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Short-term sleep disturbance-induced stress does not affect basal pain perception, but does delay postsurgical pain recovery

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Abstract

Chronic sleep disturbance-induced stress is known to increase basal pain sensitivity. However, most surgical patients frequently report short-term sleep disturbance/deprivation during pre- and post-operation periods and have normal pain perception pre-surgery. Whether this short-term sleep disturbance affects postsurgical pain is elusive. We here reported that pre- or post-exposure to rapid eye movement sleep disturbance (REMSD) 6 h daily for 3 consecutive days did not alter basal responses to mechanical, heat, and cold stimuli, but did delay recovery in incision-induced reductions in paw withdrawal threshold to mechanical stimulation and paw withdrawal latencies to heat and cold stimuli on the ipsilateral side of male or female rats. This short-term REMSD led to stress evidenced by an increase in swim immobility time, a decrease in sucrose consumption, and an elevation in the level of corticosterone in serum. Blocking this stress via intrathecal RU38486 or bilateral adrenalectomy abolished REMSD-caused delay in recovery of incision-induced

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reductions in behavioral responses to mechanical, heat, and cold stimuli. Moreover, this short-term REMSD produced significant reductions in the levels of mu opioid receptor and kappa opioid receptor, but not Kv1.2, in the ipsilateral L_{4/5} spinal cord and dorsal root ganglia on day 9 post-incision (but not post-sham surgery).

Keywords

Short-term sleep disturbance; stress; surgery; postoperative pain

Introduction

Despite intensive research into the neurobiological mechanisms of persistent pain during past decades, postsurgical pain control remains a challenge in approximately one-third of surgical patients.⁴⁹ A prospective study of patients with acute postoperative pain reports that up to 10% of the patients still experience severe and intractable postsurgical pain 1 year after surgery.²⁶ In order to improve postsurgical pain management, previous studies have identified several potential predictors of postoperative pain and several preoperative and psychological factors.^{12,22,32,41} Among several factors known to affect postsurgical pain, chronic self-reported sleep disturbances before surgery constituted the strongest determinant of pain at rest postoperatively in some surgical patients.³² Additionally, surgical patients, particularly hospitalized in the Intensive Care Unit (ICU), frequently reported short-term postoperative sleep disturbances/deprivation.^{19,39} These sleep disturbances may result from surgical stress, a disruptive environment (such as noise, lighting, and patient care activities), medical illness itself, and medical treatments (such as respiratory care, drug therapies, and mechanical ventilation). Clinical and experimental studies have shown that postsurgical sleep and pain interact bidirectionally.^{27,30,39} Sleep disturbance-induced stress is considered to a potential catalyst of postsurgical pain.^{11,39}

The substantial studies from previous clinical observations have demonstrated that chronic sleep disturbance-induced stress altered basal pain perception in healthy subjects.^{28,38} Long-term consecutive or intermittent rapid eye movement (REM) sleep disturbance/deprivation (REMSD) significantly increased behavioral responses to noxious, thermal, and electrical stimuli in naïve experimental animals.^{24,27,30,33} Indeed, in clinical settings, some surgical patients with chronic severe physical or psychological stress often reported the increased basal pain sensitivity or exacerbated existing pathological pain before surgery. However, the majority of surgical patients are under normal physiological and psychological conditions and have normal pain perception (except for pathological pain) pre-surgery although they have short-term sleep disturbances at distinct instances throughout the pre- or post-operation period. Whether or not these short-term sleep disturbances affect the recovery of postsurgical pain is elusive.

In the current study, we carried out a REMSD procedure as described previously^{18,47} and first determined the optimal time point at which short-term sleep disturbances did not alter basal pain perception. We examined whether sleep disturbances under optimal conditions augmented surgical pain induced by a hind paw incision. We then determined whether the

rats with these short-term sleep disturbances experienced stress. Finally, we defined how these short-term sleep disturbances exacerbated postsurgical pain.

Materials and Methods

Animal preparation

Male and female Sprague-Dawley rats weighing 200–300 g were obtained from Charles River Laboratories (Wilmington, MA). All rats were housed in an animal facility that was kept in a standard 12-h light/dark cycle, with standard laboratory water and food pellets available *ad libitum*. Animal experiments were conducted with the approval of the Animal Care and Use Committee at New Jersey Medical School and were consistent with the ethical guidelines of the US National Institutes of Health and the International Association for the Study of Pain. All efforts were made to minimize animal suffering and to reduce the number of animals used. To minimize intra- and inter-individual variability of behavioral outcome measures, animals were trained for 1–2 days before behavioral testing was performed. The experimenters were blinded to treatment condition during behavioral testing.

Incisional pain model

The incisional surgery was carried out with minor modification as described.⁷ Rats were anesthetized with 2% isoflurane delivered via a nose cone. The plantar aspect of the left hindpaw was prepared in a sterile manner with a 10% povidone-iodine solution. A 1-cm longitudinal incision was made with a number 11 blade, through skin and fascia of the plantar aspect of the foot, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The plantaris muscle was elevated and incised longitudinally. After hemostasis with gentle pressure, the skin was sutured with 5-0 nylon thread. The wound site was covered with a mixture of polymixin B, neomycin, and bacitracin ointment. After surgery, the animals were allowed to recover in their cages. Typically, the wounds healed well within 5–6 days.

REM sleep disturbance procedure

The pedestal-over-water or flower pot technique of REM sleep disturbance (REMSD) was carried out according to previous studies with minor modification.^{18,47} Briefly, the rats were placed on a glass platform of 5.5 cm in diameter in the middle of a Plexiglas tank filled with water (25°C, 15 cm in depth) to 1 cm below the top surface of the platform. The occurrence of REM sleep was disturbed by the muscular atonia accompanying REM sleep onset, during which the body comes into contact with water, thus awaking the animal. These rats were maintained in the tank for 6 hours per day for 3 to 5 consecutive days during the daytime (07:00 – 19:00). The control rats were placed on a glass platform of 20 cm in diameter and 1 cm in height in the middle of the same tank without water.

Intrathecal catheter implantation and drug administration

A polyethylene 10 catheter was inserted into the subarachnoid space as described previously.^{42,50} Briefly, rats were anesthetized with 2% isoflurane and the surgical area was prepared in a sterile manner with a 10% povidone-iodine solution. After a small incision was made in the back area of L4 and L5, the catheter was inserted into the subarachnoid space

between L4 and L5 vertebrae and advanced 2–2.5 centimeter to reach the lumbar enlargement of the spinal cord. The residual catheter was tunneled under skin to the neck area and the outer part of the catheter was carefully plugged and fixed onto the skin. The incision wound was sutured and 2000 U of penicillin was administered to prevent infection. Rats were observed for one week and those exhibiting postoperative neurological deficits (e.g., paralysis) or poor grooming were excluded from the experiments.

One week later, mifepristone (RU38486, 0.2 µg/µl, Sigma–Aldrich, St. Louis, Mo, USA) dissolved in a 10% ethanol solution (vehicle) or vehicle was injected intrathecally in a 10 µl volume followed by a 10 µl saline flush 1 h before REMSD daily for 3 days. The dosage of RU38486 used was based on a previous report.⁴⁶

Bilateral adrenalectomy

Bilateral adrenalectomy (ADX) was carried out through two dorsolateral midflank skin and muscular incisions as described.^{36,37} After the vessels at the base of the adrenal gland were clamped, the adrenal glands were removed, and the skin and muscle were sutured. ADX rats were supplemented with 25 µg/ml corticosterone (Sigma–Aldrich, St. Louis, Mo, USA) in their drinking saline to presumably maintain basal levels of corticosterone and its circadian rhythmicity. Fresh solution was prepared every 2 days. In sham-ADX (Sham) rats, the procedure was performed in the same manner except the adrenal glands were left intact. In addition, normal drinking saline instead of drinking water was given to the sham rats after surgery. All rats were allowed to recover for one week before the experiments.

Behavioral analysis

Paw withdrawal thresholds in response to mechanical stimuli were measured with the up–down testing paradigm as described previously.^{15,51} Briefly, rats were placed in Plexiglas chambers on an elevated mesh screen. Von Frey filaments in log increments of force (0.407, 0.692, 1.202, 2.041, 3.63, 5.495, 8.511, 15.14 g) were applied to the plantar surface of the rats' left and right hind paws. The 2.041-g stimulus was applied first. If a positive response occurred, the next smaller von Frey hair was used; if a negative response was observed, the next larger von Frey hair was used. The test was terminated when (i) a negative response was obtained with the 15.14-g hair or (ii) three stimuli were applied after the first positive response. Paw withdrawal threshold was determined by converting the pattern of positive and negative responses to the von Frey filament stimulation to a 50% threshold value with a formula provided by Dixon.⁹

Paw withdrawal latencies to noxious heat were measured with a Model 336 Analgesic Meter (IITC Inc./Life Science Instruments, Woodland Hills, CA, USA) as described previously.^{15,51} Rats were placed in a Plexiglas chamber on a glass plate. Radiant heat was applied by aiming a beam of light through a hole in the light box through the glass plate to the middle of the plantar surface of each hind paw. When the animal lifted its foot, the light beam was turned off. The length of time between the start of the light beam and the foot lift was defined as the paw withdrawal latency. Each trial was repeated five times at 5-min intervals for each side. A cut-off time of 20 s was used to avoid tissue damage to the hind paw.

Paw withdrawal latencies to noxious cold (0°C) were measured with a cold plate, which was set at 0°C as described.^{15,51} The length of time between the placement of the hind paw on the plate and the animal lifting its hindpaw, with or without paw licking and flinching, was defined as the paw withdrawal latency. Each trial was repeated three times at 10-min intervals for the paw on the ipsilateral side. A cut off time of 60 s was used to avoid paw tissue damage.

Tests of locomotor function, including placing, grasping and righting reflexes, were performed before and after incision surgery according to the previously described protocol.^{15,51} (1) Placing reflex: The rat was held with hind limbs slightly lower than the forelimbs and the dorsal surfaces of the hind paws were brought into contact with the edge of a table. Whether the hind paws were placed on the table surface reflexively was recorded; (2) Grasping reflex: The rat was placed on a wire grid and whether the hind paws grasped the wire on contact was recorded; (3) Righting reflex: The rat's back was placed on a flat surface and whether it immediately assumed the normal upright position was recorded. Scores for placing, grasping, and righting reflexes were based on counts of each normal reflex exhibited in five trials. In addition, the animal's general behaviors, including spontaneous activity (e.g. walking and running), were observed.

The forced swimming test was carried out as described.^{16,31} Briefly, the rats were placed individually for 6 min in a vertical plastic cylinder (height: 50 cm, diameter: 30 cm) filled with water ($24 \pm 1^\circ\text{C}$) at a height of 30 cm to make it impossible for the rats to reach the bottom of the cylinder. Immobility occurred when the rats made bodily movements just sufficient to maintain their heads above the water. The duration of immobility was recorded during the last 4-min of the 6-min testing period. Then, the rats were dried before being returned to their home cages. The water was changed after each session.

Sucrose preference test

A sucrose preference test was performed as described previously.⁴⁸ A two-bottle choice paradigm was used to test for differences in sucrose preference. The rats were trained for two days with two drinking bottles containing either water or 1% sucrose before REMSD. The two bottles were placed randomly on the left or right side of the cage from one trial to the next. In order to assess drinking consumption for each rat, the rats were individually housed. After REMSD on the last day, the consumption of both water and sucrose for 24 h was analyzed. The drinking bottles were weighed on a standard weight scale. Sucrose preference was calculated using the following formula: sucrose intake (g)/[sucrose intake (g) + water intake (g)].

Blood collection and corticosterone levels

To measure the level of corticosterone in the blood after REMSD, the blood was collected via retro-orbital bleeding procedures as described.⁴⁰ In brief, after the rats were lightly anesthetized with isoflurane, a sterile glass capillary tube was inserted into the retro-orbital sinus venous plexus. The blood was allowed to flow via capillary action into a collection tube. Approximately 500 µl of blood was collected within 3 minutes as the corticosterone levels were elevated after 3 min of animal handling. After the samples were centrifuged for

10 min at 4,000 rpm at 4°C, the supernatant plasma was collected and stored at -80°C. The corticosterone levels were detected using a corticosterone ELISA kit (Enzo Life Sciences, Inc. Farmingdale, NY, USA).

Measurement of body weight

Rats were weighed before REMSD or incision and on day 9 after incision.

Western blot analysis

Western blot analysis has been carried out as described previously.^{3,15,50,51} In brief, bilateral L_{4/5} dorsal root ganglia (DRG) and spinal cord were collected and rapidly frozen in liquid nitrogen. The tissues were homogenized in chilled lysis buffer (10 mM Tris, 5 mM EGTA, 2 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 40 μM leupeptin, 150 mM NaCl). The crude homogenate was centrifuged at 4°C for 15 min at 1,000 × g. The supernatant was collected and the pellet (nuclei and debris fraction) discarded. After protein concentration was measured, the samples were heated at 99°C for 5 min and loaded onto a 4% stacking/7.5% separating SDS-polyacrylamide gel (Bio-Rad Laboratories, Hercules, CA). The proteins were then electrophoretically transferred onto a polyvinylidene difluoride membrane (Immobilon-P, Millipore, Billerica, MA). The membranes were blocked with 3% nonfat milk in Tris-buffered saline containing 0.1% Tween-20 for 1 h and incubated with primary mouse anti-Kv1.2 (1:500, NeuroMab, Davis, CA), primary rabbit anti-MOR (1:1,000; Neuromics, Edina, MN), primary rabbit anti-KOR (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA) or primary mouse anti-GAPDH (1:3,000; Santa Cruz Biotechnology) overnight under gentle agitation. GAPDH was used as a loading control. The proteins were detected by horseradish peroxidase-conjugated anti-mouse secondary antibody and visualized by chemiluminescence reagents (ECL; Amersham Pharmacia Biotech, Piscataway, NJ) and exposure to film. The intensity of blots was quantified with densitometry. The blot density from the sham plus control group was set as 100%. The relative density values from other groups were determined by dividing the optical density values from these groups by the value of the sham plus control group after they were normalized to the corresponding GAPDH.

Statistical analysis

All results are collected randomly and given as means ± SEM. The data were statistically analyzed with two-tailed, paired/unpaired Student's t test and a one-way or two-way ANOVA. When ANOVA showed a significant difference, pairwise comparisons between means were tested by the *post hoc* Tukey method (SigmaStat, San Jose, CA). Significance was set at $p < 0.05$.

Results

1. Time-dependent changes in basal pain perception after repeated exposure to REMSD in male rats

Male rats were exposed to REMSD 6 h daily for 5 days. Behavioral responses to mechanical, thermal, and cold stimuli were examined prior to REMSD (baseline) and 2 h later after REMSD daily. Significant decreases in paw withdrawal thresholds in response to

mechanical stimulation on both left and right hind paws were observed only on day 5 post-REMSD as compared to the corresponding control group, a behavioral indication of mechanical pain hypersensitivity ($P < 0.01$ in Fig. 1a. $P < 0.05$ in Fig. 1b). Marked reductions in paw withdrawal latencies in response to thermal stimulation were seen on day 4 post-REMSD on the right hind paw and on day 5 post-REMSD on both left and right hind paws as compared to the corresponding control group, an indication of thermal pain hypersensitivities ($P < 0.01$ in Fig. 1c. $P < 0.05$ or 0.01 in Fig. 1d). Similarly, significant reductions in paw withdrawal latency in response to cold stimulation were detected on both days 4 and 5 after REMSD for the left hind paw as compared to the corresponding control group ($P < 0.05$ or 0.01 in Fig. 1e), an indication of cold pain hypersensitivities. It appears that basal pain perception did not change significantly during the three-day daily exposure to REMSD. Thus, the sleep disturbance/deprivation caused by REMSD 6 h daily for 3 consecutive days was defined as short-term sleep disturbance, which was carried out in the following experiments.

2. Effect of pre-exposure to short-term REMSD on postsurgical pain in male rats

To examine whether this short-term sleep disturbance before surgery affected postoperative pain, we performed unilateral hindpaw plantar incision in male rats after they were exposed to REMSD daily for 3 days. Consistent with previous reports,^{3,7,8} the incision led to persistent mechanical, thermal, and cold pain hypersensitivities on the ipsilateral (but not contralateral) side in the incision plus control group (Fig. 2). Pain hypersensitivity reached a peak on day 1, lasted for 4–7 days, and completely disappeared on day 9 post-surgery (Fig. 2). As expected, the pre-exposure to REMSD alone did not alter basal paw responses to mechanical, heat, or cold stimuli on either side of sham rats during the observation period (Fig. 2). However, the pre-exposure of REMSD delayed the recovery of surgical pain on the ipsilateral side in the incision plus REMSD group (Fig. 2a, 2b, 2c). Compared to the corresponding incision plus control group, paw withdrawal thresholds to mechanical stimulation in the incision plus REMSD group were reduced by 30% ($P < 0.05$), 32% ($P < 0.05$), and 17% ($P > 0.05$) on days 7, 9, and 12 post-surgery, respectively (Fig. 2a). Paw withdrawal latencies to thermal stimulation were decreased by 16% ($P < 0.01$), 27% ($P < 0.01$), and 9% ($P > 0.05$) on days 7, 9, and 12 post-surgery, respectively, as compared to the corresponding incision plus control group (Fig. 2b). Additionally, paw withdrawal latencies to cold stimulation were reduced by 25% ($P < 0.01$), 21% ($P < 0.01$), and 5% ($P > 0.05$) on days 7, 9, and 12 post-surgery, respectively, as compared to the corresponding incision plus control group (Fig. 2c). Paw withdrawal threshold and latencies completely returned to baseline levels on day 15 post-surgery in the incision plus REMSD group (Fig. 2). Compared to the baseline level, there were no significant decreases in paw withdrawal threshold and latencies on the ipsilateral and contralateral sides of the sham plus control rats (Fig. 2).

3. Effect of post-exposure to short-term REMSD on postsurgical pain in male rats

We next examined whether this short-term sleep disturbance after surgery affected postoperative pain in male rats. Similar to the pre-exposure of REMSD above, the post-exposure of REMSD daily for 3 days also markedly delayed the recovery of surgical pain on the ipsilateral side in the incision plus REMSD group (Fig. 3a, 3b, 3c). Paw withdrawal

thresholds to mechanical stimulation in the incision plus REMSD group were reduced by 33% ($P > 0.05$), 43% ($P < 0.05$), and 38% ($P < 0.05$) on days 4, 7, and 9 post-surgery, respectively, as compared to the corresponding incision plus control group (Fig. 3a). In addition, compared to the corresponding incision plus control group, paw withdrawal latency to thermal stimulation were decreased by 25% ($P < 0.01$), 19% ($P < 0.01$), and 26% ($P < 0.01$) on days 4, 7, and 9 post-surgery, respectively (Fig. 3b), and paw withdrawal latency to cold stimulation were reduced by 18% ($P < 0.01$), 21% ($P < 0.01$), and 23% ($P < 0.01$) on days 4, 7, and 9 post-surgery, respectively (Fig. 3c). As expected, no significant changes in paw withdrawal thresholds and latencies were detected on the contralateral side in the incision plus REMSD group or on the ipsilateral and contralateral sides in the sham plus control group or in the incision plus control group during the observation period (Fig. 3).

4. Effect of post-exposure to short-term REMSD on postsurgical pain in female rats

Gender-related differences in pain and stress have been clearly shown in experimental settings and clinical observations.^{1,5,6,44,45} We further examined whether short-term sleep disturbance after surgery had different effects on postoperative pain in female rats. Female rats were exposed to REMSD and incision in the same manner as male rats described above. Consistent with a previous study in mice^[4], the intensity and duration of incision-induced pain hypersensitivity on the ipsilateral side in female rats are similar to those in male rats (Figs. 3 and 4). The post-exposure of REMSD daily for 3 days significantly delayed the recovery of surgical pain on the ipsilateral side in the incision plus REMSD group in female rats (Fig. 4a, 4b, 4c). Paw withdrawal thresholds to mechanical stimulation in the incision plus REMSD group declined by 27% ($P > 0.05$), 28% ($P < 0.05$), and 28% ($P < 0.05$) on days 4, 7, and 9 post-surgery, respectively, as compared to the corresponding incision plus control group (Fig. 4a). Compared to the corresponding incision plus control group, paw withdrawal latency to thermal stimulation decreased by 10% ($P > 0.05$), 17% ($P < 0.01$), and 20% ($P < 0.01$) on days 4, 7, and 9 post-surgery, respectively (Fig. 4b), and paw withdrawal latency to cold stimulation decreased by 10% ($P < 0.05$), 18% ($P < 0.01$), and 22% ($P < 0.01$) on days 4, 7, and 9 post-surgery, respectively, in female rats (Fig. 4c). There were no significant changes in paw withdrawal threshold and latencies on the contralateral side in the incision plus REMSD group or on the ipsilateral and contralateral sides in the sham plus control group or in the incision plus control group during the observation period in female rats (Fig. 4).

5. Existence of stress in male rats after short-term REMSD

To confirm the existence of stress under our optimal conditions, we carried out the forced swimming test, in which stress in rats is made evident in an increased duration of immobility.^{16,31} After having received REMSD daily for 3 days, the rats in the sham plus REMSD group (1.78-fold; $P < 0.01$) and in the incision plus REMSD group (2.07-fold; $P < 0.01$) remained immobile for a significantly greater length of time compared to the sham plus control group (Fig. 5a). There was no marked difference in immobility duration between the incision plus control group and the sham plus control group (Fig. 5a). Next, we performed the sucrose preference test which operates under the supported theory that stress results in a loss of interest in pleasure, in this case a palatable sucrose solution.⁴⁷ After

having received REMSD daily for 3 days, the rats in the sham plus REMSD group (67% of the sham plus control group; $P < 0.01$) and in the incision plus REMSD group (58% of the sham plus control group; $P < 0.01$) showed significantly less sucrose consumption than those in the sham plus control group (Fig. 5b). No obvious difference in sucrose consumption was seen between the incision plus control group and the sham plus control group (Fig. 5b). We also measured the level of corticosterone in serum after the rats received REMSD daily for 3 days. The levels of serum corticosterone in the sham plus REMSD group and in the incision plus REMSD group increased by 17-fold ($P < 0.01$) and 24-fold ($P < 0.01$), respectively, as compared to that in the sham plus control group (Fig. 5c). No significant increase was observed in serum corticosterone levels from the incision plus control group (1.77-fold; $P > 0.05$) as compared to the sham plus control group (Fig. 5c). Finally, no significant differences in changes in body weight before and after REMSD were found among the four groups ($P = 0.059$, Fig. 5d).

6. Effect of intrathecal RU38486 on short-term REMSD-induced augmentation of postsurgical pain in male rats

Corticosterone acts on glucocorticoid receptors to produce stress. To determine whether the stress under our optimal conditions contributed to the observed exacerbation of postsurgical pain, we pre-administered intrathecally a selective glucocorticoid receptor antagonist RU38486 1 h before REMSD daily for 3 days. In the incision plus REMSD group, pre-treatment of vehicle did not affect significant decreases in paw withdrawal threshold to mechanical stimulation ($P < 0.01$, Fig. 6a) and paw withdrawal latencies to thermal ($P < 0.01$, Fig. 6b) and cold ($P < 0.01$, Fig. 6c) stimuli on the ipsilateral side on day 9 post-incision. However, intrathecal RU38486 completely abolished these decreases in the incision plus REMSD group (Fig. 6a, 6b, and 6c). RU38486 at the dosage used did not alter basal paw withdrawal responses to mechanical and thermal stimuli on the contralateral side of the incision plus REMSD group (Fig. 6d and 6e) and on either ipsilateral or contralateral side of the remaining treated groups (Fig. 6).

7. Effect of bilateral ADX on short-term REMSD-induced augmentation of postsurgical pain in male rats

Serum corticosterone originates mainly from adrenal glands. To further confirm RU38486's pharmacological effect observed above, we carried out bilateral ADX to eliminate the production of serum corticosterone and maintained its basal level in serum through supplementation in drinking water. In the incision plus REMSD rats, sham surgery of ADX did not markedly alter the decreases in paw withdrawal threshold to mechanical stimulation ($P < 0.01$, Fig. 7a) and paw withdrawal latencies to thermal ($P < 0.01$, Fig. 7b) and cold ($P < 0.01$, Fig. 7c) stimuli on the ipsilateral side caused by REMSD daily for 3 days on day 9 post-incision. Bilateral ADX entirely reversed these decreases in the incision plus REMSD group (Fig. 7a, 7b, and 7c). Bilateral ADX did not change basal paw withdrawal responses to mechanical and thermal stimuli on the contralateral side of the incision plus REMSD group (Fig. 7d and 7e) and on either ipsilateral or contralateral side of the remaining treated groups (Fig. 7).

8. Locomotor activities in the experimental rats

To exclude the possibility that the effects observed above were caused by impaired locomotor functions (reflexes), we examined locomotor activities of experimental rats. As shown in Table 1, none of the treatments produced any effects on locomotor functions, including placing, grasping, and righting reflexes. Convulsions and hypermobility were not seen in any of the treated rats. Additionally, we did not observe any marked difference in general behaviors, including the gait and spontaneous activity among the treated groups.

9. Effect of pre- or post-exposure to short-term REMSD on the expression of opioid receptors and Kv1.2 in DRG and spinal cord of male incisional rats

Given that endogenous opioids acting at opioid receptors exert tonic inhibitory effects on nociceptive information transmission^{13,20,35,43} and that Kv1.2 has a dominant role in neuronal excitability,^{15,51} we finally examined whether short-term REMSD-induced augmentation of postsurgical pain was related to the changes in the expression of opioid receptors and Kv1.2 in DRG and spinal cord. Because a specific delta opioid receptor antibody is unavailable commercially, we focused on the observations of mu opioid receptor (MOR) and kappa opioid receptor (KOR). Pre-exposure to short-term REMSD significantly reduced the expression of MOR and KOR, but not Kv1.2, in the L_{4/5} DRG and L_{4/5} spinal cord on the ipsilateral side 9 days post-incision (Fig. 8). Compared to the sham plus control group, the levels of MOR, KOR, and Kv1.2 proteins were decreased by 56% ($P < 0.05$), 41% ($P < 0.05$), and 6% ($P > 0.05$), respectively, in spinal cord (Fig. 8a) and by 57% ($P < 0.05$), 48% ($P < 0.05$), and by 3% ($P > 0.05$), respectively, in DRG (Fig. 8b) in the incision plus REMSD group. Post-exposure to short-term REMSD produced similar reductions of MOR and KOR in L_{4/5} spinal cord and L_{4/5} DRG on the ipsilateral side 9 days post-incision (data not shown). Neither incision alone nor REMSD alone led to the marked changes in the amounts of MOR, KOR, and Kv1.2 proteins in the ipsilateral L_{4/5} spinal cord and L_{4/5} DRG in the incision plus control group or the sham plus REMSD group (Fig. 8). As expected, basal levels of MOR, KOR, and Kv1.2 proteins in the L_{4/5} spinal cord and L_{4/5} DRG on the contralateral side were not altered in all treated groups (data not shown).

Discussion

Surgery is a common medical intervention. More than 80% of surgical patients experience acute pain after surgery.^{2,25} Approximately 10% of patients report severe and intractable chronic postsurgical pain one year after surgery.^{25,26,34} Understanding what causes the exacerbated postsurgical pain may help us to predict who will go on to delay the recovery and who will not and offer an opportunity to effectively intervene postsurgical pain. Unlike other injuries that are either gradual (e.g., fibromyalgia) or sudden but unpredictable (e.g., nerve injury from trauma), surgery presents a unique set of circumstances in which the precise timing of the physical insult and ensuing pain are known in advance. These circumstances increase preoperative state anxiety and affect preoperative sleep, resulting in stress.³² Moreover, patients after surgery often complain of sleep disturbance or deprivation, particularly while hospitalized in the ICU.^{11,19,24} Factors that are associated with sleep disruption include postsurgical state anxiety, medications, patient care interactions, patient-ventilator dyssynchrony, environmental noise and light, and medical diseases themselves.¹⁹

Most surgical patients (except some patients who have pathological pain) have normal pain perception before surgery and short-term sleep disturbances before and/or after operative surgery. Whether these short-term sleep disturbances affects postsurgical pain in surgical patients is unknown.

Previous studies demonstrated that chronic consecutive or intermittent REMSD caused abnormal nociceptive sensitivity at the basal level. REMSD for consecutive 2–4 days in naïve rats markedly reduced paw withdrawal latency in response to heat stimulation.^{14,18} This reduction persisted at least for 1 day.¹⁴ REMSD also rendered rats more sensitive to mechanical and electrical (but not chemical) stimuli for up to 1 day.^{18,47} The duration of consecutive sleep disturbance (1, 2 or 3 days) was of no influence.^{18,47} Animals subjected to sleep disturbance 1 h daily for 10 consecutive days displayed mechanical and visceral hyperalgesia.^{10,17,29} Consistently, the present study showed that REMSD 6 h daily for 4–5 consecutive days led to mechanical, thermal, and cold pain hypersensitivities in normal rats. These preclinical studies may mimic clinical observations, in which some patients with severe physical or psychological stress frequently report pain and exacerbated existing pain associated with chronic pain disorders.^{23,27,30}

The present study established a preclinical animal model of REMSD-induced stress, in which REMSD 6 h daily for 3 days did not alter basal responses to mechanical, heat, and cold stimuli in naïve rats. This model may mimic clinical conditions, in which most surgical patients have normal pain perceptions and short-term sleep disturbances during pre- and post-operation periods. We found that this short-term REMSD either before or after surgery delayed post-surgical pain recovery. Moreover, this short-term REMSD caused stress evidenced by increasing immobility time in a forced swim test, decreasing preference for sucrose, and elevated blood corticosterone levels. Additionally, intrathecally blocking glucocorticoid receptor or eliminating the production of corticosterone completely abolished the REMSD-induced delay of post-surgical pain recovery, indicating that systemic released endogenous corticosterone act cells in the dorsal root ganglion and/or spinal cord to exacerbate surgical pain hypersensitivity. REMSD-induced stress is required for exacerbating post-surgical pain hypersensitivity. It should be noted that the ADX cannot rule out the involvement of other hormones in the REMSD-induced delay of post-surgical pain recovery as both adrenal cortex and medulla are removed. Interestingly, we observed that the intensity and duration of REMSD-induced augmentation in postsurgical pain are similar between male and female rats. Sex differences in pain and stress are well known.^{1,5,6,44,45} The reason for the discrepancy between the previous and present studies is unclear but may be related to short-term REMSD-induced stress and no gender difference in incisional pain.⁴

The mechanism of how short-term REMSD-induced stress does not alter basal pain perception but does delay postsurgical pain recovery is unclear, but it may be related to expressional changes in MOR and KOR in spinal cord and DRG. The present study demonstrated that this short-term REMSD produced marked reductions in the levels of MOR and KOR in DRG and spinal cord on day 9 post-incision, at this time point short-term REMSD exacerbated postsurgical pain. Given that endogenous opioids acting at opioid receptors exert tonic inhibitory effects on nociceptive information transmission,^{13,20,35,43} it is very likely that the reductions of these two opioid receptor subtypes in DRG and spinal

cord may produce loss of tonic inhibitory effects, resulting in the exacerbation of postsurgical pain. The fact that short-term REMSD alone did not alter basal expression of MOR and KOR in the DRG and spinal cord in sham animals indicates that incisional noxious input is required for short-term REMSD-induced downregulation of MOR and KOR in the DRG and spinal cord. How the interaction between incision and short-term REMSD causes this downregulation is unknown and remains to be further investigated. Interestingly, the expression of Kv1.2, which controls neuronal excitability,^{15,51} was not changed in the DRG and spinal cord under present model conditions, suggesting that short-term REMSD plus incision have a selective effect on the expression of the receptors/channels. It should be noted that, in addition to MOR and KOR, short-term REMSD plus incision may produce changes in the expression of other proteins in the DRG and spinal cord and that these changes may occur in brain regions. These potential mechanisms may also participate in short-term REMSD-induced exacerbation of postsurgical pain and need to be studied in the future.

In summary, the current study presented an interesting observation that short-term sleep disturbances do not alter basal pain perception, but do exacerbate postsurgical pain hypersensitivity. Although the mechanism underlying the effect of this short-term sleep disturbance stress on postsurgical pain hypersensitivity is still incomplete understood, our findings suggest that prevention of short-term sleep disturbance stress before and after surgery may help the recovery of postsurgical pain in patients.

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Perspective

Our findings show that short-term sleep disturbance either pre- or post-surgery does not alter basal pain perception, but does exacerbate postsurgical pain hypersensitivity. The latter may be related to the reductions of mu and kappa opioid receptors in spinal cord and dorsal root ganglia caused by REMSD plus incision. Prevention of short-term sleep disturbance may help the recovery of postsurgical pain in patients.

Fig. 1.

Time-dependent changes in basal paw withdrawal responses to mechanical, heat, and cold stimuli after rapid eye movement sleep disturbance (REMSD) 6 h daily for consecutive days as indicated in male rats. Significant reductions were seen in bilateral paw withdrawal thresholds in response to mechanical stimulation on day 5 post-REMSD (1a, 1b), in left hind paw withdrawal latencies in response to heat stimulation on day 5 post-REMSD (1c) and on day 4 and 5 post-REMSD on right hind paw (1d), and in paw withdrawal latency in response to cold stimulation on day 4 and 5 post-REMSD (1e) in REMSD group. * $P < 0.05$, ** $P < 0.01$ vs the corresponding time points in control group. $N = 5/\text{group}$.

Fig. 2.

Effect of pre-surgical exposure to short-term rapid eye movement sleep disturbance (REMSD) on postsurgical pain in male rats. REMSD 6 h daily for 3 consecutive days did not alter basal paw withdrawal responses to mechanical (2a, 2d), heat (2b, 2e), and cold (2c) stimuli on the ipsilateral (2a, 2b, 2c) and contralateral (2d, 2e) sides in the sham plus REMSD group, but markedly delayed recovery in incision-induced reductions in paw withdrawal threshold to mechanical stimulation (2a) and paw withdrawal latencies to heat (2b) and cold (2c) stimuli on the ipsilateral side on day 7 and 9 post-REMSD in the incision plus REMSD group, compared to the incision plus control group. No changes in paw withdrawal responses were seen during the observation period in the sham plus control group. * $P < 0.05$, ** $P < 0.01$ vs the corresponding time points in the incision plus control group. $N = 5/\text{group}$.

Fig. 3.

Effect of post-surgical exposure to short-term rapid eye movement sleep disturbance (REMSD) on postsurgical pain in male rats. REMSD 6 h daily for 3 consecutive days did not alter basal paw withdrawal responses to mechanical (3a, 3d), heat (3b, 3e), and cold (3c) stimuli on the ipsilateral (3a, 3b, 3c) and contralateral (3d, 3e) sides in the sham plus REMSD group, but markedly delayed recovery in incision-induced reductions in paw withdrawal threshold to mechanical stimulation on day 7 and 9 post-REMSD (3a) and paw withdrawal latencies to heat (3b) and cold (3c) stimuli on days 4, 7, and 9 post-REMSD on the ipsilateral side in the incision plus REMSD group, compared to the incision plus control group. No changes in paw withdrawal responses were seen during the observation period in the sham plus control. * $P < 0.05$, ** $P < 0.01$ vs the corresponding time points in the incision plus control group. $N = 5/\text{group}$.

Fig. 4.

Effect of post-surgical exposure to short-term rapid eye movement sleep disturbance (REMSD) on postsurgical pain in female rats. REMSD 6 h daily for 3 consecutive days did not alter basal paw withdrawal responses to mechanical (4a, 4d), heat (4b, 4e), and cold (4c) stimuli on the ipsilateral (4a, 4b, 4c) and contralateral (4d, 4e) sides in sham plus REMSD group, but markedly delayed recovery in incision-induced reductions in paw withdrawal threshold to mechanical stimulation (4a) and paw withdrawal latency to heat stimulation (4b) on days 7 and 9 post-REMSD and paw withdrawal latency to cold stimulation (4c) on days 4, 7, and 9 post-REMSD on the ipsilateral side in the incision plus REMSD group, compared to the incision plus control group. No changes in paw withdrawal responses were seen during the observation period in the sham plus control group. * $P < 0.05$, ** $P < 0.01$ vs the corresponding time points in the incision plus control group. $N = 5/\text{group}$.

Fig. 5.

Existence of stress after short-term rapid eye movement sleep disturbance (REMSD) in male rats. REMSD 6 h daily for 3 consecutive days significantly increased immobility time in a forced swim test (5a), decreased sucrose consumption in a sucrose preference test (5b), and elevated the level of corticosterone in blood serum (5c) in both the sham plus REMSD group and the incision plus REMSD group, compared to the sham plus control group. These changes were not observed in the incision plus control group (5a, 5b, 5c). No significant differences in changes in body weight before and day 9 post-REMSD were seen among the four groups (5d). ** $P < 0.01$ vs the corresponding sham plus control group. $N = 5/\text{group}$.

Fig. 6.

Effect of intrathecal pre-administration of RU38486 (Ru) on exacerbated postsurgical pain induced by short-term rapid eye movement sleep disturbance (REMSD) in male rats. In the incision plus REMSD group, intrathecal vehicle (10 μ l of 10% ethanol) 1 h before REMSD daily for 3 days did not affect marked reductions in paw withdrawal threshold to mechanical stimulation (6a) and paw withdrawal latencies to heat (6b) and cold (6c) stimuli on the ipsilateral side on day 9 post-incision. However, RU38486 (2 μ g/10 μ l) 1 h before REMSD daily for 3 days completely abolished these reductions (6a, 6b, and 6c). RU38486 did not alter basal paw withdrawal responses to mechanical, heat, and cold stimuli on the contralateral side of the incision plus REMSD group (6d and 6e) and on either ipsilateral (6a, 6b, and 6c) or contralateral (6d and 6e) side of the incision plus control group and the sham plus control or REMSD group. Intrathecal vehicle did not affect all basal paw withdrawal responses on the contralateral side of the incision plus REMSD group (6d and 6e) and on either ipsilateral (6a, 6b, and 6c) or contralateral (6d and 6e) side of the sham

plus control group. ** $P < 0.01$ vs the corresponding sham plus control group with intrathecal vehicle. N = 5/group.

Fig. 7.

Effect of bilateral adrenalectomy (ADX) on exacerbated postsurgical pain induced by short-term rapid eye movement sleep disturbance (REMSD) in male rats. In the incision plus REMSD group, sham surgery of ADX (sham-ADX) before REMSD did not affect marked reductions in paw withdrawal threshold to mechanical stimulation (7a) and paw withdrawal latencies to heat (7b) and cold (7c) stimuli on the ipsilateral side on day 9 post-incision. However, bilateral ADX before REMSD entirely reversed these reductions (7a, 7b, and 7c). ADX did not alter basal paw withdrawal responses to mechanical, heat, and cold stimuli on the contralateral sides of the incision plus REMSD group (7d and 7e) and on either ipsilateral (7a, 7b, and 7c) or contralateral (7d and 7e) sides of the incision plus control group and the sham-incision (sham surgery of incision) plus control or REMSD group. Sham-ADX did not affect all basal paw withdrawal responses on the contralateral side of the incision plus REMSD group (7d and 7e) and on either the ipsilateral (7a, 7b, and 7c) or contralateral (7d and 7e) side of the sham plus control group. ** $P < 0.01$ vs the corresponding sham-incision plus control group with sham-ADX. $N = 5/\text{group}$.

Fig. 8.

Effect of pre-surgical exposure to short-term rapid eye movement sleep disturbance (REMSD) on the expression of MOR, KOR, and Kv1.2 proteins in the ipsilateral L_{4/5} dorsal horn (8a) and L_{4/5} DRGs (8b) on day 9 post-incision from the sham plus control group, incision plus control group, sham plus REMSD group, and incision plus REMSD group. Top panels: examples of Western blots that show the expression of MOR, KOR, and Kv1.2 proteins. GAPDH is used as a loading control. Bottom: statistical summary of MOR, KOR, and Kv1.2 protein expression. Data are presented as mean $\pm$ SEM. N = 4–5/group. * $P < 0.05$ compared to the corresponding sham plus control group.

Table 1

Mean (SEM) changes in locomotor test

Treatment	Placing reflex	Grasping reflex	Righting reflex
Control	5 (0)	5 (0)	5 (0)
REMSD	5 (0)	5 (0)	5 (0)
Sham + control	5 (0)	5 (0)	5 (0)
Incision + control	5 (0)	5 (0)	5 (0)
Sham + REMSD	5 (0)	5 (0)	5 (0)
Incision + REMSD	5 (0)	5 (0)	5 (0)
Sham + Control + Vehicle	5 (0)	5 (0)	5 (0)
Sham + Control + Ru	5 (0)	5 (0)	5 (0)
Incision + Control + Ru	5 (0)	5 (0)	5 (0)
Sham + REMSD + Ru	5 (0)	5 (0)	5 (0)
Incision + REMSD + Vehicle	5 (0)	5 (0)	5 (0)
Incision + REMSD + Ru	5 (0)	5 (0)	5 (0)
Sham-incision + Control + Sham-ADX	5 (0)	5 (0)	5 (0)
Sham-incision + Control + ADX	5 (0)	5 (0)	5 (0)
Incision + Control + ADX	5 (0)	5 (0)	5 (0)
Sham-incision + REMSD + ADX	5 (0)	5 (0)	5 (0)
Incision + REMSD + Sham-ADX	5 (0)	5 (0)	5 (0)
Incision + REMSD + ADX	5 (0)	5 (0)	5 (0)

n= 5–15/group. 5 trials. ADX: bilateral adrenalectomy. REMSD: Rapid eye movement sleep disturbance. Ru: RU38486. sham-ADX: sham surgery of ADX. Sham-incision: sham surgery of incision