An Experimental Model of Neonatal Nociceptive Stimulation in Rats

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[Abstract] In order to survive, preterm and/or sick neonates need diagnostic and therapeutic measures that may cause discomfort, stress and pain during a critical period of intense growing and modeling of the central nervous system (Anand *et al.*, 2013). Scientific interest in the long lasting effects of the Neonatal Intensive Care (NIC) experience, which provides a sensory experience completely different from the natural uterine environment, is growing (Jobe, 2014). The follow-up of critically ill newborn infants until adulthood indicated an association of early noxious stimuli with long lasting alterations in somatosensory and cognitive processing (Doesburg, 2013; Vinall *et al.*, 2014; Vinall *et al.*, 2013). However, one major limitation of the clinical studies is the difficulty to distinguish between long-term effects of pain suffered during neonatal intensive care and other confounding factors such as the presence of non-painful stress during hospital stay, the occurrence of acute and chronic morbidities, the post-natal environmental influences and family care. In this context, the understanding of the roles played by each factor and the interplay between these diverse variables require the use of animal models. The protocol described here is used to model the noxious stimulation in which premature newborns are subjected during treatment in the NIC. The current protocol models inflammatory nociceptive stimulation in neonatal rats, as previously demonstrated (Leslie *et al.*, 2011; Lima *et al.*, 2014; Malheiros *et al.*, 2014). Complete Freund's adjuvant (CFA) is a solution of antigen emulsified in mineral oil and used as an immunopotentiator, causing a painful reaction that lasts 7-8 days after subcutaneous injection. It is effective in stimulating cell-mediated immunity. The rodent model of neonatal inflammatory stimulation with CFA is advantageous because at birth the formation of the central nervous system is incomplete in rat pups and corresponds to that of 24 week intra-uterine human preterm neonates (Anand *et al.*, 1999), following similar patterns in the development of the pain system (Fitzgerald and Anand,1993). The first postnatal week in newborn rat pups corresponds to human premature infants from 24-36 weeks of gestation (Kim *et al.*, 1996; Wilson, 1995), offering a suitable condition to model and compare preterm (rat pups on P1) to full term (rat pups on P8) infants subjected to noxious stimulation. In this paper, we present our methods to induce nociceptive inflammatory stimulation in neonatal rat pups as an attractive approach to

study short- or long-term effects and the mechanisms underlying the behavioral repertoire of ex-premature infants or adolescents.

Materials and Reagents

1. Pregnant Wistar rats are acquired around the 14th day of gestation
   Note: It is important to determine the P0 (postnatal day 0).
2. Complete Freund's adjuvant (CFA) (25 µl) (Sigma-Aldrich, catalog number: F5881)
   Note: The CFA is diluted in 0.9% sterile saline just before use (2:1, CFA:saline)

Equipment

1. Plastic cages (for maintaining the animals)
2. Automatic temperature control system
3. Insulin Syringe Ultra-Fine 6 mm (15/64") x 31 G needle
4. Electronic scale (sensitivity 0.1 g)

Procedure

Ethical statement: All procedures discussed here are in accordance with and were approved by the Independent Ethics Committee of the Universidade Federal de São Paulo.

1. Obtain approval from local ethics committee.
2. Pregnant Wistar rats from the 14th day of gestation have to be housed individually in plastic cages and placed in the animal facility, equipped with an automatic temperature control system (23 ± 2 ºC), ventilation, a 12-h light-dark cycle (lights on at 7:00 AM) and unrestricted access to food and water.
3. The day of birth is designated as P0.
4. Each litter is restricted to 8 pups within 24 h after birth, excluding those pups with body weights lower than 0.5 g.
5. From P1 (postnatal day one) on, animals from all litters have their body weight recorded on a weekly basis, with an electronic scale (sensitivity 0.1 g), until the end of the experimental protocol.
6. To compare the effects of CFA injection during neural development, on postnatal days 1, 8 or 21 rat pups receive a single intraplantar subcutaneous injection of an inflammatory agent, the complete Freund's adjuvant (CFA; 25 µl) into the left hindpaw.
   Note: Take care to avoid spending more than 2 min with each pup. This would cause higher stress levels in the dam.

7. All animals in a single litter are assigned to a particular group (CFA-stimulated at P1, P8, P21 or control), to avoid the influence that handling of pups at different ages could have on maternal care towards the remainder of the litter.
8. After CFA injection, the pups return to their home-cage with their dams.
9. As a result, paws of stimulated pups show swelling, edema and redness, starting a few hours after injection, lasting 5-7 days. The edema can be visually observed, as in Figure 1.
10. Control animals are subjected to needle insertion like the experimental pups, but without fluid infusion.
11. All litters are weaned on P21, and from then on, housed in same-sex groups of 5 animals of the same experimental group at most.
12. To avoid litter effects, pups from different litter were randomly assigned to different groups.

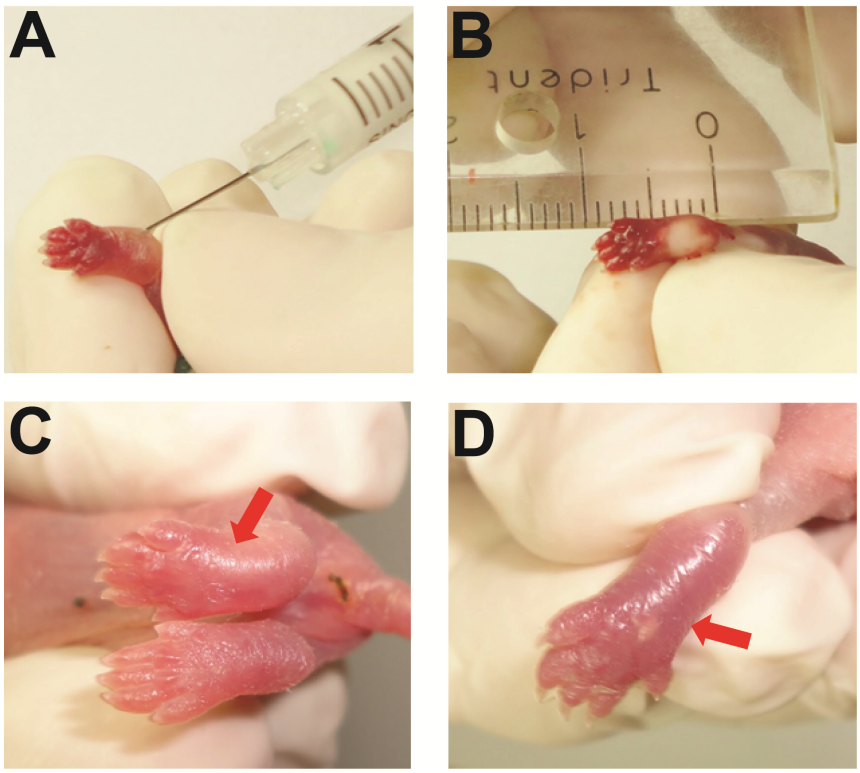

Figure 1. In A the intraplantar subcutaneous injection of CFA on postnatal day 1. Just after the injection B the bleaching and swelling under the skin of the left paw is visible. At day 3 C and day 6 D after CFA injection, the left paw is still swollen (red arrow) compared with the right paw.

Notes

All procedures are performed before 10 AM when the levels of corticosterone are low.

Acknowledgments

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