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Maternal separation stress leads to resilience against neuropathic pain in adulthood

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ABSTRACT

Early life stress (ELS) leads to a permanent reprogramming of biochemical stress response cascades that may also be relevant for the processing of chronic pain states such as neuropathy. Despite clinical evidence, little is known about ELS-related vulnerability for neuropathic pain and the possibly underlying etiology.

In the framework of experimental studies aimed at investigating the respective relationships we used the established ELS model of maternal separation (MS). Rat dams and neonates were separated for 3 h/day from post-natal day 2–12. At adulthood, noxious mechanical and thermal thresholds were assessed before and during induction of neuropathic pain by chronic constriction injury (CCI). The potential involvement of spinal glutamatergic transmission, glial cells, pro-inflammatory cytokines and growth factors was studied by using qPCR.

MS *per se* did not modify pain thresholds. But, when exposed to neuropathic pain, MS rats exhibited a marked reduction of thermal sensitivity and a delayed development of mechanical allodynia/hyperalgesia when compared to control animals. Also, MS did not alter glucocorticoid receptor mRNA levels, but prevented the CCI-induced down-regulation of NR1 and NR2 sub-units of the NMDA receptor and of the glutamate transporter EAAT3 as observed at 21 days post-surgery. Additionally, CCI-provoked up-regulation of glial cell markers was either prevented (GFAP for astrocytes) or dampened (Iba1 for microglia) by MS. Pro-inflammatory cytokine mRNA expression was either not affected (IL-6) or reduced (IL-1β) by MS shortly after CCI. The growth factors GDNF and NGF were only slightly downregulated 4 days after CCI in the MS-treated animals. The changes in glutamatergic signaling, astroglial and cytokine activation as well as neurotrophin expression could, to some extent, explain these changes in pain behavior. Taken together, the results obtained in the described experimental conditions support the mismatch theory of chronic stress where an early life stress, rather than predisposing individuals to certain pathologies, renders them resilient.

1. Introduction

The nervous and the immune system are involved in the stress response as well as in the processing of pain (Krishnan and Nestler, 2008; Schwaller and Fitzgerald, 2014). Stress-related structural or functional modifications within these systems are hence likely to impact pain sensitivity (Sandkühler, 2009). Early life is a critical period for the normal development of individuals. Preclinical (Schmidt, 2010) and clinical (Heim et al., 2010) studies have demonstrated that early life stress can have a major impact on neuronal circuits and immune system development, possibly leading to enhanced vulnerability to physio- and psychopathological states at adulthood. A well-established way of modelling early life stress in rodents is to expose new-born pups to maternal separation (MS) (Levine, 2001). MS has been shown to induce abnormal development of immune and nervous systems (Roque et al., 2015). Among these perturbations is a long term modification of the central neuronal circuitry involved in relaying noxious stimuli and in controlling pain sensitivity during normal and pathological states (Chung et al., 2007; Uhelski and Fuchs, 2010; Weaver et al., 2007). Neuropathic pain is a chronic pain state that generally occurs following nerve damage. As a consequence, significant peripheral and central remodeling leads to enhanced pain sensitivity (hyperalgesia), induction of pain by a normally non painful stimulus (allodynia) and to spontaneous pain (for review see: von Hehn et al., 2012). Despite significant advances in basic and clinical research, this condition remains difficult to treat since our understanding of the underlying pathophysiological mechanisms is still insufficient (von Hehn et al., 2012).

Although spinal synaptic transmission of noxious stimuli constitutes the first relay of this network, it has scarcely been studied in the context of stress. Among the molecular mediators involved in nociceptive transmission and in the establishment of neuropathic pain, several key players such as the glucocorticoid receptor (GR) (Ladd et al., 2004;

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Wang et al., 2005), glutamatergic receptors and transporters (Toya et al., 2014), cytokines (Alvarez et al., 2013) and neurotrophins (Faure et al., 2007) are modulated by MS. Thus, it seems plausible that MS could have an impact on neuropathic pain vulnerability at least in a subset of individuals. In addition changes of neuro-immune and/or neuroendocrine systems induced by chronic stress could facilitate or hamper the development of chronic pain independently of classical stress processing pathways. To our knowledge, no study has investigated the effect of MS on pain thresholds and potential spinal molecular mediators involved in the change of nociceptive transmission under conditions of neuropathy. In the present work we used behavioral (mechanical and thermal thresholds) and biochemical (mRNA expression of spinal markers) approaches to assess the impact of MS on the onset and the maintenance of neuropathic pain. In order to induce this pain condition, we decided to use the well-established chronic constriction injury (CCI) model initially described by Bennett and Xie (1988).

2. Material and methods

2.1. Animals

Female (nulliparous) and male Sprague Dawley rats were purchased from Harlan Laboratories (Netherland). They were then reared in our facility to provide the offspring used for the experimental studies. All animals were housed under standardized conditions: temperature controlled room (21–23 °C), relative humidity 60 ± 10%, 12 h light/dark cycle, food and water provided *ad libitum*. Rats were only briefly handled twice per week during the cage changes. Except for the maternal separation procedure in the respective groups, pups were left undisturbed until weaning at post-natal day 21. Experiments started when the animals reached the age of 8 weeks.

Animals were divided in 4 groups (see Fig. 1) depending on the stress and pain conditions they were exposed to: controls CON (no MS, no CCI; n = 10), CON + CCI (no MS but CCI; n = 15), MS (MS but no CCI; n = 15), and MS + CCI (MS and CCI; n = 14).

All animal experiments were carried out in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and met the ARRIVE guidelines. The animal procedures were approved by the Animal Experimentation Ethics Committee (AEEC) of the University of Luxembourg (Project ID 15-SPM-01-UH) and the "Ministère de l'Agriculture, de la Viticulture et de la Protection des consommateurs".

2.2. Maternal separation (MS)

Maternal separation was carried out on at least four different litters. The day pups were first seen was marked as P0. From P2 to P12 pups were separated from the dam, placed on a heated pad at 33 °C (± 2 °C) and left undisturbed for 3 h/day. At the end of each separation period pups were returned to their home cage. No other manipulation was done than indicated.

2.3. Chronic constriction injury (CCI) surgery

At two months of age, after baseline behavioral testing, rats underwent the CCI surgery. They were deeply anaesthetized with isoflurane (4.5% for induction, 2.0–2.2% for maintenance) during the entire procedure using an anesthesia apparatus (Univentor 400, Zejtun, Malta). The right sciatic nerve was exposed in the mid-thigh and three natural chromic gut 4-0 (Stoelting Europe, Dublin, Ireland) loose ligatures were placed around the nerve at a distance of 1 mm. The muscle layer was closed with 4-0 silk sutures and the skin layer with surgical skin staples.

In case animals presented signs of autotomy in the course of the experiments, they were immediately removed and sacrificed in order to minimize their suffering. For this reason 2 CON + CCI and 3 MS + CCI animals were sacrificed before the end of the neuropathy protocol and their results were discarded.

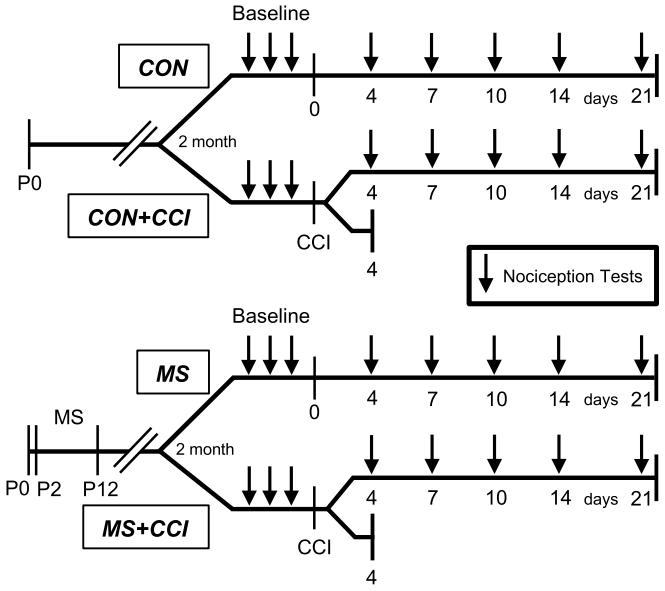

Fig. 1. Experimental design. After birth (P0), rats were separated into 2 experimental groups. The first one was left undisturbed while the second underwent the maternal separation (MS) procedure from postnatal day 2 (P2) to P12. At two months of age, the animals in each of the two groups were either assigned to a non-operated group (CON resp. MS) or to a group that underwent chronic constriction injury (CON + CCI resp. MS + CCI). All four groups were then tested for noxious mechanical and cold thresholds three days in a row to assess baseline values. At day 0, CCI was performed in the respective groups (CON + CCI and MS + CCI) and further behavioral tests were performed in all of the animals on days 4, 7, 10, 14 and 21. After the last testing, animals were sacrificed and the L4-L5 segments of the spinal cord were removed for qPCR analysis of biochemical markers. Two additional sets of animals in the CON + CCI and MS + CCI groups were included to study changes in the expression of biochemical markers early after CCI surgery. They did not undergo behavioral tests and were sacrificed 4 days post-CCI.

2.4. Behavioral tests

Noxious mechanical and thermal thresholds were assessed in 54 male rats using the von Frey monofilament test and the cold plate test respectively. All behavioral tests were done in the morning between 8:00 and 12:00 a.m. During each session, animals were moved to the experimentation room at least 1 h before the start of the experiments to allow them to habituate to the environment. Rats underwent the von Frey monofilament test, followed by the cold plate test. Baseline thresholds were assessed on three consecutive days prior to the CCI surgery. To assess the impact of maternal separation on neuropathic pain, the pain sensitivity was tested at days 4, 7, 10, 14, and 21 after the CCI surgery.

2.4.1. Von Frey monofilament test

To evaluate mechanical pain thresholds, animals were placed on a metal wire mesh floor, covered by a Plexiglas chamber (19.5 × 19.5 × 14 cm) and given at least 15 min to acclimate, until exploratory activity ceased. Filaments (OptiHair, MarstockNervTest, Germany) were applied perpendicularly on the mid-plantar region of the hind paw and pressure was gradually increased until the deflection point of the filament. Pain thresholds were determined with the ascending and descending method of limits with forces ranging from 8 to 256 mN. The threshold force was defined as the first filament evoking at least a 40% response rate (two withdrawals out of five consecutive applications). Both hind paws were tested three times in an alternative order and the mean results were defined as the respective thresholds.