Title: A1 adenosine receptor-mediated GIRK channels contributes to the resting conductance of CA1 neurons in the dorsal hippocampus

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Author contributions: C.S.K and D.J. designed research; C.S.K performed research; C.S.K. analyzed data; C.S.K. and D.J. wrote the paper.

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Running Head: Coupling of A1ARs to GIRK in dorsal CA1 neurons

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Acknowledgments: This study was supported by grants from National Institutes of Health NS084473 and NS077477 (DJ). We thank Drs. Rick Gray, Brian Kalmbach, and Darrin Brager for reviewing the manuscript and comments throughout this study. We also thank Drs. Randy Chitwood, Kelly Dougherty, and Ann Clemens for giving helpful comments.

Conflict of Interest: The authors declare no competing financial interests
Abstract

The dorsal and ventral hippocampi are functionally and anatomically distinct. Recently, we reported that dorsal CA1 neurons have a more hyperpolarized resting membrane potential (RMP), a lower input resistance (R\text{in}), and fire fewer action potentials for a given current injection than ventral CA1 neurons. Differences in the hyperpolarizing HCN conductance between dorsal and ventral neurons have been reported, but these differences cannot fully account for the different resting properties of these neurons. Here we show that coupling of A1 adenosine receptors (A1ARs) to G-protein coupled inwardly rectifying potassium (GIRK) conductance contributes to the intrinsic membrane properties of dorsal CA1 neurons but not ventral CA1 neurons. The block of GIRKs with either barium or the more specific blocker tertiapin-Q revealed that there is more resting GIRK conductance in dorsal CA1 neurons compared to ventral CA1 neurons. We found that the higher resting GIRK conductance in dorsal CA1 neurons was mediated by tonic A1 adenosine receptor activation. These results demonstrate that the different resting membrane properties between dorsal and ventral CA1 neurons are due in part to higher adenosine A1 receptor-mediated GIRK activity in dorsal CA1 neurons.

Keywords: Dorsal and ventral hippocampus; A1 adenosine receptors; GIRK
Introduction

The rodent hippocampus can be divided into dorsal and ventral regions based on biochemical, anatomical, and behavioral observations (van Groen and Wyss 1990; Moser and Moser 1998; Dong et al. 2009; Fanselow and Dong 2010). Until recently, the electrophysiological properties of CA1 pyramidal neurons along the longitudinal hippocampal axis were often thought to be uniform. However, growing evidence demonstrates that intrinsic membrane properties of CA1 neurons from the dorsal and ventral hippocampus are significantly different (Dougherty et al. 2012; Marcelin et al. 2012a). For example, dorsal neurons have a more hyperpolarized resting membrane potential (RMP) and a lower input resistance (R_{in}). As such, they fire fewer action potentials in response to a given current injection compared to ventral neurons (Dougherty et al. 2012). Although functional $I_h$ is different between dorsal and ventral CA1 neurons (Dougherty et al. 2013), the intrinsic membrane properties of these neurons cannot be fully explained by $I_h$-related differences.

Voltage-gated ion channels and neuronal morphology contribute to the intrinsic membrane properties of neurons (Hoffman et al. 1997; Magee 1998; Vetter et al. 2001; Spruston 2008). Differences in RMP and $R_{in}$ between dorsal and ventral CA1 neurons may therefore be explained in part by the expression of voltage-gated ion channels. Given the more hyperpolarized membrane potential and lower $R_{in}$ in dorsal neurons compared to ventral neurons, we hypothesized that dorsal CA1 neurons possess a greater resting potassium conductance than ventral CA1 neurons. Furthermore, inwardly rectifying K$^+$ (IRK) channels and G protein-coupled inwardly rectifying K$^+$ (GIRK) channels are highly expressed in the CA1 pyramidal neurons.
(Karschin et al. 1996; Liao et al. 1996; Ponce et al. 1996; Drake et al. 1997). G protein-coupled inwardly rectifying K\(^+\) channels (GIRKs) are activated by G\(_{i/o}\)-coupled metabotropic receptors and hyperpolarize the membrane potential and decrease R\(_{in}\), providing a decrease in neuronal excitability (Andrade et al. 1986; Mihara et al. 1987; North 1989; Luscher et al. 1997).

In this study, we investigated the resting conductances in dorsal and ventral CA1 pyramidal neurons. We found that dorsal neurons have more Ba\(^{2+}\)-sensitive conductance compared to ventral neurons. Application of tertiapin-Q revealed that this greater Ba\(^{2+}\)-sensitive conductance in dorsal CA1 neurons was mediated by GIRK channels. Finally, we found that this higher resting GIRK conductance in dorsal neurons was mediated by tonic activation of A1 adenosine receptors (A1ARs). Together, our results suggest that higher resting GIRK conductance activated by A1 adenosine receptors is present in dorsal but not ventral CA1 neurons and contributes to intrinsic membrane properties of these neurons.
**Materials and Methods**

**Animals.** Hippocampal slices from the dorsal and ventral poles were prepared from 11- to 13-week-old male Sprague Dawley rats housed 2-3 per cage on a 12 hr light schedule (on 7:00 A.M. off 7:00 P.M.) with ad libitum access to water and food. All procedures involving animals were approved by the University of Texas at Austin Institutional Animal Care and Use Committee (IACUC).

**Drugs.** Barium chloride was purchased from Fisher Scientific. Tertiapin-Q was purchased from Alomone Lab. DPCPX, ZD7288, 2'ME-CCPA, D-APV, Gabazine, CGP 55845, and DNQX were purchased from Abcam Inc.

**Slice preparation.** Rats were anesthetized with a lethal dose of a ketamine/xylazine mixture (90/10mg/ml) and transcardially perfused with ice-cold artificial cerebral spinal fluid (aCSF) composed of (in mM): 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 0.5 CaCl₂, 7 MgCl₂, 7 dextrose, 210 sucrose, 1.3 ascorbic acid, and 3 sodium pyruvate, bubbled with 95% O₂ - 5% CO₂. The brain was then removed, and hemisected along the longitudinal fissure. For dorsal hippocampal slices, a blocking cut was made at 20° - 30° from the posterior coronal plane and collected at the anterior end of the forebrain. Ventral hippocampal slices were made from horizontal section. 300 μm thick hippocampal slices were made in ice-cold aCSF using a vibrating microtome (Microslicer DTK-Zero1, DSK, Kyoto, Japan). Slices were then transferred to a holding chamber for 30 min at 35°C containing (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 2 CaCl₂, 2 MgCl₂, 12.5 dextrose, 1.3 ascorbic acid, and 3 sodium pyruvate, bubbled with 95% O₂ - 5% CO₂. Slices were then incubated for at least 45 min at room temperature.
Whole-cell current-clamp recordings.

Whole-cell current-clamp recordings were performed as previously described (Kim et al. 2012). Briefly, hippocampal slices were submerged in a recording chamber continuously perfused with aCSF containing synaptic blockers (D-APV 25 μM, Gabazine 2 μM, DNQX 20 μM) heated to 32-34 °C flowing at a rate of 1 to 2 ml/min. ACSF contained (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH$_2$PO$_4$, 25 NaHCO$_3$, 2 CaCl$_2$, 1 MgCl$_2$, and 12.5 dextrose, bubbled with 95% O$_2$ - 5% CO$_2$. CA1 pyramidal neurons were visualized using a microscope (Zeiss Axioskop) fitted with differential interference contrast optics (Stuart et al. 1993). Patch pipettes (4–7 MΩ) were prepared with capillary glass (external diameter 1.65 mm, World Precision Instruments) using Flaming/Brown micropipette puller and filled with an internal solution containing (in mM) 120 K-gluconate, 20 KCl, 10 HEPES, 4 NaCl, 7 K2-phosphocreatine, 4 Mg-ATP, 0.3 Na-GTP (pH 7.3 with KOH). Neurobiotin (Vector Labs) was used (0.1-0.2 %) for subsequent histological processing. Somatic whole-cell current-clamp recordings were performed using a Dagan BVC-700A amplifier (Dagan, Minneapolis, MN). Electrical signals were filtered at 5 kHz, sampled at 20 kHz, and digitized by an ITC-18 interface (HEKA Instruments Inc., Bellmore, NY) connected to a computer running custom software written in IGOR Pro (Wavemetrics). Experiments were terminated if the series resistance exceeded 30 MΩ. Membrane potentials were corrected for liquid junction potentials (- 8mV). For tertapiain experiments, we used log-order increases in concentration (0.03, 0.3, and 2 μM).

Immunohistochemistry. Animals were anesthetized and perfused through the
ascending aorta using ice-cold artificial cerebral spinal fluid (aCSF) composed of (in mM): 2.5 KCl, 1.25 NaH$_2$PO$_4$, 25 NaHCO$_3$, 0.5 CaCl$_2$, 7 MgCl$_2$, 7 dextrose, 210 sucrose, 1.3 ascorbic acid, and 3 sodium pyruvate, bubbled with 95% O$_2$ - 5% CO$_2$ followed by 4% paraformaldehyde (PFA) in PBS described previously (Kim et al. 2012). The brain was removed and fixed overnight in 4% PFA, and then transferred to 30% sucrose in PBS. Dorsal and ventral hippocampal slices were prepared on a cryostat and stored in cryoprotectant (30% sucrose, 30% ethylene glycol, 1% polyvinyl pyrrolidone, 0.05M sodium phosphate buffer) until processing for immunohistochemistry. Dorsal and ventral slices were briefly rinsed in PBS buffer, and incubated in 0.1% TritonX-100 for 30 min. Subsequently, slices were blocked in PBS solution containing 5% normal goat serum, 0.03% TritonX-100 for 1hr, and then incubated in primary antibody diluted in blocking solution overnight at 4°C. Slices were rinsed in PBS buffer, and then incubated in secondary antibody for 1hr at room temperature. Primary antibodies in this study were used as follows; rabbit anti-Kir3.1 (1:200, Alomone Labs), rabbit anti-Kir 3.2 (1:200, Alomone Labs), rabbit anti-A1 adenosine receptors (1:200, Alomone Labs), and mouse anti-MAP2 (1:1000, Sigma-Aldrich).

**Data analysis.** Input resistance ($R_{in}$) was determined by the slope of the linear fit of the voltage-current plot from the steady-state voltage response to the injected current ranging from +10- to -150-pA (750 ms). FI curves were determined by plotting the number of action potentials against injected current (30-300 pA, for 750 ms in 30 pA intervals).

**Statistical analyses.** All data are expressed as mean ± SEM. Statistical
comparisons were performed using ANOVA (one-factor or two-factor) followed by Bonferroni post hoc test or Student’s t test (paired or unpaired) with GraphPad Prism software. *p < 0.05 was considered as statistically significant.
Results

Contribution of Ba\textsuperscript{2+}-sensitive conductance to intrinsic membrane properties of dorsal CA1 neurons

Our initial goal in this study was to explore the resting conductances that contribute to the intrinsic membrane properties of CA1 pyramidal neurons from the dorsal and ventral hippocampus. Although functional differences in $I_h$ and distinct $\alpha$-subunit expression profiles of HCN1 and HCN2 were reported between dorsal and ventral CA1 neurons (Dougherty et al. 2013), $I_h$-related properties cannot fully account for the differences in intrinsic membrane properties between dorsal and ventral CA1 neurons (Dougherty et al. 2012). We therefore hypothesized that dorsal neurons might have a large resting potassium (K\textsuperscript{+}) conductance contributed by inward rectifier-like K\textsuperscript{+} channels (Kir). A relatively low concentration of barium (50 μM Ba\textsuperscript{2+}) is known to block Kir channels, but not many other K\textsuperscript{+} currents (Armstrong and Taylor 1980; Quayle et al. 1988; Sah 1996; Wang et al. 1998; Jin et al. 1999; Chen and Johnston 2005; Day et al. 2005). Because ZD7288 caused a gradual depolarization 10-15 min after the hyperpolarization of $V_m$ (not shown), we first applied Ba\textsuperscript{2+} and then added of ZD7288 for 4 to 5 min (Dougherty et al. 2013) before switching back to the barium-containing aCSF. Membrane potential ($V_m$) and input resistance ($R_{in}$) at RMP were monitored during baseline, application of 50 μM Ba\textsuperscript{2+} and addition of 10 μM ZD7288 application in dorsal (Fig.1B) and ventral (Fig.1F) CA1 pyramidal neurons. 50 μM Ba\textsuperscript{2+} significantly depolarized the membrane potential in dorsal neurons (Fig.1, A and C; table 1), but not in ventral neurons (Fig.1, E and G; table 1). Addition of 10 μM ZD7288 produced a significant hyperpolarization.
from baseline in ventral neurons (Fig. 1, E and G; table 1), but not in dorsal neurons (Fig. 1, A and C; table 1). $R_{in}$ at RMP for dorsal neurons was significantly increased (Fig. 1, A and D; table 1), but not for ventral neurons (Fig. 1 E and H; table 1) after 50 μM Ba$^{2+}$ application. Addition of 10 μM ZD7288 led to a significant increase in $R_{in}$ in dorsal (Fig. 1 A and D; table 1) and ventral (Fig. 1 E and H; table 1) neurons. For proper comparison between groups, $R_{in}$ was measured at the same membrane potential. At a common membrane potential (-73 mV), 50 μM Ba$^{2+}$ led to a significant increase in $R_{in}$ for dorsal neurons (Fig. 1 I; table 2), but not for ventral neurons (Fig. 1 J; table 2). $V_m$ (Fig. 1 K; table 2) and $R_{in}$ (Fig. 1 L; table 2) were significantly different between dorsal and ventral neurons. This difference was absent when either IRKs alone were blocked or were blocked in combination with $I_h$ (Fig. 1, K and L; table 2).

Because we observed significantly changes in voltage response ($V_m$) in response to the injected current from RMP and at -73 mV in the presence of Ba$^{2+}$ in dorsal neurons (Fig. 1 A and I), but not in ventral neurons (Fig. 1 E and J), steady-state voltage-current (V-I) curves in control, Ba$^{2+}$, and the difference between the two were plotted to better illustrate the Ba$^{2+}$-sensitive conductance in the hyperpolarizing direction (Figs. 1 M, N & O). The V-I curves before and after Ba$^{2+}$ application were significantly different in dorsal neurons (Fig. 1 M), but not ventral neurons (Fig. 1 N) from RMP and at -73 mV, suggesting a greater Ba$^{2+}$-sensitive conductance in dorsal neurons.

Comparison of basal GIRK activity between ventral and dorsal CA1 neurons

Given the observation that significant changes in $V_m$ and $R_{in}$ (at RMP and at a common membrane potential) were observed following blockade of a Ba$^{2+}$-sensitive
conductance in dorsal neurons (Fig. 1), we next examined which ion conductance is responsible for the resting $\text{Ba}^{2+}$-sensitive conductance. Inwardly rectifying potassium currents ($K_{ir}$) are blocked by low concentrations of $\text{Ba}^{2+}$ (Standen and Stanfield 1978; Williams et al. 1988; Gerber et al. 1991; Chen and Johnston 2005), while tertiapin-Q is a specific GIRK channel blocker at nanomolar concentration (Jin et al. 1999).

Because GIRK1 (Kir3.1) and GIRK2 (Kir3.2) heterotetrameric channels are predominantly expressed in the brain (Liao et al. 1996), we performed an immunohistochemical staining with antibodies against GIRK1 and GIRK2 in the dorsal and ventral hippocampus. To account for the longer radial axis in the CA1 region of ventral hippocampus (Dougherty et al. 2012), the quantification of GIRK1 and GIRK2 protein expression was normalized by distance (Fig. 2, A and C).

Expression of GIRK1 and GIRK2 was higher in the somatic and distal dendritic regions of dorsal CA1 compared to ventral CA1 (Fig. 2, B and D; $p<0.05$, $n=4$). Because of these differences, we tested whether dorsal neurons are more responsive to the specific GIRK channel blocker, tertiapin-Q, compared to ventral neurons. We first used three different concentrations of tertiapin-Q (0.03, 0.3, and 2 μM) and measured changes in $R_{\text{M}}$ and $R_{\text{in}}$ in dorsal and ventral neurons. Changes in $V_m$ and $R_{\text{in}}$ (at $R_{\text{M}}$) were significantly affected by bath application of 0.3 μM tertiapin-Q compared to 0.03 μM tertiapin-Q wash-in group in dorsal neurons (Fig. 2, F and G; table 3), but not in ventral neurons (Fig. 2, F and G; table 3). There were no further changes in $V_m$ (Fig. 2, F and G; table 3) and $R_{\text{in}}$ (at $R_{\text{M}}$) (Fig. 2, F and G; table 3) following bath application of 2 μM tertiapin-Q in dorsal and ventral neurons compared to 0.3 μM tertiapin-Q wash-in group. Group comparisons showed...
that there were significant differences in the changes in $V_m$ (Fig. 2, F and G; table 3) and $R_{in}$ (Fig. 2, F and G; table 3) between dorsal and ventral neurons after either 0.3 µM tertiapin-Q or 2 µM tertiapin-Q application. Based on these results, all subsequent tertiapin-Q experiments were performed using 0.3 µM.

**The Ba$^{2+}$-sensitive conductance in dorsal CA1 neurons in mostly mediated by GIRKs**

Because tertiapin-Q changed RMP and $R_{in}$ in dorsal but not ventral CA1 neurons (Fig. 2), we next sought to determine whether the Ba$^{2+}$-sensitive conductance in dorsal neurons was mediated by GIRKs. $V_m$ and $R_{in}$ (at RMP) were monitored during successive 0.3 µM tertiapin-Q and 50 µM Ba$^{2+}$ application in dorsal (Fig. 3B) and ventral (Fig. 3F) neurons. Application of tertiapin-Q significantly depolarized (Fig. 3, A and C; table 4) and increased $R_{in}$ (Fig. 3, A and D; table 4) of dorsal neurons. In contrast, tertiapin-Q had no significant effect on ventral neurons (Fig. 3, G and H; table 4). There was no further change in either dorsal or ventral neurons when 50 µM Ba$^{2+}$ was subsequently added to the bath (dorsal: Fig. 3, C and D, ventral: Fig. 3, G and H; table 4). Blockade of both GIRK and Ba$^{2+}$-sensitive conductances led to a significant depolarization (dorsal: Fig. 3, A and C; ventral: Fig. 3, E and G; table 4) and an increased $R_{in}$ (dorsal: Fig. 3, A and D; ventral: Fig. 3, E and H; table 4) in dorsal and ventral neurons. At a common membrane potential (-73 mV), a change in $R_{in}$ for dorsal neurons was significantly increased in the presence of 0.3 µM tertiapin-Q (Fig. 3I; table 5), but not for ventral neurons (Fig. 3J; table 5). When subsequent 50 µM Ba$^{2+}$ was applied after blockade of GIRK conductance, there was
no further change in $R_{in}$ in dorsal (Fig.3I; table 5) and ventral (Fig.3J; table 5) neurons. When both GIRK and $\text{Ba}^{2+}$-sensitive conductances were blocked, $R_{in}$ for dorsal neurons (Fig.3I; table 5) was significantly increased, but not for ventral neurons (Fig.3J; table 5). Action potentials evoked in response to depolarizing current steps (30-300 pA in 30 pA increments for 750 ms) were determined during successive 0.3 μM tertiapin-Q and 50 μM $\text{Ba}^{2+}$ application at a common membrane potential (-73 mV) in dorsal (Fig.3K) and ventral (Fig.3L) neurons. As expected, tertiapin-Q application significantly increased action potential firing in dorsal neurons (Fig.3K), but had no effect on firing in ventral neurons (Fig.3L) compared to baseline. Addition of 50 μM $\text{Ba}^{2+}$ had no further effects on firing in either dorsal (Fig.3K) or ventral (Fig.3L) neurons.

Comparison of basal $\text{GABA}_B$ receptor activity in dorsal and ventral CA1 neurons

The data presented thus far suggest that there was more basal GIRK activity in dorsal vs ventral neurons (Fig.2 and Fig.3). We next examined whether there was more basal activation of neurotransmitter receptors known to couple with GIRK channels in dorsal vs ventral neurons. In CA1 neurons, the activation of $\text{GABA}_B$ receptors, A1 adenosine receptors, and 5-HT$_{1A}$ serotonin receptors results in hyperpolarization of resting membrane potential by GIRK channels (Luscher et al. 1997). Thus, it is possible that there is more activity in any one of these receptor systems in dorsal compared with ventral neurons. We first examined RMP and $R_{in}$ in dorsal and ventral neurons during bath application of a potent and selective $\text{GABA}_B$
receptors antagonist, CGP52432 (5 μM) (Lanza et al. 1993). There was no significant change in $V_m$ and $R_{in}$ (at RMP) up to 30-35 min after wash-in of CGP55845 in either dorsal (Fig.4, B and C; table 6) or ventral neurons (Fig.4, E and F; table 6). Because we did not observe any changes in $V_m$ and $R_{in}$ after blockade of GABA$_B$ receptors in dorsal or ventral neurons, we next applied a GABA$_B$ receptor agonist, baclofen, to test for the presence of functional GABA$_B$ receptors. $V_m$ and $R_{in}$ (at RMP) were monitored during 100 μM baclofen application in dorsal (Fig.4G) and ventral (Fig.4J) neurons. Baclofen significantly changed $V_m$ (dorsal: Fig.4H; ventral: Fig.4K; table 6) and $R_{in}$ (dorsal: Fig.4I; ventral: Fig.4L; table 6) in both dorsal and ventral neurons. The magnitude of changes in $V_m$ (dorsal: -7.6±0.8 mV vs. ventral: -8.2±0.6 mV; p>0.05; Fig.4M; n=6) and $R_{in}$ at RMP (dorsal: -28.3±5.2 % vs. ventral: -23.2±3.6 %; p>0.05; Fig.4N; n=6) was no significantly different after activation of GABA$_B$ receptors.

Effect of A1 adenosine receptor-mediated activation on GIRK conductance in dorsal CA1 neurons

We next examined whether A1 adenosine receptors (A1ARs) are involved in the activation of the resting GIRK conductance in these neurons. 1,3-Dipropyl-8-cyclopentylxanthine (DPCPX) is a selective A1 adenosine receptor antagonist (Alzheimer et al. 1989). $V_m$ and $R_{in}$ (at RMP) were monitored during 100 nM DPCPX wash-in in dorsal (Fig.5A) and ventral (Fig.5E) neurons. Dorsal neurons had significantly depolarized membrane potential (Fig.5, B and C; table 7) and an increased $R_{in}$ at RMP (Fig.5, B and D; table 7) after blockade of A1 adenosine
receptors, whereas no changes were observed in ventral neurons (Fig.5, G and H; table 7). The magnitude of changes in $V_m$ (dorsal 4.7±0.5 mV vs. ventral 1.1±0.5 mV; p<0.05; Fig.5I) and $R_in$ at RMP (dorsal 29.5±3.4 % vs. ventral neurons 2.6±1.4 %; p<0.05; Fig.5J) were significantly greater for dorsal neurons than for ventral neurons after blockade of A1 adenosine receptors. Because the activation of GIRK conductance is mediated by A1 adenosine receptors in the hippocampal CA1 neurons (Luscher et al. 1997), we therefore performed successive Ba$^{2+}$ and DPCPX wash-in experiment to examine whether the effects of DPCPX were occluded by Ba$^{2+}$ in dorsal neurons. $V_m$ and $R_in$ (at RMP) were monitored during baseline, application of 50 μM Ba$^{2+}$ and addition of 100 nM DPCPX application in dorsal neurons (Fig.5B). 50 μM Ba$^{2+}$ led to a significant depolarization (Fig.6, A and C; table 8) and an increased $R_in$ (Fig.6, A and D; table 8) in dorsal neurons. Addition of 100 nM DPCPX had no further effects on $V_m$ (Fig.6, A and C; table 8) and $R_in$ (Fig.6, A and D; table 8) in dorsal neurons. At a common membrane potential (-73 mV), dorsal neurons had a significantly increased $R_in$ after 50 μM Ba$^{2+}$ application (Fig.6E; table 8). When DPCPX was subsequently applied in dorsal neurons, $R_in$ at -73 mV was not further changed (Fig.6E; table 8). Given the observation that ventral neurons showed no changes in $V_m$ and $R_in$ (at RMP) after blockade of A1 adenosine receptors, immunohistochemical staining with antibody against A1 adenosine receptors was carried out to examine whether ventral CA1 region express A1 adenosine receptors. The quantification of A1 adenosine receptor protein expression was again normalized by distance. The A1AR protein expression was highly expressed in somatic region in dorsal CA1 region, but not in ventral CA1 region (p<0.05; Fig.7, A
and B; n=3). Because there was a significant difference in A1AR protein expression in somatic region of dorsal and ventral CA1, we thus examined the effects by 2'ME-CCPA, a selective A1 receptor agonist. $V_m$ and $R_{in}$ (at RMP) were monitored during 1 μM 2'ME-CCPA application in the dorsal (Fig.7C) and ventral (Fig.7G) CA1 neurons. 1 μM 2'ME-CCPA significantly hyperpolarized the membrane potential (Fig.7, D and E; table 9) and decreased $R_{in}$ at RMP (Fig.7, D and F; table 9) in dorsal but not ventral neurons (Fig.7, H-J; table 9). The magnitude of changes in $V_m$ (dorsal -3.9±0.3 mV vs. ventral -0.3±0.3 mV; p<0.05; Fig.7K; n=6) and $R_{in}$ at RMP (dorsal 17.2±2.0 % vs. ventral 0.2±1.2 %; P*<0.05; Fig.7L; n=6) were significantly greater for dorsal neurons than ventral neurons after activation of A1 adenosine receptors. At a common membrane potential (-73 mV), $R_{in}$ for dorsal neurons was significantly decreased after activation of A1 adenosine receptors (Fig.7M; table 9), but not for ventral neurons (Fig.7N; table 9).
In this study, we examined the resting conductances that contribute to the different intrinsic membrane properties in dorsal and ventral CA1 neurons. We found that there was more Ba$^{2+}$-sensitive conductance in dorsal than in ventral CA1 neurons. Furthermore, Ba$^{2+}$-dependent changes in $V_m$ and $R_{in}$ were due to block of GIRK channels. We also found that this resting GIRK conductance was mediated by A1 adenosine receptors in dorsal CA1 neurons, but not in ventral CA1 neurons.

Until recently, the intrinsic electrophysiological properties of CA1 pyramidal neurons along the longitudinal hippocampal axis were assumed to be uniform. However, we and others have recently reported significant differences in the intrinsic electrophysiological properties between dorsal and ventral neurons (Dougherty et al. 2012; Marcelin et al. 2012a). Although differences in the expression of some voltage-gated ion channels between dorsal and ventral CA1 neurons have been reported (Marcelin et al. 2012b; Dougherty et al. 2013), the ionic mechanisms underlying the different intrinsic properties remain unclear. Because dorsal CA1 neurons showed a lower somatic $R_{in}$ compared with ventral CA1 neurons, this could be due to more dendritic surface area for dorsal than ventral neurons (Dougherty et al. 2013), although Marcelin et al., reported that somatic and dendritic capacitances were not different between dorsal and ventral neurons (Marcelin et al. 2012a; Marcelin et al. 2012b). A more hyperpolarized resting membrane potential in dorsal neurons compared to ventral neurons, however, could be due to dorsal CA1 neurons possessing a relatively larger resting potassium ($K^+$) conductance, which could also contribute to a decrease in $R_{in}$. Indeed, low micromolar application of Ba$^{2+}$ that
blocks inwardly rectifying $K^+$ ($K_{IR}$) channels (Chen and Johnston 2005) had
significant effects of $V_m$ and $R_m$ (at RMP and at a common membrane potential -73 mV) for dorsal neurons, but not for ventral neurons. In addition, the changes in V-I curves were greater for dorsal neurons than ventral neurons. It should be noted that higher concentrations of extracellular barium ($Ba^{2+}$, > 200 µM) block A-type potassium channels (Gasparini et al. 2007), whereas low concentrations of barium ($Ba^{2+}$, < 200 µM) are known to block inwardly rectifying potassium currents ($K_{ir}$) (Standen and Stanfield 1978; Williams et al. 1988; Gerber et al. 1991; Chen and Johnston 2005). Furthermore, when 10 µM ZD7288 was applied after blockade of $Ba^{2+}$-sensitive conductance, we observed that the change in $V_m$ for ventral neurons was larger than dorsal neurons, consistent with more $I_h$ in ventral CA1 neurons (Dougherty et al. 2013). Interestingly, the absolute values of $V_m$ and $R_m$ were similar between dorsal and ventral neurons after blockade of $Ba^{2+}$-sensitive conductance and $I_h$, suggesting a larger $Ba^{2+}$-sensitive conductance in dorsal neurons compared to ventral neurons and more $I_h$ in ventral CA1 neurons compared to dorsal CA1 neurons. Both $Ba^{2+}$-sensitive conductance and $I_h$ might account for these differences on intrinsic membrane properties between dorsal and ventral CA1 neurons.

Two classes of inwardly rectifying potassium channels, $K_{ir}$ channels, are highly expressed in hippocampal neurons: IRK channels ($K_{ir2.x}$), and GIRK channels ($K_{ir3.x}$) (Karschin et al. 1996; Luscher et al. 1997; Chen and Johnston 2005). Both IRK and GIRK channels undergo voltage-dependent intracellular Mg$^{2+}$ block, which imparts a rapid hyperpolarization-activated gating mechanism to these channels, and they become blocked and unblocked with depolarization and hyperpolarization,
respectively. Because dorsal CA1 neurons showed a larger response to a relatively low concentration of Ba\textsuperscript{2+} than ventral CA1 neurons, we used a specific GIRK channels blocker tertiapin-Q (Jin et al. 1999) in an attempt to identify molecular targets responsible for the resting Ba\textsuperscript{2+}-sensitive conductance. Interestingly, we found that dorsal neurons showed significant changes in $V_m$ and $R_{in}$ (at RMP and at a common membrane potential -73 mV), but not ventral neurons after tertiapin-Q application, indicating that there was more basal GIRK conductance. Furthermore, when action potentials were elicited from a somatic current injection at a common membrane potential (-73 mV), tertiapin-Q application significantly increased action potential firing in dorsal neurons, but not in ventral neurons. In agreement with these results, the protein expression of GIRK1 and GIRK2 was significantly enriched in somatic and dendritic CA1 region of dorsal hippocampus compared to ventral hippocampus. The somatic and dendritic protein expression of GIRK subunits in the hippocampus have also been observed by other groups. (Liao et al. 1996). Most interestingly, successive tertiapin-Q and Ba\textsuperscript{2+} application revealed that a greater Ba\textsuperscript{2+}-sensitive conductance for dorsal CA1 neurons stems from the GIRK conductance.

Hippocampal G protein-coupled inwardly rectifying K\textsuperscript{+} (GIRK) channels are activated by a number of neurotransmitters systems, including GABA\textsubscript{A} receptors, serotonergic 5HT\textsubscript{1A} receptors, and adenosine A\textsubscript{1} receptors (Andrade et al. 1986; North et al. 1987; North 1989; Luscher et al. 1997). Given that ventral neurons have a weaker GABAergic fast synaptic inhibition than dorsal CA1 neurons (Papatheodoropoulos et al. 2002), it is possible that there is more GABA\textsubscript{A}-mediated
slow tonic inhibition, which could activate more GIRK conductance in dorsal neurons compared to ventral neurons. We observed, however, that there are similar changes in $V_m$ and $R_{in}$ (at RMP) after activation of GABA$_B$ receptors in dorsal and ventral neurons, suggesting that coupling of GABA$_B$ receptors to GIRK channels might be similar. Lee et al. reported that dorsal CA1 region had a greater A1 adenosine receptor density, resulting in the suppression of extracellular excitatory postsynaptic potentials (EPSPs) in the presence of N$^6$-cyclohexyladenosine, a selective A1 adenosine receptor agonist, compared to ventral CA1 region (Lee et al. 1983). In accordance with these results, somatic whole-cell current-clamp recordings revealed that dorsal CA1 neurons showed significant changes in $V_m$ and $R_{in}$ in the presence of A1 adenosine receptor agonist or antagonist, but not in ventral neurons, suggesting again that A1 adenosine receptors are more heavily expressed in dorsal CA1 neurons. Consistent with these results, the protein expression of A1 adenosine receptor was highly present in the somatic CA1 region of dorsal hippocampus compared with ventral hippocampus. Interestingly, when A1 adenosine receptors were blocked in dorsal neurons, the absolute values of $V_m$ and $R_{in}$ at RMP were similar to ventral neurons. Furthermore, successive Ba$^{2+}$ and DPCPX application revealed that the activation of GIRK conductance is mediated by A1 adenosine receptors in dorsal CA1 neurons. These results suggest that A1 adenosine receptor-mediated GIRK conductance might contribute to intrinsic membrane properties in dorsal CA1 neurons.

Because GIRK and the adenosinergic system play important roles in the pathophysiology of diseases such as epilepsy, depression, and anxiety, our results
suggest that dorsal CA1 neurons/region (predominantly expressing A1 adenosine receptors-mediated GIRK conductance) might be important for chronic neurological disorders compared to ventral hippocampus. GIRK channels play an important role in regulating neuronal excitability (Signorini et al. 1997). The deletion of Kir3.2 (GIRK2) channels led to an increase in susceptibility to a convulsant agent and showed sporadic seizures (Signorini et al. 1997). In the present study, we found that there is a more basal GIRK activity (i.e. physiological responses to specific GIRK channels blocker at RMP) in dorsal CA1 neurons compared to ventral CA1 neurons, which might contribute to different susceptibility to a convulsant agent within the hippocampus. Adenosinergic systems have been implicated in anxiety and depression. In preclinical studies, activation of adenosine A1 receptors led to antidepressant- and anxiolytic-like behaviors, whereas blockade of A1 adenosine receptors resulted in an increase in anxiety (Kaster et al. 2004; Prediger et al. 2006; Maximino et al. 2011). In a clinical study, large amount of caffeine, a non-selective A1 adenosine receptor antagonist, are known to cause anxiety and depression in both normal and vulnerable subjects (Broderick and Benjamin 2004). Ligand-stimulated G\textsubscript{i/o} protein-coupled receptors such as A1 adenosine receptors induce an inhibition of adenylyl cyclase, which results in a decrease in cAMP concentration (van Calker et al. 1978). Because HCN channels are modulated by cAMP, resulting in an increase in \( I_h \) (Wainger et al. 2001), stimulation of A1 adenosine receptors-induced decrease in cAMP levels might be interacting with HCN channels.

**Summary**

In summary, we have investigated the resting conductances that contribute to
intrinsic membrane properties of CA1 pyramidal neurons from the dorsal and ventral hippocampus. We found that dorsal CA1 neurons possess more basal activity of GIRK conductance, which was activated by A1 adenosine receptors. Our results suggest that A1 adenosine receptor-mediated GIRK conductance contributes to the different intrinsic membrane properties in dorsal and ventral CA1 pyramidal neurons.
**Figure legends**

Fig.1. Dorsal neurons show greater response to Ba\(^{2+}\) compared with ventral CA1 neurons. (A and E) Representative voltage responses with step current commands at RMP. (B and F) Time courses of changes in \(V_m\) and \(R_{in}\) during successive Ba\(^{2+}\) (50 \(\mu\)M) and ZD7288 (10 \(\mu\)M) wash-in experiments in dorsal (B) and ventral (F) CA1 pyramidal neurons. (C and D) Dorsal CA1 pyramidal neurons showed a significantly depolarized \(V_m\) (C) and increased \(R_{in}\) (D) in the presence of Ba\(^{2+}\). Subsequent ZD7288 application resulted in a significantly hyperpolarized \(V_m\) (C) a further increase \(R_{in}\) (D). (G and H) Ventral CA1 pyramidal neurons showed no significant changes in \(V_m\) (G) and \(R_{in}\) (H) in the presence of Ba\(^{2+}\). Addition of ZD7288 led to a significantly hyperpolarized \(V_m\) (G) and an increased \(R_{in}\) (H). (I and J) The left displays representative voltage responses with step current commands at -73 mV. Dorsal CA1 neurons showed a significantly increased \(R_{in}\), but not ventral CA1 neurons at a common membrane potential (-73 mV) in the presence of Ba\(^{2+}\). (K and L) Dorsal CA1 neurons showed a more hyperpolarized RMP (K) and a lowered \(R_{in}\) at RMP (L) compared to ventral CA1 neurons. There is no difference in \(V_m\) (K) and \(R_{in}\) (L) between dorsal and ventral neurons following successive Ba\(^{2+}\) and ZD7288 application. (M and N) Dorsal neurons showed significant changes in V-I curve before and after 50 \(\mu\)M Ba\(^{2+}\) application (M), but not ventral neurons (N). (O) The differences in V-I curves before and after 50 \(\mu\)M Ba\(^{2+}\) application are indicated. (\(p < 0.05\) and \(^\#p < 0.05\) in dorsal vs. ventral groups).

Fig.2. Dorsal CA1 neurons show a more basal GIRK activity than ventral CA1 neurons. (A and C) Representative dorsal and ventral hippocampal slices
immunolabeled with antibodies against Kir 3.1 (A; GIRK1) and Kir 3.2 (C; GIRK2).

Yellow boxes depict the region of the slice used for quantification of signal intensity
(B and D) Quantification of Kir3.1 (B) and Kir3.2 (D) protein expression from
perisomatic region to the distal dendritic region of CA1 from dorsal and ventral hippocampus. The protein expression of Kir3.1 (B) and Kir 3.2 (D) was significantly
increased in somatic and distal dendritic region of CA1 from dorsal hippocampus
compared to ventral hippocampus. Vertical blue bars highlight part of the CA1 radial
axis that show significant differences in Kir 3.1 (B) and Kir 3.2 (D) protein expression
between dorsal and ventral CA1 region. (E) Representative voltage responses with
step current commands at RMP. (F and G) 0.3 μM tertiapin-Q significantly changed
V_m and R_in in dorsal but not ventral neurons. 2 μM tertiapin-Q produced similar
effects on V_m and R_in compared to 0.3 μM tertiapin-Q wash-in in dorsal and ventral
neurons. Dorsal CA1 neurons showed significant changes in V_m and R_in in the
presence of 0.3 μM or 2 μM tertiapin-Q compared to ventral CA1 neurons. *p < 0.05
and #p < 0.05 vs. ventral group.

Fig.3. Ba^{2+}-sensitive conductance-mediated changes in V_m and R_in stem from GIRK conductance in dorsal CA1 neurons. (A and E) Representative voltage responses
with step current commands at RMP are shown. (B and F) Time courses of changes
in V_m and R_in during successive 0.3 μM tertiapin and 50 μM Ba^{2+} application in dorsal
(B) and ventral (F) CA1 pyramidal neurons. (C and D) Dorsal CA1 neurons showed a
significantly depolarized V_m (C) and an increased R_in (D) following bath application of
tertiapin-Q (0.3 μM). Subsequent blockade of Ba^{2+}-sensitive conductance showed no
significant changes in $V_m$ (C) and $R_{in}$ (D) in dorsal CA1 neurons. (G and H) Ventral CA1 neurons showed no significant changes in $V_m$ and $R_{in}$ in the presence of tertiapin-Q (0.3 μM). Further changes in $V_m$ and $R_{in}$ were not observed following blockade of Ba$^{2+}$-sensitive conductance in ventral neurons. Blockade of tertiapin-Q and Ba$^{2+}$-sensitive conductance produced a significantly depolarized $V_m$ and increased $R_{in}$ compared to baseline in ventral neurons. (I and J) The left displays representative voltage responses with step current commands at -73 mV. Dorsal CA1 neurons showed a significantly increased $R_{in}$ (I), but not ventral CA1 neurons (J) at a common membrane potential (-73 mV) following successive tertiapin-Q and Ba$^{2+}$ application. (K and L) Representative membrane potential responses with depolarizing current steps (300 pA for 750 ms) at a common membrane potential (-73 mV). (K) Dorsal neurons showed significant increases in action potential firing after either tertiapin-Q or tertiapin-Q+Ba$^{2+}$ application, but not ventral neurons (L). *$p < 0.05$. 

Fig.4. Similar changes in $V_m$ and $R_{in}$ in the presence of GABA$_B$ receptor antagonist and agonist in dorsal and ventral CA1 neurons. (A and D) Time courses of changes in $V_m$ and $R_{in}$ during CGP55845 (5 μM) wash-in experiments in dorsal (A) and ventral (D) neurons. Dorsal (B and C) and ventral (E and F) neurons showed no changes in $V_m$ and $R_{in}$ in the presence of CGP55845. (G and J) Time courses of $V_m$ and $R_{in}$ in the presence of baclofen in dorsal (G) and ventral (J) neurons. Dorsal (H and I) and ventral (K and L) CA1 neurons showed a significantly hyperpolarized $V_m$ and a decreased $R_{in}$ following activation of GABA$_B$ receptors. The changes in $V_m$ and $R_{in}$
were not significantly different in dorsal and ventral CA1 neurons in the presence of CGP55845 or baclofen. *\( p < 0.05 \).

Fig.5. Dorsal CA1 neurons showed greater responses to A1 adenosine receptor antagonist compared to ventral CA1 neurons. (A and E) Time course of changes in \( V_m \) and \( R_{in} \) during DPCPX (100 nM) wash-in experiments in dorsal (A) and ventral (E) neurons. (B and F) Representative voltage responses with step current commands at RMP are shown. (C and D) 100 nM DPCPX caused a significant depolarization in dorsal neurons (C) and an increased \( R_{in} \) (D). (G and H) Ventral neurons showed no changes in \( V_m \) (G) and \( R_{in} \) (H) after DPCPX (100 nM) wash-in. (I and J) The changes in \( V_m \) (I) and \( R_{in} \) (J) after 100 nM DPCPX wash-in were greater for dorsal neurons than ventral neurons. *\( p < 0.05 \) and \#\( p < 0.05 \) vs. ventral group.

Fig.6. Dorsal CA1 neurons have an A1 adenosine receptor-mediated GIRK conductance. (A) Representative voltage responses with step current commands at RMP. (B) Time courses of changes in \( V_m \) and \( R_{in} \) during successive 50 \( \mu \)M Ba\(^{2+}\) and 100 nM DPCPX in dorsal CA1 neurons. Dorsal CA1 neurons showed a significantly depolarized \( V_m \) (C) and an increased \( R_{in} \) (D) at RMP in the presence of Ba\(^{2+}\). Subsequent bath application of DPCPX had no further effects on \( V_m \) (C) and \( R_{in} \) (D) at RMP in dorsal neurons. (E) The left displays representative voltage responses with step current commands at -73 mV. Successive Ba\(^{2+}\) and DPCPX application led to a significantly increased \( R_{in} \) at a common membrane potential (-73 mV). *\( p < 0.05 \).
Fig. 7. A1 adenosine receptors and their physiological responses predominantly exist in dorsal, but not in ventral CA1 neurons. (A) Representative dorsal and ventral hippocampal slices immunolabeled with antibodies against A1 adenosine receptors. Yellow boxes depict the region of the slice used for quantification of signal intensity.

(B) Quantification of A1ARs protein expression from perisomatic region to the distal dendritic region of CA1 from dorsal and ventral hippocampus. The protein expression of A1 adenosine receptors was highly present in somatic CA1 region of dorsal hippocampus, but not in ventral CA1 region. The blue shade indicates a significant difference in A1ARs protein expression between dorsal and ventral CA1 region. (C and G) Time courses of \( V_m \) and \( R_{in} \) during 2'ME-CCPA (1 \( \mu \)M) wash-in experiments in dorsal (C) and ventral (G) CA1 neurons. (D and H) Representative voltage responses with step current commands at RMP. (E and F) Dorsal CA1 neurons showed a significant hyperpolarization (E) and a decreased \( R_{in} \) at RMP (F) in the presence of 2'ME-CCPA (1 \( \mu \)M). (I and J) Ventral CA1 neurons showed no changes in \( V_m \) (I) and \( R_{in} \) at RMP (J) after 2'ME-CCPA (1 \( \mu \)M) wash-in. (K and L) The changes in \( V_m \) (K) and \( R_{in} \) (L) after 2'ME-CCPA (1 \( \mu \)M) application were greater for dorsal CA1 neurons than ventral CA1 neurons. (M and N) Activation of A1 adenosine receptors resulted in a significantly decreased \( R_{in} \) at a common membrane potential (-73 mV) in dorsal (M), but not in ventral (N) CA1 neurons. *\( p < 0.05 \) and #\( p < 0.05 \) vs. ventral group.
Table 1. Subthreshold properties in successive 50 μM Ba²⁺ and 10 μM ZD7288 application. (Related to Fig. 1)

<table>
<thead>
<tr>
<th></th>
<th>Dorsal CA1 Baseline</th>
<th>50 μM Ba²⁺ wash-in</th>
<th>10 μM ZD7288 50 μM Ba²⁺ wash-in</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vₘ (mV)</strong></td>
<td>-69.7±0.5 (n=7)</td>
<td>-64.4±1.0 (n=7)*</td>
<td>-71.0±0.7 (n=7)</td>
</tr>
<tr>
<td><strong>Rᵢ (MΩ)</strong></td>
<td>60.8±4.1 (n=7)</td>
<td>85.7±5.6 (n=7)*</td>
<td>166.4±9.0 (n=7)*</td>
</tr>
<tr>
<td></td>
<td>Ventral CA1 Baseline</td>
<td>50 μM Ba²⁺ wash-in</td>
<td>10 μM ZD7288 50 μM Ba²⁺ wash-in</td>
</tr>
<tr>
<td><strong>Vₘ (mV)</strong></td>
<td>-66.5±0.7 (n=7)</td>
<td>-64.8±1.0 (n=7)*</td>
<td>-71.9±1.5 (n=7)*</td>
</tr>
<tr>
<td><strong>Rᵢ (MΩ)</strong></td>
<td>73.7±8.3 (n=7)</td>
<td>84.8±8.4 (n=7)</td>
<td>162.1±12.7 (n=7)*</td>
</tr>
</tbody>
</table>

Vm: membrane potential. Rin: Steady-state input resistance. * was considered as statistically significant (P<0.05; One-Way ANOVA followed by Bonferroni Post hoc test compared to baseline).
Table 2. Change in $R_{in}$ at a common membrane potential (-73 mV) in dorsal and ventral CA1 neurons after 50 μM Ba$^{2+}$ wash-in experiments. (Related to Fig. 1)

<table>
<thead>
<tr>
<th></th>
<th>Dorsal CA1</th>
<th>Baseline</th>
<th>50 μM Ba$^{2+}$</th>
<th>Ventral CA1</th>
<th>Baseline</th>
<th>50 μM Ba$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{in}$ (MΩ)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at -73 mV</td>
<td>60.0±3.5</td>
<td>72.1±4.2*</td>
<td></td>
<td>69.1±5.1</td>
<td>72.6±7.2</td>
<td></td>
</tr>
<tr>
<td>(n=6)</td>
<td>(n=6)</td>
<td></td>
<td></td>
<td>(n=7)</td>
<td>(n=7)</td>
<td></td>
</tr>
</tbody>
</table>

*Rin*: Steady-state input resistance.

* was considered as statistically significant (P<0.05; Paired t test compared to baseline).
Table 3. Dose-response tertiapin-Q experiment in dorsal and ventral CA1 neurons.

(Related to Fig. 2)

<table>
<thead>
<tr>
<th></th>
<th>0.03 μM tertiapin-Q</th>
<th>0.3 μM tertiapin-Q</th>
<th>2 μM tertiapin-Q</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dorsal CA1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>V_m (mV)</strong></td>
<td>-69.9±1.0 (n=7)</td>
<td>-67.8±1.1 (n=7)</td>
<td>-69.0±0.5 (n=7)</td>
</tr>
<tr>
<td><strong>R_in (MΩ)</strong></td>
<td>60.2±2.8 (n=7)</td>
<td>70.5±3.5 (n=7)</td>
<td>62.9±1.2 (n=7)</td>
</tr>
<tr>
<td><strong>V_m (mV)</strong></td>
<td>-69.4±0.9 (n=6)</td>
<td>-65.4±0.9* (n=6)</td>
<td></td>
</tr>
<tr>
<td><strong>R_in (MΩ)</strong></td>
<td>64.5±3.9 (n=6)</td>
<td>85.6±4.5* (n=6)</td>
<td></td>
</tr>
<tr>
<td><strong>Ventrail CA1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>V_m (mV)</strong></td>
<td>-65.3±1.0 (n=6)</td>
<td>-64.7±1.4 (n=6)</td>
<td>-64.9±0.5 (n=6)</td>
</tr>
<tr>
<td><strong>R_in (MΩ)</strong></td>
<td>82.5±4.8 (n=6)</td>
<td>87.2±5.8 (n=6)</td>
<td>87.3±3.0 (n=6)</td>
</tr>
<tr>
<td><strong>V_m (mV)</strong></td>
<td>-64.1±0.8 (n=6)</td>
<td>-62.6±0.9 (n=6)</td>
<td></td>
</tr>
<tr>
<td><strong>R_in (MΩ)</strong></td>
<td>85.0±4.8 (n=6)</td>
<td>89.1±3.9 (n=6)</td>
<td></td>
</tr>
</tbody>
</table>

V_m: membrane potential. R_in: Steady-state input resistance. * was considered as statistically significant (P<0.0167; One-Way ANOVA with Bonferroni post hoc test compared to baseline).
Table 4. Subthreshold properties in subsequent tertiapin-Q and barium wash-in experiments. (Related to Fig. 3)

<table>
<thead>
<tr>
<th></th>
<th>Dorsal CA1</th>
<th></th>
<th>0.3 μM Tertiapin-Q wash-in</th>
<th>0.3 μM Tertiapin-Q wash-in</th>
<th>50 μM Ba²⁺ wash-in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>0.3 μM Tertiapin-Q wash-in</td>
<td>0.3 μM Tertiapin-Q wash-in</td>
<td>50 μM Ba²⁺ wash-in</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-63.3±0.7 (n=7)*</td>
<td>-61.2±1.1 (n=7)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>80.5±3.6 (n=7)*</td>
<td>92.7±4.7 (n=7)*</td>
<td></td>
</tr>
<tr>
<td>Vₘ (mV)</td>
<td>-68.0±0.6 (n=7)</td>
<td>-63.3±0.7 (n=7)*</td>
<td>-61.2±1.1 (n=7)*</td>
<td>92.7±4.7 (n=7)*</td>
<td></td>
</tr>
<tr>
<td>Rᵢ (MΩ)</td>
<td>62.4±1.5 (n=7)</td>
<td>80.5±3.6 (n=7)*</td>
<td>92.7±4.7 (n=7)*</td>
<td>102±1.9 (n=5)*</td>
<td></td>
</tr>
</tbody>
</table>

|                | Ventral CA1                              |               | 0.3 μM Tertiapin-Q wash-in | 0.3 μM Tertiapin-Q wash-in | 50 μM Ba²⁺ wash-in |
|                |                                         | Baseline     | 0.3 μM Tertiapin-Q wash-in | 0.3 μM Tertiapin-Q wash-in | 50 μM Ba²⁺ wash-in |
|                |                                         |               | -62.9±0.6 (n=5)            | -61.4±0.6 (n=5)*            |                   |
| Vₘ (mV)        | -64.6±0.5 (n=5)                         | -62.9±0.6 (n=5) | -61.4±0.6 (n=5)*            | 102±1.9 (n=5)*              |                   |
| Rᵢ (MΩ)        | 86.9±3.0 (n=5)                          | 95.0±3.4 (n=5) | 102±1.9 (n=5)*              | 102±1.9 (n=5)*              |                   |

Vm: membrane potential. Rin: Steady-state input resistance. * was considered as statistically significant (P<0.0167; One-Way ANOVA with Bonferroni post hoc test compared to baseline).
Table 5. Change in $R_{in}$ at a common membrane potential (-73 mV) in dorsal and ventral CA1 neurons in subsequent tertiapin-Q and barium wash-in experiments. (Related to Fig. 3)

<table>
<thead>
<tr>
<th></th>
<th>Dorsal CA1</th>
<th>Baseline</th>
<th>0.3 μM Tertiapin-Q wash-in</th>
<th>0.3 μM Tertiapin-Q 50 μM Ba$^{2+}$ wash-in</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{in}$ (MΩ) at -73 mV</td>
<td>52.7±1.3 (n=6)</td>
<td>60.5±1.6 (n=6)*</td>
<td>62.8±1.7 (n=6)*</td>
<td></td>
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<tr>
<td>Ventral CA1</td>
<td>Baseline</td>
<td>0.3 μM Tertiapin-Q wash-in</td>
<td>0.3 μM Tertiapin-Q 50 μM Ba$^{2+}$ wash-in</td>
<td></td>
</tr>
<tr>
<td>$R_{in}$ (MΩ) at -73 mV</td>
<td>72.5±1.6 (n=5)</td>
<td>74.1±3.4 (n=5)</td>
<td>78.1±4.1 (n=5)</td>
<td></td>
</tr>
</tbody>
</table>

$R_{in}$: Steady-state input resistance. * was considered as statistically significant (P<0.0167; One-Way ANOVA with Bonferroni post hoc test compared to baseline).
Table 6. Subthreshold membrane properties in 5 μM CGP55845 or 100 μM baclofen wash-in experiments. (Related to Fig. 4)

<table>
<thead>
<tr>
<th></th>
<th>Dorsal CA1</th>
<th>Baseline</th>
<th>5 μM CGP55845</th>
<th>Ventral CA1</th>
<th>Baseline</th>
<th>5 μM CGP55845</th>
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<tbody>
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<td></td>
<td></td>
</tr>
<tr>
<td>V_m (mV)</td>
<td>-70.0±0.8</td>
<td>-70.2±0.9</td>
<td></td>
<td>-64.7±0.4</td>
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<tr>
<td>(n=4)</td>
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<td>(n=4)</td>
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<tr>
<td>R_in (MΩ)</td>
<td>62.7±2.7</td>
<td>64.5±3.3</td>
<td></td>
<td>86.4±4.0</td>
<td>89.6±4.0</td>
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<td>(n=4)</td>
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<tr>
<td>Dorsal CA1</td>
<td>Baseline</td>
<td>100 μM baclofen</td>
<td></td>
<td>Ventral CA1</td>
<td>Baseline</td>
<td>100 μM baclofen</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>V_m (mV)</td>
<td>-69.4±1.6</td>
<td>-76.6±1.0*</td>
<td></td>
<td>-66.1±0.7</td>
<td>-74.4±1.0*</td>
<td></td>
</tr>
<tr>
<td>(n=6)</td>
<td>(n=6)</td>
<td></td>
<td></td>
<td>(n=6)</td>
<td>(n=6)</td>
<td></td>
</tr>
<tr>
<td>R_in (MΩ)</td>
<td>66.6±5.7</td>
<td>46.5±2.1*</td>
<td></td>
<td>83.2±5.7</td>
<td>63.1±3.4*</td>
<td></td>
</tr>
<tr>
<td>(n=6)</td>
<td>(n=6)</td>
<td></td>
<td></td>
<td>(n=6)</td>
<td>(n=6)</td>
<td></td>
</tr>
</tbody>
</table>

V_m: membrane potential. R_in: Steady-state input resistance. * was considered as statistically significant (P<0.05; Student’s paired t test compared to baseline).
Table 7. Subthreshold membrane properties in 100 nM DPCPX wash-in experiments.
(Related to Fig. 5)

<table>
<thead>
<tr>
<th>Dorsal CA1</th>
<th>Baseline</th>
<th>DPCPX</th>
<th>Ventral CA1</th>
<th>Baseline</th>
<th>DPCPX</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP (mV)</td>
<td>-69.0±0.7</td>
<td>-64.2±0.5</td>
<td></td>
<td>-64.7±0.7</td>
<td>-63.6±0.8</td>
</tr>
<tr>
<td>Rin (MΩ)</td>
<td>66.4±4.9</td>
<td>89.3±5.0 *</td>
<td></td>
<td>84.8±7.0</td>
<td>87.2±7.2</td>
</tr>
</tbody>
</table>

RMP: membrane potential. Rin: Steady-state input resistance. * was considered as statistically significant (P<0.05; Student’s paired t test compared to baseline).
Table 8. Subthreshold membrane properties in successive Ba\(^{2+}\) and DPCPX wash-in experiments. (Related to Fig. 6)

<table>
<thead>
<tr>
<th></th>
<th>Dorsal CA1</th>
<th>Baseline</th>
<th>50 μM Ba(^{2+}) wash-in</th>
<th>100 nM DPCPX 50 μM Ba(^{2+}) wash-in</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_m) (mV)</td>
<td>-68.5±0.6 (n=7)</td>
<td>-62.8±0.7 (n=7)*</td>
<td>-62.4±0.6 (n=7)*</td>
<td></td>
</tr>
<tr>
<td>(R_{in}) (MΩ)</td>
<td>69.8±2.5 (n=7)</td>
<td>99.7±4.9 (n=7)</td>
<td>103.7±5.0 (n=7)*</td>
<td></td>
</tr>
<tr>
<td>(R_{in}) (MΩ) at -73 mV</td>
<td>58.8±2.4 (n=7)</td>
<td>65.8±2.2 (n=7)</td>
<td>69.1±2.6 (n=7)*</td>
<td></td>
</tr>
</tbody>
</table>

\(V_m\): membrane potential. \(R_{in}\): Steady-state input resistance. * was considered as statistically significant (P<0.0167; One-Way ANOVA with Bonferroni post hoc test compared to baseline).
Table 9. Subthreshold membrane properties in 2’ME-CCPA wash-in experiments.

(Related to Fig. 7)

<table>
<thead>
<tr>
<th>Dorsal CA1</th>
<th>Baseline</th>
<th>2’ME-CCPA</th>
<th>Ventral CA1</th>
<th>Baseline</th>
<th>2’ME-CCPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_m$ (mV)</td>
<td>-68.5±0.8 (n=6)</td>
<td>-72.3±0.9* (n=6)</td>
<td>$V_m$ (mV)</td>
<td>-65.0±0.9 (n=6)</td>
<td>-65.3±0.94 (n=6)</td>
</tr>
<tr>
<td>$R_{in}$ (MΩ)</td>
<td>60.8±3.8 (n=6)</td>
<td>50.2±3.2* (n=6)</td>
<td>$R_{in}$ (MΩ)</td>
<td>75.2±5.4 (n=6)</td>
<td>75.4±6.0 (n=6)</td>
</tr>
<tr>
<td>$R_{in}$ (MΩ) at -73 mV</td>
<td>56.0±3.3 (n=6)</td>
<td>50.5±3.1* (n=6)</td>
<td>$R_{in}$ (MΩ) at -73 mV</td>
<td>60.2±3.6 (n=5)</td>
<td>60.6±3.1 (n=5)</td>
</tr>
</tbody>
</table>

$V_m$: membrane potential. $R_{in}$: Steady-state input resistance. * was considered as statistically significant (P<0.05; Student’s paired t test compared to baseline).


Drake CT, Bausch SB, Milner TA, and Chavkin C. GIRK1 immunoreactivity is present predominantly in dendrites, dendritic spines, and somata in the CA1 region of the hippocampus. *Proc Natl Acad Sci U S A* 94: 1007-1012, 1997.


North RA, Williams JT, Surprenant A, and Christie MJ. Mu and delta receptors belong to a family of receptors that are coupled to potassium channels. Proc Natl Acad Sci U S A 84: 5487-5491, 1987.


Kir3.1 (GIRK1)

A

Dorsal CA1

Ventral CA1

Kir3.2 (GIRK2)

C

Dorsal CA1

Ventral CA1

E

Dorsal CA1 neurons

0.03 μM Tertiapin-Q

Before

After

-70 mV

-68.9 mV

0.3 μM Tertiapin-Q

Before

After

-69 mV

-64.6 mV

2 μM Tertiapin-Q

Before

After

-69.4 mV

-64.8 mV

Ventral CA1 neurons

0.03 μM Tertiapin-Q

Before

After

-66 mV

-65 mV

0.3 μM Tertiapin-Q

Before

After

-65 mV

-63.7 mV

2 μM Tertiapin-Q

Before

After

-66 mV

-64.5 mV

F

Change in Vm (mV)

(Glazole - Tertiapin-Q)

Tertiapin-Q (μM)

Dorsal CA1

Ventral CA1

G

Change in Rin (%)

(Glazole - Tertiapin-Q)

Tertiapin-Q (μM)

Dorsal CA1

Ventral CA1
Dorsal CA1 neurons

![Graph A](image)

Ventrical CA1 neurons

![Graph D](image)

**B**

![Graph B](image)

**C**

![Graph C](image)

**D**

![Graph D](image)

**E**

![Graph E](image)

**F**

![Graph F](image)

**G**

![Graph G](image)

**H**

![Graph H](image)

**I**

![Graph I](image)

**J**

![Graph J](image)

**K**

![Graph K](image)

**L**

![Graph L](image)

**M**

![Graph M](image)

**N**

![Graph N](image)
Dorsal CA1 neurons

A

Baseline 50 μM Ba

100 nM DPCPX 50 μM Ba

-70 mV -64 mV -64 mV

L

10 pA 150 pA

B

V_m (mV)

50 μM Ba

100 nM DPCPX

50 μM Ba

Time (min)

C

D

E

Baseline

-73 mV

50 μM Ba

100 nM DPCPX

50 μM Ba

R_inj

10 pA 150 pA

Baseline

50 μM Ba

100 nM DPCPX

50 μM Ba

Baseline

50 μM Ba

100 nM DPCPX

50 μM Ba

10 mV

200 ms