

Corticotropin-Releasing Hormone from the Pontine Micturition Center Plays an Inhibitory Role in Micturition

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Lower urinary tract or voiding disorders are prevalent across all ages and affect >40% of adults over 40 years old, leading to decreased quality of life and high health care costs. The pontine micturition center (PMC; i.e., Barrington's nucleus) contains a large population of neurons that localize the stress-related neuropeptide, corticotropin-releasing hormone (CRH) and project to neurons in the spinal cord to regulate micturition. How the PMC and CRH-expressing neurons in the PMC control volitional micturition is of critical importance for human voiding disorders. To investigate the specific role of CRH in the PMC, neurons in the PMC-expressing CRH were optogenetically activated during *in vivo* cystometry in unanesthetized mice of either sex. Optogenetic activation of CRH-PMC neurons led to increased intermicturition interval and voided volume, similar to the altered voiding phenotype produced by social stress. Female mice showed a significantly more pronounced phenotype change compared with male mice. These effects were eliminated by CRH-receptor 1 antagonist pretreatment. Optogenetic inhibition of CRH-PMC neurons led to an altered voiding phenotype characterized by more frequent voids and smaller voided volumes. Last, in a cyclophosphamide cystitis model of bladder overactivity, optogenetic activation of CRH-PMC neurons returned the voiding pattern to normal. Collectively, our findings demonstrate that CRH from PMC spinal-projecting neurons has an inhibitory function on micturition and is a potential therapeutic target for human disease states, such as voiding postponement, urinary retention, and underactive or overactive bladder.

Key words: Barrington's nucleus; lower urinary tract symptoms; micturition; neuro-urology; optogenetics; pontine micturition center

Significance Statement

The pontine micturition center (PMC), which is a major regulator of volitional micturition, is neurochemically heterogeneous, and excitatory neurotransmission derived from PMC neurons is thought to mediate the micturition reflex. In the present study, using optogenetic manipulation of CRH-containing neurons in double-transgenic mice, we demonstrate that CRH, which is prominent in PMC-spinal projections, has an inhibitory function on volitional micturition. Moreover, engaging this inhibitory function of CRH can ameliorate bladder hyperexcitability induced by cyclophosphamide in a model of cystitis. The data underscore CRH as a novel target for the treatment of voiding dysfunctions, which are highly prevalent disease processes in children and adults.

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Introduction

Lower urinary tract dysfunction and voiding disorders affect ~20% of school aged children and >40% of adults over the age of 40 (Boyle et al., 2003; Robertson et al., 2007; Sureshkumar et al., 2009; Coyne et al., 2011; Vaz et al., 2012). These conditions negatively affect quality of life and lead to high health care costs annually for the individual and society (Robertson et al., 2007). Despite the ubiquitous nature of voiding disorders, a mechanistic model of how the central nervous system controls volitional voiding remains elusive. The main focus on brain-bladder control has been on the pontine micturition center (PMC; also known as Barrington's nucleus), which is considered the “command center” for micturition (Barrington, 1925).

PMC neurons project to the lumbosacral spinal cord where their axon terminals synapse onto parasympathetic preganglionic bladder neurons and can initiate detrusor contraction. Several supraspinal regions send afferents to PMC, including the cerebral cortex, hypothalamus, thalamus, and periaqueductal gray (Nadelhaft et al., 1992; Blok and Holstege, 1997; Sugaya et al., 1997). This diversity of signaling to the PMC may explain the variable literature findings that this nucleus plays roles in both initiating and inhibiting micturition (Holstege et al., 1986; Valentino et al., 1994; Zhang et al., 2005; de Groat et al., 2015). Another element of complexity in understanding how the PMC functions is its cellular heterogeneity, and a recent neuroanatomic study of PMC in mice identified at least five populations of neurons in and around PMC (Verstegen et al., 2017).

A prominent neuronal subpopulation in PMC expresses the stress-related neuropeptide, corticotropin-releasing hormone (CRH). CRH is present in multiple brain circuits outside of the hypothalamus-pituitary axis where it is believed to modulate neuronal activity as part of a coordinated behavioral and autonomic response to stressors. Although one study suggested that CRH is excitatory and facilitates micturition during urodynamics in awake rats (Klausner et al., 2005), convergent findings support an inhibitory function of CRH in PMC spinal projections (Pavlovich and Valentino, 1995; Kiddoo et al., 2006; McFadden et al., 2012). For example, intrathecal administration of a CRH agonist or antagonist inhibited or enhanced, respectively, bladder contractions produced by discrete chemical stimulation of PMC (Pavlovich and Valentino, 1995). Additionally, both intrathecal administration of CRH receptor agonists and adeno-associated viral mediated overexpression of CRH in PMC neurons result in decreased urinary frequency and increased bladder capacity, while intrathecal administration of a CRH₁ receptor antagonist results in increased urinary frequency and decreased bladder capacity (Kiddoo et al., 2006; McFadden et al., 2012).

An initial study, using optogenetic activation of CRH-expressing PMC neurons of anesthetized mice treated with the diuretic furosemide, demonstrated that excitation of CRH-expressing neurons in the PMC correlated with bladder contraction, supporting the role of these neurons in a pro-micturition pathway (Hou et al., 2016). However, >90% of CRH-expressing PMC neurons also produce glutamate, which could account for the excitatory effects (Hou et al., 2016; Verstegen et al., 2017). Indeed, a more recent optogenetic study in anesthetized mice used a CRH antagonist to dissect out an inhibitory role of CRH (Ito et al., 2020). In the present study, both male and female mice were bred to express either channelrhodopsin-2 (ChR2) or archaerhodopsinT (ArchT) in CRH-expressing PMC neurons. CRH-PMC neurons were optogenetically manipulated in awake and freely behaving mice in the absence of furosemide and effects on urodynamic function was determined through slow fill cystometry recordings. To define and distinguish the specific role of CRH in micturition, the effects of a CRH₁ receptor antagonist were determined. Last, we determined the effects of optogenetic stimulation of CRH-PMC neurons on urodynamics in a mouse model of cyclophosphamide-induced cystitis that is characterized by increased micturition frequency and small voids. Overall, these findings suggest that CRH and CRH-expressing PMC neurons may be potential therapeutic targets for common clinical voiding disorders in humans, such as voiding postponement, urinary retention, and underactive bladder or overactive bladder (OAB).

Materials and Methods

Animals. All experimental mice were group housed before surgery and were individually housed after surgery and for cystometry experiments. CRH^{ires-Cre} (Taniguchi et al., 2011) mice obtained from The Jackson Laboratory (#012704) and harboring an internal ribosome entry site (ires)-linked Cre recombinase gene downstream of the *Crh* locus were crossed with Cre-dependent light-activated ChR2 fused with yellow fluorescent protein (eYFP) reporter mice (Ai32, Rosa26Sor^{tm32(CAG-COP4*H134R/EYFP)}) from The Jackson Laboratory, #012569 (Madisen et al., 2012) to obtain CRH-ChR2 for experiments. To generate CRH-ArchT mice that expressed the light-activated proton pump, ArchT, selectively in CRH-expressing neurons we crossed CRH^{ires-Cre} and Cre-dependent loxP-flanked stop cassette ArchT reporter (Rosa26Sor^{tm40.1(CAG-aop3/EGFP)Hze}) mice (Chow et al., 2010; Han et al., 2011). Control mice were either single ChR2 (Ai32, Rosa26Sor^{tm32(CAG-COP4*H134R/EYFP)}) from The Jackson Laboratory, #012569) mice from ChR2 x ChR2 crossing pairs or single CRH^{ires-Cre} mice. All mice were maintained on a C57BL/6 background.

FISH. Serial coronal brain and brainstem frozen sections through the pontine micturition center from 8- to 12-week-old CRH-ChR2 mice were cut on a microtome at 10 μ m thickness and store at -80°C . FISH using separate DNA oligonucleotide probes for CRH and ChR2, which were coupled to an amplification system using two different fluorophores (RNAscope from Advanced Cell Diagnostics) were used to visualize CRH and ChR2 mRNA expression. Location of the PMC was determined based on brain anatomic landmarks, including fourth ventricle in accordance with the Franklin and Paxinos mouse brain atlas (Franklin and Paxinos, 2013). Total nuclear counts were defined by staining with DAPI. For both CRH and ChR2 expression, total number of cells counted and percentage of cells positive were calculated as well as number of cells positive for both CRH and ChR2.

Cystometry catheter placement. Eight to 10-week-old mice were anesthetized with isoflurane, and a cystometry PTF catheter with blunted end (Catamount Research) was placed via an abdominal incision and open cystotomy under aseptic technique. The catheter was sutured into place with a simple free tie at the dome of the bladder, and then the catheter was tunneled out to the nape of the back where it was secured in an Eppendorf tube that was sutured to the back of the mice. The end of the catheter in the tube was tied in a knot to prevent drainage of urine during recovery. After successful catheter placement, the mice underwent optical fiber implant as described below.

Stereotactic intracranial implantation of optical fiber. Under the same isoflurane anesthetic and immediately after cystometry catheter placement, the mice were placed in a stereotactic head frame with computer guided digital controls (Stoelting). After making a scalp incision and exposing the cranium under aseptic conditions, bregma (the union of the sagittal and coronal sutures) was identified. After drilling a small burr hole over the location of PMC, an optical fiber (200 μ m inner core diameter, 0.22 numerical aperture; Thorlabs) was unilaterally implanted just above PMC and held in place by bonding the ferrule to the scalp with epoxy glue. The coordinates used to place the PMC were obtained from the Franklin and Paxinos mouse brain atlas (Franklin and Paxinos, 2013) (Barrington's nucleus [Bar] location used for the PMC as they are equivalent) and were 5.42 mm posterior to bregma, 0.60 mm lateral to the sagittal suture line, and 3.60 mm ventral to bregma with a 0° angle. After surgical procedures, all mice were placed in single housing and allowed to recover for 3–4 d.

In vivo cystometry recording and optogenetic photostimulation. Following a 3–4 d recovery period from surgery, a swiveled fiberoptic cable was attached to the ferrule coupling on the optical fiber, and the mouse was then placed in a cystometry chamber (Catamount). Slow fill, awake, cystometry was conducted at 10 μ l/min. Micturition was also confirmed by deflection of a scale below the cystometry cage; and when possible, weight of each void was used to confirm voided volume and bladder capacity. Bladder pressure, including mean, threshold, and peak bladder pressures, was calculated using cystometry data analysis software from MedAssociates (Product Number: SOF-552). Five voiding cycles were used to establish the baseline and once stable baseline parameters

were established, optogenetic stimulation of PMC was started. For CRH-ChR2 mice, light at 470 nm was delivered by a light emitting diode (LED) (Thorlabs) in 15 ms pulses at frequencies of 2, 10, 25, and 50 Hz. These frequencies of light delivery were chosen for consistency with other published papers using optogenetics in the PMC (Hou et al., 2016; Keller et al., 2018; Ito et al., 2020) and based on previously documented burst frequencies (as high as 50 Hz) for neurons in the PMC (Rouzade-Dominguez et al., 2003; Manohar et al., 2017). Specifically, Manohar et al. (2017) noted that PMC neurons of unanesthetized rats have burst rates averaging 28 Hz in the 20 s period following each micturition event, and Rouzade-Dominguez et al. (2003) found that 33% of neurons in the PMC of anesthetized rats fire between 8 and 50 Hz. Pulses were delivered for 5 s and the light train was repeated once every 60 s, controlled with TTL signal from a Master-9 (A.M.P.I.) pulse stimulator. Light power (for 470 nm) at fiber tip was between 8 and 10 mW and was checked before each experiment using Thorlabs power meter, giving a total light intensity delivered to the PMC of between 250 and 320 mW/mm².

For CRH-ArchT mice, light at 530 nm was delivered by an LED (Thorlabs) in 15 ms pulses at 2, 25, and 50 Hz continuous. Results from 2 Hz were equivalent to baseline (photostimulation off) and results at 25 Hz were statistically equivalent to 50 Hz; therefore, for final analysis only baseline and 50 Hz were used. For 50 Hz of photostimulation, light delivery occurred for 75% of the time (duty cycle), similar to what others have described in the literature and to prevent bleaching and rebound activation (Z. V. Guo et al., 2017; Asok et al., 2018; N. Li et al., 2019). Light power was checked before each experiment and was between 0.5 and 1 mW, giving a total light intensity at the tip of the fiber optic of between 15 and 35 mW/mm². For all mice and experiments, at each frequency, three voiding cycles were repeated and cystometry pressures recorded. Micturition pressure, intermicturition interval (IMI, time between each void), and voided volume (via weight on a digital scale) were recorded into software that allowed for determination of bladder capacity, threshold pressure, voiding pressure, and voided volume. After completion of experiments, mice were killed and position of optic fiber confirmed by standard histologic techniques.

CRH antagonist injection. For experiments performed in the presence of CRH₁ receptor antagonist, 1 h before cystometry studies, mice were treated with either vehicle (10% solution of 0.1 M tartaric acid in sterile water) or NBI-30775 (10 mg/kg in 10% solution of 0.1 M tartaric acid in sterile water). Vehicle or NBI-30775 was injected subcutaneously, and this CRH receptor antagonist has been shown to have a half-life of 130 min *in vivo* and results in 75% occupancy of brain CRH₁ receptors (Heinrichs et al., 2002; Gutman et al., 2003; Fleck et al., 2012). Cystometry recordings were started 1 h after injection and repeat subcutaneous injections were performed every 1.5 h during the study.

CPM induced cystitis. Female CRH-CH2R mice underwent stereotaxic placement of a canula into the PMC. One week later, they were placed in specialized metabolism chambers (UroVoid, Med Associates) and allowed a 24 h acclimation period. Voiding patterns were measured at baseline. Cystitis was induced by injection of cyclophosphamide (150 mg/kg, i.p.) in saline as described by others (LaBerge et al., 2006; Bjorling et al., 2007, 2011; Everaerts et al., 2010; M. Guo et al., 2018); and 48 h later, the voiding patterns were measured again. At this point, optogenetic stimulation was started using 470 nm light at 50 Hz with 15 ms pulses. Light pulses were delivered for 5 s, and the light train was repeated once every 60 s for 24 h, controlled with TTL signal from a Master-9 (A.M.P.I.) pulse stimulator. Light power was calculated as

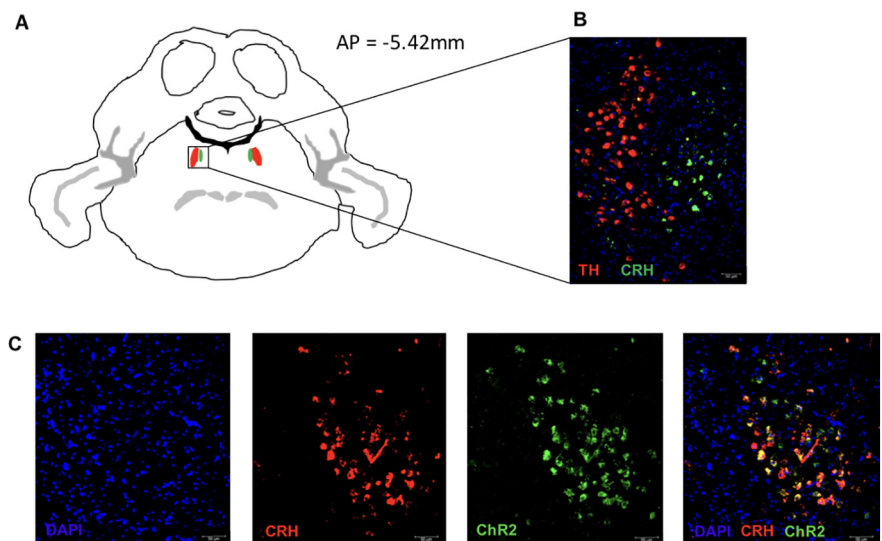


Figure 1. Cre-lox recombination results in ChR2 expression in the majority of CRH-expressing neurons in the PMC. **A**, Schematic of coronal section through mouse brain at the level of the pons illustrating the location of the pontine micturition center (green) and the adjacent locus ceruleus (red). **B**, Using RNAscope to perform ISH, probes targeting the mRNA for CRH identify PMC (green) and probes targeting the mRNA for TH identifies the LC (red). **C**, In situ hybridization (ISH) of CRH mRNA (red) and ChR2 (green) shows significant overlap of neurons with both colors in PMC (200 $\times$). Scale bar, 50 μ m. First panel shows DAPI (blue) only, second panel CRH (red) only, third panel ChR2 (green) only, and fourth panel overlap of all 3 channels.

described above. Voiding patterns were measured again during this 24 h photostimulation period. Voiding endpoints included, urinary frequency, IMI, and voided volumes.

Experimental design and statistical analysis. A sample size of 5 was calculated to have a power of 90% with a two-tailed α error set at 0.05, to determine a 50% difference in IMI and voided volume between baseline and during optogenetic stimulation for the CRH-ChR2 mice. Given this number, we sought to use 6 control mice, 6 female CRH-ChR2, and 6 male CRH-ChR2 mice for the main experiments. All data are presented as mean $\pm$ SEM. Assuming nonparametric distributions, the Kruskal-Wallis one-way ANOVA was used to compare cystometry parameters at baseline and each optogenetic frequency of stimulation. To compare cystometry parameters between controls and experimental mice at baseline and at each frequency of optogenetic stimulation, the Mann-Whitney *U* test was used. For nonparametric repeated measurements, Wilcoxon signed-rank test was used for comparison of means between two groups. Two-way ANOVA was used to compare cystometry changes at the various optogenetic stimulation frequencies by gender. For all analyses, two-tailed *p* values of <0.05 were considered significant. All statistical tests were performed using STATA (version 14.2).

Study approval

All studies and protocols were approved by the Institutional Animal Care and Use Committee of the Children's Hospital of Philadelphia and conducted in accordance with National Institutes of Health *Guide for the care and use of laboratory animals*.

Results

Cre-lox recombination results in ChR2 expression in the majority of CRH-expressing neurons in PMC

The Cre-lox recombinase system was used to generate CRH-ChR2 mice that express the light-activated cation ChR2 (Boyden et al., 2005) selectively in CRH-expressing neurons. Dual fluorescence ISH confirmed ChR2 expression in PMC CRH neurons in 8- to 12-week-old CRH-ChR2 mice; 97% of CRH-expressing neurons also express ChR2 mRNA ($n = 687$ neurons; 3 mice; variance = $\pm 1\%$; Fig. 1). CRH-expressing neurons accounted for

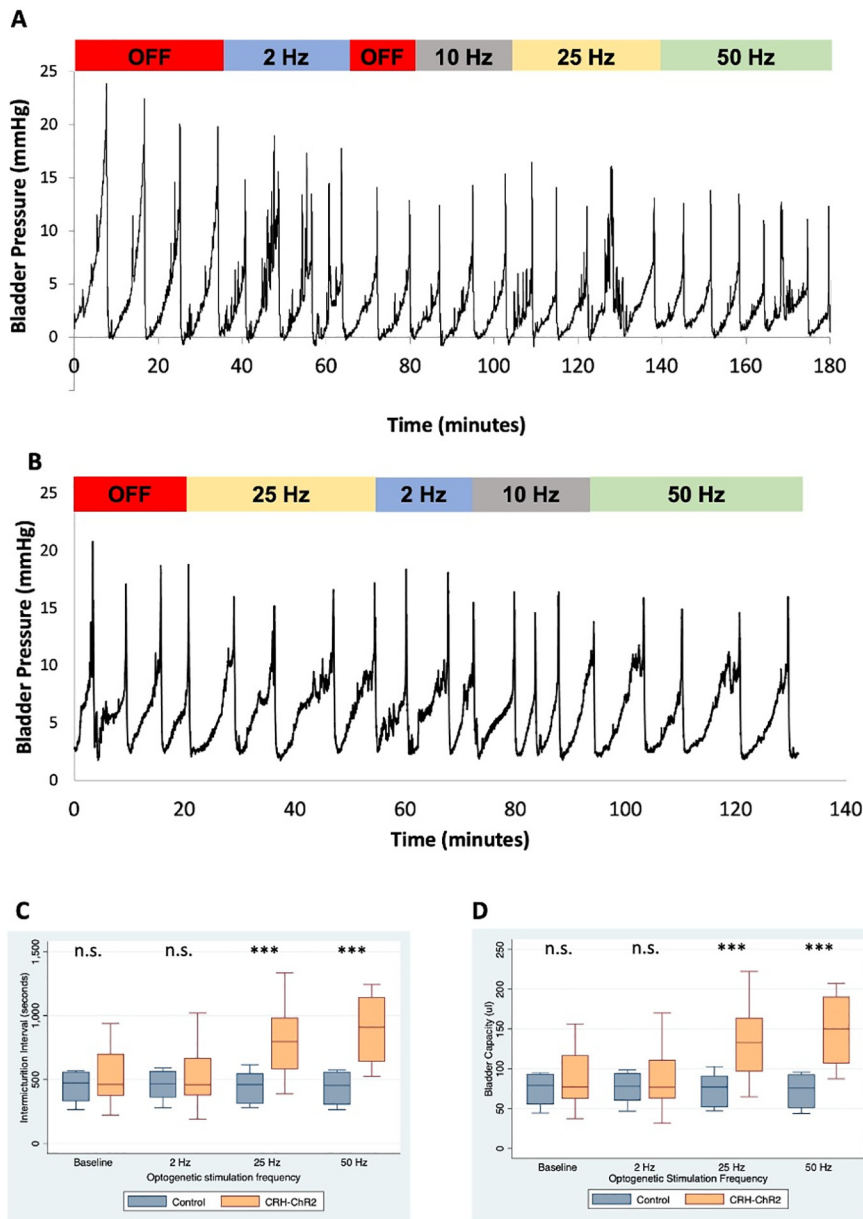


Figure 2. Effect of optogenetic stimulation of the PMC on urodynamics. **A, B**, Representative cystometry tracings of bladder pressure versus time in a control male mouse (Chr2 only) (**A**) and a double-transgenic CRH-ChR2 male mouse (**B**). Baseline cystometry pattern is obtained at an infusion rate of 10 μ l/min with the optogenetic probe turned off. After establishment of baseline IMI and bladder capacity, the LED generator is turned on and delivers light via the fiberoptic probe to PMC at the frequencies denoted above the traces. **A**, For the control male mouse, the IMI remained constant with the optogenetic stimulation off and at all frequencies of stimulation. **B**, For the CRH-ChR2 male mouse, there was an increase in both IMI and bladder capacity with 25 Hz (yellow shaded area of graph) or 50 Hz (green shaded area of graph) stimulation. **C**, Box-and-whisker plot showing the mean effects of optogenetic photostimulation on IMI in control and CRH-ChR2 mice. For control mice (Chr2 only, $n = 6$, 3 male, 3 female), there was no effect of frequency on IMI ($F_{(3,3)} = 2.1$, $p = 0.28$). In contrast, for double CRH-ChR2 mice ($n = 15$, 8 male, 7 female), there was an effect of Hz ($F_{(3,12)} = 48$, $p < 0.001$). A two-way repeated-measures ANOVA revealed an effect of genotype ($F_{(1,19)} = 5.7$, $p = 0.027$) and genotype $\times$ Hz interaction ($F_{(3,17)} = 21$, $p < 0.0001$). There were statistically significant differences in IMI between baseline and optogenetic stimulation at both 25 and 50 Hz ($p < 0.0001$; Mann–Whitney U test). **D**, Similarly, box-and-whisker plot shown for bladder capacity. There was no effect of Hz for control mice ($F_{(3,3)} = 2.1$, $p = 0.28$). In contrast, there was an effect of Hz for CRH-ChR2 mice ($F_{(3,12)} = 47$, $p < 0.0001$). A two-way repeated-measures ANOVA revealed an effect of genotype ($F_{(1,19)} = 5.7$, $p = 0.028$) and genotype $\times$ Hz interaction ($F_{(3,17)} = 20$, $p < 0.0001$). CRH-ChR2 mice had significantly larger bladder capacities at both 25 Hz and 50 Hz optogenetic stimulation compared with baseline ($p < 0.0001$; Mann–Whitney U test). *** denotes that the difference between control and CRH-ChR2 values at that Hz was significant (Mann–Whitney U test, $p < 0.001$).

36% of all cells in the PMC. Only 4.7% (variance = $\pm 0.5\%$) of PMC neurons were noted to express ChR2 and not CRH, perhaps reflecting neurons that at some point during development expressed CRH but were no longer at time of sectioning.

Frequency-dependent inhibitory effects of optogenetic stimulation on the voiding pattern

Eight- to 12-week-old CRH-ChR2 mice underwent both stereotactic placement of an optical fiber just above PMC and surgical placement of a cystometry catheter directly into the bladder. Mice were then allowed to recover for 3–4 d, and then cystometry studies were performed in awake and freely moving mice via continuous saline infusion through a bladder catheter (10 μ l/min). Bladder pressure was recorded via the cystometry catheter for at least three cycles without optogenetic stimulation (baseline) and then again for another three cycles at three frequencies of optogenetic stimulation (2, 25, and 50 Hz) with blue light via fiberoptic patch cable connected to implant. These frequencies of light delivery were chosen for consistency with other published papers using optogenetics in the PMC (Hou et al., 2016; Keller et al., 2018; Ito et al., 2020) and based on previously documented burst frequencies (as high as 50 Hz) for neurons in the PMC (Rouzade-Dominguez et al., 2003; Manohar et al., 2017).

Optogenetic stimulation had no effect on the IMI in control mice (Chr2 only; $n = 6$, 3 male, 3 female) at any frequency ($F_{(3,3)} = 2.1$, $p = 0.28$) (Fig. 2A,C). In CRH-ChR2 mice ($n = 15$, 8 male and 7 female), there was no difference in these voiding parameters at baseline compared with during 2 Hz optogenetic stimulation. However, when optogenetic stimulation frequency was increased to 25 and 50 Hz, there were increases in IMIs compared with baseline (Mann–Whitney U test, $p < 0.001$; Fig. 2B,C). For control mice, there was no effect of optogenetic stimulation frequency on IMI ($F_{(3,3)} = 2.1$, $p = 0.28$). In contrast, for CRH-ChR2 mice, there was an effect of stimulation frequency on IMI ($F_{(3,12)} = 48$, $p < 0.001$). A two-way repeated-measures ANOVA revealed an effect of genotype ($F_{(1,19)} = 5.7$, $p = 0.027$) and genotype $\times$ stimulation frequency interaction ($F_{(3,17)} = 21$, $p < 0.0001$).

Similarly, bladder capacity did not change in control mice at any of the optogenetic stimulation frequencies ($F_{(3,3)} = 2.1$, $p = 0.28$) (Fig. 2D). At higher optogenetic stimulation frequencies (25 and 50 Hz), bladder capacity increased compared with baseline in CRH-

Table 1. Mean and maximum bladder pressure at baseline and at each optogenetic stimulation frequency for double-transgenic CRH-ChR2 mice and for double-transgenic CRH-ChR2 mice after CRHR1 antagonist administration

Optogenetic stimulation frequency	Mean bladder pressure during cycle (mean $\pm$ SEM; mmHg)	<i>p</i> value (vs baseline)	Mean bladder pressure during cycle (mean $\pm$ SEM; mmHg)	<i>p</i> value (vs baseline)
Double CRH-ChR2 mice (<i>n</i> = 15) ^a				
Baseline	3.5 $\pm$ 0.5		13.5 $\pm$ 1.6	
2 Hz	3.4 $\pm$ 0.4	0.82	14.2 $\pm$ 1.7	0.40
25 Hz	3.3 $\pm$ 0.4	0.73	13.3 $\pm$ 1.6	0.55
50 Hz	3.2 $\pm$ 0.3	0.68	12.9 $\pm$ 1.6	0.78
Double CRH-ChR2 mice after CRHR1 antagonist administration (<i>n</i> = 4) ^b				
Baseline	3.0 $\pm$ 1.0		9.9 $\pm$ 2.8	
2 Hz	2.9 $\pm$ 0.7	0.72	10.8 $\pm$ 2.8	0.47
25 Hz	2.3 $\pm$ 0.7	0.36	10.1 $\pm$ 2.9	0.72
50 Hz	2.4 $\pm$ 0.4	0.45	9.4 $\pm$ 2.4	0.47

^aFor CRH-ChR2 mice (*n* = 15, 8 male, 7 female), there were no statistically significant differences between mean bladder pressure during each voiding cycle at baseline and at each optogenetic stimulation frequency. Similarly, there were no significant differences between the maximum bladder pressure reached during each cycle at baseline compared with at each optogenetic stimulation frequency.

^bFor CRH-ChR2 mice after administration of CRHR1 antagonist (*n* = 4, 2 male, 2 female), there were no statistically significant differences between mean bladder pressure during each voiding cycle at baseline and at each optogenetic stimulation frequency. Similarly, there were no significant differences between the maximum bladder pressure reached during each cycle at baseline compared with at each optogenetic stimulation frequency.

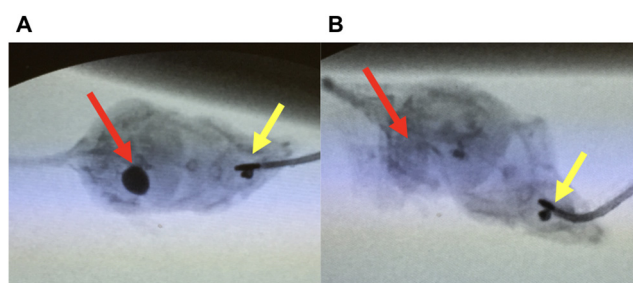


Figure 3. Fluoroscopy during cystometry study showed complete bladder emptying in mice with each voiding cycle. The male CRH-ChR2 mouse was placed in a Plexiglas cage atop the X-ray fluoroscopy detector, and cystometry studies were repeated while infusing a radiopaque contrast agent. Single or spot X-ray images were obtained at various points during the filling and voiding cycle at baseline and at each optogenetic stimulation frequency. Voids were detected by spikes in vesical pressure on the cystometry curve as well as by visually detecting voids on paper placed on the bottom of the cage. **A**, In this fluoroscopic image, the mouse has a total bladder volume of 120 μ l (red arrow). **B**, Upon voiding, the bladder was completely empty with no residual contrast seen on repeat fluoroscopic image (red arrow indicates location of bladder, now empty). Also seen in these views are the fiberoptic probe (yellow arrows) leading to the mouse's head as well as an anchoring bone screw placed to help the dental cement hold the cannula in place.

ChR2 mice (Mann–Whitney *U* test, $p < 0.001$; Fig. 2D). For bladder capacity, there was no effect of optogenetic stimulation frequency for control mice ($F_{(3,3)} = 2.1$, $p = 0.28$), while there was an effect of stimulation frequency for CRH-ChR2 mice ($F_{(3,12)} = 47$, $p < 0.0001$). A two-way repeated-measures ANOVA revealed an effect of genotype ($F_{(1,19)} = 5.7$, $p = 0.028$) and genotype $\times$ stimulation frequency interaction ($F_{(3,17)} = 20$, $p < 0.0001$).

Optogenetic stimulation did not alter bladder compliance or maximum pressure generation of CRH-ChR2 mice during the filling and voiding cycle (Table 1). Neither genotype (CRH-ChR2 or control ChR2 only) nor stimulation frequency had an effect on maximum pressure ($F_{(1,18)} = 0.24$, $p = 0.63$ and $F_{(3,16)} = 0.86$, $p = 0.48$, respectively).

To ensure that the mice were emptying their bladders completely during optogenetic stimulation, real-time bladder fluoroscopy was performed. Intermittent X-ray images were taken during cystometry while infusing with a radiopaque contrast agent. All post-void images showed an empty bladder, thus confirming that each void was complete with no residual volume left over during each cycle (Fig. 3).

The effects of PMC optogenetic activation on micturition are mediated by CRH

To test the hypothesis that the changes in voiding pattern (increases in both IMI and bladder capacity) during high-frequency optogenetic stimulation are CRH-mediated, cystometry studies were repeated in CRH-ChR2 mice after intraperitoneal injection of a vehicle or a CRH₁ receptor (CRHR1) antagonist (NBI 30775; 10 mg/kg).

As a negative control, 4 CRH-ChR2 mice (2 male, 2 female) had cystometry and optogenetic stimulation studies performed before and after injection of vehicle in volumes identical to those used for the CRH receptor antagonist. A two-way repeated-measures ANOVA revealed an effect of stimulation frequency ($F_{(3,4)} = 55$, $p = 0.001$) on IMI but no before/after vehicle control condition $\times$ stimulation frequency interaction was seen ($F_{(3,4)} = 0.4$, $p = 0.77$) (Fig. 4C,D). Therefore, the cystometric response to optogenetic stimulation in CRH-ChR2 mice was not affected by vehicle (i.p.).

Four different CRH-ChR2 mice (2 male, 2 female) underwent cystometry studies before and after CRHR1 antagonist administration. Initial cystometry studies were performed on day 1 and then repeated on day 2, 1 h after CRHR1 antagonist injection (Fig. 4A,B). For mice before CRHR1 antagonist, there was an effect of stimulation frequency on IMI ($F_{(3,4)} = 173$, $p < 0.01$) (Fig. 4C,D). A two-way repeated-measures ANOVA revealed a before/after CRHR1 antagonist condition $\times$ stimulation frequency interaction ($F_{(3,4)} = 121$, $p < 0.01$). Optogenetic stimulation frequency on day 1 (before CRHR1 antagonist) had a significant effect on both IMI and bladder capacity; this effect was lost after administration of a CRHR1 antagonist. These findings suggest that the voiding changes seen during optogenetic stimulation of PMC at higher frequencies are CRH-mediated. The presence of the CRH antagonist did not alter bladder compliance or pressure generation during the filling and voiding cycle.

Females have an enhanced response to PMC stimulation compared with males

Given reports of sex differences in CRH expression in the brain and CRH receptor activity (Iwasaki-Sekino et al., 2009; Bangasser et al., 2010; K. Li et al., 2016), we were interested in determining whether there were sex differences in responses to

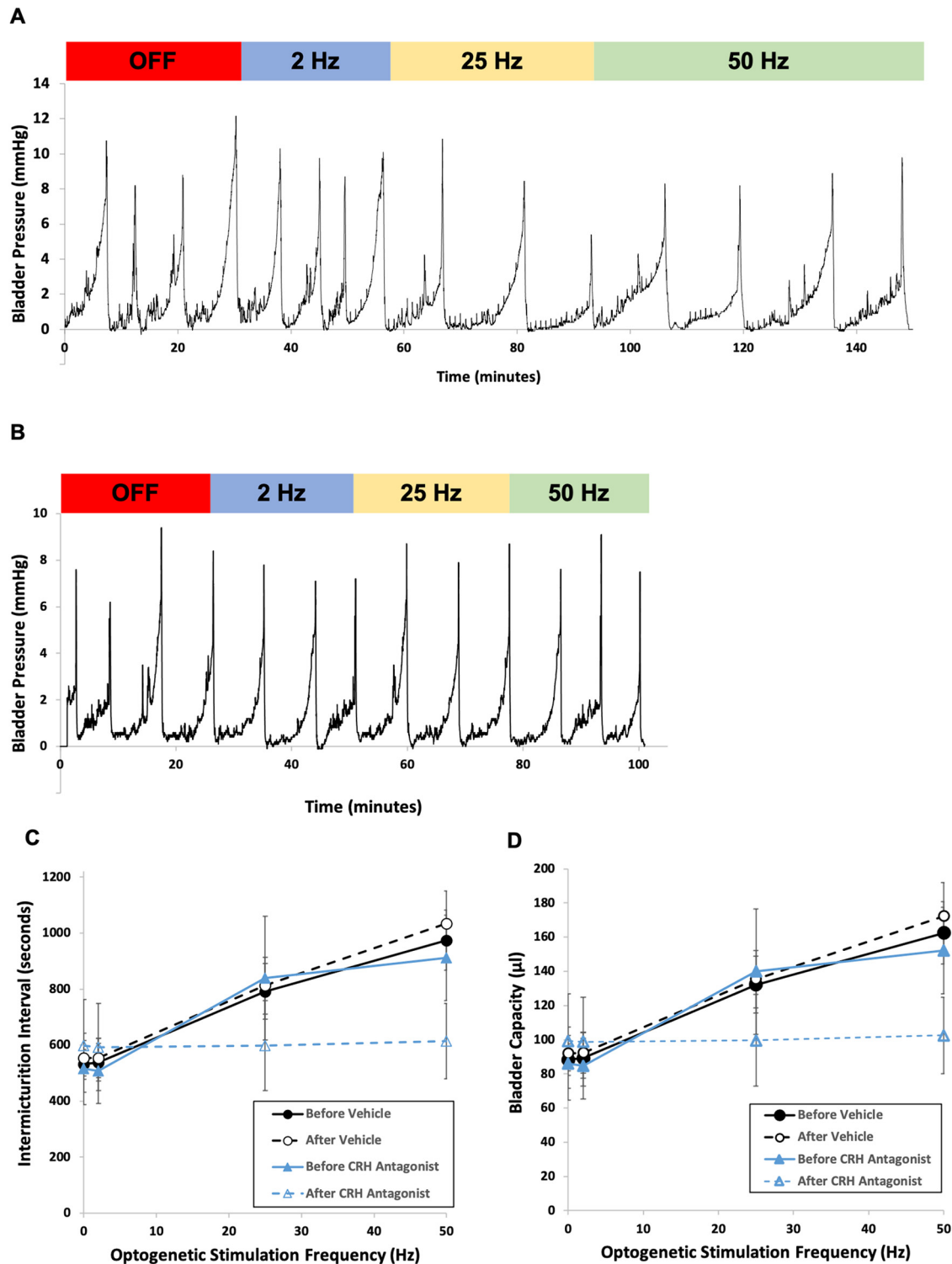


Figure 4. Effects of CRH antagonist on urodynamics during optogenetic stimulation of the pontine micturition center of a double-transgenic CRH-ChR2 female mouse. **A**, Graph showing cystometry tracing in a double-transgenic CRH-ChR2 female mouse. Baseline urodynamics were established with the optogenetic probe turned off and then at increasing stimulation frequencies as denoted above the tracing. IMI increased at both 25 and 50 Hz stimulation. **B**, On the following day, CRH antagonist NBI-30775 was administered (10 mg/kg in 10% solution of 0.1 M tartaric acid in sterile water, i.p.) to the same female mouse from **A**. One hour later, baseline urodynamics were established with the optogenetic probe turned off and then with increasing stimulation frequencies. In the presence of the CRH antagonist, no changes in IMI were noted at any optogenetic stimulation frequency. **C**, Graph showing the effects of optogenetic stimulation on IMI in double-transgenic CRH-ChR2 mice before and after vehicle control injection ($n = 4$, 2 male, 2 female; solid black and dashed black lines, respectively) and before and after CRH antagonist injection ($n = 4$, 2 male, 2 female; solid blue and dashed blue lines, respectively). A two-way repeated-measures ANOVA revealed an effect of Hz ($F_{(3,4)} = 55$, $p = 0.001$) but not before/after vehicle control condition $\times$ Hz interaction was seen ($F_{(3,4)} = 0.4$, $p = 0.77$). For mice before CRH antagonist, there was an effect of Hz on IMI ($F_{(3,4)} = 173$, $p < 0.01$). A two-way repeated-measures ANOVA revealed a before/after CRH antagonist condition $\times$ Hz interaction ($F_{(3,4)} = 121$, $p < 0.01$). **D**, Graph showing the effects of optogenetic stimulation on bladder capacity in CRH-ChR2 mice before and after vehicle control ($n = 4$; solid black and dashed black lines, respectively) and before and after CRH antagonist injection ($n = 4$; solid blue and dashed blue lines, respectively). A two-way repeated-measures ANOVA revealed an effect of Hz ($F_{(3,4)} = 53$, $p = 0.001$) on bladder capacity but not before/after vehicle control condition $\times$ Hz interaction was seen.

Table 2. Percentage change in IMI (A) and bladder capacity (B) from baseline with different optogenetic stimulation frequencies based on sex

Optogenetic stimulation	CRH-ChR2 female mice (<i>n</i> = 7)		CRH-ChR2 male mice (<i>n</i> = 8)	
	Percentage of baseline IMI ^a (mean ± SEM)	<i>p</i> value (vs baseline)	Percentage of baseline IMI ^a (mean ± SEM)	<i>p</i> value (vs baseline)
Baseline	100		100	
2 Hz	103.2 ± 2.1	0.70	96.6 ± 4.2	0.99
25 Hz	181.4 ± 13.3	0.001	141.6 ± 11.4	0.003
50 Hz	218.1 ± 14.8	<0.001	161.1 ± 11.1	<0.001
Optogenetic stimulation	CRH-ChR2 female mice (<i>n</i> = 7)		CRH-ChR2 male mice (<i>n</i> = 8)	
	Percentage of baseline bladder capacity ^b (mean ± SEM)	<i>p</i> value (vs baseline)	Percentage of baseline bladder capacity ^b (mean ± SEM)	<i>p</i> value (vs baseline)
Baseline	100		100	
2 Hz	102.9 ± 2.0	0.72	96.6 ± 4.2	0.99
25 Hz	181.8 ± 13.0	0.001	141.4 ± 11.4	0.006
50 Hz	218.4 ± 34.5	<0.001	160.8 ± 10.4	<0.001

^aUsing the baseline (no stimulation) IMI as a reference point, the change in IMI as a percentage of this baseline are shown for both female (*n* = 7) and male (*n* = 8) CRH-ChR2 mice. For both female and male mice, the mean percentage of baseline IMI increased significantly at 25 and 50 Hz but not at 2 Hz. Furthermore, the percentage change in IMI at higher frequencies for the female mice were significantly greater than the percentage change in IMI at higher frequencies in male mice (two-way repeated-measures ANOVA for effect of sex on percentage change of IMI: $F_{(1,13)} = 10.5$, $p = 0.006$). A least-squares model found an effect of sex on percentage change of IMI at 25 Hz ($p = 0.04$) and 50 Hz ($p = 0.006$).

^bSimilar results were seen comparing percentage of baseline bladder capacity at each optogenetic stimulation frequency based on sex. A two-way repeated-measures ANOVA revealed an effect of sex ($F_{(1,13)} = 10.5$, $p = 0.006$). A least-squares analysis found that female mice had larger changes in percentage of baseline bladder capacity compared with male mice at 25 Hz ($p = 0.04$) and 50 Hz ($p = 0.006$).

optogenetic stimulation of the PMC. For both female (*n* = 7) and male (*n* = 8) CRH-ChR2 mice, there were statistically significant differences in IMI between baseline and optogenetic stimulation at both 25 and 50 Hz (all $p < 0.001$; Mann–Whitney *U* test). There was a significant effect of stimulation frequency on IMI ($F_{(3,11)} = 64$, $p < 0.0001$) but no significant effect of sex on IMI ($F_{(1,13)} = 1.05$, $p = 0.32$). There was also no significant interaction found between sex and stimulation frequency ($F_{(3,11)} = 1.8$, $p = 0.2$).

Similar to IMI, both female and male mice had significantly larger bladder capacities at both 25 and 50 Hz optogenetic stimulation compared with baseline (all $p < 0.001$; Mann–Whitney *U* test). While there was a significant effect of stimulation frequency on bladder capacity ($F_{(3,11)} = 63$, $p < 0.0001$), there was no significant effect of sex on bladder capacity ($F_{(1,13)} = 1.03$, $p = 0.33$). There was no significant interaction between sex and stimulation frequency on bladder capacity ($F_{(3,11)} = 1.9$, $p = 0.19$).

The values for both IMI and bladder capacity differed significantly at baseline between males and females (Mann–Whitney *U* test, $p = 0.04$); and for this reason, we repeated analysis after normalizing values to the baseline (i.e., as a percentage of baseline) (Table 2). Analysis using percentage of baseline IMI found significant effects of both stimulation frequency ($F_{(2,12)} = 41.9$, $p < 0.0001$) and sex ($F_{(1,13)} = 11.4$, $p = 0.005$). Using a least-squares method, there were significant differences between the sexes in terms of percentage of IMI at both 25 Hz ($p = 0.04$) and 50 Hz ($p = 0.006$). Analysis with percentage of bladder capacity yielded similar results, showing significant effects of both stimulation frequency ($F_{(2,12)} = 39.3$, $p < 0.0001$) and sex ($F_{(1,13)} = 10.5$, $p = 0.006$) on bladder capacity. A least-squares model identified significant differences between the percentage change in male and female mice bladder capacities at 25 Hz ($p = 0.04$) and 50 Hz ($p = 0.006$). Thus, while males had a 54% increase in

bladder capacity at 50 Hz compared with baseline, females had over a doubling of bladder capacity (110% increase) at 50 Hz compared with baseline.

Effect of optogenetic inhibition of the CRH-PMC neurons

To investigate the effect on micturition of inhibiting the CRH-expressing neurons in the PMC, the Cre-lox recombinase system was used to generate CRH-ArchT mice that express the light-activated proton pump, ArchT, selectively in CRH-expressing neurons. CRH-Cre mice served as controls, and photostimulation with 530 nm light had no effect on bladder capacity ($F_{(1,3)} = 0.03$, $p = 0.99$) or IMI ($F_{(1,3)} = 0.03$, $p = 0.99$). For the CRH-ArchT mice (*n* = 8; 4 male, 4 female), photostimulation at 50 Hz continuously led to increased urinary frequency and decreased bladder capacity (Mann–Whitney *U* test, $p < 0.01$ for all; Fig. 5). A two-way repeated-measures ANOVA revealed an effect of genotype for both bladder volume ($F_{(1,12)} = 21$, $p < 0.001$) and IMI ($F_{(1,12)} = 23$, $p < 0.001$).

Optogenetic stimulation of CRH neurons in the PMC reverses the voiding phenotype of cyclophosphamide-induced cystitis

Six CRH-Ch2R mice (3 male, 3 female) underwent placement of an optical fiber above the PMC and were allowed a 1 week recovery period. Baseline voiding patterns were measured in metabolism chambers following a 24 h acclimation period (Fig. 6A). Cyclophosphamide was injected at doses known to produce symptoms characteristic of cystitis (150 mg/kg, i.p.) (Bjorling et al., 2007, 2011). Voiding data collected 48 h after cyclophosphamide injection revealed increased voiding frequency and diminished voided volumes (Fig. 6B). Optogenetic stimulation of CRH-PMC neurons (50 Hz) was sufficient to reverse the effect of cyclophosphamide on the voiding patterns (Fig. 6C). Repeated-measures ANOVA revealed an effect of voiding condition (baseline, CPM cystitis, or optogenetic stimulation) on voiding frequency ($F_{(2,5)} = 4.5$, $p = 0.017$), voided volumes ($F_{(2,5)} = 4.9$, $p = 0.01$), and IMI ($F_{(2,5)} = 6.2$, $p = 0.02$) (Fig. 6D–F). Further analysis revealed statistical differences between baseline and CPM cystitis, and

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($F_{(3,4)} = 0.47$, $p = 0.7$). For mice before CRH antagonist, there was an effect of Hz on bladder capacity ($F_{(3,4)} = 181$, $p < 0.01$). A two-way repeated-measures ANOVA revealed a before/after CRH antagonist condition × Hz interaction ($F_{(3,4)} = 146$, $p < 0.01$). Points in graphs represent mean values. Error bars indicate ± SEM.

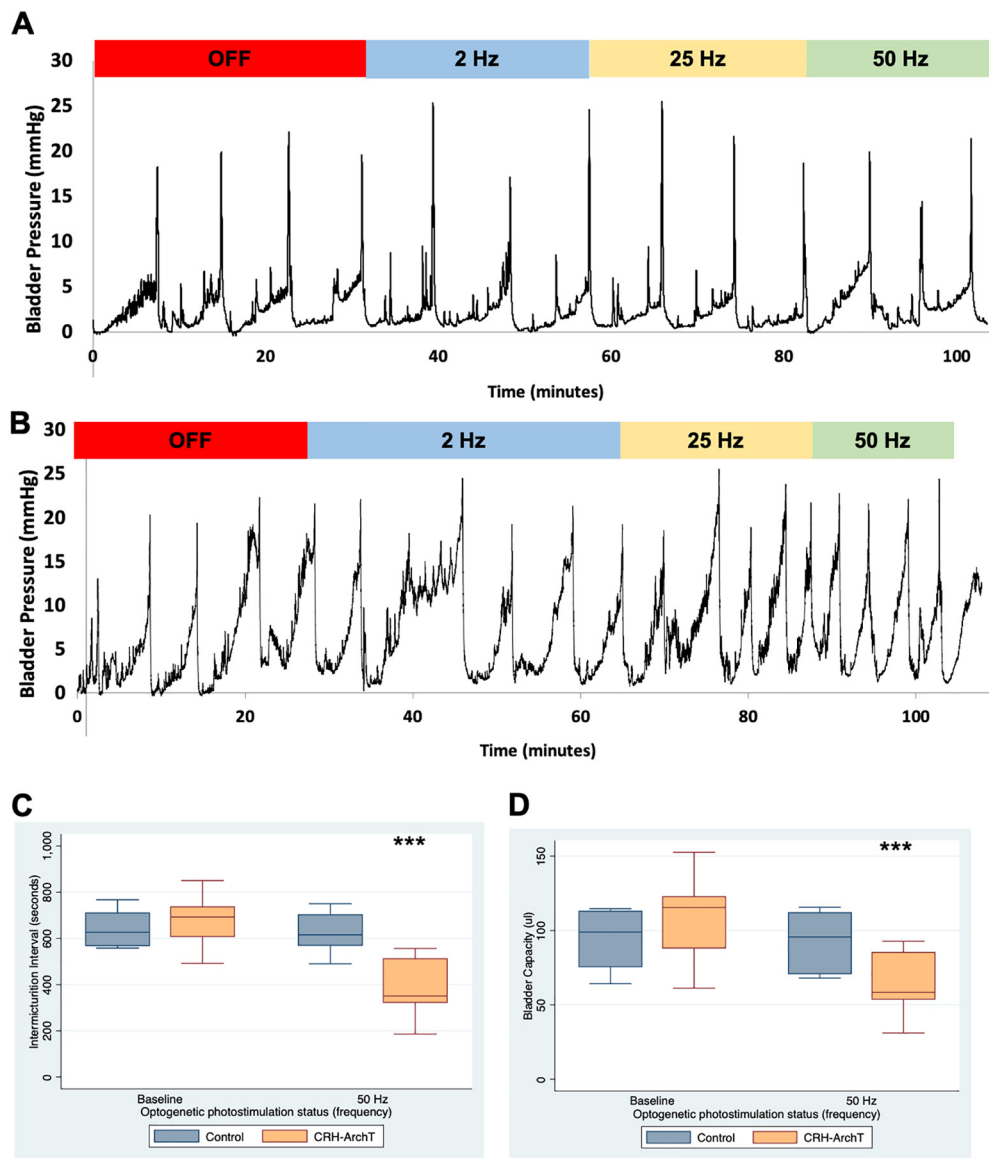


Figure 5. Effect of optogenetic inhibition of the PMC on urodynamics. **A, B**, Representative cystometry tracings of bladder pressure versus time in a control male mouse (ArchT only) (**A**) and a double-transgenic CRH-ArchT male mouse (**B**). Baseline cystometry pattern is obtained at an infusion rate of 10 μ l/min with the optogenetic probe turned off. After establishment of baseline IMI and bladder capacity, the LED generator (530 nm) is turned on and delivers light via the optic fiber to PMC at the frequencies denoted above the traces. **A**, For the control male mouse, the IMI remained constant with the optogenetic stimulation off and at all frequencies of stimulation. **B**, For the CRH-ArchT male mouse, there was a decrease in both IMI and bladder capacity with 25 Hz (yellow shaded area of graph) or 50 Hz (green shaded area of graph) stimulation. As mentioned in Materials and Methods, only photostimulation off and at 50 Hz was used in the final analysis for simplicity given equivalence of off with 2 and 25 Hz with 50 Hz. **C**, Box-and-whisker plot showing effects of optogenetic stimulation on IMI in control and CRH-ArchT mice. For control mice (CRH-Cre only, $n = 6$, 3 male, 3 female), there was no effect of photostimulation status on IMI ($F_{(1,3)} = 0.3$, $p = 0.99$). In contrast, for double CRH-ArchT mice ($n = 8$, 4 male, 4 female), there was an effect of photostimulation status ($F_{(1,5)} = 5.1$, $p = 0.006$). A two-way repeated-measures ANOVA revealed an effect of genotype ($F_{(1,12)} = 23$, $p < 0.001$). In CRH-ArchT mice, there were statistically significant differences in IMI between baseline and optogenetic photostimulation at 50 Hz ($p < 0.01$; Mann–Whitney U test). **D**, Box-and-whisker plot for bladder capacity based on mouse type and photostimulation status. There was no effect of photostimulation status for control mice ($F_{(1,3)} = 0.3$, $p = 0.99$). In contrast, there was an effect of photostimulation status for CRH-ArchT mice ($F_{(1,5)} = 4.3$, $p < 0.01$). A two-way repeated-measures ANOVA revealed an effect of genotype ($F_{(1,12)} = 21$, $p < 0.001$). CRH-ArchT mice had significantly larger bladder capacities of 50 Hz photostimulation compared with baseline ($p < 0.001$; Mann–Whitney U test). *** denotes that the difference in IMI (part **C**) and bladder capacity (part **D**) between control and CRH-ArchT mice was significant at 50 Hz (Mann–Whitney U test, $p < 0.001$ for both).

differences between CPM cystitis and optogenetic stimulation conditions, for all three parameters: voiding frequency, voided volumes, and IMI (Wilcoxon signed-rank test, all $p < 0.05$). However, there was no difference observed between baseline parameters and those measured after optogenetic stimulation (Wilcoxon signed-rank test, all $p = 0.2$ – 0.6).

Discussion

The present study used optogenetic manipulation of CRH-PMC neurons during *in vivo* cystometry in unanesthetized freely

moving mice to identify the role of CRH in the PMC in the regulation of volitional micturition. Optogenetically evoked stimulation of CRH-PMC neurons inhibited micturition; and conversely, optogenetically evoked inhibition of CRH-PMC neurons increased micturition frequency. Moreover, a CRH₁ receptor antagonist prevented the effects of optogenetic activation, indicating that the inhibition of micturition is CRH-mediated. The finding that optogenetic stimulation of CRH-PMC neurons was more effective in females compared with males is consistent with enhanced signaling of CRH₁ receptors in females (Bangasser et al., 2010). Together, the results converge with a

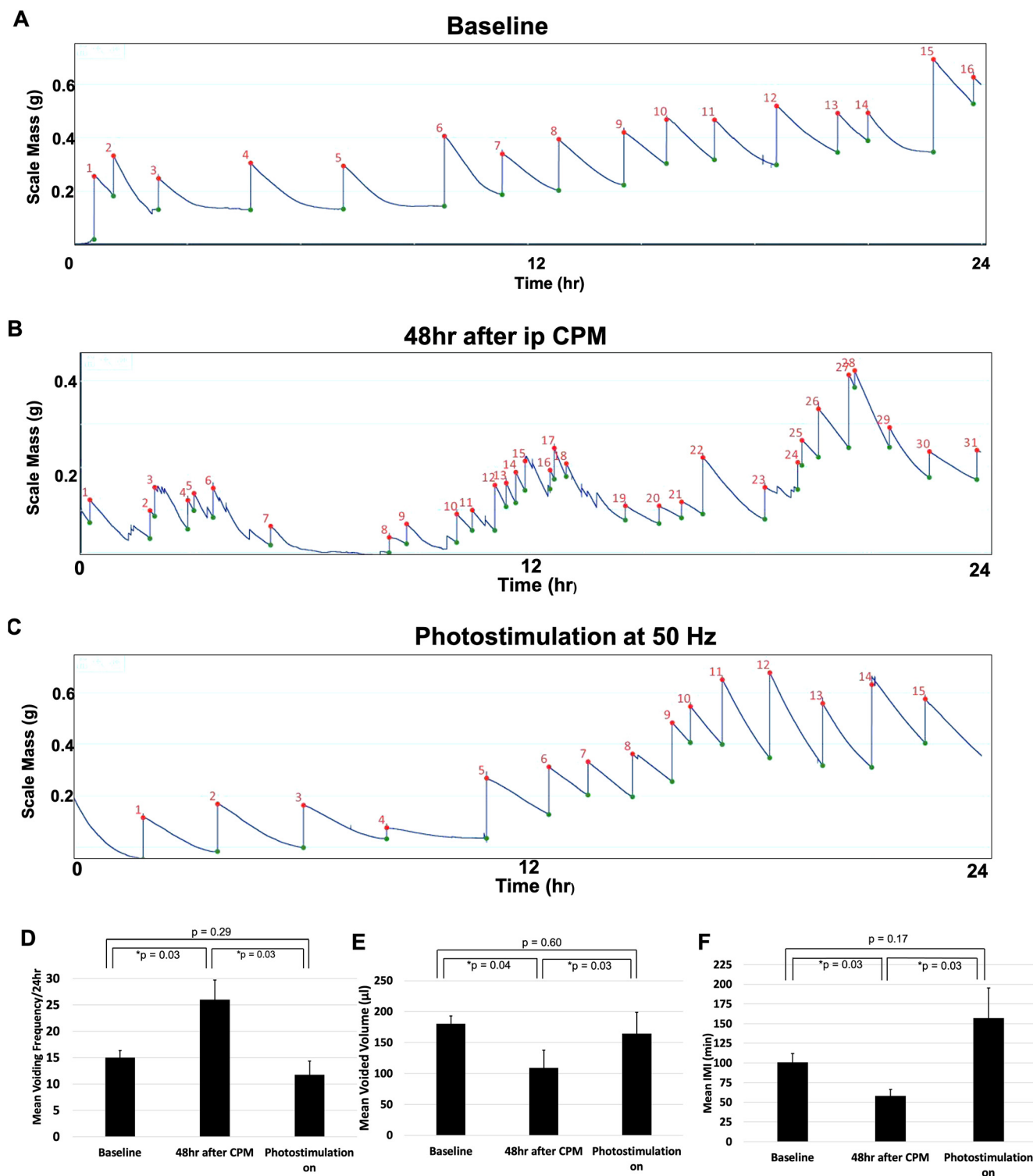


Figure 6. Optogenetic stimulation of CRH neurons in the PMC reverses the voiding phenotype of cyclophosphamide-induced cystitis. The 24 h voiding micturograms obtained using UroVoid (Med Associates) metabolic chambers from the same 8-week-old CRH-Ch2R male mouse at (A) baseline, (B) 48 h after i.p. cyclophosphamide instillation, and (C) after start of optogenetic photostimulation at 50 Hz. Graphs showing (D) voiding frequency per 24 h period, (E) mean voided volume, and (F) mean IMI at baseline, 48 h after intraperitoneal CPM administration, and during optogenetic photostimulation in CRH-Ch2R mice ($n = 6$, 3 male, 3 female).

body of pharmacological evidence supporting the concept that CRH from neurons in the PMC has an inhibitory role in the regulation of micturition. Importantly, this study demonstrated that activation of this pathway can reverse the dysfunctional urodynamic profile associated with an animal model of cystitis, underscoring the potential for targeting CRH in this pathway for the treatment of lower urinary tract dysfunction and voiding disorders.

Caveats

A transgenic approach was used to express Ch2R in CRH neurons. Although this approach has several advantages over viral injection techniques, including more selective recombinase activity (Jiang et al., 2017) and less potential for artificial synaptic depression and cytotoxicity, a consideration is that ChR2 will be expressed in all CRH-expressing neurons (Lee et al., 2020).

Notably, within our field of photostimulation, CRH is only expressed in PMC neurons. Although axons from CRH-expressing cells may pass in the vicinity of the PMC, there is no evidence that they project to spinal neurons that regulate bladder function. Finally, although activation of CRH-containing axons passing through the PMC could potentially alter activity of PMC neurons, the predominant effect of optogenetic activation in this region would be through direct activation of the PMC-CRH neurons that express ChR2. Therefore, it is unlikely that ChR2 expression in non-PMC neurons contributed to the present results.

The inhibitory effects of optogenetic stimulation on urodynamics were observed with high frequencies that could cause depolarizing block and neuronal inhibition rather than activation. However, it is unlikely that the current results could be attributed to depolarization block given that the urodynamic effects were prevented by a CRH antagonist, and opposing effects were elicited by optogenetic activation of CRH-PMC cells that express ArchT.

Relationship to other studies

Although early lesion and electrical stimulation studies identified the role of PMC spinal projections in the micturition reflex, dissecting the role of neurochemically distinct PMC populations, such as CRH neurons, remained a challenge (Noto et al., 1989; Tai et al., 2009; Gerlinger et al., 2012). One study suggested that CRH facilitates micturition (Klausner et al., 2005). However, convergent pharmacological evidence examining the effects of CRH or CRH antagonists on PMC-evoked bladder contractions or urodynamics in unanesthetized rats supported an inhibitory role in the PMC-spinal projections (Pavlovich and Valentino, 1995; Kiddoo et al., 2006; McFadden et al., 2012). Initial studies of optogenetic stimulation of CRH-PMC neurons demonstrated that this evoked bladder contractions and that chemogenetic inhibition impaired micturition (Hou et al., 2016). Another study determined that the effect was dependent on micturition cycle phase (Ito et al., 2020). Notably, our cystometry studies were performed in freely moving mice, while the previous studies were performed under urethane anesthesia (Rojas et al., 2006; Hou et al., 2016; Ito et al., 2020). In contrast to the study by Hou et al. (2016), which used a relatively fast filling rate (40 μ l/min) and used a diuretic, we performed cystometry in the absence of a diuretic and with a slow filling rate (10 μ l/min) that more closely mimics natural filling of the mouse bladder and prevents artificial detrusor contractions secondary to rapid bladder filling. Other optogenetic studies did not find a direct 1:1 correlation between stimulation and micturition in awake mice (Verstegen et al., 2017; Keller et al., 2018). Importantly, CRH-PMC neurons colocalize glutamate, which would be released by optogenetic stimulation to mediate bladder contraction and facilitate micturition. Consistent with our present findings, optogenetic stimulation in the presence of a CRH antagonist revealed an inhibitory influence of CRH (Ito et al., 2020).

Function of CRH in micturition

The findings that the inhibitory effects of optogenetic activation of CRH-PMC neurons were prevented by a CRH₁ receptor antagonist and that optogenetic inhibition facilitated micturition are consistent with the optogenetic studies of Ito et al. (2020) and earlier pharmacological studies (Pavlovich and Valentino, 1995; Kiddoo et al., 2006) that support an inhibitory role for CRH in PMC-spinal projections that regulate micturition. This is at odds with the reported lack of effect of CRH deletion from PMC

neurons (Verstegen et al., 2017). Reasons for the discrepancy between studies are not clear, although it is notable that the influence of CRH would only be apparent during high-frequency bursts as predicted for neuropeptide release or perhaps during stress, which upregulates CRH in PMC neurons (Wood et al., 2009). An inhibitory influence for CRH in the PMC-spinal pathway could provide negative feedback in the micturition cycle (Pavlovich and Valentino, 1995; Manohar et al., 2017; Ito et al., 2020). PMC neurons show high-frequency bursts (mean 25 Hz) following a void (Manohar et al., 2017). CRH release during these bursts could signal the end of voiding and facilitate the initiation of the storage phase of the micturition cycle.

CRH-mediated inhibition of micturition may also serve a conserved defensive function as a signal to avoid confrontation. Repeated social subordination stress in rodents is well documented to result in an altered voiding pattern characterized by decreased frequency, increased bladder capacity, and increased micturition volumes (Desjardins et al., 1973; Chang et al., 2009; Wood et al., 2009, 2013; Mann et al., 2015). Evidence suggests that this is mediated by CRH from the PMC because it is associated with CRH mRNA upregulation in PMC neurons, and the effects can be prevented by administration of a CRH antagonist or by preventing stress-induced upregulation of CRH using ShRNA directed against CRH (Wood et al., 2009, 2013).

Sex differences

Female mice had an almost twofold higher response to optogenetic activation compared with male mice. This gender disparity may in part be explained by the reported differences in CRH₁ receptor signaling. The CRH₁ receptor exhibits sex biased signaling in that coupling to the Gs-protein is greater in females compared with males, whereas coupling to β -arrestin 2 is greater in males (Bangasser et al., 2013). As a result, certain neuronal responses to CRH are enhanced in females compared with males, and the receptor is not internalized following stress or CRH (Valentino, 1988; Bangasser et al., 2010). Our findings here may be relevant to the higher prevalence of lower urinary tract dysfunction and many voiding disorders in females compared with males (Van Batavia et al., 2011; Tang et al., 2014; Van Batavia et al., 2014).

Translational relevance

The ability of CRH-PMC neurons to shape the voiding phenotype implicates CRH as a therapeutic target for diverse pathologic voiding conditions. Here we demonstrated that optogenetic activation of CRH-PMC neurons reversed the overactive voiding phenotype associated with cyclophosphamide (LaBerge et al., 2006; Bjorling et al., 2007, 2011; Everaerts et al., 2010; M. Guo et al., 2018). OAB, a common condition characterized by increased frequency of smaller volume voids, has a significant impact on quality of life and effective therapies are few (Tang et al., 2014). Interestingly, two recent clinical studies found significantly lower serum CRH level in children with nocturnal enuresis (i.e., bed-wetting) compared with control children (Motawie et al., 2017; Girissen et al., 2019). The present findings suggest that enhancing the influence of CRH in this pathway may be a novel therapeutic strategy for OAB and nocturnal enuresis.

Conditions that may be treated by reducing the influence of CRH include voiding postponement and underactive bladder. Lower urinary tract symptoms and voiding dysfunction affect ~20% of school aged children and >40% of adults (Sureshkumar et al., 2009; Coyne et al., 2011). One phenotype in children is characterized by habitual voiding postponement resulting in infrequent voiding of large volumes,

often associated with psychological or behavior problems (von Gontard et al., 1998, 2011). Likewise, an association between voiding dysfunction and social stress, including post-traumatic stress disorder, is seen in adults (Lettggen et al., 2002). Preclinical studies in rodents implicate stress-induced upregulation of CRH in the PMC in the pathophysiology of stress-related voiding postponement and suggest that CRH receptor antagonists might be useful. Likewise, underactive bladder is a common voiding dysfunction, particularly in the elderly; and this may be responsive to pharmacotherapies that inhibit CRH.

Given the prevalence of lower urinary tract disorders and their impact on quality of life, the present study underscores the therapeutic potential of agents that modulate CRH function in the PMC-spinal circuit and the need to consider sex differences in their action. Taken with other studies, this research highlights the need to identify the role of neurochemically distinct PMC cell types in regulation of pelvic visceral function.

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