forskolin binding presumably are related to the much smaller size of the molecular layer in *lurcher* mice compared with littermate control animals. The *reeler* mouse (rl/rl) cerebellum is characterized by a greatly reduced granule cell population and by abnormal migration of the various elements during ontogeny (Mullen and Herrup, 1979). In *reeler* mice, $^3$H-$IP_3$-associated silver grains in cerebellum are as dense as those of the phenotypically normal littermate (+/+ or rl/+) but are not in the normal location. The altered localization of $IP_3$ receptors fits with the displaced pattern of Purkinje cells in *reeler* mice.

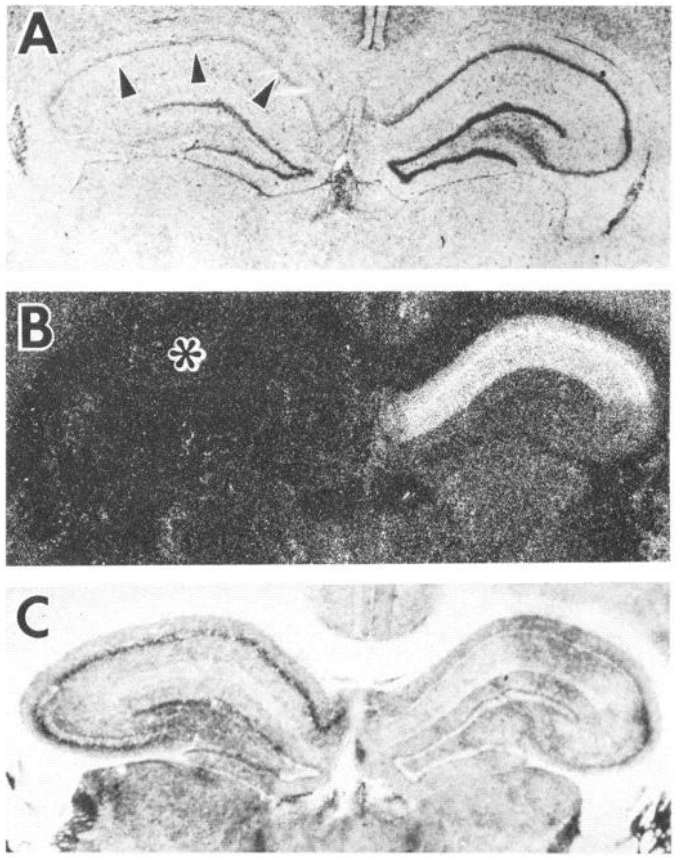


*Figure 4.* Effect of hippocampal lesion on $^3$H-$IP_3$ binding sites. *A*, Toluidine blue stain of coronal section of rat brain lesioned in the left hippocampus with 30 nmol of quinolinic acid. Pyramidal and granule cells which stain darkly in unlesioned side are clearly damaged in treated hippocampus (*arrows*). *B*, $^3$H-$IP_3$ autoradiogram of an adjacent section demonstrating striking loss of $^3$H-$IP_3$ binding in lesioned hippocampus (*asterisk*). *C*, Enhanced acetylcholinesterase staining of an adjacent section on the lesioned side indicates that the lesion spared afferent cholinergic terminals.

### *Ontogeny of $IP_3$ receptors, protein kinase C, and $^3$H-forskolin binding*

We compared the autoradiographic localization of $IP_3$ receptors, $^3$H-PDBu and $^3$H-forskolin binding in newborn and 5- and 16-d-old rats (Fig. 6, Table 1). The principal localization of $IP_3$ receptors is in the cerebellum. The molecular layer of the newborn cerebellum possesses approximately 5% of the $^3$H-$IP_3$ binding present in the adult molecular layer, while 16-d-old animals display about 90% of adult levels. The width of the molecular layer increases throughout this period, further accentuating developmental differences in total cerebellar $^3$H-$IP_3$ binding. The increase in grain density with age parallels the development of Purkinje cell dendrites in the cerebellum (Robain et al., 1981).

In a previous study we demonstrated $^3$H-PDBu binding in fetal rat brain with substantial densities in the area of the cerebellum in 18-d-old fetuses but negligible levels in higher centers (Murphy et al., 1983). In the present study we observe very little $^3$H-PDBu binding in the newborn rat cerebellum, with grain

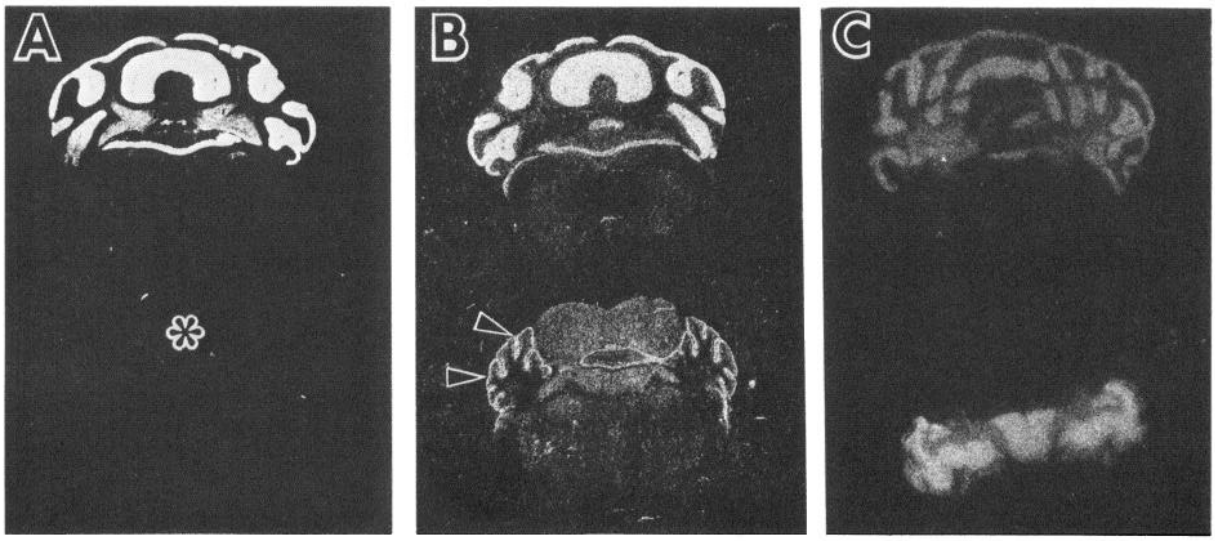


*Figure 5.* $^3$H-IP$_3$ receptor binding in cerebellar sections of mutant mice. Coronal sections at the level of the cerebellum were taken from *lurcher* or *reeler* mice and processed for $^3$H-IP$_3$ (*A* and *C*) and $^3$H-forskolin (*B*) autoradiography. Littermate control (*top*) and mutant mouse cerebella were mounted in the same tissue block to ensure identical section thickness and autoradiographic conditions. *A,* Sections from an unaffected littermate (*top*) yield strong labeling with $^3$H-IP$_3$ in the molecular layer and deep cerebellar nuclei as observed in rat. However, in the *lurcher* cerebellum (*bottom, asterisk*), no binding is observed. *B,* Adjacent sections to those used in *a* were processed with $^3$H-forskolin. Control littermate section shows typical pattern (*top*). In section from affected *lurcher* (*bottom*), labeling is present in the cerebellar molecular layer (*arrows*), which is thin due to degeneration of Purkinje cells. *C,* Section through cerebellum of affected *reeler* mouse (*bottom*) and control littermate (*top*) demonstrating preserved $^3$H-IP$_3$ binding despite loss of granule cells in this mutant.

densities appearing in 5- and 16-d-old animals in association with Purkinje cell ontogeny. Accordingly, the development of PKC in the cerebellum parallels that of IP$_3$ receptors. The ontogenetic development of $^3$H-PDBu binding in the hippocampus, caudate, and substantia nigra is fairly similar to that of the cerebellum. These autoradiographic findings fit well with biochemical measures of PKC activity during development (Turner et al., 1984).

Within the cerebellum, granule cells develop at a later stage than Purkinje cells (Altman, 1982). This accords with our observation that $^3$H-forskolin binding in the cerebellum develops somewhat later than $^3$H-PDBu or IP$_3$ receptor binding (Table 1), as $^3$H-forskolin binding is localized to granule cell processes (Worley et al., 1986b). Striatal $^3$H-forskolin binding also continues to increase between day 16 and adulthood. In contrast to the relatively late development of $^3$H-forskolin binding in the cerebellum, $^3$H-forskolin binding to the neocortex and CA1 region of the hippocampus parallels that of $^3$H-PDBu and $^3$H-IP$_3$, reaching adult levels by day 16. In the newborn rat striatum, $^3$H-forskolin binding is approximately 15% that of adult striatum and displays a patchy appearance. Similar patchiness is seen in newborn striatal $^3$H-PDBu binding. The patchiness apparent for $^3$H-forskolin and $^3$H-PDBu is also present at day 5 but is diminished at day 16 and absent in the adult striatum. DARPP-32, a protein substrate for the dopamine-sensitive adenylate cyclase shows a similar pattern of distribution in the striatum, with patchiness at birth that diminishes in the course of ontogeny (Ouimet, 1984; Foster et al., 1987).

## Discussion

In the present study we have characterized the discrete localizations of IP$_3$ receptors throughout the brain. In most areas of the brain, IP$_3$ receptor localization resembles that of PKC, though there are a number of differences. For instance, IP$_3$ receptor density is vastly higher in the cerebellum than any other brain region, whereas PKC is more uniformly distributed. Certain

**Table 1. Postnatal development of $^3$H-IP$_3$, $^3$H-PDBu, and $^3$H-forskolin binding in brain**

| | Density of ligand binding (% of adult levels) at indicated age (d) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cerebellar molecular layer | | | Neocortex | | | Hippocampus CA1 | | | Striatum | | |
| *Region* | 0 | 5 | 16 | 0 | 5 | 16 | 0 | 5 | 16 | 0 | 5 | 16 |
| *Ligand* | | | | | | | | | | | | |
| $^3$H-IP$_3$ | 5 | 36 | 89 | <15 | 45 | 115 | <10 | 33 | 116 | <12 | 32 | 120 |
| $^3$H-PDBu | 18 | 37 | 80 | 7 | 34 | 100 | 9 | 27 | 85 | 27 | 41 | 78 |
| $^3$H-forskolin | 8 | 9 | 34 | 16 | 29 | 100 | 21 | 27 | 100 | 15 | 15 | 60 |

Autoradiographic images representing $^3$H-IP$_3$, $^3$H-PDBu, and $^3$H-forskolin binding were densitized and in selected regions binding was quantified in animals 0, 5, 16, and 43 d, and 3 months old. Labeling at 43 d and 3 months was identical in all regions surveyed. Optical densities were converted to dpm using Amersham $^3$H-microscales. Binding is expressed as a percentage of adult levels for each structure. Data are from a minimum of 4 brain sections for each region and dpm bound varied by less than 10% (SEM).

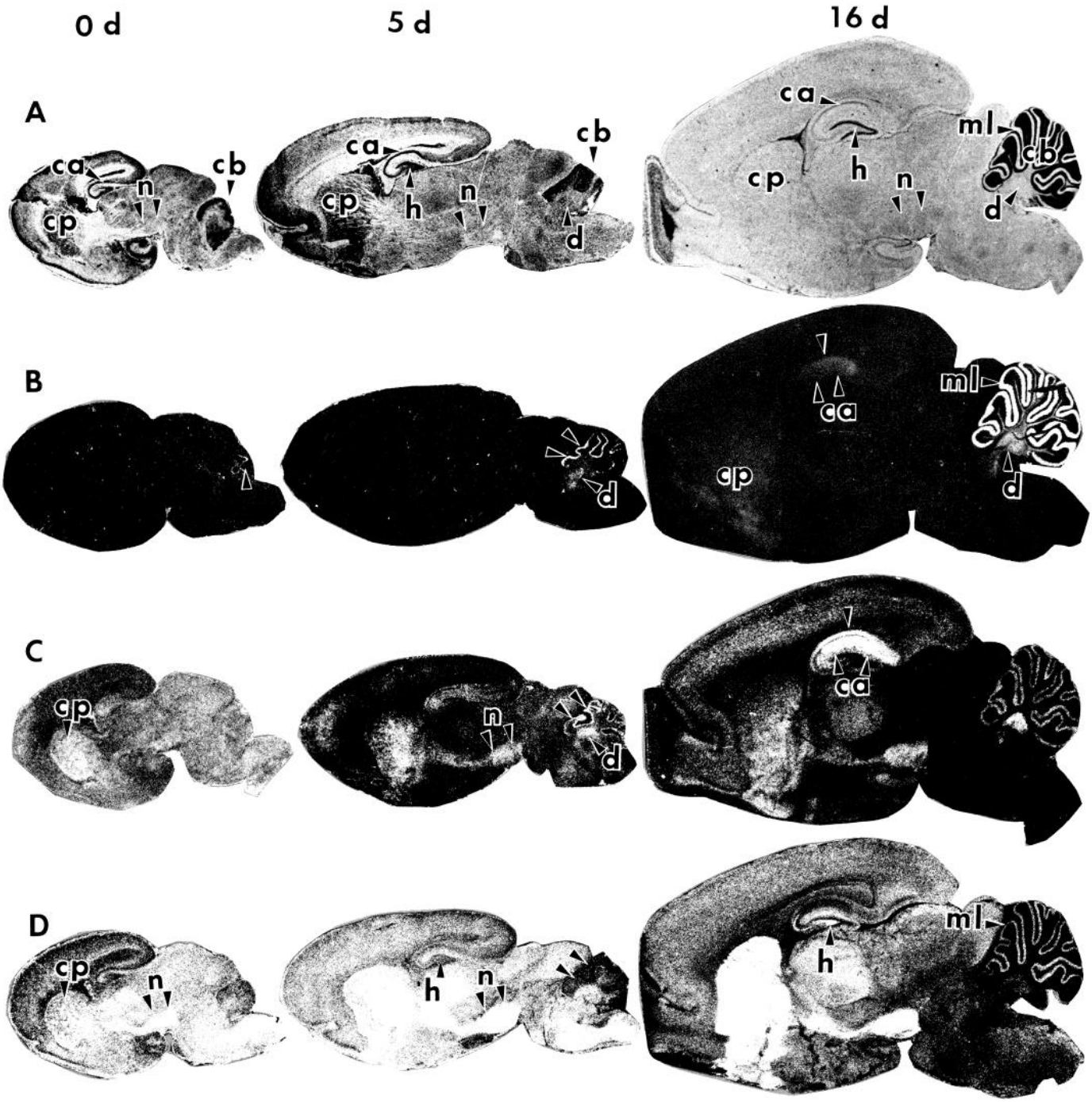


*Figure 6.* Ontogeny of second-messenger systems. Parasagittal sections through rat brains were taken at days 0, 5, and 16 and processed for Nissl stain with toluidine blue (*A*) or autoradiography with $^3$H-$IP_3$ (*B*), $^3$H-PDBu (*C*), and $^3$H-forskolin (*D*). *A,* Toluidine blue micrographs demonstrate the morphologic development of the caudate-putamen (*cp*), substantia nigra (*n*), hippocampal regions CA1 (*ca*), dentate hilus (*h*), neocortex, and cerebellum (*cb*), with the deep cerebellar nucleus (*d*) and molecular layer (*ml*). *B,* $^3$H-$IP_3$ binding is first detected at day 0 in a fine ribbon of cerebellar molecular layer (*arrows*) and becomes more prominent at day 5 in both the deep cerebellar nucleus (*d*) and the molecular layer (*arrows*). By day 16, binding is enhanced in the cerebellar molecular layer and deep cerebellar nuclei and extends into the folia. Less intensely labeled forebrain structures that demonstrate binding by day 16 include the CA1 region of the hippocampus (*ca*) and the caudate-putamen (*cp*). *C,* $^3$H-PDBu labels the caudate-putamen at day 0 with a patchy pattern, and by day 5 the substantia nigra (*n*) is well demarcated. Binding to the cerebellar molecular layer and deep cerebellar nucleus is similar to the pattern seen with $^3$H-$IP_3$ binding. By day 16, $^3$H-PDBu binding is most enriched in the hippocampal CA1 region (*ca*). Labeling of the neocortex, which at days 0 and 5 is less than the caudate-putamen, becomes comparable to the caudate-putamen at day 16. *D,* $^3$H-forskolin binding at day 0 is enriched in the caudate-putamen (*cp*), substantia nigra (*n*), and brain stem. Labeling of the caudate-putamen is patchy at day 0 and day 5 but homogeneous by day 16. The cerebellar molecular layer, which is labeled at day 5 by $^3$H-$IP_3$ and $^3$H-PDBu, is distinctly low in $^3$H-forskolin binding at day 5 (*arrows*). By contrast, at day 16, the cerebellar molecular layer is labeled. Similarly, the dentate hilus (*h*), is intensely labeled in the 16-d-old animal (and adult) but not in the 5-d-old animal.

areas of the CNS possess abundant PKC with no detectable $IP_3$ receptor binding, as exemplified by the external plexiform layer of the olfactory bulb and the substantia gelatinosa of the spinal cord (Worley et al., 1987a). This discrepancy may be related to diacylglycerol production from sources other than $PIP_2$ in these areas. It would be of interest to compare the localization of $IP_3$ receptors to the specific phospholipase C that generates $IP_3$. A recent immunohistochemical study localized 2 distinct phosphoinositide-specific phospholipase C enzymes in rat brain (Gerfen et al., 1988). One of these closely mimics the localizations we have observed of phorbol ester binding associated with protein kinase C (Worley et al., 1986a) and of the $G_o$ protein, which may be linked to the phosphinositide cycle (Worley et al., 1986c).

Based on studies of mutant mice, cerebellar $IP_3$ receptors appear to be selectively localized to Purkinje cells. Multiple types of PKC have been identified (Huang et al., 1986; Kikkawa et al., 1987; Ohno et al., 1987). Type I appears to be brain specific (Shearman et al., 1987) and selectively concentrated in Purkinje cells of the cerebellum (Brandt et al., 1987; Kitano et al., 1987), closely resembling the distribution of PKC monitored by autoradiography with $^3$H-PDBu (Worley et al., 1986a) and of $^3$H-$IP_3$ receptor binding. Other studies have demonstrated the presence of type II PKC in granule cells of the cerebellum (Brandt et al., 1987), which appear to be devoid of $^3$H-$IP_3$ binding. Thus, the localization of $IP_3$ receptor binding in cerebellum parallels that of type I PKC.

$IP_3$ receptors are not restricted to one part of neurons. For instance, in the hippocampus, $IP_3$ receptors are localized to all layers of CA1, suggesting that it is present in cell bodies and dendrites of intrinsic neurons. In the cerebellum, $IP_3$ receptors are present throughout the molecular layer, which contains Purkinje cell dendrites. In the striatonigral pathway, $IP_3$ receptors appear to be contained in axons and terminals. In the cerebellum it is probable, though not definitely established, that $IP_3$ receptors are present throughout Purkinje cell axons and extend into terminals in the deep cerebellar nuclei. These conclusions, based on lesion studies, cannot totally exclude transsynaptic effects. However, we examined animals 4 d after lesions, a period that is probably not long enough to permit major transsynaptic alterations.

Recently, we solubilized $IP_3$ receptors from the cerebellum and purified the receptor protein to homogeneity (Supattapone et al., 1988a). It is a glycoprotein with a subunit size of 270 kDa that is phosphorylated by cyclic AMP-dependent protein kinase but not by PKC or calcium-calmodulin kinase (Supattapone et al., 1988b). The $IP_3$ receptor appears to be identical to a previously isolated phosphoprotein of the cerebellum referred to as PCPP-260 (Walaas et al., 1987). Interestingly, PCPP-260 was shown to be localized to Purkinje cells in the cerebellum by experiments utilizing mutant mice similar to those in the present study. The identity of the $IP_3$ receptor with PCPP-260 also fits well with evidence that PCPP-260 is phosphorylated by cyclic GMP-dependent protein kinase (Weeks et al., 1988), as cyclic GMP-dependent protein kinase is present in extremely high levels in the Purkinje cells of the cerebellum (DeCamilli et al., 1984). A physiological link of the $IP_3$ receptor and cyclic GMP is suggested by the ability of $IP_3$ to elevate cyclic GMP levels in permeabilized neuroblastoma cells (Amar et al., 1987).

A presumed major function of $IP_3$ is to release intracellular calcium after binding to its receptors (Berridge and Irvine, 1984; Williamson et al., 1985). $IP_3$ receptor binding in brain is highly sensitive to calcium ions, which inhibit binding 50% at 300 nM (Worley et al., 1987b). Conceivably, this reflects a feedback mechanism whereby calcium released by $IP_3$ prevents further interactions of $IP_3$ with its receptor. Heparin, which binds to the $IP_3$ receptor (Supattapone et al., 1988a) and potently displaces $^3$H-$IP_3$ binding (Worley et al., 1987b), also potently blocks $IP_3$-induced calcium release (Hill et al., 1987; Ghosh et al., 1988). These observations suggest that the $IP_3$ receptor binding sites that we have characterized, and which are enriched in the cerebellum, are involved in calcium release similar to peripheral $IP_3$ receptors. The regional distribution of $^3$H-inositol phosphate generation from $^3$H-inositol added to brain slices differs from that of the $IP_3$ receptor, with the cerebellum displaying low levels (Rooney and Nahorski, 1986). However, such a comparison is meaningful only if phosphoinositide turnover in each region is stimulated by ligands that physiologically regulate such turnover in the various brain areas.

It is noteworthy that the distribution of a cytoplasmic, 28 kDa calcium binding protein (calbindin) resembles that of $^3$H-$IP_3$ binding sites (Baimbridge and Miller, 1982; Baimbridge et al., 1982; Miller and Baimbridge, 1983). For instance, in the cerebellum this calcium binding protein is also selectively localized to Purkinje cells and in the hippocampus is concentrated in $CA_1$ and the molecular layer of the dentate gyrus. Also, levels of calbindin in cerebellum are approximately 10-fold higher than in hippocampus, similar to the $IP_3$ receptor. The similar localizations of calbindin and the $IP_3$ receptor suggest mutual roles in modulating neuronal calcium signaling.

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