

Prenatal Maternal Stress Due to Repeated Exposure to A Cold Environment Affects Development of Catecholamine Neurons in Rat Offspring: An Immunohistochemical Study

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Abstract

We examined the effect of maternal repeated cold stress (RCS) on the development of catecholamine neurons in offspring using 8-day-old offspring and tyrosine hydroxylase (TH) immunohistochemistry. RCS was loaded to pregnant rats between days 10 and 20 after fertilization. The frontal and cingulate cortices tended to contain fewer TH-immunoreactive (-ir) fibers, and the density of TH-ir varicosities with a large size (more than 7 μm in diameter) was significantly ($p < 0.05$) less in rats prenatally exposed to RCS than controls. The locus coeruleus neurons of rat prenatally exposed to RCS displayed less TH immunoreactivity than controls. In the medullary C1/A1 catecholaminergic field, size of TH-ir neurons was smaller and the quantity of TH-ir fibers was less in prenatally exposed rats, although the difference was not significant. In the originating and projection fields of midbrain dopaminergic systems, we could not detect any differences in TH-ir structures between the two groups. These findings indicated that prenatal RCS impaired the development of catecholaminergic neurons, especially the noradrenergic neurons of pups.

Keywords: Cerebral cortex; Locus coeruleus; Medulla oblongata; Prenatal stress; Tyrosine hydroxylase; Dopamine; Noradrenaline; Development; Immunohistochemistry

Introduction

Prenatal stress is known to affect the emotional and behavioral development of offspring [1,2], and is thought to be a cause of mental disorders, including schizophrenia and depression [3]. Catecholamines (CA) are candidate neurotransmitters, being involved in emotion, behavior, and the pathogenesis of psychoses [4]. Furthermore, histological findings of catecholamine neuronal systems have been reported in postmortem brains of patients manifesting psychoses [5-7]. Based on these reports, morphological changes in the offsprings of prenatally stressed rats were hypothesized.

The present study investigated the affect of maternal repeated cold stress (RCS), the physiological influence of which has been well studied in rats [8-11], on the development of CA neurons in offspring using tyrosine hydroxylase (TH) immunohistochemistry [12,13].

Experimental Procedures

The study adhered current RIGOR guidelines [14,15].

Two female Wistar rats were randomly selected for loading RCS between day 10 to 20 following fertilization. The treatment group was composed of the total of 24 pups (prenatally RCS rats) borne and fostered by the RCS- loaded mother rats. Another female Wistar rat

was randomly selected, and 10 pups borne and fostered by the latter mother rat composed the control group.

The SART (specific alteration of rhythm and temperature) stress apparatus (modified M-9000 apparatus made by Advantec Toyo) consisted of a built-in heater and cooler that could be controlled by an adjustable self-timer. The size of the interior of the apparatus was 120 cm in height and 105 X 65 cm in width [8]. The environmental temperature in this apparatus was altered from 24 to -3 at 1 cycle / 2 h from 1000 to 1800 hours by switching the heater and cooler and was kept at -3 from 1800 hours until 1000 hours the following morning [8]. This sequence was repeated four times between 1000 and 1800 hours. The lighting was maintained on a 12: 12 light-dark cycle (light, 0700 to 1900 hours; dark, 1900 to 0700 hours). RCS was loaded to 2 pregnant Wistar rats between day 10 and 20 after fertilization.

A total of 24 prenatally SART-stressed rats (prenatal RCS rats, = treatment group) and 10 control rats (= control group) were examined. At postnatal day 8, under deep anesthesia with 10mg / kg of sodium

pentobarbital (Nembutal, Dainippon Pharm.), each neonate rat was perfused through the cardiac ventricle with 5 ml of saline (0.9% of NaCl) followed by 20 ml of fixative containing 4% paraformaldehyde or 5% glutaraldehyde [12]. Thirty or 50 μm thick cryostat coronal sections were made from each brain. Detailed procedures for TH immunohistochemistry have been described elsewhere [12]. Details of the production, characterization and specificity of TH antiserum have been described elsewhere [13]. Some sections were counterstained with neutral red. Neurons immunoreactive for TH were observed

under a light microscope. The atlas by Hokfelt et al. [16] was used to determine the anatomical localization of TH-positive neurons.

Image analyses were performed using a software, Win ROOF (version 5.0, Mitani Corporation, Japan) and a self-made PC program to quantify the size and number of varicosities and neural fibers.

Results

Average body weights of 8-day-old pups were 13.6 ± 1.32 g (n=10) for control rats and 9.90 ± 1.54 g (n=24) for prenatal RCS rats, and wet weights of the brain were 0.67 ± 0.07 g (n=10) for control rats and 0.66 ± 0.06 g (n=24) for prenatal RCS rats, but there were no significant differences in either body or brain weight (t-test, $p < 0.05$). Although there were some individual differences between the findings in RCS rats and control rats, some evident differences between the two groups were noted.

The frontal and cingulate cortices of prenatal RCS rats, especially layers II and III, contained fewer TH-immunoreactive (-ir) fibers than controls (Figure 1A and B), though there were no significant differences. In these areas, TH-ir fibers demonstrated apparently less TH-ir varicosities (Figure 1C, D). Image analysis demonstrated that the density of large varicosities (more than $7\mu\text{m}$ in diameter) in the TH-ir fibers was significantly ($p < 0.05$) less in prenatal RCS rats than in controls.

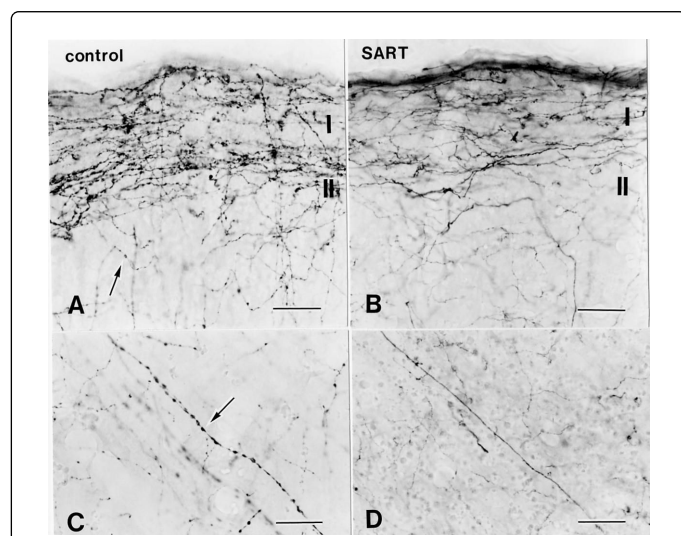


Figure 1: TH-immunoreactive (-ir) fibers in the frontal cortex, area 2 of 8-day-old pup following maternal exposure to RCS. (A, C) A control rat. (B, D) A prenatal RCS rat. The quantity of TH-ir fibers is lower in the prenatally RCS rat, especially in layers II and III. Cortical TH-ir fibers of RCS show a reduced number of large varicosities. An arrow indicates a varicose fiber of the control rat. Bar: $25\mu\text{m}$.

In the substantia nigra (SN, A9) and ventral tegmental nucleus (VTA, A10), the originating nuclei of midbrain dopaminergic neurons, and in the striatum, their major projection field, there were no apparent differences in stainability or cellular sizes between the two groups. The locus coeruleus (LC), the originating nucleus of noradrenergic neurons, of the prenatal RCS rats showed less intense TH immunoreactivity (Figure 2A, B).

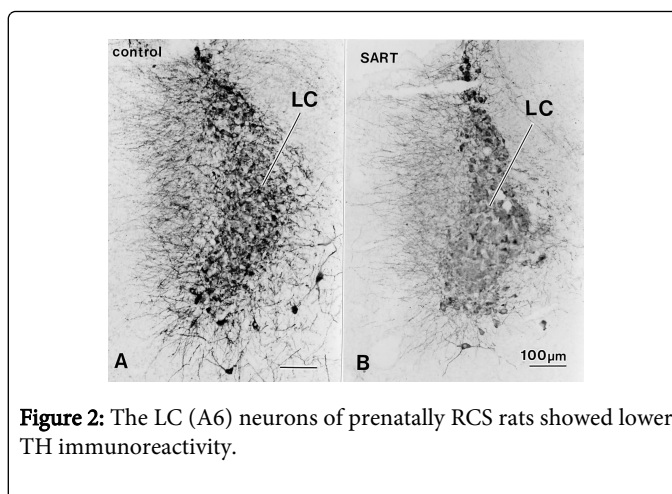


Figure 2: The LC (A6) neurons of prenatally RCS rats showed lower TH immunoreactivity.

In the medullary A1 / C1 catecholaminergic region (VLM: ventrolateral medulla) of the prenatal RCS rats, TH-ir neurons were likely to be smaller (the major axis: $11 \sim 30\mu\text{m}$) than those in the controls (the major axis: $15 \sim 33\mu\text{m}$), and TH-ir fibers were fewer as shown in Figure 3A, B. However, the comparison of the total areas of TH-ir structures in A1 / C1 CA fields in each section by image analysis did not show any significant differences between the two groups ($p < 0.05$).

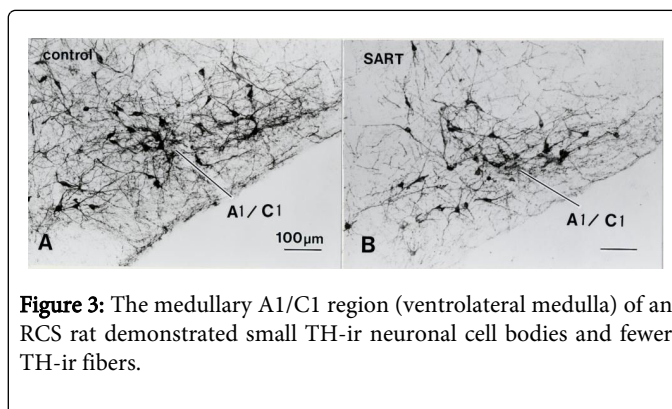


Figure 3: The medullary A1/C1 region (ventrolateral medulla) of an RCS rat demonstrated small TH-ir neuronal cell bodies and fewer TH-ir fibers.

Discussion

By examining TH-ir structures of 8-day-old pups, we demonstrated that maternal stress by repeated exposure to a cold environment affected the fetal development of CA neurons. This is the first morphological evidence showing the developmental influence of prenatal stress on the central nervous systems.

In the prenatal RCS group, the LC and VLM, the originating nuclei of noradrenergic neurons showed decreased TH-immunoreactivity, smaller TH-ir cell bodies and decreased TH-ir neural fibers, suggesting that prenatal exposure to RCS affects the fetal development of noradrenergic neurons. The SN and VTA, the major originating nuclei of dopamine (DA) neurons, and the striatum, the projection field, did not show apparent morphological differences between the two groups. It suggested that prenatal RCS impaired the development of noradrenaline (NA) neurons rather than that of DA neurons in pups.

Findings in the frontal and cingulate cortices of prenatal RCS pups, such as reduction on TH-ir fibers in all layers, especially in layers II~III, morphologically coincide with findings of the NA neurons in the LC and VLM [17]. The reduction of large TH-ir varicosities (more than 7µm in diameter) in RCS pups implied impaired function of CA neurons. Such morphological changes in CA neurons of RCS pups do not transient change, but are likely to have long-term functional influences [18]. A recent report using restraint stress showed that intense prenatal stress reduced reactivity of NA neuronal systems for stress in adulthood [19]. Alteration of noradrenergic modulation of LTP in hippocampal slice by prenatal stress has also been shown [20].

Though numerous animal studies on prenatal stress using various methods including restraint stress have focused on metabolism and / or turn over of monoamines [18,19,21], to the authors' knowledge, there are no other studies focused on morphological changes in the CA neuronal systems.

Our recent studies demonstrated that prenatal RCS rats showed altered emotional development [2], and significantly smaller cingulate cortices on the coronal plane (unpublished data) similar to morphological findings in schizophrenia [22]. The cingulate cortex is likely to be a brain area vulnerable to prenatal stress, pathogenesis of psychoses, or a cause of developmental impairment.

In the present study, the analyses were limited only in 8-day-old pups. It remains to be elucidated whether these changes persist until an adolescent or adult stage. The genetic and/or epigenetic involvement producing the present findings should also be investigated.

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