Dopamine Affects Parvalbumin Expression during Cortical Development \textit{In Vitro}

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This study was undertaken to determine how dopamine influences cortical development. It focused on morphogenesis of GABAergic neurons that contained the calcium-binding protein parvalbumin (PV). Organotypic slices of frontoparietal cortex were taken from neonatal rats, cultured with or without dopamine, harvested daily (4–30 d), and immunostained for parvalbumin. Expression of parvalbumin occurred in the same regional and laminar sequence as \textit{in vivo}. Expression in cingulate and entorhinal preceded that in lateral frontoparietal cortices. Laminar expression progressed from layer V to VI and finally II–IV. Somal labeling preceded fiber labeling by 2 d.

Dopamine accelerated PV expression. In treated slices, a dense band of PV-immunoreactive neurons appeared in layer V at 7 d \textit{in vitro} (DIV), and in all layers of frontoparietal cortex at 14 DIV, whereas in control slices such labeling did not appear until 14 and 21 DIV, respectively. The laminar distribution and dendritic branching of PV-immunoreactive neurons were quantified. More labeled neurons were in the superficial layers, and their dendritic arborizations were significantly increased by dopamine. Treatment with a D1 receptor agonist had little effect, whereas a D2 agonist mimicked dopamine’s effects. Likewise, the D2 but not the D1 antagonist blocked dopamine-induced changes, indicating that they were mediated primarily by D2 receptors.

Parvalbumin expression was accelerated by dopaminergic reinnervation of cortical slices that were cocultured with mesencephalic slices.

Key words: organotypic; tissue culture; corticogenesis; interneuron; GABA; dopamine; development

Early perturbations to neurotransmitters that regulate cortical development change the time course and outcome of corticogenesis. Prenatally altered catecholamine levels cause persistent changes in dendritic morphology of pyramidal neurons, numbers of GABAergic neurons, and dendritic lengths of interneurons (Wang et al., 1995, 1996; Jones et al., 1996). Neonatal monoamine depletion delays normal cortical lamination (Osterheld-Haas and Hornung, 1996). These developmental influences may be mediated by transmitter-induced alterations of neuronal activity.

Subsets of GABAergic neurons contain calcium-binding proteins (Celio, 1986, 1990; Hendry et al., 1989; van Brederode et al., 1990, 1991; DeFelipe, 1993; Conde et al., 1994) that may protect developing neurons against fluctuating levels of metabolic activity by buffering intracellular calcium (Seto-Ohshima et al., 1990; Lukas and Jones, 1994). Parvalbumin (PV), a calcium-binding protein in basket and chandelier cells (Hendry et al., 1989; DeFelipe, 1993), is thought to regulate intracellular free calcium fluctuations caused by membrane depolarization (Chard et al., 1993). Parvalbumin expression occurs late in rodent and primate development through a sequence of postnatal events (Hendrickson et al., 1991; Alcantara et al., 1993; Yan et al., 1995; Berger and Alvarez, 1996): somata appear first in layer V, then in layer VI, and finally in superficial layers. Fiber labeling, including pericellular baskets, succeeds that of somata by \textasciitilde{} 2 d.

Parvalbumin expression is linked to neuronal activity in adult and developing cortex. Parvalbumin-containing neurons occur in areas of high metabolic activity, such as cytochrome oxidase-rich blobs of visual cortex (Celio et al., 1986) and barrels of somatosensory cortex (Cohen et al., 1993). Conversely, chronic monocular deprivation in adult primates causes decreases in PV and cytochrome oxidase staining in the deprived ocular dominance column (Gutierrez and Cusick, 1994; Carder et al., 1996). Individual neurons in barrel cortex stain intensely for 2-deoxyglucose and PV (Maier and McCasland, 1997). Furthermore, PV is localized in fast spiking cells of rodent cortex (Kawaguchi et al., 1987; Kawaguchi and Kubota, 1993, 1997). In the developing monkey striate and rat visual cortex, PV expression begins in late fetal life but increases with postnatal onset of visual function (Hendrickson et al., 1991; Solbach and Celio, 1991). Conversely, decreased activity induced by monocular deprivation during the critical period of development in rats leads to reduced PV expression in the cortex contralateral to the deprived eye (Cellerino et al., 1992). It seems then that neurotransmitter-induced increases in cortical activity will enhance PV expression. Dopamine (DA) may increase cortical activity by activation of adenylate cyclase, which enhances glutamate channel conductivity (McMahon et al., 1994). Dopamine innervation of rodent cortex begins prenatally, but peaks at approximately postnatal day (PN) 14 (Berger et al., 1983). D1 and D2 receptor mRNA expression also peaks late (PN...
The dense band of PV-ir neuronal somata with labeled dendrites is evident in layer V, and a few lightly labeled cells are evident in layer VI.

An untreated sister culture harvested at 14 DIV. The pattern of labeling for PV is similar to that in the 7 DIV DA-treated culture.

A slice culture, treated with DA and harvested at 14 DIV. The dense band of PV-ir neuronal somata in layer V and the neurons in layer VI have extensive dendritic labeling. PV-ir neurons are seen throughout the superficial laminae, and some of these cells have labeled dendrites.

An untreated sister culture harvested at 21 DIV. The pattern of PV labeling is similar to that in the 14 DIV DA-treated culture.

The laminar distribution of PV-ir neurons in a low magnification of the slices shown in C and D, respectively. Roman numerals denote laminar boundaries. Scale bars: in D for A–D, 50 μm; in F for E, F, 600 μm.

Table 1. Culture treatments

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<td>1 μM DA</td>
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<td>10 μM DA and 1 μM eticlopride&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10 μM DA and 1 μM MK 801&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>Ascorbic acid&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1 μM SK&amp;F 38393&lt;sup&gt;f&lt;/sup&gt;</td>
<td>10 μM DA and 1 μM SCH23390&lt;sup&gt;g&lt;/sup&gt;</td>
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<td>100 μM AP5&lt;sup&gt;h&lt;/sup&gt;</td>
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Stock solutions were stored for a maximum of 1 week at 4°C and diluted to working concentrations in control medium at the time of medium exchanges. Each group consists of 12–16 slices, half of which were harvested at 7 or 14 DIV.

<sup>a</sup>DA HCl in 0.01% ascorbic acid in H<sub>2</sub>O, stock solution = 10 mM.

<sup>b</sup>Quinpirole, a selective D2 agonist, in H<sub>2</sub>O, stock solution = 1.0 mM.

<sup>c</sup>Eticlopride, a selective D2 antagonist, in H<sub>2</sub>O, stock solution = 1.0 mM.

<sup>d</sup>MK801, an NMDA receptor antagonist, in H<sub>2</sub>O, stock solution = 1.0 mM.

<sup>e</sup>Ascorbic acid 0.01% in H<sub>2</sub>O.

<sup>f</sup>SK&F, a selective D1 agonist, in H<sub>2</sub>O, stock solution = 1.0 mM.

<sup>g</sup>SCH 23390, a selective D2 antagonist, in H<sub>2</sub>O, stock solution = 1.0 mM.

<sup>h</sup>AP5, an NMDA receptor antagonist, in H<sub>2</sub>O, stock solution = 100 mM.
Coronal plane at a thickness of 250 μm, whereas subcortical structures were cut away. The overlying pia was removed mid-sagittally. Coronal cuts were made to remove portions of the rostral and caudal poles, leaving the frontoparietal region intact. Midline and lateral regions of the cortex were included in the slices.

The varicosities are heavily labeled and the intervaricose segments are sparsely labeled. The pericellular basket pattern is visible, but weakly defined at this time point. Pericellular baskets are prominent in varicosities than in the intervaricose segments. This study was undertaken to determine whether DA affects the morphogenesis of GABAergic neurons that contain PV, and the mechanisms mediating these changes.

**MATERIALS AND METHODS**

Organotypic slice cultures were prepared from neonatal rats aged PN 2 or 3. All animal procedures were approved by the Institutional Committee for Evaluation of Animal Care and Use and were in compliance with National Institutes of Health Guidelines for the Care and Use of Animals. The pups were anesthetized by hypothermia and then quickly decapitated under aseptic conditions. The brains were rapidly removed and placed into cold sterile MEM (Life Technologies, Gaithersburg, MD) with 200 mM Tris buffer (dissecting medium). The brains were placed into a Millicell-CM (Millipore, Bedford, MA) insert that was immersed in dissecting medium (Life Technologies) and 200 mM glutamine. Slices were maintained in medium at a level that provided an air–medium interface (Stoppini et al., 1991), in a humid atmosphere at 35°C, in 5% CO2. Medium was refreshed every 2–3 d by removing half of the existing medium and replacing it with fresh medium. Serum levels were reduced to 15% after 1 week. Culture medium alone was used as control medium in all trials. The following pharmacological treatments were applied: for Trial 1, slices were treated with DA (1 μM, 10 μM) or the D1 (SK&F 38393; 1 μM) or D2 (quinpirole; 1 μM) receptor agonist. For Trial 3, slices were treated with DA (10 μM) or the D1 (SCH 23390; 1 μM) or the D2 (eticlopride; 1 μM, 10 μM), or both receptor antagonists. For Trial 4, slices were treated with DA (10 μM), DA and MK801 (1 μM), DA and AP5 (100 μM), MK801, or AP5. Treatments are summarized in Table 1.

In all instances, the treatments were applied throughout the entire cultivation period. Each trial was repeated at least four times using different litters. Fresh stock solutions were prepared at 1 week intervals. Initially, cortical slice cultures grown in control medium or treated with DA were harvested daily at 4 through 14, 21, 26, and 30 d in vitro (DIV). Subsequently, for Trials 1–4, slices were harvested at 7 and 14 DIV only, by removing two of four inserts from each group at these time points.

Cortical and mesencephalic slices were cocultured to allow dopaminergic reinnervation of the cortex. Slices of the midbrain, containing substantia nigra pars compacta, were collected from brains prepared as described above, cut in the coronal plane at 300 μm, and placed in a Millicell insert adjacent to a cortical slice taken from a litter mate. The ventrolateral surface of the midbrain was placed ~0.5–1 mm away from the pial surface of the cortex. Control cocultures were prepared by placing the cortex and midbrain slices in separate inserts but in the same medium.
Table 2. Sequence of parvalbumin expression

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<th>DIV</th>
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1. Somata in layers V–VI of cingulate/perirhinal cortex; 2, somata in layers II–III of cingulate/perirhinal cortex; discontinuous band of somata in layer V of frontoparietal cortex 3, continuous band of somata in layer V of frontoparietal cortex; 4, sparse fibers in layer V of frontoparietal cortex; 5, somata with dendrites and dense fibers in layer V of frontoparietal cortex; 6, somata in layer VI of frontoparietal cortex; 7, somata with dendrites in layer VI of frontoparietal cortex; 8, pericellular axonal baskets in layer V of frontoparietal cortex; 9, somata in layers II–III of frontoparietal cortex; 10, sparse fibers in layers II–III of frontoparietal cortex; 11, dense fibers and pericellular baskets in layers II–III of frontoparietal cortex; 12, somata with dendrites in layers II–III of frontoparietal cortex; bilaminar distribution of somata in II and V–VI of frontoparietal cortex; 14, dense axons and extensive dendrites in layers I–VI of frontoparietal cortex.

RESULTS

Dopamine specifically modulates the expression of PV in cortical neurons during development

To screen for neuronal proteins whose expression would be affected by the presence of DA, an immunocytochemical analysis of DA-treated and control slices at 14 DIV was performed, using markers for interneurons (calbindin, parvalbumin, calretinin) and pyramidal neurons (neurofilaments, MAP2a+b). All proteins were readily detected in the cultures (data not shown) and were localized in the predicted cell types. Of all the markers, only PV appeared to have an expression and distribution altered by the DA treatment, as described below. Therefore, the quantitative assessments focused only on PV-labeled slices.

In the control slices, PV-ir neurons first appeared in the deep laminae of cingulate and perirhinal cortices at 6 DIV. At this time, these neurons had only their somata immunolabeled. Both PV-ir dendrites and axons (as evidenced by beaded fibers in the neuropil) were observed around these neurons at 8 DIV. Parvalbumin-ir somata appeared next in the superficial layers of the cingulate and perirhinal cortices at 7 DIV, and a band of sparse PV-ir somata in layer V first appeared in frontoparietal cortex at 8 DIV. Dendritic branches on labeled somata and well labeled fibers in the neuropil began to appear 2 d after the appearance of labeled somata in each region and layer, and fiber labeling in the surrounding neuropil continued to increase in density over the next several days. The PV-ir somata appeared next in deeper portions (layer VI) of frontoparietal cortex at 12 DIV. Labeling of dendrites of deeper layer VI and axonal specializations in more superficial layer V neurons were not apparent until 14 DIV. Thus, after 14 d in culture, PV-ir somata, dendrites, and axons were observed throughout all layers of the cingulate and perirhinal regions but were mostly limited to the deep laminae of the frontoparietal regions (Fig. 1B). Pericellular axonal...
baskets were found in the deep laminae of frontoparietal cortex at this time, but they were extremely difficult to distinguish, even in deeper portions of the superficial laminae (Fig. 2A, B), and axons were not visible in upper layer III and layer II. By 21 DIV a dense population of extensively labeled neurons and fibers were noted in layers V and VI, and both well labeled neurons (somata and dendritic label) and fibers had extended throughout the superficial layers II–IV (Fig. 1D, F). At 26 DIV, fiber staining in superficial laminae was more dense (data not shown).

The DA-treated slices exhibited a similar pattern of emergence of PV expression, but labeled profiles appeared at consistently earlier time points. At 4 DIV, the earliest time examined, labeled somata with dendrites were already present in the deep layers of cingulate and perirhinal cortices. By 6 DIV, some labeled somata were also present in the superficial layers of cingulate and perirhinal cortex, and a few scattered PV-ir neurons were present in layer V of frontoparietal cortex. On the following day, 7 DIV, a band of neurons containing label in both their somata and dendrites was observed in layer V throughout the frontoparietal cortex. Lightly labeled neuronal somata were also observed in layer VI at this time point (Fig. 1A). The layer VI neurons exhibited labeled dendrites by 9 DIV. The number of labeled neurons and the density of labeled fibers in both layers V and VI increased gradually over the next several days. Parvalbumin-ir somata appeared in the superficial laminae at 13 DIV, with a few labeled axons and dendrites also apparent at this time. Dense dendrites and axonal specializations in the surrounding neuropil were first noted to be immunostained at 14 DIV. The density of labeled profiles continued to increase over the next several days. Thus, at 2 weeks, neurons with labeled dendrites and labeled axons in the neuropil were clearly evident throughout all cellular layers of the frontoparietal region (Fig. 1C, E), with the most extensive dendritic labeling and fiber labeling in the deep layers. Intricate pericellular baskets were observed in the deep laminae, but distinguishable baskets were commonly observed throughout the superficial laminae as well (Fig. 2C, D). At 21 DIV, the density of label was further increased, and a dense band of horizontal fibers was evident in layer I (data not shown). These features did
not show significant changes when examined at later times. The developmental sequence of events for cortical expression of PV and the time of their emergence in the control and DA-treated slices are summarized in Table 2. The lower dose of DA (1 μM) was only minimally effective in mediating changes in PV expression. Although our culture conditions (tissue and ascorbic acid) may be optimal for slowing the decomposition rate of DA (Heikkila and Cabbat, 1983; Marien et al., 1984), this lower dose may not have been stable for a sufficient time to be effective, as was the 10 μM DA treatment. Vehicle (ascorbic acid)-treated slice cultures showed the same time course of PV expression as did the control slices.

The effects of DA on PV expression are differentially mediated by the dopamine D1 and D2 receptors

The laminar distribution and dendritic branching patterns of PV-ir neurons in control slices were compared with the slices treated with DA, vehicle, D1 or D2 receptor agonists, and DA + D1 or DA + D2 antagonists at 14 DIV. In the DA-treated slices, a greater proportion of labeled neurons appeared to be in the superficial layers, and PV-ir neurons appeared to have more extensive dendritic arbors than those of control slices (Fig. 3). Although the D1 agonist treatment did not mimic the effects of DA, the D2 agonist-treated slices showed a shift in the distribution of PV-ir neurons toward the superficial layers and an increase in dendritic labeling similar to that seen with the DA treatment (Fig. 4). A quantitative analysis showed that DA significantly altered the laminar distribution of labeled neurons across all intervals (Student’s t test, p = 0.00065) as compared with untreated slices. In the DA-treated slices, a higher percentage of PV-ir neurons were located at intervals of cortical depth that corresponded to the superficial layers (Fig. 5A). The D1 agonist treatment did not cause significant changes in laminar distribution of labeled neurons, but the D2 agonist treatment caused a shift in distribution of PV-ir neurons toward the superficial layers in comparison to control slices (Student’s t test, p = 0.014).

Ascorbic acid treatment had little effect on laminar distribution of PV-ir neurons.

Figure 4. Micrographs of PV-ir neurons in 14 DIV sister cultures. A, Labeled neurons in an untreated culture form a band in layer V with a few scattered neurons also visible in VI and II–III. B, Labeled neurons in a DA-treated culture are dense in all layers. C, Labeled neurons in a D1 agonist-treated culture form a band in layer V with a few scattered neurons also visible in VI. D, Labeled neurons in a D2 agonist-treated culture form a band in layer V with numerous neurons also visible in VI and III. E, High magnification of PV-ir neurons in layer V of the slice shown in C. Many somata are labeled, but only a few neurons show extensive dendritic branching. F, High magnification of PV-ir neurons in layer V of the slice shown in D. Many somata with extensive dendritic labeling are visible. Fiber labeling in the neuropil is dense. Scale bars: in D for A–D, 50 μm; in F for E, F, 20 μm.
Dopamine treatment also affected the dendritic arborizations of PV-ir neurons. This effect appeared to be mediated primarily by D2 receptors. Dopamine treatment resulted in a significant increase in dendritic length overall and at all individual concentric radii compared with controls. Quinpirole treatment showed a slight increase in dendritic lengths at all radial distances but was significantly different only at 100 μm from the soma. The number of intersections at concentric radii from the soma are shown. DA- and quinpirole-treated slices showed significant increases in dendritic branching of PV-ir neurons overall and at many individual radial distances. Dendritic intersections with concentric radii were noted up to 520 μm from the soma in the DA-treated group but only up to 360 μm in other groups (*p < 0.05; Student’s t test).

Figure 5. Histograms of the depth distribution and the dendritic branching patterns of PV-ir neurons in slices from the agonist trials that were maintained for 14 DIV. A. The distribution of PV-ir neurons is shown relative to the depth from the pial surface. The DA-treated slices had a higher percentage of neurons in the superficial laminae than in control slices. The other treatment groups were not significantly different from controls. B. The lengths of labeled dendrites at concentric radii from the soma are shown. Neurons in the DA-treated slices showed an increase in dendritic lengths overall and at all individual concentric radii compared with controls. Quinpirole treatment showed a slight increase in dendritic lengths at all radial distances but was significantly different only at 100 μm from the soma.

C. The number of intersections at concentric radii from the soma are shown. DA- and quinpirole-treated slices showed significant increases in dendritic branching of PV-ir neurons overall and at many individual radial distances. Dendritic intersections with concentric radii were noted up to 520 μm from the soma in the DA-treated group but only up to 360 μm in other groups (*p < 0.05; Student’s t test).
the density and laminar distribution of PV-ir neurons appeared to be similar to that of untreated slices (Fig. 8). Indeed, quantitative analysis showed no significant differences between controls and the dual antagonist-treated group. The laminar distribution of PV-ir neurons for these trials is shown in Figure 9.

**Changes in PV expression may be mediated by DA–glutamate interactions**

Cortical slices were treated with the glutamate NMDA receptor antagonists MK801 or AP5 to determine whether the effects of DA on PV expression were linked to DA-mediated changes in cortical excitability. Both MK801 and AP5 were effective in substantially blocking the DA-induced changes in laminar distribution and dendritic branching patterns of PV-ir neurons. The increase in percentage of PV-ir neurons in the superficial cortical layers at 14 DIV induced by DA treatment was attenuated in slices treated with both DA and MK801. Treatment with the antagonists alone had little effect on the laminar distribution of labeled neurons as compared with controls. Slices from the MK801 trials illustrate these effects (Fig. 10). A quantitative analysis of the depth distribution of PV-ir neurons showed that DA alone induced a significant increase in the percentage of PV-ir neurons in the superficial layers, concomitant with a decrease in the deeper layers. Although the addition of MK-801 with DA attenuated the laminar shift, it did not fully block the effect, in that the portion of labeled neurons in the superficial layers remained significantly increased compared with controls in the superficial layers (20–40% uppermost part of total cortical depth) and decreased in portions of the deeper layers (60% of depth) (Fig. 11A). MK801 was effective in blocking the DA-induced increases in dendritic arborizations of PV-ir neurons as illustrated in Figure 11B, C. Similar findings were observed with the AP-5 trials. Coapplication of AP5 with DA effectively blocked the laminar shift in PV-ir neuron distribution. In addition, the density of neurons in each lamina of the AP5 + DA-treated slices was reduced in comparison to DA-treated slices but similar to that in controls (Fig. 12). The number of neurons per layer was significantly lower in both of these groups than in the DA-only slices ($p = 0.0004$ for DA vs DA + AP5 and $p = 0.00038$ for DA vs NHS).

**Parvalbumin expression is accelerated by the ingrowth of dopaminergic fibers**

Dopaminergic reinnervation to the developing cortex was established with the mesencephalon–cortical cocultures. The characteristics of DA fiber ingrowth at daily time points were examined. At DIV 1–2, the axotomized DA fibers that were present at the time of culture preparation remained in the deep laminae of the cortex. By DIV 3 all of these fibers had virtually disappeared, and TH-positive fibers from the midbrain slice had reached the pial surface of the cortical slice. Fibers crossed from the mesencephalic slice, entered the cortex over the next few days (Fig. 13), and initially ran in two bundles: one bundle of fibers ran radially down to enter the white matter, and a second bundle ran horizontally in layer I. From DIV 5 to 8, TH-positive fibers entered the deep laminae from the white matter and extended along the horizontal axis of the slice. These fibers arborized extensively over the next few days, and some branches reached into the superficial layers where they also began to branch. The deep laminae remained the most densely innervated. The bundle of fibers that extended into layer I early in the cocultures remained in this position. Its horizontal extent lagged behind that of the deeply running fiber bundle. Eventually a mature bilaminar pattern of fibers emerged.

The reinnervation of cortical slices with DA axons accelerated PV expression in comparison to cortical slices cultured without an adjacent midbrain slice (Student’s $t$ test, $p = 0.00054$ for laminar distribution). At 14 DIV, the laminar distribution of PV-ir neurons was comparable to that of cortical slices treated with DA (no significant difference) in that labeled neurons were observed not only in the deep, but also in the superficial cortical layers (Fig. 14). Furthermore, the increased density of labeled neurons seen with DA-treated cortical slices was evident in the reinnervated cocultures as well (Fig. 15).
DISCUSSION

Parvalbumin expression follows similar sequential events in vitro and in vivo. Dopamine, acting preferentially at D2 receptors, accelerates developmental expression of PV. Ingrowth of dopaminergic axons in the coculture system also accelerates PV expression. MK801 and AP5 block the effects of DA on PV expression.

Technical considerations

Organotypic slice cultures are ideal for studying developmental events mediated by connectivity and exogenous influences in isolated systems. Features of intrinsic organization such as neuronal morphology and cytoarchitecture are maintained (Bolz et al., 1990; Stoppini et al., 1991; Gähwiler et al., 1997). Parvalbumin-ir neurons, in particular, maintain similar densities in organotypic cultures as in vivo (Weisenhorn et al., 1998). Moreover, neurons cocultured with target tissues form appropriate efferent connections (Bolz et al., 1990, 1992). Afferents distribute to and synapse with appropriate targets (Frotscher et al., 1995). Although difficult to assess morphologically, functional intrinsic connections are detectable physiologically: long-term potentiation in hippocampal cultures (Stoppini et al., 1991, Müller et al., 1996). Developmental events occur in response to the same influences and in the same manner as in vivo: downregulation of neuropeptide Y in vivo and in organotypic cultures that receive specific (thalamic/cortical) afferents, but not in deafferented slices (Obst and Wahle, 1997).

Our measurements of dendritic arbors were taken from portions of immunoreactive dendrites that express PV. Because observations were made during a period of sharp increases in PV expression, it was not possible to determine whether observed dendritic changes reflected actual morphological changes or merely differences in PV expression that yield incomplete dendritic filling. Therefore, although our observed differences in dendritic measures can be attributed to direct effects of DA on PV expression, they only suggest that dendritic growth is altered by DA.

Developmental expression of PV in vitro

The developmental sequence of PV expression is similar in organotypic cultures and in vivo. In the intact rodent cortex, PV
expression appears first in somata of cingulate and retrosplenial cortices at PN 8–9 and of occipital, frontoparietal, and temporal cortices at PN 10–11 (Soriano et al., 1992; Alcantara et al., 1993). Our findings show similar in vitro events. Cingulate–perirhinal precedes frontoparietal labeling. Parvalbumin appears sequentially in layers V, VI, and then II–III, with somal preceding axonal and dendritic labeling. The earlier appearance in layer V than in VI indicates that expression is not concordant with neuronal migration and subsequent maturation, because migration occurs earlier and progresses from layer VI to II (Miller, 1988, Ignacio et al., 1995). Weisenhorn and colleagues (1998) were able to elicit mature patterns of PV expression only in organotypic slices of parietal cortex explanted at PN 7 or later, and not in slices explanted at PN 3. They concluded that extrinsic influences, such as late onset of tactile responsiveness in sensory cortex, were needed for normal PV expression. Different findings may have arisen from variable conditions (serum deprivation does not alter results in either study), but Plenz and Aertsen (1996) also achieved mature PV expression patterns in early organotypic explants (PN 0–2). Our observation that PV expression is delayed but not prevented in early, untreated cultures suggests that intrinsic factors trigger PV expression, whereas extrinsic influences, including DA, accelerate it.

**Dopamine accelerates PV expression**

If PN age at explant plus time in culture are considered equivalent to age in vivo, then the timing of PV expression in DA-treated slices, rather than controls, corresponds more closely to that in intact animals. Slices from PN 2–3 pups maintained for 7 DIV would be considered 10 d cortices. The PV expression pattern (dense band in frontoparietal layer V) in these DA-treated slices corresponds to that in intact animals at PN 10 (Soriano et al., 1992; Alcantara et al., 1993). This pattern does not occur until cortical age 16–17 (PN 2–3 donors + 14 DIV) in controls. Parvalbumin-ir somata appear in all frontoparietal layers by cortical age 16 d in DA-treated slices and 15 DIV. The appearance of labeled neurons in cingulate cortex and of somata, fibers, and baskets in specific laminae also occurs at similar times in vivo and in DA-treated cultures (Soriano et al., 1992; Alcantara et al., 1993). This observation is not surprising considering that the intact cortex is exposed to DA during development. Indeed, prenatal exposure to cocaine, which increases DA levels by interfering with uptake, increases PV-ir dendrites on interneurons in cingulate cortex. Interestingly, PV-containing interneurons in visual cortex, where DA innervation is sparse, are unaffected (Wang et al., 1996). These studies focused on morphogenesis of one subset of GABAergic neurons, but DA may affect others. Neurite extension of cultured primary neurons, which comprise a mixed neuronal population, is also altered by DA (Reinoso et al., 1996).

**Dopamine reinnervation accelerates PV expression**

Although development of the DA system varies across cortical regions, the pattern of DA innervation in our organotypic cocol-
The system mimics that of similar cortical regions in vivo (Lindvall et al., 1978). The ingrowth of DA axons in the mesencephalon–cortex cocultures accelerated cortical PV expression in the same manner as in slices treated with exogenous DA. This observation suggests that perturbations to the developing DA system occurring in intact animals may modulate circuit formation during the period of PV expression.

**D2 receptor mediation of DA-induced effects**

Our findings indicate that D2 receptor activation preferentially, but not exclusively, enhances PV expression. Coapplication of D1 and D2 antagonists is more effective in blocking DA-induced increases in dendritic length at all concentric radii examined compared with controls. MK801 completely blocked the DA-induced increases in dendritic length. The dendritic intersections at concentric radii from the soma are shown. DA treatment resulted in an increase in number of intersections at nearly all radial distances. This effect was blocked by MK801 at all radial distances, except 40 μm from the soma (*p < 0.05; Student’s t-test).

Figure 11. Histograms of the laminar distribution and the dendritic branching patterns of PV-ir neurons in slices from the MK801 trials that were maintained for 14 DIV. A. The distribution of PV-ir neurons is shown relative to the depth from the pial surface. A higher percentage of neurons is in the superficial laminae of DA-treated slices in comparison with controls. Treatment with MK801 in addition to DA did not block this effect of DA. B, The lengths of PV-ir dendrites at concentric radii from the soma are shown for each group. Neurons in the DA-treated slices showed increased dendritic lengths at all concentric radii examined compared with controls. MK801 completely blocked the DA-induced increases in dendritic length. C, The dendritic intersections at concentric radii from the soma are shown. DA treatment resulted in an increase in number of intersections at nearly all radial distances. This effect was blocked by MK801 at all radial distances, except 40 μm from the soma (*p < 0.05; Student’s t-test).

Although the D2 receptor agonist is implicated in enhancing PV expression, its mechanism of action is uncertain. Both facilitatory and inhibitory responses to D2 receptor activation occur in the cortex (Bunney and Aghajanian, 1976; Reader et al., 1979; Sawaguchi et al., 1986; Penit-Soria et al., 1987; Pirot et al., 1992; Geijo-Barrientos and Pastore, 1995). Facilitatory responses of glutamatergic neurons could increase cortical excitability, which has been linked with increased PV expression (Celio et al., 1986; Hendrickson et al., 1991; Solbach and Celio, 1991; Cohen et al., 1993), but further studies are needed to determine the mechanisms of D2-mediated effects.
Dopamine and glutamate interactions

Our observation that the NMDA receptor antagonists MK801 and AP5 effectively block DA-induced effects on PV expression suggests that DA alters glutamatergic transmission. DA–glutamate interactions have been observed in slices of human neocortex where DA facilitates glutamate-induced responses in layer V pyramidal neurons by increasing depolarization and firing rate and decreasing latency of NMDA-induced EPSPs (Cepeda et al., 1992). Conversely, DA decreases depolarization and firing frequency of quisqualate-induced EPSPs (Cepeda et al., 1992). Thus, modulatory effects of DA depend on the glutamate receptor subtype involved. In layer V pyramidal neurons of rodent cortex, D1 agonists decrease both NMDA and AMPA components of EPSPs (Law-Tho et al., 1994). Interactions may differ during development. In prefrontal cortex of young but not of aged rats, nonspecific DA receptor activation increases extracellular glutamate (Porras et al., 1997). Perhaps this age-related alteration in DA receptor function reflects unique developmental roles for receptor subtypes. The predicted presynaptic effects of DA on glutamatergic transmission (Pralong and Jones, 1993; Law-Tho et al., 1994) have a putative morphological correlate. Triads, composed of a DA axon terminal, a postsynaptic spine, presumably of a pyramidal neuron, and an unlabeled asymmetric, presumably glutamatergic terminal, have been observed in the cortex (Goldman-Rakic et al., 1989).

Onset of synapse formation is another candidate for regulation of PV expression. Although the pattern of synaptogenesis in cortical slices is unknown, synaptic responses are detected early and increase dramatically at 2–3 weeks in hippocampal cultures (Stoppini et al., 1991). A recent study shows that synaptic maturation is accelerated by DA (Rizzo et al., 1998) and supports the notion that increased activity enhances PV expression.

Although PV expression occurs regardless of the presence of DA, it is increased and accelerated by DA in vivo (Wang et al., 1996) and in vitro (present study). The extent of dopaminergic innervation varies between species, projecting preferentially to medial frontal cortex in rodents but to the entire frontal lobe in primates (Berger and Gaspar, 1994). Thus, perturbations to the DA system might have broader effects on primate cortical development than that in rodents.
REFERENCES


Porter et al. • Dopamine Affects Parvalbumin Expression


