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Profiling the Functional Phenotype of Dorsal Root Ganglia Sensory Neurons from the K/BxN Murine Rheumatoid Arthritis Model

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Background

Rheumatoid arthritis (RA) is characterized by chronic and symmetric inflammation of synovial joints leading to joint destruction and a pain phenotype which often persists after resolution of the active inflammatory component. The murine K/BxN serum transfer model of arthritis displays an early inflammatory component and pain that occurs during and persists long after resolution of the inflammatory state. The late phase displays characteristics of a neuropathic phenotype (dorsal horn and DRG activation and an associated increases in synovial innervation as evidenced by increased CGRP+, TH+, and GAP-43+ fiber density). To assess the role of afferent excitability in this phenotype, DRG sensory neurons were prepared from K/BxN mice exhibiting allodynia 4 weeks post injection.

Methods

Groups of adult K/BxN transgenic mice were bled, the sera pooled, and transferred to recipient mice by intraperitoneal (IP) injection (100 µl on days 0 and 2). Tactile thresholds were measured with a series of von Frey filaments ranging from 2.44-4.31 (0.02-2.00 g). The 50% probability of withdrawal threshold was recorded. Joint inflammation in the paws was evaluated by visual inspection and rated on a scale of 1 to 28 where one point was given for each swollen digit and two points for each swollen ankle or wrist. Dorsal root ganglia were enzymatically and mechanically dissociated, and neurons were plated on glass coverslips. Standard patch clamp recording methods were used to profile the electrophysiological properties of sensory neurons prepared from K/BxN and naïve mice.

K/BxN Mice Exhibited Allodynia

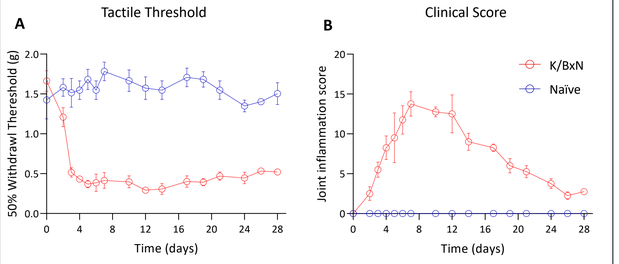
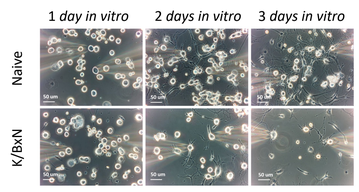


Figure 1. Characterization of K/BxN arthritis pain behavior. **(A)** K/BxN mice developed persistent significant tactile allodynia on days 2-28. **(B)** K/BxN mice developed significant clinical signs of arthritis on days 2-12 that decayed gradually after day 12. Joint inflammation in the paws was rated on a scale of 1 to 28 and shown as arthritis score in graph.

DRGs from K/BxN mice and naïve mice exhibited similar passive properties

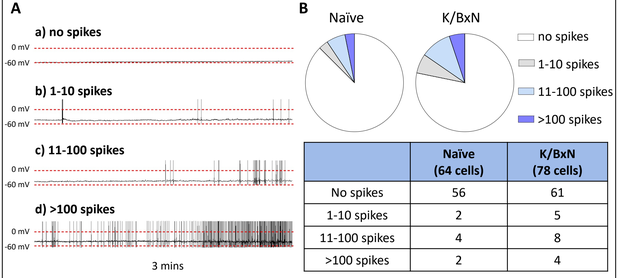


B

	Naïve	K/BxN	Total
Cm	34.7 ± 1.8 pF (57)	37.0 ± 1.6 pF (85)	36.1 ± 1.2 pF (142)
Rm	558 ± 65 MΩ (60)	603 ± 59 MΩ (89)	585 ± 44 MΩ (149)
Vm	-51.0 ± 1.0 mV (60)	-51.4 ± 1.0 mV (89)	-51.3 ± 0.7 mV (149)

Figure 2. **(A)** Sensory neurons from K/BxN and naïve mice exhibited similar size and shape. **(B)** Table of capacitance (Cm), input resistance (Rm) and resting membrane potential (Vm) from sensory neurons recorded from naïve and K/BxN mice. Values represent average ± sem.

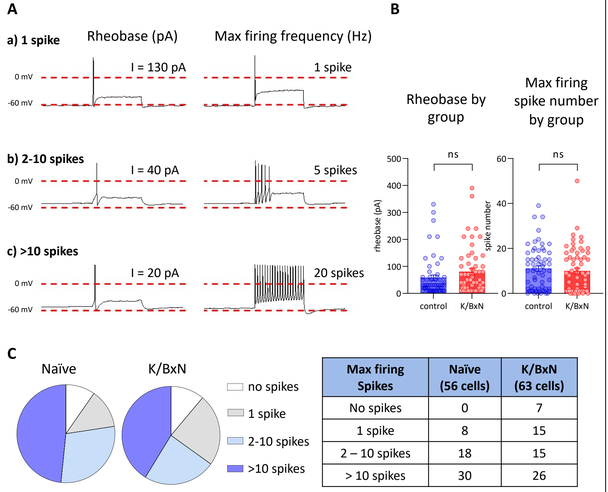
DRGs from K/BxN mice and naïve mice exhibited similar spontaneous activity



	Naïve (64 cells)	K/BxN (78 cells)
No spikes	56	61
1-10 spikes	2	5
11-100 spikes	4	8
>100 spikes	2	4

Figure 3. Most sensory neurons *in vitro* did not exhibit spontaneous activity. Only 14% (8 of 56) of naïve and 22% (17 of 78) K/BxN neurons fired spontaneous action potentials. **(A)** Representative sweeps of spontaneous action potentials (3 min) from four different neurons exhbiting a) no spontaneous activity, b) 1-10 spikes, c) 11-100 spikes, d) >100 spikes. **(B)** Frequency distribution of spontaneous activity.

DRGs from K/BxN mice and naïve mice exhibited similar evoked activity



Max firing Spikes	Naïve (56 cells)	K/BxN (63 cells)
No spikes	0	7
1 spike	8	15
2 – 10 spikes	18	15
> 10 spikes	30	26

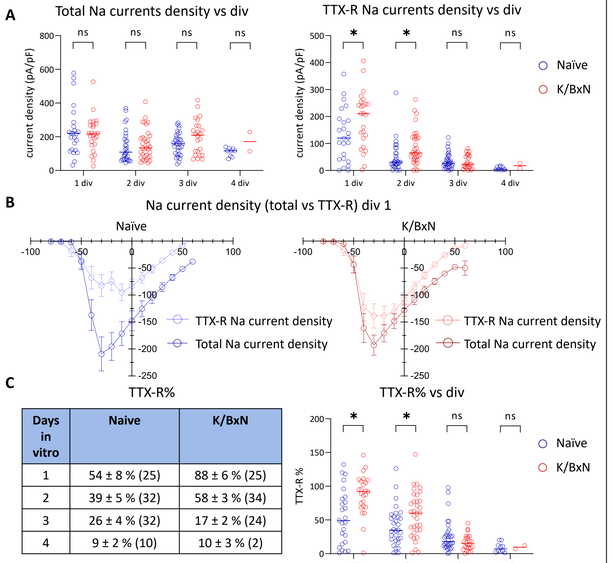
Figure 4. **(A)** Representative spike trains elicited by a 1 sec depolarizing current injection from three different neurons. **(B)** The average rheobase was 58.0 ± 9.9 from naïve DRGs and was 80.2 ± 11.6 from K/BxN DRGs. The maximum number of spikes was 11.0 ± 1.2 from naïve DRGs and was 10.0 ± 1.3 from K/BxN DRGs. **(C)** Pie charts showing the frequency distribution of the maximum number of spikes train elicited by a 1 sec current injection.

References

Christianson, C.A., Corr, M., Firestein, G.S., Mobargha, A., Yaksh, T.L., and Svensson, C.I. (2010). Characterization of the acute and persistent pain state present in K/BxN serum transfer arthritis. Pain 151: 394–403.

Park HJ, Sandor K, McQueen J, Woller SA, Svensson CI, Corr M, Yaksh TL. (2016). The effect of gabapentin and ketorolac on allodynia and conditioned place preference in antibody-induced inflammation. Eur J Pain.20(6):917-25.

DRGs from K/BxN mice exhibited greater TTX-resistant Na currents



Days in vitro	Naive	K/BxN
1	54 ± 8 % (25)	88 ± 6 % (25)
2	39 ± 5 % (32)	58 ± 3 % (34)
3	26 ± 4 % (32)	17 ± 2 % (24)
4	9 ± 2 % (10)	10 ± 3 % (2)

Figure 5. **(A)** Both total and TTX-R Na current density decreased with time in culture from 1 to 4 days in vitro. TTX-R current density was greater in sensory neurons from K/BxN mice than naïve mice at 1 and 2 days in vitro. **(B)** I-V plot of total and TTX-R Na current density (average ± sem) at 1 day in vitro. **(C)** The proportion of TTX-R Na currents was greater in sensory neurons from K/BxN mice than naïve mice at 1 and 2 days in vitro.

Conclusions

These results suggest that increased synovial innervation and gain of function of the voltage-gated sodium channel might contribute to the chronic pain phenotype in this preclinical model of RA.