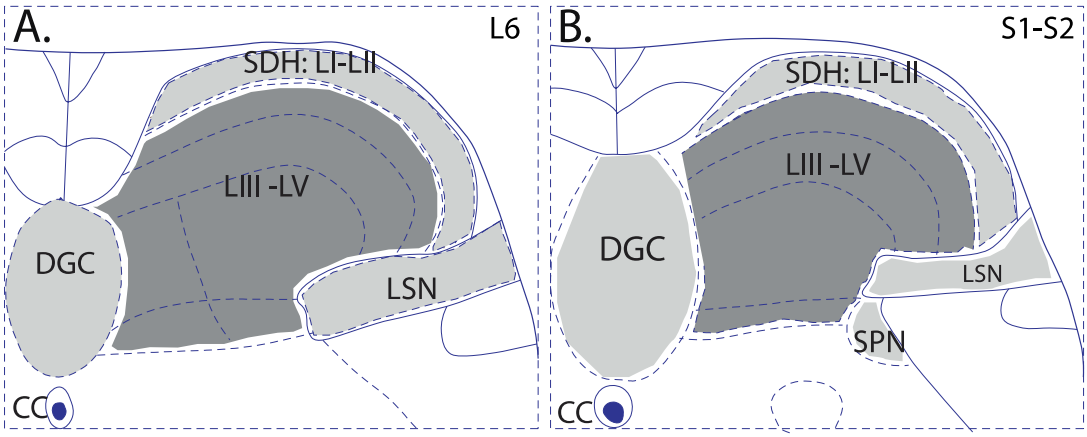
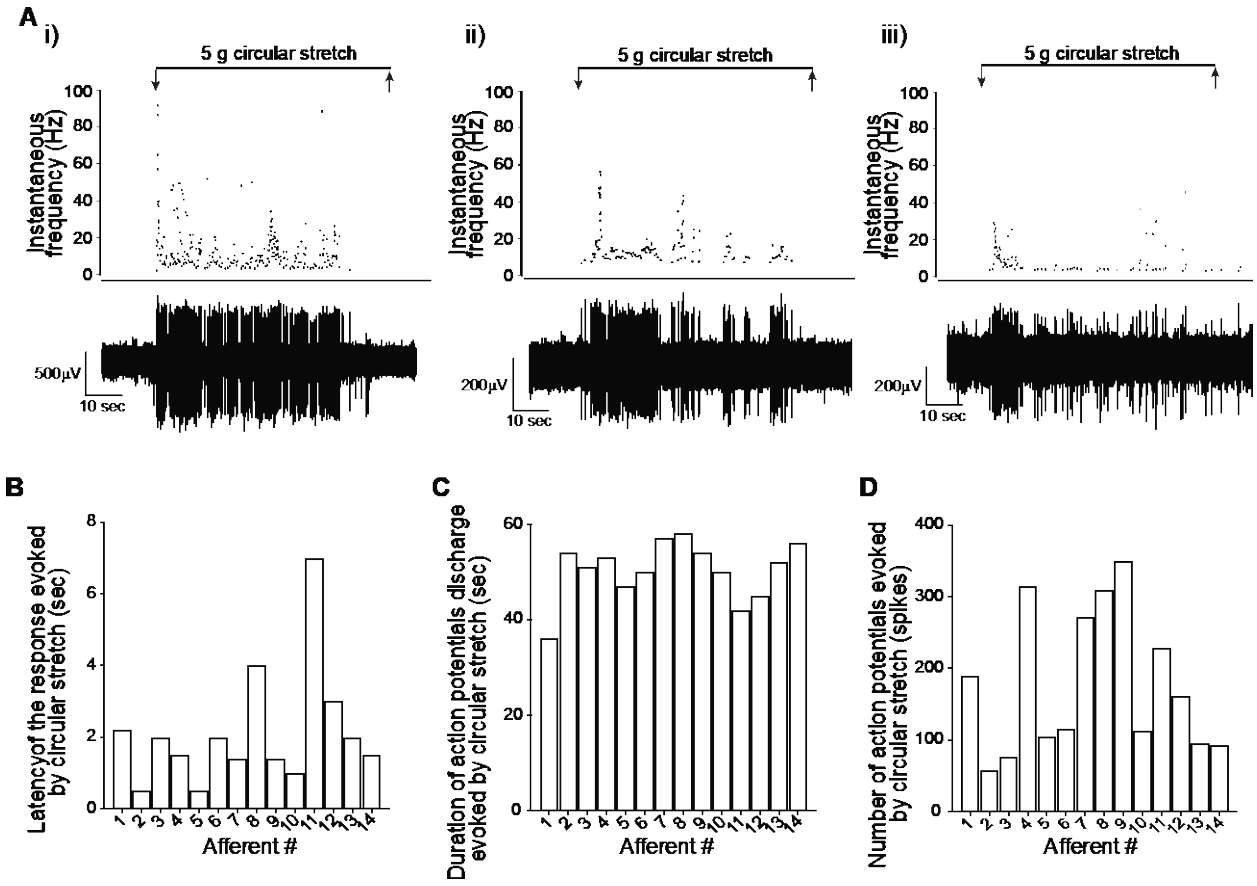
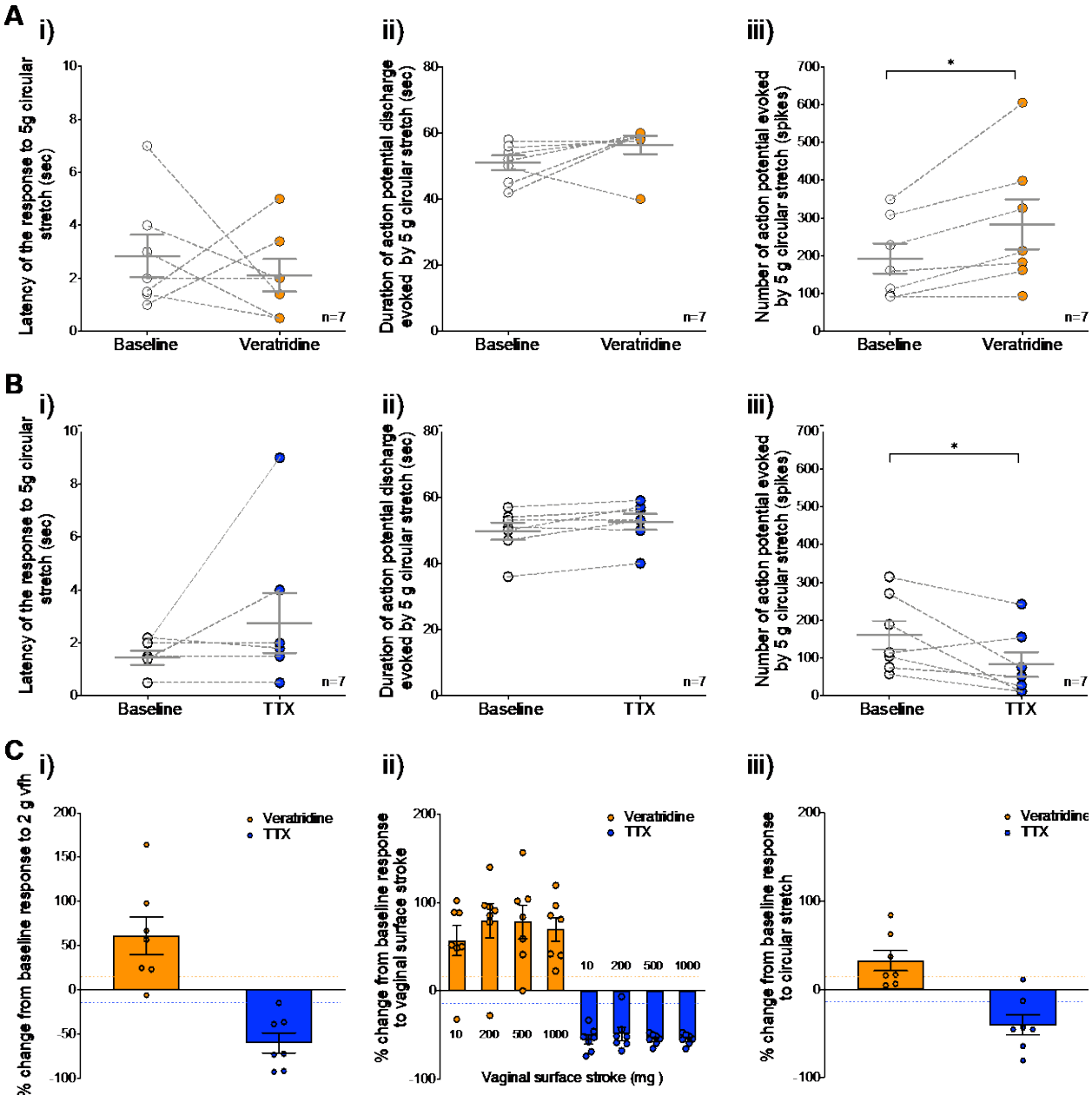
Supplementary Figures:



Supplementary Figure 1: Schematic overview of the mouse lumbosacral spinal cord (L6-S2). Illustration of a cross section of the lumbosacral spinal cord (L6-S2) showing dorsal horn laminae and regions in which the number of pERK-IR neurons was compared between experimental groups. Map adapted from 2008 Allen Institute for Brain Science©. Allen Spinal Cord Atlas. Available from: https://mousespinal.brain-map.org.



Supplementary Figure 2: Wide dynamic range of pelvic vaginal afferent responses to circular stretch of the vagina. (A, i-iii) Representative traces showing heterogeneity in the profile of pelvic afferent action potential discharges in response to circular stretch of the vagina (5 g stretch for 1 minute). **(B)** Time for each individual afferent to fire its first action potential upon stimuli application (latency of the response). **(C)** Duration of action potential discharge from each individual vaginal afferent. **(D)** Number of action potentials fired by each individual vaginal afferent throughout the duration of the stimuli.

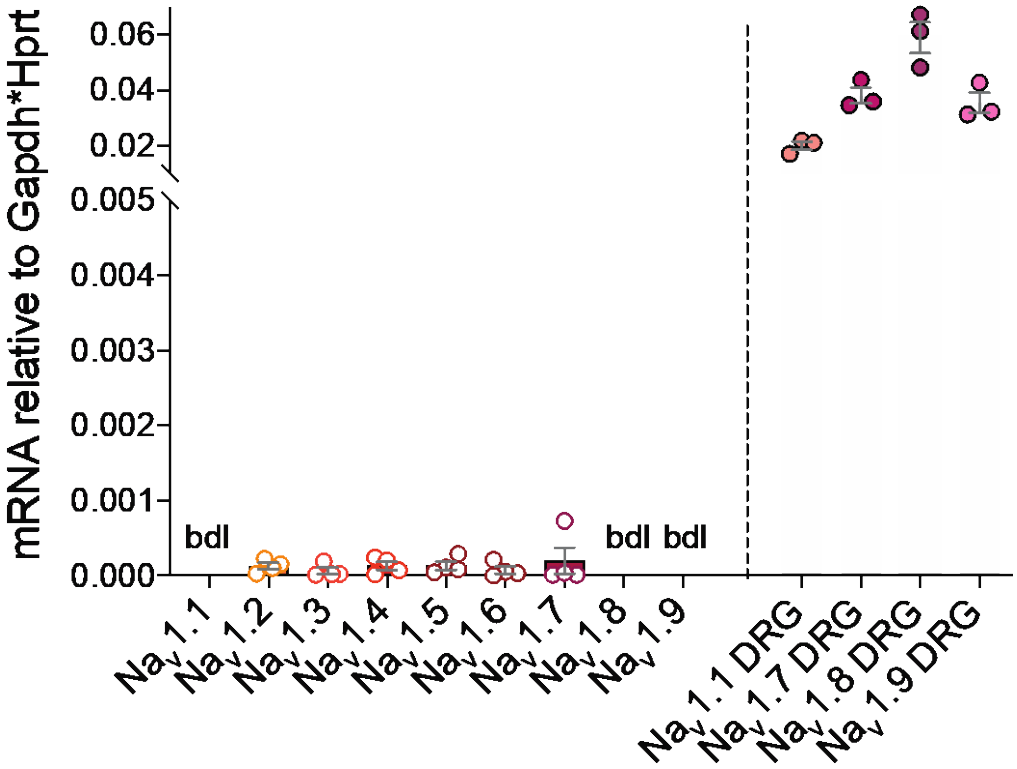


Supplementary Figure 3: Response of pelvic vaginal afferents to circular stretch of the vagina can be modulated *ex vivo* by targeting Na_V channels.

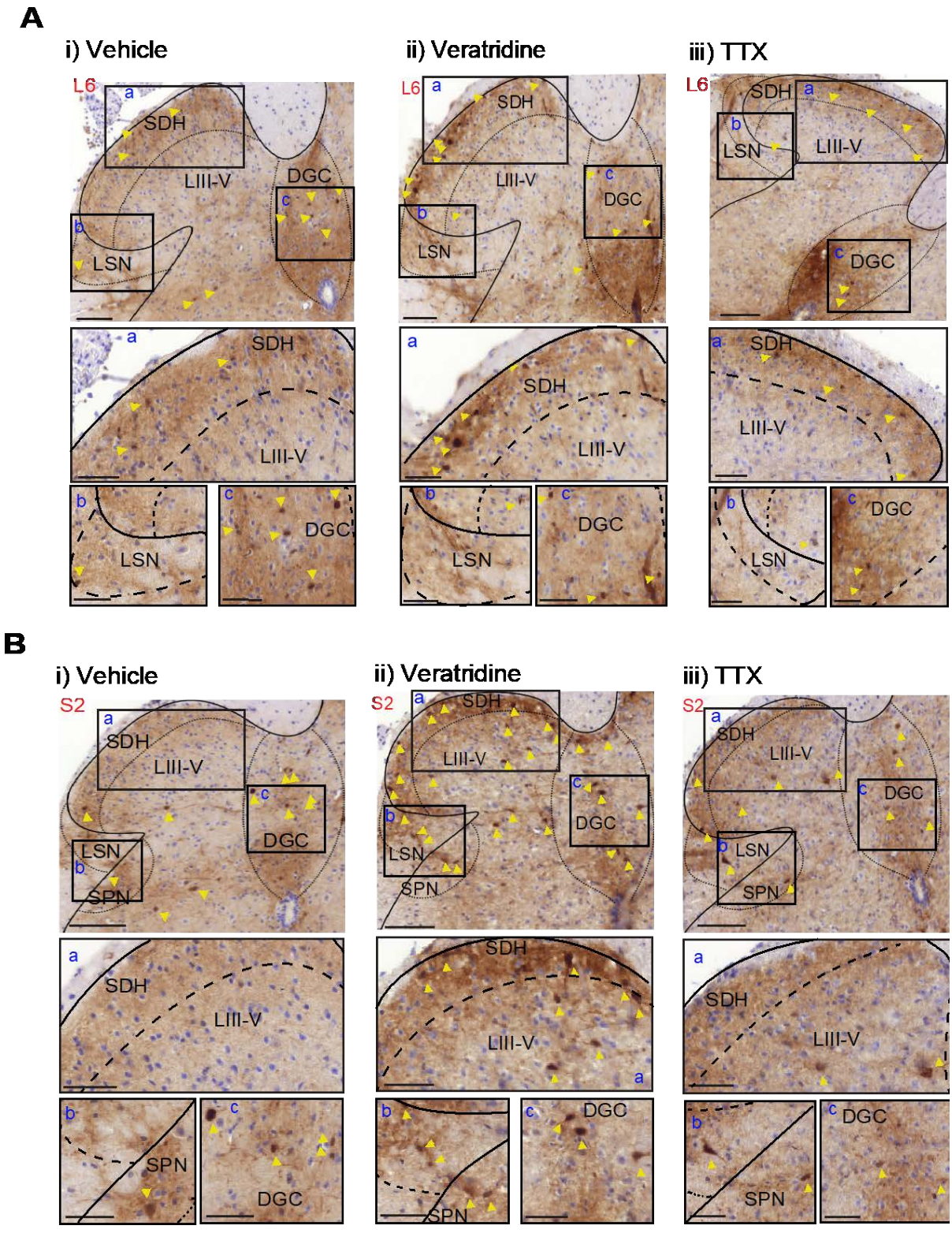
(A) Individual data showing the response profile of pelvic vaginal afferents to circular stretch at baseline (open circles) and after afferent receptive field exposure to veratridine (orange circles). Veratridine application had no overall effect on **(i)** the time between stimulus application and the

firing of the first action potential (latency of the response, P>0.05, paired Student t-test), and **(ii)** the duration of afferent firing (P>0.05, paired Student t-test). **(iii)** There was a significant increase in the total number of action potentials fired by pelvic vaginal afferents after veratridine incubation (*P<0.05, paired Student t-test). Data was obtained from n=7 afferents from N=6 mice **(B)** Individual data showing the response profile of pelvic vaginal afferents to circular stretch at baseline (open circles) and after afferent receptive field exposure to TTX (blue circles). TTX application had no overall effect on **(i)** the time between stimulus application and the firing of the first action potential (latency of the response) (P>0.05, paired Student t-test), and **(ii)** the duration of afferent firing (P>0.05, paired Student t-test). **(iii)** TTX incubation significantly reduced the total number of action potentials fired by pelvic vaginal afferents (*P<0.05, paired Student t-test). Data was obtained from n=7 afferents from N=5 mice.

(C) Grouped data obtained with the ex vivo single-unit extracellular nerve recording preparation showing veratridine- and TTX-induced changes relative to the baseline mechanosensory response of vaginal afferents. Changes are expressed as percentage change from baseline response to **(i)** a 2 g vfh, **(ii)** graded stroke of the vaginal surface and **(iii)** circular stretch of the vagina. Horizontal dotted lines indicate a 15% change in mechanosensory response relative to baseline for both veratridine (orange) and TTX (blue).



Supplementary Figure 4: mRNA expression profile of Na_V transcripts within the mouse vagina and whole LS DRG. Na_V channel ($Na_V1.1$- $Na_V1.9$) mRNA expression in vaginal tissue relative to the housekeeper genes Gapdh*Hprt. $Na_V1.7$ is the most expressed Na_V transcript in the vagina. $Na_V1.2$-1.6 are barely detected, whilst $Na_V1.1$ and $Na_V1.8$-1.9 are below the level of accurate detection. Vaginal tissues were obtained from N=4 mice. $Na_V1.1$ and $Na_V1.7$-1.9 expression in LS DRG are provided as a reference. LS DRG were bilaterally obtained from N=3 mice.



Supplementary Figure 5: Intravaginal administration of Na_V channel modulators alters

signalling in lumbosacral regions (LS: L6 and S2) of the spinal cord in response to vaginal distension *in vivo*.

Representative images of cross-sections from the **(A)** lumbar (L6) and **(B)** sacral (S2) spinal cord labelled for pERK-IR from mice treated with **(i)** saline, **(ii)** 50 µM veratridine or **(iii)** 0.5 µM TTX prior to vaginal distension. Yellow arrowheads indicate pERK-IR neurons. Scale bars in upper panels represent 100 µm. The bottom panels for both **A** and **B** are higher magnification images of the different spinal area areas highlighted in the top panels**.** Yellow arrowheads indicate pERK-IR neurons. Scale bars in lower panel represent 50 µm. Abbreviations: dorsal grey commissure (DGC), lateral spinal nucleus (LSN), sacral parasympathetic nucleus (SPN), superficial dorsal horn (SDH).