

Mesirca et al., <http://www.jgp.org/cgi/content/full/jgp.201310996/DC1>

mRNA extraction and RT-PCR protocol

Isolation of SAN and cardiac chambers tissues as well as mRNA extraction were performed according to a protocol that we have previously published (Marionneau et al., 2005). In brief, cardiac and neuronal tissues were dissected, and mRNA was purified using the RNAlater and RNeasy kits (QIAGEN) according to the manufacturer's instructions. For cardiac samples, tissues undergoing mRNA extraction came from six isolated hearts for each genotype, as described previously (Marionneau et al., 2005). Expression of Girk channel isoform mRNA was studied by RT-PCR using the following amplification primers. mGIRK1: RefSeq accession no. (Kcnj3) NM_008426; amplicon length 208 bp; mGIRK1S203, 5'-GCAATGTGCAGCATGGTAAC-3'; mGIRK1R411, 5'-GGGAGTGTAGTTGCCGACAT-3'. mGIRK2:

RefSeq accession nos. (Kcnj6; these primers amplify four Girk2 isoforms) NM_001025584 (Girk2-1), NM_001025585 (Girk2B), NM_001025590 (Girk2C), and NM_010606 (Girk2A-1); amplicon length 177 bp; mGIRK2S651, 5'-GTACGTGAGGAAGGATGGGA-3'; mGIRK2R828, 5'-AATCAGCCACCAGATCATCC-3'. mGIRK3: RefSeq accession no. (Kcnj9) NM_008429; amplicon length 243 bp; mGIRK3S8539, 5'-AGAAGGACGGTCGCTGTAAC-3'; mGIRK3R8782, 5'-AAGCCGTTGAGGTTGTTGAC-3'. mGIRK4: RefSeq accession no. (Kcnj5) NM_010605; amplicon length 273 bp; mGIRK4S12505, 5'-GGACCACAAAGAAGATTCCC-3'; mGIRK4R12778, 5'-AATGAGCCACCAATGAAGC-3'. For the PCR reaction, samples were thermal cycled at 94°C for 3 min, followed by 35 cycles at 94°C for 45 s, 55°C for 45 s, 72°C for 30 s, and 72°C for 10 s.

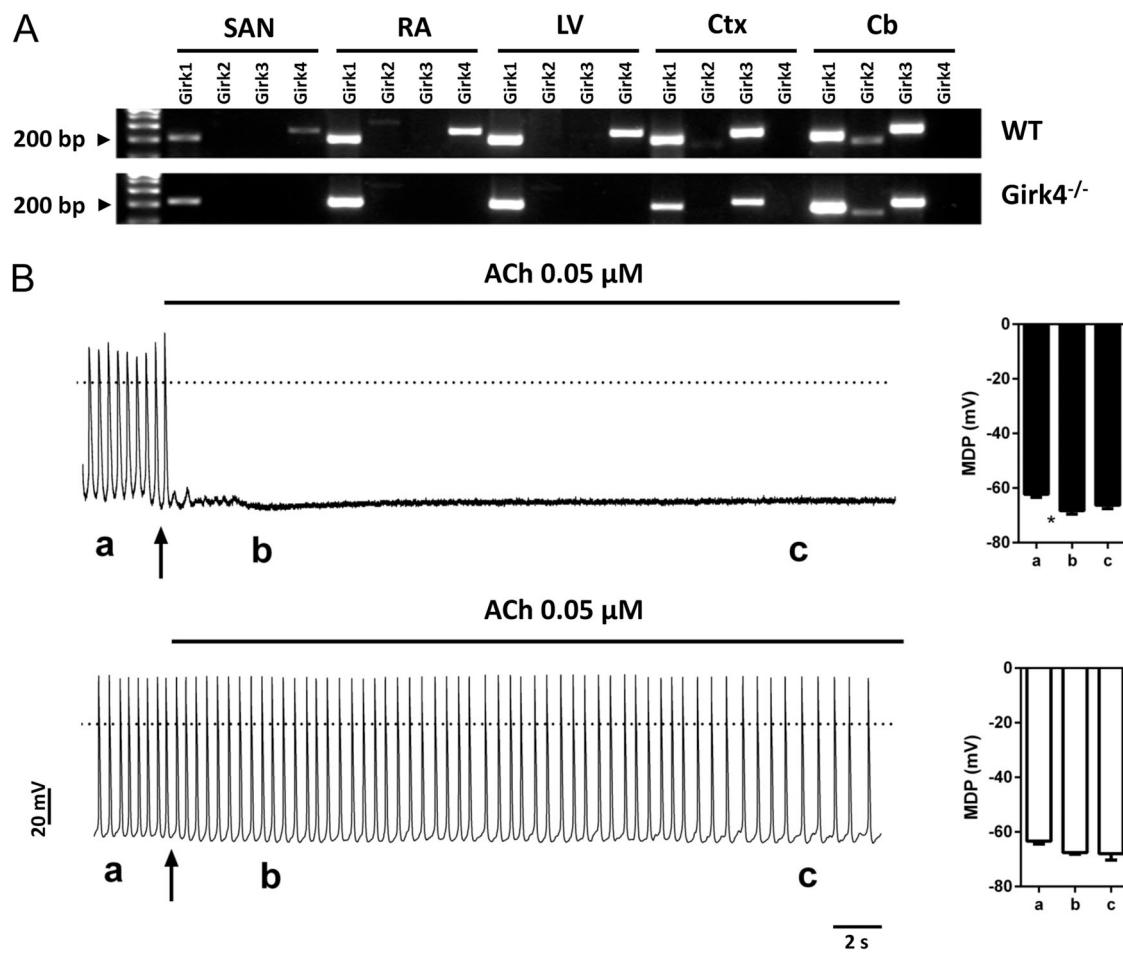


Figure S1. Expression and distribution of Girk subunits in different mouse tissues by RT-PCR. (A) In WT mice, Girk4 and Girk1 mRNA were detected in SAN, right atrium (RA), and left ventricle (LV). Girk2 and Girk3 mRNA were present in brain cortex (Ctx) and cerebellum (Cb) but not in myocardial tissues. No Girk4 mRNA was detected in SAN preparations from Girk4^{-/-} mice. (B) Sample traces (left) and averaged maximum diastolic potentials (right) of spontaneous action potentials of SAN cells from WT (top) and Girk4^{-/-} (bottom) mice. The arrow in each record indicates the moment of ACh superfusion. The letter "a" identifies pacemaker activity before ACh, "b" indicates hyperpolarization of the membrane potential, and "c" the membrane potential at the steady state during ACh superfusion. The value in "c" has been reported and averaged in Table S1. Significant hyperpolarization of MDP was observed only in WT SAN cells at 0.05 μM ACh (top, right). A slight nonsignificant trend was observed also in Girk4^{-/-} cells. This slight membrane hyperpolarization in WT and Girk4^{-/-} cells is probably caused by inhibition of I_f by ACh. Hyperpolarization of the MDP has been observed after inactivation of HCN4 channels, which reduces I_f by ~70% (Herrmann et al., 2007). Error bars represent SEM. Statistical symbol: *, P < 0.05.

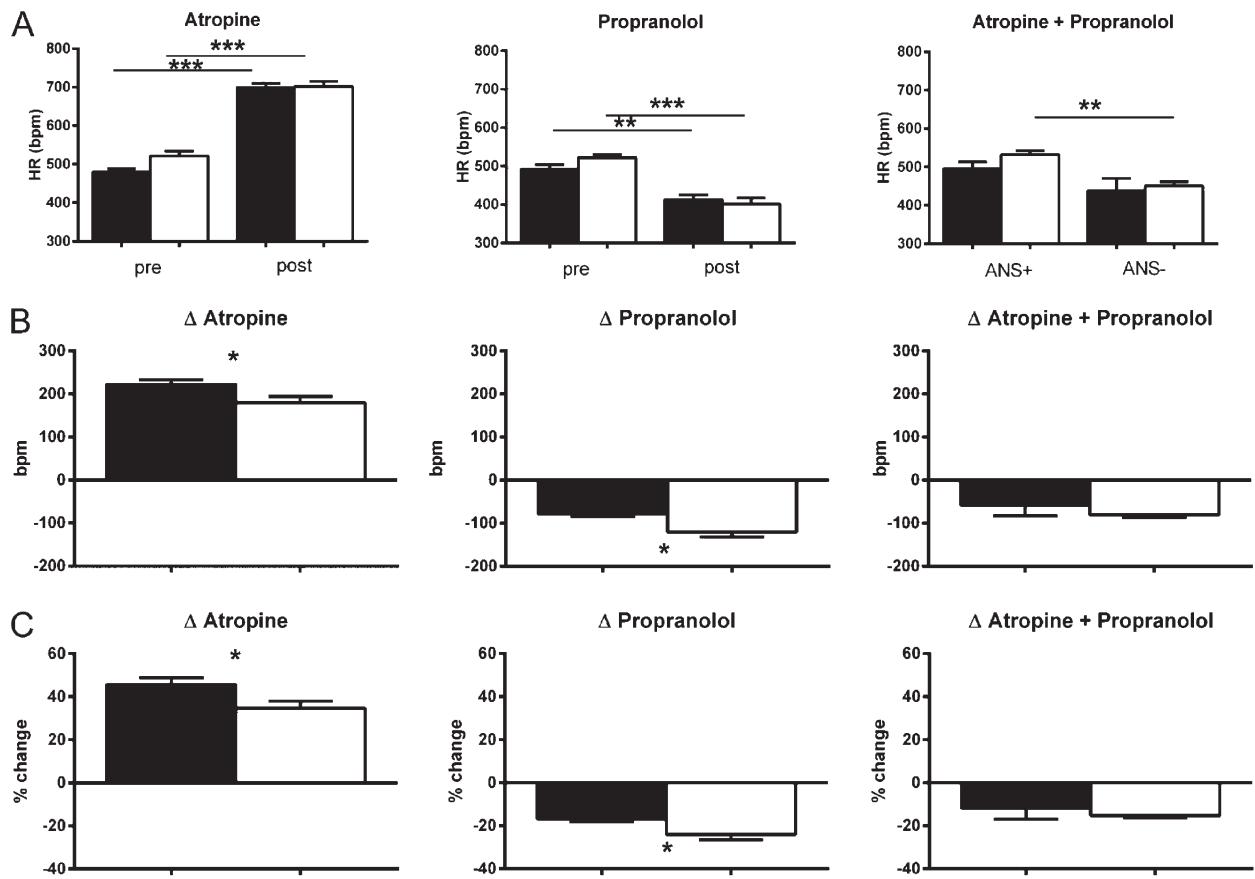
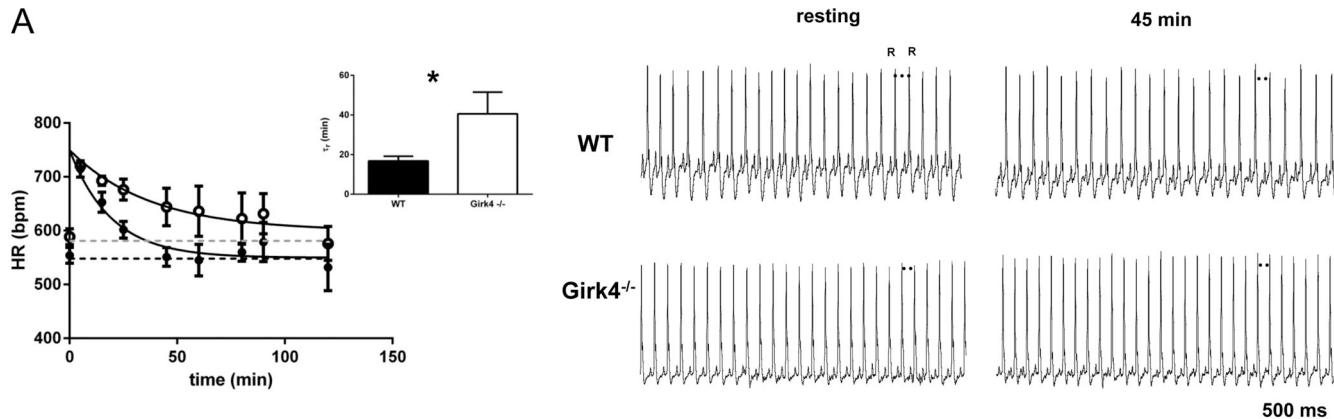


Figure S2. Heart rate variation in Girk4^{-/-} mice after administration of atropine, propranolol, and atropine plus propranolol. (A) Bar graphs of heart rate (HR) in WT and Girk4^{-/-} before and after intraperitoneal injection of propranolol (WT $n = 6$; Girk4^{-/-} $n = 7$), atropine (WT $n = 16$; Girk4^{-/-} $n = 15$), and combined injection of atropine and propranolol (WT $n = 7$; Girk4^{-/-} $n = 8$). ANS, autonomic nervous system. (B and C) Relative differences (Δ) expressed in heart rate (B) and as percentages of change (C) before and after injection of propranolol, atropine, and atropine plus propranolol. Error bars represent SEM. Statistical symbols: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

A



B

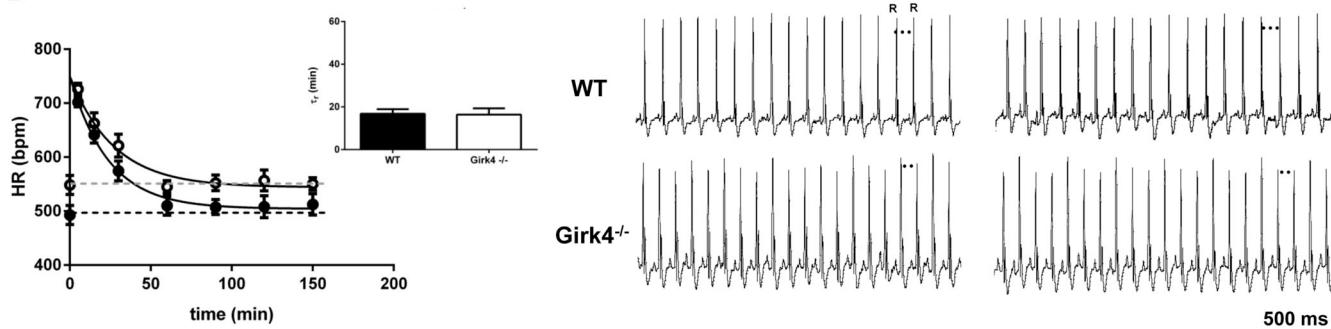


Figure S3. Recovery of heart rate in *Girk4^{-/-}* mice after treadmill test or intraperitoneal injection of atropine. (A, left) Treadmill test (5 min) was used to monitor heart rate (HR) during physical exercise of WT ($n = 6$; closed circles) and *Girk4^{-/-}* ($n = 4$; open circles) mice. Dashed lines represent the basal heart rate before treadmill test in WT (black) and *Girk4^{-/-}* (gray) mice. Averaged τ_r for WT and *Girk4^{-/-}* mice is reported in the inset. (right) Sample ECG traces from WT and *Girk4^{-/-}* mice in resting condition (before treadmill test) and 45 min after treadmill test. (B) Recovery of basal heart rate after intraperitoneal injection of 0.5 mg/kg atropine in WT ($n = 16$; closed circles) and *Girk4^{-/-}* ($n = 15$; open circles) mice. Dashed lines represent the basal heart rate before injection of atropine in WT (black) and *Girk4^{-/-}* (gray) mice. Averaged τ_r of recovery in WT and *Girk4^{-/-}* mice is reported in the inset. (right) Sample ECG recordings during basal conditions and 60 min after injection of atropine in WT and *Girk4^{-/-}* mice. Note that after 60 min, both WT and *Girk4^{-/-}* mice completely recovered basal heart rate. Error bars represent SEM. Statistical symbol: *, P < 0.05.

Table S1

Action potential parameters in WT and *Girk4^{-/-}* SAN cells before and after perfusion of 0.003, 0.01, and 0.05 μM ACh

Parameter	WT (A)	n	<i>Girk4^{-/-}</i> (B)	n	P-values						
					A vs. B	D vs. E (A)	D vs. E (B)				
0.003 μM ACh											
Before perfusion (D)											
Rate (bpm)	152 \pm 14	6	157 \pm 13	8	NS	NS	NS				
MDP (mV)	-62 \pm 1	6	-63 \pm 3	8	NS	NS	NS				
Eth (mV)	-45 \pm 1	6	-46 \pm 2	8	NS	NS	NS				
SLDD (mV/ms)	0.03 \pm 0.01	6	0.04 \pm 0.01	8	NS	NS	NS				
APA (mV)	86 \pm 6	6	86 \pm 6	8	NS	NS	NS				
dV/dt (mV/ms)	20 \pm 4	6	24 \pm 3	8	NS	NS	NS				
APD (ms)	220 \pm 20	6	230 \pm 10	8	NS	NS	NS				
After perfusion (E)											
Rate, ACh (bpm)	124 \pm 6	6	140 \pm 9	8	NS						
MDP, ACh (mV)	-63 \pm 2	6	-64 \pm 3	8	NS						
Eth, ACh (mV)	-45 \pm 2	6	-47 \pm 2	8	NS						
SLDD, ACh (mV/ms)	0.02 \pm 0.01	6	0.03 \pm 0.01	8	NS						
APA, ACh (mV)	87 \pm 7	6	88 \pm 6	8	NS						
dV/dt, ACh (mV/ms)	21 \pm 4	6	22 \pm 3	8	NS						
APD, ACh (ms)	240 \pm 30	6	230 \pm 20	8	NS						
0.01 μM ACh											
Before perfusion (D)											
Rate (bpm)	152 \pm 14	13	158 \pm 14	10	NS	<0.001	<0.001				
MDP (mV)	-62 \pm 2	13	-63 \pm 2	10	NS	NS	NS				
Eth (mV)	-46 \pm 1	13	-47 \pm 2	10	NS	NS	NS				
SLDD (mV/ms)	0.033 \pm 0.005	13	0.04 \pm 0.01	10	NS	<0.01	<0.05				
APA (mV)	87 \pm 5	13	87 \pm 5	10	NS	NS	NS				
dV/dt (mV/ms)	20 \pm 5	13	19 \pm 4	10	NS	NS	NS				
APD (ms)	220 \pm 10	13	230 \pm 10	10	NS	NS	NS				
After perfusion (E)											
Rate, ACh (bpm)	48 \pm 13	13	99 \pm 17	10	<0.05						
MDP, ACh (mV)	-65 \pm 1	13	-65 \pm 3	10	NS						
Eth, ACh (mV)	-50 \pm 2	13	-48 \pm 2	10	NS						
SLDD, ACh (mV/ms)	0.009 \pm 0.003	13	0.02 \pm 0.01	10	NS						
APA, ACh (mV)	91 \pm 3	13	90 \pm 11	10	NS						
dV/dt, ACh (mV/ms)	19 \pm 2	13	24 \pm 6	10	NS						
APD, ACh (ms)	260 \pm 30	13	260 \pm 10	10	NS						
0.05 μM ACh											
Before perfusion (D)											
Rate (bpm)	157 \pm 11	7	156 \pm 11	10	NS	<0.001	<0.001				
MDP (mV)	-62 \pm 2	7	-63 \pm 3	10	NS	NS	NS				
Eth (mV)	-45 \pm 1	7	-48 \pm 2	10	NS						
SLDD (mV/ms)	0.03 \pm 0.01	7	0.04 \pm 0.01	10	NS		<0.05				
APA (mV)	89 \pm 7	7	90 \pm 11	10	NS						
dV/dt (mV/ms)	20 \pm 4	7	24 \pm 6	10	NS						
APD (ms)	210 \pm 10	7	240 \pm 20	10	NS						
After perfusion (E)											
Rate, ACh (bpm)	3 \pm 2	7	70 \pm 18	10	<0.05						
MDP, ACh (mV)	-65 \pm 1	7	-68 \pm 5	10	NS						
Eth, ACh (mV)			-49 \pm 3	10							
SLDD, ACh (mV/ms)			0.008 \pm 0.003	10							
APA, ACh (mV)			91 \pm 7	10							
dV/dt, ACh (mV/ms)			25 \pm 5	10							
APD, ACh (ms)			260 \pm 20	10							

APA, action potential amplitude; APD, action potential duration; SLDD, slope of diastolic depolarization. Values have been measured at the steady state of the ACh-induced negative chronotropic effect (point "c" in Fig. S1 B).

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