Manipulating placebo analgesia and nocebo hyperalgesia by changing brain excitability

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Harnessing placebo and nocebo effects has significant implications for research and medical practice. Placebo analgesia and nocebo hyperalgesia, the most well-studied placebo and nocebo effects, are thought to initiate from the dorsal lateral prefrontal cortex (DLPFC) and then trigger the brain's descending pain modulatory system and other pain regulation pathways. Combining repeated transcranial direct current stimulation (tDCS), an expectancy manipulation model, and functional MRI, we investigated the modulatory effects of anodal and cathodal tDCS at the right DLPFC on placebo analgesia and nocebo hyperalgesia using a randomized, double-blind and sham-controlled design. We found that compared with sham tDCS, active tDCS could 1) boost placebo and blunt nocebo effects and 2) modulate brain activity and connectivity associated with placebo analgesia and nocebo hyperalgesia. These results provide a basis for mechanistic manipulation of placebo and nocebo effects and may lead to improved clinical outcomes in medical practice.

placebo and nocebo effects | transcranial direct current stimulation | expectancy manipulation | dorsolateral prefrontal cortex | mechanistic manipulation

Placebo (and nocebo) effects are salubrious benefits (or negative outcomes) attributable to the nonspecific symbolic components of health care. They have profound implications for basic and clinical research as well as medical practice. Thus, harnessing placebo and/or reducing nocebo effects is an important issue with broad implications for human self-healing and self-harming (1).

Placebo analgesia and nocebo hyperalgesia are the most well-studied placebo and nocebo effects (2, 3). In the past decade, brain imaging studies have identified complicated brain networks that may serve as a basis for modulating placebo analgesia and nocebo hyperalgesia by directly changing the excitability of key brain regions (4, 5). The most consistent placebo-related increases in response to pain were reported in the dorsolateral prefrontal cortex (DLPFC) and ventromedial prefrontal cortex (vmPFC, including the rostral and pregenual cingulate and medial orbitofrontal cortex), and responses in these regions were correlated with magnitudes of placebo analgesia (5). In particular, studies suggest that these cognitive modulations of pain may initiate from the DLPFC (6, 7), a cognitive-executive–control brain region processing expectancy (i.e., the key component for placebo and nocebo effects), which then triggers the descending pain modulatory system (DPMS; e.g., anterior cingulate cortex [ACC], insula, thalamus) (8, 9) and reward system (e.g., ventral striatum) (10) to diminish or intensify one's pain experience depending on context (5). In addition, placebo analgesia and nocebo hyperalgesia may be associated with opposite responses of the DPMS and reward system (11), and anxiety may specifically affect nocebo hyperalgesia (12, 13).

Although many studies have investigated the neural mechanisms, few have explored the feasibility of modulating the placebo and nocebo effects with noninvasive brain stimulation (NIBS). An early behavioral study found that low-frequency repetitive transcranial magnetic stimulation (TMS) at the DLPFC could block placebo analgesia as measured by pain threshold and tolerance increases (14). In a following behavioral study, we tested the modulatory effects of another neuromodulation method, transcranial direct current stimulation (tDCS). Specifically, we applied single-session anodal and cathodal (without a sham tDCS control) tDCS at the right DLPFC (rDLPFC) immediately after a visual-cue conditioning paradigm and found significant placebo- and nocebo-like conditioning effects only in the anodal group (15). These behavioral studies provided early evidence for modulating placebo and nocebo effects with NIBS, but the neurobiological mechanisms linking the manipulated brain activity and behaviors are still unknown. Investigating how NIBS at the rDLPFC can change brain activity/connectivity and modulate placebo analgesia and nocebo hyperalgesia may advance the field from observation of neural responses to their mechanistic manipulation and may facilitate its application in medical practice (16).

Combining an expectancy manipulation paradigm, repeated tDCS (anodal, cathodal, and sham) paradigm, and functional MRI (fMRI), we investigated the modulation effects of changing the excitability of the rDLPFC on placebo analgesia and nocebo hyperalgesia from 81 healthy subjects using a randomized, double-blind and sham-controlled design. We hypothesized that repeated

Significance

Although previous studies have extensively studied the neurobiological mechanisms of placebo and nocebo effects, ways to manipulate these effects to yield further benefits remain relatively unknown. We combined simultaneous repeated transcranial direct current stimulation (tDCS)-fMRI, an expectancy manipulation model, and task-based fMRI to investigate the modulatory effects of anodal and cathodal tDCS at the right dorsolateral prefrontal cortex on placebo analgesia and nocebo hyperalgesia. Our results suggest that active tDCS has the potential to boost placebo and blunt nocebo effects and modulate brain activity and connectivity associated with placebo analgesia and nocebo hyperalgesia. These findings provide deeper understanding of mechanistic manipulation of placebo and nocebo effects and may lead to improved clinical outcomes in medical practice.

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active tDCS (compared with sham tDCS) 1) would modulate behaviors and brain responses associated with placebo analgesia and nocebo hyperalgesia and 2) would modulate rDLPFC functional connectivity with regions associated with placebo analgesia and nocebo hyperalgesia.

Results

Behavioral Results. A total of 81 healthy participants were randomly assigned to either the anodal, cathodal, or sham tDCS group (Table 1) and completed the study, which was comprised of five study sessions (Fig. 1*A* and see *Materials and Methods* for details).

For all groups, we used an expectancy manipulation model (17–20) to induce expectations of three inert creams, with one cream labeled "lidocaine" (inducing expectations for pain decreases), one labeled "capsaicin" (inducing expectations for pain increases), and one labeled "neutral" (for control). Conditioning was accomplished by surreptitiously decreasing (low pain), increasing (high pain), or using an intermediate (moderate pain) noxious stimulus intensity on each of the nine test spots on the forearm (Fig. 1*B*). We found that participants had significantly different pain perception (rated using a 0 to 20 Gracely scale; Table 1 and *SI Appendix*, Fig. S1*A*) across the three stimulus intensities ($F_{(2,52)}$ = 416.5, $P < 0.001$) but not across the three tDCS groups ($F_{(2,52)}$ = 0.82, $P = 0.44$) or across the interaction of stimulus and group ($F_{(4,104)} = 1.67$, $P = 0.16$). In addition, participants' expectations of both the analgesic effect of lidocaine cream and the hyperalgesic effect of capsaicin cream (rated using a 0 to 10 scale before and after expectation manipulation) were significantly boosted in all three groups after expectancy manipulation (Table 1 and *SI Appendix*, Fig. S2), but the changes in expectation were not significantly different among the three groups (lidocaine: $F_{(2,78)} = 0.48$, $P = 0.62$; capsaicin: $F_{(2,78)} = 0.62$, $P = 0.54$). These results indicate that participants in the three groups had similar levels of conditioning/learning effects during expectancy manipulation.

Afterward, participants received different rDLPFC tDCS interventions, based on their randomization, in three sessions over 3 d (the first and last sessions were conducted inside the scanner, and fMRI data were collected before, during, and after tDCS application). Repeated tDCS was used because recent studies have suggested that repeated sessions may have cumulative effects on behaviors and brain activities (21–23). After the last tDCS session, participants performed placebo and nocebo tests while fMRI data were collected. Similar to the expectancy manipulation session, the three different creams (in reality, all one inert cream) were applied to the forearm. Similar to our previous studies (12, 17), we only delivered different noxious stimuli, which produced significantly different pain perception ($P < 0.001$ for the comparisons between low and moderate, as well as between high and moderate), on the three spots in the most lateral column (Fig. 1*C* and *SI Appendix*, Fig. S3). This was done to recondition or reinforce expectations and ensure that placebo and nocebo effects could be detected (24), as the conditioning in Session 2 was approximately performed 3 to 7 d before Session 5. After reconditioning, participants' expectations of both the analgesic effect of lidocaine cream and the hyperalgesic effect of capsaicin cream remained at the same level as they had after conditioning in Session 2 (*SI Appendix*, Fig. S2). To address the potential confounding effect of tDCS on sensory perception, we compared the differences in pain ratings and found that they were not significantly different across the three tDCS groups (low painful stimuli: $F_{(2,78)} = 2.27$, $P = 0.10$; high painful stimuli: $F_{(2,78)} = 0.03$, $P = 0.97$; and moderate painful stimuli: $F_{(2,78)}$ = 0.63, $P = 0.53$). This result indicates that different tDCS interventions did not have an effect on subjective pain perception/sensitivity.

We then delivered identical moderate stimuli to all remaining regions with all three creams (Fig. 1*C* and *SI Appendix*, Fig. S3). Participants in the two active tDCS groups had significant placebo analgesia (defined as the difference between perceived intensity to identical moderate painful stimuli applied on areas of skin with lidocaine cream and those applied on areas of skin with neutral cream; $P < 0.05$ for anodal and $P < 0.001$ for cathodal, paired-sample t test) and nonsignificant nocebo hyperalgesia (defined as the difference between perceived intensity to identical moderate painful stimuli applied on areas of skin with capsaicin cream and those applied on areas of skin with neutral cream; $P = 0.22$ for anodal and $P = 0.07$ for cathodal, paired-sample t test). It is worth mentioning that participants in the sham tDCS group had both significant placebo analgesia and nocebo hyperalgesia, indicating the validity of our experimental design in inducing placebo and nocebo effects (Table 1). Comparisons among the three groups (Table 2) showed significant main effects of tDCS groups on placebo analgesia (i.e., lidocaine versus neutral; $F_{(2,71)} = 3.55$, $P = 0.034$) and nocebo hyperalgesia (i.e., capsaicin versus neutral; $F_{(2,68)} = 3.27$, $P = 0.044$). Post hoc two-sample t tests showed that cathodal tDCS significantly boosted placebo analgesia compared with sham tDCS (mean difference = 1.16, Cohen's d = 0.75, $P_{Tukey} = 0.028$), while anodal tDCS significantly inhibited nocebo hyperalgesia compared with sham tDCS (mean difference = −1.39, Cohen's d = −0.77, $P_{Tukey} = 0.041$).

Table 1. Demographics and behaviors

	Anodal tDCS	Cathodal tDCS	Sham tDCS
Demographics			
Age, mean (SD), y	27.4 (6.3)	26.9 (5.9)	27.9 (7.1)
Male (female), No.	14 (13)	16 (11)	14 (13)
Behaviors in Session 2			
Pain ratings for low painful stimuli, mean (SD)	6.05 (2.67)	4.54 (2.04)	5.08 (2.14)
Pain ratings for moderate painful stimuli, mean (SD)	9.31 (1.97)	8.84 (2.54)	8.97 (2.34)
Pain ratings for high painful stimuli, mean (SD)	14.52 (2.67)	14.56 (2.33)	14.52 (2.20)
Changes of expectation for pain decrease, mean (SD)	1.70 (2.89)**	2.30 (2.45)***	1.67 (2.59)**
Changes of expectation for pain increase, mean (SD)	1.78 (2.22)***	2.00 (2.47)***	2.48 (2.41)***
Behaviors in Session 5			
Placebo analgesia, mean (SD)†	0.81 (1.54)*	1.55 (1.81)***	0.50 (1.47)*
Nocebo hyperalgesia, mean (SD)†	0.44 (1.82)	0.84 (2.32)	1.13 (1.46)***

P values were corrected for multiple comparisons using FDR. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, two-tail t test; participants in the sham tDCS group had significant placebo analgesia ($P < 0.05$ for one-tail t test).

†Placebo analgesia (or nocebo hyperalgesia) was defined as the difference between perceived intensity (as indicated by pain rating) to identical moderate painful stimuli applied on lidocaine (or capsaicin) cream and those applied on neutral cream.

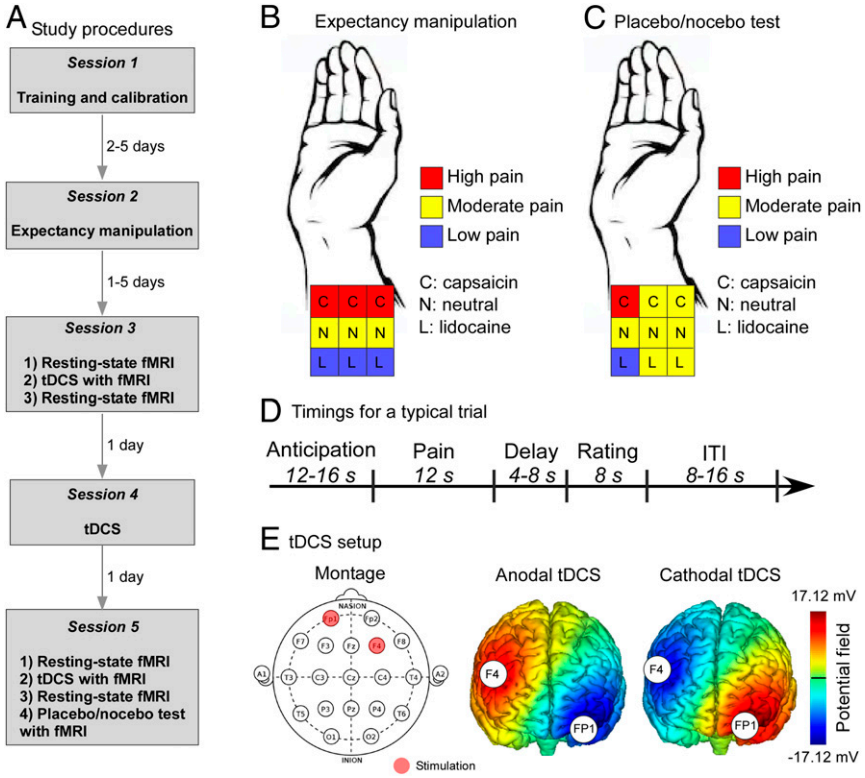

Fig. 1. Study design. (*A*) The procedures in the present study. (*B*) Inert lidocaine, capsaicin, and neutral creams were applied at different spots. Different intensities of heat pain were applied at corresponding spots to manipulate subjects' expectancy on the analgesic and hyperalgesic effects of the creams. (*C*) Placebo and nocebo tests. Inert lidocaine, capsaicin, and neutral creams were applied at different spots. Different intensities of heat pain were applied at the three spots in the left column, while the same moderate intensity painful stimuli were applied to the remaining six spots. (*D*) Timings for a typical trial. (*E*) tDCS setup. Subjects received 20 min tDCS in Sessions 3, 4, and 5. The anodal electrode was placed over F4 and the cathodal electrode over FP1 for rDLPFC excitability enhancement. The anodal electrode was placed over FP1 and the cathodal electrode over F4 for rDLPFC excitability inhibition. For sham tDCS treatment, stimulation was applied only at ramp-up/ramp-down periods at the beginning and end of sham stimulation to mimic the somatosensory effect of real tDCS for 15 s.

Modulatory Effects of tDCS during Pain. In the analyses of task fMRI during placebo and nocebo tests, we first identified a wide range of brain regions activated by painful stimuli, including the bilateral insula, thalamus, caudate, putamen, ACC, presupplementary motor area (pre-SMA), and primary somatosensory cortex (S1) (Fig. 2*A* and *SI Appendix*, Table S1). Pain-evoked deactivations were observed in the precuneus and visual cortex (Fig. 2*A* and *SI Appendix*, Table S1).

Next, we identified regions that displayed neural representations of placebo analgesia and nocebo hyperalgesia. During stimulation, the placebo contrast (lidocaine versus neutral) showed increased brain activations in the caudate, pre-SMA, ACC, vmPFC, and rDLPFC and decreased brain activations in the SMA and S1 (Fig. 2*B* and *SI Appendix*, Table S2). The nocebo contrast (capsaicin versus neutral) showed increased brain activations in the pregenual and subgenual ACC (pgACC and sgACC), bilateral insula, and caudate and decreased brain activations in the precuneus (Fig. 2*C* and *SI Appendix*, Table S2).

Because behavioral placebo analgesia was significantly stronger in the cathodal group as compared with the sham group (Table 2 and Fig. 2 *D* and *E*), we investigated how cathodal tDCS modulated placebo at the neural level. The fMRI comparison of placebo effect between the cathodal and sham tDCS groups showed that subjects in the cathodal tDCS exhibited greater activations in the vmPFC (Fig. 2*F* and *SI Appendix*, Table S3). Specifically, within-group comparisons showed that subjects in the cathodal group had significantly higher activations in the vmPFC when painful stimuli were applied on areas of skin with lidocaine cream as compared with neutral cream ($P = 0.003$, paired-sample t test), but this difference was not observed in subjects from the sham tDCS group. Between-group comparisons showed that subjects in the cathodal group had significantly higher activations in the vmPFC than those

Table 2. Modulatory effects of tDCS on placebo analgesia and nocebo hyperalgesia

Placebo analgesia	Sum of squares	F	*P*	Nocebo hyperalgesia	Sum of squares	F	*P*
Main effect				Main effect			
tDCS group	16.83	3.55	0.034	tDCS group	21.41	3.27	0.044
Post hoc *t* test	Mean difference	Cohen's d	*P*	Post hoc *t* test	Mean difference	Cohen's d	*P*
Anodal versus sham	0.36	0.24	0.68	Anodal versus sham	−1.39	−0.77	0.041
Cathodal versus sham	1.16	0.75	0.028	Cathodal versus sham	−0.99	−0.55	0.18
Anodal versus cathodal	−0.80	−0.52	0.19	Anodal versus cathodal	−0.40	−0.22	0.75

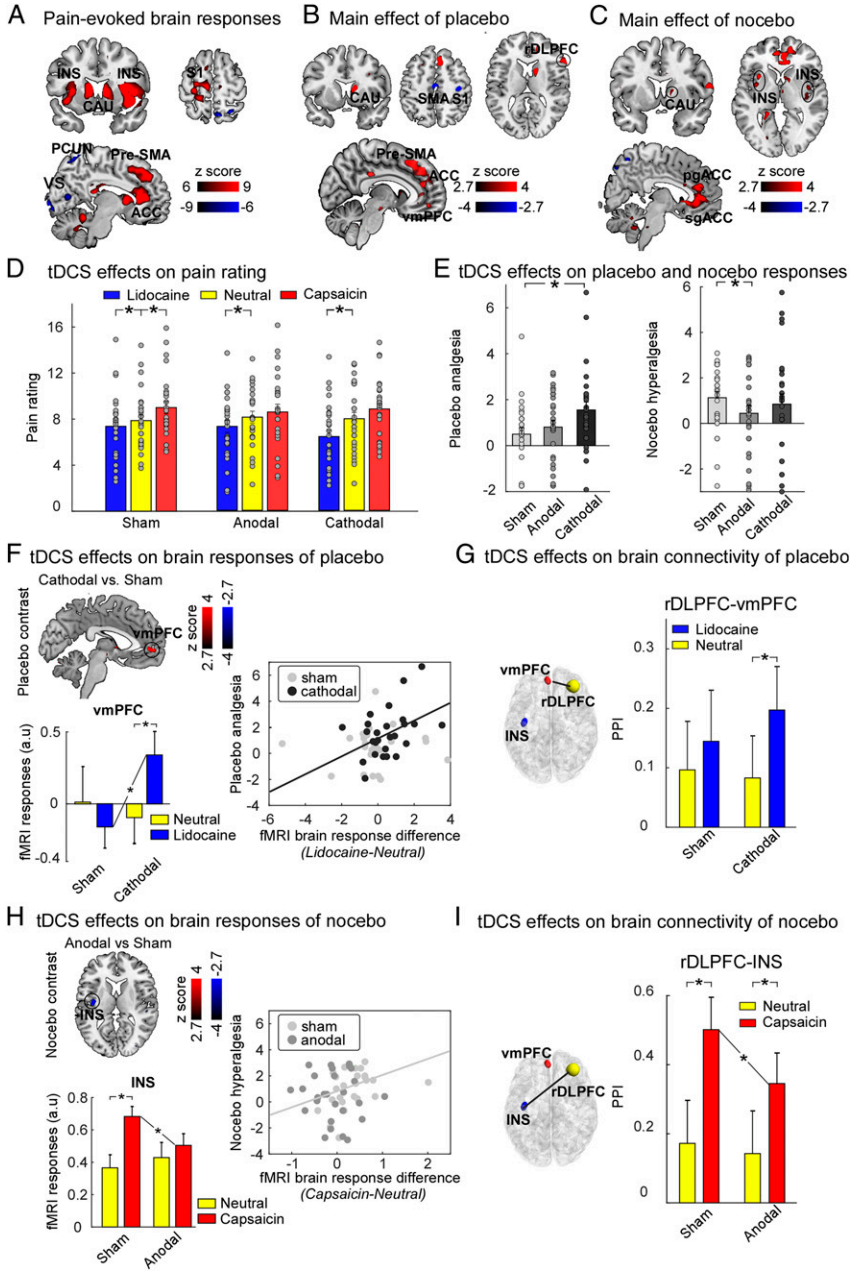

Fig. 2. Pain and conditioned pain-related brain responses. (*A*) Heat painful stimuli elicited brain activations in the bilateral insula (INS), caudate (CAU), anterior cingulate cortex (ACC), presupplementary motor area (pre-SMA), and primary somatosensory cortex (S1) and decreased brain activations in the precuneus (PCUN) and visual cortex (VS). (*B*) During pain experience, the expectancy of pain relief with lidocaine induced increased brain activations in the rDLPFC, CAU, pre-SMA, and ACC and decreased brain activations in the SMA and S1. (*C*) During pain experience, the expectancy of pain exacerbation with capsaicin induced increased brain activations in the bilateral INS, pregenual and subgenual ACC (pgACC and sgACC), and CAU and decreased brain activations in the PCUN. (*D*) Pain ratings corresponding to identical moderate painful stimuli applied on different creams. The sham group had both significant placebo analgesia and nocebo hyperalgesia, while both anodal and cathodal groups had significant placebo analgesia and nonsignificant nocebo hyperalgesia. (*E*) Compared with sham tDCS, cathodal tDCS significantly boosted placebo analgesia, and anodal tDCS significantly inhibited nocebo hyperalgesia. Asterisks indicate two-tail $P_{tukey} < 0.05$ for post hoc comparisons of adjusted placebo and nocebo responses by covariates in the ANCOVAs. (*F*) Subjects who received cathodal tDCS showed significantly higher brain activations in the vmPFC for placebo contrast (lidocaine versus neutral). Cathodal tDCS significantly increased activations in the vmPFC when experiencing painful stimuli on the lidocaine cream, and the fMRI brain response difference (lidocaine neutral) was significantly correlated with placebo analgesia in the cathodal group. (*G*) The task-based connectivity (measured by PPI) between the rDLPFC and vmPFC was increased when experiencing pain on the lidocaine cream in the cathodal group. (*H*) Subjects who received anodal tDCS showed decreased brain activations in the left INS for nocebo contrast ("capsaicin versus neutral"). Anodal tDCS significantly inhibited activations in the INS when experiencing painful stimuli on the capsaicin cream and disrupted the significant association between the fMRI brain response difference (lidocaine neutral) and nocebo hyperalgesia observed in the sham group. (*I*) The task-based connectivity between the rDLPFC and insula was increased when experiencing pain on the capsaicin cream in both sham and anodal groups, but such connectivity in the anodal group was significantly lower than the sham group. Note, results in *A*–*C* were corrected for multiple comparisons at the whole-brain level, and results in *E* were corrected within the mask consisting of typical regions in the DPMS (i.e., the ACC, mPFC, insula, and SMA). Statistical tests between bars were threshold as $P < 0.05$ and were corrected from multiple comparisons using FDR. Error bars represent SE of mean.

in the sham group when painful stimuli were applied on the lidocaine cream ($P = 0.04$, two-sample t test), whereas the brain responses were not significantly different between the two groups when painful stimuli were applied on the neutral cream. The differences in fMRI responses in the vmPFC between painful stimuli on the lidocaine cream and the neutral cream were significantly positively correlated with the magnitudes of placebo analgesia in the cathodal group ($r = 0.43$, $P = 0.02$) but not in the sham group, suggesting the associated effects on brain response and behavior from cathodal tDCS. Using psychophysiological interaction (PPI) to evaluate the task-based connectivity between the stimulated site (i.e., rDLPFC) and the vmPFC (see *Materials and Methods* for technical details), we found increased connectivity between the rDLPFC and vmPFC when painful stimuli were applied on lidocaine cream as compared with neutral cream in the cathodal group ($P = 0.02$, paired-sample t test). This difference was not observed in subjects from the sham tDCS group (Fig. 2*G*).

In contrast, behavioral nocebo hyperalgesia was significantly blunted in the anodal group as compared with sham group (Table 2 and Fig. 2 *D* and *E*), in which participants had reduced activations in the left insula (Fig. 2*H* and *SI Appendix*, Table S3). Within-group comparisons showed that subjects in the sham group had significantly elevated activations in the insula when painful stimuli were applied on areas of skin with capsaicin cream as compared with neutral cream ($P < 0.001$, paired-sample t test), but this phenomenon was not observed in subjects from the anodal tDCS group. Between-group comparisons showed that subjects in the anodal group had significantly blunted brain responses in the insula compared with those in the sham group when painful stimuli were applied on the capsaicin cream ($P = 0.03$, two-sample t test), whereas the brain responses were not significantly different between the two groups when painful stimuli were applied on the neutral cream. The differences in fMRI responses in the insula between painful stimuli on the capsaicin cream and the neutral cream were significantly associated with the magnitudes of nocebo hyperalgesia in the sham group ($r = 0.36$, $P = 0.04$) but not in the anodal group, suggesting that anodal tDCS may disrupt the brain-behavior association and blunt the nocebo effect. Further task-based connectivity analysis (i.e., PPI, Fig. 2*I*) showed increased connectivity between the rDLPFC and insula when painful stimuli were applied on the capsaicin cream for both sham ($P = 0.007$, paired-sample t test) and anodal groups ($P = 0.03$, paired-sample t test). However, between-group comparison showed that subjects in the anodal group had significantly lower rDLPFC–insula connectivity than those in the sham group when painful stimuli were applied on the capsaicin cream ($P = 0.02$, two-sample t test).

Modulatory Effects of tDCS on Intrinsic Brain Connectivity. We then investigated how repeated tDCS modulated intrinsic brain connectivity and consequently modulated placebo and nocebo effects. Eight fMRI scans were collected during Session 3 (the first tDCS session) and Session 5 (the third tDCS session), including one before the application of tDCS (pre-tDCS), two during the tDCS (one during the first 6 min [tDCS-early] and another during the last 6 min [tDCS-late]), and one immediately after the application of tDCS, for each session respectively. Based on the results from task-based fMRI, we calculated the functional connectivity between the rDLPFC and the identified insula and vmPFC in different fMRI scans for different tDCS groups respectively. In Fig. 3*A*, we observed that the anodal tDCS significantly decreased rDLPFC–insula connectivity in Session 3 (pre-tDCS versus post-tDCS: $P = 0.02$, paired-sample t test) and Session 5 (pre-tDCS versus post-tDCS: $P = 0.01$), as well as across two sessions (pre-tDCS in Session 3 versus post-tDCS in Session 5: $P = 0.001$). Moreover, the modulatory effect on rDLPFC–insula connectivity was cumulative when comparing pre-/post-tDCS in Sessions 3 and 5 ($P = 0.005$, Friedman test). In the other two groups, we did not observe significant and reliable modulatory effects in the two sessions. We further investigated whether the decreased rDLPFC–insula connectivity after all anodal tDCS sessions (i.e., post-tDCS in Session 5) was associated with subsequent task-fMRI brain responses in the insula as well as subjective pain ratings (Fig. 3*B*). Results showed that the rDLPFC–insula connectivity was significantly correlated with the brain responses ($r = 0.47$, $P = 0.01$) and pain ratings ($r = 0.40$, $P = 0.04$) when painful stimuli were applied on the capsaicin cream, while such associations were not observed on the neutral cream ($r = 0.24$, $P = 0.23$, and $r = 0.27$, $P = 0.18$ for brain response and pain ratings, respectively).

Similar analyses found that cathodal tDCS significantly increased rDLPFC–vmPFC connectivity in Session 3 (pre-tDCS versus post-tDCS: $P = 0.03$, paired-sample t test) and Session 5 (pre-tDCS versus post-tDCS: $P = 0.02$), as well as across two sessions (pre-tDCS in Session 3 versus post-tDCS in Session 5: $P = 0.005$). Moreover, the modulatory effect on rDLPFC–vmPFC connectivity was cumulative when comparing pre-/post-tDCS in Sessions 3 and 5 ($P = 0.01$, Friedman test). In the other two groups, we did not observe significant and reliable modulatory effects in the two sessions. In the cathodal group, the rDLPFC–vmPFC connectivity after all tDCS sessions (i.e., post-tDCS in Session 5) was significantly correlated with the brain responses in the vmPFC ($r = 0.44$, $P = 0.02$) and pain ratings ($r = -0.45$, $P = 0.02$) when painful stimuli applied on the lidocaine cream but not on the neutral cream ($r = -0.04$, $P = 0.85$, and $r = -0.29$, $P = 0.15$ for brain response and pain ratings, respectively).

Discussion

In the present study, we investigated the causal relationship between brain excitability and placebo/nocebo effects using repeated tDCS on the rDLPFC. We found that 1) inert lidocaine and capsaicin creams produced a significant pain-rating decrease (placebo effect) and increase (nocebo effect), respectively, in response to identical heat painful stimuli as compared with a within-subject control neutral cream; 2) compared with sham tDCS, cathodal tDCS showed significant effects in increasing placebo analgesia and brain responses in the vmPFC, while anodal tDCS showed significant effects in inhibiting nocebo hyperalgesia and brain responses in the insula; and 3) repeated active tDCS significantly and cumulatively modulated intrinsic functional connectivity between the rDLPFC and vmPFC (cathodal tDCS) as well as between the rDLPFC and insula (anodal tDCS) to have the "priming effect" for the upcoming placebo analgesia and nocebo hyperalgesia.

Importance of Modulating Placebo and Nocebo Effects. Placebo effects are essential components of medical practice and efficacy research. In fact, a significant amount of clinical improvement, especially with subjective symptom outcomes, is directly attributable to placebo effects (25). Nocebo effects are also a major concern for clinical care since patients discontinue prescribed medications, make unnecessary medical visits, and take additional medications to counteract adverse effects that are actually nocebo effects (26). The potential to enhance salubrious placebo effects and/or diminish treatment-interfering nocebo effects may have clinical significance. For example, clinical studies have suggested that expectancy is positively associated with chronic pain improvement (27), and using conditioning-like expectancy manipulation, we have shown that significantly boosting expectancy can improve treatment outcome (28). Individual differences and/or characteristics of patient/clinician relations are critical factors in the efficacy of expectancy modulation (29) and therefore limit the manipulation of placebo and nocebo effects in clinical settings.

The scientific literature has suggested that both left and right DLPFC are involved in placebo effects, but findings have been quite mixed. A previous meta-analysis found that the right and

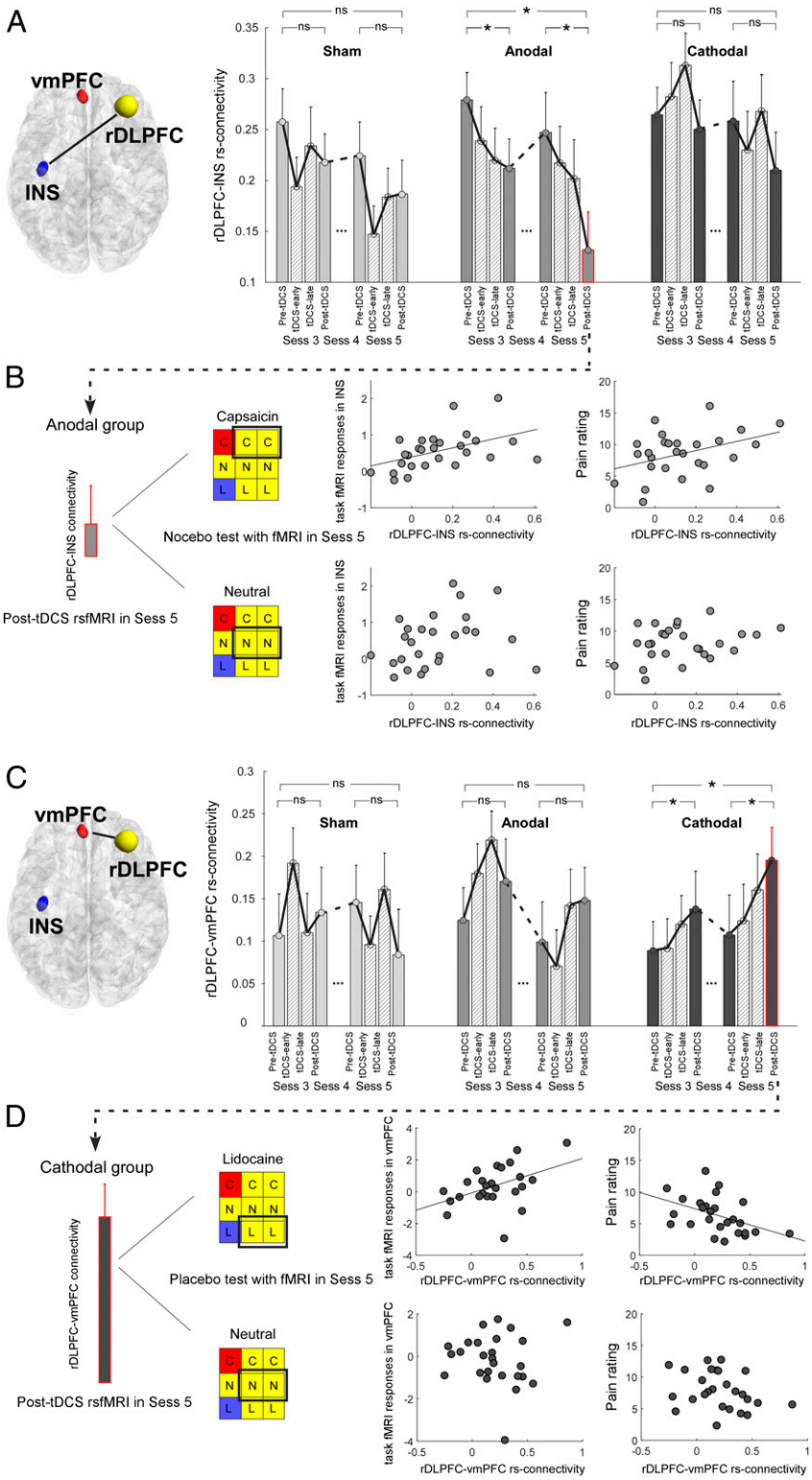

Fig. 3. Modulatory effects of tDCS on intrinsic brain connectivity. (*A*) Anodal tDCS significantly decreased functional connectivity between the rDLPFC and insula in Sessions 3 and 5. (*B*) In the anodal group, the rDLPFC-insula connectivity at the post-tDCS fMRI scan of Session 5 was significantly associated with subsequent brain responses in the insula and pain ratings when experiencing pain on the capsaicin cream but not on the neutral cream. (*C*) Cathodal tDCS significantly increased functional connectivity between the rDLPFC and vmPFC in Sessions 3 and 5. (*D*) In the cathodal group, the rDLPFC-vmPFC connectivity at the post-tDCS fMRI scan of Session 5 was significantly associated with subsequent brain responses in the vmPFC and pain ratings when experiencing pain on the lidocaine cream but not on the neutral cream. vmPFC: ventromedial prefrontal cortex; INS: insula; Pre-/Post-tDCS: the resting-state fMRI scan before/after the application of tDCS; tDCS early/late: the simultaneously collected fMRI during the first/last 6 min of tDCS application; rsfMRI: resting-state fMRI. Asterisks indicate two-tail $P_{FDR} < 0.05$. Error bars represent SEM.

left DLPFC were involved in the placebo effects during expectation anticipation and pain administration, respectively (30), while another meta-analysis found placebo-induced activations in bilateral DLPFC during pain administration (31). In the present study, we targeted the rDLPFC based on two previous behavioral studies investigating the feasibility of using neuromodulation to harness placebo and nocebo effects. One study used low-frequency repetitive TMS to disrupt left or right DLPFC function and block placebo analgesia, and the results showed that TMS on left and right DLPFC produced similar modulation effects on placebo analgesia (14). In another study, we used anodal and cathodal rDLPFC tDCS to modulate brain excitability immediately after a visual-cue conditioning paradigm and found significant placebo and nocebo effects in the anodal group (15). Partly in line with previous studies, the present study included a sham control, more tDCS sessions, and brain imaging to reveal how anodal and cathodal tDCS modulate placebo and nocebo effects as compared with sham tDCS. The present study only modulated rDLPFC. Further studies are needed to compare and investigate the tDCS effects with other target areas (including the left DLPFC).

Although still under investigation, we believe that in the experimental setting, there exists large individual variability to placebo and nocebo responses. One interesting question that remains to be answered is if tDCS can enhance all individuals in general or just target on responder or nonresponder. Unfortunately, our study cannot answer this question. Future studies with cross-over design may be helpful to test if the tDCS 1) can modulate placebo/nocebo effects in all participants, 2) modulate primarily on the responders, or 3) transform nonresponders into responders.

Repeated tDCS on Conditioned Pain and Pain-Related Brain Responses. Literature suggests that placebo and nocebo can modulate brain circuits to confer therapeutic effects (32–34). Brain-imaging studies suggest that there are unique brain mechanisms associated with placebo and nocebo effects (4, 17). It has been proposed that placebo analgesia and nocebo hyperalgesia, the cognitive modulations of pain (i.e., expectancy), may be initiated from the prefrontal cortex (e.g., DLPFC and vmPFC) (7) and then modulate activations in pain-associated regions in the cortex including the ACC, insula, and thalamus (8, 10, 12, 35, 36). A recent study directly comparing placebo and nocebo responses in the same cohort of subjects showed that they had shared neural responses in pain-related regions (e.g., ACC) and distinct neural responses in reward- and anxiety-related brain regions (17). Consistent with these previous studies, we observed that both placebo and nocebo effects modulated the ACC. Lidocaine cream also modulated the vmPFC and DLPFC, while capsaicin cream modulated the insula.

As a noninvasive neuromodulation technique, tDCS offers a way to modulate brain excitability and allows us to explore causal relationships between a target brain area and its perceptual, cognitive, and motor functions (37, 38). Since the processing of positive expectancy (placebo analgesia) and negative expectancy (nocebo hyperalgesia) of pain may initiate from the DLPFC and then trigger the DPMS to diminish or intensify pain depending on context (6, 39), we used repeated tDCS to stimulate the rDLPFC and found that cathodal stimulation could boost placebo analgesia and anodal stimulation could inhibit nocebo hyperalgesia. It is worth mentioning that although we tried to target rDLPFC in the study, the return electrode of tDCS may also produce effects in the brain. Specifically, the anodal tDCS enhanced rDLPFC and inhibited vmPFC, while the cathodal tDCS inhibited rDLPFC and enhanced vmPFC (Fig. 1*E*). We found that the cathodal tDCS enhanced placebo-effect (behavior) and fMRI responses in vmPFC as well as brain connectivity between rDLPFC and vmPFC (brain). This result is consistent with the literature, suggesting that the vmPFC/rACC involved the neural processing of placebo effect, and showed the casualty of the brain circuit (i.e., top-down processing) and may forge a new direction for placebo studies.

We also observed the same trend in anodal and cathodal tDCS of modulating placebo (enhancing) and nocebo (inhibiting) effects compared with sham tDCS. Similar modulations by anodal and cathodal tDCS have been previously reported in the visual cortex (40) and motor cortex (41). We also acknowledge the possible floor/ceiling effect of the placebo analgesia and nocebo hyperalgesia, which might have prevented differential tDCS effects on anodal and cathodal groups.

Although it has been hypothesized that anodal tDCS could depolarize (increase excitability) and cathodal tDCS could hyperpolarize (inhibit excitability) the neurons, subsequent observations indicated that anodal and cathodal stimulations might have variable and interchangeable excitatory/inhibitory effects, depending on factors such as 1) intensity applied (i.e., low-intensity [1 mA] stimulation causes conventional polarity-specific modulation of neural excitability, while higher-intensity [2 mA] stimulation may lead to increased excitability from both stimulation polarities) (42); 2) stimulation duration (i.e., longer stimulation may reverse effects) (43); 3) parallel versus perpendicular orientation of axons (which vary according to cortical folding) in relation to current flow (44); and 4) individuals' baseline brain states (45). Therefore, the physiological changes by anodal and cathodal stimulation may not be as simple as their hypothesized polarity (16). Results from our task fMRI suggest that cathodal tDCS enhanced brain activations in the vmPFC and the connectivity between the rDLPFC and vmPFC to boost placebo analgesia [placebo-related increases in rDLPFC and vmPFC have been consistently observed in previous studies (5) and our present study], while anodal tDCS inhibited brain activations in the insula and the connectivity between the rDLPFC and insula to blunt nocebo hyperalgesia [nocebo-related increase in the insula has been consistently observed in previous studies (12) and our present study]. It is interesting to note that the tDCS only modulated brain responses on the conditioned creams (i.e., lidocaine cream for positive expectancy, capsaicin cream for negative expectancy) but not on the neutral cream. This finding suggests that tDCS does not influence our pain perception in general but rather regulates the expectancy modulation of pain.

Repeated tDCS on Intrinsic Brain Networks. Subjects in the three groups had similar learning performance during the conditioning stage (Session 2, as reflected by expectations for relief scale (ERS) changes for lidocaine and capsaicin, as well as pain rating differences between high, low, and moderate stimuli). In addition, subjects' pain perceptions to high, low, and moderate stimuli were not modulated by tDCS. Therefore, we believe our findings were derived from repeated tDCS between Session 2 and Session 5, which may modulate intrinsic brain activity/connectivity and consequently modulate cognitive performance (i.e., placebo and nocebo effects) other than pain perception.

We found that repeated anodal tDCS significantly and cumulatively inhibited functional connectivity between the rDLPFC and insula, and the strength of intrinsic rDLPFC–insula connectivity after all tDCS sessions was correlated with subjective pain ratings and pain-related brain responses when experiencing pain on the capsaicin cream. In contrast, cathodal tDCS significantly and cumulatively enhanced the functional connectivity between the rDLPFC and vmPFC, and the strength of intrinsic rDLPFC–vmPFC connectivity after all tDCS sessions was correlated with subjective pain ratings and pain-related brain responses when experiencing pain on the lidocaine cream. These results are converged with task fMRI, suggesting that repeated tDCS may have priming effects. That is, cathodal tDCS enhances synchrony between the rDLPFC and vmPFC to facilitate the functioning of

these two regions, which are involved in the subsequent cognitive modulation of positive expectancy on pain. At the same time, anodal tDCS disrupts the synchrony between the rDLPFC and insula to inhibit the functioning of these two regions, which are involved in the subsequent cognitive modulation of negative expectancy on pain.

In this study, we only focused on the functional connectivity of three brain regions based on the result derived from pain-related brain responses. Nevertheless, tDCS may produce more extensive effects, that is, influencing the functional connectivity of other brain areas. Future studies are needed to apply a more comprehensive functional connectivity to investigate modulation effect of tDCS on different brain networks.

Limitations. There are several limitations in the present study. First, although the sham tDCS setup has been widely used in previous studies, one potential concern is that subjects in the sham group may have been aware that they were receiving sham stimulation since they were only stimulated during the first and last 15 s of tDCS. We measured subjective sensations during tDCS on the last 31 participants (n = 10, 10, and 11 for sham, anodal, and cathodal groups) using a questionnaires of sensations related to transcranial electrical stimulation (46). Results showed that these sensations were not significantly different in all three sessions ($F_{(2,28)} = 0.35$, $P = 0.71$; $F_{(2,28)} = 2.25$, $P = 0.12$; and $F_{(2,28)} = 1.56$, $P = 0.23$) across the three treatment groups (reference *SI Appendix* for details). Since a previous study tested the same tDCS setup and also found that participants were not able to distinguish between active and sham tDCS (47), we believe our blinding was effective. Future studies can include an active sham control to exclude the potential confounder of tDCS sensation. Second, although we included 81 participants in the study, the sample size (n = 27 for each group) for detecting group differences is relatively small. A future study with a larger sample size is needed to validate our findings and to maximize the effects to reach clinical significance. Third, although we targeted rDLPFC, the field map indicates that tDCS might change the excitability of a large area in the lateral frontal cortex due to the technical limitation of tDCS. Finally, in clinical settings, placebo and nocebo interventions are typically applied with ongoing pain (i.e., chronic pain), which is different from the brief experimental pain applied in this study. Future studies are needed to test if the findings observed in this study can be extended to a clinical setting.

Conclusion. We investigated the ability of tDCS to modulate placebo analgesia and nocebo hyperalgesia, as well as mechanisms linking manipulated brain activity and behaviors. Results showed that anodal tDCS could inhibit the nocebo effect and cathodal tDCS could boost the placebo effect using a well-tested placebo analgesia/nocebo hyperalgesia experimental paradigm, and these modulation effects were accompanied by altered brain activations during pain and changes in intrinsic functional connectivity. These results suggest that changing the excitability of the DLPFC and the surrounding area using tDCS may modulate placebo and nocebo effects, which may have the potential to improve clinical outcomes in medicine.

Materials and Methods

Participants. A total of 103 healthy participants without psychiatric or neurologic disorders were enrolled in the study. Recruitment and data collection were conducted at Massachusetts General Hospital (MGH) between September 2016 and March 2019. Before the randomization, 18 participants were dropped from the study, and 4 participants were dropped after the randomization (n = 3 and n = 1 for cathodal and sham tDCS groups, respectively; due to scheduling issues or device error). The final sample consisted of 81 participants (37 females, mean ± SD age: 27.4 ± 6.4). Participants were randomly assigned to one of three tDCS groups (n = 27 for each group), and they were not different in age ($F_{(2,78)} = 0.17$ and $P = 0.84$) or gender ($\chi^2 = 0.40$ and $P = 0.82$). This study was approved by the MGH Institutional Review Board, and informed consent was obtained from all participants.

Pain Administration. Noxious heat stimuli were delivered using a PATHWAY advanced thermal stimulator (Medoc Advanced Medical Systems). The square activation area of the contract thermode was 3 × 3 cm. All stimuli were initiated from a baseline temperature of 32 °C and increased to a target temperature. Each stimulus was presented for 12 s, including 2.5 s to ramp-up to the target temperature and 2.5 s to ramp down to baseline again. Heat stimuli were applied to the right forearm.

Study Procedures. Subjects participated in five experimental sessions: a training and familiarity session, a contextual learning/expectancy manipulation session, two tDCS sessions, and a session combining tDCS and testing for placebo/nocebo effects (Fig. 1*A*). Sessions 1 and 2 were separated by 2 to 5 d, sessions 2 and 3 were separated by 1 to 5 d, and sessions 3, 4, and 5 were conducted on three consecutive days. After expectancy manipulation (session 2), subjects were randomized into one of three groups using a centrally generated variable-size block design: cathodal tDCS, anodal tDCS, and sham tDCS groups. The randomization and double-blinded setup of tDCS was conducted by a team member who was not involved in the experiments and analyses of the study, before initiating the very first experiment. The tDCS modes were configured in the StarStim system software and blinded to both operators/analysts and participants. Participants were informed that they would be in one of three tDCS groups.

Session 1: Training, familiarity, and calibration. Subjects were trained to use the Gracely Sensory Scale (0 to 20) (48) to rate pain experiences. They first received an ascending heat stimulus sequence (started from 38 °C with a step of 1 °C and ended at 50 °C). The three temperatures that each subject rated as ∼5 to 6 (low pain), 10 to 11 (moderate pain), and 14 to 15 (high pain) were selected. Then they received three random pain sequences (three trials for low, moderate, and high pain, respectively, in each sequence) and three identical pain sequences (six trials for low, moderate, or high pain in each sequence) to test the validity of calibrated temperatures. The State-Trait Anxiety Inventory (STAI) was used to assess subjects' state anxiety and trait anxiety levels (49).

Session 2: Expectancy manipulation. At the beginning of the session, all subjects were informed that the aim of this study was to test how a neuromodulation tool (tDCS) can modulate the analgesic effects of lidocaine cream and the hyperalgesic effects of capsaicin cream using a neutral cream as a control. We first applied sham tDCS for 20 min and then applied all three creams to different spots on each participants' right forearm. In reality, only sham tDCS was applied and an inert cream was used for all three creams. The cream was a fragrance-free moisturizing lotion dyed three different colors (blue for lidocaine, pink for capsaicin, and white for neutral).

After explanation, nine unique regions of the subject's arm were demarcated for each stimulus (Fig. 1*B*). We drew a 3 × 3 grid composed of 3 × 3 cm squares on the subject's right forearm, starting the grid approximately one inch below the subject's elbow crease. For subjects with narrow arms, the sides of the grid occasionally extended to the sides of the forearm. The creams were then applied with each cream spread onto a unique set of three adjacent squares (i.e., one cream for each row). The row placement of the neutral control, lidocaine, and capsaicin creams was randomized between subjects.

Using methods similar to those of previous studies (17, 24), subjects were then told that to test the hyperalgesic effect of capsaicin and the analgesic effect of lidocaine, six identical painful stimuli would be applied to each of the nine spots. However, to boost subjects' expectancy, six moderate stimuli were applied to each of the neutral control cream spots, six mild stimuli were applied to each of the placebo lidocaine cream spots, and six high stimuli were applied to each of the nocebo capsaicin cream spots (Fig. 1*B*). The Gracely scale was used after the painful stimuli were applied to rate experienced pain during the stimulation. Subjects were asked to rate their expectancy of both the analgesic effect of lidocaine cream and the hyperalgesic effect of capsaicin cream using a 0 to 10 scale (0 indicating an expectation of "does not work at all" and 10 indicating an expectation of "very effective") before and after expectancy manipulation. Ratings on the ERS scale were obtained before and after expectancy manipulation to assess how much they expected the cream to reduce or enhance their pain.

Sessions 3 and 4: The first and second tDCS sessions. In session 3, 20 min of tDCS at 2 mA was administered using the StarStim system (https://www.neuroelectrics.com/solutions/starstim/) in the MRI scanner. The MRI-compatible electrodes consisted of radiotranslucid materials (a sponge covering and a carbon rubber core with a contact area of 8 cm^2) and were used to stimulate the

rDLPFC. For the rDLPFC excitability enhancement group (Fig. 1*D*), the anodal electrode was placed over F4 and the cathodal electrode over the left orbitofrontal cortex (FP1). For the rDLPFC excitability inhibition group, the cathodal electrode was placed over F4 and the anodal electrode over the left orbitofrontal cortex (FP1). Stimulation started and finished with a 15 s gradual current ramp-up and ramp down to decrease subjects' discomfort. For sham tDCS, the electrodes were placed at the same positions, but the current was applied only for the 15 s ramp-up phase at the beginning and ramp-down phase at the end of a 20 min sham-stimulation period. This was done to simulate the experience of a local tingling sensation that real stimulation produces but without sustained effect on cortical activity. This setup of sham tDCS is widely accepted as a way to blind subjects in tDCS studies, and subjects in this study were not able to distinguish between active and sham tDCS (47). The impedance was kept below 10 k-Ω. Resting-state fMRI data were collected shortly before, during, and shortly after tDCS in Session 3. Subjects only received tDCS in Session 4 to obtain cumulative effects.

Session 5: The third tDCS session and placebo/nocebo tests. At the beginning of the session, subjects were told that the Session 2 procedure would be repeated in the fMRI scanner. Specifically, the three different creams (in reality all one inert cream) were administered to each row of squares, with lidocaine and capsaicin cream administered to the same rows as determined in Session 2. The STAI was used to assess subjects' state anxiety and trait anxiety levels. Then, tDCS was applied based on subjects' randomization. Resting-state fMRI data were collected shortly before, during, and after tDCS.

Afterward, calibrated painful stimuli were applied during the fMRI scan. Because this session tested placebo/nocebo effects, we only administered different heat stimuli to the three regions in the most lateral column of the 3×3 spots to further boost expectancy of the subjects. Subjects were asked to rate their expectancy of both the analgesic effect of lidocaine cream and the hyperalgesic effect of capsaicin cream using a 0 to 10 scale before and after this reconditioning procedure. Afterward, we administered identical moderate stimuli to all remaining regions with all three creams (Fig. 1*C*). The location of lidocaine, neutral, and control creams was randomized across subjects to eliminate the confound of stimulation order. The timings of a typical trial are detailed in Fig. 1*D*. Our outcome measurements were the subjective pain ratings and fMRI signal changes to identical calibrated heat painful stimuli.

MRI Acquisition. All MRI data were acquired using a 32-channel radiofrequency head coil in a 3T Siemens scanner at the MGH Martinos Center for Biomedical Imaging. High-resolution structural brain images were also acquired with a T1-weighted three-dimensional multiecho magnetization-prepared rapid gradient-echo sequence (voxel size: $1 \times 1 \times 1$ mm^3, repetition time: 2,500 ms, echo time: 1.69 ms, slice thickness 1 mm, flip angle: 7°, and 176 slices).

In Sessions 3 and 5, we collected four resting-state fMRI scans including one before the application of tDCS, two during the application of tDCS (one for the first 6 min and one for the last 6 min), and one shortly after the application of tDCS. During the resting-state fMRI, subjects were asked to keep their eyes open and to blink normally while looking at a darkened screen for ∼6 min. A whole-brain gradient-echo echo-planar-imaging sequence was used for functional scanning (voxel size: $3 \times 3 \times 3$ mm^3, repetition time: 3,000 ms, echo time: 30 ms, slice thickness: 2.6 mm, flip angle: 90°, and 44 slices), and a total of 125 volumes were collected. The simultaneous tDCS-fMRI scans were collected with the use of "Neuroelectrics" "Multi-Channel MRI Extension Kit," which enabled us to connect the tDCS device (outside the MRI room) to the subjects in the MRI scanner safely and with high-quality scanning during stimulation. We performed quality control for each fMRI run using MRIQC (https://mriqc.readthedocs.io/en/stable/) to 1) compare the data quality between tDCS groups and 2) compare the data quality between the runs with tDCS off and tDCS on. We focused on two typical metrics, temporal signal-to-noise ratio and mean framewise displacement (measures head motion during scan), for quality control (reference *SI Appendix* for details).

Task fMRI images were acquired while subjects performed placebo/nocebo tests in Session 5 using the gradient-echo echo-planar-imaging sequence (voxel size: $3 \times 3 \times 3$ mm^3, repetition time: 2,000 ms, echo time: 30 ms, slice thickness: 4.0 mm thick, flip angle: 85°, and 32 slices). We used a sequence with faster repetition time to allow for a higher temporal resolution of task-based fMRI analyses.

Behavioral Data Analysis. Placebo analgesia was defined as the difference between perceived intensity to identical moderate painful stimuli applied on areas of skin with lidocaine cream and those applied on areas of skin with neutral cream. Nocebo hyperlagesia was defined as the difference between perceived intensity to identical moderate painful stimuli applied on areas of skin with capsaicin cream and those applied on areas of skin with neutral cream. We analyzed the placebo and nocebo responses using analysis of covariance (ANCOVA), with tDCS group (i.e., anodal, cathodal, and sham tDCS) as the fixed factors (ANCOVA was performed separately for placebo and nocebo responses). Covariates included 1) age, 2) cream randomization (i.e., location of lidocaine, neutral, and control cream on the forearm), and 3) the difference in ERS for lidocaine or capsaicin before and after expectancy manipulation (which represented how well the expectancy was modulated) in Session 2. In addition, we added the STAI state and trait anxiety scores as covariates when assessing the main effect of tDCS group on the nocebo response (*SI Appendix*, Fig. S6), as previous studies have suggested that anxiety level could affect nocebo hyperalgesia (12, 13). When the main effect of ANCOVA was significant, post hoc two-sample *t* tests with Tukey correction were performed (corrected across three comparisons: anodal versus sham, anodal versus cathodal, and cathodal versus sham; adjusted for covariates as in the ANCOVAs) and two-tailed *P* values were reported. All computations were performed in R (https://www.r-project.org/).

fMRI Data Analysis. Both resting-state and task fMRI data were preprocessed using SPM12 (Wellcome Trust Center for Neuroimaging). The first five volumes were discarded to allow for signal equilibration. Images were slice-timing corrected using the middle slice and realigned to the first scan. The resulting images were normalized to the Montreal Neurological Institute (MNI) space (resampling voxel size = $3 \times 3 \times 3$ mm^3) (50) and smoothed by a 6 mm full width at half maximum isotropic Gaussian kernel. To minimize the effect of head motion in the following fMRI analyses, six motion estimates and two physiological time series (white matter and cerebrospinal fluid) were regressed out of the normalized images. For resting-state fMRI data, artifact detection tool (https://www.nitrc.org/projects/artifact_detect/) was also applied to detect motion during the scans. Time points in subjects' images were marked as outliers if the global signal exceeded three SDs from the mean or if scan-to-scan motion deviation exceeded 0.5 mm and were scrubbed from the data. The fMRI data were then bandpass filtered from 0.01 Hz to 0.1 Hz.

Single-subject task fMRI data, including functional runs on each of the nine sites, were analyzed using a general linear model approach to model each painful stimulus (i.e., identical moderate painful stimuli applied at lidocaine, capsaicin, and neutral creams) and rating scale as events. Regressors representing the experimental paradigm were then modeled by convolving boxcar functions for each regressor with a canonical hemodynamic response function. After model estimation, we defined contrasts to test 1) overall pain, 2) placebo effect (i.e., lidocaine versus neutral), and 3) nocebo effect (i.e., capsaicin versus neutral).

The resulting contrast images were then fed to random effects group-level statistical analyses with 1) a one-sample *t* test to identify pain-evoked brain responses; 2) paired-sample *t* tests for the main effect of placebo and main effect of nocebo when identical painful stimuli were applied; and 3) two-sample *t* tests for the placebo contrast between cathodal tDCS and sham groups (i.e., cathodal $_{<\text{lidocaine-neutral}>}$ versus sham$_{<\text{lidocaine-neutral}>}$; because behavioral results showed that cathodal could significantly enhance placebo analgesia), as well as for nocebo contrast between anodal tDCS and sham groups (i.e., anodal $_{<\text{capsaicin-neutral}>}$ versus sham$_{<\text{capsaicin-neutral}>}$; because behavioral results showed that anodal could significantly blunt nocebo hyperalgesia). The significance threshold was set as $P < 0.005$ at the voxel level and $P_{FDR} < 0.05$ at the cluster level (false discovery rate [FDR] correction for multiple comparisons) in the whole-brain for analyses one and two. The threshold was set as $P_{FDR} < 0.05$ at the cluster level within regions of interest (ROIs), which are involved in descending pain modulation (i.e., *SI Appendix*, Fig. S7, a mask consisting of the ACC, mPFC, insula, and SMA; defined by the automatic anatomical labeling atlas); these regions were widely reported as neural representations of placebo and nocebo effects (19, 51) and were also found in the present study in Fig. 2 *B* and *C*, for analysis three. In SPM12, we performed statistical analyses on both sides (i.e., anodal tDCS larger than sham tDCS, as well as anodal tDCS weaker than sham tDCS) and reported two-tailed *P* values. In analysis three, when the brain regions showing significant differences were identified, we extracted the means of beta contrast estimates within the identified regions as the fMRI brain responses and compared between different creams and tDCS groups (two-tailed *P* values were FDR corrected and reported). The differences in fMRI brain responses between different creams (i.e., lidocaine-neutral or capsaicin-neutral) were correlated with individuals' placebo or nocebo magnitudes within each tDCS group separately.

To study the interaction between the fMRI signal in the rDLPFC and the identified brain regions (i.e., vmPFC and insula; see Fig. 2 *E* and *G* for details) when experiencing pain on different creams, we performed the PPI analysis (52) using the rDLPFC as the seed. The coordinates (x = 36, y = 44, and z = 32) of the rDLFPC seed were defined by the 10 to 20 electrode system (F4 electrode) on the MNI cortical space (53, 54). The seed was a sphere with a 10 mm radius, comparable to that used by Miranda et al. (55), and areas outside the cortex were rejected. Unlike conventional whole-brain exploratory PPI, we focused on the interaction between rDLPFC and vmPFC/insula on a ROI-to-ROI basis (56). For each subject, PPI effects were estimated for the rDLPFC and vmPFC with the placebo contrast (lidocaine > neutral), as well as for the rDLPFC and insula with the nocebo contrast (capsaicin > neutral). A positive PPI effect indicated that the regression slope indexing the relationship between the rDLPFC and vmPFC/insula was more positive when experiencing pain on the lidocaine/capsaicin cream than on the neutral cream. Individual PPI values were compared within tDCS groups (i.e., lidocaine > neutral, capsaicin > neutral) and between tDCS groups (i.e., anodal versus sham, cathodal versus sham). *P* values were corrected for multiple comparisons using FDR, and one-tailed *P* values were reported for within-group comparisons while two-tailed *P* values were reported for between-group comparisons.

To investigate the modulatory effects of repeated tDCS on intrinsic brain connectivity, we calculated the resting-state functional connectivity between the rDLPFC and the vmPFC and insula in eight fMRI scans (including four simultaneous tDCS-fMRI scans) collected in Sessions 3 and 5. We extracted the ROI-to-ROI connectivity strength for each scan and statistically compared 1) the pre-tDCS and post-tDCS in the two sessions and 2) the pre-tDCS in Session 3 and post-tDCS in Session 5, within each tDCS group using a paired-sample *t* test. To explore whether repeated tDCS had cumulative effects, we used the Friedman test to evaluate the ranks of pre-tDCS and post-tDCS in Sessions 3 and 5 (i.e., pre-tDCS Session 3 > post-tDCS Session 3 > post-tDCS Session 5 or the opposite trend) within each tDCS group.

Finally, we argue if the tDCS-modulated connectivity (i.e., rDLPFC–vmPFC and rDLPFC–insula connectivity in the post-tDCS fMRI scan of Session 5) before the placebo/nocebo tests was associated with the subsequent task-based brain responses and subjective pain ratings. For the anodal group, we correlated individuals' rDLPFC–insula connectivity with brain responses and pain ratings when experiencing pain on the capsaicin and neutral creams, respectively. For the cathodal group, we correlated individuals' rDLPFC–vmPFC connectivity with brain responses and pain ratings when experiencing pain on the lidocaine and neutral cream, respectively. For each group, *P* values for the correlation analysis were corrected for multiple comparisons.

Data Availability. The dataset in this manuscript is part of a multiphase project which is still under investigation. The data will eventually be made available in the institutional storage once the project is complete. Reasonable requests with clear research purpose can be sent to the corresponding author. Codes used in the analyses can be found in the Statistical Parametric Mapping (SPM) toolbox (GLM analyses) and CONN toolbox (functional connectivity analyses) (SPM link: https://www.fil.ion.ucl.ac.uk/spm/; CONN toolbox link: https://web.conn-toolbox.org/).

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