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Persistent, comorbid pain and anxiety can be uncoupled in a mouse model

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HIGHLIGHTS

- We compare the interaction between chronic pain and anxiety in two mice strains.
- Pain and anxiety may not necessarily exacerbate one another in mouse models.
- FVB mice were more resilient in behavioral responses to social stress than C57 mice.

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ABSTRACT

Clinically, pain and anxiety frequently coexist; however, these two conditions' interaction is limited and contradictory in animal studies. In this study, we combined social defeat (SD) stress with Freund's adjuvant (CFA)-induced persistent inflammatory pain to investigate the reciprocal relationship between anxiety-like and nociceptive behaviors in two mouse strains. C57BL/6J mice subjected to the 10-day period of SD stress by repeated CD-1 mice aggression exhibited significant social interaction avoidance behaviors in the social interaction (SI) test, which is believed to represent the symptoms of anxiety. These mice also displayed anxiety-like behaviors in elevated plus maze (EPM) and open field (OF) tests. Compared to C57BL/6J mice, FVB/NJNju mice showed less basal social contact, but their behavioral responses to 10-day SD stress were more resilient. CFA-inflammatory mice showed robust mechanical allodynia and thermal hyperalgesia in both strains, but did not develop obvious social avoidance and anxiety-like behaviors 10 days after CFA-inflammation. Interestingly, CFA-inflammatory mice exposed to SD stress were not accompanied by a worsening of pain and anxiety-like behaviors in most tests. In contrast, the SD stress-induced social avoidance was significantly antagonized by combining with CFA-inflammatory pain. These findings suggest that persistent inflammatory pain and SD stress-induced anxiety may not necessarily exacerbate one another in animal models of comorbidity.

1. Introduction

Clinical comorbidity of pain and mood disorders (anxiety or depression) is widely recognized. Epidemiological studies have reported that chronic pain and mood disorders are reciprocally linked. The prevalence of pain in subjects with anxiety or depression, and that of anxiety or depression in subjects with pain, is higher than in the cohort with either condition alone [1–3]. In addition to the large amounts of literature on comorbidity of pain and mood disorders in human subjects, there have been many studies investigating this issue in animal models [4]. Although the general data from patient studies suggest that comorbidity provokes a general worsening in both conditions [5,6], animal studies found conflicting results, as the relationship between chronic pain and mood disorders depends on the differences in animal species/strains, models, test periods and behavioral test paradigms [4,7]. For example, a study by Mao showed that in social stress-induced anhedonic rats (Wistar), arthritic pain worsened depressive-like behaviors in forced swimming and tail suspension tests and was associated with exacerbated thermal hyperalgesia and mechanical allodynia [8]. In contrast, Bravo and colleagues showed that the combination of chronic constriction injury (CCI, a model of neuropathic pain) and unpredictable chronic mild stress (CMS) had no effect on the CCI-induced mechanical allodynia and CMS-induced anhedonia and behavioral despair in Sprague–Dawley rats [9].

Chronic social defeat (SD) stress in rodents induces social interaction avoidance and the anxious/depressive-phenotype [10,11]. This ethologically relevant stressor involves forcing a rodent to intrude on the home cage of a more aggressive rodent, which overpowers it until

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a rapidly submissive phenotype emerges [12]. Social defeat bears anxiogenic consequences, as assessed by unconditioned anxiety tests [13,14]. The previous studies from our and other laboratories showed that C57BL/6J mice subjected to a 6- or 10-day period of SD stress induced by repeated CD-1 mouse aggression displayed significant social interaction avoidance behaviors representing symptoms of anxiety [15–17]. In the present study, by combining SD stress with complete Freund's adjuvant (CFA)-induced chronic inflammatory pain models, we further investigated the reciprocal relationship between pain and anxiety-like behaviors. Because of the known variability among animal strains in their expression of either pain or anxiety/depression-like behaviors [18–20], we studied two strains (C57BL/6J and FVB/NJNju) in the present study.

2. Materials and methods

2.1. Experimental animals

Adult male C57BL/6J mice (7–9 weeks) from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences and FVB/NJNju mice (7–9 weeks) from the Model Animal Research Center of Nanjing University (Nanjing, China) were housed in groups in a temperature- and humidity-controlled room with a 12:12 light–dark cycle (lights on 07:00) and water available ad libitum. CD-1 mice that were retired breeders (male, 8–10 months) from Vital River Laboratories (Beijing, China) were used as the aggressors. The aggressors were screened every 3 months to ensure their antagonistic interactions. Six groups were tested in both strains of mice (C57BL/6J and FVB/NJNju): sham-SD control, SD stress, sham-CFA control, CFA-inflammation, CFA plus SD, and sham-CFA plus SD. To control for possible effects of time of day, mice were trained and tested at approximately the same time of day (3–6 h after lights on). All of the behavioral tests were performed by the same experimenter blinded to the group assignment to minimize the differences between-experimenters. All experimental protocols and animal handling procedures were permitted by the Shanghai Animal Care and Use Committee and Animal Ethical Committee of Fudan University, and were consistent with the policies issued by the International Association for the Study of Pain.

2.2. Social defeat stress and social interaction test

Social defeat (SD) stress was conducted as previously described [16]. Briefly, the C57BL/6J or FVB/NJNju mouse was individually introduced to the home cage of an unfamiliar aggressive CD-1 resident mouse for 6–10 min and physically defeated. During the exposure, when the C57BL/6J or FVB/NJNju mouse showed signs of subordination (freezing or upright submissive postures) or when three attacks on the CD-1 mouse were observed, the defeated C57BL/6J or FVB/NJNju mouse was separated from the aggressive CD-1 mouse by introducing a perforated plastic divider into the cage to allow visual, auditory and olfactory contacts for the remainder of 24 h. If the aggressive CD-1 mouse did not initiate an attack within 10 min, a new aggressive CD-1 mouse was introduced. The next day, the experimental mouse was transferred to a new cage where another unfamiliar aggressive CD-1 mouse resided. During a period of SD stress, these mice were subjected to social defeat for 10 consecutive days (exposure to 10 different CD-1 mice). Considering that the damage by the social aggression may influence nociceptive responses, experimental mice that showed obvious visible wounds resulting from the SD stress procedure were excluded from the experiments. Control animals were kept in identical home cages in pairs (separated by the shielding board) during the 10 days. The schematic diagram of the experimental apparatus is shown in Fig. 1A.

The social interaction (SI) test was performed on day 11 in a clean, open arena (42 cm × 42 cm). Each test consisted of two 2.5-min sessions, separated by an interval of 30–60 s. In the first (also called No target) session, the experimental (C57BL/6J or FVB/NJNju) mouse was introduced to the arena with an empty mesh cage (10 cm × 6 cm). In the second (Target) session, a mesh cage with an unfamiliar CD-1 mouse replaced the empty cage. The mesh cage allowed visual and olfactory interactions (but not physical contact) between the experimental and target mice. The area (14 cm × 26 cm, meaning 8 cm around the mesh cage) was defined as the interaction zone (IZ) (Fig. 1B). The time spent in the IZ was measured. A social interaction ratio (SIR) was calculated as follows: dividing the interaction time of the second (Target) session by the first (No target).

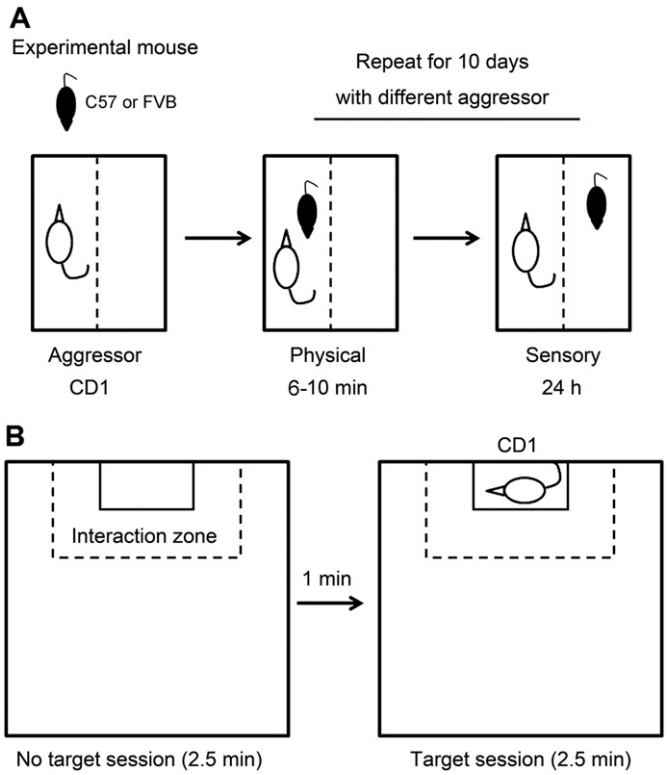

Fig. 1. Social defeat paradigm. (A) A schematic representation of the experimental process for social defeat (SD) stress. An experimental mouse (C57BL/6J or FVB/NJNju) was introduced to the cage of a CD-1 resident mouse (aggressor) for 6–10 min and physically defeated; then, they were housed together but separated by perforated plastic divider to allow visual, olfactory and auditory contact for the remainder of 24 h. (B) The social interaction test was performed in an open field like. The area around the cage (8 cm from the mesh) was the interaction zone. The time spent in the interaction zone was recorded.

2.3. Model of inflammatory persistent pain

Complete Freund's adjuvant (CFA, 10 μl, Sigma, USA) was injected into the plantar surface of the unilateral hindpaw under brief isoflurane anesthesia. Sham control animals received 10 μl of incomplete Freund's adjuvant. Local redness and swelling were observed in CFA-injected mice but not sham-CFA mice during the experimental period.

2.4. Comorbidity of persistent inflammatory pain and SD stress

One day after intraplatar injection of CFA (or sham), experimental mice received the SD stress as described above. Behavioral tests were performed on day 12.

2.5. Nociceptive behavioral tests

2.5.1. von Frey test

Mechanical allodynia was assessed by measuring paw withdrawal thresholds (PWTs) in response to a calibrated series of von Frey hairs