Sleep and Pain: A Relationship that Begins in Early Life

A complex interaction of genetic and environmental factors modulates pain sensitivity. The early years' experiences are an important environmental factor that has been associated to predispose an individual to pain conditions. Prolonged postnatal brain maturation makes the early years a critical period for adequate development and suggests that insults in infancy may cause long-term alterations in pain sensitivity (1,2). The vulnerability of the pain system during development indicates the need to investigate infant's experiences as a possible determinant of pain conditions.

Sleep quality and quantity can modulate pain sensitivity. Reduction in sleep time increases pain response and chronic pain conditions are able to alter sleep pattern (3,4). This relationship is well documented in adults, but very little is known about how pain and sleep interacts in early life. Sleep is a predominant behavior during infancy and evidence indicates that sleep is essential for brain development (5-7). Whether sleep is crucial for the maturation of several physiological systems, including the pain system, the bidirectional relation between sleep and pain begins early in life.

To better elucidate the function of sleep in early years and the relation between sleep and pain we conducted an investigation in mice to evaluate the effect of neonatal sleep deprivation on nociceptive behavior, an important component of the pain system. We hypothesized that neonatal sleep loss could be a threat to the developing organism, leading to long-term changes in pain sensitivity. An animal model is a reliable tool to explore behavioral impairment induced by sleep deprivation under controlled experimental conditions.

Neonatal mice at postnatal day (PND) 12 were randomly assigned to the following groups: control (CTRL), sleep deprivation (SD) or maternal separation (MS). Sleep deprivation was conducted using the gentle handling method. The method of gentle handling involves touching the animals with the hand or a brush, or shaking the cage, when pups showed signs of sleep onset. Sleep was behaviorally detected by motor activity quiescence, eye closure and myoclonic twitching. The gentle handling method is effective in producing SD in neonatal rodents, being able to eliminate up to 91% of total sleep time (8). The manipulation of the pups occurred in the absence of the mother. To control for the possible effects of the absence of the mother, the MS group was used. In the MS group, the litters were removed and placed in separate cages, which were put on a heating pad set in an adjacent room. During the separation period, food and water was not available, pups were not manipulated and could sleep. The SD and MS were only conducted for a period of 6 hours (from 8 a.m. to 2 p.m.). The CTRL litters remained undisturbed with dams in the colony room. All pups in the same litter received the same treatment. Weaning occurred at PND 22, when all pups were group housed by litter and sex until adolescence (PND 35, n = 7-9/group/sex) or adulthood (PND 90, n = 6-8/group/sex). At each developmental time-point (PND 35 or PND 90), the mice were tested for nociceptive response using the hot plate test. In this test mice were placed singly on a hot plate apparatus (Ugo Basile, Italy) that consists of a temperature-controlled metal surface (53 ± 1°C) encased by a cylindrical Plexiglas chamber. A timer was started when all 4 paws were in contact with the surface. The latency to withdraw the paw (licking either a hind paw or forepaw) to avoid thermal stimulus was measured in seconds, at which point the mouse was immediately removed from the hot plate and the timer was stopped. The paw withdrawal threshold to avoid thermal stimulus is a widely used measure of nociceptive response in rodents. Sixty second cut-off latency was determined for non-responsive animals (9). The experimental protocol was in accordance with the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals (10) and it was approved by the University's Ethics Committee (#1960/09). Data were evaluated using two-way analysis of variance (ANOVA), with group and sex as main factors. Post-hoc comparisons were performed using the Tukey's test whenever necessary. Results were expressed as the mean±S.D. The level of significance was set at $P < 0.05$. All analyses were carried out using SPSS 20.

The long-term effects of neonatal manipulations on nociceptive response showed their main effect in the adolescent mice ($F(2,42) = 3.97$; $P = 0.02$; eta = 0.15; power = 0.68). Six hours of SD at PND 12 was able to increase nociceptive sensitivity at PND 35, with adoles-

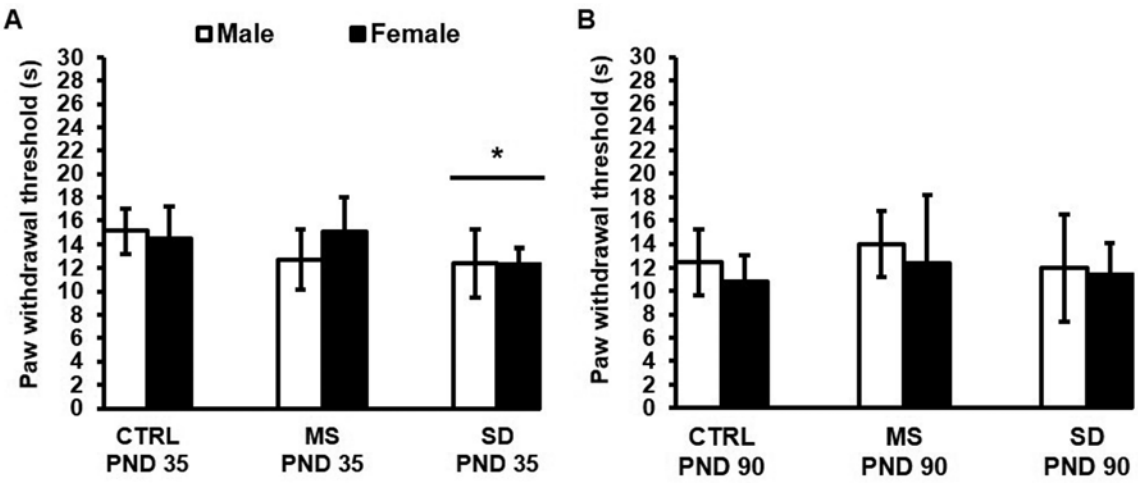

Fig. 1. *Long-term effect of sleep deprivation on nociceptive response. A: Latency to withdraw the paw (licking either a hind paw or forepaw) to avoid thermal stimulus (hot plate) in mice at PND 35 (A, N = 7 -9/sex/group, total N = 48) and PND 90 (B, N = 6-8/sex/group, total N = 46). CTRL: control; MS: maternal separation; SD: sleep deprivation; PND: postnatal day. Data are presented as mean ± S.D. *vs. CTRL, P < 0.05.*

cent mice exhibiting a lower paw withdrawal threshold compared with CTRL mice (12.3 vs. 14.8 sec, $P = 0.01$; Fig. 1A). Sleep loss in a critical period for pain system development reduced the nociceptive threshold by 17%. There was no significant main effect or interactions on nociceptive response in adult mice. The paw withdrawal threshold at PND 90 was statistically similar between groups (Fig. 1B).

Nociception is an essential component of the pain system and dysfunctions of the nociceptive response, such as hypersensitivity, could be associated with increased risk for chronic pain conditions. In the present study, sleep deprived pups on PND 12 showed hypersensitivity during adolescence revealed by the lower paw withdrawal threshold on hot plate test, even after 3 weeks of normal sleep conditions. The increased sensitivity could reflect a brain that is more responsive and vulnerable to noxious stimuli as a result of neonatal sleep loss. However, this change in nociceptive response was not statistically different from the MS group and it was not present in adult mice. It is possible that our results reflect a short-term effect of the accumulation of manipulations (summation of sleep deprivation associated to maternal separation), not only sleep loss per se. Despite this limitation, our data add evidence to a scarce literature related to sleep and pain interactions in early-life.

We believe that our data will encourage more animal and clinical studies to investigate the bidirectional relation between sleep and pain at different developmental time-points. Given the importance of sleep for health and well being, perhaps improved children's sleep could be the key to improve pain condition in pediatric populations.

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