

Background and aim

A variety of clinical and basic research studies have shown a close relationship between inflammatory responses and cardiovascular diseases, and in particular arrhythmias. Interleukin-6 (IL-6) is a multifunctional cytokine involved in the regulation of immune responses, inflammation, and hematopoiesis. These studies suggest a possible implication of IL-6 in promoting/sustaining dysfunctions of the sinoatrial node (SAN) and atrioventricular node (AVN). Understanding the molecular pathways through which IL-6 may influence SAN and AVN functions could provide new therapeutic targets for managing conduction dysfunctions associated with inflammatory diseases.

Materials & Methods

Immunofluorescences assays and **Patch-clamp** experiments were performed on single SAN/AVN cells isolated from the mouse heart. Two experimental conditions were used. **Acute treatment**: the cells were freshly isolated and IL-6 50 ng/ml was tested to detect short-term effects on beating rate. **Prolonged treatment**: before cells isolation tissues were exposed for about 60 hrs to either Vehicle or IL-6 at 50 ng/ml (15 hrs Vehicle+45 hrs IL6) to highlight the long-term effects of the cytokine.

Results

1. SAN and AVN cells express IL-6 membrane receptor

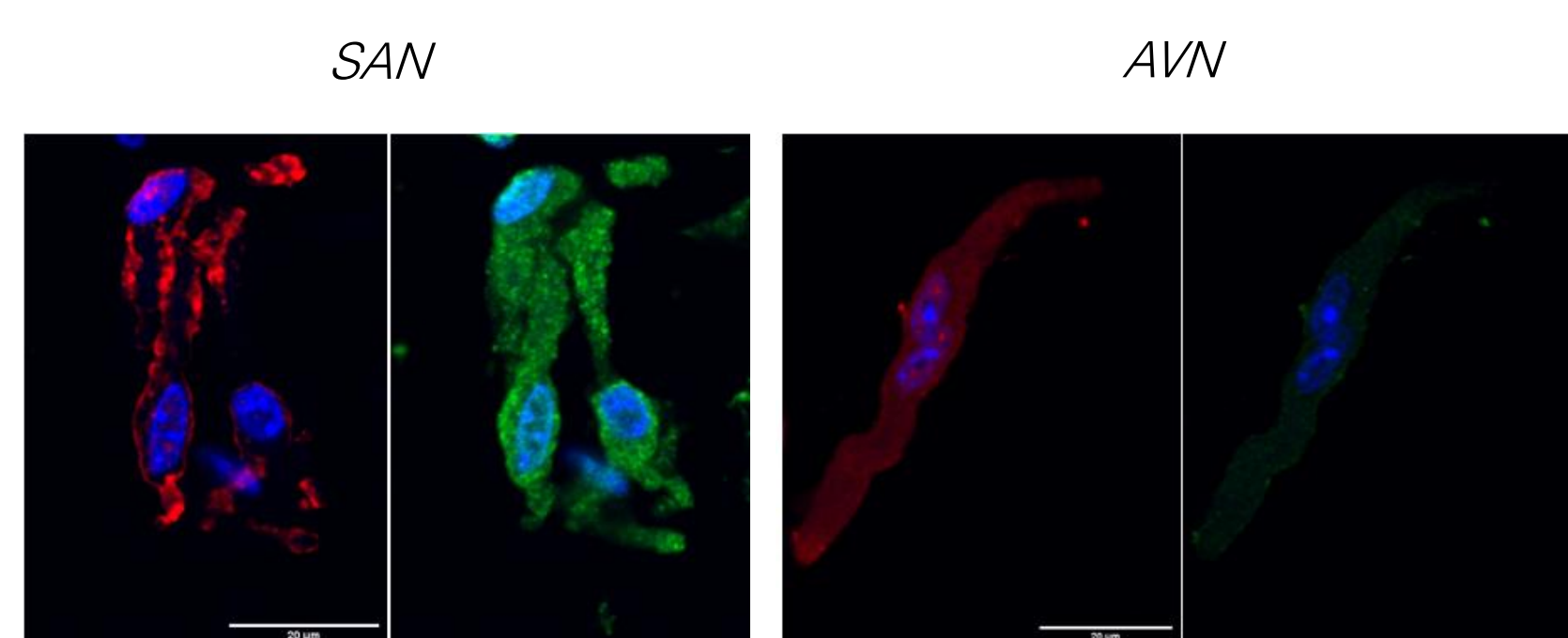


Fig. 1 SAN and AVN cells labeled with anti-HCN4 antibody (red) and anti-mIL-6R antibody (green); nuclei marked with Hoechst.

2. Acute treatment of IL-6 on SAN cells does not alter APs

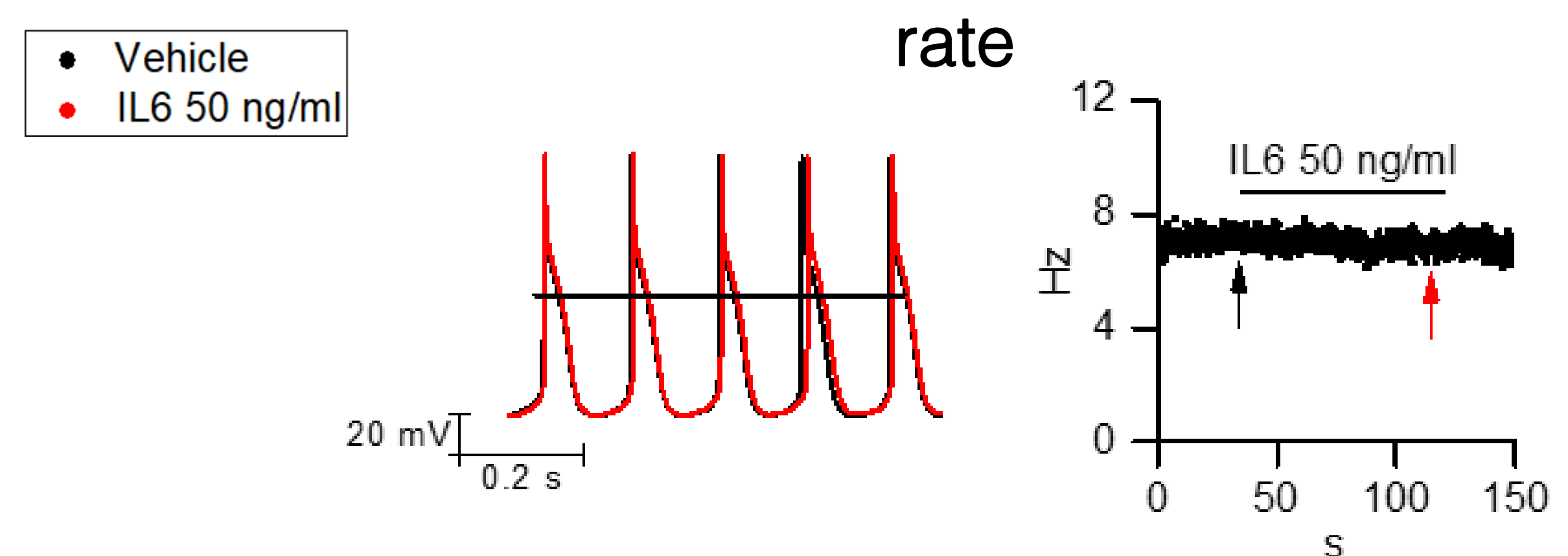


Fig. 2 Representative SAN action potentials (APs) in control solution (black) and during IL-6 perfusion (red) 50 ng/ml; Time course of the APs rate of a sample SAN cell in the absence and presence of IL-6. A similar behavior was observed in another cell.

3. Prolonged IL-6 treatment decreases the spontaneous activity of SAN cells

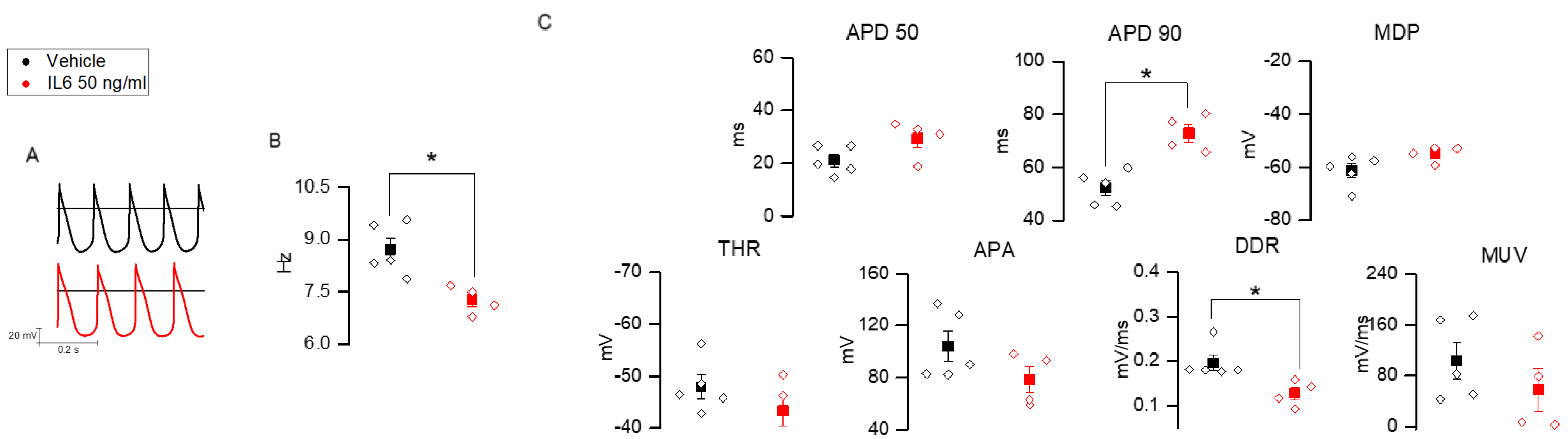


Fig. 3. A. Representative SAN APs recorded after incubation with vehicle and IL-6; B. Boxplot showing individual and mean rate data in the two conditions. Empty diamonds represent single spontaneous AP rates; filled squares represent the mean values and bars the sem. C. AP parameters analyzed with the ParamAP software. APD 50/90, AP duration at 50/90% of repolarization; MDP: maximum diastolic potential; THR: threshold potential; APA: action potential amplitude; DDR: slow diastolic depolarization rate; MUV: maximum upstroke velocity. * $P < 0.05$ (T-test).

4. Prolonged IL-6 treatment decreases the I_f in SAN cells

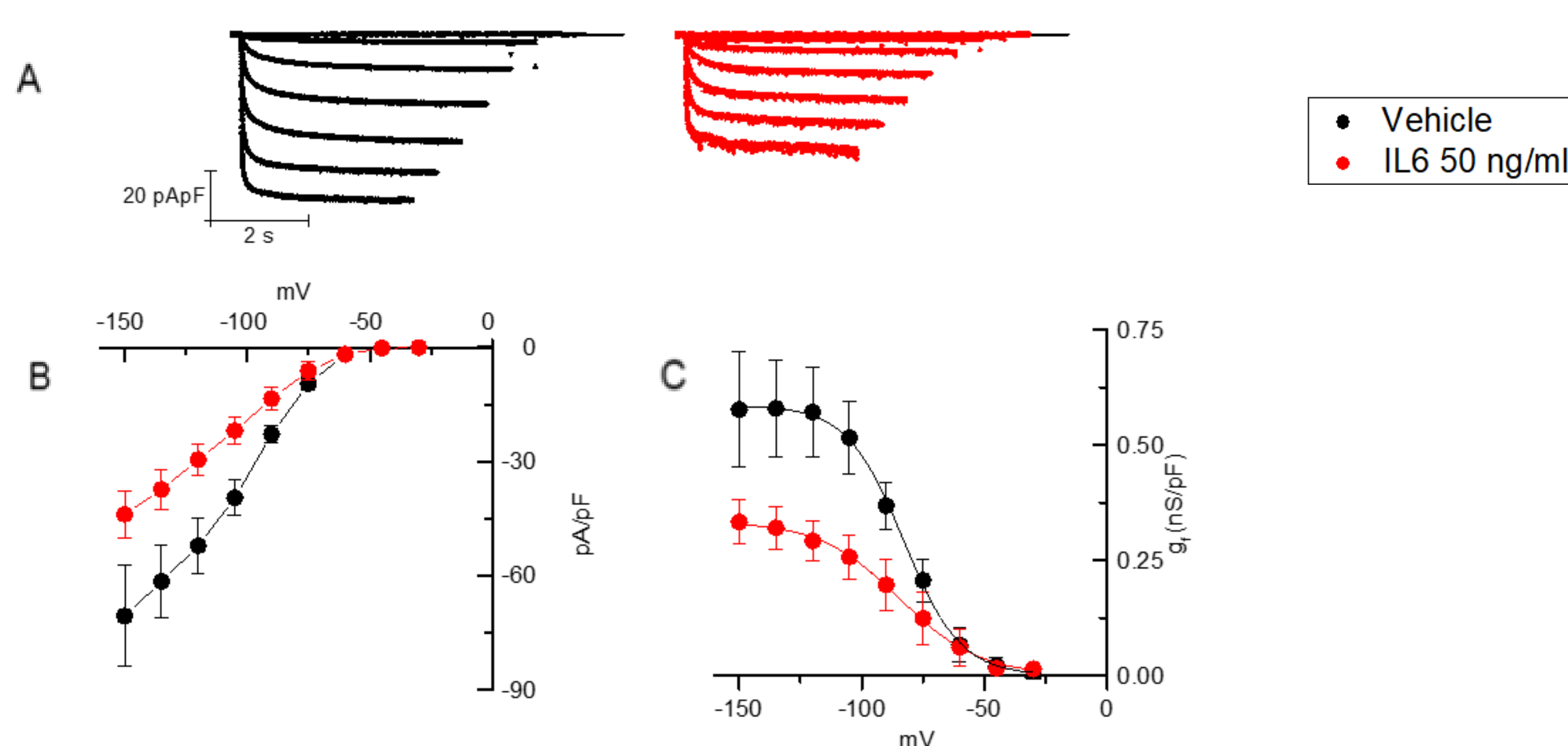


Fig. 4. A. Representative traces of I_f current recorded in SAN cells after incubation with vehicle and IL-6. Traces were obtained by applying hyperpolarizing steps from -30 mV to -150 mV ($\Delta V = -15$ mV, HP = -30 mV). B. Mean steady-state current-voltage curves obtained in the two conditions (vehicle $n = 7$, IL6 $n = 8$). C. Mean conductance curves obtained in the two conditions. Mean $V_{1/2}$, slope, and g_{fmax} values (Boltzmann fitting) are: -83.5 mV, 11.8 mV, 0.55 nS/pF and -82.0 mV, 15.6 mV, 0.30 nS/pF for vehicle and IL-6, respectively. The decrease of the g_{fmax} induced by IL-6 is significant ($P < 0.001$ Extra sum-of-squares F test).

5. Prolonged IL-6 treatment tends to decrease the I_f in AVN cells

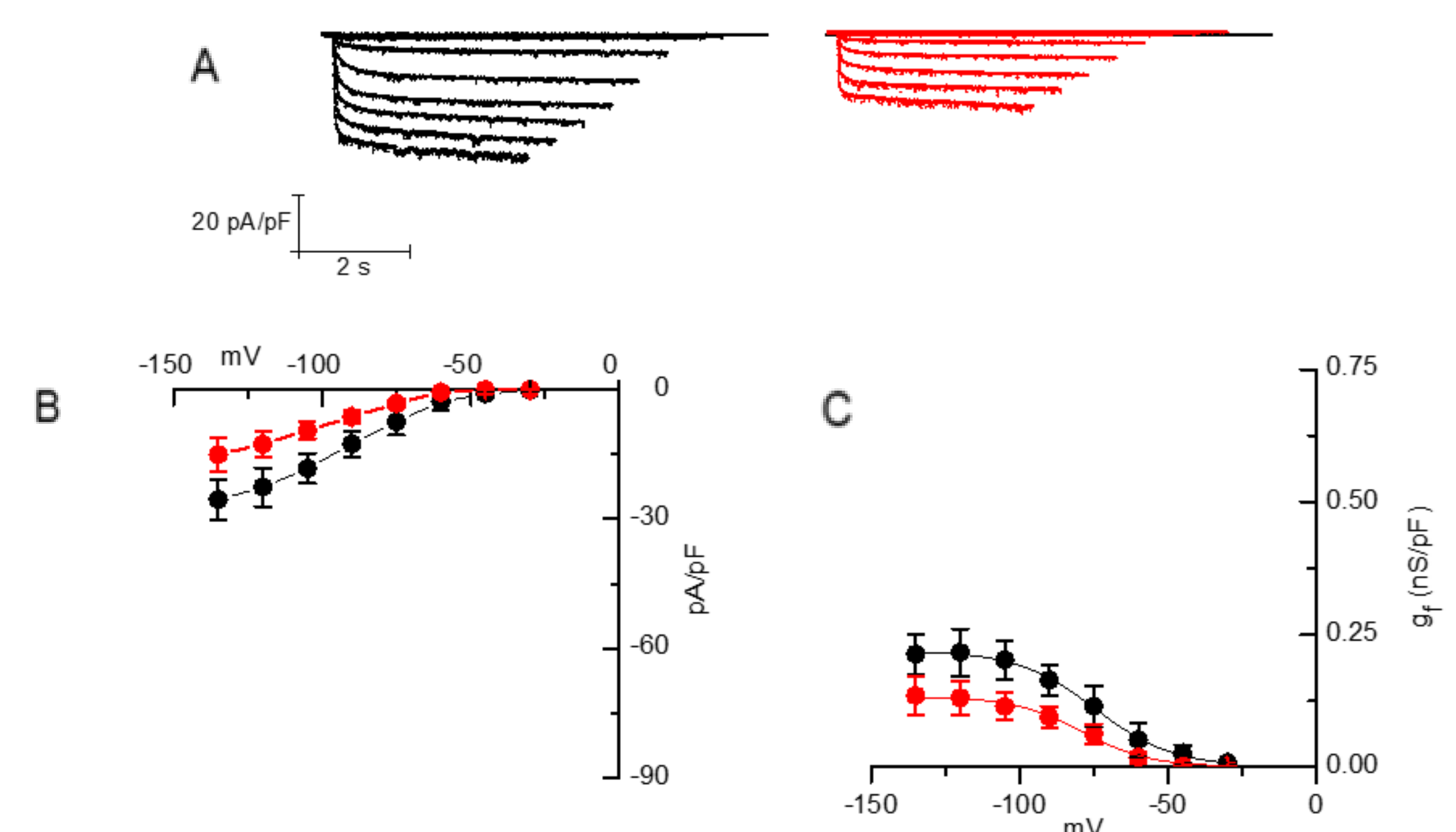


Fig. 5. A. Representative traces of I_f current recorded in AVN cells after incubation with vehicle and IL-6. Traces were obtained by applying hyperpolarizing steps from -30 mV to -135 mV ($\Delta V = -15$ mV, HP = -30 mV). B. Mean steady-state current-voltage curves obtained in the two conditions (vehicle $n = 4$, IL6 = 6). C. Mean conductance curves obtained in the two conditions. Mean $V_{1/2}$, slope, and g_{fmax} values (Boltzmann fitting) are: -74.5 mV, 13.1 mV, 0.21 nS/pF and -78.8 mV, 11.4 mV, 0.13 nS/pF for vehicle and IL-6, respectively. The decrease of the g_{fmax} induced by IL-6 is not significant ($P = 0.0059$ Extra sum-of-squares F test).

Conclusions

- SAN and AVN cells express the IL-6 membrane receptor.
- Prolonged exposure to IL-6 lowers the spontaneous rate of SAN cells by decreasing the I_f current and the DDR.
- Although not significant a reduction of the I_f current was also observed in AVN cells treated with IL-6. Future studies must address this issue.
- These experiments support an effective role of IL-6 in promoting SAN and AVN dysfunction related to inflammation.