# Analysis of Rat Ultrasonic Vocalizations in a Preclinical Model for Inflammatory Pain

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### Introduction

Rodents have the ability to communicate in a broad range of situations in the ultrasonic range, over 20 kHz. These ultrasonic vocalizations (USV) are emitted in varying durations and frequencies, and can reveal their emotional state.

In rats, USV frequencies ranging 18-32 kHz are associated with negative affect situations, like distress and pain.

In this study we aimed to better understand the USV emission of rats in naïve and inflammatory pain conditions. Particularly, we compared the USV properties between Sprague-Dawley (SD) and Han Wistar (HW) rat strains.

## Methods

USVs were recorded during 5-10 min inside noise-isolated recording chambers (Sonotrack System, Metris, NL). Female rats, either SD or HW were habituated to the chambers for 3 days prior recordings by placing them inside for the same time and conditions as for recordings. The CFA model of local inflammatory pain was used. Recordings of USV were made at baseline (1d before), 2h, 24h and 48h after model induction. The total number of USV calls, as well as the duration and frequency of calls were analyzed. Treatment with a standard of care compound (SoC, Rofecoxib 15 mg/kg) was compared against vehicle. Data were plotted and analyzed with the software GraphPad Prism 8.02 (GraphPad Software Inc, USA).

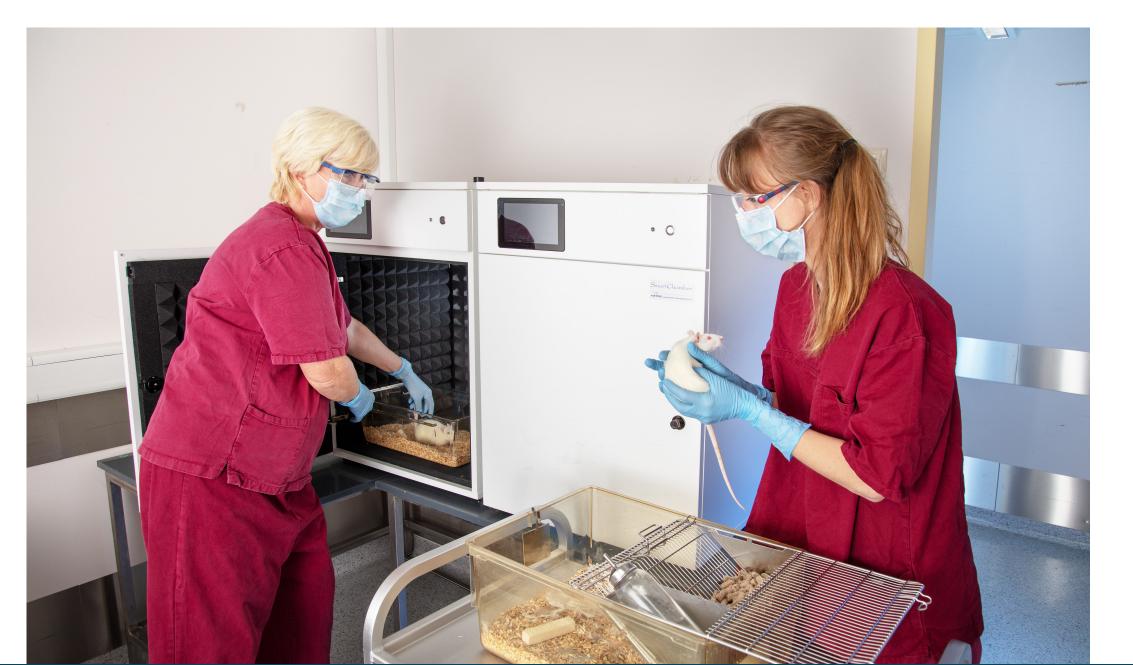
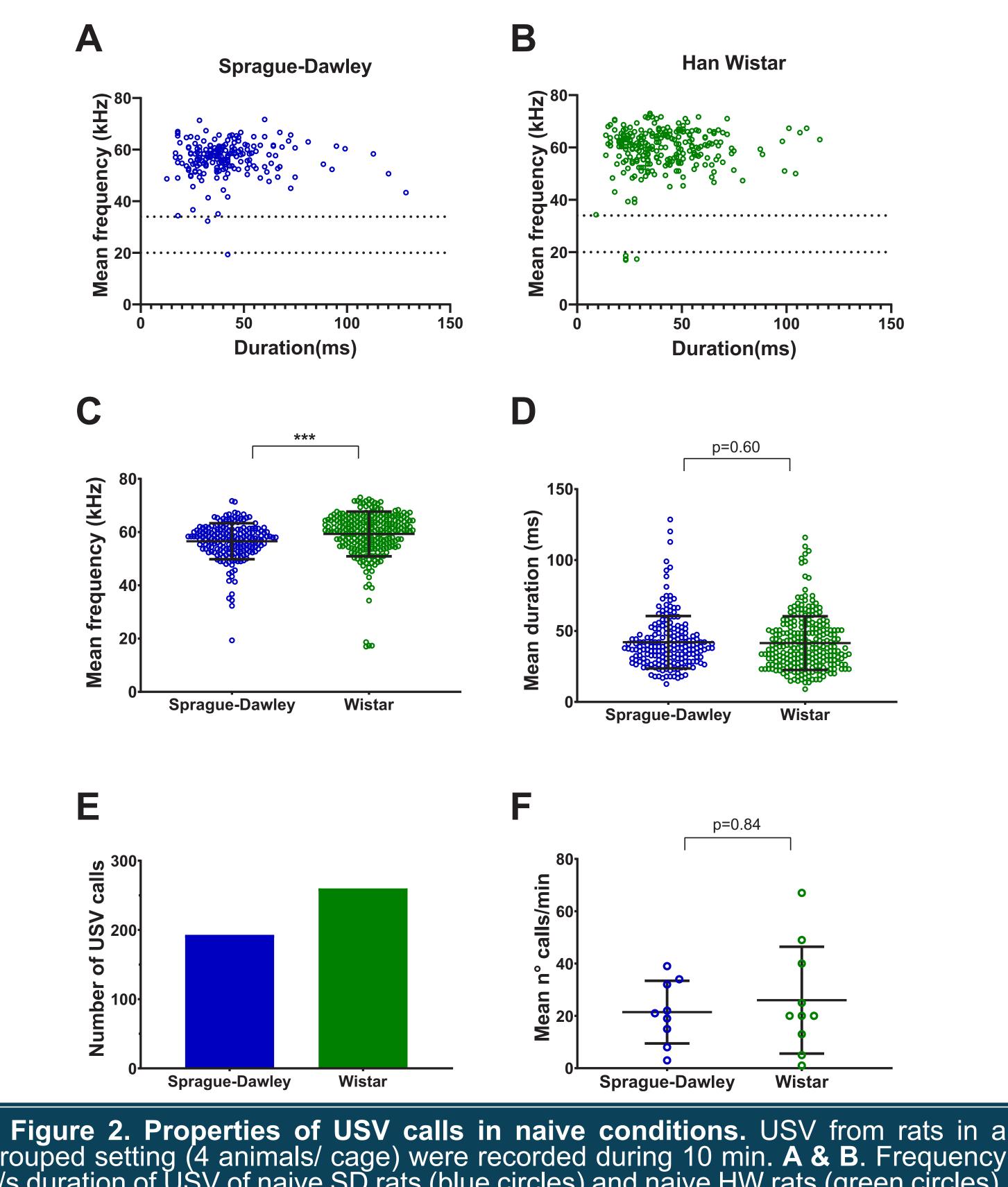


Figure 1. Noise-isolated chambers used for recording USV. These are equiped with internal light, ventilation and a videocamera for live observation of animals activity.

### Results

#### **1. Naïve rats vocalized in frequencies and durations** associated with positive emotional states.

In naive conditions, USV from both strains were emmitted out of the 22-kHz range. HW rats vocalized in average at a slightly higher frequency (59.3 ± 8.4 v/s 56.6 ± 6.8 kHz, p<0.001), and vocalized more (260 v/s 193 calls) than SD rats. No differences in duration or periodicity of calls were found.



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SD rats (blue circles) and naive HW rats (green circles) uencies of USV emmited by SD v/s HW rats. D. Durations of USV emmited /s HW rats. E. Number of USV calls. F. Periodicity of emmited calls (mean number of calls/min). In blue: SD data. In green: HW data.

#### 2. Local paw inflammation decreased USV frequencies in **SD & HW rats**.

In inflammatory pain conditions, the frequency of USV calls decreased over time. Particularly 24h after CFA injection, USV frequencies were on average 23.5% and 40.8% lower than its baseline values in SD and HW rats, respectively. This effect remained significant 48h after model induction. Treatment with SoC reversed these observations.

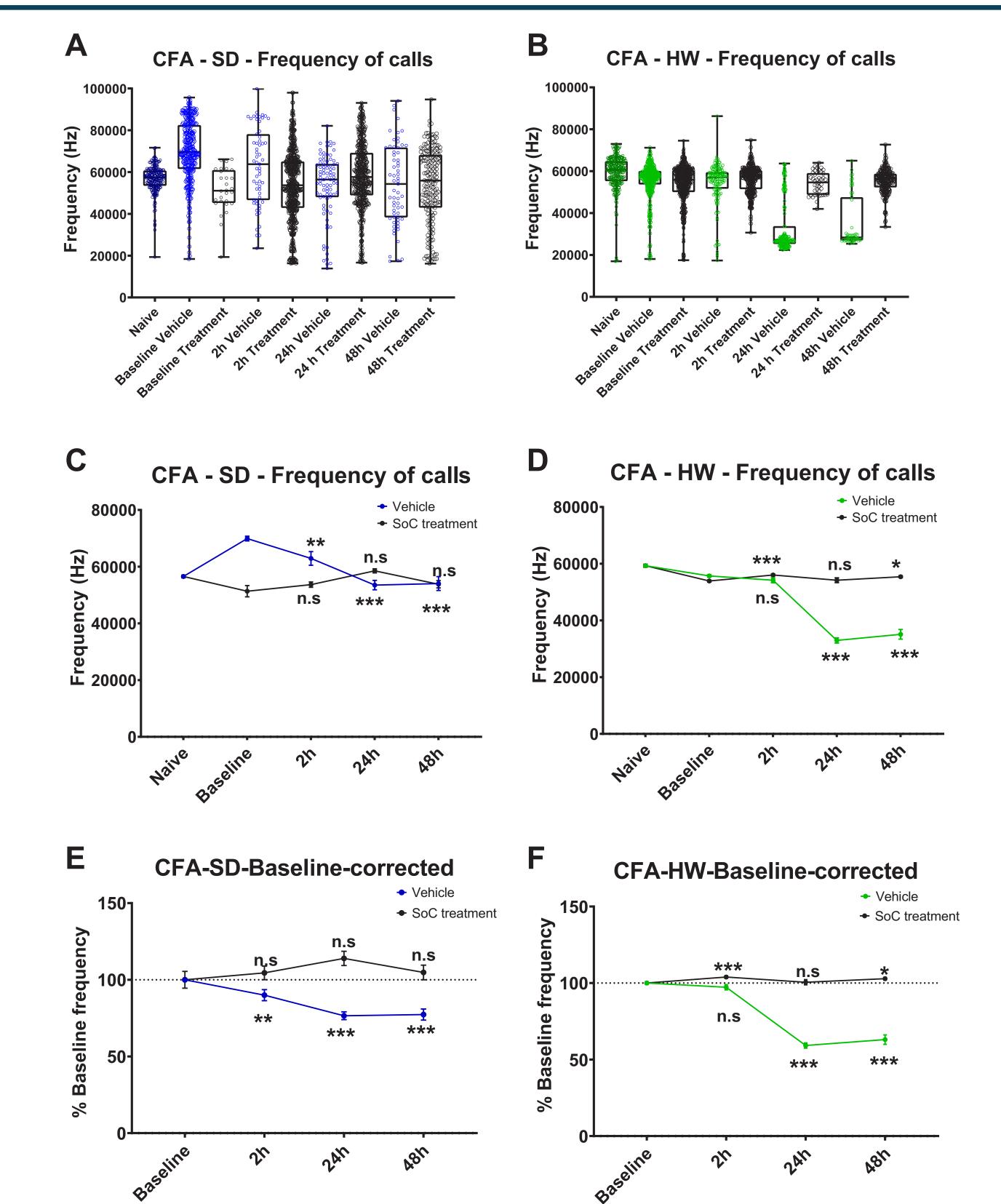


Figure 3. USV frequencies from SD and HW rats decrease in inflammatory pain conditions. A. and B. Frequencies of USV recorded from CFA-induced SD and HW rats treated with vehicle (blue and green circles) or with standard of care (SoC Boxes represent 95% CI and whiskers represent min to max values C. and D. Méan ± SEM frequencies of USV recorded from CFA-induced SD and HW rats treated with vehicle (blue and green lines) or standard of care (SoC, black lines). E. and F. Baseline-corrected values from C and D.





#### 3. Local paw inflammation increased USV duration in HW but not SD rats.

In HW, but not SD rats we observed a consistent increased duration of USV calls starting 24 h after intraplantar CFA injection. This increade was 12 times longer than baseline (330 ± 23 ms to 27 ± 1 ms), and remeined significant 48h after CFA injection. Treatment with SoC reversed these observations.

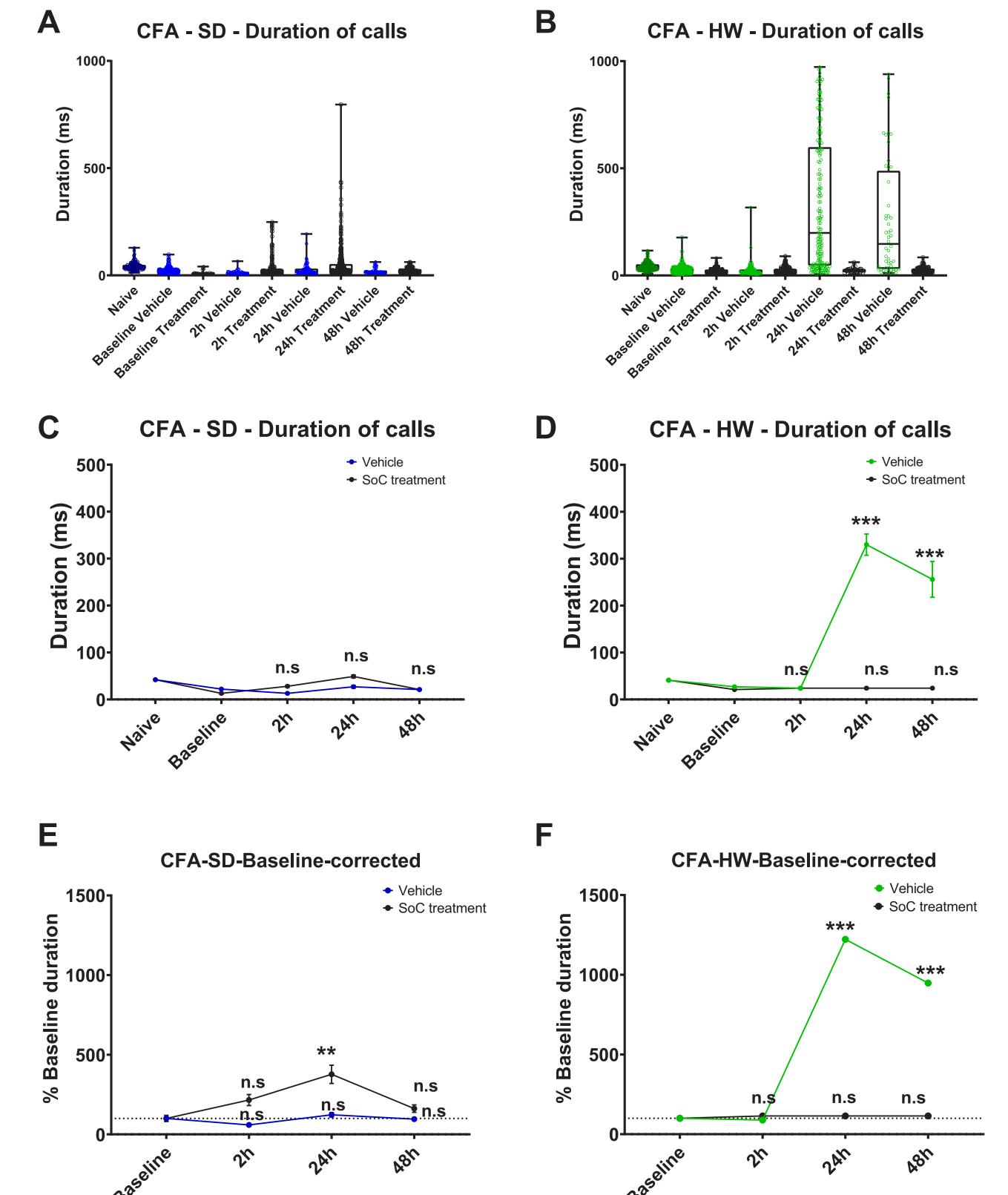


Figure 4. USV durations from HW but not SD rats increase in inflammatory pain conditions. A. and B. Duration of USV calls recorded from CFA-induced SD and HW rats treated with vehicle (blue and green circles) or with standard of care lack circles). Boxes represent 95% CI and whiskers represent min to max and D. Mean + SEM duration of USV calls from CFA-induced SD and HW rats treated with vehicle (blue and green lines) or SoC (black lines). E. and F. Baseline-corrected values from C and D.

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### Conclusions

Recording USV in rats can be used as a noninvasive, spontaneous pain readout in a model of local inflammatory pain

Changes in USV properties, particularly frequency and duration of calls are reliable outcomes for pain states.

The results presented here support the use of HW over SD rats for recording USV for in vivo pain research.

Further research is needed to determine to which extent this tool can be used in other chronic pain models, like endometriosis-associated pain (EAP).

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