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Brain-network mechanisms underlying the divergent effects of depression on spontaneous versus evoked pain in rats: A multiple single-unit study

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Abstract

Studies have reported divergent behavioral effects of depression on spontaneous vs. stimulus-evoked pain. However, the underlying neurobiological mechanisms are still unclear. The present study used a depression model of unpredictable chronic mild stress (UCMS) and pain models for spontaneous pain (i.e., the formalin test) and acute evoked pain (i.e., noxious thermal stimulation) in rats. The activity of neurons within thalamo-cortical circuits in the lateral and medial pain pathways was recorded by a multiple-channel recording technique, and behaviors were observed simultaneously. The results confirmed our previous findings that rats exposed to UCMS tended to exhibit decreased pain sensitivity to experimental stimuli but increased behavioral responses to ongoing pain. Based on the analysis of single-unit responses, the results demonstrated that the processing of spontaneous vs. evoked pain in a depressive-like state was altered in the opposite direction (activation vs. inhibition). The ensemble encoding analysis revealed that exposure to UCMS gave rise to enhanced inter-regional functional connectivity in spontaneous pain processing, but did not influence that of evoked pain. In addition, different brain activation patterns underlying the processing of spontaneous vs. evoked pain were observed. These findings revealed that the distinct response patterns of neurons within the pain-related brain circuits, especially in the affective pain pathway, mediate the divergent effects of depression on spontaneous vs. evoked pain. This is also the first report on the electrophysiology of depression models that provides direct evidence that the effect of depression on spontaneous and evoked pain may involve different brain mechanisms.

Keywords

Cortex; Depression; Pain pathway; Rat; Single unit; Thalamus

Introduction

Depression is a mood state that can affect perception processes, such as sight, hearing, and pain. The complex interactions between depression and pain increase the suffering of patients (Stahl, 2002). Nevertheless, experimental studies have found bidirectional effects of depression on pain. For example, Tang et al. observed that experimental negative mood increased self-reported pain in chronic back pain patients (Tang et al., 2008), while Bar et al. reported that subjects with depressive disorders displayed an elevated threshold and tolerance during exposure to thermal- and electrical-evoked pain (Bar et al., 2003, 2005). Using animal models of unpredictable chronic mild stress (UCMS) and olfactory bulbectomy, our previous work demonstrated that depression can enhance the tonic inflammatory pain response but reduce the noxious stimulus-evoked withdrawal behavior in rats (Shi et al., 2010a, 2010b; Su et al., 2010; Wang et al., 2010). The differential modulatory effects of depression on spontaneous versus evoked pain may be attributed to the differences between the two pain models. The formalin test is believed to initiate spontaneous and persistent pain (Bai et al., 2007), triggering internal processing mechanisms that are not dependent on the initial stimulation. Conversely, the acute thermal pain test primarily evokes a withdrawal response and produces transient pain, which is avoidable and depends on the application of an external stimulus (Woolf and Costigan, 1999).

Although the effects of depression on pain have been widely explored in recent years, the underlying neural mechanisms are still unclear. There is growing evidence that depression and pain share genetic factors, biological pathways, and neurotransmitters (Bair et al., 2003; Gambassi, 2009). Studies indicate that the activities of pain-related brain areas are especially stronger in depressive patients than in those without depression (Giesecke et al., 2005). Imaging and physiological studies have revealed that the interaction between depressive and pain disorders is associated with the affective (medial) pain pathway (Tripp et al., 2011; Wiech and Tracey, 2009), including the anterior cingulate cortex (ACC). In patients with chronic pain, Giesecke et al. have found that the severity of depression correlates with the magnitude of brain activation in the medial region of the brain, but not with the pain-related sensory ratings (Giesecke et al., 2005). In depressed patients, decreased activation of cortical regions, such as the dorsolateral prefrontal cortex (DLPFC) and ACC (Drevets et al., 1997; Mayberg et al., 1999), and increased activation of limbic regions, such as the medial thalamus and amygdala (Koenigs et al., 2008; Moulton et al., 2011; Sheline et al., 2001), have also been reported.

Although clinical observations support a high prevalence of pain and amplified pain responses in patients with depression, contradictory findings, i.e., facilitatory vs. inhibitory effects of depression on pain, have been reported in laboratory experiments. These discrepancies may be due to the different evaluation measures (e.g., spontaneous vs. evoked pain) during the experimental studies as well as the intrinsic differences between the two types of pain. Spontaneous pain may involve more emotional responses, such as fear and distress, due to its unavoidability (Goncalves et al., 2008), while evoked pain primarily measures the behavioral reflex to an external stimulus and reflects the nociceptive aspect of pain perception (Terhaar et al., 2011).

The present study was designed using a multiple single-unit recording technique to observe the altered processing of pain (spontaneous vs. evoked) in both sensory and affective pain pathways in rats with a UCMS-induced depression-like phenotype. We analyzed the neuronal and population coding patterns in the parallel pain pathways under depression and comorbid pain conditions. Our working hypothesis is that the affective (medial) and sensory (lateral) pain pathway neurons play different, or even opposite, roles in mediating the impact of depression on spontaneous versus evoked pain. That is, the medial pathway neural activity primarily mediates changes in spontaneous pain while the lateral pathway contributes more to thermal-evoked pain.

Materials and methods

Animals

Twenty-four male Wistar rats (220–250 g) from the Laboratory Animal Center of the Academy of Military Medical Science were used in this study. Data from four of the animals were excluded from analysis due to misplacement of the recording electrode arrays. All rats were housed individually with food and water freely available, and were kept in separated rooms in order to manipulate the environments independently. The rooms were maintained at 22 ± 2 °C with a standard 12-h light–dark cycle (lights on at 07:00 am). Testing was performed during the light cycle. Animals were allowed to habituate to the environment for 1 week before the experiments, and they were softly handled 3 to 5 min per day by the experimenter. All experimental procedures were approved by the Institutional Review Board of the Institute of Psychology, Chinese Academy of Sciences (confirmation number: A12013).

Experimental procedure

Rats underwent microelectrode implantation into the target brain areas. Then they were randomly assigned to 2 groups: the UCMS group ($n = 12$) and the control group ($n = 12$). The rats received chronic stress exposure and normal (non-stress) treatment for 6 consecutive weeks, respectively. The control and UCMS-exposed animals were kept in separate rooms in order to manipulate the environments independently. Body weights and sucrose preferences were measured once a week before and during the UCMS/control procedure.

At the end of the 6th week of the procedure, pain tests (acute thermal stimulation and formalin injection, one day apart) were performed on all rats. The formalin test was preceded by the thermal-evoked pain session. The electrophysiological recordings and behavioral testing were simultaneously conducted. The experimental protocol is depicted in Fig. 1.

Surgery for microelectrode implantation

Following anesthesia with sodium pentobarbital (50 mg/kg, i.p.), animals were transferred to a Kopf stereotaxic apparatus. Supplementary doses (one-third of the original) of pentobarbital were given when necessary. Four, small, left-sided craniotomies were made for microelectrode array implantation. According to the atlas of Paxinos and Watson

(Paxinos and Watson, 1998), the stereotaxic coordinates were as follows: (1) Primary somatosensory cortex (SI), 1.0 mm posterior to bregma (−1.0 A), 2.0 mm lateral to midline (L), and 2.0 mm ventral to the skull surface (V); (2) Anterior cingulate cortex (ACC), 3.2 A, 0.8 L, and 2.8 V; (3) Medial dorsal thalamus (MD), −2.3 A, 0.8 L, and 5.5 V; and (4) Ventral posterior lateral thalamus (VPL), −3.0 A, 3.0 L, and 6.0 V. Four arrays of eight, stainless steel, Teflon-insulated microwires (50-μm diameter, Biographics, Winston-Salem, USA) were slowly lowered into the target areas. The microelectrode arrays were secured onto the cranium with stainless steel skull screws and dental cement. Animals were administered with penicillin (16,000 U, i.m.) before surgery to prevent infection. Rats were allowed 1 week to recover from this surgical procedure.

UCMS procedure

The UCMS animal model is one of the classic depression models (Willner et al., 1987). The procedure consisted of two to five different unpredictable stressors per day and lasted for several weekly cycles. Stressors were presented in a pseudo-random order during each weekly cycle (see Table 1). The following stressors were used: water deprivation (22- and 40-h periods), food deprivation (20- and 22-h periods), empty water bottle (1-h period, exposure to empty water bottle immediately after one 40-h period of water deprivation), restricted access to food (3-h period, two small pieces of pellet in each cage following one 20-h period of food deprivation), cage tilt (45°, 8- and 16- h periods), strobe light (7- and 8- h periods), soiled bedding (16-h period, twice), overnight illumination (16-h period, twice), intermittent white noise (75 dB, 2- and 5-h periods), novel odor (16-h periods, twice), exposure to a hot room (40 °C, 30-min period, twice), and exposure to a cold room (10 °C, 30-min period, twice).

Sucrose preference test

During the 6-week UCMS procedure, sucrose consumption was monitored weekly. The sucrose preference test was conducted at 2:00 pm on Sunday in the home cage for 1 h, following a 22-h period of food and water deprivation. Two bottles (one containing sucrose solution (1%) and the other containing water) were presented simultaneously to each rat. Sucrose preference was calculated according to the formula: % sucrose preference = sucrose solution consumption / (sucrose solution consumption + water consumption) × 100. Sucrose intake before the UCMS procedure (i.e., at week 0) was measured as baseline.

Pain tests

Testing sessions were carried out in a quiet room, with the room temperature kept at 25 ± 1 °C. Rats were placed in a plastic test chamber (44 cm × 44 cm × 44 cm), and acclimatized to the environment for at least 20 min before the pain tests. Animal behaviors during the experimental sessions were videotaped, with an imbedded time line synchronized with the neuronal activity data for offline analysis.

The noxious radiant heat stimulus was applied through a glass floor (1 mm thick) to the plantar surface of the rats' right hind paws (contralateral to the implanted electrode arrays). The thermal pain thresholds were measured by paw withdrawal latency (PWL) to the radiant heat. PWL was defined as the time length between the heat onset and the paw lift. A cutoff

time of 15 s was used to prevent tissue damage. Rats received a total of 20 noxious thermal stimuli. The inter-stimulus interval was no less than 3 min. Painful stimuli were delivered only when the animal was quiet and showed no voluntary motor activity. A time stamp series (resolution, 1 ms) marking the start and end of the noxious stimulus was recorded and synchronized with the neural spike recording.

Rats received subcutaneous injections of 5% formalin (50 μ l, 1:20 dilution of a 37% formaldehyde solution in 0.9% saline) into the plantar surface of the right hind paw (contralateral to the electrode implantation). The spontaneous licking behavior induced by formalin was observed during a 60-min period following injection. Pain intensity was determined by measuring the time spent in licking the affected paw for each 5-min period.

Electrophysiological data acquisition

Single-unit activity was recorded using a 128-channel data acquisition system (Cerebus, Blackrock Microsystems, Salt Lake City, USA). The neuronal activity was detected by the microwires and passed from the headset assemblies to a preamplifier via a light-weight cable and a rotating commutator (allowing for the free movement of animals). The sampled analog signals were amplified and filtered at cut-off frequencies of 0.3 Hz and 7.5 kHz, and digitized with a 16-bit resolution at 30 kHz by the amplifier. Then the signals from each microelectrode were bandpass filtered (250 Hz to 5 kHz) by a neural signal processor and sorted using spike-sorting programs. The time stamps of the spike activities were saved into a database file for offline analysis. The quality of single unit isolation was assessed with the following criteria: (1) the separation of waveform clusters in projections onto the first three principle component axes, (2) the homogeneity of waveforms, and (3) the presence of a refractory period in the interspike interval (ISI). Each recording session lasted approximately 1.0–1.5 h (for both the thermal-evoked pain test and the formalin test).

Data analysis

Statistica 5.1 (Statsoft, Inc., Tulsa, USA) and GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, USA) were used for statistical analyses and graph generation. The body weight, sucrose consumption, and duration of licking data were analyzed using two-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test. The Student's *t*-test was employed when two groups were compared for data on the PWL, peak latency, normalized firing rates (z-score) of neurons, and PDC. The *Chi*-square test was used to detect significant differences in the number of responsive neurons and correlated neuronal pairs between groups. The data were presented as the mean $\pm$ standard error of the mean (SEM). The statistical significance was set at $P < 0.05$.

For the formalin test and the thermal-evoked pain test, discharges of single neurons were counted in 20-s and 0.1-s bins, respectively. The analysis program NeuroExplorer (Plexon, Dallas, USA) was used to construct peri-stimulation time histograms (PSTH) with a Gaussian filter of three bins. The results were exported to Matlab (The MathWorks, Inc.) in a spread sheet form. The firing rates for each unit and each time bin were transferred into z-scores: $z = (x - m) / s$, where x is the raw firing rate of the neuron obtained from PSTH, and m and s are the mean and standard deviation of the baseline firing rate of that neuron,

respectively. An increase or decrease in firing rate was considered significantly excitatory or inhibitory when it was more than two standard deviations from the baseline (i.e., z -score > 2 or < -2 , respectively) for at least five consecutive bins. Peak latency was defined as the time delay from the stimulus onset to the response peak.

The difference in the dynamic neural response to formalin injection between groups was evaluated using a sliding-window averaging technique (Schultz and Romo, 1992), in which a 10-bin window was slid through the entire observation period in 1-bin steps. The bin counts of each window were compared between groups by the Student's t -test. The differences were considered significant only when it reached a significance level of $P < 0.005$ for three consecutive steps, which achieved a global significance of $P < 0.05$ (Wang et al., 2003).

Cross-correlation analysis was performed to reveal the functional connectivity between neuronal ensembles. Cross-correlation histograms were created by NeuroExplorer, in which one neuron within a given region was selected as the reference neuron, and all neurons from the other region were defined as partner neurons. The time of occurrence of spikes from the reference neuron was set at 0 s, and the partner neuron's firing 0.5 s before and after the spike of the reference neuron was plotted using a 5-ms bin width and a 3-bin Gaussian smooth. The significance level of the cross-correlograms was tested using 95% confidence intervals for at least five successive bins. Data from the pain tests were calculated for 0–60 min after formalin injection and for 0 to 20 s following thermal stimulation.

Partial directed coherence (PDC) analysis was performed to detect the direct influence of a certain neuronal group on another. The process of PDC analysis has been described in detail elsewhere (Baccala and Sameshima, 2001; Sameshima and Baccala, 1999; Yang et al., 2005). Briefly, PDC is a frequency domain representation of the key concept of Granger causality. If knowledge of $x(n)$'s past significantly improves prediction of $y(n)$, we could then state that an observed time-series $x(n)$ Granger-causes series $y(n)$. For PDC analysis, we first performed principal component analyses (PCA) throughout the recording session for neurons in each brain area in NeuroExplorer. Then the first principal component (PC1) of the brain area that had the largest response was exported into MatLab to calculate the value of PDC across a 1–50 Hz range. These values were then averaged around the stimulation events, and normalized to z -scores relative to the baseline (–20 min to 0 for the formalin test, and –5 s to 0 for the thermal pain test).

Histology

After completion of the recording, the subjects were overdosed with pentobarbital, and 10–20 μ A of anodal current was passed for 10–20 s through the recording electrodes to deposit iron ions. The animals were then sacrificed and perfused with 4% paraformaldehyde and 5% potassium ferricyanide solution, and their brains were extracted. The brains were post-fixed with the same solution used for the perfusion. Coronal sections (40 μ m thick) were cut through the SI, ACC, and thalamus. Recording sites were determined under a light microscope. The iron deposits were easily identified as blue dots. Data from rats with any of the sites outside the target areas were removed from the analysis. The percentage of correct electrode placements was 96% (92/96), and four rats were excluded.

Results

Depression-like behaviors in rats

The body weights and sucrose preferences of the rats were measured weekly during the UCMS procedure. Over the 6-week observation period, the body weights of the control rats increased steadily (Fig. 2A), and the sucrose intake remained unchanged (Fig. 2B). However, the rats treated with UCMS had significantly lower body weights over time (two-way ANOVA, group $\times$ time interaction: $F_{(5, 90)} = 23.0$, $P < 0.001$; group effect: $F_{(1, 18)} = 91.5$, $P < 0.001$; time effect: $F_{(5, 90)} = 71.9$, $P < 0.001$), and they exhibited a concomitant decrease in sucrose consumption (two-way ANOVA, group $\times$ time interaction: $F_{(5, 90)} = 3.74$, $P = 0.004$; group effect: $F_{(1, 18)} = 32.0$, $P < 0.001$; time effect: $F_{(5, 90)} = 3.22$, $P = 0.010$), as compared to the control rats. These results suggest that the animal receiving stress exposure developed depressive-like behaviors.

Effect of UCMS exposure on the brain processing of experimental evoked pain

UCMS exposure produced a significant increase in the thermal nociceptive thresholds of rats, i.e., it significantly prolonged paw withdrawal latency to radiant heat stimuli, when compared to the non-stress condition (5.58 ± 0.31 s vs. 4.28 ± 0.27 s, t -test, $t_{(18)} = 3.20$, $P = 0.005$, Fig. 3A).

Neuronal responses accompanying behavioral changes were recorded and analyzed. The number of recorded neurons is shown in Table 2. The acute thermal stimulation evoked exclusively excitatory responses in all of the recorded areas, both in the control and stress groups. No inhibitory responses were observed. Fig. 3B depicts the spatial and temporal distribution patterns of neuronal responses after the thermal stimulation. There was a significant reduction in the excitatory neuronal activity as indicated by the number of responding neurons in the ACC and SI cortical areas in the stress-exposed rats in comparison to the control rats (Table 3). The firing rate data, represented as mean z-scores, showed that the UCMS treatment produced significant inhibitory effects on the magnitude of the neuronal responses within the SI and ACC cortices as well as in the VPL as compared with the control group (Fig. 3C). In terms of the temporal aspects, the peak latency of the neuronal responses in the UCMS rats was significantly delayed in the lateral pathway (SI and VPL) neurons as compared to the control rats (Fig. 3D). In contrast, no difference was found between the two conditions in the medial pathway (Fig. 3D).

Pairwise cross-correlation analysis revealed that the neuronal correlation in the lateral pathway (SI-VPL correlation) was remarkably stronger than that in the medial pathway (ACC-MD correlation) for both the control and UCMS groups (Table 4). However, no significant difference was observed between the two conditions in the correlated neuronal activity, suggesting that the functional connectivity of the neuronal population during the brief stimulus-evoked pain is not influenced by chronic stress exposure. Consistently, the PDC analysis demonstrated that there was no difference in the neural information flow between the UCMS and control groups, either in the medial pathway or in the lateral pathway, during thermal-evoked pain (data not shown here).

Effect of UCMS exposure on the brain processing of spontaneous pain

For both the UCMS group and the control group, subcutaneous injection of formalin into the hind paw produced a typical biphasic pattern of licking behavior (phase I: 0 to 5 min; interphase: 5 to 15 min; phase II: 15 to 60 min), as shown in Fig. 4A. Compared with the control rats, the ongoing licking behaviors in the UCMS-treated rats were significantly enhanced throughout the 60-min observation period (two-way ANOVA: group $\times$ time interaction: $F_{(11, 198)} = 0.497$, $P = 0.904$; group effect, $F_{(1, 18)} = 18.8$, $P < 0.001$; time effect: $F_{(11, 198)} = 14.2$, $P < 0.001$), consistent with our previous findings.

Single-unit discharges were recorded in the medial (ACC and MD) and lateral (SI and VPL) pain pathways during the pain tests for both groups. The number of recorded neurons is shown in Table 2. The dynamic neuronal responses under UCMS and control non-stress conditions following formalin injection are illustrated in Fig. 4B. In general, formalin injection resulted in primarily excitatory responses for both groups. There were significantly more neurons exhibiting excitatory responses in the UCMS group than in the control group (UCMS vs. control: 50.1% vs. 35.6%, *Chi-square* test, $P = 0.001$). By contrast, there was a significant decrease in the number of neurons showing inhibitory response in the UCMS group, compared with the control group (UCMS vs. control: 8.36% vs. 13.6%, *Chi-square* test, $P = 0.028$). Calculating the mean z-scores for all neurons revealed a double-peak response pattern in both groups (Fig. 4D, left panel), with the first peak within 0–5 min and the second within 15–60 min post-formalin. Notably, the mean firing rates in all of the three phases (phase I, II, and interphase), manifested as z-scores, were significantly higher in the UCMS group than in the control group (Fig. 4D, right panel).

To reveal the nociceptive coding patterns of medial and lateral pathway neurons in the depressive-like state, we explored the spatial and temporal distributions of neuronal responses within each recorded area after the formalin injection (Fig. 5). For both the UCMS and control states, excitation was observed within all of the recorded regions while inhibition was found mainly in the medial pain pathway (i.e., ACC and MD) (Fig. 5A). In particular, the excitation in the lateral pathway (including the SI and VPL neurons) was significantly stronger, and the inhibition in the medial pathway (only MD neurons) was weaker, in the UCMS condition than in the control condition (Table 5). Fig. 5B illustrates the difference between the UCMS and control groups in the magnitude of neuronal responses (computed as z-score) to the formalin-induced tonic pain. The lateral pathway (i.e., SI and VPL) neurons in the UCMS group reacted more strongly than those in the control group, especially during the second phase of the formalin test. By contrast, the medial pathway (i.e., ACC and MD) neurons did not respond consistently, and the response intensity was merely enhanced in the MD neurons, but not in the ACC neurons.

To clarify the relative contributions of the medial and lateral pathway neurons in coding spontaneous pain in the depressive-like state, functional connectivity based on pairwise cross-correlation was calculated for the simultaneously recorded neurons (Table 6). Overall, the correlated activity in the lateral pathway was significantly higher than that in the medial pathway for both the control and UCMS conditions. Most importantly, only the correlation

in the medial pathway was intensified by UCMS treatment; no changes were observed in the lateral pain pathway after exposure to chronic stress.

PDC analysis was used to determine the direction and amount of neural information flow from one brain region to another and to examine the reciprocal influence between them. Fig. 6 illustrates the results of PDC analysis between the thalamic nucleus and cortical area within the parallel pain pathways (i.e., VPL-SI and MD-ACC) after formalin injection. There was no difference in the amount of normalized PDC between the UCMS and control groups in the lateral pathway (*t*-test, all $P > 0.05$; Fig. 6A). However, there were significant changes in the UCMS group in the medial pathway, i.e., an increase in the ACC to MD direction and a decrease in the MD to ACC direction (*t*-test, all $P < 0.05$; Fig. 6B), as compared to the control group.

Histology

The location of the microwires, as revealed by the iron deposits at the tips of selected microwires, is depicted in Fig. 7. The black dots label the recording sites.

Discussion

In the present study, the UCMS model was employed to explore the effects of depression on pain-related behaviors and simultaneous neuronal activity within the lateral and medial pain pathways. The present data confirm our previous findings that UCMS exposure produces divergent effects on spontaneous and evoked pain behaviors (facilitation vs. inhibition) in rats (Shi et al., 2010a, 2010b). More importantly, distinct patterns of neuronal activity underlying these effects were detected using multiple data analysis techniques for the first time. The analysis of single-unit responses demonstrated that the processing of spontaneous vs. evoked pain in the depressive-like state was altered in the opposite direction (activation vs. inhibition), and involved both the lateral and medial pain pathways (summarized in Table 7). The ensemble encoding analysis revealed that the UCMS exposure influenced the inter-regional functional connectivity in spontaneous pain processing but not in evoked pain processing.

Additionally, different brain activation patterns underlying the processing of spontaneous vs. evoked pain were observed in the non-stress control group (summarized in Table 8). In the spontaneous pain condition, the lateral and medial pathway neurons were activated to a similar degree. Meanwhile, in the evoked-pain condition, the activation was stronger in the lateral pathway than in the medial pathway. Only excitatory responses were observed during evoked pain, while both inhibition and activation were observed during spontaneous pain.

The current findings did not seem to support our initial working hypothesis in that the dissociable effects of chronic stress exposure on spontaneous and evoked pain were not reflected in the modulation of pathway activity. That is, the lateral and medial pain pathways were activated in a largely similar pattern under both pain conditions. Instead, fundamental differences in the brain processing of spontaneous versus evoked pain, represented as completely opposite response patterns (increase vs. decrease in the neuronal activity; and positive vs. negative in the changes in between-region functional connectivity), were found

in rats with the depressive phenotype. These patterns perfectly matched the external behavioral responses of the animals.

The present study found that the inhibitory responses were only observed in the formalin pain condition. There is evidence demonstrating that the central mechanism underlying spontaneous pain differs from that of evoked pain in that the former involves supraspinal processing and activates the descending pain inhibitory system (Nandi et al., 2003; Schaible et al., 2002; Urban and Gebhart, 1999). Formalin injection could cause the release of endogenous opioids into periaqueductal gray matter (PAG) and induce a decrease of gamma-aminobutyric acid (GABA) in the PAG, thus disinhibiting PAG neurons and results in the activation of descending inhibitory pathway (de Novellis et al., 2003; Maione et al., 1999, 2000). Thus, it is possible that the inhibitory responses during spontaneous pain may indicate the activation of descending pain inhibitory systems, such as the diffuse noxious inhibitory controls (DNIC) (Lapicot et al., 2009).

Depression, as an emotional disorder, is characterized by functional abnormalities in the fronto-limbic brain circuitry. Clinical observations have found that the exacerbation of pain is closely associated with abnormal functioning of medial pathway structures (Grande et al., 2004; Maarrawi et al., 2011; Seifert et al., 2013). In our study, rats with the depressive-like phenotype plus spontaneous pain showed a prominent neuronal change, namely the reduction of inhibitory responses in the medial thalamus. As previously mentioned, the inhibition during spontaneous pain may reflect the activation of the descending inhibitory system. Accordingly, the reduction in inhibition during the comorbidities of depression and pain may indicate the disinhibition of spinal nociceptive transmission. Additionally, the low level of monoamine neurotransmitters and impairment in brain monoamine pathways may lead to the weakening of supraspinal descending inhibition and thus aggravates pain (Burke et al., 2010).

Another effect of depression on the neural activity of rats with spontaneous pain was that the inter-regional connectivity was enhanced in the affective (medial) pain pathway, as manifested by the strengthened functional relationship (both cross-correlation and PDC results) between the ACC and MD in the present study. The ACC has long been reported to be involved in the activation of descending pain suppression mechanisms (Krout and Loewy, 2000; Mantyh, 1982). A previous study revealed a negative correlation between depression and PAG connectivity strength with the thalamus and ACC in patients with fibromyalgia (Cifre et al., 2012). As we discussed above, depression can lead to the dysfunction of descending modulation, such as the suppression of inhibitory responses in the medial thalamus. Thereby, the increased functional connectivity between the ACC and MD may be driven by negative feedback in which the ACC sent descending control to the thalamic neurons to compensate for the deficit in descending modulation. Additionally, resting-state studies have found reduced connectivity between the MD and ACC in depressed subjects as compared to healthy controls (Anand et al., 2005; Greicius et al., 2007), further indicating that the enhanced functional relationship in the medial pathway is most likely associated with the endogenous modulation of pain.

Contrary to the enhanced impact of depression on spontaneous pain, stress exposure produced inhibition in thermal-evoked pain, represented as the elevation of paw withdrawal thresholds and the delay in peak latency of single-unit responses in the pain pathways. In contrast to the medial pathway (only involving the ACC), the prolongation of peak latency was found more in neurons of the lateral pathway (both the SI and VPL). The thalamic nuclei in the lateral pathway receives A δ - and C-fiber inputs via spinal projection neurons and transmits information to the somatosensory cortex, participating in the sensory discrimination of pain (Basbaum et al., 2009; Brooks and Tracey, 2005; Talbot et al., 1991). The decreased neuronal responses in the depressive-like condition in this study may indicate that the ability of encoding the physical properties of noxious stimuli was weakened to and the process velocity was slowed down. According to Lautenbacher et al., the hypoalgesia to phasic experimental pain was due to diminished spinal and brainstem transmission (Lautenbacher et al., 1994). Similarly, by recording laser-evoked potential (LEP) in depressed patients (Terhaar et al., 2011; Weiss et al., 2011), Terhaar et al. found decreased A δ -LEP amplitudes and delayed latencies of C-LEP components.

From the psychological perspective, the lowered attention capacity and reduced avoidance motive in depressed subjects may also explain the abnormalities in somatosensory processing (Bora et al., 2012). Attention has been thought to mediate the transformation from stimuli processing into behavioral responses (Koetsier et al., 2002). In the evoked pain condition, the attention deficit induced by depression may lead to a decrease in cognitive abilities (e.g., judgment) to respond to external stimuli with precision. In addition, weak avoidance motivation may play a role in the reduced withdrawal behavior.

A limitation of the present study is that the modulation of neural activity by stress did not result in consistent response patterns between regions within the same pathway, especially in the medial pathway, in either pain condition. This may cause some confusion when interpreting the current results. However, the phenomenon can be understood when the different roles of the lateral and medial pathways are taken into account. The lateral pathway is a relatively simple system that participates in processing the physical characteristics of sensation and involves a specific sensory projection system (Brooks and Tracey, 2005; Chen et al., 2009). By contrast, the function of the medial pathway is more complicated. The ascending projections are diffuse and multisynaptic, and the connections of the feedback circuits are slow and loose in the medial thalamo-cortical pathway (Wang et al., 2007). It does not only process the emotion-related information, but also has roles in maintaining arousal (attention), decision making, and top-down modulation of pain (Bryden et al., 2011; Descalzi et al., 2012; Holroyd et al., 2004). Given that pain is a multi-dimensional phenomenon comprised of several interacting components (i.e., sensory, affective, and cognitive), it is difficult for the medial pathway regions to maintain a high consistency in the response patterns to peripheral inputs. Future studies are needed to clarify the underlying mechanisms of our observations.

Another limitation is the depressive test chosen in this study. The most widely used tool for measuring depression-like behavior is forced swimming test (FST), whose reliability has been proven across laboratories (Cryan et al., 2005; Elizalde et al., 2008; Elsayed et al., 2012; Porsolt et al., 1977). However, it cannot be used here due to the fact that water does

not combine with electrodes and electricity. Thus, the sucrose preference test (SPT) was selected for compensation. Although the validity of SPT has been questioned by several studies (Forbes et al., 1996; Hatcher et al., 1997;; Matthews et al., 1995) for they consider that the decrease in sucrose intake is related to the effects of stress on body weights, a large number of studies have demonstrated that the UCMS-induced decrease in sucrose drinking, as well as the reversal of those effects by antidepressant drugs, is independent of changes in body weights (Harkin et al., 2002; Konkle et al., 2003; Pothion et al., 2004; Willner et al., 1996).

Conclusion

The present study demonstrated that the distinct response patterns of neurons within the pain-related brain circuits, especially in the affective pain pathway, mediate the divergent behavioral effects of depression on spontaneous vs. evoked pain.

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Fig. 1.

Experimental protocol. Rats were randomly assigned to two groups, receiving either chronic stress exposure or control treatment for six consecutive weeks. Bodyweight and sucrose preference were measured once a week. Then pain tests, including acute thermal stimulation and formalin injection, were conducted. Simultaneous recording of multiple single units were performed. UCMS: unpredictable chronic mild stress.

Fig. 2.

Depressive-like behaviors after the 6-week UCMS exposure. (A) The rats treated with UCMS had significantly lower body weights than the control rats. (B) There was a significant reduction in sucrose consumption in the UCMS-exposed rats in comparison to the control rats. ** $P < 0.01$, *** $P < 0.001$.

Fig. 3.

The effect of chronic mild stress on behavioral responses and neuronal activity due to acute thermal pain. (A) The paw withdrawal latency (PWL) to noxious heat stimuli was significantly prolonged in the stress-exposed rats ($n = 10$). (B) The spatial and temporal distribution patterns of neuronal activity in response to radiant heat stimulation. No inhibitory response was found across all areas. Each line in the image represents the normalized firing rates (i.e., z-score) of one neuron. (C) Comparison of normalized firing rates of neurons to noxious radiant heat between the UCMS and control groups. The UCMS treatment produced significant inhibitory effects on the z-scores of neurons, which were averaged across all neurons within each brain area over the post-stimulus period (0–20 s). (D) The peak latency of neuronal responses in the UCMS rats was significantly delayed in the lateral pathway (SI and VPL) neurons. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared to the control group.

Fig. 4.

Comparison of behavioral responses and neuronal activity to formalin injection between the UCMS and control rats. (A) Formalin-induced licking behavior. The licking behaviors in the UCMS-treated rats were significantly enhanced as compared to the control rats. (B) Temporal distribution patterns of neuronal activity in response to formalin injection. Neurons were sorted by their z-score value (i.e., normalized firing rate). Each line in the pseudocolor plot represents the z-score of one neuron. The neurons with z-scores > 2 were accepted as significantly excited, and those with z-scores < -2 were significantly inhibited. There were more excitatory responses and less inhibitory responses in the UCMS group than in the control group. (C) Examples of neurons showing excitatory or inhibitory responses. (D) Averaged single-unit response to formalin injection. The difference in the dynamic neural response between groups was evaluated with the sliding-window method. The firing rates in all of the three phases (phase I, II, and interphase) were significantly higher in the UCMS group than in the control group. The rectangular markers along the x-axis (left panel) indicate the statistically significant difference between the control group and the UCMS group. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Fig. 5.

Comparison of neural activity in the lateral and medial pain pathways between the UCMS and control rats during the formalin test. (A) The temporal patterns of the neuronal responses to formalin injection in the ACC, MD, SI, and VPL. Each line in the image represents the normalized firing rates (i.e., z-score) of one neuron. (B) The averaged z-scores of neurons in each brain area. The rectangular markers along the x-axis indicate the statistically significant difference between groups, as evaluated with the sliding-window method. Time = 0 on the axis corresponds to the time of formalin injection.

Fig. 6. Results of partial directed coherence analysis within the lateral (A) and medial (B) pain pathways after formalin injection. Significant changes in the UCMS rats were found in the medial pathway as compared to the control group. * $P < 0.05$; compared to the control.

Fig. 7.
A schematic drawing indicating the location of recording sites in the ACC, SI, MD, and VPL. The black dots label the positions of iron deposits at the tips of selected microwires.

Table 1

Procedure of unpredictable chronic mild stress.

Time	Type of stress
<i>Monday</i>	
09:00	Clean cages, commence strobe light
16:00	End strobe light and commence hot room (40 °C) for 30 min
17:00	Commence water deprivation
<i>Tuesday</i>	
09:00	Commence cage tilting
14:00	Commence intermittent white noise for 2 h
17:00	End cage tilting and expose to novel odor. Switch lights to on overnight
<i>Wednesday</i>	
09:00	Remove novel odor and exposed to empty water bottle for 1 h
10:00	Restore water
12:00	Commence cold room (10 °C) for 30 min
17:00	Soil cage
<i>Thursday</i>	
07:00	Reverse the light/dark cycle for 24 h
09:00	Clean cage, commence food deprivation and exposed to hot room (40 °C) for 30 min
<i>Friday</i>	
09:00	Commence strobe light
17:00	End strobe light, restricted access to food (2 small pieces of pellet in each cage) for 3 h
20:00	Restore food and switch lights to on overnight
<i>Saturday</i>	
09:00	Commence cold room (10 °C) for 30 min
15:00	Commence intermittent white noise for 2 h
16:00	Remove water and food
17:00	Commence cage tilting and exposure to novel odor
<i>Sunday</i>	
09:00	End cage tilting and remove novel odor
14:00	Sucrose preference test for 1 h
15:00	Restore water and food
17:00	Soil cage

Table 2

Number of simultaneously recorded neurons in each brain region during the thermal-evoked pain test and formalin test.

	Thermal-evoked pain test		Formalin test	
	Control	UCMS	Control	UCMS
SI	85	82	88	83
VPL	85	86	83	85
ACC	86	83	86	85
MD	84	85	80	82
Total	340	336	337	335

UCMS: unpredictable chronic mild stress. ACC: anterior cingulate cortex. MD: medial dorsal thalamic nucleus. SI: primary somatosensory cortex. VPL: ventral posterolateral thalamus.

Table 3

Number and percentage of neurons showing excitatory responses during the thermal-evoked pain test.

	Control	UCMS
SI	62 (72.9)	43 (52.4)**
VPL	69 (81.2)	68 (79.1)
ACC	26 (30.2)	12 (14.4)*
MD	24 (28.6)	18 (21.2)

Numbers outside and inside the parentheses represent the number and percentage of responsive neurons, respectively.

* $P < 0.05$, chi-square test, compared to the control.

** $P < 0.01$, chi-square test, compared to the control.

Table 4

Correlated activity between neuronal populations during the thermal-evoked pain test.

	Control		UCMS	
SI-VPL	*	280/705(39.7%)	*	313/709(44.1%)
ACC-MD	}	44/716(6.1%)	}	34/720(4.7%)

Number and percentage of correlated neuronal pairs in the lateral (SI-VPL) and medial (ACC-MD) pain pathways are shown here. Statistical differences were examined by the chi-square test.

* $P < 0.001$, comparison between the pathways.

Table 5

Number and percentage of neurons showing excitatory or inhibitory responses during the formalin test.

	Excitatory response		Inhibitory response	
	Control	UCMS	Control	UCMS
SI	31 (35.2)	44 (53.0)*	4 (4.55)	2 (2.41)
VPL	39 (47.0)	62 (72.9)**	5 (6.02)	1 (1.18)
ACC	23 (26.7)	28 (32.9)	13 (15.1)	19 (22.4)
MD	27 (33.8)	34 (41.5)	24 (30.0)	6 (7.32)**

Numbers outside and inside the parentheses represent the number and percentage of responsive neurons, respectively.

* $P < 0.05$, chi-square test, compared to the control.

** $P < 0.001$, chi-square test, compared to the control.

Table 6

Correlated activity between neuronal populations during the formalin test.

	Control		UCMS	
SI-VPL	**	284/731(38.9%)	**	291/709(41.0%)
ACC-MD	{	64/688(9.3%)	{	117/700(16.7%)*

Number and percentage of correlated neuronal pairs in the lateral (SI-VPL) and medial (ACC-MD) pain pathways are shown here. Statistical differences were examined by the chi-square test.

* $P < 0.001$, compared to the control.

** $P < 0.001$, comparison between the pathways.

Table 7

Changes in neural activity by UCMS exposure as compared to the control condition.

	Spontaneous pain		Thermal-evoked pain	
	Lateral pathway	Medial pathway	Lateral pathway	Medial pathway
<i>Single-unit level</i>				
Response proportion	↑(E)	↓(I)	↓(E)	↓(E)
Response intensity	↑	↑	↓	↓
<i>Ensemble level</i>				
Cross-correlation	No	Yes	No	No
PDC	No	Yes	No	No

E: excitatory response; I: inhibitory response. Medial pathway: including anterior cingulate cortex and medial dorsal thalamic nucleus. Lateral pathway: including primary somatosensory cortex and ventral posterolateral thalamus.

Table 8

Comparison of ratios of excitation or inhibition in the parallel pain pathways between spontaneous versus thermal-evoked pain in the non-stress control group.

	Spontaneous pain		Thermal-evoked pain	
	Lateral pathway	Medial pathway	Lateral pathway	Medial pathway
Excitatory response	35–47%	27–34%	73–81%	29–30%
Inhibitory response	4.5–6.0%	15–30%	0	0