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High-frequency stimulation of the medial prefrontal cortex decreases cellular firing in the dorsal raphe

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Abstract

High-frequency deep brain stimulation (HFS-DBS) of the subcallosal cingulate (SCC) region has been investigated as a treatment for refractory forms of depression with a ~50% remission rate in open label studies. However, the therapeutic mechanisms of DBS are still largely unknown. Using anaesthetized Sprague Dawley rats, we recorded neuronal spiking activity in 102 neurons of the dorsal raphe (DR) before, during and after the induction of a 5-min HFS train in the infralimbic region (IL) of the medial pre-frontal cortex (mPFC), the rodent homologue of the human SCC. The majority of DR cells (82%) significantly decreased firing rate during HFS ($P < 0.01$, 55.7 ± 4.5% of baseline, 35 rats). To assess whether mPFC-HFS mediates inhibition of DR cellular firing by stimulating local GABAergic interneurons, the $GABA_A$ antagonist bicuculline (Bic, 100 μM) was injected directly into the DR during HFS. Neurons inhibited by HFS recovered their firing rate during Bic+HFS ($P < 0.01$, $n = 15$, seven rats) to levels not different from baseline. Cells that were not affected by HFS did not change firing rate during Bic+HFS ($P = 0.968$, $n = 7$, three rats). These results indicate that blocking $GABA_A$ reverses HFS-mediated inhibition of DR neurons. As the cells that were not inhibited by HFS were also unaffected by HFS+Bic, they are probably not innervated by local GABA. Taken together, our results suggest that mPFC-HFS may exert a preferential effect on DR neurons with $GABA_A$ receptors.

Keywords

cellular firing; depression; dorsal raphe; high-frequency stimulation; medial prefrontal cortex; rat

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Introduction

Deep brain stimulation (DBS) for treatment-resistant depression involves the delivery of a continuous high-frequency electrical stimulus (HFS) to the subcallosal cingulate (SCC) region through surgically implanted electrodes (Mayberg *et al.*, 2005; Lozano *et al.*, 2008; Kennedy *et al.*, 2011). Open label studies have shown that over 50% of patients had a positive clinical response 1 year after surgery (i.e. were 50% better when assessed with validated scales) (Lozano *et al.*, 2008; Holtzheimer *et al.*, 2012). Subsequent follow-ups have shown that these results were stable after 2 and 3 years (Lozano *et al.*, 2008; Kennedy *et al.*, 2011). Our group has recently been able to shed some light on local HFS-mediated plasticity in the SCC of depression patients (Srejic *et al.*, 2014), although the electrophysiological and neurochemical mechanisms underlying downstream effects of DBS are still not well understood. To better understand these mechanisms, research has focused on stimulating homologous regions in experimental animals (Hamani *et al.*, 2010; Challis *et al.*, 2014; Veerakumar *et al.*, 2014). According to neuroanatomical projections and cytoarchitectural structures, the infralimbic (IL) region of the medial prefrontal cortex (mPFC) in rodents has been most commonly suggested as the anatomical correlate of the human SCC (Gabbott *et al.*, 2003; Uylings *et al.*, 2003; Hamani & Temel, 2012). Antidepressant effects of mPFC electrical stimulation in animals have been investigated in some detail (Hamani *et al.*, 2012, 2014; Warden *et al.*, 2012; Veerakumar *et al.*, 2014). However, to our knowledge there have been no *in vivo* studies examining the real-time effects of high frequency prefrontal stimulation on cellular activity in descending mood pathways implicated in mood disorders.

The mPFC has been shown to exert descending control of the cortical projecting neurons in the dorsal raphe (DR), which suggests an important role for DR afferent systems in the modulation of emotional responses (Peyron *et al.*, 1998; Lee *et al.*, 2003; Vertes, 2004). *In vivo* studies conducted in anaesthetized rodents showed that brief (0.2 ms) electrical stimulation of the mPFC results in immediate inhibition of putative serotonin (5-HT) neurons in the DR (Celada *et al.*, 2001; Varga *et al.*, 2001). However, these studies did not examine longer train stimulation. To fully clarify DBS effects on the descending prefrontal projections, it is necessary to examine long stimulation trains at clinically relevant high frequencies (100 Hz). Electrophysiological and histological studies also showed that GABAergic interneurons in the DR are preferentially targeted by the glutamatergic projections from the mPFC and exert an inhibitory tone over DR neurons (Celada *et al.*, 2001; Varga *et al.*, 2001, 2003; Jankowski & Sesack, 2004). On the basis of these results, we hypothesize that HFS mediates an inhibitory effect on DR neurons by enhancing the local GABAergic tone. Using microelectrode recordings, we describe the cellular profile of neurons in the DR and demonstrate local inhibition of cellular firing during mPFC-HFS. Lastly, to examine the role of GABA in stimulation-mediated DR neuronal inhibition, we injected the $GABA_A$ receptor antagonist bicuculline (Bic) during HFS and show a recovery of cellular firing to baseline levels.

Materials and methods

Animal surgeries

Animal experiments were conducted using 35 adult male Sprague–Dawley rats (280–400 g) in compliance with the ethics protocol of the University Health Network Animal Care Committee (UHN ACC) and in accordance with EU Directive 2010/63/ EU on the protection of animals used for scientific purposes. Every effort was made to minimize the number of experimental animals used. Rats were housed in the animal care facility at the Toronto Western Hospital with food and water available *ad libitum*. Experiments were performed in the light phase of a 12/12-h light–dark cycle.

Anaesthesia was induced using isoflurane (Pharmaceutical Partners of Canada, Richmond Hill, ON, Canada) and maintained with an intraperitoneal injection of urethane (Sigma, Oakville, ON, Canada) at a dose of 1.2 g/kg. Urethane anaesthesia allows continuous monitoring of cortical electrophysiology over a prolonged period with minimal cardiovascular and respiratory complications (De Wildt *et al.*, 1983). Furthermore, spontaneous potentials measured in rats anaesthetized with urethane have similar characteristics to those generated in the conscious animal (Ebenezer, 1986). Adequate levels of anaesthesia were confirmed every 15 min by absence of a withdrawal reflex to hindlimb pinch and maintained as required by supplemental doses (20% of initial dose) of urethane. Body temperature was maintained at 36–37 °C.

Extracellular recordings of DR neurons

Extracellular recordings were performed with three microelectrodes assembled from Parylene-C-insulated tungsten wires (Micro Probe, Gaithersburg, MD, USA), with a 20-μm tip length. To decrease the initial impedance of 1 MΩ for recording, the electrode tips were electroplated in 24-carat yellow gold electroplating solution (Krohn Technical Products, Carlstadt, NJ, USA) and followed by platinizing solution (VWR Scientific Products, Mississauga, ON, Canada) using a stimulus isolator (A360; World Precision Instruments, Sarasota, FL, USA) and 1 μA of cathodal direct current applied to the electrode for approximately 10 s, giving final impedances of 200–400 kΩ. Finally, the microelectrodes were insulated by a sleeve of polyimide Kapton (Micro ML Tubing, Midway, MA, USA).

To avoid the artery at the sagittal sinus, the microelectrodes were positioned on the midline, 12 mm posterior to bregma. To reach the DR, the trajectory was adjusted and microelectrode angled 32° in the anteroposterior plane, advanced 7–8 mm below dura. The recorded signals were digitized at 12.5 kHz with a CED 1401 data acquisition system (Cambridge Electronic Design, Cambridge, UK) and saved to a computer hard-drive running SPIKE2 software (Cambridge Electronic Design). The acquired neuronal signals were monitored continuously during acquisition by computer display.

Stimulation of the mPFC

Stimulations were performed using a blunted bipolar electrode placed into the infralimbic region of the ventral mPFC (ML +0.5 mm, AP +3.2 mm and DV +5.4 mm relative to bregma), which was modified from previous studies (Hajós *et al.*, 1998; Hamani *et al.*,

2010). Stimulation parameters consisted of clinically relevant high frequency trains (HFS, 100 Hz) of single, square-wave current pulses (60-μA amplitude, 0.2-ms pulse width, 5-min duration). The positions of the stimulating electrodes were verified histologically.

Drug administration

Drugs (dissolved in saline) were infused through a 10-μm stainless steel cannula (Small Parts Inc., Miami, FL, USA), and the microelectrodes were then positioned around the cannula, with the electrode tips extended 200–300 μm from the end of the cannula and were spaced approximately 600 mm from one another. Drugs were injected by using a 10-mL microsyringe (Hamilton Co Inc., Whittier, CA, USA) connected to the microinjection cannula by a ~30-cm length of 26-gauge Teflon tubing (Small Parts Inc.). The stylet of the microsyringe was removed, the assembly was forward-filled with deionized water and visually inspected for leaks, and the stylet was then replaced. An air bubble (~0.5 mL) was introduced into the cannula by drawing back the stylet of the microsyringe and then 3–10 mL of drug solution was back-loaded into the injection cannula. The location of the bubble in the Teflon tubing was marked and used to confirm movement of the solution during microinjection.

The $GABA_A$ receptor antagonist bicuculline methobromide (3.25 nM) (Sigma-Aldrich, St Louis, MO, USA) was used to see whether blockade of $GABA_A$ receptors in the DR blocks the inhibitory effects of mPFC-HFS on neuronal firing in DR. Bicuculline was prepared in 0.9% saline in a final dose of 75 ng/0.5 μL (100 μM) and infused into the DR 2 min after the onset of mPFC-HFS for a total of 1 min of perfusion time. The doses were chosen on the basis of previous publications as well as pilot studies, using the criteria of pharmacological and behavioural efficacy (Tao & Auerbach, 2003; Tao & Ma, 2012).

Histology

At the end of each experiment, the final position of the stimulating and recording electrode tips was marked with an electrolytic lesion (10 mA for 10 s) to verify location in the tissue. Animals underwent transcardiac perfusion with heparinized saline followed by 4% weight/volume paraformaldehyde in phosphate-buffered saline (PBS). Brains were extracted, post-fixed in 20% sucrose and 4% paraformaldehyde in PBS, processed for 40-μm-thick coronal sections using a cryostat (1720 digital; Leica Microsystems, Wetzlar, Germany) and stained with Cresyl Violet. Recording site locations were confirmed in the DR and stimulating sites in the infralimbic region of the mPFC. Placements of cannulae were verified by comparing the slides with a rat brain atlas (Paxinos & Watson, 2007).

Offline analysis of neuronal activity

The recorded neurons (spikes) were sorted offline by template-matching software (SPIKE2; Cambridge Electronic Design). Neurons were accepted for further analysis if the spikes were of a consistent, distinct shape that could be separated with a high degree of certainty from the spike waveforms of other neurons and background noise or cardiac pulsations. In this study, the artefacts produced by microstimulation were relatively short lasting (0.6 ms) and were removed from the signal, starting from the onset of the artefact to the end. This dead space was then replaced with an equivalent period of neural data immediately prior to the

stimulus artefacts but in a reversed order. This had the advantage of producing a smooth transition in the signal at the site where the artefact was removed. The 'cleaned' neuronal recordings were then used for spike sorting with the template-matching algorithm, as spike band-pass filtering without removal of the artefact will increase the duration of the artefacts (Bar-Gad *et al.*, 2004; Liu *et al.*, 2012).

The w_hist script in SPIKE 2.0 was used to analyse frequency changes during HFS and upon pharmacological manipulation. Two-minute time windows of stable cellular spiking were taken in for each condition (baseline, HFS, HFS+Bic and post-HFS). Changes in firing rates were assessed on each isolated neuron using Student's *t*-test in w_hist. A cell was deemed inhibited or facilitated when *P* values were greater than 0.05 (significant). Changes in firing rates between each condition across recording sites and animals were compared using one-way ANOVA, Student's *t*-test or the Wilcoxon signed rank test.

Results

Cellular characteristics

Extracellular single-unit recordings were taken from a total of 102 well-isolated neurons in the DR of 35 urethane-anaesthetized rats (Fig. 1). This large group of neurons showed a considerable range in firing rate and spike shape. The firing frequency varied widely between 0.2 and 45 spikes per second (average = 11 ± 1 Hz) and spike duration between 0.5 and 1.9 ms (average = 1.08 ± 0.03 ms) (Fig. 2). The distribution of frequencies revealed a significant correlation between neuronal firing rate and spike width, where slower firing cells tended to have broader spikes, while fast spiking neurons were found to have shorter spike width (Fig. 2, $R = 0.330$, $P < 0.001$). Neurons firing > 5 Hz were termed 'fast' and < 5 Hz as 'slow', as previously described (Varga *et al.*, 2003). Recently, multiple studies have shown that the classic electrophysiological features (Vandermaelen & Aghajanian, 1983) extensively used to identify 'putative' serotonergic neurons (slow, regular firing rates and wide spike waveforms) were highly variable among both 5-HT and non-5-HT cells (Allers & Sharp, 2003; Kirby *et al.*, 2003; Kocsis *et al.*, 2006; Calizo *et al.*, 2011). Because electrophysiological and pharmacological tests have proven to be insufficient in discerning 5-HT from non-5-HT cells, we examined all DR cells and tested their responses to mPFC-HFS and Bic.

Effects of mPFC-HFS on neuronal firing in DR

The effect of HFS of the mPFC was studied on all 102 stable firing DR neurons (Table 1). The vast majority of the recorded cells (84 of 102; 82%) responded to the 5-min mPFC-HFS train with a significant decrease in firing rate ($P < 0.01$, 55.7 ± 4.5% of baseline, 35 rats) (Fig. 3). Following HFS, the firing rate recovered to levels not significantly different from baseline ($P = 0.389$, 88.6 ± 7.1% of baseline), but significantly different from HFS ($P < 0.05$). Thirteen neurons (12%) did not show any significant response to HFS and only six neurons (6%) increased firing rate (Table 1). Facilitation of firing might be a non-specific effect of stimulus spread and a motor-like response that activated the cell due to arousal and subsequent movements of the animal. If the animals were administered an additional neuromuscular blockade or ventilated, the facilitation might not have occurred. HFS was not

found to preferentially inhibit slow over fast neurons. In total, 24 out of 29 (83%) slow neurons decreased firing rate during HFS ($P < 0.05$, 53.4 ± 5.7% of baseline, 16 rats), which recovered to baseline after HFS ($P = 0.263$, 87.5 ± 9.8% of baseline) (Fig. 4A and C). Two slow neurons (7%) increased firing rate, and three (10%) displayed no change during HFS. Sixty out of 75 (80%) fast neurons were inhibited during HFS ($P < 0.001$, 56.7 ± 4.7% of baseline, 24 rats) and returned to baseline after HFS ($P = 0.185$, 84.1 ± 7.2% of baseline) (Fig. 4B and D). Ten fast neurons (13%) were facilitated and HFS had no effect on three (5%).

mPFC-HFS mediates inhibition of DR neurons via local $GABA_A$ receptors

To examine whether mPFC-HFS-mediated inhibition of cellular firing in the DR occurred through the stimulation of local GABAergic interneurons, we tested the effect of focal injections of the $GABA_A$ antagonist Bic (100 μM, 75 ng/0.5 μL) during HFS-induced DR inhibition. After a stable baseline of neuronal firing was obtained, HFS was induced for 5 min. Bic was injected in the middle of the HFS train for a total of 1 min of diffusion (Fig. 5A and B). Out of a total of 22 neurons tested, 15 responded by inhibition during HFS (Fig. 5A), one was facilitated and six did not change firing rates (Fig. 5B). Thirteen out of the 15 neurons inhibited by HFS significantly increased their firing rate in response to Bic+HFS ($P < 0.01$, seven rats) to levels not different from baseline ($P = 0.783$, 96.8 ± 13% of baseline) (Fig. 5C). The remaining neurons that were not affected by HFS did not change firing rate in response to Bic+HFS ($P = 0.968$, 93.7 ± 12% of baseline, three rats) (Fig. 5D), indicating that blocking $GABA_A$ receptors worked only to cancel HFS-mediated inhibition of DR neurons.

Discussion

In the present study we used microelectrodes to record neurons in the region of the DR in anaesthetized rats and report a wide range of firing rates and spike widths. Classic electrophysiological criteria first used to identify putative 5-HT neurons according to their slow-firing, regular patterns and wide spikes (Vandermaelen & Aghajanian, 1983) have become insufficient for gross classification of raphe cells (Allers & Sharp, 2003; Kocsis *et al.*, 2006; Calizo *et al.*, 2011). Several intracellular (Li *et al.*, 2001; Kirby *et al.*, 2003) and juxtacellular labelling (Allers & Sharp, 2003) studies reported that neuronal identification based on electrophysiological criteria alone produced a significant number of false-negative and false-positive results. Allers & Sharp (2003) found that half of the slow-firing DR neurons were non-serotonergic and 20% of fast-spiking putative non-5-HT neurons were actually serotonergic (Allers & Sharp, 2003). Spike width has also been shown to be a poor method of identifying 5-HT neurons, where using morphological and neurochemical evidence found that 45% of non-5-HT neurons were misidentified as 5-HT and 35% of 5-HT neurons were misidentified as non-5-HT (Calizo *et al.*, 2011). In the same study, the authors also looked at the topographical characteristics of raphe neurons and found that homogeneity between 5-HT and non-5-HT neurons also varied according to their location within the DR. Within the ventromedial DR and the lateral wing of the DR, non-5-HT cells had electrophysiological characteristics that were similar to 5-HT neurons. In contrast, the difference in cellular characteristics between 5-HT and non-5-HT cell populations was

greatest and most consistent within dorsomedial DR (Calizo *et al.*, 2011). The future use of optogenetic methods to unequivocally identify 5-HT neurons by their response to light stimulation would offer a clear advantage to classic electrophysiological techniques.

The prelimbic (PL) and infralimbic (IL) subdivisions of the mPFC are commonly thought of as the major prefrontal regions that send projections to the DR (Gonçalves *et al.*, 2009). Some retrograde labelling studies show that the PL provides the greatest source of inputs to the DR (Vertes, 2004; Gabbott *et al.*, 2005), whereas others show that a significant glutamatergic innervation to the DR originates from the IL (Hajós *et al.*, 1998; Peyron *et al.*, 1998; Groenewegen & Uylings, 2000; Gonçalves *et al.*, 2009). As the IL has been identified as the closest physiological correlate of the human SCC (Uylings & van Eden, 1990), we chose this region as our stimulation target. We demonstrate that a significant majority of both slow- and fast-firing DR neurons were inhibited during mPFC-HFS. Previous studies used single pulse stimulation of the mPFC and measured post-stimulus latency and duration of inhibition of DR neurons (Hajós *et al.*, 1998; Celada *et al.*, 2001; Varga *et al.*, 2001). Our study is the first to show persistent inhibition of DR firing rates with much longer trains at a clinically relevant stimulation frequency and current intensity (100 Hz, 60 μA). Stimulation of the mPFC has also been shown to elicit preferential inhibition of 5-HT neurons in the DR (Celada *et al.*, 2001; Varga *et al.*, 2001). As electrophysiological methods have proven insufficient to identify 5-HT neurons, we cannot with certainty say that the HFS-induced inhibition did not preferentially target 5-HT neurons in our study. However, because 82% of all cells were inhibited and 5-HT is thought to make up at most 50% of all DR neurons (Fu *et al.*, 2010; Bang & Commons, 2012), it is very unlikely that mPFC-HFS inhibition in our study is 5-HT-specific. Initially, fast firing cells in the DR were thought to be GABAergic (Varga *et al.*, 2003), but subsequent immunohistological studies showed that DR contains many excitatory glutamatergic neurons, which heavily regulate local 5-HT (Pan & Williams, 1989; Lee *et al.*, 2003; Tao & Auerbach, 2003; Lemos *et al.*, 2006; Calizo *et al.*, 2011). Therefore, it is possible that the inhibitory effect of the mPFC-HFS affects a broad range of raphe neurons including glutamatergic neurons, which then in turn modulate 5-HT firing and transmission.

Neuroanatomical and pharmacological studies offer evidence that, together with 5-HT autoreceptors, GABA neurons are crucial mediators of inhibitory control over 5-HT systems (Liu *et al.*, 2000; Varga *et al.*, 2001). Using histological and ultrastructural methods it has been shown that the glutamatergic projections from the mPFC to the DR preferentially target GABA neurons (Jankowski & Sesack, 2004). This has recently been confirmed using whole-cell recording and cFos mapping after direct optogenetic photoactivation of glutamatergic mPFC terminals in the DR (Challis *et al.*, 2014). Various studies have also shown that GABA neurons in the DR may serve as local inhibitory relays for major glutamatergic excitatory inputs originating from the mPFC (Celada *et al.*, 2001; Varga *et al.*, 2001, 2003). Most recently, using optogenetic Archaerhodopsin-mediated silencing, Challis *et al.* (2013) showed that DR *GAD2*$^{+}$ GABA neurons synapse directly onto local 5-HT neurons, providing a source of postsynaptic inhibition. However, it should also be noted that a recent study showed evidence of direct glutamatergic inputs from the mPFC to 5-HT cells in the DR (Pollak Dorocic *et al.*, 2014). In the present study we report that 6% of neurons increased firing rate during HFS, which may indicate that these cells were directly synapsed

by the excitatory glutamatergic projections. Taken together, these results point to the heterogeneous nature of inputs from the mPFC to the DRN.

To determine the mechanism of mPFC-HFS-mediated inhibition of DR neurons, we administered the $GABA_A$ antagonist Bic during HFS. Bic recovered cellular firing of neurons that were originally inhibited by mPFC-HFS, but did not affect the firing rate of cells that were unchanged or facilitated during HFS. This indicates that HFS-induced inhibition in the DR is mediated by exciting local GABAergic connections (Fig. 6). The cells that were not inhibited by HFS were also unaffected by HFS+Bic, which suggests that these cells are neither synapsed by GABA nor directly influenced by the mPFC. Taken together, our results suggest that mPFC-HFS may have a preferential effect on DR neurons that have $GABA_A$ receptors. Another source of inhibition in the DR are $5\text{-}HT_{1A}$ receptors located on the soma and dendrites of serotonin neurons (Riad *et al.*, 2000), where they exert inhibitory feedback on the presynaptic neuron in response to local serotonin release (Wang & Aghajanian, 1977). Release of serotonin in the DR region of the cell body leads to a decrease in firing rate of 5-HT neurons and inhibition of 5-HT synthesis (Blier & de Montigny, 1987). As mPFC-HFS inhibited a large majority of all cells and HFS+Bic recovered firing rates of inhibited neurons, it is unlikely that $5\text{-}HT_{1A}$-mediated autoinhibition played a significant role in the HFS-mediated inhibition of DR neurons observed in this study.

The mPFC in rodents and humans has been described as a critical node of emotional, executive and cognitive functions that serves as a regulatory region for affective control of descending inputs to subcortical regions, such as the brainstem raphe (Roy *et al.*, 2012). Multiple animal studies using pharmacological, electrical and optogenetic manipulation have implicated the mPFC as a region that exerts a top-down modulation of DR circuits and regulates behavioural responses to stress (Hamani *et al.*, 2010; Slattery *et al.*, 2011; Warden *et al.*, 2012; Veerakumar *et al.*, 2014). Clinical studies from our group have shown that SCC stimulation is therapeutically beneficial to the chronically depressed population (Hamani *et al.*, 2011; Kennedy *et al.*, 2011; Lozano *et al.*, 2012) and that it may be mediated through the release of 5-HT in forebrain regions (Hamani *et al.*, 2010). We know from our work in human patients that HFS can inhibit cell bodies locally via release of inhibitory transmitters, but also at a significant distance from the stimulation site through the excitation of fibres of passage of inhibitory pathways (Liu *et al.*, 2012). Therefore, although stimulation of the mPFC in the present study inhibited neurons in the DR, it also probably excited the terminals that released 5-HT in the forebrain. Along the same lines, because blocking GABA disinhibited the DR, it stands to reason that it also blocked HFS-mediated 5-HT release in the forebrain.

A recent study demonstrated that selectively activating only those mPFC glutamatergic cells projecting to the DR induced a rapid behavioural reaction during forced swim in defeated rats (Warden *et al.*, 2012). However, disinhibition of 5-HT neurons by pharmacologically blocking DR $GABA_B$ but not $GABA_A$ receptors has been shown to increase 5-HT output in the mPFC and promote social approach behaviours in defeated mice (Takahashi *et al.*, 2010, 2012). Our results show that HFS of the mPFC has a preferential effect on DR neurons with $GABA_A$ receptors, which may indicate that modulating $GABA_A$ as one of the factors

involved in the anti-depressant mechanism of mPFC-HFS. While these results clearly point to GABA as a mediator of mPFC-HFS-induced inhibition, the involvement of 5-HT is highly probable but remains unproven. Because there is a complicated relationship between 5-HT cellular firing in the DR and 5-HT release in associated regions, future work needs to unequivocally identify 5-HT in the DR and characterize its concentration and reuptake.

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Abbreviations

Bic	bicuculline
DBS	deep brain stimulation
DR	dorsal raphe
HFS	high-frequency stimulation
IL	infralimbic region
mPFC	medial prefrontal cortex
PL	prelimbic region
SCC	subcallosal cingulate

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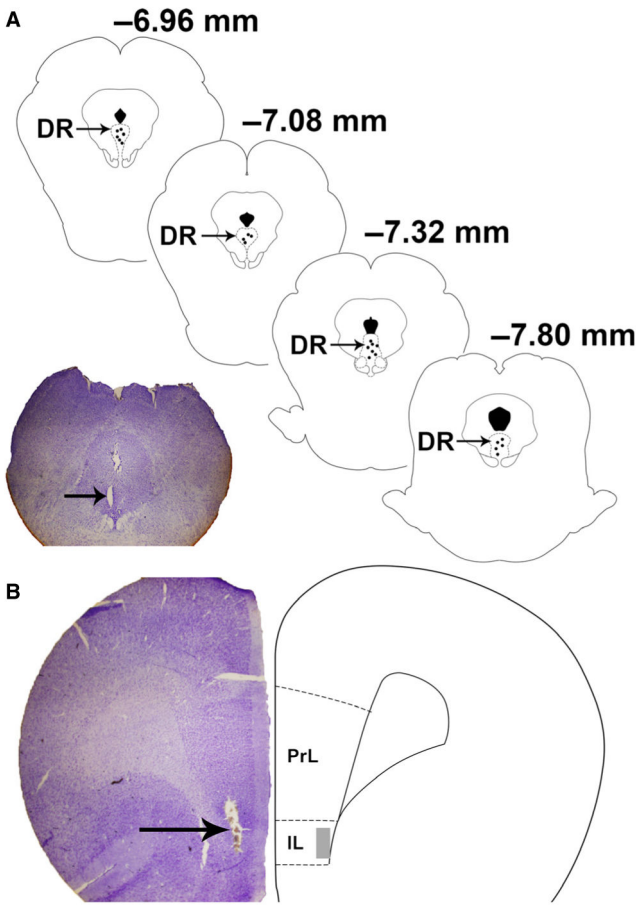

Fig. 1.

(A) Diagram illustrating the location of recording electrodes in the dorsal raphe (DR) (ML 0 mm, AP +12 mm, and DV +7–8 mm relative to bregma in the 32° plane). The section of the brain on the left (40 μm) has been stained with cresyl violet and illustrates the recording electrode lesion (arrow). (B) Illustration (right) of the stimulating electrode location in the infralimbic region (IL) of the medial prefrontal cortex (mPFC) along with cresyl violet histological section (left) of the same region with electrode lesion (arrow).

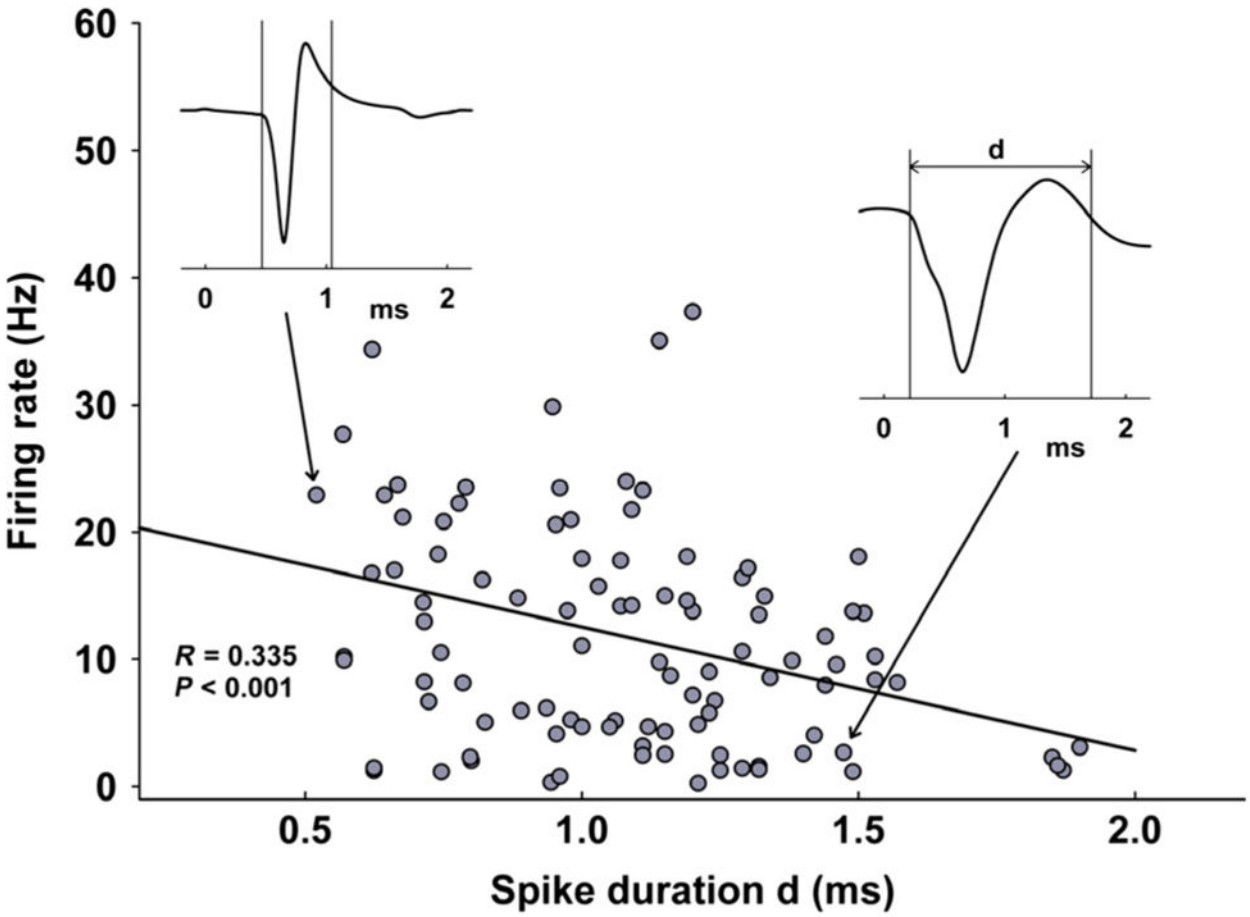

Fig. 2.

All recorded neurons in the dorsal raphe plotted based on spike duration (*d* in waveform inset, *x*-axis, in ms) and baseline firing rate (*y*-axis, Hz). The two insets are examples of a slow-firing (right) and fast-firing neuron (left). The distribution of frequencies revealed a significant correlation between cellular firing rate and spike width, where slower firing cells tended to have broader spikes, while fast spiking neurons were found to have shorter spike width ($R = 0.330$, $P < 0.001$, $n = 102$, 35 rats).

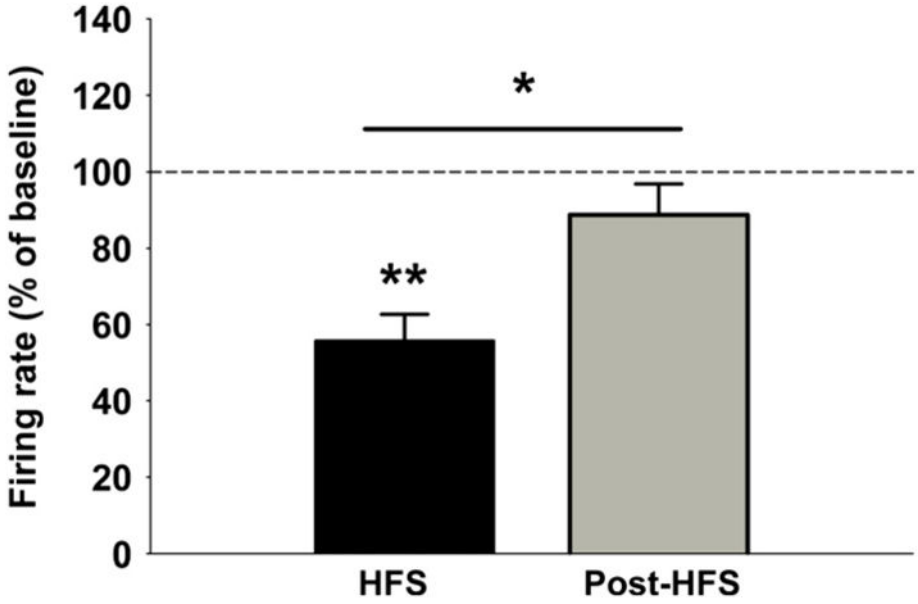

Fig. 3.

Histograms showing significant decrease in firing rate of cells in the DR during 5 min of mPFC-HFS ($P < 0.01$, $n = 102$, 35 rats). Following HFS, the firing rate recovered to levels not significantly different from baseline ($P = 0.389$), but significantly different from HFS ($P < 0.05$). *$P < 0.05$, ** $P < 0.01$.

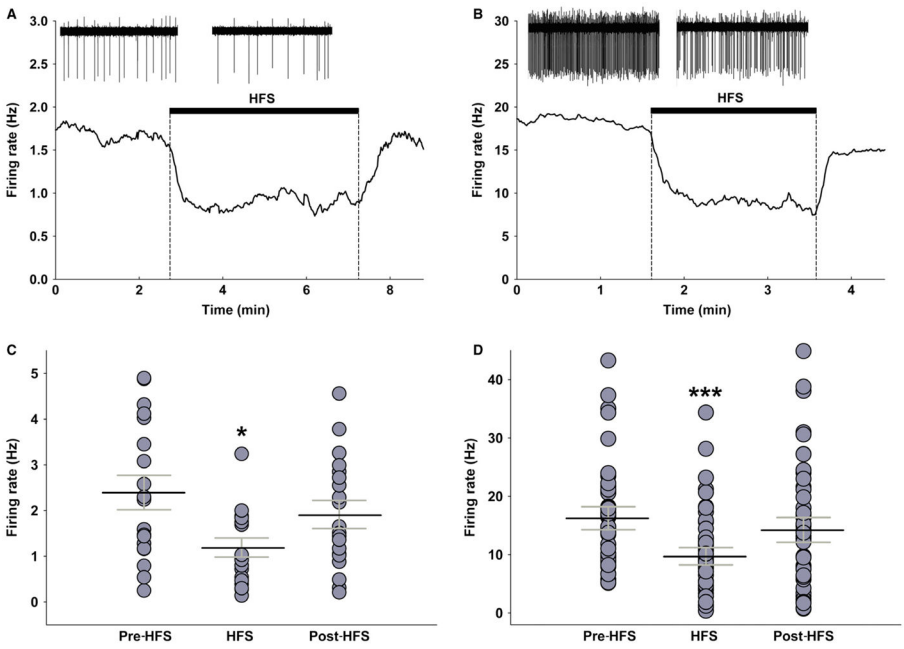

Fig. 4.

Examples of slow-firing (A) and fast-firing (B) cells before, during and after HFS. Insets are 30-s segments of raw waveforms illustrating the change in spiking frequency. Note a significant decrease in firing rate during HFS and a recovery to baseline levels in both slow and fast neurons. Bottom graphs are vertical plots of all slow (C) and fast (D) neurons before, during and after HFS. Black lines in the middle of each group indicate mean firing rate, while grey lines are error bars (SEM). Note a significant decrease in firing rate during HFS in both slow ($P < 0.05$, $n = 29$) and fast neurons ($P < 0.001$, $n = 73$). *$P < 0.05$, ***$P < 0.001$.

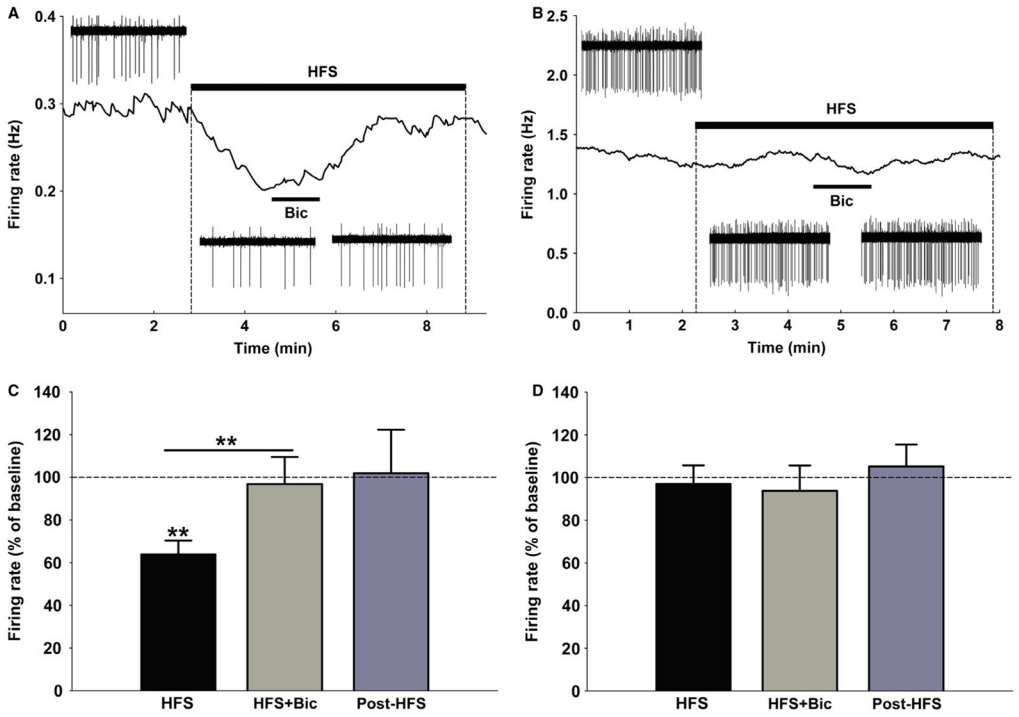

Fig. 5.

(A) Illustration of the effect of bicuculline (Bic, 100 μM direct microinjection) on a DR neuron that was inhibited by mPFC-HFS. Insets are 30-s segments of raw waveforms illustrating the change in spiking frequency. Note the recovery of the firing rate to near-baseline levels upon Bic administration. (B) Illustration of the lack of any effect of Bic on a DR neuron that was not affected by mPFC-HFS. (C) Histogram showing that neurons inhibited by HFS significantly increased their firing rate in response to Bic+HFS ($P < 0.01$, $n = 15$, seven rats) to levels not different from baseline ($P = 0.783$). (D) The neurons that were not inhibited by HFS did not change firing rate in response to Bic+HFS ($P = 0.968$, $n = 7$, three rats). $^{**}P < 0.01$.

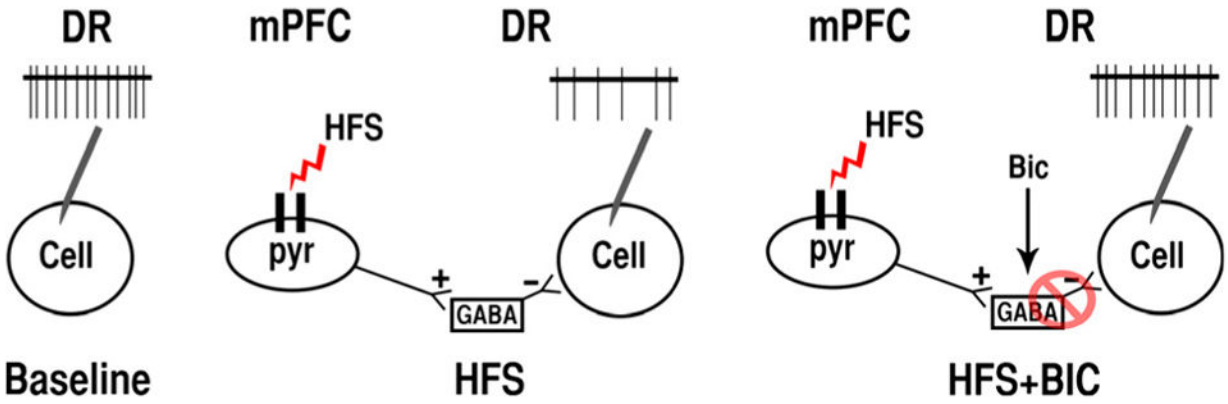

Fig. 6.

Illustration of the effects of bicuculline (Bic) on neurons in the dorsal raphe (DR) that were inhibited by high-frequency stimulation (HFS) of the medial prefrontal cortex (mPFC). HFS activates the excitatory glutamatergic mPFC projections that preferentially innervate GABA neurons in the DR, thus increasing inhibitory GABAergic tone and decreasing cellular firing of recorded neurons (middle illustration). Local administration of Bic blocks $GABA_A$ receptors on the recorded cells, disinhibiting the firing rate of the recorded cell, and thereby cancelling the inhibitory effect of HFS.

Table 1

Recordings of all neurons were taken from dorsal raphe (DR) during high-frequency stimulation (HFS) of the medial prefrontal cortex (mPFC); the table lists the responses in firing rate during HFS of slow (< 5 Hz) and fast (> 5 Hz) cells

	Neurons		
Response	**All**	**Slow**	**Fast**
Inhibition	84 (82%)	24 (83%)	60 (80%)
Facilitation	13 (12%)	2 (7%)	10 (13%)
No change	6 (6%)	3 (10%)	3 (4%)