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ScienceDirect
Behavioural Brain Research 186 (2008) 91–97
BEHAVIOURAL BRAIN RESEARCH
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Research report

Neonatal tactile stimulation reverses the effect of neonatal isolation on open-field and anxiety-like behavior, and pain sensitivity in male and female adult Sprague–Dawley rats

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Received 19 March 2007; received in revised form 23 July 2007; accepted 28 July 2007
Available online 6 August 2007

Abstract

It is well known that early life events induce long-lasting psychophysiological and psychobiological influences in later life. In rodent studies, environmental enrichment after weaning prevents the adulthood behavioral and emotional disturbances in response to early adversities. We compared the behavioral effect of neonatal isolation (NI) with the effect of NI accompanied by tactile stimulation (NTS) to determine whether NTS could reverse or prevent the effects of NI on the adulthood behavioral and emotional responses to environmental stimuli. In addition, we also examined the sex difference of the NTS effect. Measurements of body weights, an open-field locomotor test, an elevated plus maze test, a hot-plate test, and a contextual fear-conditioning test were performed on postnatal day 60. As compared with rats subjected to NI, rats subjected to NTS showed significantly higher activity and exploration in the open-field locomotor test, lower anxiety-like behavior in the elevated plus maze test, and significantly prolonged latencies in the hot-plate test, and this effect was equal among males and females. In the contextual fear-conditioning test, whereas NTS significantly reduced the enhanced freezing time due to NI in females, no significant difference in the freezing time between NI and NTS was found in males. These findings indicate that adequate tactile stimulation in early life plays an important role in the prevention of disturbances in the behavioral and emotional responses to environmental stimuli in adulthood induced by early adverse experiences.

Keywords: Neonatal isolation (NI); Neonatal tactile stimulation (NTS); Rat; Open-field locomotor test; Elevated plus maze test; Hot-plate test; Contextual fear-conditioning test

1. Introduction

It is well known that early experiences induce long-lasting psychophysiological and psychobiological changes in later life. Numerous studies have demonstrated that early adverse experiences such as maternal separation (MS) or neonatal isolation (NI), which interrupt dam–pup interactions, affect the development of the central nervous system and subsequently lead to enhanced susceptibility to stress in adulthood, both behaviorally and endocrinologically [8,10,12,14,17,37]. NI and MS differ with respect to isolation of individual offspring. With most MS procedures, only the dams are removed to separate cages, while the pups remain in their home cage. With NI procedures, pups are isolated and placed individually into containers separately from their dams and littermates. In contrast, the protective or therapeutic effects of early intervention on the development of stress vulnerability during the interruption of the dam–pup relationship has not been as thoroughly examined. For example, brief handling of neonatal rats during maternal separation was reported to induce resistance of the hypothalamo-pituitary-adrenal (HPA) axis to stress in adult rats [17]. Several studies have demonstrated that adult rats subjected to neonatal handling exhibited less anxiety-like behaviors in the elevated plus maze as compared with nonhandled rats [19,27].

Another type of neonatal handling, neonatal tactile stimulation (NTS), also has a distinct effect on the development of stress reactivity. Rats subjected to NTS show increased curiosity and problem-solving ability, and exhibit less emotionality in stressful situations [16]. NTS prevents the rise of serum corticosterone

Abbreviations: NI, neonatal isolation; NTS, neonatal tactile stimulation

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0166-4328/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2007.07.039

levels typically associated with maternal deprivation stress and causes elevated corticosterone levels to return to normal [25]. NTS also protects against maternal deprivation-induced shortening of hot-plate latencies [35]. Furthermore, it has been reported that NTS alleviates the reduction of hippocampal volume in rats subjected to neonatal hypoxia–ischemia [29]. These findings indicate that subjecting animals to NTS can facilitate their ability to cope with stressful environmental conditions, decrease the enhanced HPA axis in response to stress in adulthood, and protect against brain damage induced by neonatal manipulation.

Postnatal handling, which involves only a brief period (15 min) of mother–pup separation, dampens HPA responses to stress [15,20,21]. In contrast, postnatal MS (3 h/day; PN days 2–14) or (6 h/day; PN days 2–10) enhances HPA responses to stressors [18,28]. In addition, 1 h-neonatal isolation on postnatal days 2–9 also enhances HPA responses to stressors [3]. It has also been shown that early adverse experiences have sex-specific effects on the development of HPA-axis reactivity [4,36]. Similarly, gender differences exist with respect to the effects of neonatal isolation and neonatal handling on the development of anxiety-like behavior in the elevated plus maze [8,19] and the conditioned fear test [1,11]. Although there was no significant sex differences in anxiety-like behavior between rats subjected to neonatal handling and neonatal handling with tactile stimulation [32], it is unclear whether sex differences exist with respect to the ability of NTS to prevent or reverse the enhancement of susceptibility to environmental stimuli in response to early adversities.

In this context, the present study was undertaken to assess whether NTS can prevent or reverse the enhanced susceptibility to environmental stimuli due to NI in adult rats. We compared the effect of NI with the effect of NTS during NI on body weights, locomotor activities in the open-field test, anxiety-like behavior in the elevated plus maze test, pain sensitivity in the hot-plate test, and the fear responses in a contextual fear test on postnatal day 60. We also examined sex-specific effects of NI and NTS on these behavioral tests.

2. Materials and methods

2.1. Animals

Pregnant female Sprague–Dawley rats were purchased from Charles River (Yokohama, Japan). The rats were housed individually in the breeding colony at constant room temperature (23 ± 2 °C) and humidity (60%) with a 12/12 h light–dark cycle (lights on at 08:00). Food (Rodent Lab Diet EQ 5L37, Japan SLC Inc.) and water, conforming to the Water Quality Standard required by the Japanese Waterworks Law, were provided ad libitum. Male (n = 153) and female (n = 151) SD rats were used, and no more than two pups from the same dam were used in behavioral experiments. The experimental animals were divided into the following groups: (1) sham-treatment, (2) NI, and (3) NTS. Prior to birth, litters from each dam were randomly assigned to the sham, NI, and NTS groups. Litters were weaned on postnatal (PN) day 22. After weaning, male and female rats were housed in same-sex, same-treatment groups of three per cage (38 cm × 23 cm × 20 cm stainless steel cage) and maintained under normal conditions until the behavioral experiments; these included the open-field locomotor test (males; sham: n = 10, NI: n = 10, NTS: n = 10, females; sham: n = 10, NI: n = 10, NTS: n = 10), elevated plus maze test (males; sham: n = 12, NI: n = 16, NTS: n = 12, females; sham: n = 12, NI: n = 13, NTS: n = 15), hot-plate test (males; sham: n = 12, NI: n = 12, NTS: n = 15, females; sham: n = 12, NI: n = 11, NTS: n = 12), and contextual fear-conditioning test (males; sham: n = 14, NI: n = 14, NTS: n = 16, females; sham: n = 15, NI: n = 16, NTS: n = 15), which were undertaken on PN day 60 (Fig. 1). A different set of rats was used for each of these experiments. All animal procedures were approved by the Hiroshima University Medical Science Animal Care Committee.

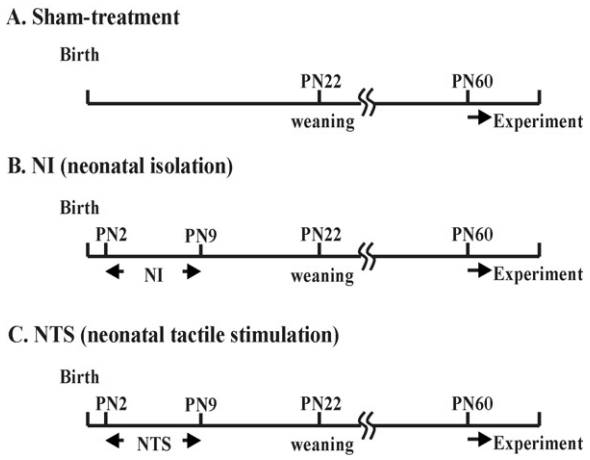

Fig. 1. Animal treatment paradigms. Prior to birth, litters from each dam were randomly assigned to (A) sham, (B) NI, and (C) NTS groups depending on neonatal treatments. All litters were weaned on postnatal (PN) day 22. After weaning, male and female rats in all groups were housed in same-sex, same-treatment groups of three per cage. Behavioral experiments were performed on PN day 60.

2.2. Neonatal isolation (NI)

After birth, the pups and mothers were housed together in their home cages (38 × 23 × 20 cm clear plastic cages) until weaning. Kehoe and Bronzino's method [9] was used for NI treatment. The first 24-h period after birth was designated PN day 1. Only litters with 11–14 pups were used in this study, and there were no differences in mean litter size among the three groups (NI, NTS, sham-treatment). The number of male and female pups was equal or almost equal in each litter (e.g., five males, six females). In the NI group, pups were isolated from the dam, nest, and siblings, and placed in individual opaque round containers (7 cm diameter and 8 cm deep) without bedding in a temperature- and humidity-controlled chamber, for 1 h per day on PN days 2–9. This microenvironment temperature was 30 ± 2 °C, similar to nest temperature, and humidity was 60%. Containers were placed 20 cm apart. Isolation was carried out between 09:00 and 12:00 each day. The rats in the sham group was housed under normal conditions and left undisturbed, except for weekly cage cleaning, until weaning. The rats in the sham group were similar to what are usually designated as animal facility-reared (AFR) animals.

2.3. Neonatal tactile stimulation (NTS)

Pups were isolated from the dam, nest, and siblings, and placed in individual round containers, as described above for NI. All pups were then gently handled dorsally from head to tail for 1 h per day by an investigator whose hands were covered with fine latex gloves. After handling, all pups were returned to the home cage at the same time. The duration of each handling session was approximately 30 s per pup and each pup was handled for a total of 5 min. This procedure was conducted on PN days 2–9.

2.4. Body weight

Body weight (g) was measured on the day of weaning (PN day 22), PN day 40, and on PN day 60.