Supplementary Figure Legends

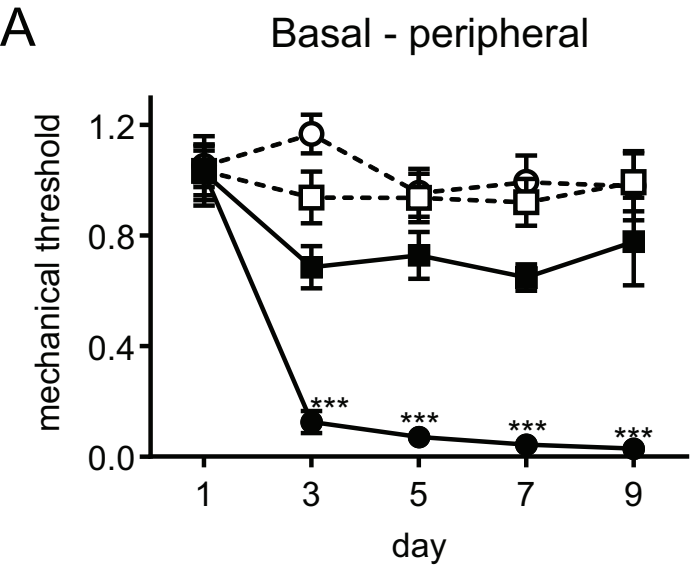
Supplementary Figure 1: Repeated SNC80 prevents the development of acute and chronic NTG-induced peripheral allodynia. A separate group of mice were tested as described in Figure 1, but hind paw mechanical responses were measured. (A) Over 9 days, NTG-VEH mice developed a chronic peripheral allodynia as measured before treatment on that day, an effect not observed in NTG-SNC80 treated mice. $p<0.001$ effect of treatment, time, and interaction, three-way ANOVA, Holm-Sidak post hoc analysis, ***p<0.001 relative to VEH-VEH on that day. (**D**) On each test day, NTG evoked acute allodynia, 2 hours post-injection, an effect that was not observed in the NTG-SNC80 treated groups. n=8/group. DOP activation prevents the development of acute and chronic peripheral allodynia associated with chronic migraine.

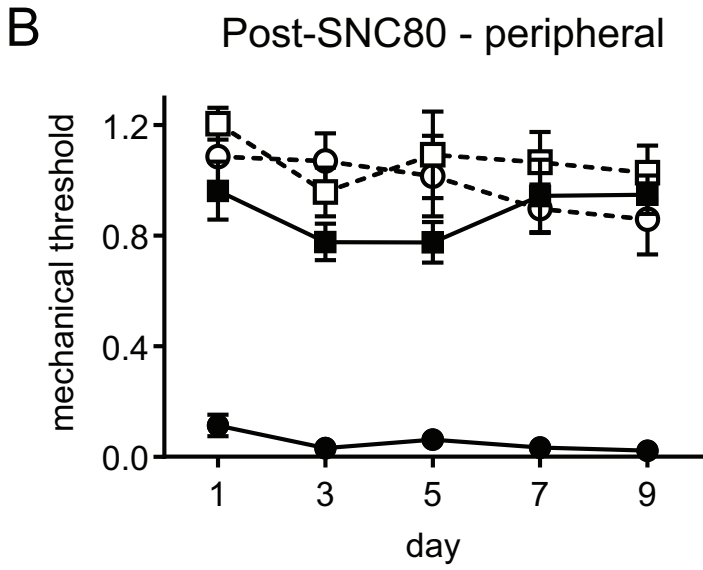
Supplementary Figure 2: Chronic NTG results in acute and chronic cephalic allodynia in DOPeGFP mice. A separate group of DOPeGFP mice were tested with chronic vehicle (VEH) or NTG (10 mg/kg IP). (A) Over 9 days, NTG mice developed a chronic cephalic allodynia as measured before treatment on days 1, 5, and 9. p<0.05 treatment and interaction, two-way RM ANOVA, Holm-Sidak post hoc analysis, ***p<0.001 relative to VEH on that day. (b) On each test day, NTG also evoked acute allodynia, 2 hours post-injection. **$p<0.001$ effect of treatment, two-way RM ANOVA, n=5/group.

Supplementary Figure 3: DOP is co-expressed with NF200, a marker of myelinated afferents. Representative image of DOP, IB4, and NF200 in trigeminal ganglia from DOPeGFP mice. Filled in arrowhead show DOP+ cells, outlined arrowhead show IB4+

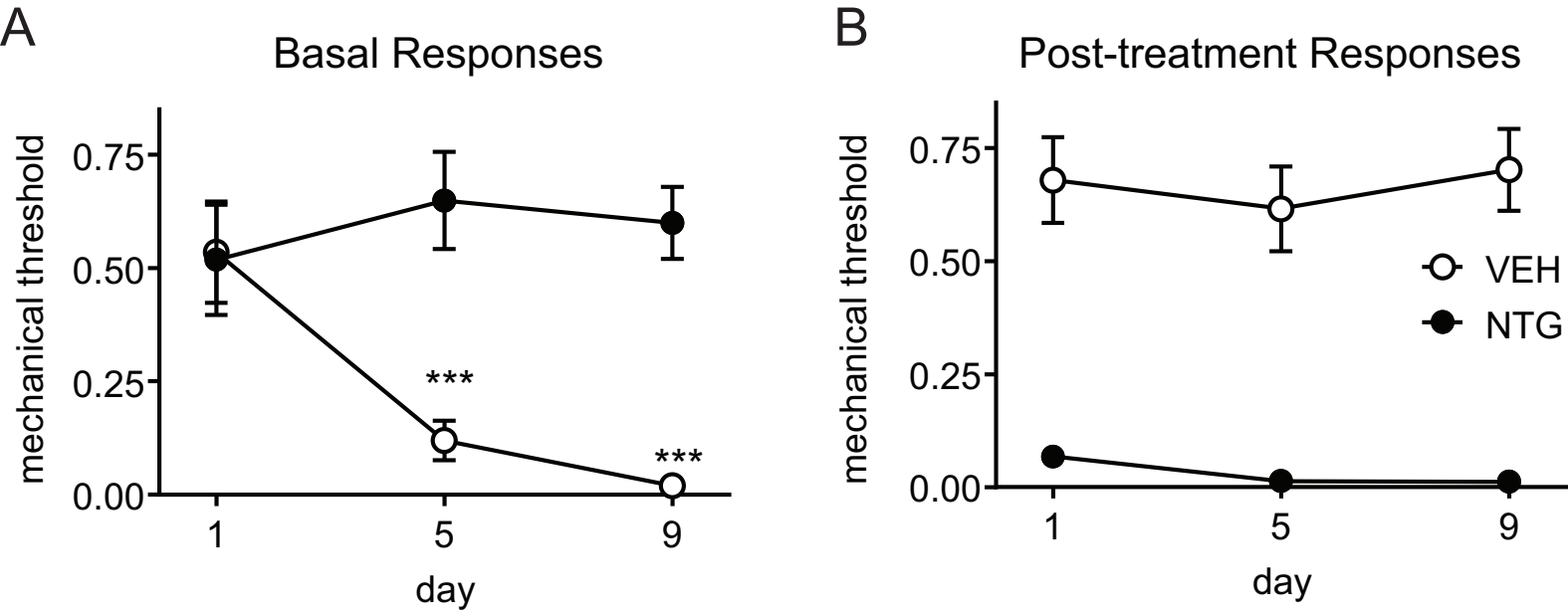
cells, filled arrows show NF200+ cells, and outlined chevrons show DOP+/NF200+ cells. There is a high co-expression between DOP and NF200, and little co-expression of IB4 with either of these markers.

Supplementary Figure 4: A proposed mechanism through which DOP agonists could inhibit migraine-associated pain. (A) The scheme summarizes the expression profiles of DOP, CGRP and CGRP receptor components observed in this study. We found co-expression between DOP and CRGP and DOP and the CGRP receptor component RAMP1 in trigeminal ganglia. We also observed that in the trigeminal nucleus caudalis CGRP was highly expressed in superficial lamina while DOP was expressed throughout the TNC. In the TNC there was high co-expression between DOP and the CGRP receptor components RAMP1 and/or CRLR. (B) We propose that inhibition of CGRP release and CGRP receptor signal propagation by DOP agonists could prevent the positive feedback loop which results in increased CGRP and chronic migraine associated pain.

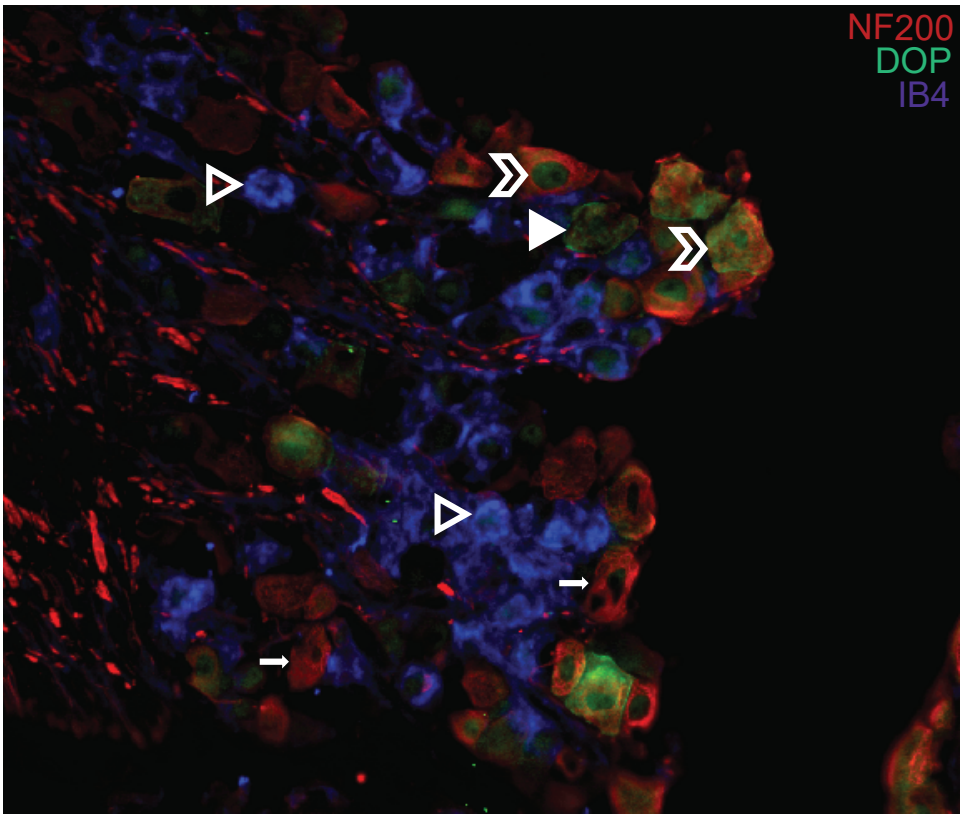




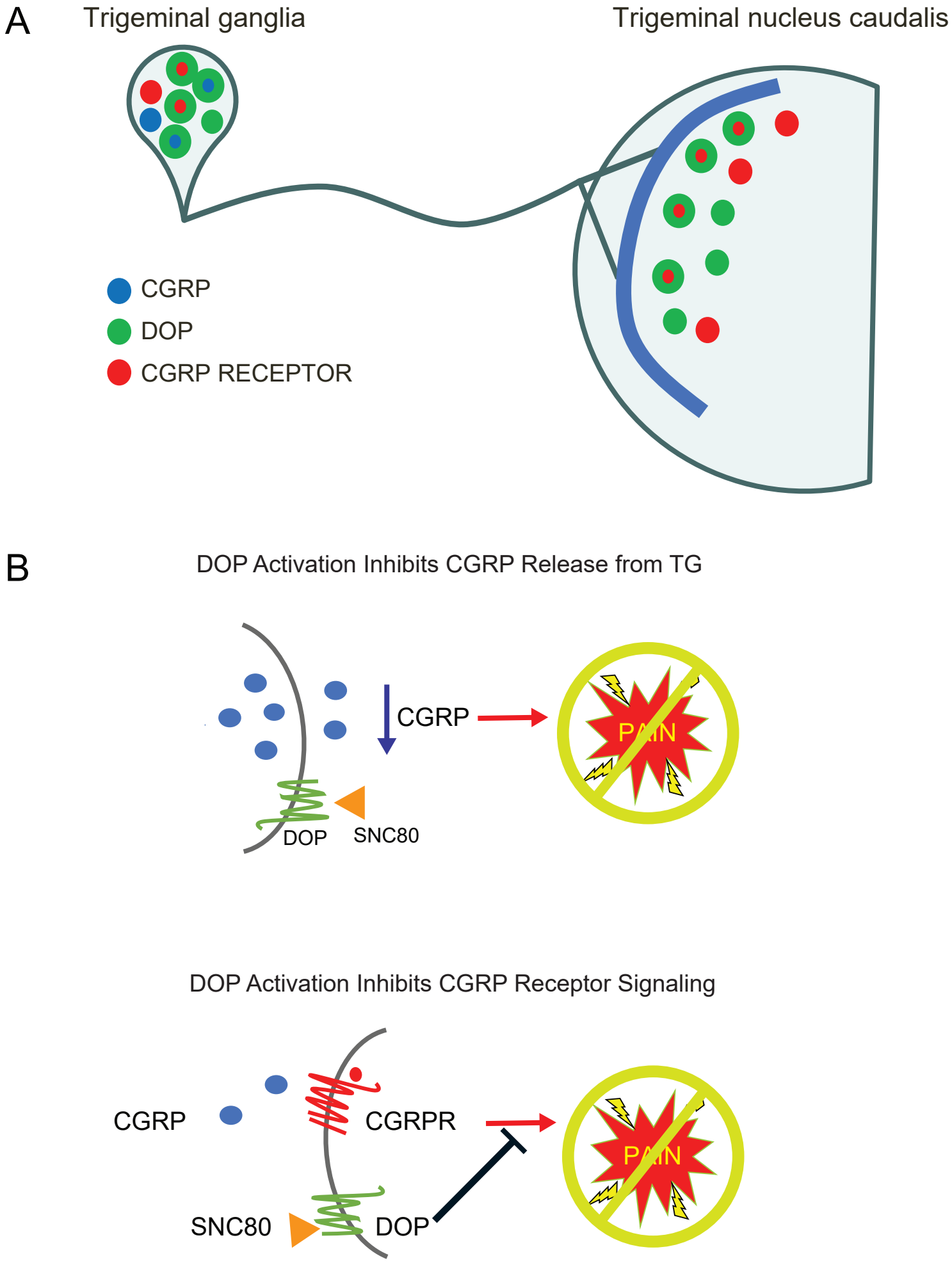
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

Supplementary Table 1: Statistical Analysis

Mixed Effects Model

Figure	Time			Veh-NTG			Saline-SNC80			Time Veh-NTG			Time Saline-SNC80			Veh-NTG Saline-SNC80			Interaction		
	Dfn,dfd	F	p	Dfn,dfd	F	p	Dfn,dfd	F	p	Dfn,dfd	F	p	Dfn,dfd	F	p	Dfn,dfd	F	p	Dfn,dfd	F	p
1C	2, 15	6.794	p=0.0079	1, 15	51.77	p<0.0001	1, 15	56.01	p<0.0001	2,15	12.53	p=0.0006	2,15	14.06	p=0.0004	1,14	76.54	p<0.0001	2,14	19.38	p<0.0001
1D	2, 15	2.031	p=0.1658	1, 15	206.9	p<0.0001	1,15	148.0	p<0.0001	2,15	0.1321	p=0.8773	2,15	2.577	p=0.1091	1, 14	198.6	p<0.0001	2, 14	0.01287	p=0.9872

3M+3F in each group

Three-Way ANOVA

Figure	Time			Veh-NTG			Saline-SNC80			Time Veh-NTG			Time Saline-SNC80			Veh-NTG Saline-SNC80			Interaction		
	Dfn,dfd	F	p	Dfn,dfd	F	p	Dfn,dfd	F	p	Dfn,dfd	F	p	Dfn,dfd	F	p	Dfn,dfd	F	p	Dfn,dfd	F	p
Supp 1A	4,35	11.28	p<0.0001	1,35	157.9	p<0.0001	1,35	33.43	p<0.0001	4,35	9.399	p<0.0001	4, 35	3.110	P=0.0273	1,35	60.06	p<0.0001	4,35	3.601	P=0.0146
Supp 1B	4, 35	2.229	p=0.0858	1,35	214.9	p<0.0001	1,35	84.88	p<0.0001	4,35	0.8626	p=0.4959	4,35	0.7926	P=0.5380	1,35	74.47	p<0.0001	4,35	0.1643	p=0.9551

3M+3F in each group

Two-Way ANOVA

Figure	Vehicle vs NTG			Vehicle vs SNC80			Interaction			M/F
	Dfn,dfd	F	p	Dfn,dfd	F	p	Dfn,dfd	F	p	
2B	1, 16	5.290	0.0352	1, 16	9.349	0.0075	1, 16	5.202	0.0366	3/3 each group
2C	1, 16	25.70	0.0001	1, 16	13.59	0.0020	1, 16	12.00	0.0032	
2E	1, 12	5.012	0.0449	1, 12	19.44	0.0009	1, 12	4.072	0.0665	
	Veh/NTG			Time			Interaction			
	Dfn,dfd	F	p	Dfn,dfd	F	p	Dfn,dfd	F	p	
Supp 2A	1, 8	9.545	0.0149	2, 16	2.320	0.1304	2, 16	3.397	0.0589	3/2 each group
Supp 2B	1, 8	86.20	<0.0001	2, 14	0.2956	0.7125	2, 16	0.1519	0.8603	

Unpaired t-test; two-tailed

Figure	F test to compare variances			Unpaired t Test			M/F
	dfn, dfd	F	P	P	T	df	
3C	5,5	1.340	0.7562	0.0041	3.703	10	3/3 each group
3D	5,5	13.84	0.0119	0.0004	5.143	10	
3E	5,5	44.02	0.0008	0.0442	2.300	10	
3F	5,5	36.66	0.0012	0.0121	3.055	10	
3G	5,5	1.2	0.8647	0.9979	0.0027	10	
3H	5,5	1.8	0.5379	0.9203	0.10	10	
4C	5,5	2.5	0.3360	0.0258	2.6	10	3/3 each group
4D	5,5	2.7	0.3002	0.8532	0.19	10	
4F	5,5	2.6	0.3100	<0.0001	7.1	10	
4G	5,5	1.1	09062	0.0100	3.2	10	
5B	5,5	4.4	0.1294	0.0762	2.0	10	3/3 each group
5C	5,5	1.1	0.9373	0.4797	0.73	10	
5D	5,5	3.8	0.1684	0.3347	1.0	10	
6A	5,5	4.1	0.1494	0.9555	0.057	10	4/2 vehicle; 2/4 NTG
6B	5,5	2.568	0.3238	0.0617	2.103	10	
6C	5,5	1.508	0.6632	0.2227	1.300	10	
6D	5,5	1.506	0.6644	0.9531	0.0604	10	