

# **Veratridine produces distinct calcium response profiles in mouse Dorsal Root Ganglia neurons**

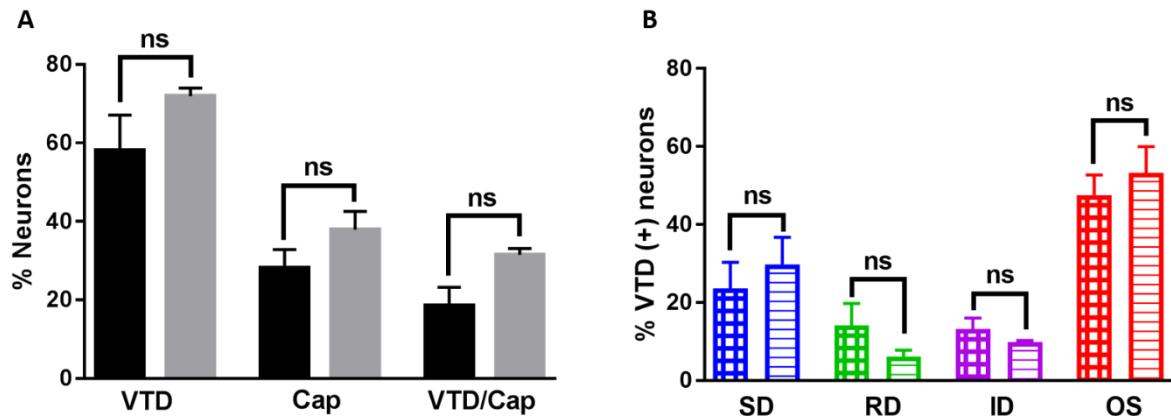
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Supplementary figures to Zainab et al

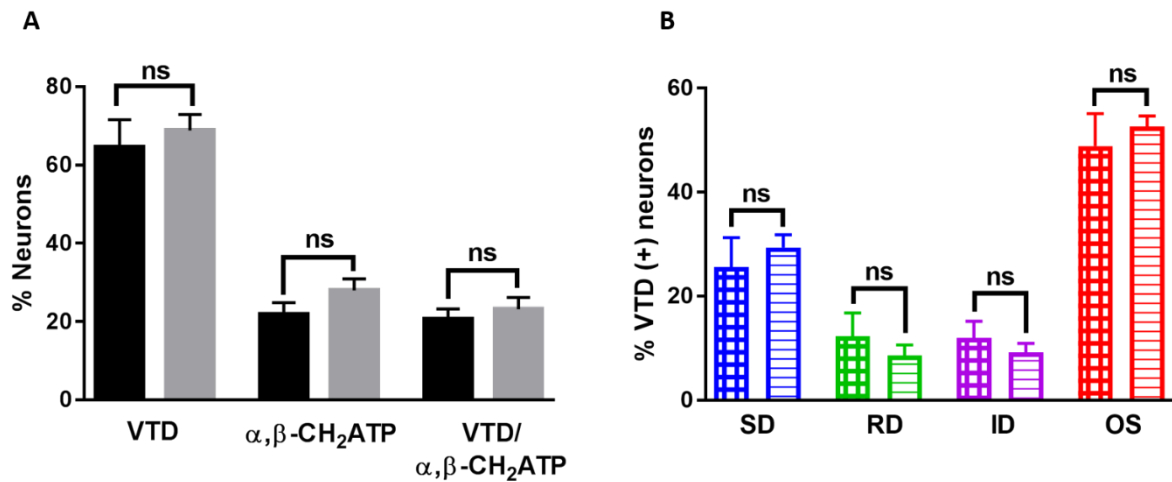
Figure S1:



**Supplementary S1: Order of veratridine and capsaicin application does not affect VTD response profiles.**

**A.** Percentage of neurons that responded to VTD, capsaicin (CAP) and VTD plus capsaicin (VTD+/CAP+) when VTD was applied before 1  $\mu$ M capsaicin (black bars; N = 5 mice, n = 200 cell) and when VTD was applied after 200 nM capsaicin (grey bars; N = 3 mice, n = 379 cell). Percent responses between VTD first and VTD second protocols were not significantly different. Percent of neurons responsive to VTD ( $58 \pm 9$  %, 112 cell vs.  $71.8 \pm 2$  %, n = 274 cell); percent of neurons sensitive to capsaicin ( $28.1 \pm 5$  %, n = 59 cell vs.  $37.9 \pm 4.6$  %, n = 164 cell) and percent of neurons responsive to both VTD and capsaicin ( $31.5 \pm 1.6$  %, n = 119 cell vs.  $18.5 \pm 5$  %, n = 37 cell) were not different. **B.** The frequency of occurrence of the four VTD profiles is not different when VTD is applied first (crossed bars, N = 3 mice) and second (horizontal lines, N= 3 mice). The frequencies for SD response profile are ( $23.6 \pm 7$  %, n = 29 cell vs.  $29.7 \pm 7$  %, n = 82 cell), for RD response profile are ( $14.1 \pm 6$  %, n = 13 cell vs.  $6.3 \pm 2$  %, n = 17 cell), for ID response profile are ( $13.2 \pm 3$  %, n = 15 cell vs.  $9.8 \pm 0.4$  %, n = 27 cell) and for OS response profile are ( $47.5 \pm 5$  %, n = 53 cell vs.  $53.3 \pm 7$  %, n = 145 cell). Data shown are mean  $\pm$  SEM. One-way analysis of variance with Sidak's post-test.

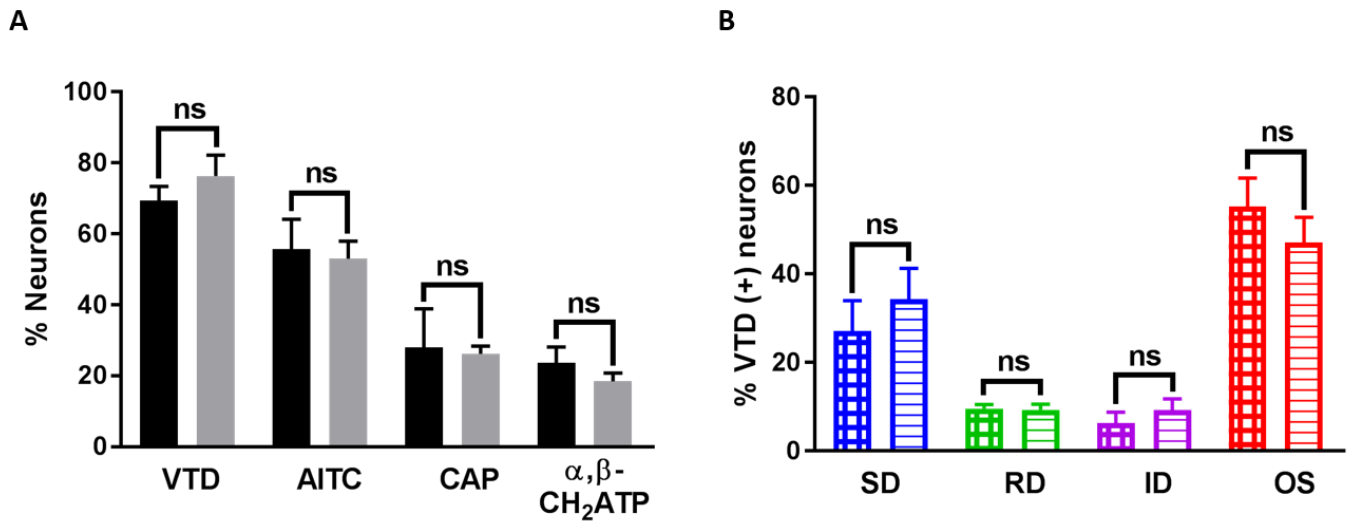
Figure S2:



**Supplementary S2: the order of veratridine and  $\alpha, \beta$ -methylene ATP application does not affect**

**VTD response profiles. A.** Percentage of neurons that responded to VTD,  $\alpha, \beta$ -methylene ATP ( $\alpha, \beta$ -CH<sub>2</sub>ATP) and VTD plus  $\alpha, \beta$ -methylene ATP (VTD/ $\alpha, \beta$ -CH<sub>2</sub>ATP) when VTD was applied before  $\alpha, \beta$ -methylene ATP (black bars; N = 3 mice, n = 294 cell) and when VTD was applied after  $\alpha, \beta$ -methylene ATP (grey bars; N = 6 mice, n = 1051 cell). Percent responses between VTD first and VTD second protocols were not significantly different. Percent of neurons responsive to VTD ( $64.7 \pm 7$ , n = 201 cell vs.  $68.8 \pm 4$  %, n = 711 cell); percent of neurons sensitive to  $\alpha, \beta$ -methylene ATP ( $21.8 \pm 3$  %, n = 69 cell vs.  $27.9 \pm 3$  %, n = 275 cell); and percent of neurons sensitive to both VTD and capsaicin ( $20.7 \pm 3$  %, n = 65 cell vs.  $23.2 \pm 3$  %, n = 224 cell) are not different. **B.** The frequency of occurrence of the four VTD profiles is not different when VTD is applied first (crossed lines, N = 3 mice) and second (horizontal lines, N= 6 mice). The frequencies for the SD response profile are ( $25.6 \pm 6$ , n = 50 cell vs.  $29.4 \pm 3$  %, n = 208 cell), for the RD response profile are ( $12.3 \pm 5$  %, n = 20 cell vs.  $8.6 \pm 2$  %, n = 62 cell), for the ID response profile are ( $12 \pm 3$  %, n = 20 cell vs.  $9.2 \pm 2$  %, n = 70 cell) and for the OS response profile are ( $48.9 \pm 6$  %, n = 108 cell vs.  $52.7 \pm 2$  %, n = 370 cell). Data shown are mean  $\pm$  SEM. One-way analysis of variance with Sidak's post-test.

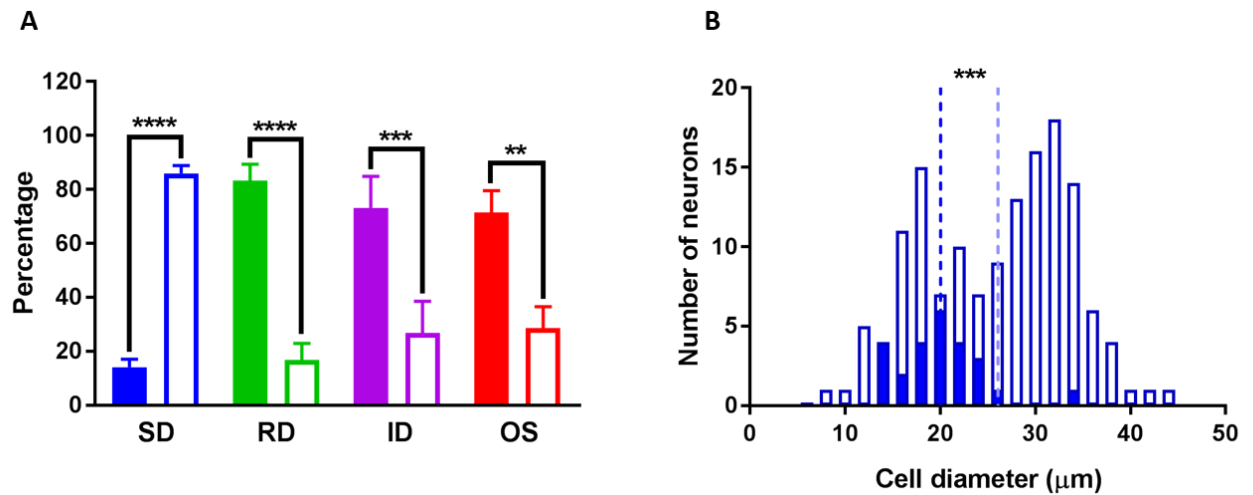
Figure S3:



**Supplementary S3: The order of Veratridine,  $\alpha$ ,  $\beta$ -methylene ATP, capsaicin and AITC application does not affect VTD response profiles. A.**

Percentage of neurons responding to VTD, allylthiocyanate (AITC), capsaicin (CAP), and  $\alpha$ ,  $\beta$ -methylene ATP ( $\alpha$ ,  $\beta$ -CH<sub>2</sub>ATP) when VTD was applied first (black bars; N = 3 mice, n = 220 cell) and when VTD was applied third (grey bars; N = 4 mice, n = 451 cell). Percent responses between VTD first and VTD second protocols were not significantly different. Percent of neurons responsive to VTD ( $69.4 \pm 4$  %, n = 150 cell vs.  $76.3 \pm 6$  %, n = 361 cell), AITC ( $55.7 \pm 8.5$  %, n = 128 cell, vs.  $53.1 \pm 5$  %, n = 229 cell), CAP ( $28 \pm 11$  %, n = 58 cell vs.  $26.2 \pm 2.1$  %, n = 115), and  $\alpha$ ,  $\beta$ -methylene ATP ( $23.7 \pm 4.4$  %, n = 54 cell vs.  $18.5 \pm 2.3$  %, n = 87 cell) are not different. **B.** The frequency of occurrence of the four VTD profiles is not different when VTD is applied first (crossed bars, N = 3 mice) and third (horizontal lines, N = 4 mice). The frequencies for the SD response profile are ( $27.1 \pm 6.9$  %, n = 13 cell vs.  $34.3 \pm 7$  %, n = 38 cell), for the RD response profile are ( $9.5 \pm 1$  %, n = 15 cell vs.  $9.3 \pm 1.3$  %, n = 26 cell), for the ID response profile are ( $6.3 \pm 2.5$  %, n = 9 cell vs.  $9.3 \pm 2.5$  %, n = 26 cell) and for the OS response profile are ( $55.2 \pm 6.5$  %, n = 71 cell vs.  $47.2 \pm 5.6$  %, n = 135 cell). Data shown are mean  $\pm$  SEM. One-way analysis of variance with Sidak's post-test.

Figure S4:



**Supplementary S4: Relationship between AITC sensitivity and the four veratridine profiles. A.** This data is extracted from data presented in figure 6. The proportion of AITC-sensitive (closed bars) and AITC-insensitive neurons (open bars) within each of the four VTD response profiles. Significantly a larger proportion of neurons with the SD (86 vs. 14 + 3 %) profile is insensitive to AITC. In contrast, significantly a larger proportion of neurons with the RD (83.2 vs. 16.8 + 6.2 %), ID ( 73.2 vs. 16.8 + 11.7 %) and OS (71.5 vs. 28.5 + 8 %) profiles are sensitive to AITC. Percentages were calculated from total number of neurons in each profile ( $n_{(SD)} = 168$ ,  $n_{(RD)} = 44$ ,  $n_{(ID)} = 48$ , and  $n_{(OS)} = 251$  neuron). Data shown are mean  $\pm$  SEM. One-way analysis of variance with Sidak's post-test, \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and \*\*\*\* $P < 0.0001$ . **B.** Histogram of diameter of neurons with the SD response profile showing that neurons sensitive to AITC (closed bars; mean 20 + 0.9  $\mu\text{m}$ , median 19.5  $\mu\text{m}$ ,  $n = 25$  cell) are significantly smaller than neurons insensitive to AITC (open bars; mean 26.1 + 0.6  $\mu\text{m}$ , median 27.9  $\mu\text{m}$ ,  $n = 143$  cell). Dotted lines indicate the mean. Two-tailed unpaired Student's t-test, \* $P < 0.05$ .