# eNeuro

# Refining the Identity and Role of Kv4 Channels in Mouse Substantia Nigra Dopaminergic Neurons

*This Early Release article has been peer-reviewed and accepted, but has not been through the composition and copyediting processes. The final version may differ slightly in style or formatting and will contain links to any extended data.*

**Alerts:** Sign up at www.eneuro.org/alerts to receive customized email alerts when the fully formatted version of this article is published.

1 **REFINING THE IDENTITY AND ROLE OF KV4 CHANNELS IN MOUSE**

2 **SUBSTANTIA NIGRA DOPAMINERGIC NEURONS**

3 **Abbreviated Title**: Kv4 channels in substantia nigra dopaminergic neurons

4 Alexis HADDJERI-HOPKINS[1,*], Mónica TAPIA[1,*], Jorge RAMIREZ-FRANCO[1], Fabien TELL[1,2],

5 Béatrice MARQUEZE-POUEY[1], Marianne AMALRIC[3] and Jean-Marc GOAILLARD[1,2,§]

6

7 [1] UMR_S 1072, Aix Marseille University, INSERM, Faculté de Médecine Secteur Nord, 51 boulevard

8 Pierre Dramard, 13015 Marseille, FRANCE

9 [2] Current address: Institut de Neurosciences de la Timone, UMR 7289, Aix-Marseille Université and

10 Centre National de la Recherche Scientifique, Marseille, FRANCE.

11 [3] UMR7291, Aix Marseille University, CNRS, LNC, 3 place Victor Hugo, 13331 Marseille, FRANCE

12 [*] These authors contributed equally to this work

13 [§] Corresponding author

14 **Corresponding author**: Jean-Marc GOAILLARD. jean-marc.goaillard@univ-amu.fr

1

23

24 **ABSTRACT**

25 Substantia nigra pars compacta (SNc) dopaminergic (DA) neurons display a peculiar electrical

26 phenotype characterized *in vitro* by a spontaneous tonic regular activity (pacemaking activity), a

27 broad action potential and a biphasic post-inhibitory response. The transient A-type current ($I_A$) is

28 known to play a crucial role in this electrical phenotype, and so far this current was considered to

29 be carried exclusively by Kv4.3 potassium channels. Using Kv4.3$^{-/-}$ transgenic mice, we

30 demonstrate that the constitutive loss of this channel is associated with increased exploratory

31 behavior and impaired motor learning at the behavioral level. Consistently it is also associated

32 with a lack of compensatory changes in other ion currents at the cellular level. Using antigen

33 retrieval immunohistochemistry, we then demonstrate that Kv4.2 potassium channels are also

34 expressed in SNc DA neurons, even though their contribution to $I_A$ appears significant only in a

35 minority of neurons ($\sim$5-10%). Using correlative analysis on recorded electrophysiological

36 parameters and multi-compartment modeling, we then demonstrate that, rather than its

37 conductance level, $I_A$ gating kinetics (inactivation time constant) appear as the main biophysical

38 property defining post-inhibitory rebound delay and pacemaking frequency. Moreover, we show

39 that the hyperpolarization-activated current ($I_H$) has an opposing and complementary influence on

40 the same firing features.

41

42

43

**SIGNIFICANCE STATEMENT**

Substantia nigra pars compacta (SNc) dopaminergic (DA) neurons are characterized by pacemaking activity, a broad action potential and biphasic post-inhibitory response. The A-type transient potassium current ($I_A$) plays a central role in this electrical phenotype. While it was thought so far that Kv4.3 ion channels were fully responsible for $I_A$, using a Kv4.3[-/-] transgenic mouse and antigen retrieval immunohistochemistry we demonstrate that Kv4.2 channels are also expressed in SNc DA neurons, although their contribution is significant in a minority of neurons only. Using electrophysiological recordings and computational modeling, we then demonstrate that $I_A$ gating kinetics and its functional complementarity with the hyperpolarization-activated current are major determinants of both pacemaking activity and post-inhibitory response in SNc DA neurons.

55

## INTRODUCTION

While the expression of only two types of voltage-gated ion channels in the squid giant axon allowed Hodgkin and Huxley to dissect the biophysical processes underlying action potential (AP) genesis and conduction (Hodgkin and Huxley, 1952), most neuronal types express a multitude of ion channel subtypes underlying their electrical activity (Cembrowski et al., 2016; Fuzik et al., 2016; Tapia et al., 2018; Northcutt et al., 2019). In spontaneously active neurons, a variety of voltage- and calcium-gated ion channels are not only responsible for the AP, but also govern the subthreshold oscillations leading to AP firing, determine firing frequency and control its regularity (Atherton and Bevan, 2005; Swensen and Bean, 2005; Bean, 2007; Gantz et al., 2018). Substantia nigra pars compacta (SNc) dopaminergic (DA) neurons spontaneously generate a regular tonic pattern of activity, also known as "pacemaking" activity (Grace and Onn, 1989; Gantz et al., 2018). Over the past 40 years, many studies contributed to the identification of the specific ion channels involved in shaping pacemaking activity (Nedergaard and Greenfield, 1992; Liss et al., 2001; Seutin et al., 2001; Wolfart et al., 2001; Neuhoff et al., 2002; Liss et al., 2005; Chan et al., 2007; Puopolo et al., 2007; Guzman et al., 2009; Putzier et al., 2009; Ji et al., 2012; Gantz et al., 2018). In particular, several studies have suggested that the transient A-type potassium current ($I_A$) plays an essential role in controlling pacemaking rate and post-inhibitory firing delay in these neurons (Liss et al., 2001; Putzier et al., 2008; Amendola et al., 2012; Tarfa et al., 2017). In addition, single-cell PCR, in situ hybridization and immunohistochemistry experiments suggested that the A-type current is carried exclusively by Kv4.3 ion channels (Serodio and Rudy, 1998; Liss et al., 2001; Ding et al., 2011; Dufour et al., 2014a; Tapia et al., 2018). Interestingly, several studies also suggested that the H-type current ($I_H$, carried by HCN channels) displays strong functional interactions with $I_A$, having for instance an opposite

4

79    influence on post-inhibitory rebound delay (Amendola et al., 2012; Tarfa et al., 2017). The gating

80    properties of these two currents were also shown to be co-regulated in rat SNc DA neurons

81    (Amendola et al., 2012).

82        In the current study, we used in particular electrophysiological recordings from wild-type

83    (WT) and Kv4.3$^{-/-}$ mice to refine the identity and role of Kv4 channels in the firing of SNc DA

84    neurons. Using this mouse model, we show that the constitutive loss of Kv4.3 is associated with

85    increased exploratory activity and impaired motor learning. Consistently, it is also associated

86    with a lack of compensatory changes in other ion currents at the cellular level. We then

87    demonstrate that the Kv4.2 subunit is expressed in SNc DA neurons, although its functional

88    contribution is minor in most SNc DA neurons. Finally, we also demonstrate that pacemaking

89    frequency and post-inhibitory rebound delay are mainly determined by $I_A$ time constant of

90    inactivation and $I_H$ amplitude.

91

## MATERIAL AND METHODS

### *Animals*

Female and male P15-P80 WT (n=68 animals) and Kv4.3$^{-/-}$ (n=40, Deltagen) mice from C57BL6/J genetic background were housed with free access to food and water in a temperature-controlled room (24°C) on a 12:12 h dark–light cycle (lights on at 07:00 h). All efforts were made to minimize the number of animals used and to maintain them in good general health, according to the European (Council Directive 86/609/EEC) and institutional guidelines for the care and use of laboratory animals (French National Research Council).

### *Behavioral Experiments*

A group of female and male WT (n=11) and Kv4.3$^{-/-}$ (n=13) mice aged P56-P63 at the start of the behavioral testing were used to evaluate changes in motor function, using in particular locomotor activity chambers and the rotarod test.

### Locomotor and exploratory activities

Actimetry was monitored in individual activity chambers (20 cm × 11.2 cm × 20.7 cm) housed within a sound-attenuating cubicle and under homogeneous illumination (Imetronic, Pessac, France). Each chamber was equipped with two pairs of infrared photobeams located 1.5 and 7.5cm above the floor level of the chamber. The number of back-and-forth movements (animals breaking the lower photobeams) as well as the number of vertical movements (animals breaking the upper photobeams) were recorded in 5-min bins over 90 min. Numbers of back-and-forth movements (locomotion) and vertical movements (rearing) are shown as mean ± SEM for each

6

112   time bin over the whole period of recording time. Locomotion and rearing activities over the

113   whole 90-min period were normalized and displayed as a mean percentage of control littermates.

114   Motor learning

115   Motor learning was evaluated on the accelerating rotarod (10cm diameter rod) test at a speed of 5

116   to 40 rotations per min (RPM) for 5 min. On the first day, mice were allowed to freely explore

117   the non-rotating apparatus for 60s and subsequently trained to hold on the rotating rod (5 RPM)

118   for at least two 60s trials, each trial being separated by a 10min break. Mice were allowed to

119   recover for one hour before the first test. The testing phase consisted in 10 consecutive trials on

120   the accelerating rod separated by 15-min breaks that allowed consolidation of performance.

121   Results are shown as the average latency to fall off the rod (mean ± SEM) at each trial. A

122   *performance index* was calculated for each individual and consisted in the average latency of the

123   last 3 trials divided by the average latency of the first 3 trials multiplied by 100

124   $(\frac{average\ latency\ 8-10}{average\ latency\ 1-3} \times 100)$.

125   ***Electrophysiology***

126   119 neurons from 39 WT mice and 109 neurons from 24 Kv4.3$^{-/-}$ mice were recorded (current-

127   clamp and voltage-clamp).

128   Acute midbrain slice preparation

129   Acute slices were prepared from P15-P25 animals of either sex. Mice were anesthetized with

130   isoflurane (CSP) in an oxygenated chamber (TEM SEGA) before decapitation. After decapitation

131   the brain was immersed briefly in oxygenated ice-cold low-calcium aCSF containing the

132   following (in mM): 125 NaCl, 25 NaHCO3, 2.5 KCl, 1.25 NaH2PO4, 0.5 CaCl2, 4 MgCl2, and

133  25 D-glucose, pH 7.4, oxygenated with 95% O2/5% CO2 gas. The cortices were removed and

134  then coronal midbrain slices (250 μm) were cut in ice-cold oxygenated low-calcium aCSF on a

135  vibratome (Leica VT1200S and vibrating microtome 7000smz, Camden Instruments, UK).

136  Following 20-30 min incubation in oxygenated low-calcium aCSF at 33°C, the acute slices were

137  then incubated for a minimum of 30 min in oxygenated aCSF (containing in mM: 125 NaCl, 25

138  NaHCO3, 2.5 KCl, 1.25 NaH2PO4, 2 CaCl2, 2 MgCl2, and 25 glucose, pH 7.4, oxygenated with

139  95% O2/5% CO2 gas) at room temperature before electrophysiological recordings.

140  Drugs

141  Kynurenate (2 mM, Sigma-Aldrich) and picrotoxin (100 μM, Sigma-Aldrich) were used to block

142  excitatory and inhibitory synaptic activity, respectively. AmmTX3 (1μM, Alomone) was used to

143  block the transient potassium current ($I_A$) carried by Kv4 channels. Drugs were bath applied via

144  continuous perfusion in aCSF.

145  Electrophysiology recordings and analysis

146  All recordings (228 neurons from 63 mice) were performed on midbrain slices continuously

147  superfused with oxygenated aCSF at 30–32°C. Picrotoxin and kynurenate were systematically

148  added to the aCSF for all recordings to prevent contamination of the intrinsically generated

149  activity by glutamatergic and GABAergic spontaneous synaptic activity. Patch pipettes (1.9–2.7

150  MOhm) were pulled from borosilicate glass (GC150TF-10, Harvard Apparatus) on a DMZ-

151  Universal Puller (Zeitz Instruments) and filled with a patch solution containing the following (in

152  mM): 20 KCl, 10 HEPES, 0.5 EGTA, 2 MgCl2, 0.4 Na-GTP, 2 Na$_2$-ATP, 4 Mg-ATP,0.3 CaCl2,

153  SUPERase RNase inhibitor (0.1 U/μl) and 115 K-gluconate, pH 7.4, 290–300 mOsm. For

154  AmmTX3 experiments, patch pipettes (3.2-4.0 MOhm) were filled with a patch solution

155   containing the following (in mM): 20 KCl, 10 HEPES, 0.5 EGTA, 2 MgCl2, 2 Na-ATP, and 120

156   K-gluconate, pH 7.4, 290–300 mOsm. Whole-cell recordings were made from SNc DA neurons

157   visualized using infrared differential interference contrast videomicroscopy (QImaging Retiga

158   camera; Olympus BX51WI microscope), and were identified based on their location, large soma

159   size (>25μm), and electrophysiological profile (regular slow pacemaking activity, large spike

160   half-width, large sag in response to hyperpolarizing current steps). For voltage-clamp

161   experiments, only whole-cell recordings with an uncompensated series resistance <7 MOhm

162   (compensated 85–90%) were included in the analysis. For current-clamp pharmacology

163   experiments, higher series resistances were tolerated as long as the bridge compensation was

164   properly adjusted to 100%. Liquid junction potential (-13.2 mV) and capacitive currents were

165   compensated on-line. Recordings were acquired at 50kHz and were filtered with a low-pass filter

166   (Bessel characteristic 2.8kHz cutoff). For current-clamp recordings, 1s hyperpolarizing current

167   steps were injected to elicit a hyperpolarization-induced sag (due to $I_H$ activation).

168   <u>Current-clamp recordings and protocols</u>

169   The spontaneous firing frequency was calculated from a minimum of 30 s of stable recording in

170   cell-attached mode and from current-clamp recording (with no injected current) within the first 5

171   min after obtaining the whole-cell configuration. The coefficient of variation of the interspike

172   interval ($CV_{ISI}$) was extracted from the same recording. Action potentials (APs) generated during

173   this period of spontaneous activity were averaged and several parameters were extracted: AP

174   threshold, AP amplitude, AP duration at half of its maximal height (AP half-width), AHP trough

175   voltage, AHP latency. Hyperpolarizing current steps and depolarizing current steps were used to

176   characterize the post-inhibitory rebound and the excitability properties. The gain start, gain end

9

177   and spike frequency adaptation (SFA) index used to define excitability were calculated as

178   described before (Dufour et al., 2014b).

179   <u>Voltage-clamp recordings</u>

180   The $I_A$ current was elicited by a protocol consisting in a 500 ms prestep at -100 mV (to fully de-

181   inactivate $I_A$) followed by a 500 ms voltage step to -40 mV (to activate $I_A$ without eliciting

182   delayed rectifier potassium currents). The current generated by the same protocol using a prestep

183   at -40mV (to fully inactivate $I_A$) was subtracted to isolate $I_A$. $I_A$ properties (peak amplitude and

184   total charge) were measured after subtracting the baseline at -40mV. Total charge was calculated

185   by integrating the current over the whole duration (500ms) of the voltage step. The peak of the

186   current elicited at -40mV was then plotted against the voltage of each corresponding prestep, and

187   was fitted with a Boltzmann function to obtain $I_A$ half-inactivation voltage ($V_{50}$ $I_A$) (see

188   (Amendola et al., 2012)). The inactivation time constant ($I_A$ tau) was extracted from a mono-

189   exponential fit of the decay of the current. A two-step voltage-clamp protocol was used to

190   determine the voltage-dependence of activation of $I_H$ ($V_{50}$ $I_H$) and obtain the maximum $I_H$

191   amplitude (see (Amendola et al., 2012) for details). For voltage-clamp recordings of delayed

192   rectifier current ($I_{KDR}$), tetrodotoxin (1 μM, Alomone), nickel (200 μM, Sigma-Aldrich) and

193   cadmium (400 μM, Sigma-Aldrich) were also added to the aCSF. $I_{KDR}$ was elicited by using a

194   protocol consisting in a prestep at -40mV (to fully inactivate $I_A$) followed by incremental

195   depolarizing voltage steps up to +40mV.

196   <u>Data acquisition</u>

197   Data were acquired using an EPC 10 USB patch-clamp amplifier (HEKA) and the Patchmaster

198   software acquisition interface (HEKA). Analysis was performed using FitMaster v2x73 (Heka).

10

199 ***Immunohistochemistry***

200 Adult (P21-P28) C57BL/6 WT mice (n=2) or Kv4.3$^{-/-}$ littermates (n=2) of either sex were

201 euthanized with ketamine-xylazine mix (100mg/Kg ketamine, 10mg/Kg xylazine), and

202 transcardially perfused with PBS and ice-cold 4% paraformaldehyde in PBS. Brains were

203 removed and post-fixed overnight (o/n) at 4°C in the same fixative solution. Coronal brain slices

204 of 50 μm were obtained using a vibratome (vibrating microtome 7000smz, Camden Instruments,

205 UK) and collected as floating sections. When indicated, antigen retrieval was performed by

206 incubating the slices in sodium citrate (10mM, Sigma-Aldrich) during 30 min at 80°C (Jiao et al.,

207 1999). Subsequently, slices were blocked for 1 h 30 min at room temperature (RT) in a solution

208 containing 0.3% Triton X-100 (SIGMA) and 5% Normal Goat Serum (NGS, Vector

209 Laboratories) in PBS. After blocking, sections were incubated with primary antibodies in a

210 solution containing 0.3% Triton X-100 and 1% NGS in PBS (o/n; 4°C). The following primary

211 antibodies were used in this study: Chicken anti-TH (1:1000; Abcam, ab76442,

212 RRID:AB_1524535), Rabbit anti Kv4.3 (1:500, Alomone Labs, APC-017, RRID:AB_2040178),

213 Mouse anti Kv4.2 (1:200, Neuromab, 75-361, clone L28/4, RRID:AB_23155873). After three

214 washes (15 min/each) in PBS containing 0.3% Triton X-100, the floating sections were incubated

215 with the following secondary antibodies: Alexa 488-Goat anti-mouse (1:200, Jackson

216 ImmunoResearch), Alexa 488-Goat anti-rabbit (1:200, Jackson ImmunoResearch) and Alexa

217 594-Goat anti-chicken (1:200, Jackson ImmunoResearch) in a PBS solution containing 0.3%

218 Triton X-100 and 1% NGS for 2 h at RT. Finally, sections were washed three times

219 (15min/each), incubated with DAPI (1.5 μg/mL; Sigma-Aldrich) for 10 min, and mounted in

220 Vectashield (Vector Laboratories). Sections were stored at 4°C, and images were acquired on a

221 Zeiss LSM-780 confocal scanning microscope. All experiments involving WT and Kv4.3$^{-/-}$

222    comparisons were performed in parallel applying the same acquisition settings to both genotypes.

223    Images were processed and analyzed with ImageJ (NIH). Kv4.2-positive cells were visually

224    identified in both genotypes on the basis of a perimembranous-like Kv4.2 staining and expressed

225    as a percentage of the total number of TH+ cells. In order to compare the labeling pattern of

226    Kv4.2 and Kv4.3, the line selection tool was used to trace 3µm-length lines perpendicular to the

227    cell perimeter in individual optical sections. In each cell, 3 regions were analyzed, and 5 cells

228    were used to calculate the average profile in each condition. Raw intensity values were collected,

229    normalized (0-1 range) to the maximal value, and plotted as a function of distance (0

230    corresponding to Kv4 peak fluorescence signal, negative distances to extracellular space and

231    positive distances to intracellular space; see **Figure 5B**). All the images shown are one single

232    optical slice. For the sake of representation and to overcome differences regarding levels of TH

233    expression in different DA neurons, different minimum and maximum display settings were

234    applied in **Figure 5C** exclusively for the TH channel.

235    *Modeling*

236    Simulations were performed using NEURON 7.5 software (Hines and Carnevale, 2001) as

237    previously described (Moubarak et al., 2019). Realistic morphologies of 22 rat SNc DA neurons

238    obtained previously were used to build multicompartment models (Moubarak et al., 2019). For

239    each compartment, membrane voltage was obtained as the time integral of a first-order

240    differential equation:

$$\frac{dV}{dt} = -\frac{1}{C_m} * \sum [g_i * (V_m - E_{rev})] - I_{axial}$$

241    where $V_m$ is the membrane potential, $C_m$ the membrane capacitance, $g_i$ are ionic conductances

242    and $E_{rev}$ their respective reversal potentials. The axial flow of current ($I_{axial}$) between adjacent

12

243    compartments is calculated by the NEURON simulation package (Hines and Carnevale, 2001).

244    Cytoplasmic resistivity, specific membrane capacitance and specific membrane resistance were

245    set to 150 Ohm.cm, 0.75 µF/cm², and 100,000 Ohm.cm², respectively, with $E_{rev}$ for the leak

246    conductance set at -50 mV. Six active conductances were included in the model: fast sodium

247    ($I_{Na}$), delayed rectifier potassium ($I_{KDR}$), transient potassium ($I_A$), L-type calcium ($I_{CaL}$),

248    hyperpolarization-activated ($I_H$) and small conductance calcium-activated potassium ($I_{SK}$)

249    currents (Moubarak et al., 2019). Active conductances followed activation-inactivation Hodgkin-

250    Huxley kinetics (**Table 2**). Parameters for $I_A$, $I_{CaL}$, $I_{SK}$, $I_{Na}$, $I_{KDR}$ and $I_H$ were based on our

251    previous model and published values for SNc DA neurons (Gentet and Williams, 2007; Seutin

252    and Engel, 2010; Amendola et al., 2012; Philippart et al., 2016; Moubarak et al., 2019).

253    Intracellular calcium uptake was modeled as a simple decaying model according to Destexhe

254    (Destexhe et al., 1993). Conductance values were set according to our own measurements or

255    published values (see **Table 2**). Consistent with the literature (Kole and Stuart, 2008; Hu et al.,

256    2009), $g_{Na}$ and $g_{KDR}$ were set to higher values in the axon initial segment (AIS) than in the rest of

257    the neuron so that the action potential always initiated in the AIS. For sake of simplicity,

258    activation and inactivation kinetics of $I_A$ were voltage-independent but coupled to each other,

259    such that activation rate was 50 times faster than inactivation rate. In addition the inactivation and

260    activation $V_{50}$s were also coupled (50 mV shift). As $I_A$ and $I_H$ voltage-dependences have been

261    shown to be positively correlated in rat SNc DA neurons (Amendola et al., 2012), both values

262    were forced to co-vary in the model according to the equation $V_{50}$ inact. $I_A = 0.814*(V_{50}$ act.

263    $I_H)+3.36$.

264    Initializing potential was set at -70 mV and pacemaking frequency was let to stabilize (4 spikes)

265    before further analysis. Each simulation run had a duration of 8000 ms with a dt of 0.02 ms.

13

266  Spatial discretization followed the "d_lambda rule" (Hines and Carnevale, 2001). All dendritic

267  compartments and the axon-start compartment contained all currents whereas AIS and axon only

268  contained fast sodium ($I_{Na}$) and delayed rectifier potassium ($I_{KDR}$) currents. To measure post-

269  inhibitory rebound delay, current injection was performed by inserting a virtual electrode in the

270  soma. A 1s pulse of current was injected into the model. Negative current amplitude was adjusted

271  to achieve a peak hyperpolarization around -120 mV in each neuron and condition. Firing

272  frequency, rebound delay and action potential property analyses were computed online by

273  handmade routines directly written in NEURON hoc language (Hines and Carnevale, 2001). This

274  model is derived from a previous model available at model DB database under the number

275  245427.

276  ***Statistics***

277  All statistical analyses were conducted under the R environment with appropriate packages. For

278  behavioral experiments, normality was assessed by visual inspection of quantile-quantile (Q-Q)

279  plots for the different scores per animal (ggpubr package). Nearly all the data points did not

280  depart for normality estimated within a 95% coefficient interval. For electrophysiology

281  experiments, normality was checked using the Shapiro-Wilk normality test (stat package).

282  <u>Behavior</u>

283  To assess locomotor and exploratory behavior, number of horizontal (locomotion) and vertical

284  (rearing) photobeam breaks was measured per 5-min bin over 90 min and compared between

285  genotypes. As no significant difference was found between males and females in any of the

286  behavioral tests, both sexes were pooled and analyzed as a single sample. Data are represented as

287  line and scatter plots for the number of horizontal and vertical photobeam breaks per 5-min bin.

14

288    The number of total movements was then normalized to the level of WT spontaneous

289    locomotion. To assess the locomotor phenotype of Kv4.3$^{-/-}$ mice, two-way repeated measures

290    ANOVA tests with groups (WT/Kv4.3$^{-/-}$) as the independent between-factor and time as the

291    within-factor (training sessions or time-bins for locomotion) were performed (stat package).

292    When the ANOVA was significant, multiple comparisons (false-discovery rate (fdr) adjustment,

293    multicomp and emmeans packages) were used to evaluate differences between groups at different

294    time points (Benjamini and Hochberg, 1995). P-values $<0.05$ were considered as statistically

295    significant for all analyses. For motor learning, the average latency to fall off the accelerating

296    rotarod was measured for each trial. Statistical difference in motor learning was assessed by

297    comparing the *performance index*. Data are represented as line and scatter plots for the average

298    latency to fall off the rod. Data are represented as mean ± SEM.

299    Electrophysiology and immunohistochemistry

300    The univariate statistical analysis of electrophysiological data, performed according to the

301    distribution properties of the data using a Shapiro-Wilk normality test, included paired t-test or

302    Wilcoxon signed rank test; t-test or Mann-Whitney Wilcoxon test with $p < 0.05$ considered to be

303    statistically significant (stat package). In most figures, data are represented as scatter plots or box

304    and whisker plots, with all individual points appearing on the graphs and dotted lines indicating

305    the distribution of data (violin plots). For pharmacological experiments, data are represented as

306    mean ± SEM (scatter or bar plots). Correlation, linear regression and multiple linear regression

307    analysis (**Figure 1**, **9**) were performed in R. For every pair of variables, correlation parameters,

308    rho (Spearman correlation factor) or r (Pearson correlation factor), were selected after performing

309    a Shapiro-Wilk normality test on the linear regression residuals and p values were corrected for

310    multiple comparisons by an fdr adjustment (stat package). For multiple linear regression,

15

311    variables (extracellular ISI, rebound delay, $I_A$ tau, $I_H$ amplitude and $I_A$ amplitude) were first log

312    transformed, and then dependent variables were standardized (subtracting the mean and dividing

313    by the SD). A selection of the best subsets of dependent variables for each model size (1 to 4 for

314    the model and 1 to 5 for real data) was first performed (leaps package) according to several

315    criteria (adjusted $R^2$, AIC, BIC). The best model was then selected by comparing the prediction

316    error of each model after performing a repeated (20 times) 10-fold cross-validation on test data

317    (caret package). The best linear model, corresponding to the minimum cross-validation error (i.e.

318    a model with the best predictive power) was then obtained. Multicolinearity was assessed by

319    computing a score called the variance inflation factor (VIF package) and VIF was < 1.5 for all

320    variables retained in the different models. For the immunohistochemistry experiments, a Fisher

321    exact test was used to compare the proportions of Kv4.2-positive cells among TH-positive cells

322    in WT and Kv4.3$^{-/-}$ mice. Figures were prepared using R, SigmaPlot 11.0, GraphPad Prism 6 and

323    Adobe Illustrator CS5/CS6.

324

325    **RESULTS**

326    *Motor learning impairment of the Kv4.3$^{-/-}$ transgenic mice*

327    In order to define the precise contribution of Kv4.3 channels to SNc DA neuron

328    electrophysiological phenotype, we used Kv4.3 constitutive KO mice (Niwa et al., 2008;

329    Carrasquillo et al., 2012; Granados-Fuentes et al., 2012). These mice have been used previously

330    to study Kv4.3 function in cardiac ventricles (Niwa et al., 2008), in neurons of the

331    suprachiasmatic nucleus (Granados-Fuentes et al., 2012) and in neocortical pyramidal neurons

332    (Carrasquillo et al., 2012). No major defect in cardiac function or circadian locomotor behavior

16

333     was reported in these studies. As Kv4.3 is also strongly expressed in the SNc and in the ventral

334     tegmental area (VTA) (Serodio and Rudy, 1998), we first sought to determine whether Kv4.3

335     loss could affect SNc or VTA-related behaviors, such as locomotion and motor learning (**Figure**

336     **1**). As illustrated in **Figure 1A**, horizontal locomotor activity assessed in photocell activity

337     chambers was similar between Kv4.3$^{-/-}$ mice and their WT littermates ($F_{1,21}$=0.31, p=0.58,

338     between factor effect), both strains displaying a significant decrease of locomotion over time

339     ($F_{17,357}$=20.5, p=2•$10^{-16}$, within factor effect). The exploratory behavior was higher in Kv4.3$^{-/-}$

340     mice than WT littermates in particular during the first part of the session (**Figure 1A**), although

341     the overall exploratory activity was not significantly different between the two strains ($F_{1,21}$=1.42,

342     p=0.25, between factor effect). Again, both strains displayed a strong run-down of activity over

343     the whole 90-min testing time as a function of habituation to the locomotor activity chambers

344     ($F_{17,357}$=13.97, p=2•$10^{-16}$, within factor effect). Consistent with the increased exploratory activity

345     at the start of the session for the Kv4.3$^{-/-}$ mice, the two-way repeated measures ANOVA revealed

346     a significant interaction between strains and time ($F_{17,357}$=1.87, p=0.019). Motor learning abilities

347     were then assessed using the rotarod test for ten consecutive trials (**Figure 1B**). While WT mice

348     improved their performance across trials, as shown by the increase in the latency-to-fall, Kv4.3$^{-/-}$

349     mice were not able to adjust their performance over time. While the latency averaged over trials

350     was not different between the two strains ($F_{1,22}$=1.26, p=0.28, between factor effect, two-way

351     repeated measures ANOVA), performance changed significantly across trials ($F_{9,\ 198}$=4.5,

352     p=2•$10^{-5}$, within factor effect), especially for WT mice. Consistent with the differences in latency

353     for the late trials (**Figure 1B**), the difference in learning between the two strains was revealed by

354     the strains*trials interaction statistics ($F_{9,198}$=2.95, p=0.0026) and significant differences in fall-

355     off latencies of Kv4.3$^{-/-}$ and WT mice was found for the late trials (7 to 9, **Figure 1B**). In order to

356     better quantify the difference in learning between strains, we then calculated a performance index

357  based on the difference in latency for the first 3 trials against the last 3 trials. Consistent with the

358  ANOVA results, the performance index in this motor learning task was significantly lower for

359  the Kv4.3[-/-] mice (WT, 176.9 ± 21.4, n=11 *vs* Kv4.3[-/-], 111.2 ± 8.3, n=13, p=0.006, unpaired t-

360  test, **Figure 1B**).

361  ***Changes in electrophysiological phenotype of Kv4.3[-/-] SNc DA neurons***

362  Following the approach already used in a previous study (Dufour et al., 2014b), we then

363  performed an exhaustive current-clamp characterization of the firing properties of SNc DA

364  neurons to determine changes in phenotype associated with Kv4.3 deletion (**Table 1**). Passive

365  properties, spontaneous activity, post-inhibitory rebound, action potential shape and excitability

366  were assessed by measuring 16 different electrophysiological parameters in 75-101 WT and 66-

367  77 Kv4.3[-/-] neurons. The first obvious electrophysiological change observed was that spontaneous

368  activity (extracellularly recorded in cell-attached mode) was dramatically modified in Kv4.3[-/-]

369  SNc DA neurons (**Figure 2A**, **B**). Spontaneous firing rate was increased by ~2-fold in Kv4.3[-/-]

370  mice, as demonstrated by the significant decrease in interspike interval (ISI, **Figure 2A**, **B**; **Table**

371  **1**). Pacemaking regularity, measured by the coefficient of variation of the ISI ($CV_{ISI}$) was also

372  significantly different in Kv4.3[-/-] mice, although $CV_{ISI}$ values were very low (<20%) in both WT

373  and Kv4.3[-/-] mice, indicating a highly regular tonic activity. Post-inhibitory rebound delay was

374  also dramatically decreased in Kv4.3[-/-] mice (**Figure 2C**, **D**; **Table 1**). However, the $I_H$-mediated

375  voltage sag observed during prolonged hyperpolarization was not modified (**Figure 2C**, **D**; **Table**

376  **1**). Interestingly, most action potential parameters were unchanged in Kv4.3[-/-] mice, except for

377  action potential half-width, which was slightly larger (**Figure 3A**; **Table 1**). We also analyzed

378  neuronal excitability by measuring the responses of the neurons to increasing depolarizing

379  current steps (**Figure 3B**). Excitability was slightly increased in the DA neurons of Kv4.3[-/-] mice,

18

380    although this change only affected the initial response of neurons (gain start) to current injection

381    (**Figure 3B**; **Table 1**). Consistently, the frequency of the response of Kv4.3[-/-] neurons to a 100pA

382    step was also found to be significantly higher (**Figure 3B**, **Table 1**).

383    *Voltage-clamp characterization of $I_A$ in Kv4.3[-/-] SNc DA neurons*

384    We then directly investigated changes in the properties of $I_A$ by performing voltage-clamp

385    recordings in WT and Kv4.3[-/-] SNc DA neurons (**Figure 4**). A dramatic decrease in $I_A$ amplitude

386    was observed in Kv4.3[-/-] mice (**Figure 4A**, **B**; **Table 1**). However, a small transient residual

387    current with $I_A$-like properties (voltage-dependent inactivation) was still present in all Kv4.3[-/-]

388    recordings (**Figure 4A**). Most interestingly, this residual current was completely blocked by the

389    Kv4-specific toxin AmmTX3 (n=4, no measurable residual current after toxin application;

390    **Figure 4A**), suggesting that a Kv4 subunit other than Kv4.3 is expressed at a low level in Kv4.3[-/-]

391    SNc DA neurons. We then measured its time constant of inactivation ($I_A$ tau) and calculated the

392    overall charge carried by the current (**Figure 4C-E**). Both parameters were strongly decreased in

393    Kv4.3[-/-] SNc DA neurons (**Figure 4C**, **D**; **Table 1**), although a minority of cells (n=5/42)

394    displayed values similar to the WT measurements for both of these parameters. Plotting $I_A$ charge

395    versus $I_A$ tau revealed the clear separation of values between the Kv4.3[-/-] and WT measurements,

396    except for the 5 cells identified before (**Figure 4E**). Based on these voltage-clamp data, it appears

397    that, although the Kv4.3 subunit by far predominates in WT SNc DA neurons, another Kv4

398    subunit is also expressed, at least in the Kv4.3[-/-] neurons. Although in most cases, the expression

399    level of this unidentified subunit is too low to compensate for the loss of Kv4.3, it generates an

400    A-type current that provides a minority of Kv4.3[-/-] SNc DA neurons (5/42=12%) with a "wild-

401    type" voltage-clamp phenotype.

19

*Using antigen retrieval to reveal the expression of Kv4.2 channels by SNc DA neurons*

403　Several studies have investigated the expression of A-type Kv channels in SNc DA neurons,

404　using in situ hybridization (Serodio et al., 1996; Serodio and Rudy, 1998), single-cell quantitative

405　PCR (Liss et al., 2001; Ding et al., 2011; Tapia et al., 2018) and immunohistochemistry (Liss et

406　al., 2001; Dufour et al., 2014a). A high level of expression for Kv4.3 (Liss et al., 2001; Dufour et

407　al., 2014a; Tapia et al., 2018) and the absence of Kv4.1 (Serodio and Rudy, 1998; Liss et al.,

408　2001; Ding et al., 2011) were consistently reported, while the presence of Kv4.2 is still debated.

409　In particular, while Kv4.2 mRNA has been detected in several studies (Ding et al., 2011; Tapia et

410　al., 2018), the protein was not detected by classical immunohistochemistry (Liss et al., 2001;

411　Dufour et al., 2014a). Interestingly, it has been shown that, depending on the brain region and the

412　subcellular location of the ion channel of interest, an antigen retrieval (AR) procedure may be

413　required to uncover potassium channel antigen epitopes before performing immunolabeling

414　(Lorincz and Nusser, 2008). We first confirmed that Kv4.3 was strongly expressed in SNc DA

415　neurons in WT mice, with the expected membrane profile of immunostaining, and that it was

416　absent from Kv4.3$^{-/-}$ SNc DA neurons (**Figure 5A**). We then performed Kv4.2 immunolabeling

417　with or without AR on the neocortex and the CA1 region of the hippocampus where this ion

418　channel is highly expressed (Serodio and Rudy, 1998). As can be seen in **Figure 5-1**, Kv4.2

419　immunostaining was greatly improved by AR, revealing a strong perisomatic and dendritic

420　staining of pyramidal cells in both regions. Therefore we implemented AR before performing

421　Kv4.2 immunostaining on WT midbrain slices (**Figure 5B**). Similar to what we observed in the

422　hippocampus and in the cortex, Kv4.2 immunostaining in the SNc was greatly improved by AR,

423　although only a minority of DA neurons (TH-positive) displayed a clear perisomatic Kv4.2 signal

424　compatible with membrane expression of the channel. In fact, the AR Kv4.2 staining profile of

425  Kv4.2-positive cells was very similar to the membrane staining profile observed for Kv4.3

426  (**Figure 5B**). This distinctive staining profile was then used to quantify the percentage of Kv4.2-

427  positive SNc DA neurons in both WT and Kv4.3$^{-/-}$ mice (**Figure 5C, D**). The percentage of

428  Kv4.2-positive cells was not significantly different between WT and Kv4.3$^{-/-}$ mice (WT

429  20/423=4.7% *vs* Kv4.3$^{-/-}$ 19/382=5%, p=1, Fisher exact test, **Figure 5D**), suggesting that Kv4.2

430  pattern of expression is not modified by the loss of Kv4.3. This percentage is not statistically

431  different from the percentage of Kv4.3$^{-/-}$ SNc DA neurons presenting an atypically large and slow

432  $I_A$ reported in **Figure 4C-E** (5/42=11.9% vs 19/382=4.6%, p=0.077, Fisher exact test). Moreover,

433  the percentage of Kv4.2-positive DA neurons was very similar in the medial and lateral SNc of

434  both WT (SNc medial 11/227=4.8% *vs* SNc lateral 9/196=4.6%, p=1, Fisher exact test) and

435  Kv4.3$^{-/-}$ mice (SNc medial 13/218=6% *vs* SNc lateral 6/164=3.7%, p=0.35, Fisher exact test).

436

437  ***Lack of compensation in the face of Kv4.3 loss***

438  Genetic deletion of Kv4.2 channels in cortical pyramidal neurons is associated with

439  compensatory modifications in a delayed rectifier-like ($I_{KDR}$-like) potassium current (Nerbonne et

440  al., 2008). Moreover, $I_H$ and $I_A$ have complementary influences on post-inhibitory firing in SNc

441  DA neurons (Amendola et al., 2012; Tarfa et al., 2017). In order to reveal putative homeostatic

442  compensations of Kv4.3 deletion in SNc DA neurons, we first performed a series of current-

443  clamp recordings on a subset of neurons (n=18 for WT, n=32 for Kv4.3$^{-/-}$) to compare the effect

444  of acutely blocking Kv4 channels using the scorpion toxin AmmTX3 (Vacher et al., 2002) to the

445  changes observed in the Kv4.3$^{-/-}$ mouse. Consistent with previous reports (Amendola et al., 2012;

446  Tarfa et al., 2017), AmmTX3 strongly increased pacemaking frequency and dramatically reduced

447    post-inhibitory rebound delay (**Figure 6**). Most interestingly though, the magnitude of the effects

448    of the toxin was very similar to that observed in the Kv4.3$^{-/-}$ neurons: firing frequency was

449    increased by ~68% in both conditions (**Figure 6B**), while rebound delay was decreased by 87%

450    after AmmTX3 and by 82% in Kv4.3$^{-/-}$ mice (**Figure 6D**). Consistent with the data presented

451    earlier, these results strongly suggest that the Kv4-mediated A-type current is virtually

452    completely abolished in Kv4.3$^{-/-}$ SNc DA neurons and that its loss is not compensated by changes

453    in other Kv channels (and associated currents).

454    We then used voltage-clamp recordings to directly assess whether a decrease in $I_A$ could be

455    compensated by a parallel decrease in $I_H$ or a compensatory increase in $I_{KDR}$ (Nerbonne et al.,

456    2008) (**Figure 7**). Unlike what has been described in cortical neurons following Kv4.2 deletion,

457    $I_{KDR}$ was not modified in Kv4.3$^{-/-}$ SNc DA neurons (**Figure 7A**). $I_H$ was found to be slightly

458    larger in Kv4.3$^{-/-}$ SNc DA neurons (**Figure 7B**, **Table 1**), but its voltage sensitivity was

459    unchanged. Altogether, the voltage-clamp recordings of $I_A$, $I_{KDR}$ and $I_H$ and the AR Kv4.2

460    immunostaining suggest that Kv4.3 loss is not compensated by changes in expression and/or

461    function of functionally-overlapping channels. These data provide a biophysical explanation for

462    the observation made earlier that the acute blockade of Kv4 channels produces an

463    electrophysiological phenotype qualitatively and quantitatively virtually identical to the Kv4.3

464    genetic deletion (**Figure 6**).

465    ***Bridging the gap between biophysical changes in $I_A$ and $I_H$ and variation in***

466    ***electrophysiological phenotype***

467    We then decided to investigate whether the cell-to-cell variations in $I_A$ biophysical properties in

468    WT and Kv4.3$^{-/-}$ SNc DA neurons were predictive of variations in electrophysiological

469    phenotype (**Figure 8**). We first looked at potential correlations between firing parameters. As

470    already presented in **Figure 2**, the most significant alterations in firing observed in Kv4.3[-/-] SNc

471    DA neurons are a strong increase in spontaneous firing frequency (strong decrease in the

472    extracellularly-measured ISI) and a strong decrease in post-inhibitory rebound delay. We found

473    that extracellular ISI and rebound delay (log transformed) were strongly positively correlated

474    with each other in both WT and Kv4.3[-/-] neurons (**Figure 8B**), although the slope of this

475    relationship seemed slightly different between the two genotypes. We therefore tried to determine

476    whether specific $I_A$ biophysical properties were better predictors of these variations in ISI or

477    rebound delay (**Figure 8C**). We also analyzed the relationship between $I_H$ properties and ISI or

478    rebound delay. Out of the 5 biophysical parameters analyzed ($I_A$ tau, $I_H$ amplitude, $I_A$ amplitude,

479    $I_A$ inactivation $V_{50}$, $I_H$ activation $V_{50}$), only two parameters were significantly correlated with ISI

480    or rebound delay: $I_A$ tau and $I_H$ amplitude (log transformed) were positively and negatively

481    correlated, respectively, with both ISI and rebound delay in both WT and Kv4.3[-/-] neurons

482    (**Figure 8C**). Surprisingly, neither $I_A$ amplitude (measured at -40mV) nor its voltage-dependence

483    (inactivation $V_{50}$) were predictive of variations in ISI or rebound delay (**Figure 8C**). $I_H$ activation

484    $V_{50}$ was also unable to predict variations in these firing parameters (data not shown). In addition,

485    reminiscent of the observation made in rat neurons (Amendola et al., 2012), $I_H$ activation and $I_A$

486    inactivation $V_{50}$s were found to be positively correlated (**Figure 8D**). $I_A$ tau and $I_H$ amplitude

487    were also found to be negatively correlated (**Figure 8D**). Based on these observations, we then

488    tested whether combining several of these 5 biophysical parameters could improve the prediction

489    of ISI or rebound delay using multiple linear regression (**Figure 8E**). We first standardized these

490    parameters (subtracting the mean and dividing by the SD), and then looked for the best subset of

491    variables predictive of ISI or rebound delay. While ISI was best predicted by a multiple linear

492    regression involving only $I_A$ tau and $I_H$ amplitude, rebound delay was better predicted when $I_A$

23

493    tau, $I_H$ amplitude and $I_A$ inactivation $V_{50}$ were included in the linear regression (**Figure 8E**).

494    Rebound delay prediction was much more accurate than ISI prediction ($r^2$=0.771 compared to

495    $r^2$=0.421). Based on the scaling factors given by the multiple linear regression, it is important to

496    note that both ISI and rebound delay in real neurons seem to be most sensitive to variations in $I_A$

497    tau.

498    *Influence of $I_A$ biophysical properties on SNc DA neuron firing*

499    Many conductances other than $I_A$ and $I_H$ may vary in expression level and biophysical properties

500    from neuron to neuron, potentially compensating or enhancing the effect of variations in the

501    properties of these two currents on firing (Gentet and Williams, 2007; Puopolo et al., 2007;

502    Seutin and Engel, 2010; Amendola et al., 2012; Philippart et al., 2016; Moubarak et al., 2019). In

503    order to isolate the influence of specific biophysical properties of $I_A$ and $I_H$ on SNc DA neuron

504    activity, we used a realistic multi-compartment Hodgkin-Huxley model of rat SNc DA neurons

505    (Moubarak et al., 2019). Based on measurements obtained by different groups (Liss et al., 2001;

506    Gentet and Williams, 2007; Amendola et al., 2012; Tarfa et al., 2017), each of the biophysical

507    properties of $I_A$ (maximal conductance $g_A$, voltage-dependence $I_A$ $V_{50}$, inactivation rate $I_A$ tau)

508    and $I_H$ maximal conductance ($g_H$) were varied over a 10-fold range (20mV range for the voltage-

509    dependence) using 5 equi-distributed values (**Figure 9A**). $I_A$ and $I_H$ voltage-dependences were

510    forced to co-vary in the model, based on our previous observations (Amendola et al., 2012).

511    Using a sample of 22 realistic models and 5 independently-varying values for each biophysical

512    property, a database of 13750 models ($22*5^4$) was generated (**Figure 9**). The average interspike

513    interval (ISI) during spontaneous activity and the post-inhibitory firing delay in response to a

514    hyperpolarizing pulse (rebound delay) were measured for each model and their average values

515    were calculated for each combination of values of the 4 biophysical parameters (n=625).

24

516   Dimensional stacking (Taylor et al., 2006) was then used to represent the influence of the 4

517   biophysical parameters on these electrophysiological features in 2-dimensional heatmaps,

518   allowing us to visually determine which parameters were most critical in controlling ISI and

519   rebound delay (**Figure 9B**, **C**): $g_A$ and $I_A/I_H$ $V_{50}$ strongly modulated both ISI and rebound delay

520   while $I_A$ tau and $g_H$ had a weaker influence on these firing properties. To quantify the

521   contribution of each biophysical parameter to ISI and rebound delay variations, we then used the

522   same strategy already presented in **Figure 8E** for experimental measurements: standardized

523   parameters were used to run multiple linear regression against ISI or rebound delay (**Figure 9D**).

524   Consistent with the visualization provided in **Figure 9B**, this sensitivity analysis revealed that the

525   influence of $g_A$ and $I_A/I_H$ $V_{50}$ on ISI and rebound delay was 2-3 times stronger than that of $g_H$ and

526   $I_A$ tau. While the results of this computational modeling are consistent with the general influence

527   of $I_A$ and $I_H$ reported in these neurons (Liss et al., 2001; Seutin et al., 2001; Neuhoff et al., 2002;

528   Puopolo et al., 2007; Amendola et al., 2012; Gantz et al., 2018), they reveal specific effects of $I_A$

529   biophysical properties contrasting with the correlations identified in our experimental data.

530

531   **DISCUSSION**

532   The current study provides important elements regarding the identity of the ion channels

533   underlying $I_A$ in SNc DA neurons and the relative influence of specific biophysical parameters of

534   $I_A$ (voltage-dependence, gating kinetics, maximal conductance) on neuronal output. In particular,

535   in contrast with previous studies (Serodio and Rudy, 1998; Liss et al., 2001; Dufour et al.,

536   2014a), we show that the Kv4.2 subunit is expressed in SNc DA neurons, although its functional

537   influence appears negligible in most neurons, due to a very low level of expression. In spite of

25

538　Kv4.2 presence, we show that the constitutive loss of Kv4.3 is not compensated by

539　complementary intrinsic conductances (Kv4.2-mediated $I_A$, $I_{KDR}$, $I_H$). In addition, while previous

540　studies (Liss et al., 2001; Putzier et al., 2008) and the computational modeling performed here

541　suggest a strong role of $I_A$ conductance in controlling firing frequency, we demonstrate that $I_A$

542　gating kinetics appear as the major determinant of both pacemaking frequency and post-

543　inhibitory rebound delay. Our results also highlight the functional complementarity and

544　correlation of biophysical properties of $I_A$ and $I_H$ in these neurons.

545　*Kv4.2 is expressed in SNc DA neurons*

546　One of the important results of the present work is the demonstration that Kv4.2 is expressed in

547　mouse SNc DA neurons. So far, it was thought that only Kv4.3 was expressed and entirely

548　responsible for the large A-type current observed in these neurons (Liss and Roeper, 2008; Gantz

549　et al., 2018). Our results however unambiguously demonstrate that Kv4.2 is expressed by SNc

550　DA neurons. First, in the absence of Kv4.1 (Liss et al., 2001; Ding et al., 2011), the presence of

551　an AmmTX3-sensitive residual A-type current in the Kv4.3$^{-/-}$ neurons is only compatible with the

552　expression of Kv4.2. While the Kv4.2-mediated residual $I_A$ is very small and fast in most

553　neurons, in 12% of the voltage clamp-recorded neurons it is large enough to confer a "wild type"

554　phenotype (see **Figure 5, 9**). Even though this residual $I_A$ current is much faster than its WT

555　counterpart in most neurons, it still influences firing, particularly rebound delay, as suggested by

556　the highly significant correlation between $I_A$ tau and rebound delay. Second, antigen retrieval

557　immunohistochemistry confirmed that a Kv4.2 staining is observed in a minority (~5%) of SNc

558　DA neurons in both Kv4.3$^{-/-}$ and WT mice. While the percentage of cells displaying a "high"

559　expression of Kv4.2 is too small to allow a combined voltage-clamp/AR immunohistochemistry

560　approach, the similarity in the proportion of AmmTX3-sensitive "large residual" $I_A$ (~12%) and

26

561 Kv4.2-positive neurons (~5%, not statistically different) strongly suggests that Kv4.2 is

562 responsible for a large $I_A$ in a minority of SNc DA neurons. The presence of a small AmmTX3-

563 sensitive residual $I_A$ in the rest of the Kv4.3[-/-] SNc DA neurons suggests that Kv4.2 is expressed

564 and functional in all SNc DA neurons, although its influence might be minor in the presence of

565 Kv4.3. The fact that the residual A-type current in Kv4.3[-/-] neurons inactivates faster than its WT

566 counterpart is also consistent with the reported differences in inactivation kinetics of Kv4.2 and

567 Kv4.3 (Serodio et al., 1994; Serodio et al., 1996). Altogether, these results suggest that, in

568 contrast to the widely accepted view, a small subpopulation of SNc DA neurons (~5-10%)

569 display an A-type current likely mediated by both Kv4.3 and Kv4.2 channels. Interestingly, these

570 two subunits are very similar and co-immunoprecipitate from mouse brain lysates (Marionneau et

571 al., 2009), suggesting that they could form heteromeric $I_A$ channels. At this point however, it is

572 difficult to determine whether the Kv4.2-positive neurons in WT mice express Kv4.3/Kv4.2

573 heteromers or distinct Kv4.3 and Kv4.2 homomers.

574 ***Lack of homeostatic compensation in Kv4.3[-/-] SNc DA neurons***

575 Intriguingly, these results not only suggest that Kv4.2 expression is very low in most SNc DA

576 neurons but also that it is not modified in the Kv4.3[-/-] SNc DA neurons. The structural and

577 functional similarities between Kv4.3 and Kv4.2 means that in theory Kv4.2 channels should be

578 able to compensate for the loss of Kv4.3. Despite this, the Kv4.2 pattern of expression is not

579 modified in Kv4.3[-/-] SNc DA neurons. This is reminiscent of previous studies performed on the

580 Kv4.2[-/-] mouse demonstrating that Kv4.3 expression pattern, assessed by western-blot or

581 immunohistochemistry, is not modified following Kv4.2 loss (Menegola and Trimmer, 2006;

582 Nerbonne et al., 2008). In addition, other currents (such as $I_{KDR}$ or $I_H$) also appear to not be

583 regulated in a compensatory direction in Kv4.3[-/-] SNc DA neurons. The current-clamp

27

584 comparison between the acute blockade of Kv4 channels in WT neurons and the Kv4.3[-/-] neurons

585 also supports this idea of a lack of compensatory modifications in functionally-overlapping

586 currents. This is surprising in the light of the results obtained on neonatal cortical pyramidal cells

587 (Nerbonne et al., 2008), where Kv4.2 genetic deletion is almost "fully" compensated by an

588 increase in sustained potassium currents. To explain this difference, we may hypothesize that,

589 although the alteration in electrophysiological phenotype observed in Kv4.3[-/-] neurons is striking

590 (see **Figure 3** in particular), the change in calcium dynamics associated with the elevated

591 spontaneous activity may not be sufficient to trigger homeostatic regulatory mechanisms

592 (O'Leary et al., 2014). Alternatively, modifications in the properties of incoming excitatory or

593 inhibitory synaptic inputs (not analyzed in the current study) may compensate for the changes in

594 intrinsic activity reported here, such that the overall *in vivo* activity of the SNc network is

595 maintained in Kv4.3[-/-] animals. However, behavioral alterations suggest that the change in firing

596 of SNc DA neurons is not totally compensated at the network level. Kv4.3[-/-] mice were more

597 active than WT littermates in exploring the environment (increased rearing behavior) and could

598 not adjust their motor control over sessions on the rotarod. This suggests that the changes in

599 midbrain DA neuron tonic firing observed in the Kv4.3[-/-] SNc DA neurons might only be

600 revealed when animals are exploring their environment or challenged in a motor learning task.

601 *$I_A$ gating kinetics play a central role in SNc DA neuron output*

602 The use of $I_A$ voltage-clamp measurements and current-clamp recordings on a large number of

603 neurons in WT and Kv4.3[-/-] mice allowed us to determine the impact of cell-to-cell variations in

604 $I_A$ biophysical properties on spontaneous activity and rebound delay. Interestingly, while realistic

605 multi-compartment modeling suggested that $I_A$ maximal conductance and voltage-dependence

606 were the two factors most strongly influencing these electrophysiological features, our recordings

28

607    revealed that $I_A$ inactivation rate was the dominant factor defining pacemaking frequency and

608    rebound delay (**Figure 9E**). $I_H$ amplitude (proportional to $I_H$ maximal conductance in our

609    measurements) was also found to play an important role in real neurons, while its influence was

610    minor in the model. These differences may be explained by several factors. First, the database

611    approach used for our simulations implies that all the tested biophysical properties are varied

612    independently (except for the strict correlation applied to $I_A$ and $I_H$ $V_{50}$s). While the independence

613    between these biophysical parameters allowed us to precisely quantify the sensitivity of

614    spontaneous activity and rebound delay to each parameter, it does not correspond to the

615    observations made in real neurons: for instance $I_H$ amplitude and $I_A$ inactivation rate are

616    negatively correlated, i.e. not independent from each other. On the other hand, reminiscent of a

617    previous study performed on rat neurons (Amendola et al., 2012), we demonstrated that $I_A$ and $I_H$

618    $V_{50}$s are also positively correlated in WT mouse neurons. However, this correlation is much

619    lower than the one applied in the model (r=0.658, $r^2$=0.43 compared to $r^2$=1) and this difference

620    may explain why this biophysical parameter appears as one of the most efficient in modulating

621    firing in the model. Thus, the differences in independence of the biophysical properties may

622    partly explain why the model and the experimental observations give different answers. Another

623    factor may explain why $I_A$ maximal conductance has a strong effect on firing in the model, but

624    not in real neurons. In order to isolate the effect of $I_A$ and $I_H$ biophysical properties on firing, all

625    other conductances included in the model were held at fixed values. However, every ion current

626    displays significant cell-to-cell variations in its properties (gating, conductance density) in a same

627    neuronal population (Swensen and Bean, 2005; Schulz et al., 2006; Amendola et al., 2012;

628    Moubarak et al., 2019). If happening at random, these variations in other currents would most

629    likely dampen the effect of the variations in $I_A$ or $I_H$ specific properties on firing. In fact, we

630    demonstrated in a previous study (Tapia et al., 2018) that the level of expression of Kv4.3 (at the

29

631　mRNA level) in midbrain DA neurons co-varies with the expression levels of multiple

632　somatodendritic ion channels, including Nav1.2, SK3 and GIRK2. If this co-variation is retained

633　at the protein level, it would mean that cell-to-cell variations in $I_A$ maximal conductance occur in

634　parallel with variations in density of other ion channels. Whether correlated or not, cell-to-cell

635　variations in other conductances may thus explain why $I_A$ amplitude does not predict pacemaking

636　and rebound delay in real neurons, and why $I_A$ inactivation rate appears as the main predictor of

637　these electrophysiological features. In contrast with our findings, the results obtained by Liss and

638　colleagues (2001) suggested that Kv4.3 expression level and channel density predicted

639　pacemaking frequency in mouse neurons. Interestingly, it is noteworthy that $I_A$ inactivation rate

640　showed restricted cell-to-cell variations in their recordings (2-fold range). On the other hand, $I_A$

641　charge density showed a 10-fold range of variation, suggesting that most of the variation in $I_A$

642　function was due to variations in $I_A$ maximal conductance (Liss et al., 2001). In our recordings

643　however, the levels of variability observed for $I_A$ (and $I_H$) biophysical properties were rather

644　similar, which led us to apply a 10-fold range to each parameter in our model. Thus, we postulate

645　that the differences in our conclusions may be essentially related to differences in the cell-to-cell

646　variability range of $I_A$ and $I_H$ biophysical parameters recorded in our samples.

647　***Functional complementarity and co-regulation of $I_A$ and $I_H$ in SNc DA neurons***

648　While our results confirm the well-established influence of $I_A$ on SNc DA neuron firing (Liss et

649　al., 2001; Gentet and Williams, 2007; Putzier et al., 2008; Amendola et al., 2012; Tarfa et al.,

650　2017), they also emphasize the functional complementarity between $I_A$ and $I_H$ in these neurons,

651　and reinforce the idea that the channels underlying these currents are co-regulated (Amendola et

652　al., 2012). Indeed, we confirm that $I_A$ and $I_H$ voltage-dependences are positively correlated and

653　show that $I_H$ amplitude and $I_A$ inactivation rate are negatively correlated. While we do not have a

30

654 mechanistic explanation for this latter correlation, these results are reminiscent of the

655 observations made by Tarfa and colleagues on nigrostriatal and mesoaccumbal DA neurons

656 (2017). At the functional level, our results demonstrate that these two parameters are the main

657 predictors of the cell-to-cell variations in pacemaking frequency and rebound delay, reinforcing

658 the idea that $I_A$ and $I_H$ function as a complementary pair of currents tightly controlling post-

659 inhibitory rebound delay in SNc DA neurons ($r^2$=0.77). The fact that pacemaking rate is not as

660 accurately predicted by $I_A$ and $I_H$ properties ($r^2$=0.42) is consistent with the documented role of

661 many other conductances and morphological parameters in defining this firing feature

662 (Nedergaard and Greenfield, 1992; Wilson and Callaway, 2000; Wolfart et al., 2001; Liss et al.,

663 2005; Puopolo et al., 2007; Putzier et al., 2009; Gantz et al., 2018; Moubarak et al., 2019).

664

665 **REFERENCES**

666 Amendola J, Woodhouse A, Martin-Eauclaire MF, Goaillard JM (2012) Ca(2)(+)/cAMP-

667 sensitive covariation of I(A) and I(H) voltage dependences tunes rebound firing in

668 dopaminergic neurons. J Neurosci 32:2166-2181.

669 Atherton JF, Bevan MD (2005) Ionic Mechanisms Underlying Autonomous Action Potential

670 Generation in the Somata and Dendrites of GABAergic Substantia Nigra Pars Reticulata

671 Neurons In Vitro. Journal of Neuroscience 25:8272-8281.

672 Bean BP (2007) The action potential in mammalian central neurons. Nat Rev Neurosci 8:451-

673 465.

674 Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate - a Practical and

675 Powerful Approach to Multiple Testing. J R Stat Soc B 57:289-300.

676  Carrasquillo Y, Burkhalter A, Nerbonne JM (2012) A-type K+ channels encoded by Kv4.2,
677      Kv4.3 and Kv1.4 differentially regulate intrinsic excitability of cortical pyramidal
678      neurons. J Physiol 590:3877-3890.

679  Cembrowski MS, Bachman JL, Wang L, Sugino K, Shields BC, Spruston N (2016) Spatial Gene-
680      Expression Gradients Underlie Prominent Heterogeneity of CA1 Pyramidal Neurons.
681      Neuron 89:351-368.

682  Chan CS, Guzman JN, Ilijic E, Mercer JN, Rick C, Tkatch T, Meredith GE, Surmeier DJ (2007)
683      'Rejuvenation' protects neurons in mouse models of Parkinson's disease. Nature 447:1081-
684      1086.

685  Destexhe A, Babloyantz A, Sejnowski TJ (1993) Ionic Mechanisms for Intrinsic Slow
686      Oscillations in Thalamic Relay Neurons. Biophysical Journal 65:1538-1552.

687  Ding S, Matta SG, Zhou FM (2011) Kv3-like potassium channels are required for sustained high-
688      frequency firing in basal ganglia output neurons. J Neurophysiol 105:554-570.

689  Dufour MA, Woodhouse A, Goaillard JM (2014a) Somatodendritic ion channel expression in
690      substantia nigra pars compacta dopaminergic neurons across postnatal development. J
691      Neurosci Res 92:981-999.

692  Dufour MA, Woodhouse A, Amendola J, Goaillard JM (2014b) Non-Linear Developmental
693      Trajectory of Electrical Phenotype in Rat Substantia Nigra Pars Compacta Dopaminergic
694      Neurons. eLife 3:e04059.

695  Fuzik J, Zeisel A, Mate Z, Calvigioni D, Yanagawa Y, Szabo G, Linnarsson S, Harkany T (2016)
696      Integration of electrophysiological recordings with single-cell RNA-seq data identifies
697      neuronal subtypes. Nat Biotechnol 34:175-183.

698 Gantz SC, Ford CP, Morikawa H, Williams JT (2018) The Evolving Understanding of Dopamine

699 Neurons in the Substantia Nigra and Ventral Tegmental Area. Annu Rev Physiol 80:219-

700 241.

701 Gentet LJ, Williams SR (2007) Dopamine gates action potential backpropagation in midbrain

702 dopaminergic neurons. J Neurosci 27:1892-1901.

703 Grace AA, Onn SP (1989) Morphology and electrophysiological properties of

704 immunocytochemically identified rat dopamine neurons recorded in vitro. J Neurosci

705 9:3463-3481.

706 Granados-Fuentes D, Norris AJ, Carrasquillo Y, Nerbonne JM, Herzog ED (2012) I(A) channels

707 encoded by Kv1.4 and Kv4.2 regulate neuronal firing in the suprachiasmatic nucleus and

708 circadian rhythms in locomotor activity. J Neurosci 32:10045-10052.

709 Guzman JN, Sanchez-Padilla J, Chan CS, Surmeier DJ (2009) Robust pacemaking in substantia

710 nigra dopaminergic neurons. J Neurosci 29:11011-11019.

711 Hines ML, Carnevale NT (2001) NEURON: A tool for neuroscientists. Neuroscientist 7:123-135.

712 Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its

713 application to conduction and excitation in nerve. J Physiol 117:500-544.

714 Hu W, Tian C, Li T, Yang M, Hou H, Shu Y (2009) Distinct contributions of Na(v)1.6 and

715 Na(v)1.2 in action potential initiation and backpropagation. Nat Neurosci 12:996-1002.

716 Ji H, Tucker KR, Putzier I, Huertas MA, Horn JP, Canavier CC, Levitan ES, Shepard PD (2012)

717 Functional characterization of ether-a-go-go-related gene potassium channels in midbrain

718 dopamine neurons - implications for a role in depolarization block. Eur J Neurosci

719 36:2906-2916.

720    Jiao Y, Sun ZQ, Lee T, Fusco FR, Kimble TD, Meade CA, Cuthbertson S, Reiner A (1999) A

721        simple and sensitive antigen retrieval method for free-floating and slide-mounted tissue

722        sections. Journal of Neuroscience Methods 93:149-162.

723    Kole MHP, Stuart GJ (2008) Is action potential threshold lowest in the axon? Nature

724        Neuroscience 11:1253-1255.

725    Liss B, Roeper J (2008) Individual dopamine midbrain neurons: functional diversity and

726        flexibility in health and disease. Brain Res Rev 58:314-321.

727    Liss B, Franz O, Sewing S, Bruns R, Neuhoff H, Roeper J (2001) Tuning pacemaker frequency

728        of individual dopaminergic neurons by Kv4.3L and KChip3.1 transcription. EMBO J

729        20:5715-5724.

730    Liss B, Haeckel O, Wildmann J, Miki T, Seino S, Roeper J (2005) K-ATP channels promote the

731        differential degeneration of dopaminergic midbrain neurons. Nat Neurosci 8:1742-1751.

732    Lorincz A, Nusser Z (2008) Cell-Type-Dependent Molecular Composition of the Axon Initial

733        Segment. Journal of Neuroscience 28:14329-14340.

734    Marionneau C, LeDuc RD, Rohrs HW, Link AJ, Townsend RR, Nerbonne JM (2009) Proteomic

735        analyses of native brain K(V)4.2 channel complexes. Channels (Austin) 3:284-294.

736    Menegola M, Trimmer JS (2006) Unanticipated region- and cell-specific downregulation of

737        individual KChIP auxiliary subunit isotypes in Kv4.2 knock-out mouse brain. J Neurosci

738        26:12137-12142.

739    Moubarak E, Engel D, Dufour MA, Tapia M, Tell F, Goaillard JM (2019) Robustness to Axon

740        Initial Segment Variation Is Explained by Somatodendritic Excitability in Rat Substantia

741        Nigra Dopaminergic Neurons. Journal of Neuroscience 39:5044-5063.

34

742    Nedergaard S, Greenfield SA (1992) Subpopulations of Pars Compacta Neurons in the
743        Substantia-Nigra - the Significance of Qualitatively and Quantitatively Distinct
744        Conductances. Neuroscience 48:423-437.

745    Nerbonne JM, Gerber BR, Norris A, Burkhalter A (2008) Electrical remodelling maintains firing
746        properties in cortical pyramidal neurons lacking KCND2-encoded A-type K+ currents. J
747        Physiol 586:1565-1579.

748    Neuhoff H, Neu A, Liss B, Roeper J (2002) I(h) channels contribute to the different functional
749        properties of identified dopaminergic subpopulations in the midbrain. J Neurosci
750        22:1290-1302.

751    Niwa N, Wang W, Sha Q, Marionneau C, Nerbonne JM (2008) Kv4.3 is not required for the
752        generation of functional Ito,f channels in adult mouse ventricles. J Mol Cell Cardiol
753        44:95-104.

754    Northcutt AJ, Kick DR, Otopalik AG, Goetz BM, Harris RM, Santin JM, Hofmann HA, Marder
755        E, Schulz DJ (2019) Molecular profiling of single neurons of known identity in two
756        ganglia from the crab Cancer borealis. P Natl Acad Sci USA 116:26980-26990.

757    O'Leary T, Williams AH, Franci A, Marder E (2014) Cell types, network homeostasis, and
758        pathological compensation from a biologically plausible ion channel expression model.
759        Neuron 82:809-821.

760    Philippart F, Destreel G, Merino-Sepulveda P, Henny P, Engel D, Seutin V (2016) Differential
761        Somatic Ca2+ Channel Profile in Midbrain Dopaminergic Neurons. J Neurosci 36:7234-
762        7245.

763    Puopolo M, Raviola E, Bean BP (2007) Roles of subthreshold calcium current and sodium
764        current in spontaneous firing of mouse midbrain dopamine neurons. J Neurosci 27:645-
765        656.

766    Putzier I, Kullmann PHM, Horn JP, Levitan ES (2008) Dopamine Neuron Responses Depend
767            Exponentially on Pacemaker Interval. Journal of Neurophysiology 101:926-933.

768    Putzier I, Kullmann PHM, Horn JP, Levitan ES (2009) Cav1.3 Channel Voltage Dependence,
769            Not Ca2+ Selectivity, Drives Pacemaker Activity and Amplifies Bursts in Nigral
770            Dopamine Neurons. Journal of Neuroscience 29:15414-15419.

771    Schulz DJ, Goaillard JM, Marder E (2006) Variable channel expression in identified single and
772            electrically coupled neurons in different animals. Nat Neurosci 9:356-362.

773    Serodio P, Rudy B (1998) Differential expression of Kv4 K+ channel subunits mediating
774            subthreshold transient K+ (A-type) currents in rat brain. J Neurophysiol 79:1081-1091.

775    Serodio P, Kentros C, Rudy B (1994) Identification of Molecular-Components of a-Type
776            Channels Activating at Subthreshold Potentials. Journal of Neurophysiology 72:1516-
777            1529.

778    Serodio P, Vega-Saenz de Miera E, Rudy B (1996) Cloning of a novel component of A-type K+
779            channels operating at subthreshold potentials with unique expression in heart and brain.
780            Journal of Neurophysiology 75:2174-2179.

781    Seutin V, Engel D (2010) Differences in Na+ conductance density and Na+ channel functional
782            properties between dopamine and GABA neurons of the rat substantia nigra. J
783            Neurophysiol 103:3099-3114.

784    Seutin V, Massotte L, Renette MF, Dresse A (2001) Evidence for a modulatory role of Ih on the
785            firing of a subgroup of midbrain dopamine neurons. Neuroreport 12:255-258.

786    Swensen AM, Bean BP (2005) Robustness of burst firing in dissociated purkinje neurons with
787            acute or long-term reductions in sodium conductance. J Neurosci 25:3509-3520.

788    Tapia M, Baudot P, Formisano-Treziny C, Dufour MA, Temporal S, Lasserre M, Marqueze-
789            Pouey B, Gabert J, Kobayashi K, Goaillard JM (2018) Neurotransmitter identity and

790       electrophysiological phenotype are genetically coupled in midbrain dopaminergic

791       neurons. Sci Rep 8:13637.

792   Tarfa RA, Evans RC, Khaliq ZM (2017) Enhanced Sensitivity to Hyperpolarizing Inhibition in

793       Mesoaccumbal Relative to Nigrostriatal Dopamine Neuron Subpopulations. Journal of

794       Neuroscience 37:3311-3330.

795   Taylor AL, Hickey TJ, Prinz AA, Marder E (2006) Structure and visualization of high-

796       dimensional conductance spaces. J Neurophysiol 96:891-905.

797   Vacher H, Alami M, Crest M, Possani LD, Bougis PE, Martin-Eauclaire MF (2002) Expanding

798       the scorpion toxin alpha-KTX 15 family with AmmTX3 from Androctonus mauretanicus.

799       Eur J Biochem 269:6037-6041.

800   Wilson CJ, Callaway JC (2000) Coupled oscillator model of the dopaminergic neuron of the

801       substantia nigra. J Neurophysiol 83:3084-3100.

802   Wolfart J, Neuhoff H, Franz O, Roeper J (2001) Differential expression of the small-

803       conductance, calcium-activated potassium channel SK3 is critical for pacemaker control

804       in dopaminergic midbrain neurons. J Neurosci 21:3443-3456.

805

806   **FIGURE LEGENDS**

807   **Figure 1. Behavioral assessment of the Kv4.3$^{-/-}$ transgenic mouse**. **A**, locomotion measured in

808   actimetry chambers. Left, line and scatter plot showing the mean number of horizontal

809   movements per 5-min bin (± SEM) in Kv4.3$^{-/-}$ mice (red) compared to their WT littermates

810   (black). Right, line and scatter plot showing the mean number of rearing events per 5-min bin in

811   Kv4.3$^{-/-}$ mice (red) compared to their WT littermates (black). Significant differences between

812  strains for specific time bins (two-way repeated measures ANOVA) are indicated by an asterisk.

813  **B**, changes in motor learning measured using a rotarod assay. The latency to falling off the

814  rotating rod (with increasing rotating speed) was assessed over 10 consecutive trials (left).

815  Significant differences between strains for specific trials (two-way repeated measures ANOVA)

816  are indicated by an asterisk. The performance index ((average latency 8-10)/(average latency 1-

817  3)*100, right) was used to evaluate the learning ability of Kv4.3$^{-/-}$ mice (red) compared to their

818  WT littermates (black). * $p<0.05$, ** $p<0.01$.

819

820  **Figure 2. Spontaneous activity and post-inhibitory rebound are profoundly altered in**

821  **Kv4.3$^{-/-}$ SNc DA neurons**. **A**, representative cell-attached recordings showing the spontaneous

822  pattern of activity in WT (black trace, left) and Kv4.3$^{-/-}$ SNc DA neurons (red trace, right). **B**, box

823  and whisker plots showing the distribution of values for extracellularly recorded spontaneous ISI

824  (Extracellular ISI, left) and ISI coefficient of variation (CV$_{extra. ISI}$, right) in WT and Kv4.3$^{-/-}$ SNc

825  DA neurons. **C**, representative current-clamp recordings showing the voltage response of SNc

826  DA neurons to a current step (gray trace) hyperpolarizing membrane voltage to ~ -120mV in WT

827  (black trace, left) and Kv4.3$^{-/-}$ mice (red trace, right). The recordings come from the same

828  neurons as the cell-attached recordings presented in **A**. **D**, box and whisker plots showing the

829  distribution of values for voltage sag amplitude (left) and post-inhibitory rebound delay (right) in

830  WT and Kv4.3$^{-/-}$ SNc DA neurons. *** $p<0.001$. Dotted lines in the box and whisker plots

831  indicate the distribution of data (violin plots). Scale bars: **A**, horizontal 1s; **C**, top, horizontal

832  500ms, vertical 40mV; bottom, vertical 100pA; horizontal dotted lines indicate -60mV.

833

834  **Figure 3. Action potential and excitability properties in Kv4.3$^{-/-}$ SNc DA neurons**. **A**, left,

835  current-clamp recordings showing the shape of the action potential in the WT (black traces) and

836  Kv4.3$^{-/-}$ mice (red traces) on a slow (top) and fast time-scale (bottom). Right, box and whisker

837  plots showing the distribution of values for action potential threshold (top left), amplitude (top

838  right), half-width (AP half-width, bottom left) and afterhyperpolarization trough (AHP trough,

839  bottom right) in WT and Kv4.3$^{-/-}$ SNc DA neurons. **B**, left, current-clamp recordings showing the

840  voltage response of SNc DA neurons to 100 and 200pA depolarizing current steps (gray traces)

841  in WT (top, black traces) and Kv4.3$^{-/-}$ mice (bottom, red traces). Gray dotted rectangles indicate

842  the interspike interval used to calculate the gain start and gain end on each train of action

843  potentials. Right, box and whisker plots showing the distribution of values for gain start (top left),

844  gain end (top right), spike frequency adaptation (SFA) index (bottom left) and start frequency at

845  100pA (bottom right) in WT and Kv4.3$^{-/-}$ SNc DA neurons. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

846  Dotted lines in the box and whisker plots indicate the distribution of data (violin plots). Scale

847  bars: **A**, top, horizontal 50ms, vertical 20mV; **A**, bottom, horizontal 2ms, vertical 20mV,

848  horizontal dotted lines indicate -40mV; **B**, voltage, horizontal 500ms, vertical 40mV; current,

849  vertical 100pA.

850

851  **Figure 4. Voltage-clamp analysis of I$_A$ in wild-type and Kv4.3$^{-/-}$ SNc DA neurons**. **A**, voltage-

852  clamp traces showing representative I$_A$ recordings obtained from a WT (black trace) and a Kv4.3$^{-}$

853  $^{/-}$ SNc DA neuron (red trace) in response to a voltage step to -40mV (gray trace). The small

854  residual current present in the Kv4.3$^{-/-}$ mice is blocked by AmmTX3 (inset, orange trace). **B**, box

855  and whisker plot showing the distribution of values for I$_A$ amplitude in WT and Kv4.3$^{-/-}$ SNc DA

856  neurons. **C**, box and whisker plot showing the distribution of values for I$_A$ time constant of

39

857　　inactivation ($I_A$ tau) in WT and Kv4.3$^{-/-}$ SNc DA neurons. The green dotted rectangle highlights 5

858　　Kv4.3$^{-/-}$ outliers displaying unusually large values for $I_A$ tau. **D**, box and whisker plot showing the

859　　distribution of values for $I_A$ charge in WT and Kv4.3$^{-/-}$ SNc DA neurons. The green dotted

860　　rectangle highlights 5 Kv4.3$^{-/-}$ outliers displaying unusually large values for $I_A$ charge (same cells

861　　as in **C**). **E**, left, scatter plot showing the relationship between $I_A$ tau and charge in WT (gray

862　　dots) and Kv4.3$^{-/-}$ SNc DA neurons (red dots). Please note that 5 of the Kv4.3$^{-/-}$ measurements lie

863　　in the WT region of space (green dotted ellipse). Right, voltage-clamp traces showing one

864　　example of the atypical $I_A$ recording (green trace, corresponding to the large green circle in the

865　　scatter plot) encountered in one of the 5 Kv4.3$^{-/-}$ outliers highlighted in panels **C** and **D**,

866　　compared to the typical recording obtained in Kv4.3$^{-/-}$ neurons (red trace, same as in panel **A**,

867　　corresponding to the large red circle in the scatter plot). *** $p < 0.001$. Dotted lines in the box and

868　　whisker plots indicate the distribution of data (violin plots). Scale bars in **A** and **E**: horizontal

869　　200ms, vertical 1nA.

870

871　　**Figure 5. Kv4.2 channels are expressed by a minority of wild-type and Kv4.3$^{-/-}$ SNc DA**

872　　**neurons**. **A**, Top, low-magnification pictures of the SNc showing (from left to right) TH (red),

873　　Kv4.3 (green) and DAPI (blue) stainings in WT (top row) and Kv4.3$^{-/-}$ mice (bottom row).

874　　Bottom, high-magnification pictures of SNc DA neurons showing (from left to right) TH (red),

875　　Kv4.3 (green) and DAPI (blue) stainings in WT (top row) and Kv4.3$^{-/-}$ mice (bottom row). **B**,

876　　effect of the antigen retrieval procedure on Kv4.2 detection in the SNc. Top, low-magnification

877　　pictures showing (from left to right) TH (red), Kv4.2 (green) and DAPI staining (blue) without

878　　(top row) or with antigen retrieval (bottom row). Bottom, left, high-magnification pictures of the

879　　insets depicted in the merged low-magnification picture and corresponding to a Kv4.2-negative

880    (left) and a Kv4.2-positive SNc DA neuron (middle), for which inset pictures (right) illustrate the

881    region selected to characterize the immunofluorescence profile of Kv4.2 and TH stainings (3μm

882    yellow bar). Bottom, right, immunofluorescence profiles of Kv4.2 (green, top graph), Kv4.3

883    (green, bottom graph) and TH staining (red, both graphs), showing that both Kv4.2 and Kv4.3

884    profiles are very similar and strongly suggestive of specific plasma membrane expression. The

885    average profiles (n=5) shown here were defined over 3μm selected regions of (yellow bar above

886    the graph), such as the one shown on the inset pictures on the left. **C**, left, high-magnification

887    pictures showing (from left to right) TH (red), Kv4.2 (green) and DAPI staining (blue) after

888    antigen retrieval in WT (top row) and Kv4.3$^{-/-}$ mice (bottom row). Right, expanded view of the

889    Kv4.2-positive cells (1, 2) highlighted in the merged pictures on the left. **D**, bar plot showing the

890    counts of Kv4.2-negative (dark colors) and -positive (light colors) cells observed in the SNc of

891    WT (black bar) and Kv4.3$^{-/-}$ mice (red bar). n.s. non-significant. Scale bars: **A**, top row 100μm,

892    bottom row 10μm; **B**, top row 10μm, bottom row 10μm, inset 2μm; **C**, 10μm.

893

894    **Figure 5-1. Validation of antigen retrieval Kv4.2 immunohistochemistry on neocortical and**

895    **hippocampal neurons. A**, low-magnification (top rows) and high-magnification pictures

896    (bottom rows) showing the effect of the antigen retrieval procedure on Kv4.2 immunostaining

897    (green) in the cortex. DAPI staining (blue) is also shown. **B**, low-magnification (top rows) and

898    high-magnification pictures (bottom rows) showing the effect of the antigen retrieval procedure

899    on Kv4.2 immunostaining (green) in the CA1 region of the hippocampus. DAPI staining (blue) is

900    also shown. **A**, top row 100μm, bottom row 10μm; **B**, top row 100μm, bottom row 10μm.

901

902

**Figure 6. Comparing the alterations in electrophysiological phenotype after acute blockade of Kv4 channels with the Kv4.3$^{-/-}$ mouse model**. **A**, current-clamp recordings showing the spontaneous pattern of activity of a WT SNc DA neuron in control condition (black trace, left) and after AmmTX3 application (red trace, right). **B**, left, line and scatter plot showing the change in spontaneous firing frequency induced by AmmTX3 application in individual WT SNc DA neurons. Right, bar plot comparing the average change in spontaneous firing frequency after AmmTX3 application (left, light colors) or Kv4.3 channel deletion (right, dark colors). **C**, current-clamp recordings showing the voltage response of a WT SNc DA neuron to a hyperpolarizing current step (bottom gray traces) in control condition (left, black trace) and after AmmTX3 application (right, red trace). **D**, left, line and scatter plot showing the change in rebound delay induced by AmmTX3 application in individual WT SNc DA neurons. Right, bar plot showing the average change in rebound delay after AmmTX3 application (left, light colors) or Kv4.3 channel deletion (right, dark colors). ** $p<0.01$, *** $p<0.001$. Scale bars: **A**, horizontal 1s, vertical 20mV, horizontal gray dotted lines indicate -60mV; **C**, horizontal 500ms, vertical 20mV.

918

**Figure 7. Absence of compensatory changes in delayed rectifier and I$_H$ currents in the Kv4.3$^{-/-}$ SNc DA neurons**. **A**, properties of the delayed rectifier potassium current (I$_{KDR}$) in SNc DA neurons in WT and Kv4.3$^{-/-}$ mice. Left, voltage-clamp recordings of I$_{KDR}$ obtained in a WT (black trace) and a Kv4.3$^{-/-}$ mouse (red trace) in response to a voltage step to +20mV (gray trace). The peak and steady-state components of I$_{KDR}$ are indicated by arrowheads. Right, line and

42

924 scatter plots representing the average current-voltage relationships of the peak (left) and steady-

925 state $I_{KDR}$ (right) obtained from WT (gray dots) and Kv4.3[-/-] SNc DA neurons (red dots). **B**,

926 properties of $I_H$ in SNc DA neurons in WT and Kv4.3[-/-] mice. Left, voltage-clamp recordings of

927 $I_H$ obtained in a WT (black traces) and a Kv4.3[-/-] mouse (red traces) in response to increasingly

928 hyperpolarized voltage steps. Right, box and whisker plot showing the distribution of values for

929 $I_H$ amplitude (left) and voltage sensitivity (right) in WT and Kv4.3[-/-] SNc DA neurons. ***

930 p<0.001. Dotted lines in the box and whisker plots indicate the distribution of data (violin plots).

931 Scale bars: **A**, horizontal 200ms, vertical 1nA; **B**, horizontal 2s, vertical 500pA.

932

933 **Figure 8. Linking biophysical changes in $I_A$ to changes in electrophysiological phenotype in**

934 **wild-type and Kv4.3[-/-] SNc DA neurons**. **A**, recordings representative of the variation in

935 spontaneous activity, post-inhibitory rebound and $I_A$ in WT and Kv4.3[-/-] SNc DA neurons. Left,

936 cell-attached voltage-clamp recordings of spontaneous pacemaking activity in 2 WT (black

937 traces) and 2 Kv4.3[-/-] neurons (red traces). The value of the average ISI is indicated above the

938 trace. Middle, current-clamp recordings of the post-inhibitory rebound obtained in the same

939 neurons. The value of rebound delay is indicated above the trace. Right, $I_A$ voltage-clamp

940 recordings obtained at -40mV in the same neurons. The values of $I_A$ amplitude and tau are

941 indicated above the trace. **B**, scatter plot showing the significant positive correlation between

942 extracellularly recorded ISI and rebound delay observed in WT (gray dots) and Kv4.3[-/-] SNc DA

943 neurons (red dots). The plain black and red lines correspond to the linear regression of the data (r,

944 n and p values are shown on the graph), while the gray and pink dotted lines indicate the

945 regression confidence intervals. The diamond and square symbols correspond to the recordings

946 presented in panel **A**. **C**, scatter plots showing the relationships between biophysical variables

947    and neuronal output. Correlations between $I_A$ tau, $I_H$ amplitude, $I_A$ amplitude or $I_A$ $V_{50}$ (from left

948    to right) and rebound delay or extracellular ISI (from top to bottom) were tested in both WT (gray

949    dots) and Kv4.3[-/-] neurons (red dots). Please note that only $I_A$ tau and $I_H$ amplitude were

950    significantly correlated with both ISI and rebound delay in WT and Kv4.3[-/-] neurons. The plain

951    black and red lines correspond to the linear regression of the data (r/rho, n and p values are shown

952    on the graph), while the gray and pink dotted lines indicate the regression confidence intervals.

953    Dashed black and red lines indicate non-significant correlations. The diamond and square

954    symbols correspond to the recordings presented in panel **A**. **D**, scatter plots showing the

955    significant correlations between $I_A$ and $I_H$ properties. Left, scatter plot showing the positive

956    correlation between $I_A$ inactivation $V_{50}$ and $I_H$ activation $V_{50}$ in WT neurons. Right, scatter plot

957    showing the negative correlation between $I_A$ tau and $I_H$ amplitude observed in both WT (gray

958    dots) and Kv4.3[-/-] neurons (red dots). The plain black and red lines correspond to the linear

959    regression of the data (r/rho, n and p values are shown on the graph), while the gray and pink

960    dotted lines indicate the regression confidence intervals. **E**, left, scatter plot showing the multiple

961    linear regression of extracellular ISI *vs* $I_A$ tau and $I_H$ amplitude (predicted ISI) in WT neurons.

962    Right, scatter plot showing the multiple linear regression of rebound delay *vs* $I_A$ tau, $I_A$

963    inactivation $V_{50}$ and $I_H$ amplitude (predicted rebound delay) in WT neurons. The corresponding

964    equations are indicated below the X axis of each graph. The plain black lines correspond to the

965    linear regression of the data (r, n and p values are shown on the graph), while the gray dotted

966    lines indicate the regression confidence intervals. Scale bars: **A**, left horizontal 1s; middle

967    horizontal 500ms, vertical 50mV; right horizontal 250ms, vertical 2nA.

968

969   **Figure 9. Modeling the effect of the biophysical properties of $I_A$ and $I_H$ on spontaneous**

970   **activity and post-inhibitory rebound**. **A**, table presenting the 5 values tested for each

971   biophysical parameter ($g_A$, $g_H$, $I_A/I_H$ $V_{50}$ and $I_A$ inactivation tau) in the multi-compartment model.

972   Each property was varied independently, leading to 625 ($5^4$) versions of the model. **B**,

973   dimensionally stacked heatmaps showing the variation in ISI (displayed as log(ISI), left) and in

974   post-inhibitory rebound delay (displayed as log(rebound delay), right) as a function of $g_A$ (X axis,

975   big scale), $I_A/I_H$ $V_{50}$ (Y axis, big scale), $I_A$ tau (X axis, small scale) and $g_H$ (Y axis, small scale).

976   **C**, example traces of spontaneous pacemaking (left) and post-inhibitory rebound (right) obtained

977   for the minimal and maximal values of $g_A$ and $I_A$ tau (corresponding to the 4 squares surrounded

978   in the panel **B** heatmaps). The gray horizontal bars above the traces help visualize the change in

979   ISI (left) or rebound delay (right) as a function of changes in $g_A$ and $I_A$ tau. **D**, multiple linear

980   regression reveals the relative contribution of each biophysical property to ISI (left) or rebound

981   delay variation (right). The scatter plots show the relationship between the measured values of

982   log(ISI) and log(rebound delay) and the values predicted using a linear combination of the 4

983   biophysical variables listed in panel **A** (the corresponding equations are shown above each

984   graph). Scale bars: **C**, horizontal 500ms, vertical 50mV.

985

986   **Table 1. Statistical analysis of electrophysiological parameters in wild-type and Kv4.3[-/-] SNc**

987   **DA neurons.** The values for 16 electrophysiological parameters measured under current-clamp

988   (corresponding to passive properties, spontaneous activity, post-inhibitory rebound, action

989   potential and excitability) and 8 electrophysiological parameters measured under voltage-clamp

990   (corresponding to $I_A$ and $I_H$ properties) are presented for WT and Kv4.3[-/-] SNc DA neurons. Mean

991   and SD (black text) are reported for normally-distributed data, while Median and interquartile

45

992    range (IQR) are reported otherwise (gray text). Accordingly, statistical differences between WT

993    and Kv4.3$^{-/-}$ neurons were tested using a t-test or a Mann Whitney test, depending on the

994    normality of the data. Asterisks indicate statistically significant differences (* $p<0.05$, ** $p<0.01$,

995    *** $p<0.001$).

996

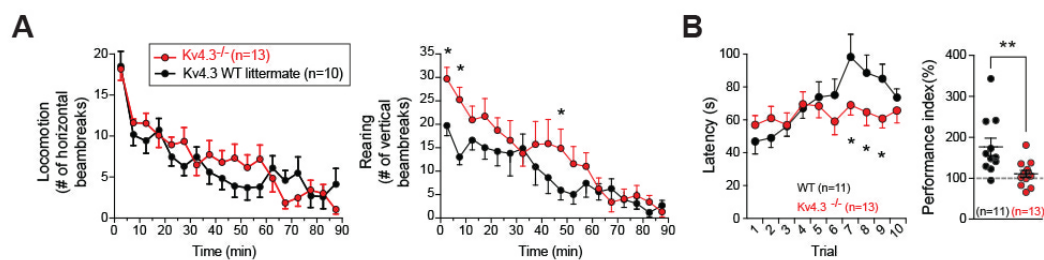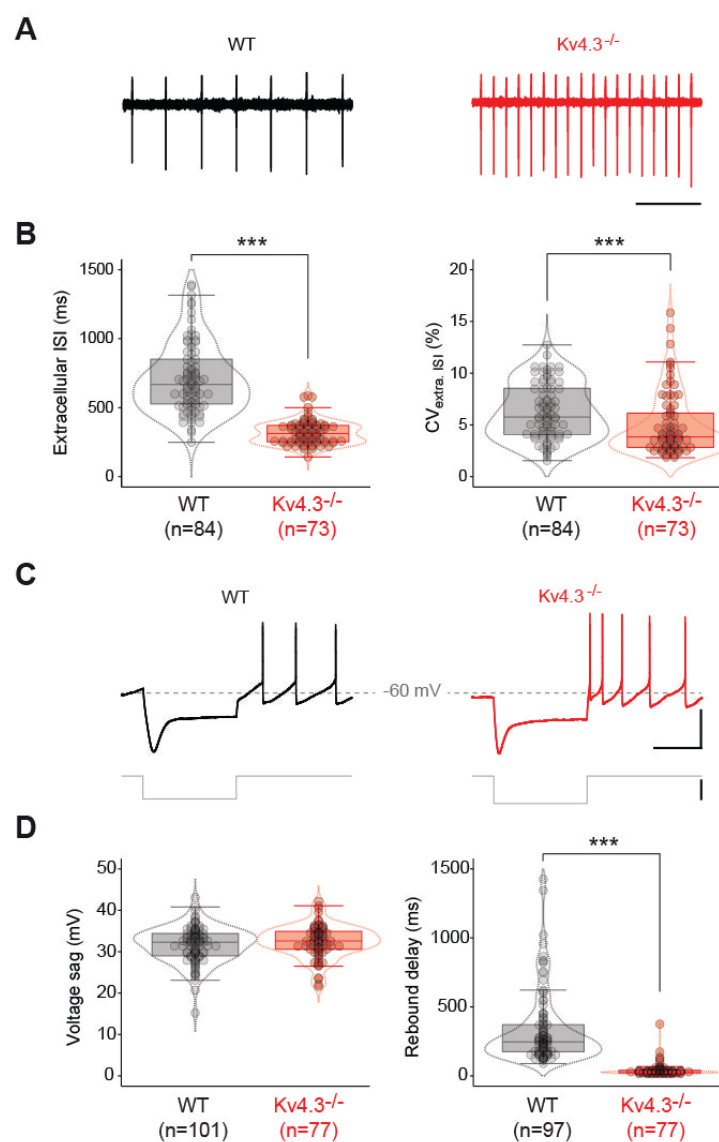997    **Table 2. Equations governing the voltage dependence and kinetics of currents in the model**

998

**A**

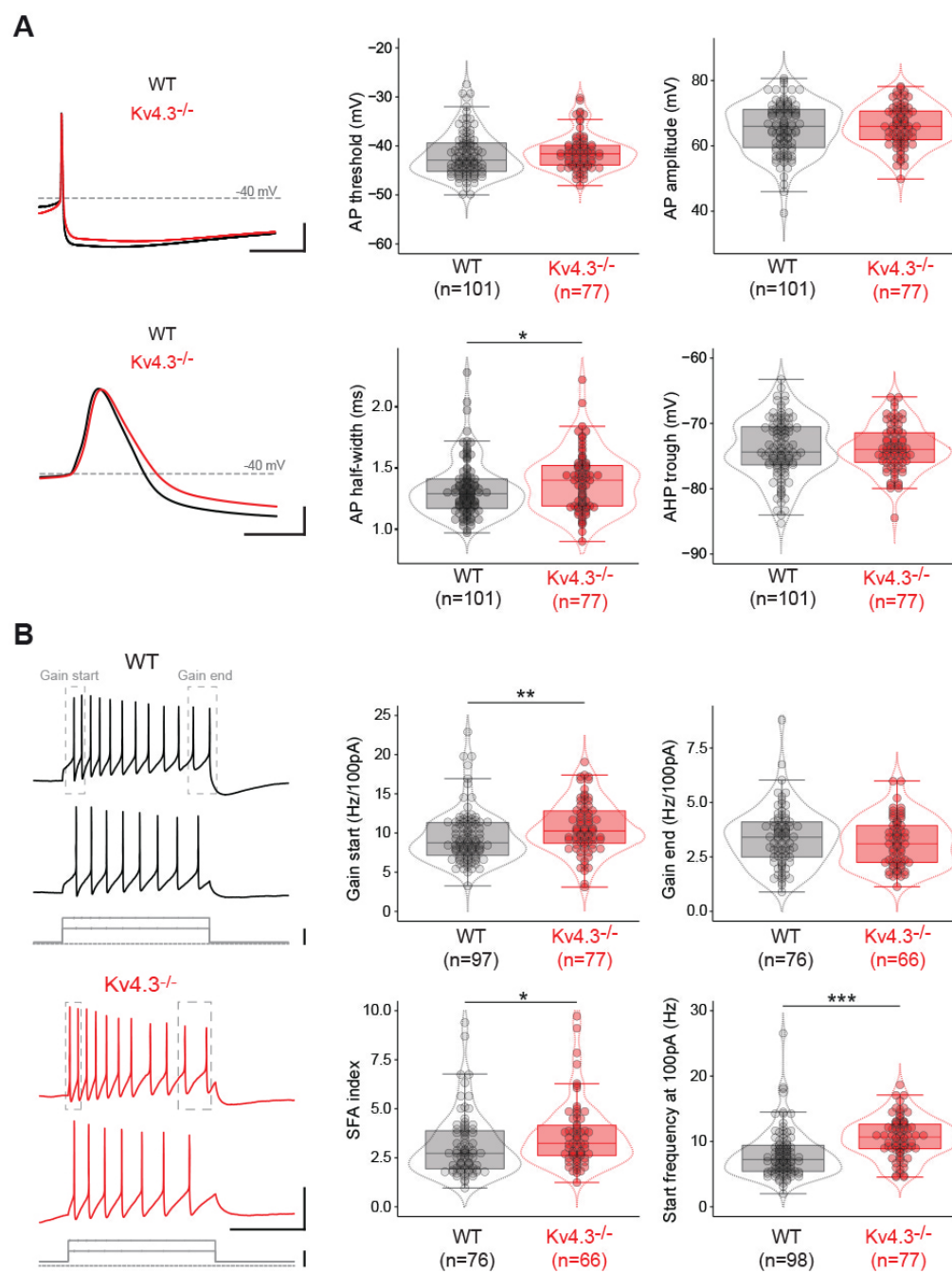

**B**



**FIGURE 1**

**A**

WT                    Kv4.3⁻/⁻

**B**



**C**

WT                    Kv4.3⁻/⁻

-60 mV

**D**
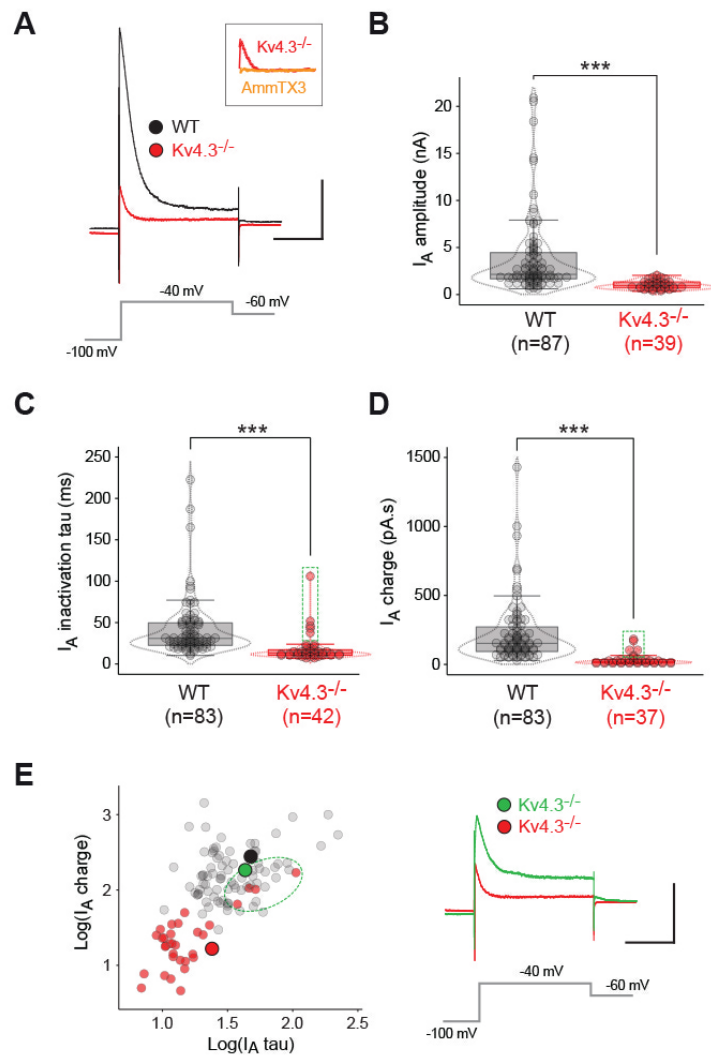


**FIGURE 2**

**FIGURE 3**

**FIGURE 4**
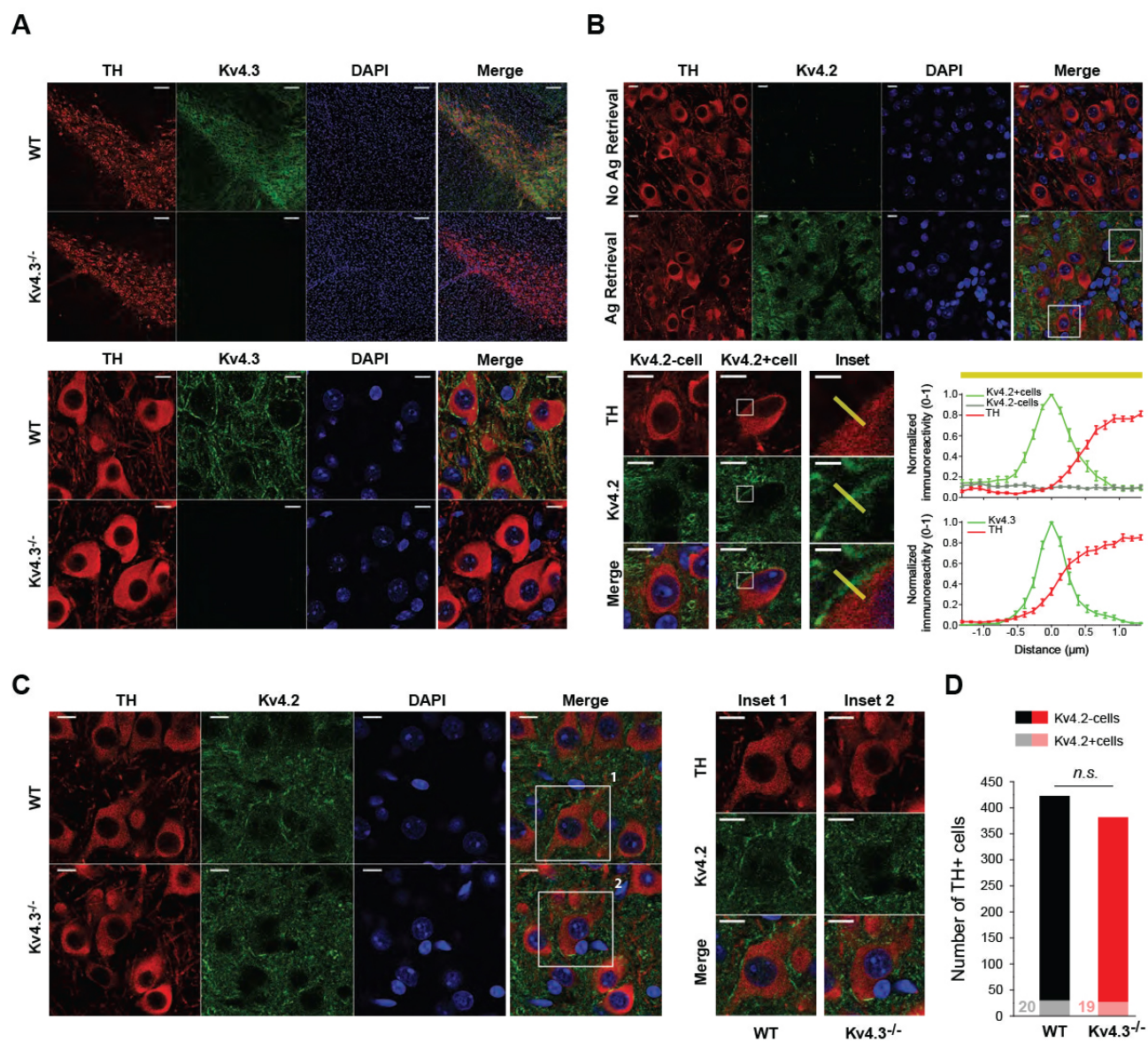
**FIGURE 5**
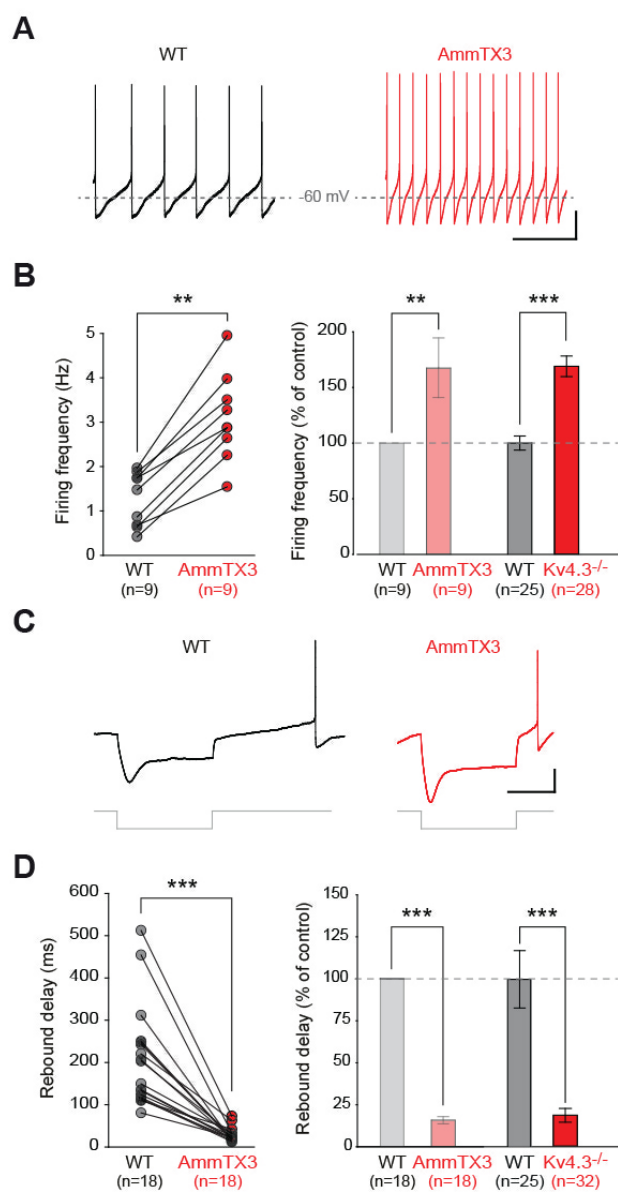
**FIGURE 6**

**FIGURE 7**

**FIGURE 8**

**A**

| Parameter | Values | | | | | Range |
|---|---|---|---|---|---|---|
| $g_A$ (pS/μm$^2$) | 15 | 49 | 83 | 116 | 150 | 10-fold |
| $g_H$ (pS/μm$^2$) | 0.25 | 0.8 | 1.35 | 1.9 | 2.5 | 10-fold |
| $I_A/I_H$ V$_{50}$ (mV) | -78/-100 | -74/-95 | -70/-90 | -66/-85 | -62/-80 | 20mV |
| $I_A$ tau (ms) | 15 | 49 | 83 | 116 | 150 | 10-fold |

**B**



**C**



**D**

Predicted log(ISI)
=
$2.132 - 0.183 * V_{50} + 0.156 * g_A - 0.076 * g_H + 0.05 * tau_A$

Predicted log(rebound delay)
=
$1.841 - 0.268 * V_{50} + 0.228 * g_A - 0.125 * g_H + 0.116 * tau_A$



adj. $r^2 = 0.875$
n=625
p<2·10$^{-16}$

adj. $r^2 = 0.888$
n=625
p<2·10$^{-16}$

**FIGURE 9**