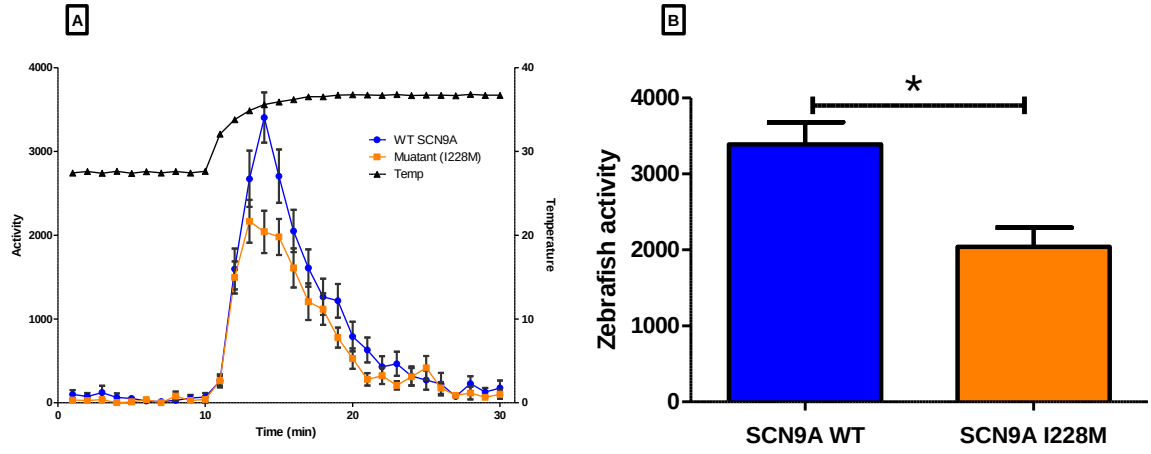

Figure 1. Zebrafish activity in response to temperature change.

Zebrafish locomotor (swimming) activity in response to temperature change was quantified by using a Zebrabox system (Viewpoint). Before the temperature interval was started, a baseline of zebrafish activity was recorded for 10 min. During this baseline measurement the temperature of the water is 28.5°C (black line). After the start of the temperature interval the maximum temperature of 35°C is reached within 3-4 minutes. (A) Overview of the activity of WT zebrafish (Blue line) and the mutant scn8aa Loss of function (LOF) line (orange) in response to temperature change (black line). (B) Quantification of the activity of WT (blue bar) and scn8aa LOF mutant zebrafish (orange bar) for a period of 5 minutes from the moment when the maximum temperature is reached. Statistical analysis was performed using a Student's t-test (P ≤ 0.01)

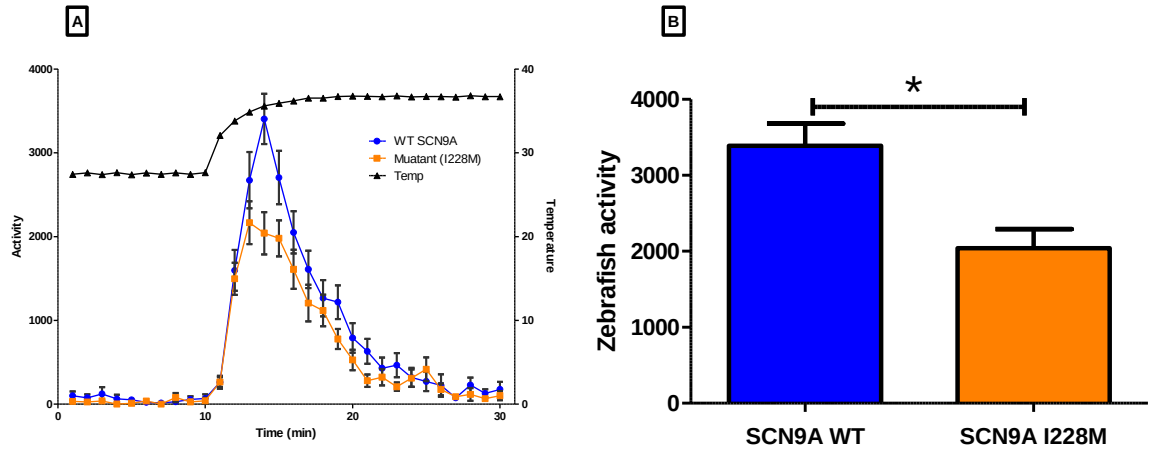

***Figure 2. Zebrafish activity in response to temperature change**.*

Zebrafish activity @ 4dpf in response to temperature change was quantified by using the Zebrabox system (Viewpoint). Before the temperature interval was started, a baseline of zebrafish activity was recorded for 10 min. During this baseline measurement the temperature of the water is 28.5°C (black line). After the start of the temperature interval the maximum temperature of 36°C is reached within 3-4 minutes. (A) Overview of the activity of WT zebrafish (Blue line) and the mutant (I228M) line (orange) in response to temperature change (black line). (B) Quantification of the activity of WT (blue bar) and mutant zebrafish (orange bar) at the maximum temperature. Statistical analysis was performed with a Student's t-test (P ≤ 0.05)

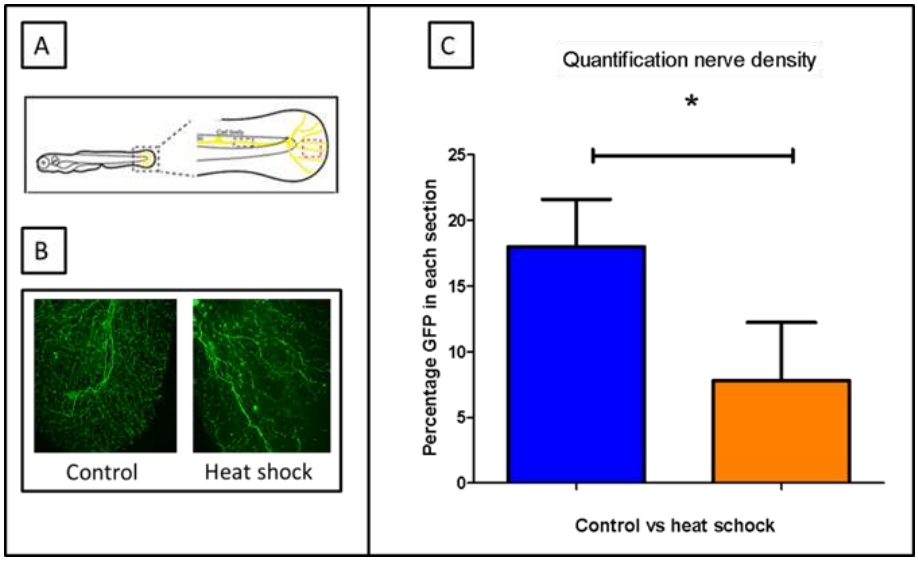

Figure 3: Quantification of the density of sensory neurons in zebrafish embryo's after heat shock.

The tail section of the zebrafish (A) is a very thin section and gives a good representation of the nerve fibers in the zebrafish (Campbell, P. D. et al. J. Neurosci. 34, 14717–14732 (2014)). After a heat shock of 5 seconds at 42°C the nerve density of the zebrafish is significantly reduced (B and C). Quantification is performed with the ImageJ plugin particle analyzer after thresholding. For each recording the percentage of GFP was calculated in 3 at random areas (C).

Figure 4. Confocal recordings WT (left panel) vs I228M-Mutant (right panel)

Confocal recordings of the tail section of WT (left) and Mutant (right) 2-day-old zebrafish; maximal projection of Z-stack.

Table 1: Functional assessment of variants in voltage-gated sodium channels

Mutation	Voltage-clamp	Current-clamp	Reference
		Nav1.6	
Leu1284Gln	Not Done	No Change	unpublished
		Nav1.7	
Met130Ile	No Change	Not Done	unpublished
Lys665Arg	No Change	Not Done	unpublished
Val810Met	Not Done	No Change	unpublished
Thr920Asn	No Change	Not Done	unpublished
Met1230Thr	No Change	Not Done	unpublished
Asn1245Ser	No Change	Not Done	unpublished
Met1852Thr	Gain-of-Function	Not Done	Pending
		Nav1.8	
Pro721Leu	Gain-of-Function	Pending	Unpublished
Arg1582His	Loss-of-function	Increased Excitability	Unpublished
		Nav1.9	
Ile381Thr	Gain-of-Function	Increased Excitability	Huang et al 2014
Ala681Asp	No current	Not Done	Unpublished
Lys682Ile	Not Done	No Change	Unpublished
Gly699Arg	Gain-of-Function	Increased Excitability	Han et al 2015
Ile807Val	Gain-of-Function	Pending	Unpublished
Ala864Pro	No Major change	No Major Change	Unpublished
Leu1158Pro	Gain-of-Function	Increased Excitability	Huang et al 2014
Val1437Met	Loss-of-Function	Not Done	Unpublished
Phe1689Leu	Mixed Phenotype	pending	Unpublished
		Na2	
Asp109Asn	Pending	Increased Excitability	Unpublished

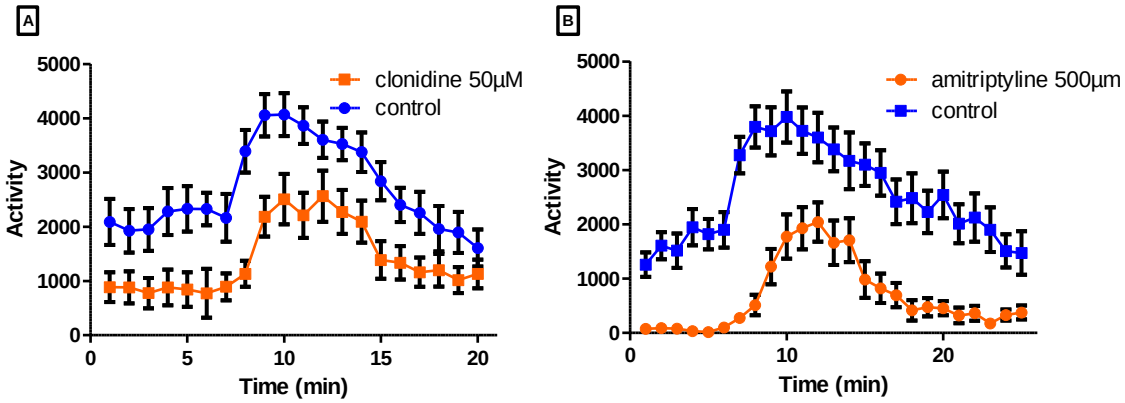

Figure 5. The effect of high concentrations of clonidine and amitriptyline on zebrafish activity in response to noxious temperatures.

The left panel represents the activity of 5-day-old zebrafish larvae in response to a temperature increase starting at 6 minutes and reaching a maximum of 36.5 °C. During these experiments, zebrafish larvae were incubated in Clonidine 50µM (A) and Amitriptyline 500µM (B).

Compound	Concentration
Carbamazepine	121µM
Lamotrigine	150µM
Amitriptyline	0.5µM
Clonidine	5µM

Table 2. Used concentrations of the compounds

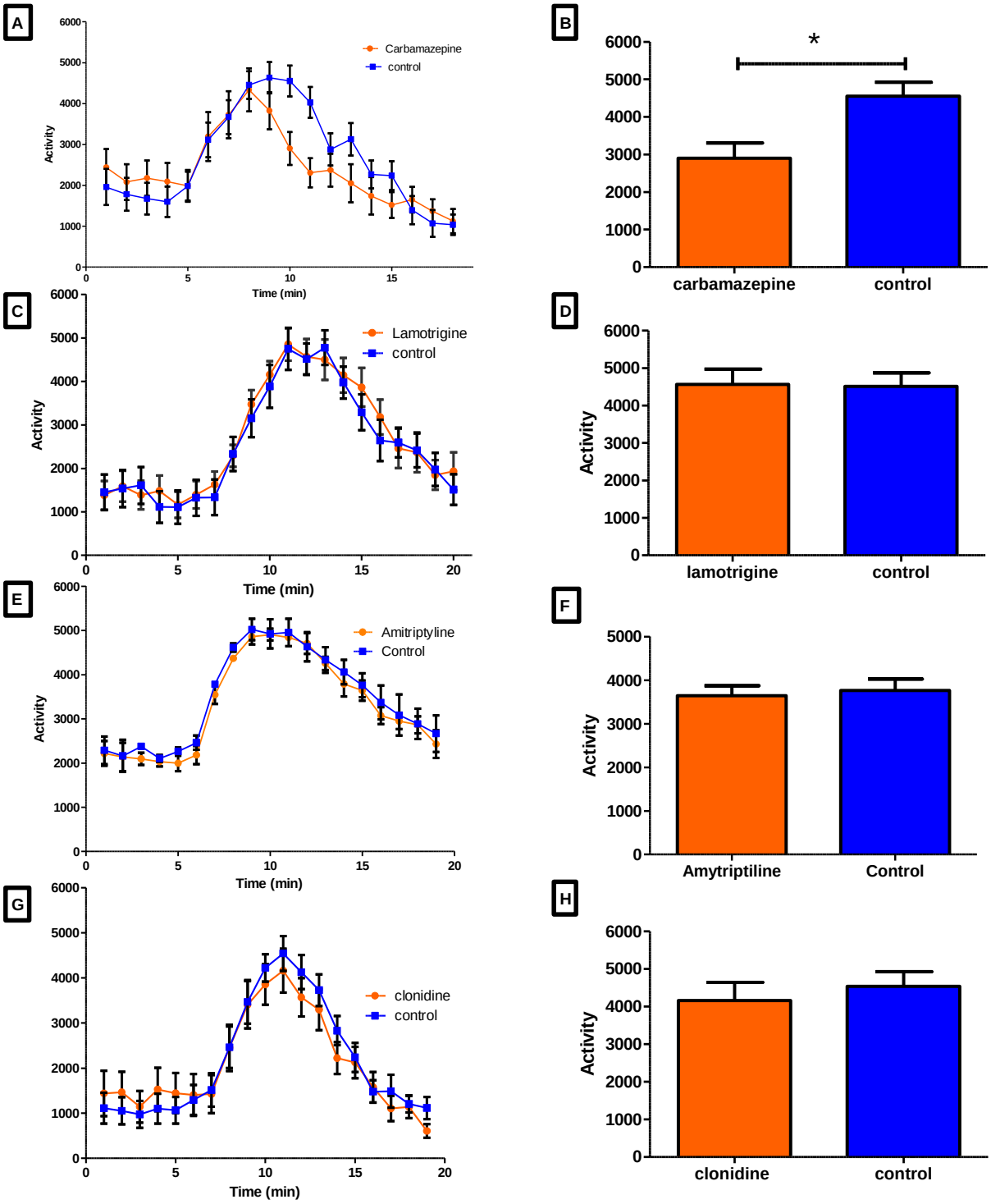

Figure 6. The effects of pain medication on zebrafish activity in response to noxious temperatures.

The left panel represents the activity of zebrafish in response to a temperature increase starting after 6 minutes and reaching a maximum of 36.5 °C. The right panel presents the activity of 5-day-old zebrafish at the maximal temperature (36.5 °C). The zebrafish were during the experiments incubated in carbamazepine (A,B), Lamotrigine (C,D), Amitriptyline (E,F) and Clonidine (G,H).

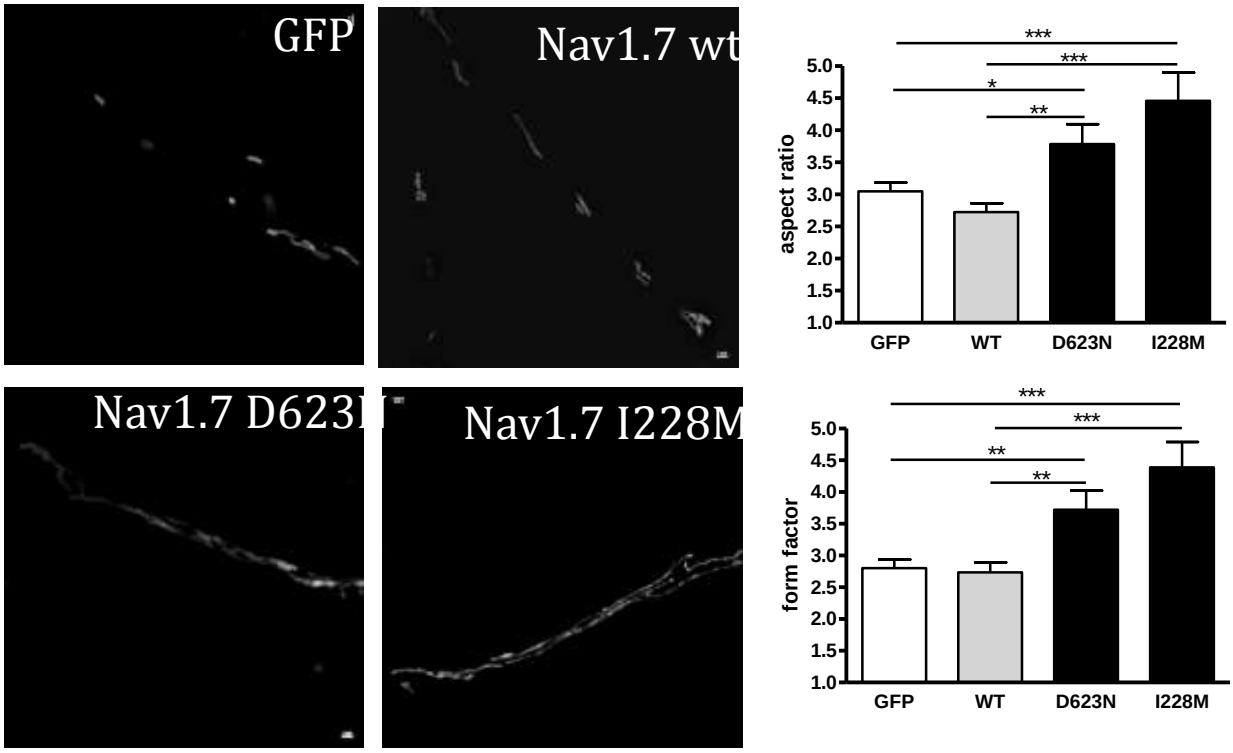

Figure 7. The effects of NaV1.7 mutation on mitochondrial shape.
Quantitative analysis of mitochondrial morphology in rat DRG neurons co-transfected with pDsRed2-Mito and GFP plasmid, Nav1.7 wt or mutant D623N and I228M. Representative confocal images show mitochondrial shape. The histograms show AR and FF media values for all mitochondria (>300 mitochondria particles, 10 cells analyzed per condition).