Surgical Incision-Induced Nociception Causes Cognitive Impairment and Reduction in Synaptic NMDA Receptor 2B in Mice

Xiaoqin Zhang,^{1,2*} Xin Xin,^{1,3*} Yuanlin Dong,¹ Yiyang Zhang,¹ Buwei Yu,² Jianren Mao,⁴ and Zhongcong Xie¹

¹Geriatric Anesthesia Research Unit, Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts, 02129-2060, ²Department of Anesthesia, Rui Jin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, P.R. China, ³Department of Anesthesia, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, P.R. China, and ⁴Massachusetts General Hospital Center for Translational Pain Research, Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts 02129-2060

Postoperative cognitive dysfunction (POCD) is associated with impairments in daily functioning, and increased morbidity and mortality. However, the causes and neuropathogenesis of POCD remain largely unknown. Uncontrolled pain often occurs postoperatively. We therefore set out to determine the effects of surgical incision-induced nociception on the cognitive function and its underlying mechanisms in 3- and 9-month-old mice. The mice had surgical incision in the hindpaw and then were tested for nociceptive threshold, learning, and memory. Brain levels of NMDA receptor and cyclin-dependent kinase 5 (CDK5) were also assessed. We found that surgical incision-induced nociception in mice led to a decreased freezing time in the tone test (which assesses the hippocampus-independent learning and memory function), but not the context test, of Fear Conditioning System at 3 and 7 d, but not 30 d post incision in 9-month-old, but not 3-month-old mice. Consistently, the surgical incision selectively decreased synaptic NMDA receptor 2B levels in the medial prefrontal cortex, and increased levels of tumor necrosis factor- α and CDK5 in the cortex, but not hippocampus, of the mice. Finally, eutectic mixture of local anesthetics and CDK5 inhibitor, roscovitine, attenuated the surgical incision-induced reduction in the synaptic NMDA receptor 2B levels and learning impairment. These results suggested that surgical incision-induced nociception reduced the synaptic NMDA receptor 2B level in the medial prefrontal cortex of mice, which might lead to hippocampus-independent learning impairment, contributing to POCD. These findings call for further investigation to determine the role of surgical incision-induced nociception in POCD.

Introduction

Postoperative cognitive dysfunction (POCD) (Moller et al., 1998; for review, see Terrando et al., 2011) is associated with increased morbidity and mortality (Monk et al., 2008; Deiner and Silverstein, 2009) and impairments in daily functioning (Phillips-Bute et al., 2006). The causes and neuropathogenesis of POCD remain largely to be determined. More than 80% of patients who have surgery in the United States have postoperative pain (Apfelbaum et al., 2003). Clinical investigations have shown that pain associated with surgery could contribute to POCD (Wang et al., 2007).

NMDA receptor 2B (NR2B) is involved in learning, memory, and pain (for review, see Qiu et al., 2011). The long-term potentiation activation is dependent on NMDA receptor (Bauer et al., 2002; Berberich et al., 2007; Miwa et al., 2008). Genetic overexpression of NR2B in forebrains causes an enhanced central synaptic potentiation (Tang et al., 1999; Liu et al., 2004). Moreover, NR2B antagonist ifenprodil and Ro25–6981 induce fear conditioning learning impairment (Rodrigues et al., 2001; Dalton et al., 2008). NR2B transgenic mice have enhanced responsiveness to peripheral injection of formalin and complete Freund's adjuvant (Wei et al., 2001), neuropathic pain by transection of spinal nerve induces phosphorylation of NR2B at Tyr1472, and the NR2B-selective antagonist CP-101,606 attenuates the NR2B phosphorylation and the neuropathic pain (Abe et al., 2005). Synaptic- and extrasynaptic NMDA receptors may have different roles in neurological functions (for review, see Hardingham and Bading, 2010). Finally, cyclin-dependent kinase 5 (CDK5; P35 and P25 are its activators) regulates NR2B levels (Sato et al., 2008; Zhang et al., 2008a).

Fear Conditioning System (FCS) can test associative learning and memory, and is among the most commonly used behavioral tests to assess learning and memory function (Satomoto et al., 2009; Cibelli

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*X.Z. and X.X. contributed equally to this work.

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Correspondence should be addressed to Dr. Zhongcong Xie, Associate Professor of Anesthesia, Geriatric Anesthesia Research Unit, Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, 149 13th Street, Room 4310, Charlestown, MA 02129-2060. E-mail: xzie@partners.org.

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et al., 2010; Saab et al., 2010; Terrando et al., 2010). Moreover, context and tone test of FCS can assess the hippocampus-dependent and hippocampus-independent learning and memory function, respectively (Kim and Fanselow, 1992; Anagnostaras et al., 1999; Kitamura et al., 2009; Wiltgen et al., 2010). The fear conditioning learning and memory pathway involves the hippocampus, amygdala, and medial prefrontal cortex (for review, see Maren et al., 2013 and Johansen et al., 2011). The Morris Water Maze (MWM) is another commonly used behavior procedure to assess learning and memory function in rodents.

Our previous study has shown that sleep disturbance, another potential perioperative risk factor of POCD, may selectively induce neuroinflammation in the hippocampus and may lead to hippocampus-dependent learning impairment in the mice (Zhu et al., 2012). However, whether surgical incision-induced nociception can cause neurotoxicity and neurobehavioral deficits in mice is unknown.

Together, we used an established pre-clinical acute nociception model (Pogatzki and Raja, 2003) in 3- and 9-month-old mice to test a hypothesis that acute nociception following surgical incision increases brain CDK5 levels, which then reduces brain synaptic NR2B levels, leading to learning impairment. We further assessed the effects of surgical incision-induced nociception on NR2B levels in different brain regions (e.g., medial prefrontal cortex vs hippocampus) and the brain region-associated cognitive function in the mice.

Materials and Methods

Animals. The animal protocol was approved by the Institutional Animal Care and Use Committee at Massachusetts General Hospital, Boston, Massachusetts. Wild-type mice (C57BL/6J, 3 and 9 months old; The Jackson Laboratory) of either sex were randomly assigned to a surgical incision or sham group. The mice were housed in a controlled environment (20–22°C; 12 h light/dark on a reversed light cycle) for 1 week before the studies. The maintenance and handling of mice were consistent with the guideline of National Institutes of Health, and all of the efforts were made to minimize the number of animals in the studies.

Surgical incision. The surgical incision in the right hindpaw of each mouse was made under a brief anesthesia (1.4% isoflurane anesthesia for 3 min) as described in a previous study (Pogatzki and Raja, 2003). Briefly, a 0.5 cm longitudinal incision was made through skin and fascia of the right plantar foot with a number 10 scalpel blade under sterile preparation. The incision was started 0.2 cm from the proximal edge of the heel and extended distally. The underlying muscle was elevated with curved forceps and incised longitudinally, leaving the muscle origin and insertion intact. Then, the skin overlying the muscle was closed with a 5.0 nylon mattress suture. After the procedure, the mice were allowed to recover in cages with sterile bedding. Control mice underwent a sham procedure that consisted of anesthesia and sterile preparation but without the incision.

Drug treatments. Eutectic mixture of local anesthetics (EMLA) cream (2.5% lidocaine and 2.5% prilocaine) was applied on the wound in the

Figure 1. Surgical incision in mice hindpaw induces nociception in the mice. **A**, The surgical incision (black bar) reduces the nociception threshold of the mice as compared with the sham condition (white bar) at 1 d after the incision. **B**, The surgical incision (black bar) reduces the nociception threshold of the mice as compared with the sham condition (white bar) at 3 d after the incision. **C**, The surgical incision (black bar) does not reduce the nociception threshold of the mice as compared with the sham condition (white bar) at 7 d after the incision. **D**, The surgical incision (black bar) does not significantly affect the distance of movement of the mice in the open field test as compared with the sham condition (white bar) at 1 d after the incision. **E**, The surgical incision (black bar) does not significantly affect the speed of movement of the mice in the open field test as compared with the sham condition (white bar) at 1 d after the incision. $N = 5-10$.

paw (surgical incision group) or just the paw (control group) as analgesia every 8 h for 1 week post incision for the mice in the EMLA treatment group. To apply the cream, each mouse was allowed to walk through the EMLA cream, thus EMLA was attached to each paw of the mouse, including the hindpaw with the surgical incision. The control condition of EMLA in the current study was no EMLA treatment. Roscovitine (R-1234; LC Laboratories) 10 mg/kg or equal volume vehicle (sterile oil with 0.2% dimethylsulfoxide) was injected through intraperitoneal route daily for 3 d post incision in the interaction studies.

Open field test. Locomotor activity was tested in the open field as described in a previous study (Weaver et al., 2006) with modification. Briefly, the mice were placed directly into one corner of the open field (120 × 120 cm), which was divided into a grid of 3 × 3 squares. Movement of the animal in the area was recorded during a 10 min testing session. The total distance traveled and mean speed were recorded and quantified by Any-Maze (Stoelting).

Nociception threshold determination. Nociception threshold was determined by using nylon von Frey filaments as described in a previous study (Chaplan et al., 1994). Mice were placed on a wire mesh platform in clear cylindrical plastic enclosures (8 cm diameter and 10 cm in height). After 20 min of stay on the platform, the filaments were applied (bending force range from 0.008–26 g; North Coast Medical) on the wound edge of the incised hindpaw for ~5 s with a 10 s interval between each stimulation. Withdrawal of the hindpaw from the floor was scored as a positive re-

Figure 2. Surgical incision in mice hindpaw induces a learning impairment in learning new things in the mice. **A**, The surgical incision decreases freezing time in the tone test of the FCS as compared with the sham condition at 3 d post incision in the mice. **B**, The surgical incision does not decrease freezing time in the context test of the FCS as compared with the sham condition at 3 d post incision in the mice. **C**, The surgical incision decreases freezing time in the tone test of the FCS as compared with the sham condition

at 7 d post incision in the mice. **D**, The surgical incision does not decrease freezing time in the context test of the FCS as compared with the sham condition at 7 d post incision in the mice. **E**, The surgical incision does not decrease freezing time in the tone test of the FCS as compared with the sham condition at 30 d post incision in the mice. **F**, The surgical incision does not decrease freezing time in the context test of the FCS as compared with the sham condition at 30 d post incision in the mice. Interestingly, when the pairing of mice in the FCS was performed at 1 d before the incision (aiming to assess whether nociception could prevent mice from remembering old tasks), the incision does not reduce freezing time of mice in tone test (**G**) and context test (**H**) of FCS at 1 d post incision, and at 5 d post incision in tone test (**I**) and context test (**J**) of FCS. The surgical incision does not increase the escape latency (**K**) and does not decrease the platform crossing times (**L**) in the MWM test as compared with the sham condition in the mice. $N = 15$.

sponse. When no response was obtained, the next stiffer filament in the series was applied to the same paw. Each monofilament was applied in the hindpaw five times. The hindpaw withdrawal threshold or the nociception threshold was obtained as the force (in grams) at which foot withdrawal occurred at least three of the five stimulations.

FCS. FCS has been often used to detect associative learning and memory function induced by anesthesia alone (Saab et al., 2010; Zhang et al., 2012), anesthesia with surgery (Terrando et al., 2010; Wan et al., 2010), and sleep disturbance (Zhu et al., 2012). We performed the FCS experiments as described in our previous studies (Zhang et al., 2012; Zhu et al., 2012) and other studies (Saab et al., 2010; Terrando et al., 2010). Specifically, different groups of mice were exposed for training in the FCS (Stoelting) 24 h before and after the surgical incision, respectively. Each mouse was allowed to explore the chamber for 180 s before presentation of a 2 Hz pulsating tone (80 dB, 1500 Hz) that persisted for 60 s. The tone was followed immediately by a mild foot shock (0.8 mA for 0.5 s). Context (no tone period) and tone (tone period) learning and memory were probed at 3, 7, and 30 d post incision in sequence. The mice were tested in the context test first, then in the tone test 1 h later. For the context test, each mouse (from either the control group or surgical incision group) was allowed to stay in the chamber for 180 s, followed by another 180 s period without a tone, and finally 30 s for recovery. For the tone test, each mouse (from either the control group or surgical incision group) was allowed to stay in the chamber for 180 s, followed by another 180 s period with a tone, and finally 30 s for recovery. Learning and memory function was assessed by measuring the amount of time the mouse demonstrated “freezing behavior,” defined as a completely immobile posture except for respiratory efforts, during the test period (the second 180 s period), which was analyzed by Any-Maze software (Stoelting). The first 180 s period allowed mice to adjust for the environment before the counting of freezing time in the second 180 s period.

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at 7 d post incision in the mice. **D**, The surgical incision does not decrease freezing time in the context test of the FCS as compared with the sham condition at 7 d post incision in the mice. **E**, The surgical incision does not decrease freezing time in the tone test of the FCS as compared with the sham condition at 30 d post incision in the mice. **F**, The surgical incision does not decrease freezing time in the context test of the FCS as compared with the sham condition at 30 d post incision in the mice. Interestingly, when the pairing of mice in the FCS was performed at 1 d before the incision (aiming to assess whether nociception could prevent mice from remembering old tasks), the incision does not reduce freezing time of mice in tone test (**G**) and context test (**H**) of FCS at 1 d post incision, and at 5 d post incision in tone test (**I**) and context test (**J**) of FCS. The surgical incision does not increase the escape latency (**K**) and does not decrease the platform crossing times (**L**) in the MWM test as compared with the sham condition in the mice. $N = 15$.

MWM. MWM was performed as previously described (Bianchi et al., 2008) to assess spatial learning and memory function. Briefly, all mice were trained to swim to a hidden platform in four trials per day for 7 d (day 1–7) at 3 d after the surgical incision. The mice were given 90 s to find the platform and allowed to stay for 10–15 s before being removed from the pool. If a mouse could not find the platform within 90 s, it was gently guided to the platform and allowed to remain there for 10–15 s. The platform was placed in the target quadrant for all trials, but the starting points were random for each mouse. We trained the mice by using a cue on the wall (cued training). We measured the time it took for each mouse to reach the platform (escape latency) and used this as the learning score. On the seventh day, we also tested the memory of each mouse by removing the platform and measuring the number of times the mouse crossed the platform area with the same cue to obtain the memory score. We compared the learning and memory score and swimming speed between the mice in the surgical incision group and sham group.

Brain tissue harvest and protein level quantification. Three and seven days post incision, the mice were killed by decapitation. The brain cortex (medial prefrontal cortex, lateral prefrontal cortex, and cingulate cortex), hippocampus, and amygdala were harvested separately. For the Western blot analysis, the harvested brain cortex, hippocampus, or amygdala was homogenized on ice using immunoprecipitation buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, and 0.5% Nonidet P-40) plus protease inhibitors (1 μ g/ml aprotinin, 1 μ g/ml leupeptin, and 1 μ g/ml pepstatin A). The lysates were collected, centrifuged at 12,000 rpm for 10 min, and quantified for total proteins with Bicinchoninic Acid protein assay kit (Pierce).

Separation of synaptic and extrasynaptic membranes. The separation of synaptic and extrasynaptic fractions was performed as described in previous studies (Goebel-Goody et al., 2009; Li et al., 2011) with modifications. The separation was based on the principle that the postsynaptic density (PSD) protein-associated or synaptic fraction is insoluble in Triton X-100, but the non-PSD protein-associated or extrasynaptic fraction is soluble in Triton X-100. All of the following procedures were conducted at 4°C to minimize enzymatic activity. The harvested brain tissues were put in an ice-cold sucrose homogenization buffer containing the following (in mM): 320 sucrose, 10 Tris, pH 7.4, 1 Na₃VO₄, 5 NaF, 1 EDTA, and 1 EGTA. The tissues were then homogenized in a glass grinding vessel using a rotating Teflon pestle (2000 rpm) with at least 12 passes to create a dounce homogenate. The dounce homogenate was centrifuged at 1000 g for 10 min to remove nuclei and incompletely homogenized material (P1). The resulting supernatant (S1) was spun at 10,000 g for 15 min to obtain a P2. The P2 was subsequently resuspended in 120 μ l sucrose buffer using a Fisher Vortex (Fisher Scientific). The P2 was then subjected to detergent extraction by adding eight volumes of a nonionic detergent Triton X-100 buffer (final: 0.5% volume/volume) containing the following reagents (in mM): 10 Tris, pH 7.4, 1 Na₃VO₄, 5 NaF, 1 mM EDTA, and 1 EGTA, which was described in a previous study (Cotman and Taylor, 1972). This suspension was incubated at 4°C for 20 min with gentle rotation and then centrifuged at 32,000 g for 20 min. We operationally defined the pellet as the PSD protein-associated or synaptic fraction and the supernatant as the non-PSD protein-associated or extrasynaptic fraction, based on the fact that synaptic fraction, which is bound to PSD proteins, is not soluble in Triton X-100. For every centrifugation step above, the pellets were rinsed twice with sucrose buffer to avoid potential contamination between fractions. Final pellets (synaptic fraction) were resuspended in sucrose buffer, and proteins were solubi-

Figure 3. EMLA attenuates the surgical incision-induced nociception and learning impairment. **A**, The surgical incision (gray bar) reduces nociception threshold of the mice as compared with the sham condition (white bar) at 8 h after the incision. The EMLA treatment attenuates the surgical incision-induced reduction in the nociception threshold (net bar). **B**, The surgical incision (gray bar) reduces nociception threshold of the mice as compared with the sham condition (white bar) at 3 d after the incision. The EMLA treatment attenuates the surgical incision-induced reduction in the nociception threshold (net bar). **C**, The surgical incision (gray bar) decreases freezing time in the tone test of the FCS as compared with the sham condition (white bar) at 3 d post incision in the mice. The EMLA treatment (net bar) attenuates the surgical incision-induced reduction in the freezing time in the tone test of the FCS. **D**, The surgical incision (gray bar) decreases the freezing time in the tone test of the FCS as compared with the sham condition (white bar) at 7 d post incision in the mice. The EMLA treatment (net bar) attenuates the surgical incision-induced reduction in the freezing time in the tone test of the FCS. $N = 6–15$.

lized by the addition of SDS to a final concentration of 1% SDS-treated fractions, which were then thoroughly sonicated, boiled at 99°C for 5 min, and frozen at -80°C until further analysis. Eight volumes of 100% acetone (stored at -20°C) were added to the supernatant (extrasynaptic fraction), and the mixture was gently vortexed and incubated at -20°C overnight. The concentrated protein precipitate was then collected by 3000 g spin for 15 min. The acetone supernatant was decanted and the precipitate was centrifuged for an additional 1 min at 3000 g to remove any excess acetone. The precipitate was air dried at room temperature for at least 15 min and then resuspended and sonicated in 1% SDS, boiled at 99°C for 5 min, and frozen at -80°C until further analysis.

Western blot analysis. NR2B antibody (1:1000; Millipore) was used to recognize NR2B (180 kDa). CDK5 antibody (1:1000; Millipore) was used to detect CDK5 (33 kDa). P35 antibody (1:250; Santa Cruz Biotechnology) was used to detect P35 (35 kDa) and P25 (25 kDa). Antibody anti- β -actin (1:10,000; Sigma) was used to detect β -actin (42 kDa). Western blot quantification was performed as described by Xie et al. (2008). Briefly, signal intensity was analyzed using a Bio-Rad image program (Quantity One). We quantified the Western blots in two steps. First, we used β -actin levels to normalize (e.g., determining the ratio of S-NR2B to β -actin amount) protein levels and control for loading differences in the total protein amount. Second, we presented the protein level changes in mice undergoing surgical incision as a percentage of those in the sham group. One hundred percent of protein level changes refer to control levels for the purpose of comparison to experimental conditions.

Statistics. Non-normally distributed data were expressed using median and interquartile range (25–75%) and analyzed by nonparametric analysis. These data included nociception threshold and platform crossing time in MWM. Mann–Whitney test and Kruskal–Wallis nonparametric ANOVA (*post hoc* Dunn test) were used to analyze the difference between sham and incision, and control and EMLA. In assessing the interaction effect of the block (sham and incision) and treatment (EMLA and saline), the generalized linear model (Friedman nonparametric two-way

Figure 4. Surgical incision in mice hindpaw reduces S-NR2B levels in the medial prefrontal cortex of mice. **A**, The surgical incision (lanes 5 and 6) decreases the levels of S-NR2B in the cortex of mice as compared with sham condition (lanes 1 and 2) in the Western blot analysis. EMLA itself (lanes 3 and 4) does not increase the levels of S-NR2B in the cortex of mice as compared with the control condition (lanes 1 and 2), but the EMLA treatment attenuates the surgical incision-induced reduction in the S-NR2B levels in the cortex of mice (lanes 7 and 8) in the Western blot analysis. **B**, Quantification of the Western blot shows that the surgical incision (gray bar) decreases the S-NR2B levels as compared with sham condition (white bar). EMLA itself (black bar) does not increase the levels of S-NR2B in the cortex of mice as compared with the control condition (white bar), but the EMLA treatment attenuates the surgical incision-induced reduction in the S-NR2B levels (net bar) in the mice. **C**, The surgical incision (lanes 6–10) does not decrease the levels of Es-NR2B in the cortex of mice as compared with the sham condition (lanes 1–5) in the Western blot analysis. **D**, Quantification of the Western blot shows that the surgical incision (black bar) does not decrease the Es-NR2B levels as compared with the sham condition (white bar). **E**, The surgical incision (lanes 6–10) does not decrease the levels of total NR2B in the cortex of mice as compared with the sham condition (lanes 1–5) in the Western blot analysis. **F**, Quantification of the Western blot shows that the surgical incision (black bar) does not decrease the total NR2B levels as compared with the sham condition (white bar). **G**, The surgical incision (lanes 5 and 6) decreases the levels of S-NR2B in the medial prefrontal cortex of mice as compared with sham condition (lanes 1 and 2) in the Western blot analysis. EMLA itself (lanes 3 and 4) does not increase the levels of S-NR2B in the medial prefrontal cortex of mice as compared with control condition (lanes 1 and 2), but the EMLA treatment

ANOVA) was used after data were ranked within their respective block. Normally distributed data were expressed as mean $\pm$ SD and analyzed by using parametric analysis. These data included distance and speed in the open field studies, freezing time in the FCS studies, escape latency in the MWM studies, and protein amounts in the quantitative Western blotting studies (levels of S-NR2B, Es-NR2B, total NR2B, P35, P25, and tumor necrosis factor (TNF)- α). Student's *t* test was used to analyze the difference between sham and incision, and treatment with control, EMLA, and roscovitine. Two-way ANOVA was used to analyze the interaction of EMLA or roscovitine and control under sham or incision condition. The number of samples varied from 5 to 15; *p* values <0.05 or 0.01 were considered statistically significant. Degree of freedom from parametric data analysis was calculated and presented. The significance testing was two tailed, and Prism 6 software and SAS (SAS Institute) software (version 9.2) were used to analyze the data.

Results

Surgical incision in the hindpaw of mice induced nociception in the mice

We used an established preclinical model in mice (Pogatzki and Raja, 2003) to assess whether surgical incision in the hindpaw of mice can induce nociception, leading to neurotoxicity and neurobehavioral deficits. We found that the surgical incision reduced nociception threshold as compared with the sham condition at 1 d [Fig. 1A; 0.31 (0.28–0.31), median and interquartile range (25–75%), vs 0.69 (0.64–0.76), ***p* = 0.0002] and 3 d [Fig. 1B; 0.57 (0.46–0.70) vs 0.88 (0.83–0.95), **p* = 0.002], but not 7 d [Fig. 1C; 0.80 (0.75–0.83) vs 0.78 (0.69–0.82), *p* = 0.917, NS], post incision in the mice. We also found that the surgical incision did not significantly affect the distance (Fig. 1D) and the

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attenuates the surgical incision-induced reduction in the S-NR2B levels in the medial prefrontal cortex of mice (lanes 7 and 8) in the Western blot analysis. **H**, Quantification of the Western blot shows that the surgical incision (gray bar) decreases the S-NR2B levels as compared with sham condition (white bar). EMLA itself (black bar) does not increase the levels of S-NR2B in the medial prefrontal cortex of mice as compared with the control condition (white bar), but the EMLA treatment attenuates the surgical incision-induced reduction in the S-NR2B levels (net bar) in the medial prefrontal cortex of mice. **I**, The surgical incision (lanes 6–10) does not decrease the levels of Es-NR2B in the medial prefrontal cortex of mice as compared with the sham condition (lanes 1–5) in the Western blot analysis. **J**, Quantification of the Western blot shows that the surgical incision (black bar) does not decrease the Es-NR2B levels in the medial prefrontal cortex of mice as compared with the sham condition (white bar). *N* = 6–8.

speed of movement of the mice (Fig. 1E) in the open field test as compared with the sham condition at 1 d post incision. These data suggested that the surgical incision in the hindpaw of mice was able to induce nociception in the mice without impairment of locomotor activity.

The surgical incision induced hippocampus-independent learning impairment in the mice

Next, we investigated whether the surgical incision might induce learning impairment in the mice in the FCS. The pairing of FCS was performed 1 d after the surgical incision. The surgical incision reduced the freezing time of mice in the tone test of FCS at 3 d (Fig. 2A; 24.17 ± 15.76 vs 46.09 ± 25.92 , $^{**}p = 0.009$) and 7 d (Fig. 2C; 26.08 ± 13.24 vs 38.10 ± 18.03 , $^{*}p = 0.047$), but not 30 d (Fig. 2E; 14.98 ± 3.46 vs 18.66 ± 3.98 , $p = 0.490$, NS) post incision. The surgical incision did not reduce the freezing time of mice in the context test of FCS at 3 d (Fig. 2B; $p = 0.458$, NS), 7 d (Fig. 2D; $p = 0.666$, NS), and 30 d (Fig. 2F; $p = 0.869$, NS) post incision. The tone and context test of the FCS have been reported to assess hippocampus-independent and hippocampus-dependent learning and memory function, respectively (Kim and Fanselow, 1992; Anagnostaras et al., 1999; Kitamura et al., 2009; Wiltgen et al., 2010). Therefore, these data suggested that the surgical incision in the hindpaw of mice induced hippocampus-independent learning impairment, which was detected by the tone test of FCS. In a different experiment, the pairing of FCS was performed 1 d before the surgical incision. In these studies, the surgical incision did not induce learning impairment in the mice (Fig. 2G–J). Together, these results suggested that the surgical incision-induced nociception could cause fearing learning impairment, i.e., impairing the mice from learning new tasks; but not fear extinction impairment, i.e., not impairing the mice from remembering old tasks.

In addition, we assessed the effects of the surgical incision on cognitive function in the MWM. We found that the surgical incision did not increase the escape latency (Fig. 2K), did not reduce the platform crossing times in MWM (Fig. 2L), and did not alter swimming speed (data not shown). These data suggested that the surgical incision might not induce spatial learning and memory impairment in the mice.

The surgical incision-induced learning impairment was dependent on the surgical incision-induced nociception in the mice

Given that the surgical incision induced nociception and learning impairment in the mice, next we asked whether the surgical incision-induced learning impairment was dependent on the

Figure 5. Surgical incision in mice hindpaw increases levels of CDK5, P35, and P25 in the cortex of mice. **A**, The surgical incision (lanes 5 and 6) increases the levels of CDK5 in the cortex of mice as compared with the sham condition (lanes 1 and 2) in the Western blot analysis. EMLA itself (lanes 3 and 4) does not decrease the levels of CDK5 in the cortex of mice as compared with the control condition (lanes 1 and 2), but the EMLA treatment attenuates the surgical incision-induced increase in CDK5 levels in the cortex of mice (lanes 7 and 8) in the Western blot analysis. **B**, Quantification of the Western blot shows that the surgical incision (gray bar) increases CDK5 levels as compared with the sham condition (white bar). EMLA itself (black bar) does not decrease the levels of CDK5 in the cortex of mice as compared with the control condition (white bar), but the EMLA treatment attenuates the surgical incision-induced increase in the CDK5 levels (net bar). **C**, The surgical incision (lanes 5 and 6) increases the levels of P35 and P25 in the cortex of mice as compared with the sham condition (lanes 1 and 2) in the Western blot analysis. EMLA itself (lanes 3 and 4) does not decrease the levels of P35 and P25 in the cortex of mice as compared with the control condition (lanes 1 and 2), but the EMLA treatment attenuates the surgical incision-induced increase in P35 and P25 levels in the cortex of mice (lanes 7 and 8) in the Western blot analysis. Quantification of the Western blot shows that the surgical incision (gray bar) increases P35 (**D**) and P25 (**E**) levels as compared with the control condition (white bar). EMLA itself (black bar) does not decrease the levels of P35 and P25 in the cortex of mice as compared with the control condition (white bar), but the EMLA treatment attenuates the surgical incision-induced increase in the P35 and P25 levels (net bar). $N = 6$.

surgical incision-induced nociception in the mice. We used local anesthetic cream EMLA (2.5% lidocaine and 2.5% prilocaine) in the interaction studies. We were able to show that there was a significant interaction between surgical incision and EMLA treatment, and that the treatment with EMLA attenuated the surgical incision-induced reduction in the nociception threshold in the mice at 8 h (Fig. 3A; $F = 4.72$, $p = 0.037$, Friedman nonparametric two-way ANOVA; $^{**}p = 0.001$, Kruskal–Wallis nonparametric ANOVA *post hoc* Dunn test) and 3 d post incision (Fig. 3B; $F = 6.45$, $p = 0.016$, Friedman nonparametric two-way ANOVA; $^{**}p = 0.006$, Kruskal–Wallis nonparametric ANOVA *post hoc* Dunn test). Moreover, two-way ANOVA showed that there was a significant interaction of the surgical incision and EMLA in the tone test of FCS at 3 d (Fig. 3C; $F = 6.19$, $p = 0.017$, $df = 1$) and 7 d (Fig. 3D; $F = 7.94$, $p = 0.007$, $df = 1$) post incision. *Post hoc* Bonferroni test showed that whereas EMLA treatment alone did not significantly change the freezing time of the tone test of FCS as compared with control condition (Fig. 3C; $p = 0.9999$, NS; Fig. 3D; $p = 0.426$, NS), the EMLA treatment attenuated the surgical

Figure 6. Roscovitine attenuates the surgical incision-induced learning impairment and reduction in the levels of S-NR2B in cortex of mice. **A**, The surgical incision (gray bar) decreases freezing time in the tone test of the FCS as compared with the sham condition (white bar) at 3 d post incision in the mice. Roscovitine (net bar) attenuates the surgical incision-induced reduction in freezing time in the tone test of the FCS. **B**, The surgical incision (gray bar) decreases freezing time in the tone test of the FCS as compared with the sham condition (white bar) at 7 d post incision in the mice. Roscovitine (net bar) attenuates the surgical incision-induced reduction in freezing time in the tone test of the FCS. **C**, The surgical incision (lanes 7–9) decreases levels of S-NR2B in the cortex of mice as compared with the sham condition (lanes 1–3) in the Western blot analysis. Roscovitine itself (lanes 4–6) does not increase the levels of S-NR2B in the cortex of mice as compared with the control condition (lanes 1–3), but roscovitine attenuates the surgical incision-induced reduction in the S-NR2B levels in the cortex of mice (lanes 10–12) in the Western blot analysis. **D**, Quantification of the Western blot shows that the surgical incision (gray bar) decreases the S-NR2B levels as compared with the sham condition (white bar). Roscovitine itself (black bar) does not increase the levels of S-NR2B in the cortex of mice as compared with control condition (white bar), but the roscovitine treatment attenuates the surgical incision-induced reduction in the S-NR2B levels (net bar). $N = 6$.

Figure 7. Surgical incision in mice hindpaw increases TNF- α levels in the cortex of mice. **A**, The surgical incision (lanes 5 and 6) increases levels of TNF- α in the cortex of mice as compared with the sham condition (lanes 1 and 2) in the Western blot analysis. EMLA itself (lanes 3 and 4) does not decrease the levels of TNF- α in the cortex of mice as compared with the control condition (lanes 1 and 2), but the EMLA treatment attenuates the surgical incision-induced elevation in the TNF- α levels in the cortex of mice (lanes 7 and 8) in the Western blot analysis. **B**, Quantification of the Western blot shows that the surgical incision (gray bar) increases TNF- α levels as compared with the sham condition (white bar). EMLA itself (black bar) does not decrease the levels of TNF- α in the cortex of mice as compared with the control condition (white bar), but the EMLA treatment attenuates the surgical incision-induced increase in the TNF- α levels (net bar). $N = 6$.

incision-induced reduction in the freezing time of the tone test of FCS as compared with control condition (Fig. 3C; $^{**}p < 0.0001$; Fig. 3D; $^{*}p = 0.044$). The EMLA-treated incision mice had a higher nociception threshold (Fig. 3A,B) and more freezing time (Fig. 3C,D) as compared with the control-treated incision mice. These findings suggested that the surgical incision-induced learning impairment was dependent on the surgical incision-induced nociception in the mice.

The surgical incision-induced nociception reduced synaptic NR2B levels and increased the levels of CDK5, P35, and P25 in mice cortex

Synaptic NR2B (S-NR2B) has been shown to be involved in both cognitive function and pain (for review, see Hardingham and Bading, 2010; Zhou and Sheng, 2013), we therefore assessed the effects of surgical incision-induced nociception on the S-NR2B levels. Immunoblotting of S-NR2B showed that there were visible reductions in the bands representing S-NR2B in the cortex of mice following the surgical incision as compared with the sham condition (Fig. 4A). Moreover, the surgical incision-induced reductions in S-NR2B levels were attenuated by the treatment of EMLA (Fig. 4A). There was no significant difference in the β -actin levels among these treatments (Fig. 4A). The quantification of the Western blot, based on the ratio of S-NR2B to β -actin, showed that there was a significant interaction between the surgical incision and the EMLA treatment on S-NR2B levels, and that the EMLA treatment attenuated the surgical incision-induced reduction in S-NR2B levels in mice cortex (Fig. 4B; $F = 21.41, p = 0.0004, df = 1$). *Post hoc* Bonferroni test showed that whereas EMLA treatment alone did not significantly change the S-NR2B levels as compared with control condition (Fig. 4B; $p = 0.380, NS$), the EMLA treatment attenuated the surgical incision-induced reduction in the S-NR2B levels as compared with control condition (Fig. 4B; $^{*}p = 0.003$). The surgical incision did not reduce extrasynaptic NR2B (Es-NR2B) levels in the cortex of mice (Fig. 4C,D). Finally, the surgical incision did not significantly affect the total NR2B levels in mice cortex (Fig. 4E,F).

Next, we assessed the effects of the surgical incision on the NR2B levels in specific brain regions. We found that the surgical incision decreased the S-NR2B levels in medial prefrontal cortex (Fig. 4G,H), but not in lateral prefrontal cortex, cingulate cortex, and amygdala (data not shown) of the mice. Moreover, the surgical incision-induced reductions in S-NR2B levels were attenuated by the treatment of EMLA (Fig. 4G,H; $F = 10.10,$

$p = 0.004, df = 1, ^{**}p = 0.002$, two-way ANOVA and *post hoc* Bonferroni test). Finally, the surgical incision did not reduce Es-NR2B levels in medial prefrontal cortex (Fig. 4I,J). These data suggested that the surgical incision-induced nociception could reduce S-NR2B (but not Es-NR2B) levels in specific brain regions (e.g., medial prefrontal cortex but not cingulate cortex).

CDK5 has been shown to regulate NR2B levels (Sato et al., 2008; Zhang et al., 2008a), and both P35 and P25 are activators of

CDK5 (Patrick et al., 1999; Kusakawa et al., 2000; Lee et al., 2000; Cruz et al., 2003; Asada et al., 2008). We were able to show that the surgical incision increased the levels of CDK5, P35, and P25 in the cortex (entire cortex; Fig. 5A,C) of mice at 3 d post incision in the Western blot analysis (Fig. 5A,C). The EMLA treatment attenuated the surgical incision-induced elevation of CDK5, P35, and P25 levels in the cortex (entire cortex) of mice (Fig. 5A,C). There was no significant difference in the β -actin levels of these treatments (Fig. 5A,C). The quantification of the Western blots, based on the ratio of CDK5, P35, and P25 to β -actin, showed that there was a significant interaction between the surgical incision and the EMLA treatment (Fig. 5B; $F = 18.17$, $p = 0.0006$, $df = 1$; Fig. 5D; $F = 10.97$, $p = 0.004$, $df = 1$; Fig. 5E; $F = 19.44$, $p = 0.0004$, $df = 1$, two-way ANOVA). *Post hoc* Bonferroni test showed that whereas EMLA treatment alone did not significantly change the levels of CDK5 (Fig. 5B), P35 (Fig. 5D), and P25 (Fig. 5E) as compared with control condition, the EMLA treatment attenuated the surgical incision-induced increases in the levels of CDK5 (Fig. 5B), P35 (Fig. 5D), and P25 (Fig. 5E) as compared with control condition. The EMLA-treated incision mice had lesser levels of CDK5 (Fig. 5B), P35 (Fig. 5D), and P25 (Fig. 5E) as compared with the control-treated incision mice. These data suggested that the surgical incision could decrease S-NR2B levels and increase the levels of CDK5, P35, and P25 in the cortex of mice, and such effects were dependent on the surgical incision-induced nociception.

CDK5 inhibitor roscovitine mitigated the surgical incision-induced learning impairment and reduction in synaptic NR2B levels in mice cortex

Given that the surgical incision increased the levels of CDK5 (as well as P35 and P25), reduced S-NR2B levels in the mice cortex, and caused learning impairment in the mice, next, we determined whether CDK5 inhibitor roscovitine could ameliorate these effects of the surgical incision. Two-way ANOVA and *post hoc* Bonferroni test showed that there was a significant interaction between the surgical incision and the roscovitine treatment (Fig. 6A; $F = 5.59$, $p = 0.023$, $df = 1$; Fig. 6B; $F = 6.82$, $p = 0.014$, $df = 1$), and that the roscovitine treatment ameliorated the surgical incision-induced reduction in freezing time of mice in tone test of FCS at 3 d (Fig. 6A; $*p = 0.037$) and 7 d (Fig. 6B; $*p = 0.043$) post incision. Moreover, quantitative Western blot showed that the roscovitine treatment attenuated the surgical incision-induced reduction in the S-NR2B levels in the cortex of mice (Fig. 6C,D; $F = 30.01$, $p = 0.0006$, $df = 1$, $**p = 0.001$, two-way ANOVA and *post hoc* Bonferroni test). The roscovitine-treated incision mice had more freezing times (Fig. 6A,B) and higher levels of S-NR2B (Fig. 6D) as compared with the control-treated incision mice. These data suggest that the surgical incision-induced learning impairment and reduction in S-NR2B

Figure 8. Effects of surgical incision on the levels of S-NR2B, CDK5, P35, P25, and TNF- α in mice cortex. The surgical incision does not reduce the S-NR2B levels (A), does not increase the levels of CDK5 (B), P35 and P25 (C), and TNF- α (D) as compared with the sham condition in mice cortex at 7 d post incision.

Figure 9. Effects of surgical incision on the levels of S-NR2B, CDK5, P35, P25, and TNF- α in mice hippocampus. The surgical incision does not reduce the S-NR2B levels (A), does not increase the levels of CDK5 (B), P35 and P25 (C), and TNF- α (D) as compared with the sham condition in mice hippocampus at 3 d post incision.

levels might be dependent on the surgical incision-induced elevation of CDK5.

The surgical incision-induced nociception increased TNF- α levels in mice cortex

TNF- α has been shown to regulate CDK5 activity, contributing to inflammation-induced pain signaling (Utreras et al., 2009). We therefore determined the effects of the surgical incision on brain TNF- α levels in the mice. We found that the surgical incision increased the levels of TNF- α in the cortex (Fig. 7A). The EMLA treatment attenuated the surgical incision-induced elevation in TNF- α levels in the cortex (Fig. 7A). Quantification of the Western blot, based on the ratio of TNF- α to β -actin, showed that the surgical incision was able to increase the TNF- α levels in the cortex of mice, which was mitigated by the EMLA treatment (Fig. 7B; $F = 7.47$, $p = 0.026$, $df = 1$, two-way ANOVA). The EMLA-treated incision mice had lesser levels of TNF- α (Fig. 7B) as compared with the control-treated incision mice ($*p = 0.030$, *post hoc* Bonferroni test).

The surgical incision-induced nociception did not lead to the biochemistry and behavioral changes at different times post incision, in different brain regions, or with different ages of the mice

The surgical incision did not significantly alter the levels of S-NR2B, CDK5, P35, and P25 in the cortex of mice at 7 d post incision (Fig. 8) nor in the hippocampus of mice at 3 d post incision (Fig. 9).

Finally, we asked whether the surgical incision might also induce learning impairment in the younger mice. We used

Figure 10. Effects of surgical incision on nociception threshold, cognitive function, and the cortex levels of NR2B, CDK5, P35, P25, and TNF- α in 3-month-old mice. **A**, The surgical incision (gray bar) reduces the nociception threshold of the mice as compared with the sham condition (white bar) at 3 d after the incision. **B**, The surgical incision (gray bar) does not reduce the nociception threshold of the mice as compared with the sham condition (white bar) at 7 d after the incision. **C**, The surgical incision does not decrease freezing time in the tone test of the FCS as compared with the sham condition at 3 d post incision in the mice. **D**, The surgical incision does not decrease freezing time in the context test of the FCS as compared with the sham condition at 7 d post incision in the mice. **E**, The surgical incision does not increase the escape latency in the MWM test as compared with the sham condition in the mice. **F**, The surgical incision does not decrease the platform crossing times in the MWM test as compared with the sham condition in the mice. The surgical incision does not decrease the levels of S-NR2B (**G**) and Es-NR2B (**H**), and does not increase the levels of CDK5 (**I**), and P35 and P25 (**J**). **K**, The surgical incision (lanes 4–6) increases the levels of TNF- α in the cortex of mice as compared with the sham condition (lanes 1–3) in the Western blot analysis. **L**, Quantification of the Western blot shows that the surgical incision (black bar) increases the TNF- α levels as compared with the sham condition (white bar). $N = 6–15$.

3-month old mice in the experiments and found that the surgical incision in the hindpaw of mice reduced nociception threshold as compared with the sham condition at 3 d (Fig. 10A), but not 7 d (Fig. 10B), post incision. However, the surgical incision did not significantly reduce the freezing time in the tone test of FCS at 3 d or 7 d after the incision (Fig. 10C,D). The surgical incision did not reduce escape latency (Fig. 10E) and did not increase the platform crossing times (Fig. 10F) of the MWM test. The surgical incision increased the levels of TNF- α , but not CDK5, F35, F25, and S-NR2B, and the surgical incision did not reduce the levels of S-NR2B and Es-NR2B in the mice cortex (Fig. 10G–L).

Collectively, these data implied that the surgical incision-induced nociception in the 9-month-old, but not 3-month-old mice might enhance TNF- α levels in the cortex of mice, which increased CDK5 levels, leading to a reduction in S-NR2B levels in the medial prefrontal cortex of mice, and ultimately a hippocampus-independent learning impairment in the mice (Fig. 11).

Discussion

We demonstrated that the surgical incision-induced nociception caused learning impairment in mice at 3 and 7d, but not 30 d, post incision. Moreover, the surgical incision only caused learning impairment in the tone test of FCS, which assesses hippocampus-independent learning and memory function (Kim and Fanselow, 1992; Anagnostaras et al., 1999; Kitamura et al., 2009; Wiltgen et al., 2010; Zhu et al., 2012), but not in the context test of FCS, which assesses hippocampus-dependent learning and memory function (Kim and Fanselow, 1992; Anagnostaras et al., 1999; Kitamura et al., 2009; Wiltgen et al., 2010; Zhu et al., 2012; Fig. 2). These findings suggested that the surgical incision could selectively cause hippocampus-independent learning impairment. Furthermore, the surgical incision only caused learning impairment when the pairing of FCS occurred after the incision but not before the incision (Fig. 2). These data suggested that the surgical incision might only impair mice from learning new tasks, but not from retaining old tasks. The surgical incision did not significantly alter escape latency or platform crossing times in the MWM (which also assess the hippocampus-dependent learning impairment; Fig. 2K,L). These data suggested that the surgical incision might not induce spatial learning and memory impairment in the mice.

Figure 11. Hypothesized pathway of surgical incision-induced learning impairment. Surgical incision causes nociception in the mice, which may increase the levels of TNF- α in the mouse cortex, leading to elevation of CDK5 levels in the mouse cortex. The elevated CDK5 levels then reduce synaptic NR2B levels in the mouse cortex, ultimately leading to hippocampus-independent learning impairment.

EMLA treatment mitigated the surgical incision-induced nociception (Fig. 3*A,B*), and learning impairment (Fig. 3*C,D*) in the mice. These findings suggested that the surgical incision could have induced nociception, which then caused learning impairment in the mice. However, it is still possible that the surgical incision induced inflammation, which caused the learning impairment given the anti-inflammatory effect of local anesthetics. The surgical incision reduced the levels of S-NR2B (but neither total NR2B nor Es-NR2B) specifically in the medial prefrontal cortex (Fig. 4), but not in the lateral prefrontal cortex, cingulate cortex, amygdala (data not shown), and hippocampus (Fig. 9) of the mice. Amygdala and medial prefrontal cortex are two brain regions that play an important role in the fear conditioning learning and memory (Tan et al., 2010; Marek et al., 2013). Hippocampus also contributes to the fear conditioning learning and memory (Koseki et al., 2009). The findings that the surgical incision-induced nociception caused hippocampus-independent learning impairment and reduced S-NR2B levels in medial prefrontal cortex (but not other brain regions, e.g., amygdala and hippocampus) suggested that reduction in S-NR2B levels specifically in the medial prefrontal cortex could be, at least partially, the underlying mechanisms by which surgical incision-induced nociception caused learning impairment.

A recent study has reported that neuropathic pain may alter medial prefrontal cortex populational firing activity patterns, leading to impairment of spatial memory (Cardoso-Cruz et al., 2013). Consistently, the current study showed that the NR2B in the medial prefrontal cortex may be responsible for the associative cognitive impairment caused by surgical incision-induced nociception. However, the studies by Cardoso-Cruz et al. (2013) assessed the spatial memory and neural activity signals, whereas the current study focused on spatial and associative memory, as well as NR2B levels in the medial prefrontal cortex.

NR2B in hippocampus and/or cortex has been reported to contribute to fear conditioning learning and memory (Tang et al., 1999; Rodrigues et al., 2001; Zhao et al., 2005; Dalton et al., 2008; Zhang et al., 2008*b*) but not spatial memory in rodents tested in MWM (Guscott et al., 2003; for review, see Qiu et al., 2011). NR2B in hippocampus and/or cortex is also involved in

chronic pain (Wei et al., 2001, 2002; Liauw et al., 2005; Wu et al., 2005; for review, see Qiu et al., 2011). TNF- α has been shown to contribute to pain (Narita et al., 2008; Gao and Ji, 2010) and learning and memory function (Terrando et al., 2010; Shen et al., 2013). Consistently, our current data suggested that the surgical incision increased TNF- α levels and decreased NR2B levels in mouse brain tissues, and caused fear conditioning learning impairment in the mice. Moreover, the surgical incision only reduced the levels of S-NR2B, but not the Es-NR2B, in the cortex (Fig. 4). S-NR2B is involved in nociception (Wei et al., 2001), learning, and memory (Rodrigues et al., 2001; Zhuo, 2009), whereas Es-NR2B is involved in cell damage (Hardingham et al., 2002; for review, see Hardingham and Bading, 2010). We therefore postulate that S-NR2B is specifically responsible for the learning impairment associated with the surgical incision-induced nociception.

Interestingly, the surgical incision-induced nociception caused learning impairment at both 3 and 7 d post incision. However, the surgical incision-induced nociception only decreased the S-NR2B levels in the cortex at 3 d (Fig. 4), but not 7 d (Fig. 8), post incision. The underlying mechanisms of the observations remain unknown. It is possible that the surgical incision-induced nociception reduced the S-NR2B levels at 3 d post incision, which then caused other changes associated with learning and memory, e.g., synaptic dysfunction, at 7 d post incision, leading to the observed fearing learning impairment.

Furthermore, the surgical incision-induced nociception increased the levels of CDK5 and its activators P35 and P25 (Patrick et al., 1999; Kusakawa et al., 2000; Lee et al., 2000; Cruz et al., 2003; Asada et al., 2008) in the cortex (Fig. 5). CDK5 has been shown to regulate NR2B levels (Sato et al., 2008; Zhang et al., 2008*a*), and CDK5 inhibitor roscovitine attenuated the surgical incision-induced hippocampus-independent learning impairment and reduction in S-NR2B levels in the cortex (Fig. 6). Finally, the surgical incision-induced nociception increased the levels of TNF- α in the cortex (Fig. 7). TNF- α regulates CDK5 activity and contributes to inflammation-induced pain signaling (Utreras et al., 2009). Collectively, these data suggest that the surgical incision-induced nociception may increase TNF- α levels in the cortex of mice, which increases CDK5 levels, leading to reduction of S-NR2B levels in the medial prefrontal cortex, and ultimately hippocampus-independent learning impairment (Fig. 11). However, it is still possible that the fear conditioning learning impairment following the surgical incision is not associated with these changes because EMLA or roscovitine treatment could not provide direct causal relationship between S-NR2B and fear conditioning learning impairment. Nevertheless, NR2B has been reported to contribute to both cognition and nociception (for review, see Qiu et al., 2011), and other studies have illustrated that brain ischemia may also induce cognitive impairment through alterations in brain NR2B levels (Okiyama et al., 1997; Huang et al., 2008).

The surgical incision-induced nociception did not significantly alter the levels of S-NR2B, Es-NR2B, CDK5, P35, and P25 in brain, and did not cause learning and memory impairment (Fig. 10) in 3-month-old mice. The surgical incision-induced nociception only increased the TNF- α levels in the cortex of the younger mice. The reason why the surgical incision-induced nociception only caused fear conditioning learning impairment in 9-month-old, but not 3-month-old mice is unknown at this time. It is hypothesized that the other changes in brain, e.g., aging-associated accumulation of β -amyloid in brain (Fukumoto et al., 2004), may facilitate the neurotoxic effects of surgical incision-

induced nociception, leading to neurobehavioral deficits. The current findings have established a system to embark on future studies to test this hypothesis.

Sleep disturbance in mice may cause hippocampus-dependent learning and memory impairment (Zhu et al., 2012). In the current studies, however, the surgical incision-induced nociception might cause hippocampus-independent learning impairment (Fig. 2). Therefore, it is unlikely that the sleep disturbance, which might be associated with the surgical incision-induced nociception, may contribute to the surgical incision-induced hippocampus-independent learning impairment, although such a possibility cannot be totally excluded.

The studies have several limitations. First, we only assessed the effects of the surgical incision-induced nociception on the brain levels of NR2B and cognitive function. It is likely that nociception induced by different insults (e.g., thermal properties and chemistry stimulation) may lead to different profiles of neurobiochemistry and neurobehavioral changes. Nevertheless, the current findings have established and characterized a preclinical system of surgical incision-induced nociception and learning impairment, and furthermore have shown that the surgical incision-induced nociception may lead to hippocampus-independent learning impairment and reduction in NR2B levels in medial prefrontal cortex. Second, we did not assess the effects of the surgical incision-induced nociception on other domains of cognitive function, e.g., executive function. However, the findings that the nociception can impair learning function, the primary domain of cognitive function, suggest that the surgical incision-induced nociception could contribute to POCD, pending further studies.

In conclusion, we found that the surgical incision-induced nociception increased the levels of TNF- α , CDK5, P35, and P25 in cortex, and reduced the S-NR2B levels in the medial prefrontal cortex, but not hippocampus. The surgical incision-induced nociception impaired hippocampus-independent learning in the mice. Finally, a CDK5 inhibitor attenuated the learning impairment and reduction in S-NR2B levels in the mice. These results suggest that surgical incision-induced nociception might cause learning impairment via reduction in S-NR2B levels, pending further studies. These findings would promote more studies to further determine the role of pain in cognitive function decline, leading to elucidation of the underlying mechanisms of POCD, which could ultimately improve the outcomes of surgical care in patients.

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