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SELECTIVE ACETYLCHOLINE AND DOPAMINE LESIONS IN NEONATAL RATS PRODUCE DISTINCT PATTERNS OF CORTICAL DENDRITIC ATROPHY IN ADULTHOOD

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Abstract—Acetylcholine and dopamine afferents reach their cortical targets during periods of synaptogenesis, and are in position to influence the cytoarchitectural development of cortical neurons. To determine the effect of removing these afferents on dendritic development, we lesioned rat pups at 7 days of age with the selective immunotoxins 192 IgG-saporin, or 6-hydroxydopamine, or both. One group of rats was killed in adulthood for neurochemistry and another was prepared for morphology using Golgi-Cox staining. Changes in morphology were compared in layer V pyramidal cells from medial prefrontal cortex, which sustained the greatest dopamine depletion, and in layer II/III pyramidal cells from retrosplenial cortex, which sustained the greatest choline acetyltransferase depletion. In rats with acetylcholine lesions, layer V medial prefrontal cells had smaller apical tufts and fewer basilar dendritic branches. Both apical and basilar spine density was substantially reduced. Layer II/III retrosplenial cells also had smaller apical tufts and substantially smaller basilar dendritic trees. Apical and basilar spine density did not change. In rats with dopamine lesions, layer V medial prefrontal cells had fewer oblique apical dendrites and atrophied basilar trees. Layer II/III retrosplenial cells had fewer apical dendritic branches. In neither area were spine densities significantly different from control. Neurons from rats with combined lesions were always smaller and less complex than those from singly lesioned rats. However, these cells were simple, additive composites of the morphology produced by single lesions. These data demonstrate that ascending acetylcholine and dopamine afferents play a vital role in the development of cortical cytoarchitecture. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: pyramidal cell, morphology, dendritic spines, 192 IgG-saporin, 6-hydroxydopamine, development.

Diffusely projecting neurotransmitter systems may have a role in cortical development that differs from their usual synaptic function. For example, some neurotransmitters can be detected before the appearance of synaptic machinery and some receptors are expressed only transiently during development (Daval et al., 1987; Lankford et al., 1988; Lauder, 1988; Taylor et al., 1990). Subcortical cholinergic and dopaminergic afferents arrive relatively late in the development of the rat cortex, either just before or just after birth (Verney et al., 1982; Berger et al., 1985; Kalsbeek et al., 1988; Koh and Loy, 1989; Kiss and Patel, 1992). The fact that these axons are making connections during a period of intense synaptogenesis suggests that they may be in a position to influence the synaptic circuitry of their cortical targets (Berger-Sweeney and Hohmann, 1997). Accordingly, an absence or hypofunction of appropriate input during critical periods of cortical development could produce morphological abnormalities in these neurons.

The relationship between these transmitters, neural structure, and function may be particularly relevant to the understanding of neurodevelopmental disorders such as Rett and Down syndrome. Both are characterized by perinatal cholinergic (Casanova et al., 1985; Coyle et al., 1986; Godridge et al., 1987; Wenk et al., 1991; Wenk and Mobley, 1996; Wenk and Hauss-Wegrzyniak, 1999) and dopaminergic (Mann et al., 1987; Reiss et al., 1993; Kitt and Wilcox, 1995; Wenk, 1995, 1997; Subramaniam et al., 1997; Satoi et al., 2000) hypofunction, as well as reduced dendritic branching and spine density of cortical pyramidal neurons (Takashima et al., 1981; Becker et al., 1986; Belichenko et al., 1994; Armstrong et al., 1995, 1998; Kaufmann and Moser, 2000). Decreasing synapse density restricts a neuron's ability to transfer information. As such, dendritic atrophy is believed to underlie at least some of the severe cognitive disability associated with these disorders.

A handful of studies in rodents supports this hypothesis. For example, atrophy of layer V pyramidal neurons has been reported in the somatosensory (Hohmann et al., 1991) and visual cortices (Robertson et al., 1998) after acetylcholine (ACh) lesions made within two days of birth. Postnatal day 1 (P1) lesions of the dopamine (DA) projection from the ventral tegmental area are associated with atrophy of the basilar aspects of layer V pyramidal cells of the medial prefrontal cortex (Kalsbeek et al., 1989). The dendritic atrophy is believed to permanently alter synaptic connectivity, resulting in behavioral impairments in adulthood (e.g. Berger-Sweeney and Hohmann, 1997). However, our studies using the selective cholinergic immunotoxin 192 IgG-saporin indicate that neonatal ACh lesions produce only subtle behavioral impairments in adulthood (Pappas et al., 1996, 2000, 2005; Sherren et al., 1999). Given that neither of the ACh lesion studies

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Abbreviations: ACh, acetylcholine; Cg3, medial prefrontal cortex, after Zilles (1985); ChAT, choline acetyltransferase; DA, dopamine; NE, norepinephrine; NGF, nerve growth factor; P, postnatal day; RSG, granular retrospinal cortex, after Zilles (1985); 192SAP, 192 IgG-saporin; 5-HT, serotonin; 6-OHDA, 6-hydroxydopamine.

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examined cell morphology past the second week of life, and the DA lesion study examined morphology only during the juvenile period, at P36, we felt that an assessment of morphological changes in adulthood was warranted. A study of this type provides a more tangible link between altered cell structure and the functional consequences of these lesions.

We injected rat pups intraventricularly with either 192 IgG-saporin (192SAP) or 6-hydroxydopamine (6-OHDA) to lesion ACh and DA terminals respectively. 192SAP is an immunotoxin directed against p75, the low affinity nerve growth factor (NGF) receptor, which is expressed almost exclusively on basal forebrain cholinergic neurons in adulthood (Batchelor et al., 1989; Woolf et al., 1989). During development, p75 expression is widespread, so we performed our lesions at P7: at this time, p75 mRNA expression is peaking in the basal forebrain but is decreasing in other areas (Koh and Higgins, 1991; Chen et al., 1994). 6-OHDA is a neurotoxin that accesses catecholaminergic neurons through their endogenous reuptake mechanisms. Selective DA lesions were achieved by blocking noradrenergic terminals with desmethylimipramine prior to toxin administration. Catecholamine levels and choline acetyltransferase (ChAT) activity were quantified in adulthood, and guided the selection of cortical regions for dendritic analysis using Golgi-Cox staining. Previous studies have shown that neonatal ACh lesions produce only subtle behavioral impairments in adulthood (Leanza et al., 1996; Pappas et al., 1996, 2000, 2005; Sherren et al., 1999). In contrast, neonatal DA depletion causes marked behavioral deficits (Heffner and Seiden, 1983; Takasuna and Iwasaki, 1996; Luthman et al., 1997). Since functional changes have been associated with structural alterations in cortical neurons (Greenough et al., 1979, 1985; Kleim et al., 1997; Kolb et al., 1998), we predicted that the DA lesion would induce more dramatic morphological changes than the ACh lesion. A group of rats with combined ACh and DA lesions was also included in the analysis. We wondered whether the combined loss of ACh and DA afferents would catastrophically affect cell morphology, causing changes that would not be anticipated from the effect of either lesion alone.

EXPERIMENTAL PROCEDURES

Animals

Female Sprague–Dawley rats were obtained 14–16 days pregnant from Charles River Laboratories (St.-Constant, Quebec, Canada) and maintained on a reversed 12-h light/dark schedule with free access to water and standard rat chow. The day of birth was designated P1, and the litters were culled to four males and four females each on P2. Each lesion type was assigned to one male and one female rat within each litter. The four lesion conditions were thereby evenly distributed across litters. Surgery was performed on P7. Beginning at P12–P14, each maternity cage was supplemented with rat chow moistened with water and a palatable protein supplement (chocolate-flavored Boost™). Supplementation was discontinued after weaning for all but a few of the smallest rats—these were given rat chow moistened with water for an additional two weeks.

The rats were weaned between P23 and P28 and then housed in pairs. After this, the experimenter was blind to group membership until all experimental procedures were complete.

The care and handling of the animals conformed strictly to the guidelines for humane treatment set forth by the Canadian Council of Animal Care. The Council is in compliance with NIH and Society for Neuroscience standards on the ethical use of animals in experimental research. This project received prior approval from the Carleton University Animal Care Committee. Every effort was made to minimize animal numbers and reduce their pain and suffering.

Surgery

On P7, the dam was removed from the home cage and not returned until all the pups had fully regained consciousness. The home cage was placed on a heating pad in the interim. To block the uptake of 6-OHDA by noradrenergic terminals, 10 mg/kg of desmethylimipramine (Sigma-Aldrich) was administered s.c. one hour prior to surgery. The pups were brought to a surgical plane of anesthesia with a 3–4% halothane/oxygen mixture, and were maintained with 1.5–2.5% halothane/oxygen. Bilateral intraventricular injections of each toxin were made with a Hamilton microsyringe (Fisher Scientific) at AP$\pm$0, ML$\pm$1.8, DV $-$3.5 (for a detailed description of the procedure, see Sherren et al., 1999). The pups were warmed by hand and returned to the home cage when they had regained consciousness.

The neurotoxins consisted of 1) a 0.2 μg/μl solution of 192SAP (Advanced Targeting Systems Inc., CA, USA) in vehicle (total dose 600 ng of 192SAP); 2) a 33.3 μg/μl solution of 6-OHDA hydrobromide (Sigma-Aldrich) in vehicle (total dose 100 μg of 6-OHDA, free base); 3) a cocktail of 0.2 μg/μl (600 ng) of 192SAP and 33.3 μg/μl (100 μg) solution of 6-OHDA in vehicle. Control rats received 1.5 μl/ventricle of vehicle only. The vehicle was the same for each toxin, and consisted of phosphate-buffered saline plus 0.2 mg/ml ascorbic acid to prevent oxidation of the 6-OHDA. The 192SAP was diluted to the appropriate concentration and stored in aliquots at $-$80 °C. It was thawed immediately before use and stored at 4 °C between surgeries. The 6-OHDA solution was made immediately before surgery for each litter, and was stored at 4 °C in the dark between surgeries.

The control group consisted of 14 male and 14 female pups who received vehicle only. The ACh group contained 14 male and 14 female pups who received 192SAP alone. The DA group had 13 male and 16 female pups who received 6-OHDA alone. The dual group consisted of 16 male and 16 female pups who received the 192SAP/6-OHDA cocktail.

Neurochemistry

At about 3.5 months of age, five male and five female control, seven male and seven female ACh, five male and six female DA and five male and six female dual rats were briefly anesthetized with CO_2 and decapitated for the determination of brain monoamines and ChAT activity. The brains were quickly removed and placed on ice. The hippocampus and caudate were dissected out first, and then the cortex was laid flat and dissected into three parts. A coronal cut was made immediately caudal to the last visible piece of lateral olfactory tract to separate the frontal region from the rest of the cortex. This piece was designated the frontal cortex, and contained Zilles' (1985) areas Cg3, Cg1, and Cg2, lateral frontal areas, as well as a small amount of anterior parietal cortex. Sagittal cuts were made to the remaining piece approximately 2 mm to either side of the midline. This area was designated the retrosplenial cortex, and contained Zilles' area RSG (retrosplenial cortex), as well as a small amount of the surrounding occipital cortex. The remaining piece of cortex contained temporal, parietal and occipital areas, and was designated the temporal cortex. Samples were immediately frozen in isopentane and

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