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Research article

Environmental enrichment mitigates the impact of ancestral stress on motor skill and corticospinal tract plasticity

J. Keiko McCreary, Zachary T. Erickson, Gerlinde A.S. Metz (PhD)*

Canadian Centre for Behavioural Neuroscience, Department of Neuroscience, University of Lethbridge, Lethbridge, AB, T1K 3M4, Canada

HIGHLIGHTS

- Ancestral stress impaired skilled walking abilities in rats.
- Ancestral stress reduced axonal density of corticospinal projections.
- Enriched environment mitigated adverse consequences of ancestral stress in the motor system.

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ABSTRACT

Background: An adverse fetal environment in utero has been associated with long-term alterations in brain structure and function, and a higher risk of neurological disorders in later life. A common consequence of early adverse experience is impaired motor system function. A causal relationship for stress-associated impairments and a suitable therapy, however, have not been determined yet.
Objective: To investigate the impact of ancestral stress on corticospinal tract (CST) morphology and fine motor performance in rats, and to determine if adverse programming by ancestral stress can be mitigated by environmental enrichment therapy in rats.
Methods: The study examined F3 offspring generated by three lineages; one with prenatal stress only in the F1 generation, one with compounding effects of multigenerational prenatal stress, and a non-stress control lineage. F3 offspring from each lineage were injected with biotinylated dextran amine (BDA) into the motor cortex for anterograde tracing of the CST.
Results: Examination of the CST revealed reduced axonal density in the ancestrally stressed lineages. These anatomical changes were associated with significant impairments in skilled walking, as indicated by reduced foot placement accuracy and disturbed inter-limb coordination. Therapeutic intervention by environmental enrichment reduced the neuromorphological consequences of ancestral stress and restored skilled walking ability.
Conclusions: The data suggest a causal relationship between stress-induced abnormal CST function and loss of fine motor performance. Thus, ancestral stress may be a determinant of motor system development and motor skill. Environmental enrichment may represent an effective intervention for the adverse programming by ancestral stress and trauma.

1. Introduction

Central nervous system development is a dynamic and complex process that begins in utero and continues prominently throughout infancy, adolescence, and even lasts into adulthood. The pre- and postnatal environment represents a significant impact on brain development and maturation [28]. Notably, environmental factors can both positively and negatively affect brain structure and function through altering neuronal plasticity throughout life [27]. Consequently, a hostile condition in utero, such as prenatal stress (PS), has been associated with long-term changes in neuroplasticity and subsequent susceptibility to neurodevelopmental disorders and neurological diseases [33,36]. An ideal model system to study the impact of stress is the motor system and associated pathways, since motor outputs, such as skilled movement, are easily quantifiable [23,32]. The corticospinal tract (CST) represents the major descending white matter pathway controlling voluntary

* Corresponding author at: Canadian Centre for Behavioural Neuroscience, Department of Neuroscience, University of Lethbridge, 4401 University Drive, Lethbridge, Alberta, T1K 3M4, Canada.
E-mail address: gerlinde.metz@uleth.ca (G.A.S. Metz).

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movement and is critically involved in skilled reaching and skilled walking [10,19,34]. Little information is available however, on the influence of stress on CST development and function.

It is reasonable to expect that adverse early life experience modulates CST development. Earlier findings suggest that preterm birth and low birth weight, both a corollary of PS [54] and ancestral stress [55], may determine the developmental trajectory of the CST. Specifically, illness severity of the very preterm neonate along with pain-related stress exposure has been linked to slower microstructural development of the CST [41,58]. Moreover, prenatal and postnatal malnutrition in the rat has been associated with neuronal loss, reduced brain weight, and a reduction in conduction velocity along the CST [40,45,46]. PS has been shown to modulate neuronal development of the frontal cortex [44] and thus it may also potentially affect the motor cortex and voluntary motor control [57]. Further experimental rodent and non-human primate studies indicated that an adverse intrauterine environment during pregnancy impairs neuromotor behavioural development in offspring [13,37,47]. PS-induced motor deficits may include disabilities in fine motor skills [50], reflexes [37] and disturbed coordination and balance [7].

Beyond the direct impact of PS on fetal development, recent findings also showed that remote ancestral stress influences motor system development. Some of the biological sequelae of early life stress can propagate through subsequent generations [11,20,55]. Notably, recurrent PS across four consecutive generations can compromise skilled movement especially in males [1]. Defects in CST function and associated systems may be one mechanism to explain these impairments. Reduced CST integrity has been linked to loss of skilled movement capacity [48], and gross and skilled movement impairments have been associated with PS [7,37]. In turn, beneficial experience, such as housing in an enriched environment (EE) that provides animals with rich social, motor, cognitive and sensory stimulation, has been shown to reverse neuromorphological deficits due to stress [4,31].

Here we proposed that transgenerational and multigenerational ancestral stress alters voluntary motor control, and that deficits in skilled movement ability are associated with altered CST neuromorphology. Moreover, we tested to see if the consequence of ancestral stress on motor function can be mitigated by beneficial experience through exposure to environmental enrichment.

2. Materials and methods

2.1. Animals and experimental design

Forty-eight adult male Long-Evans rats were taken from one of three lineages: non-stress controls (n = 16), transgenerational prenatal stress (TPS; n = 16), and multigenerational prenatal stress (MPS; n = 16). TPS rats were the F3 generation of a filial line in which only the F1 generation was stressed prenatally and MPS rats were the F3 generation of a filial line in which each consecutive generation was prenatally stressed [1,55]. Two offspring per litter of each sex were randomly selected to be included in the experiments. Each experimental group included offspring from four different litters.

Half of the TPS and MPS groups were randomly assigned to one of two housing conditions: standard housing or enriched environment (EE). In the standard housing condition rats were housed in non-sibling pairs in a standard shoebox Plexiglass cage. In the EE condition, rats were housed in groups of four in standard shoebox cages from postnatal day (P) 21–35. At P 35, each group of four EE rats was moved to large circular condominiums (8 rats/house). In addition to the increased social interactions and living space, the EE was filled with multiple shelters and enrichment toys to provide rich social, physical and sensory stimulation.

All animals were tested in the ladder rung walking task at P110. Two weeks later, five animals per group (30 animals in total) received intracortical injections of the tract tracer biotinylated dextran amine (BDA). Two weeks after the BDA injection all animals were euthanized.

2.2. Gestational stress procedures

Chronic maternal stress was induced through social isolation, which has been shown to result in mild psychosocial stress in rats and other mammals (Hawkley et al., 2012). Social isolation represents a survival threat to social species, including female rats, and physiology has been shaped to deliver an appropriate stress response. Therefore, each dam was housed alone starting preconception on P 90 until her offspring was weaned. Control rats were housed in pairs until gestational day 21.

2.3. Skilled walking task

Skilled fore- and hind limb coordination and limb placement was assessed using the ladder rung walking task [34,35]. Rats were trained to cross a 1-m long horizontal ladder with metal rungs randomly arranged at distances between 1 and 5 cm. Rats were pre-trained to cross the ladder for two days, followed by a test session in which five trials were videotaped for further analysis. Seven categories of limb placement on the rungs were analyzed [9,34] including (0) Total Miss, (1) Deep Slip, (2) Slight Slip, (3) Replacement, (4) Correction, (5) Partial Placement, and (6) Correct Placement. An error was counted if a total miss, a deep slip or a slight slip occurred. Foot fault scores were averaged across 3–5 trials, and time needed to cross the length of the ladder was measured. The number of errors was recorded by calculating the mean number of errors (score of 0, 1 or 2) per step, which was averaged over five trials.

Skilled walking was recorded manually by using a Canon ZR50 MD camcorder set at a shutter speed of 1/500 s. During filming, additional light was supplied (Lowel-light Mtg Inc, New York, USA). Frame-by-frame analysis and scoring by a blind investigator was performed using a Sony GV-D1000 NTSC miniDV player.

2.4. Anterograde tract tracing using BDA

High molecular weight BDA (10 k) is an anterograde tracer which yields sensitive and detailed labeling of axons and terminals, [42]. BDA can be visualized for light microscopy and combined with other tract tracing or immunohistochemical methods [42]. In this study, BDA was injected into the primary motor cortex M1, which then travelled via the CST through the internal capsule and midbrain, decussated at the level of the medulla oblongata and reached the spinal cord.

Two weeks prior to euthanization, rats underwent anterograde tract tracing of the CST with BDA (n = 5 rats/group, total of 30 animals). Under isoflurane anesthesia, rats were positioned in a stereotaxic frame. Using a Hamilton syringe, 1 ml of BDA (10%, 10,000 MW; Invitrogen, Eugene, OR) was slowly (over 5 min) injected bilaterally into the forelimb areas of the motor cortex using the following coordinates based on Paxinos and Watson [38]: (1) 1 mm anterior and 2 mm lateral; (2) 1 mm anterior and −2 mm lateral (in reference to Bregma). Injections were placed 1.5 mm from the surface of the cortex. The syringe remained in place for another 3 min following the injection. The area of incision was sutured, and the animal was left to recover overnight on a heating pad and monitored until recovery.

Previous protocols have indicated that a two-week interval between BDA labeling and animal euthanization is sufficient for