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SUPPLEMENTARY INFORMATION

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Voluntary urination control by brainstem neurons that relax the urethral sphincter

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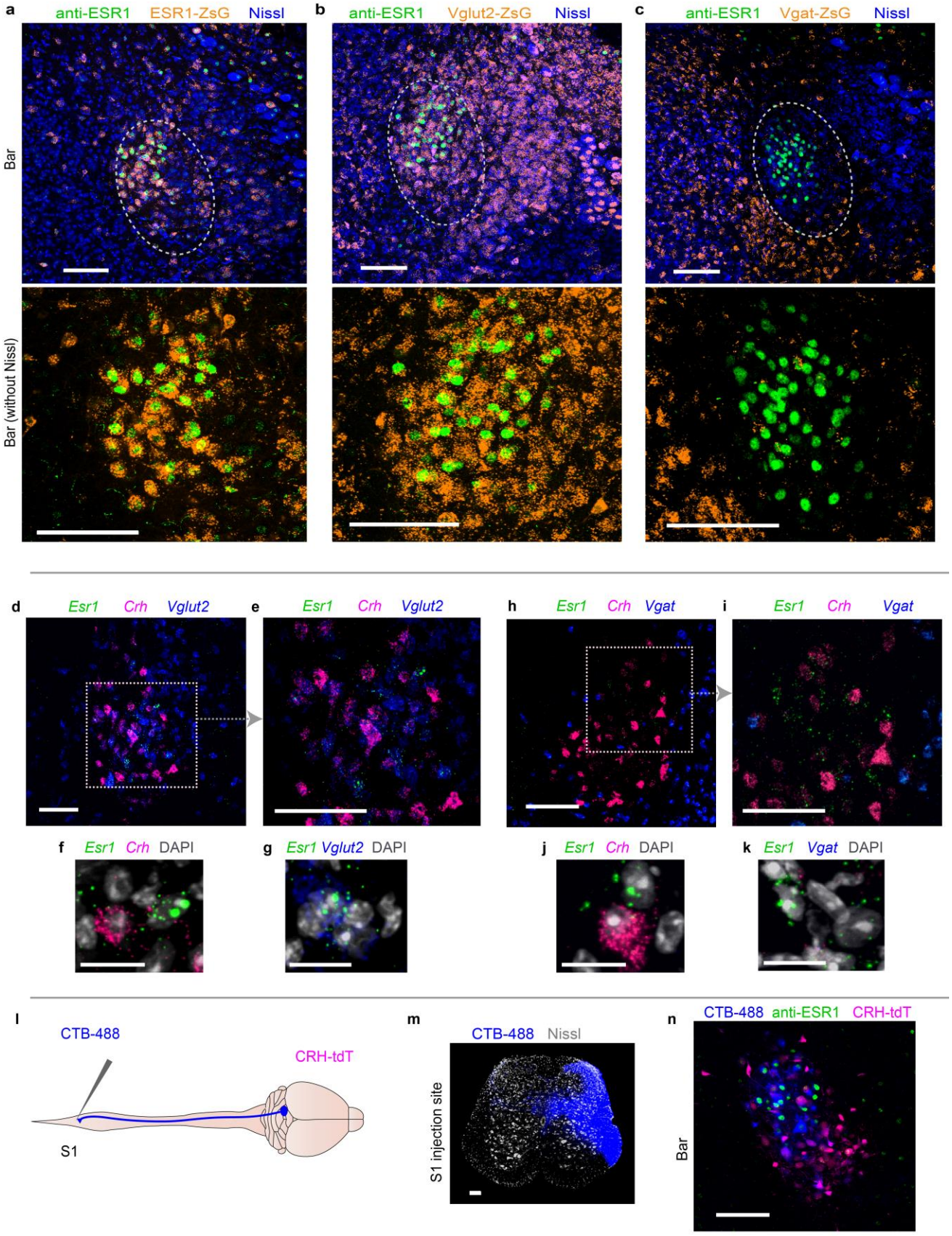
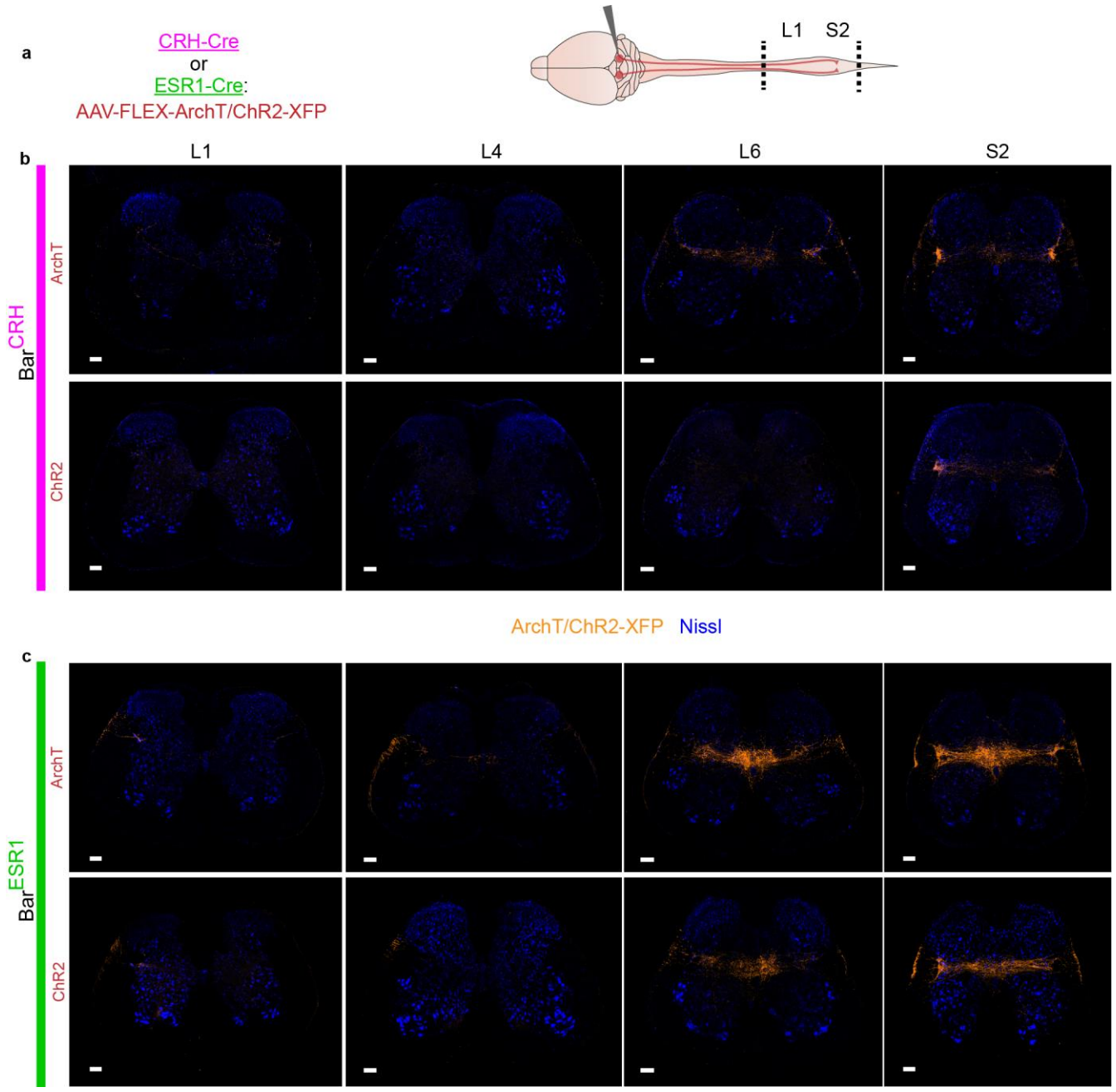

a
anti-ESR1 ESR1-ZsG Nissl
b
anti-ESR1 Vglut2-ZsG Nissl
c
anti-ESR1 Vgat-ZsG Nissl
Bar
Bar (without Nissl)
d
Esr1 Crh Vglut2
e
Esr1 Crh Vglut2
h
Esr1 Crh Vgat
i
Esr1 Crh Vgat
f
Esr1 Crh DAPI
g
Esr1 Vglut2 DAPI
j
Esr1 Crh DAPI
k
Esr1 Vgat DAPI
l
CTB-488
CRH-tdT
S1
m
CTB-488 Nissl
S1 injection site
n
CTB-488 anti-ESR1 CRH-tdT
Bar

Supplementary Figure 1

Neurotransmitter identity and direct spinal projections of Bar^{ESR1} neurons.

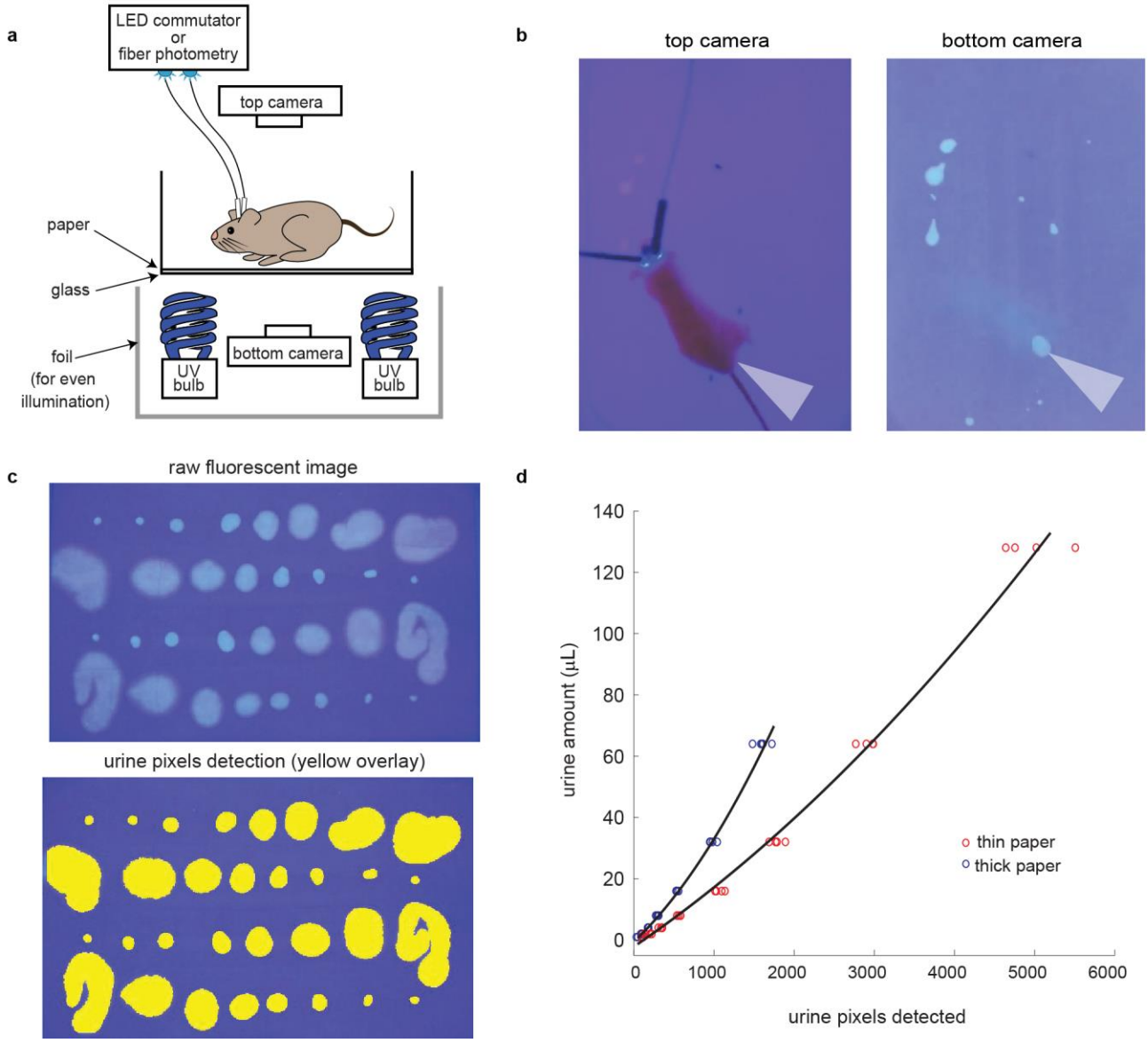
a, Anti-ESR1 overlap with ESR1-ZsGreen (Ai6) genetic reporter. Bottom is larger view without Nissl. **b,** Anti-ESR1 overlap with Vglut2-ZsGreen genetic reporter. **c,** Anti-ESR1 overlap with Vgat-ZsGreen genetic reporter. **d,** RnaScope in-situ hybridization of *Crh/Esr1/Vglut2* mRNA in Bar region of a wild-type male mouse, 20X objective. **e,** Larger view of dotted area in (d), 40X objective. **f, g,** Close-up views of individual cells in (e), with DAPI counterstain. **h-k,** same as (d)-(g), but with *Vgat* mRNA probe. **l,** Schematic of CTB injection into S1 spinal cord. **m,** CTB injection site. **n,** Retrograde CTB labeling in Bar with anti-ESR1 and CRH-tdT. Dotted ovals delineate Bar. Scale bars = 100 μm, except panels f/g/j/k, scale bars = 20 μm.



Supplementary Figure 2

Bar^{ESR1} and Bar^{CRH} projections to urinary nuclei in the spinal cord.

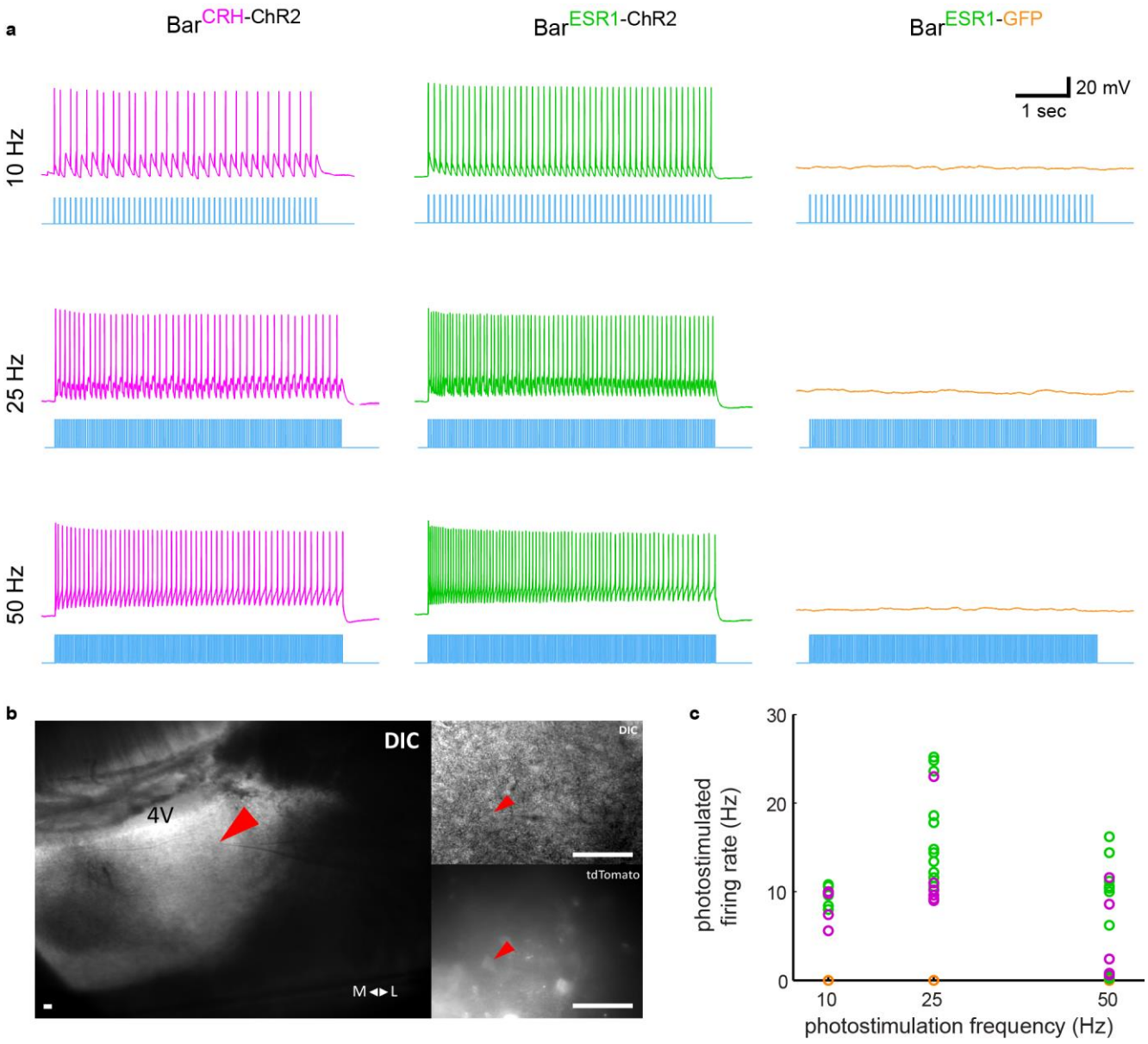
a, Schematic for testing Bar cell type axonal projections to spinal cord. **b,** Representative Bar^{CRH} axon projections at the L1/L4/L6/S2 spinal cord levels, separate from example in Fig. 1g-i. Top is ArchT virus, bottom is ChR2 virus. **c,** Same as (b), but for Bar^{ESR1} axon projections. Scale bars = 100 μm.



Supplementary Figure 3

Visualizing and quantifying urination behavior.

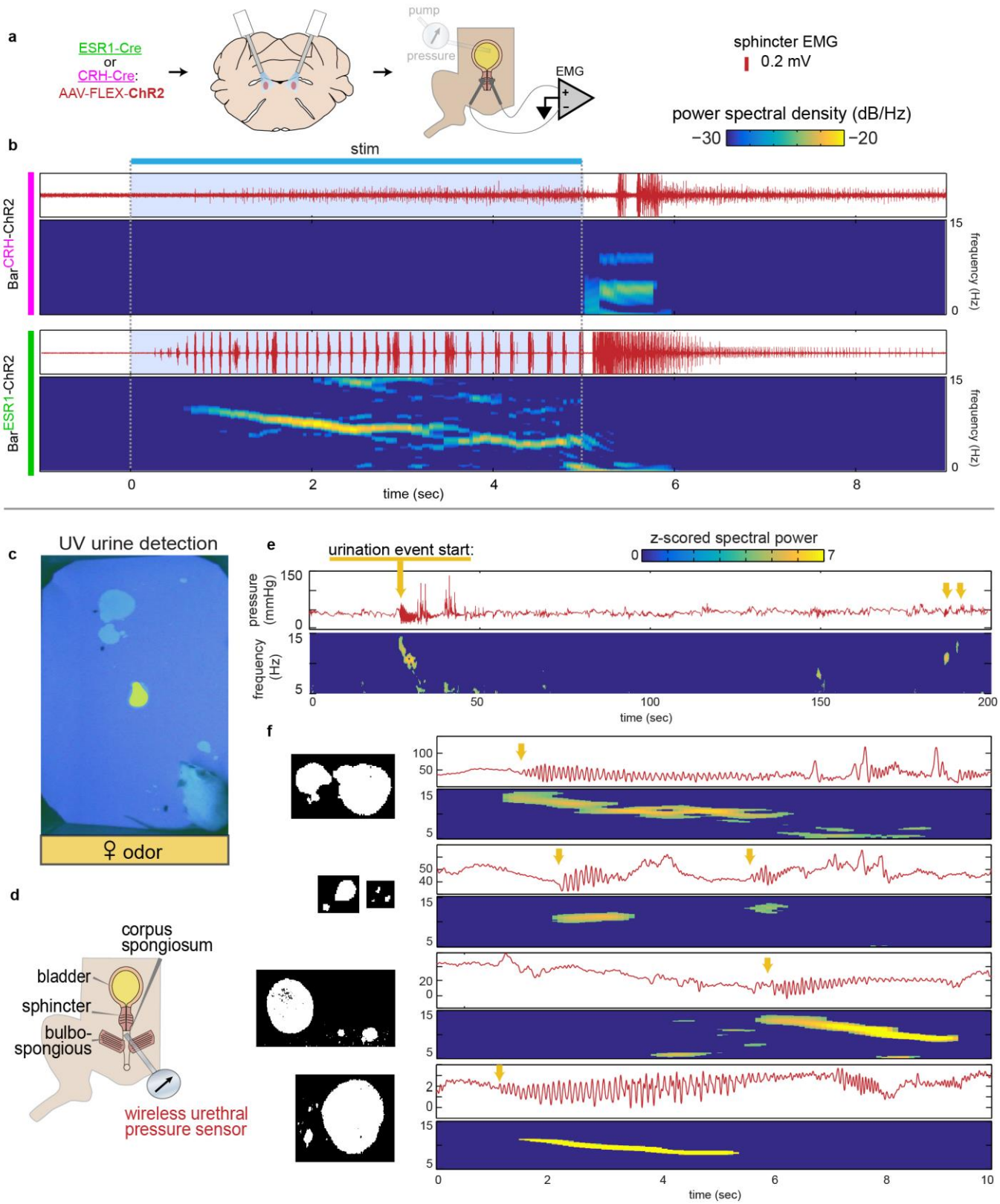
a, Schematic of behavioral setup for simultaneous optogenetics / fiber photometry, video recording, and analysis of urine excretion of awake behaving mice. **b,** Left: top camera records mouse position, right: urine fluoresces under UV light enabling excretion to be visualized throughout assay. Grey carrot indicates position between synchronized images from top and bottom cameras. **c,** Example of automated urine pixel detection with calibration data consisting of 4 replicates of 8 different volumes of male mouse urine on thin chromatography paper. **d,** Second order polynomial fit to calibration data on thick and thin paper; coefficients from these were used to calculate all urine amounts reported in microliters.



Supplementary Figure 4

Whole-cell recordings of Bar$^{CRH\text{-}ChR2}$ and Bar$^{ESR1\text{-}ChR2}$ neurons during photostimulation.

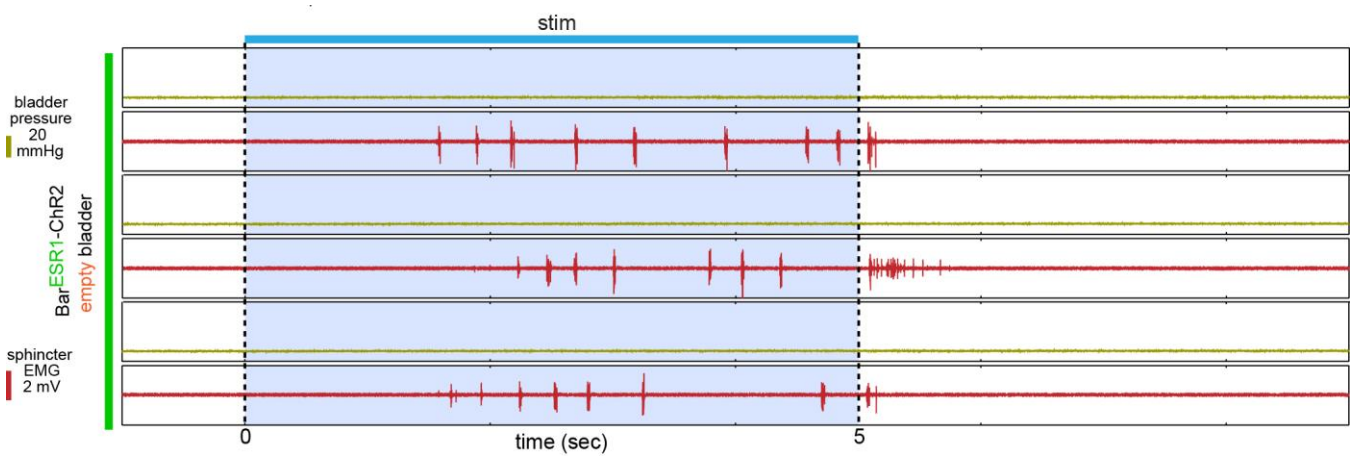
a, Example current clamp traces from representative Bar$^{CRH\text{-}ChR2}$ (magenta), Bar$^{ESR1\text{-}ChR2}$ (green), and Bar$^{ESR1\text{-}GFP}$ (orange) neurons during 5 sec photostimulation bouts at 10/25/50 Hz. **b,** Visualization of recording location of Bar$^{ESR1\text{-}ChR2}$ neuron in (a) showing ChR2-tdTomato expression. **c,** Stimulated firing rates (each circle represents the mean firing rate during the stimulation period for an individual neuron) versus photostimulation frequency for all recorded Bar$^{CRH\text{-}ChR2}$ (magenta, n=6), Bar$^{ESR1\text{-}ChR2}$ (green, n=12), and Bar$^{ESR1\text{-}GFP}$ (orange, n=4) neurons. Most neurons, particularly Bar$^{CRH\text{-}ChR2}$, are affected by depolarization block at 50 Hz. Scale bars = 100 μm.



Supplementary Figure 5

Frequency characteristics of urethral sphincter bursting during natural behavior and after Bar photostimulation.

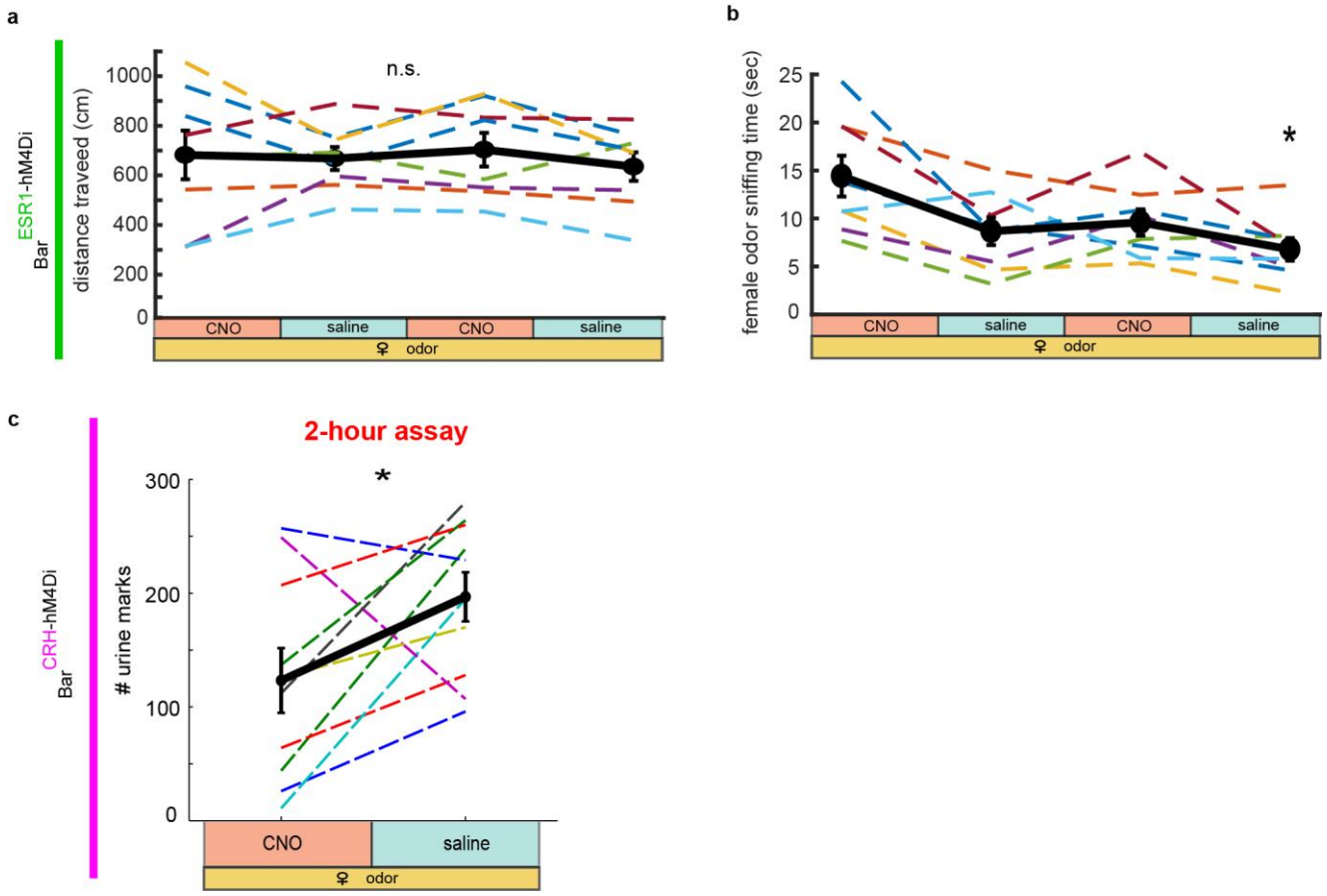
a, Schematic for optogenetic Bar stimulation during EUS EMG recording. **b,** Example raw EUS EMG and corresponding spectral power in the 5-15 Hz band for photostimulated burst responses in $Bar^{CRH\text{-}ChR2}$ (top) and $Bar^{ESR1\text{-}ChR2}$ (bottom) mice. $Bar^{CRH\text{-}ChR2}$ burst is preceded by an increase in tonic activity during the photostimulation period, whereas $Bar^{ESR1\text{-}ChR2}$ burst occurs at low latency without preceding tonic activity, and displays decreasing frequency characteristic of natural bursts in (f). **c,** Example video frame from wireless corpus spongiosum pressure recording in the presence of female odor (yellow shading). **d,** Schematic of corpus spongiosum recording setup. **e,** Corpus spongiosum pressure recording after presentation of female odor. Top, raw pressure; bottom, spectral power in the 5-15 Hz band. Yellow arrows mark approximate start times for urination events. **f,** Shorter timescale recordings as in (e), for 5 urination events across 2 mice. Binary images on the left show relative sizes of thresholded urine marks corresponding to bursts on the right. Frequency typically decreases over a large burst lasting a few seconds, although shorter bursts with less urine were also observed (2^{nd} from top).



Supplementary Figure 6

Bar$^{ESR1\text{-}ChR2}$ photostimulation can enable urethral sphincter relaxation without bladder contraction.

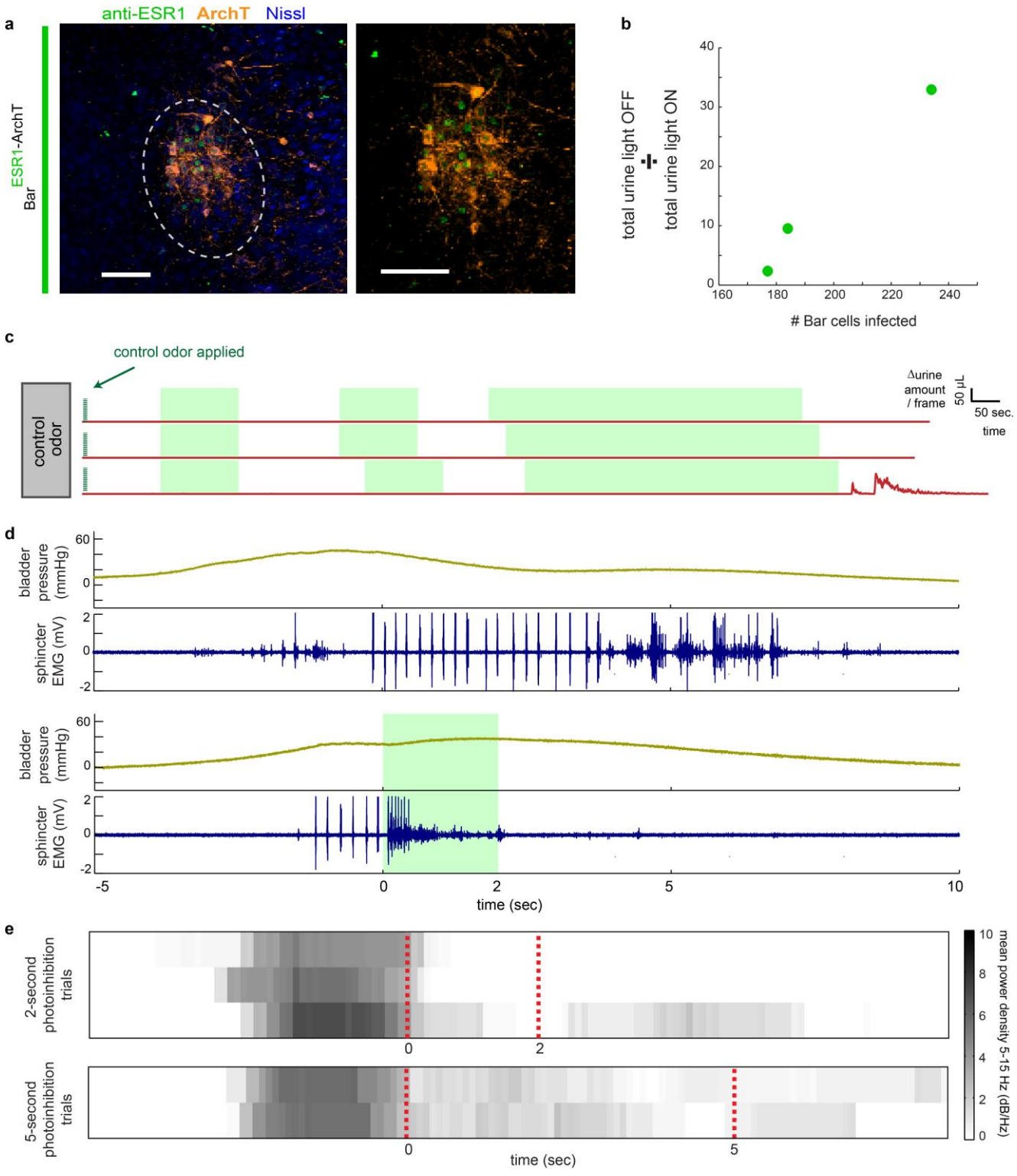
a, Three example Bar$^{ESR1\text{-}ChR2}$ photostimulation trials in the empty bladder condition (from heatmap in Figure 5b), in which burst-like EMG activity was observed in the absence of bladder response. Top/yellow traces are bladder pressure, bottom/red traces are raw EMG. Blue shading and dotted lines delineate photostimulation periods.



Supplementary Figure 7

Behavioral controls for Bar$^{ESR1\text{-}hM4Di}$ and Bar$^{CRH\text{-}hM4Di}$ chemogenetic inhibition.

a, Total distance traveled during the assay shown in Figure 7a-e for Bar$^{ESR1\text{-}hM4Di}$ mice (n=8). Thin dotted lines are individuals, thick black line is mean ± s.e.m. p=0.58 Friedman's test. **b,** Same as (b), but for total female urine odor sniffing time. p=0.021 Friedman's test, *day 4 saline p=0.012 Dunn-Sidak posthoc differences from CNO day 1. **c,** Analysis of Bar$^{CRH\text{-}hM4Di}$ mice (n=10) injected with either saline or CNO on consecutive days prior to a 2-hour urination assay, similar to that previously published[15] and which is not limited to odor-evoked, voluntary urination. *p=0.049, Mann-Whitney U test for difference between CNO and saline treatment days.

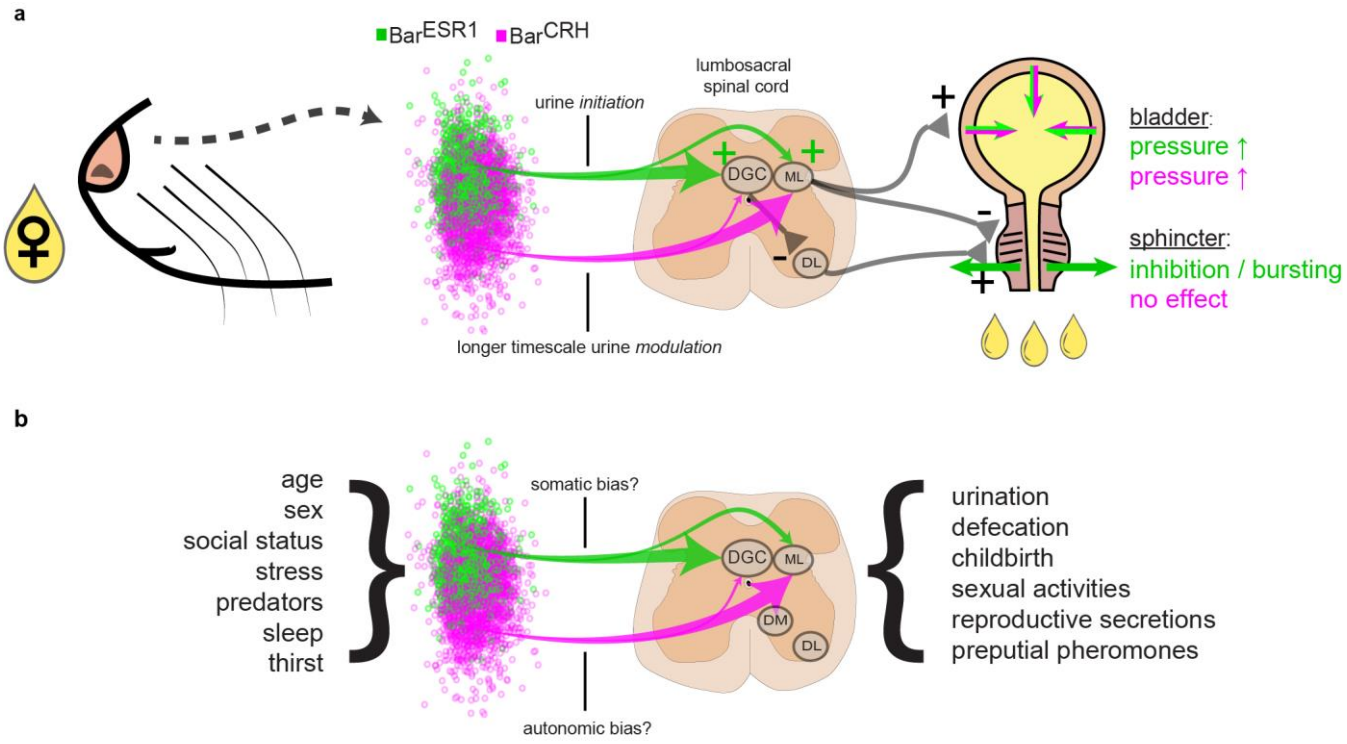


Supplementary Figure 8

Bar$^{ESR1\text{-}ArchT}$ photoinhibition terminates sphincter bursting during cystometry and does not result in rebound urination in awake mice.

a, Example ArchT-GFP expression in Bar of ESR1-Cre mouse; right, larger views minus Nissl. **b,** Number of Bar cells

infected with ArchT virus versus total urine with light OFF (not inhibited) divided by that with light on (while inhibited) for all mice (n=3). **c,** ∆urine amount around two 30 second photoinhibition periods followed by one 2 min. photoinhibition period, during which control odor only was present. n=9 total photoinhibition bouts from 3 mice. **d,** Bladder pressure and sphincter EMG during cystometry, top, with natural unimpeded cycling, and bottom, in which 2 seconds of $Bar^{ESR1\text{-}ArchT}$ photoinhibition was triggered as soon as filling evoked bursting was detected, which terminates bursting within ~100ms, often followed by tonic contractions such as with cessation of ChR2 photostimulation. **e,** Heatmap of mean EMG power density at bursting frequencies (5-15 Hz) during $Bar^{ESR1\text{-}ArchT}$ photoinhibition as in bottom of panel d (top: 2 second inhibition trials; bottom: 5 second inhibition trials; n=2 mice). Green shading or red dotted lines mark photoinhibition periods. Scale bars = 100 μm.



Supplementary Figure 9

Simplified summary of a nose-to-sphincter circuit and other potential Bar functions.

a, Bar^{ESR1} (green) and Bar^{CRH} (magenta) neurons are intermingled (cell overlay from Fig. 1d), and the minority Bar^{ESR1} population projects to both the mediolateral column (ML) and heavier to the dorsal grey commissure (DGC), which directly inhibits sphincter motoneurons in the dorsolateral nucleus (DL). Activation of Bar^{ESR1} neurons increases bladder pressure and simultaneously inhibits the sphincter via bursting, thus driving efficient urine excretion, whereas activation of Bar^{CRH} neurons produces a focal increase in bladder pressure and either no effect at the sphincter or a tonic excitation resembling the guarding reflex. Thoracolumbar projections of Bar^{ESR1} neurons are not shown, as well as afferent feedback connections from bladder and urethra. **b,** Many factors influence a variety of pelvic functions, and heterogeneity in Bar may allow differential coordination of both somatic and autonomic targets for various behaviors.