Perinatal Distress Leads to Lateralized Medial Prefrontal Cortical Dopamine Hypofunction in Adult Rats

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Obstetric complications involving anoxia or prolonged hypoxia are suspected to increase the risk for such mental disorders as schizophrenia and attention deficit–hyperactivity disorder. In previous studies, we reported evidence of enhanced nucleus accumbens (NAcc) dopamine (DA) function in adult rats subjected to intrauterine anoxia during cesarean (C) section birth. In the present study, we used voltammetry and monoamine-sensitive electrodes to investigate the possibility that this functional hyperactivity of the meso-NAcc system is attributable to a loss of inhibitory control from the medial prefrontal cortex (PFC). We monitored the DA responses to repeated once-daily stress in the right or left PFC of adult male rats born vaginally (VAG) or by C-section, either with (C + 15) or without (C + 0) an additional 15 min of intrauterine anoxia. In C + 15 animals, we observed a pronounced and persistent blunting of stress-induced DA release in the right PFC but not in the left; with repeated testing, a similar pattern of dampened right PFC DA stress responses emerged in C + 0 animals. In addition, C + 15 animals were spontaneously more active than VAG and C + 0 animals and displayed an increase in PFC DA transporter density that was also lateralized to the right hemisphere. There was no evidence, however, that PFC D1 and D2 receptor levels differed between birth groups or hemisphere. These findings suggest a mechanism by which perinatal complications involving anoxia might contribute to the etiology of mental disorders that have been linked to disturbances in central DA transmission and lateralized PFC dysfunction.

Key words: stress; voltammetry; cesarean section; anoxia; obstetric complications; dopamine transporter; asymmetry; attention deficit/hyperactivity disorder

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MATERIALS AND METHODS

Animals and intrauterine anoxia. All procedures were performed in accordance with the guidelines established by the Canadian Council on Animal Care and the Society for Neuroscience Policy on the Use of Animals in Research. Rats were delivered via C section and asphyxiated according to methods modified from those first reported by Bjelke et al. (1991); a detailed description of the procedure has been published previously (Brake et al., 1997a,b). On the day of parturition, Sprague Dawley dams (Charles...

al., 1989; Doherty and Gratton, 1996). The PFC plays a pivotal role in so-called executive functions. Specialized neurons within the PFC are involved in maintaining task-relevant information “on line” for brief periods (Fuster, 1997). These neurons are part of the circuitry that subserves processes related to working memory and sustained attention, both essential components for structuring goal-directed behaviors. Dopamine plays a modulatory role here by optimizing the activity of PFC neurons and the functions they subserve (Williams and Goldman-Rakic, 1995; Murphy et al., 1996). Not surprisingly, disorders of higher executive function often reflect disruptions of prefrontal cortical and DA systems.

Thus, the purpose of the present study was to investigate how PFC DA function is affected in adult animals subjected to intrauterine anoxia during C section delivery. Based on our recent findings in neonatally PFC-lesioned rats (Brake et al., 2000), we hypothesized that the enhanced NAcc DA stress response seen in these animals reflects, at least in part, impaired DA transmission in PFC. To test this idea, we used voltammetry to monitor the acute PFC DA responses to repeated, once-daily stress. We also used quantitative receptor autoradiography to examine possible changes in PFC DA D1 and D2 receptors and DA transporter (DAT) levels. There is accumulating evidence that the PFC is functionally lateralized with respect to the regulation of stress responses (Sullivan and Gratton, 1999), and the left and right mesocortical DA projections have been shown to be differentially involved in numerous aspects of stress-related processes (Carlson et al., 1993, 1996; Sullivan and Szechtman, 1995; Sullivan and Gratton, 1998; Bertridge et al., 1999). Consequently, we examined DA stress responses and DAT D1 and D2 receptor binding in left and right PFC separately.
The extent of asphytic insult in C + 15 animals is less severe than it appears; compared with humans, the rat brain at birth is more resistant to anoxia (Jlick et al., 1970). It is worth noting, however, that although anoxia during the period of fetal origin is a well-recognized risk factor for adverse neurological outcome in preterm infants without observable changes in breathing or heart rate (Hales et al., 1993; Poets et al., 1995). It should be emphasized also that rats are born at an earlier ontogenetic stage than humans and would more closely parallel the premature infant (Romijn et al., 1991).

Because of the inherent dif-
ficencies with sex differences. Upon weaning at 21 d, animals were randomly paired and housed on a 12 hr light/dark cycle (lights on at 8:00 A.M.) with ad libitum water and food.

Histology. At the conclusion of the experiment, animals were deeply anesthetized with sodium pentobarbital (75 mg/kg, i.p.) and transcardially perfused with 0.9% saline, followed by a 10% formalin solution. The brains were stored in 10% formalin and subsequently cryoprotected in a 30% sucrose–formalin solution before being sliced. Electrode placements in sections with no added period of anoxia (C + 0). These animals were delivered immediately after removing the uterus from the dam. Both groups of pups delivered by C section were placed on a heating pad until they had fully recovered. The third group of animals comprised pups that were born vaginally (VAG).

Animals were tested in a sound-attenuating recording chamber with a built-in computer that extended 50–100 m beyond the sealed tip of a pulled glass capillary. The exposed fiber bundle was repeatedly coated with a 5% solution of the ion exchange polymer Nafion (Aldrich, Milwaukee, WI). Electrodes were calibrated immediately before implantation to determine their sensitivity to changes in the oxidation current recorded with different electrodes (in different animals) cannot be assumed to be equivalent. Thus, valid comparisons are possible only if the sensitivity of each electrode is calibrated against a standard and the electrochemical data are expressed as standard equivalent values. In the present study, DA was used as the standard to calibrate electrode sensitivity. Accordingly, in vivo changes in oxidation current are expressed as micromolar equivalent values of DA concentration. Averaged data are presented as means ± SEM. Statistical analyses were done using one-way analysis of variance (ANOVA) with the Bonferroni/Dunn post hoc test. Differences between groups were assessed using Tukey's honestly significant difference (HSD) test.
ANOVA, followed by planned comparisons based on the results of the electrochemical recording experiment.

RESULTS

PFC dopamine response to stress

Nineteen animals with histologically confirmed electrode placements in the left PFC (VAG, \(n = 6\); C + 0, \(n = 6\); C + 15, \(n = 7\)) and 17 animals with confirmed placements in the right PFC (VAG, \(n = 6\); C + 0, \(n = 6\); C + 15, \(n = 5\)) were included in the data analysis (Fig. 1). Based on the atlas of Paxinos and Watson (1985), the point of deepest electrode penetration was estimated in all cases to be within the infralimbic or ventral prelimbic region of the PFC.

Figure 2 shows for the three birth groups of animals the mean increases in DA signal recorded in the left and right PFC on each of the 5 test days. Analysis of the stress-induced DA signal increases recorded on day 1, when animals were naïve to experimental conditions, revealed a significant interaction between birth group and hemisphere (\(F(2,30) = 3.504, p = 0.0429\)). Post hoc comparisons indicated that stress-induced signal increases recorded in the right PFC of C + 15 animals were significantly smaller than those recorded in the same hemisphere of C + 0 and VAG animals (\(p < 0.05\)). There were no such group differences in the magnitude of the stress responses recorded in the left PFC.

A four-way ANOVA of the entire data set (days 1–5) indicated that there was no significant effect of test day, nor was there a significant interaction between test day and any of the other variables (birth group, hemisphere, and time from stress onset). Thus, the data collected from each animal were collapsed across test days, and a three-way ANOVA was conducted with birth group and hemisphere as between-subject factors and time from stress onset as the within-subject factor. This analysis uncovered a significant three-way interaction between birth group, hemisphere, and time from stress onset (\(F(26, 2262) = 3.788, p < 0.0001\)). Post hoc analysis revealed that DA stress responses recorded in the right PFC of both C + 0 and C + 15 animals were significantly attenuated compared with the right PFC stress responses of VAG animals (\(p < 0.05\)). No such group differences were observed in the left hemisphere where stress-induced DA signal increases were often greater but also considerably more variable than in the right PFC; although unexplained, this difference has been reported by others (Maisonneuve et al., 1990).

Spontaneous locomotor activity

Figure 3 presents the within-session changes in spontaneous locomotor activity of VAG, C + 0, and C + 15 animals. Compared with animals of the other two birth groups, C + 15 animals were, overall, significantly more active during the initial 50 min of the session (\(F_{(2,1947)} = 2.107, p = 0.0019\)).

DA receptors and transporter

Significant birth group differences in DAT binding were observed in the right but not the left PFC (\(F_{(2,21)} = 3.686, p = 0.037\)).

Specifically, the density of [3H]BTCP-labeled sites in the right PFC was higher in C + 15 animals than in VAG controls (\(p < 0.05\); Tukey's HSD). However, on the first test day, right PFC DA responses were significantly attenuated only in C + 15 animals (\(p < 0.05\); Tukey's HSD). Length of horizontal bar corresponds to the duration of the stress period.
Figure 3. Mean ± SEM spontaneous locomotor activity scores of VAG, C + 0, and C + 15 animals (n = 12 per group). Across the 5 test days, C + 15 animals were more active than VAG and C + 0 animals during the initial 50 min of the session (\( p < 0.05 \); \( p < 0.01 \); Tukey’s HSD).

Table 1. Birth group differences in left and right PFC DA D1- and D2-like receptor and transporter levels

<table>
<thead>
<tr>
<th></th>
<th>Left PFC</th>
<th>Right PFC</th>
</tr>
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<tbody>
<tr>
<td>D1-like receptors [^{[H]}\text{Raclopride}]</td>
<td>( 1.92 ± 0.22 )</td>
<td>( 1.99 ± 0.38 )</td>
</tr>
<tr>
<td>VAG</td>
<td>( 2.49 ± 0.48 )</td>
<td>( 2.46 ± 0.45 )</td>
</tr>
<tr>
<td>C + 15</td>
<td>( 3.24 ± 1.06 )</td>
<td>( 2.12 ± 0.58 )</td>
</tr>
<tr>
<td>D2-like receptors [^{[H]}\text{Raclopride}]</td>
<td>( 16.7 ± 1.98 )</td>
<td>( 16.2 ± 1.74 )</td>
</tr>
<tr>
<td>VAG</td>
<td>( 23.3 ± 4.76 )</td>
<td>( 21.6 ± 3.98 )</td>
</tr>
<tr>
<td>C + 0</td>
<td>( 21.5 ± 2.10 )</td>
<td>( 19.5 ± 1.77 )</td>
</tr>
<tr>
<td>DA transporter [^{[H]}\text{B-HTCP}]</td>
<td>( 33.5 ± 4.97 )</td>
<td>( 25.7 ± 5.89 )</td>
</tr>
<tr>
<td>VAG</td>
<td>( 47.7 ± 7.43 )</td>
<td>( 31.9 ± 7.85 )</td>
</tr>
<tr>
<td>C + 0</td>
<td>( 39.5 ± 7.04 )</td>
<td>( 53.1 ± 10.40^* )</td>
</tr>
</tbody>
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Dopamine receptor and transporter levels are expressed in femtomoles per milligram wet tissue. Values are means ± SEM of six to eight animals from each birth group (*\( p < 0.05 \); Scheffe’s C + 15 vs VAG in right PFC).
also significant in this regard. The standard treatment for ADHD is methylphenidate (Ritalin), which acts at the DAT by blocking DA uptake, thus increasing the availability of synaptic DA. In humans, genetic studies have shown links between anomalies in the DAT gene and ADHD (Cook et al., 1995; Gill et al., 1997), and knock-out mice lacking the DAT gene have been proposed as an animal model of ADHD based primarily on their profound hyperactivity and response to psychostimulants (Gainetdinov, 1999). The present results suggest that far more subtle, localized, and lateralized alterations in DAT may be associated with a spectrum of changes characteristic of ADHD. Such changes are induced by early developmental insult and may become manifest through similar, if not common, mechanisms as those that are affected in genetically individualized disease state, parallels with the clinical features of HDAD. The implications are predictive of ADHD diagnosis in both familial and nonfamilial forms of the disorder (Milberger et al., 1997).

The present birth manipulations could alter cortical development and maturation in several ways. The timing of any developmental insult is important in determining the type of long-term damage incurred and the particular afferent systems affected. In the rat, cortical DA afferents begin reaching their cortical targets earlier (approximately the final trimester of gestation) and achieve their adult innervation pattern later (2 months postnatal) than other major cortical afferent systems (Berger-Sweeney and Hohmann, 1997). The window of vulnerability of this system to developmental insults is therefore particularly long. Moreover, this system develops faster in females than males, possibly conferring a prolonged vulnerability to insult in the latter; presumably, this might contribute to the higher incidence among males of neurodevelopmental disorders such as ADHD.

Additionally, the same perinatal manipulation as presently used is known to cause dysregulation of plasma hormone (glucocorticoid) physiology in the first weeks of life (Boksa, 1997). There is a high density of steroid receptors in PFC and hippocampus, which are overexpressed at approximately the time of birth (MacLusky et al., 1979; Meaney et al., 1985; McEwen, 1992). Glucocorticoids affect both cell death and neurogenesis in the hippocampus during this developmental phase (Gould et al., 1991a,b), although such actions have not been investigated in PFC. Cesarean section birth with anoxia, however, does increase cell death in PFC by postnatal day 8 in the rat (Dell’Anna et al., 1997). Finally, glucocorticoid receptors are also colocalized in DA-containing cell bodies of the ventral tegmental area, the origin of the mesocortical DA innervation (Harfstrand et al., 1986), potentially affecting development of this projection system.

The asymmetrical nature of the cortical changes reported here is striking, although not surprising. The mesocortical DA system in the adult rat can exhibit considerable functional lateralization in such things as regulation of stress responses (Carlson et al., 1993; Sullivan and Gratton, 1998; Berridge et al., 1999), drug self-administration (Glick et al., 1994; Nielsen et al., 1999), and subcortical DA function (Sullivan and Szechtman, 1995; Carlson et al., 1996). Cerebral DA asymmetries are present from the time of birth (Afonso et al., 1993; Rodriguez et al., 1994; Varrlinskaya et al., 1995). Also, given the different rates of human cortical maturation of the two hemispheres (Geshwind and Galaburda, 1987), it should not be surprising that long-term sequelae of early developmental insults would be asymmetrical as well.

That perinatal anoxia affected only the right PFC may reflect the intrinsic specialization of this structure in regulating physiological stress responses in the rat (Sullivan and Gratton, 1999). Differences in the degree of fetal or neonatal physiological distress, either acutely or cumulatively, might therefore preferentially impact on maturational processes within this brain region. Mesocortical DA innervation, particularly in the right PFC, is thought to play an important role in optimizing adaptive responses to stressful conditions (Sullivan and Szechtman, 1995; Sullivan and Gratton, 1998; Berridge et al., 1999). It is perhaps then not a coincidence that blunted or suboptimal plasma glucocorticoid stress responses are seen not only in adult rats subjected to perinatal anoxia (Boksa et al., 1996) but also in ADHD subjects with “developmentally persistent” forms of the disorder (King et al., 1998). Hence, compromising the development of the right PFC would ultimately be expected to disrupt those processes normally subserved by this region, be they adapting to stress, behavioral inhibition, regulation of subcortical systems, or higher executive and attentional functions.

In summary, perinatal distress involving anoxia induces lasting changes in mesocortical DA function and behavior. Together with other findings, it is suggested here that highly selective and lateralized changes in PFC may be primary in contributing to an observed spectrum of changes associated with this developmental challenge. Although the present manipulations are not intended to model specific genetic disorders such as the neuropsychiatric conditions, our observations point to the potential role of inflammation in the pathogenesis of the disturbances such as ADHD are numerous and intriguing. These findings implicate mechanisms by which perinatal distress could lead to clinically reported outcomes and generate testable hypotheses concerning the long-term behavioral consequences of perinatal trauma.

**REFERENCES**


