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# The impact of early repeated pain experiences on stress responsiveness and emotionality at maturity in rats

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**Abstract**

The intensive care necessary for premature newborns is characterized by multiple procedures, many of which are painful. Given emerging evidence that such early pain during this time of high brain plasticity may affect long-term neurodevelopmental and social–emotional functioning, this study explored the impact of early repeated pain on emotionality and stress responsivity at maturity. From birth through postnatal day 7, Fischer 344 pups underwent either paw needle prick every day versus every other day or daily paw touch, or were left unperturbed. Each paw received the designated perturbation once per day. At maturity, some animals underwent emotionality testing: either a 4-day series of open field exposures or a single elevated plus-maze (EPM) exposure. The paw prick groups exhibited less open field habituation and occupied the EPM open arms more. Two weeks later, all animals were either subjected to forced swim or not. At 1 h post-swim, animals underwent either blood withdrawal for plasma corticosterone (CS) levels and ex vivo natural killer cell activity (NKCA) or were injected intravenously with radiolabeled NK-sensitive syngeneic MADB106 tumor cells and assessed for lung tumor retention. Sex was a major factor in the manifestation of perturbation-related differences in the biologic outcomes. Whereas postnatal pain differentially affected baseline tumor retention between males and females, only males exhibited perturbation-related differences in swim stress-induced increases in tumor retention and CS. Finally, male-female differences were evident in CS, NKCA, and tumor responses to swim stress. These findings suggest that early pain affects neurodevelopmental function in the mature organism; however, these relationships are complicated by sex differences, the postnatal pain schedule, and the outcome measured.


*Keywords:* Natural killer cell activity; Tumor; Corticosterone; MADB106; Fischer 344; Neonatal

## 1. Introduction

Premature and critically ill newborns are among the most vulnerable of populations, requiring intensive care characterized by multiple procedures, many of which are painful (Johnston et al., 1997; Porter et al., 1999; Stevens et al., 2003). Pain at this very young age is a profound physiologic stressor (e.g., Anand, 2000; Goldman and Koren, 2002), and emerging evidence suggests such repeated early pain experiences in premature newborns at this time of high plasticity in the developing brain may affect long-term neurodevelopmental and social–emotional functioning (Bhutta et al., 2002; Grunau, 2002). By postnatal day (PND) 10, the somatosensory function of the rat approximates that of the full term human infant (Fitzgerald, 1995; Fitzgerald et al., 1988) and pain experiences through the first postnatal week have been suggested to correspond to those experienced by extremely low birthweight infants during their stay in the neonatal intensive care unit (Johnston et al., 2002; Lidow, 2002). Although animal models can only approach in a very limited way the human experience, this study sought to explore in the rat the effects of postnatal repeated pain experiences during the first week of life on emotionality and stress responsiveness in the mature organism. Emotionality was assessed as elevated plus-maze (EPM) behavior and habituation to an open field. Stress responsivity was assessed as changes in plasma

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corticosterone (CS) levels and natural killer cell (NKCA) activity after forced swim.

Studies in rats support the hypothesis that stressful neonatal perturbations can exact a significant behavioral and biological toll in the mature animal; however, they have largely focused upon nonpainful early stressors such as maternal deprivation, neonatal handling, and infection. There is substantial agreement that such early nonpainful perturbations alter hypothalamic–pituitary–adrenal (HPA) axis responsivity (Anisman et al., 1998; Durand et al., 1998; Hodgson et al., 2001; Shanks et al., 1995), behavioral responses to novel environments (Kalinichev et al., 2002; McIntosh et al., 1999; Meerlo et al., 1999; Vallée et al., 1997; Wigger and Neumann, 1999) as well as measures of immune function (Shanks et al., 2000) and disease resistance (Hodgson and Knott, 2002; Hodgson et al., 2001) in the mature animal. The impact of early needle prick pain experiences on later HPA axis function and responses to novelty in the adult animal are much less studied (Anand et al., 1999; Walker et al., 2003).

NK cells are a subpopulation of lymphocytes that spontaneously recognize and kill virally infected cells and play a key role in controlling a variety of tumor cells during the metastatic process (Brittenden et al., 1996). We and others have shown both painful and nonpainful stress to result in robust suppression of NK activity in both humans and animals (e.g., Ben-Eliyahu, 2003; Page et al., 2001; Pollock et al., 1991). Resistance against the MADB106 tumor, a mammary adenocarcinoma cell line syngeneic to the inbred Fischer 344 (F344) rats used in this study sensitively reflects in vivo NK activity. Following intravenous injection, MADB106 cells seed and colonize only in the lungs; both processes have been shown to be highly controlled by NK cells, but only during the first 24 h after injection (Barlozzari et al., 1985; Ben-Eliyahu and Page, 1992; Page and Ben-Eliyahu, 1999). Thus, the lung retention of MADB106 cells provides an indicator of both in vivo levels of NK activity and host susceptibility to tumor development.

## 2. Methods

### 2.1. Animals

Mature F344 females, 8–10 per breeding, cohabitated with mature F344 males, 2 females per male, for 21 days. Subsequently, females were single housed until their litter was weaned on PND 22. The animal room was maintained on a 12 h dark/light cycle and animals had free access to food and water. Day of birth was assigned when delivery of the litter was completed by lights off. On the day of birth, PND 0, each litter was randomly assigned to one of the three postnatal perturbation groups, paw needle prick every day, paw needle prick every other day or paw touch, or remained unperturbed until weaning. All groups were represented by at least one litter within each breeding. Dams of perturbed litters were not rebred. Postnatal perturbation litters were weighed after the first perturbation session of PND 4; all animals were weighed on PND 22, weekly until week 12, and biweekly thereafter.

### 2.2. Postnatal treatments

From PND 0 through PND 7, each pup in the litter underwent either paw needle prick or touch, such that each paw was perturbed one time per day between 1.5 and 5.5 h after dark onset, one paw per hour. Paw order was counterbalanced. An additional litter of pups underwent paw needle prick every other day and was perturbed only on postnatal days 0, 2, 4, and 6 on the same hourly schedule, and left undisturbed on odd numbered postnatal days. This group was included to assess whether a difference in schedule and total number of neonatal pain experiences would have an impact on the outcomes assessed at maturity.

For each perturbation session, the dam was gently moved from the nest to a waiting cage and remained in the vivarium throughout the 5–10 min procedure. In the laboratory, upon removal from the nest, each pup underwent the designated perturbation and was placed in a warmed holding box until each pup in the litter had been perturbed. Pups were returned to the nest then rejoined by the dam in the vivarium. Paw needle prick consisted of a single poke with a 25 gauge needle, followed by gentle pressure between a cotton swab and cotton ball as necessary to stop bleeding. Paw touch consisted of four gentle touches of the paw with a cotton swab (Anand et al., 1999).

### 2.3. Tumor cell maintenance and radiolabeling

Both MADB106 and YAC-1 cells were maintained in 5% $CO_2$ at 37 °C in complete medium [RPMI 1640 media (Mediatech) supplemented with 10% heat-inactivated fetal calf serum (FCS), 0.05 mg/ml gentamycin, 2 mM L-glutamine, 0.1 mM nonessential amino acid, and 1 mM sodium pyruvate]. The adherent MADB106 cells were separated from the flask using trypsin 0.25%.

The DNA of MADB106 cells was labeled by adding 0.4 μCi [$^{125}$I]iododeoxyuridine per milliliter of medium to the growing MADB106 cell culture 24 h before cell harvest. After separation from the flask, cells were washed and reconstituted in phosphate-buffered saline (PBS). The cytoplasm of YAC-1 cells was labeled by a 90 min co-incubation with 200 μCi ($^{51}NaCrO_4$), 200 μl FCS, and 150 μl medium.

### 2.4. Testing at maturity

Animals were age-matched within each assay throughout the testing conducted at maturity, and were a