Neuronal Excitability

Noradrenergic Suppression of Persistent Firing in Hippocampal CA1 Pyramidal Cells through cAMP-PKA Pathway

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Abstract
Persistent firing is believed to be a cellular correlate of working memory. While the effects of noradrenaline (NA) on working memory have widely been described, its effect on the cellular mechanisms of persistent firing remains largely unknown. Using in vitro intracellular recordings, we demonstrate that persistent firing is supported by individual neurons in hippocampal CA1 pyramidal cells through cholinergic receptor activation, but is dramatically attenuated by NA. In contrast to the classical theory that recurrent synaptic excitation supports persistent firing, suppression of persistent firing by NA was independent of synaptic transmission, indicating that the mechanism is intrinsic to individual cells. In agreement with detrimental effects of cAMP on working memory, we demonstrate that the suppressive effect of NA was through cAMP-PKA pathway. In addition, activation of β1 and/or β3 adrenergic receptors, which increases cAMP levels, suppressed persistent firing. These results are in line with working memory decline observed during high levels of NA and cAMP, which are implicated in high stress, aging, and schizophrenia.

Key words: β1 adrenoceptors; cAMP; M1 receptor; M2/4 receptors; persistent firing; PKA

Significance Statement
While cholinergic modulation supports working memory, high concentrations of noradrenaline (NA), which occurs under high stress for example, are detrimental for working memory. However, cellular and molecular mechanisms underlying such working memory deficit remain largely unclear. In this paper, we studied the effect of these two neuromodulators on persistent firing, the cellular correlate of working memory. We demonstrate that a cholinergic receptor activation supports, while a noradrenergic activation strongly inhibits persistent firing because of the PKA activation through specific NA receptor types which upregulate cAMP. These data are in line with working memory deficits in aging and schizophrenia in which cAMP levels are altered, and indicate potential intrinsic cellular mechanism of working memory impairment.

Introduction
Persistent firing is a cellular response characterized by repetitive spiking that outlasts triggering stimulus, and is believed to be a neural base of working memory (Goldman-Rakic, 1995; Major and Tank, 2004). Persistent firing observed in humans and animals in vivo during working memory tasks correlate with the task performance (Funahashi et al., 1989; Colombo and Gross, 1994; Hampson and Deadwyler, 2003; Rakic, 1995; Major and Tank, 2004). Persistent firing observed in humans and animals in vivo during working memory tasks correlate with the task performance (Funahashi et al., 1989; Colombo and Gross, 1994; Hampson and Deadwyler, 2003; Rakic, 1995; Major and Tank, 2004). Persistent firing observed in humans and animals in vivo during working memory tasks correlate with the task performance (Funahashi et al., 1989; Colombo and Gross, 1994; Hampson and Deadwyler, 2003; Rakic, 1995; Major and Tank, 2004). Persistent firing observed in humans and animals in vivo during working memory tasks correlate with the task performance (Funahashi et al., 1989; Colombo and Gross, 1994; Hampson and Deadwyler, 2003; Rakic, 1995; Major and Tank, 2004). Persistent firing observed in humans and animals in vivo during working memory tasks correlate with the task performance (Funahashi et al., 1989; Colombo and Gross, 1994; Hampson and Deadwyler, 2003; Rakic, 1995; Major and Tank, 2004). Persistent firing observed in humans and animals in vivo during working memory tasks correlate with the task performance (Funahashi et al., 1989; Colombo and Gross, 1994; Hampson and Deadwyler, 2003; Rakic, 1995; Major and Tank, 2004). Persistent firing observed in humans and animals in vivo during working memory tasks correlate with the task performance (Funahashi et al., 1989; Colombo and Gross, 1994; Hampson and Deadwyler, 2003; Rakic, 1995; Major and Tank, 2004). Persistent firing observed in humans and animals in vivo during working memory tasks correlate with the task performance (Funahashi et al., 1989; Colombo and Gross, 1994; Hampson and Deadwyler, 2003; Rakic, 1995; Major and Tank, 2004).
Bukalo et al., 2004; Kamiński et al., 2017). In contrast to the classical hypothesis that persistent firing is supported by recurrent excitatory synaptic connections, recent studies have shown that persistent firing can also be supported in individual cells (Egorov et al., 2002; Jochems and Yoshida, 2013; Knauer et al., 2013). These studies in general used cholinergic receptor activations to induce persistent firing, which is in agreement with the supportive role of acetylcholine on working memory (Reboredo et al., 2018). However, roles of other neuromodulators such as the noradrenaline (NA) on this type of persistent firing largely remain to be studied.

While cholinergic modulation generally supports working memory (Kaneko and Thompson, 1997; Weiss et al., 2000), NA could be detrimental for working memory (Arnsten, 2009; Roosendaal and McCaugh, 2011). High levels of NA are usually present during states of stress (Bremner et al., 1996), which are known to impair working memory (Diamond et al., 1996; Schools et al., 2008; Qin et al., 2009; Arnsten et al., 2012). At the receptor subtype levels, β1 and α2 adrenoreceptors, which are positively and negatively coupled to cAMP signaling, impair and facilitate working memory, respectively (Jäkälä et al., 1999; Li et al., 1999; Ma et al., 2003; Amsten and Li, 2005; Ramos et al., 2005). In addition, elevated and decreased cAMP levels impair and enhance, respectively, working memory performance (Arnsten et al., 1999; Taylor et al., 1999; Runyan et al., 2005; Wang et al., 2007). Moreover, upregulated cAMP levels are associated with working memory impairment in aging and schizophrenia (Millar, 2005; Wang et al., 2011; Barch and Ceaser, 2012). Raising cAMP levels would increase PKA activation that also is negatively correlated to working memory performance (Kobori et al., 2015), together supporting the importance of NA and cAMP–PKA in working memory.

At the cellular level, a supportive role of NA on persistent firing through a reduction of cAMP levels has been observed in the prefrontal cortex (PFC; Zhang et al., 2013). In addition, elevated cAMP levels caused a suppression of the calcium-activated nonspecification cation (CAN) current and persistent firing in the PFC and hippocampus (SidiroPOULOU et al., 2009; EL-HASSAR et al., 2011; ZHANG et al., 2013). However, it remains unclear whether NA has a supportive or detrimental effect on persistent firing in the hippocampus.

We therefore tested the effects of cholinergic and noradrenergic receptor activation in both induction and modulation of persistent firing in individual hippocampal CA1 pyramidal cells in mice. We find that a noradrenergic stimulation strongly inhibits persistent firing while a cholinergic activation supports it. In addition, the noradrenergic suppression of persistent firing is independent of ionotropic synaptic transmission, but is through the cAMP–PKA pathway. We further demonstrate specific NA receptor subtypes involved in this suppression. These observations indicate that high NA and cAMP conditions, which often suppress working memory through PKA activation, also suppress cellular mechanisms for persistent firing in the hippocampus.

Materials and Methods
All the experimental designs were approved by the local ethic committee (Der Tierschutzbeauftragte, Ruhr-Universität Bochum, and Deutsches Zentrum für Neurodegenerative Erkrankungen) and conducted in accordance with the European Communities Council Directive of September 22, 2010 (2010/63/EU).

Slice preparation
Acute hippocampal slices were obtained from adult (two to four months) C57BL6 female mice (Charles River). Animals were deeply anesthetized (intraperitoneal injection of 120 mg/kg of ketamine and 16 mg/kg of xylazin) and intracardiac perfusion with ice-cold modified artificial CSF (mACSF) was conducted directly without or after cerebral dislocation. The mACSF contained 87 mM NaCl, 25 mM NaHCO3, 10 mM glucose, 2.5 mM KCl, 1.25 mM NaH2PO4, 0.375 mM CaCl2, 3.28 mM MgCl2, 3 mM pyruvate acid, and 1 mM ascorbic acid or 87 mM NaCl, 25 mM NaH2CO3, 10 mM glucose, 75 mM sucrose, 1.25 mM KCl, 1.25 mM NaH2PO4, 0.188 mM CaCl2, 1.64 mM MgCl2, 3 mM pyruvate acid, and 1 mM ascorbic acid (pH was adjusted to 7.4 by saturation with 95% O2-5% CO2). Following the decapitation, horizontal hippocampal slices (350 μm) were obtained with a vibrating-blade microtome (Leica VT1000S) in ice-cold mACSF. Brain slices were then incubated for 30 min at 37°C in normal ACSF (nACSF) containing 124 mM NaCl, 1.25 mM NaH2PO4, 1.8 mM MgO2S, 3 mM KCl, 10 mM glucose, 26 mM NaHCO3, and 1.2 mM CaCl2 or 124 mM NaCl, 1.25 mM NaH2PO4, 1.8 mM MgO2S, 1.5 mM KCl, 10 mM glucose, 26 mM NaHCO3, and 0.6 mM CaCl2 (pH was adjusted to 7.4 by saturation with 95% O2-5% CO2). The second nACSF with 1.5 mM KCl was used in an attempt to reduce synaptic effect but was used only in seven cells in which ICI-118,551 was applied. In all other 105 cells, the first nACSF with 3 mM KCl was used. Brain slices were maintained at room temperature for at least 30 min before recording.

Whole-cell recordings
Whole-cell procedures were performed as described previously (Valero-Aracama et al., 2015). Briefly, CA1 cells were visually identified using an upright microscope (Zeiss Axioskop 2FS), and whole-cell patch was obtained using glass pipettes filled with an intracellular solution containing 120 mM Kgluc, 10 mM HEPES, 0.2 mM EGTA,
20 mM KCl, 2 mM MgCl₂, 7 mM PhCrea, 0.1 mM Na₂ATP, and 0.3 mM Tris GTP (pH adjusted to 7.3 with KOH). Electrical signals were amplified with an Axoclamp 2A amplifier (Molecular Devices), low-pass filtered at 10 kHz and sampled at 20 kHz, using WinWCP (John Dempster, University of Strathclyde) software. Liquid junction potential (~10 mV) was not corrected. To prevent pharmacological contamination, only one cell per slice was recorded. Recordings were performed at 34 ± 1°C.

**Drugs**

All drugs except for carbachol (Cch; Alfa Aesar) were purchased from Sigma-Aldrich. Water-based stock solutions were made for all drugs except picrotoxin and kynurenic acid (freshly dissolved in nACSF). Except for the Cch stock solution, which was stored at 4°C, all other solutions were aliquoted and stored at –20°C. Stock solutions were diluted at least 1000 times in the final solution. Recording solutions with Cch were freshly made each day of experiment and solutions containing other drugs were freshly made from stock solutions minutes before being used. Recordings started at least 5 min after the application of the experimental drug.

**Data analysis**

Analysis was conducted with MATLAB R2009b (MathWorks) and Anaconda (Python 3.6). Only pyramidal cells with a silent resting membrane potential lower than –55 mV, and spike amplitudes that overshoot 0 mV were included into the analysis. Persistent firing was induced by a current injection (2 s, 100 pA) from a membrane potential below spiking threshold. The membrane potential and frequency of persistent firing were measured during the first 10 s after the termination of the stimulation. General excitability was measured from the current-frequency curve (50–350 pA, 50-pA increment, 1 s) starting from the static potential of −65 mV. Input resistance (IR) was computed from the voltage drop peak in response to a 50-pA negative current injection from –65 mV. Sag ratio was computed from voltage response to a negative current step (1 s, –300 pA). Medium and slow after-hyperpolarizing potential (AHP) were measured after the induction of six spikes (0.1–1 nA, 3 ms, 50 Hz) from a membrane potential of –60 mV. In cases the AHP was positive compared with the baseline (as in conditions with Cch) this property is referred to as ADP. Comparisons were made using paired t tests. Significance level α < 0.05 (p < 0.05, **p < 0.01, ***p < 0.001) was used. Data are expressed as mean ± SEM.

**Results**

Cch supports persistent firing through G₄q/11 and Gᵢₒ G-protein-coupled receptors (GPCRs)

First, we tested the effects of cholinergic stimulation alone on the generation of persistent firing in CA1 pyramidal cells. Once a whole-cell recording was obtained, induction of persistent firing was tested by using a brief (2 s, 100 pA) current injection at a membrane potential right below the spike threshold. In the nACSF, none of the cells tested continued spiking after the termination of the stimulation (n = 34; Fig. 1A, left). However, in the presence of Cch (5 μM), persistent firing was induced in 62% (21 out of 34) of the same set of cells (Fig. 1A, right). This is in agreement with previous reports of similar persistent firing in rat and mouse CA1 pyramidal cells in Cch (Knauer et al., 2013; Arboit et al., 2020). For the quantification of persistent firing, spike frequency and membrane potential after the stimulation were measured: poststimulus frequency and poststimulus potential. The gray line below each voltage trace indicates the period during which these measurements were taken. Cch significantly increased the poststimulus frequency (n = 34; T(33) = −5.65, p < 0.001; Fig. 1B, left) and the poststimulus membrane potential (n = 34; T(33) = −6.67, p < 0.001; Fig. 1B, right).

Cch activates both G₄q/11 and Gᵢₒ GPCRs through M1 and M2/4 receptor subtypes, respectively. To study the role of each receptor subtype on persistent firing, we selectively blocked M1 or M2/4 receptors. Bath application of the M1 antagonist pirenzepine (10 μM), in addition to Cch, resulted in a complete blockade of persistent firing (Fig. 1C,D). The poststimulus frequency (n = 5; T(4) = 5.94, p < 0.01; Fig. 1D, left) and depolarization (n = 5; T(4) = 7.10, p < 0.01; Fig. 1D, right) were significantly reduced. Similarly to this, M2/4 antagonist himbacine (1 μM) suppressed persistent firing significantly (Fig. 1E) reducing both the frequency (n = 6; T(5) = 6.59, p < 0.01; Fig. 1F, left) and the depolarization (n = 6; T(5) = 7.83, p < 0.001; Fig. 1F, right). These results point out that both G₄q/11-coupled and Gᵢₒ-coupled receptors are necessary to trigger persistent firing. Furthermore, a control experiment was conducted to exclude the possibility that the suppressive effect of pirenzepine and himbacine was because of a run-down of cellular mechanisms supporting persistent firing during the whole-cell recording. In this experiment, persistent firing recorded at two different time points after the break-in was compared in the presence of Cch. The first and second time points were comparable to the times at which persistent firing was tested in Cch alone and Cch + pirenzepine (or himbacine), respectively, in the above experiment. Persistent firing was not reduced, as shown in Figure 1G,H (frequency: n = 8, T(7) = 0.388, p = 0.709; potential: n = 8, T(7) = 0.730, p = 0.489), indicating that the suppression of persistent firing was not an artifact of our experimental procedure.

NA does not support persistent firing

We next tested the effects of NA (5–10 μM; n = 14) on the induction of persistent firing. Persistent firing was not observed in any of the cells recorded and the poststimulus firing frequency was unchanged (Fig. 2A). While the poststimulus membrane hyperpolarization (AHP) was reduced by NA (n = 14; T(13) = −3.068, p = 0.009; Fig. 2C), the membrane potential did not reach levels above the baseline (averaged voltage before the stimulation) unlike Cch.

It has been reported that intrinsic excitability of CA1 pyramidal cells is increased by both cholinergic and noradrenergic modulation (Benardo and Prince, 1982; Madison and Nicoll, 1982). In agreement with these, the numbers of spikes elicited during the stimulation (2 s, 100 pA) were significantly larger in both Cch and NA compared with those in the nACSF (Cch: nACSF 23 ± 1, Cch 31 ± 2,
NA has modulatory effects on persistent firing under cholinergic activation. In this experiment, persistent firing was tested first in nACSF, then in Cch (5 μM), and finally in a solution containing Cch and NA (5–10 μM). To ensure a full effect of NA, persistent firing was tested ~15 min after the initiation of the NA application. In ten neurons that showed persistent firing in Cch condition, persistent firing was completely blocked in six, suppressed in three, and slightly increased in one cell by NA (Fig. 2B). Poststimulus firing frequency and membrane potential were both significantly decreased by NA (frequency: \( n = 12, T_{(11)} = 4.118, p = 0.002 \); potential: \( n = 12, T_{(11)} = 5.647, p < 0.001 \); Fig. 2D,E). Cells that did not exhibit persistent firing in Cch did not show it in NA either (\( n = 3 \); data not shown).

Next, to assess whether the reduction of persistent firing was synaptic or intrinsic, the effect of NA on cholinergically induced persistent firing was examined in the presence of synaptic blockers (SBs) that blocked the

**Figure 1.** Cholinergic induction of persistent firing in CA1 hippocampal neurons. A, Representative cell’s responses in nACSF (left) and in Cch (right). The gray line indicates the 10-s period in which characteristics of persistent firing were analyzed. Trace at the bottom shows the current step applied to test persistent firing. B, Poststimulus frequency (left) and poststimulus potential (right) in nACSF (gray) and Cch (white). C, Representative trace of the effect of muscarinic M1 antagonist pirenzepine on the cholinergic persistent firing. D, Poststimulus frequency (left) and poststimulus potential (right) in Cch (gray) and Cch and pirenzepine (white). E, Representative trace of the effect of muscarinic M2/M4 antagonist himbacine on the cholinergic persistent firing. F, Poststimulus frequency (left) and poststimulus potential (right) in Cch (gray) and Cch and himbacine (white). G, Representative responses from timed control experiment. H, Poststimulus frequency (left) and poststimulus potential (right) in the first test in Cch (gray) and the second test in Cch (white).
Intracellular mechanisms underlying the suppression of persistent firing

Cholinergic and noradrenergic systems act through different receptors coupled to mainly three types of GPCRs. While Ach activates only Gq/11 (M1, M3, M5) and Gi/o (M2, M4) pathways, NA can activate Gα (β1, β2, β3) in addition to Gq/11 (α1) and Gi/o (α2, partially β2) pathways. The Gα pathway, unlike other two pathways, increases the cAMP level, which is detrimental to working memory (Dash et al., 2007). In addition, an elevated cAMP level has been shown to suppress persistent firing in vivo in the PFC, and suppresses the CAN current in the hippocampus as mentioned above (El-Hassar et al., 2011). Therefore, we tested whether NA suppressed persistent firing by a cAMP activation through the Gα pathway.

First, we evaluated the effect of an elevated cAMP concentration on persistent firing using forskolin, a compound that activates the adenylate cyclase and therefore increases intracellular levels of cAMP. Persistent firing was first tested in Cch (5 μM) and then forskolin (10 μM) was bath applied in addition to Cch. This and other experiments in this section were conducted in the presence of SBs. Forskolin clearly suppressed persistent firing (frequency: n = 8, T(7) = 3.789, p = 0.007). We found that forskolin also decreased the number of spikes during the stimulation (Cch: 23.56 ± 1.5; Cch+forskolin: 19 ± 1.8; n = 8; T(7) = 3.799, p = 0.007). To study whether the suppression of persistent firing was simply because of the smaller number of spikes elicited during the stimulation, response of the cells to a fixed number (six) of induced spikes were additionally tested (Fig. 3C). Spikes were induced by using six brief current injections (0.1–1 nA, 3 ms, 50 Hz) at −60 mV, and the poststimulus potential was measured as
Table 1: Effect of Cch and NA on general cellular properties

| Part I | Number of spikes elicited by 1-s current steps. Part II, Input resistance (IR), spike threshold (TH), sag ratio (SAG), medium AHP (mAHP), afterdepolarizing potential (ADP). Values correspond to mean ± SEM. Significance level: *p < 0.05, **p < 0.01, ***p < 0.001.

| Condition | 50 pA | 100 pA | 150 pA | 200 pA | 250 pA | 300 pA | 350 pA |
|-----------|-------|--------|--------|--------|--------|--------|--------|
| nACSF n = 47 | 1 ± 0 | 11 ± 2 | 21 ± 2 | 31 ± 2 | 37 ± 2 | 42 ± 2 | 46 ± 2 |
| Cch | 6 ± 2** | 23 ± 2*** | 38 ± 2*** | 41 ± 2*** | 46 ± 2*** | 49 ± 2*** | 53 ± 3** |
| nACSF n = 13 | 1 ± 1 | 7 ± 3 | 15 ± 3 | 22 ± 4 | 27 ± 5 | 27 ± 5 | 27 ± 5 |
| NA | 2 ± 1 | 12 ± 3** | 23 ± 4** | 31 ± 4** | 36 ± 4** | 36 ± 4** | 36 ± 4** |

| Part II | IR (MΩ) | TH (mV) | SAG | mAHP (mV) | ADP (mV) |
|---------|---------|--------|-----|-----------|---------|
| nACSF | 48 105 ± 4 | 51 | −47.9 ± 0.6 | 48 | 0.166 ± 0.01 | 42 | −1.16 ± 0.11 | 42 | −0.27 ± 0.07 |
| Cch | 139 ± 6*** | −48.9 ± 0.7** | 24 | 0.151 ± 0.01 | 42 | −0.26 ± 0.14*** | 42 | 1.24 ± 0.15*** |
| nACSF | 15 90 ± 6 | 9 | −46.4 ± 2.0 | 15 | 0.173 ± 0.01 | 9 | −1.47 ± 0.34 | 9 | −0.40 ± 0.25 |
| NA | 115 ± 8*** | −47.3 ± 1.8 | 42 | 0.115 ± 0.01** | 42 | −1.33 ± 0.27 | 42 | 0.23 ± 0.13* |

Table 2: Effect of NA on top of Cch on general cellular properties

| Part I | 50 pA | 100 pA | 150 pA | 200 pA | 250 pA | 300 pA | 350 pA |
|--------|-------|--------|--------|--------|--------|--------|--------|
| Cch n = 13 | 6 ± 3 | 26 ± 4 | 37 ± 3 | 41 ± 3 | 47 ± 3 | 50 ± 3 | 53 ± 3 |
| Cch + NA | 19 ± 5 | 29 ± 5 | 42 ± 4 | 48 ± 4 | 54 ± 5 | 57 ± 6 | 60 ± 5 |

| Part II | IR (MΩ) | TH (mV) | SAG | mAHP (mV) | ADP (mV) |
|---------|---------|--------|-----|-----------|---------|
| Cch | 192 ± 10* | 142 ± 11 | −51.0 ± 1.6 | 0.147 ± 0.02 | −0.14 ± 0.29 | 1.28 ± 0.26 |
| Cch + NA | 129 ± 10* | −53.7 ± 1.6 | 0.145 ± 0.01 | −0.27 ± 0.22 | 0.84 ± 0.12 |

Part I, Number of spikes elicited by 1-s current steps. Part II, Input resistance (IR), spike threshold (TH), sag ratio (SAG), medium AHP (mAHP), after depolarizing potential (ADP). Values correspond to mean ± SEM.
T_{9} = 1.234, p = 0.252; Fig. 5A,B). As for the adrenergic β receptors, although all β receptors (β1, β2, β3) are coupled to Gα, recent studies pointed out that β2 activates the Gβ in addition to the Gα cascade (Hall, 2004; Schutsky et al., 2011a,b). Therefore, we aimed to activate β1 and β3 receptors without activating β2 by using the general β receptor agonist isoproterenol (ISO; 1 µM) along with the β2 blocker ICI-118,551 (ICI; 1 µM) based on a previous report (Schutsky et al., 2011a). Combination of ISO and ICI significantly inhibited persistent firing (frequency: n = 7, \( T_{9} = 4.634, p = 0.004 \); potential: n = 7, \( T_{9} = 4.488, p = 0.004 \); Fig. 5C,D). In contrast, a sole application of the general β agonist ISO did not affect persistent firing either at 1 µM (frequency: n = 7, \( T_{9} = 1.788, p = 0.124 \); potential: n = 7, \( T_{9} = 0.777, p = 0.467 \)) or at 10 µM (frequency: n = 8, \( T_{9} = 0.008, p = 0.994 \); potential: n = 8, \( T_{9} = 0.729, p = 0.128 \); Fig. 5E,F), indicating that, in line with previous literature, β2 might induce actions that counteract the increase of cAMP. In summary, these results suggest that NA suppresses cholinergically-induced persistent firing via PKA activation downstream of a β1 and/or β3 receptors mediated increase of cAMP.

**Discussion**

Based on the differential modulation of working memory by acetylcholine and NA, we investigated effects of these neuromodulators on the induction and modulation of persistent firing in hippocampal CA1 pyramidal cells in brain slices taken from adult female mice. Results from this study indicate that only the cholinergic agonist Cch, but not NA, is able to support induction of persistent firing. However, when NA was applied in addition to Cch, NA strongly suppressed cholinergically triggered persistent firing. We have further shown that the suppression of persistent firing by NA was not a result of decreased intrinsic excitability or ionotropic synaptic transmissions. In contrast, forskolin, the specific activation of β1 and PKA blockade with Rp-cAMPS indicated that cAMP elevations through the Gα-protein cascade activating PKA caused the suppression of the cholinergic persistent firing.

**Molecular mechanisms underlying suppression of persistent firing**

Persistent firing similar to our study is shown to be supported by intrinsic cellular mechanisms in multiple brain areas involved in working memory (Fraser and MacVicar, 1996; Haj-Dahmane and Andrade, 1998; Egorov et al., 2002; Tahvildari et al., 2008; Navaroli et al., 2012; Jochems and Yoshida, 2013; Knauer et al., 2013). These studies have pointed out that this type of persistent firing is supported by the CAN current, which is believed to be supported by TRPC channels (Reboreda et al., 2011; Zhang et al., 2011; Arboit et al., 2020). In line with our data, the CAN current and TRPC channels are inhibited by the cAMP (Partridge et al., 1990; El-Hassar et al., 2011; Sung et al., 2011). In particular, El-Hassar et al. (2011) used forskolin in the hippocampal CA1 pyramidal cells as in our study to indicate that TRPC mediated CAN current is suppressed. Suppression of similar persistent firing by cAMP was also reported in the entorhinal cortex and PFC (Sidiropoulou et al., 2009; Zhang et al., 2013). Therefore, CAN current suppression is a feasible mechanism for the suppression of persistent firing in our study. However, it is possible that other mechanisms were additionally involved. One possibility could be that an increase in GABAergic input mediated by NA (Kawaguchi and Shindou, 1998; Sessler et al., 1995) suppressed persistent firing. Inhibitory synaptic input is known to be higher in the presence of NA (Kawaguchi and Shindou, 1998; Sessler et al., 1995). Inhibitory synaptic input is effective in terminating persistent firing in the dentate gyrus.
This interpretation was in line with the decreased IR we observed in NA, which could be caused by the opening of GABAergic receptors. To gain insight into this, we used the same SBs as in Zhang et al. (2013), which demonstrated facilitated persistent firing by NA in PFC neurons. However, in the presence of the SBs, IR was still reduced and persistent firing was suppressed by NA. Based on these, we suggest that changes in synaptic transmission were not the main cause of persistent firing suppression in our study.

Another possibility is that the elevation of cAMP has modulated the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. In the PFC, adrenergic stimulation of α2 receptors improved working memory performance by suppressing HCN channels through cAMP downregulation (Wang et al., 2007). This is in line with facilitated persistent firing in the PFC neurons under

Figure 4. Inhibition of PKA by Rp-cAMPS blocks the NA and forskolin mediated reduction of persistent firing. A, Representative traces of the effect of Rp-cAMPS on the NA-mediated inhibition of the cholinergic persistent firing. B, Poststimulus frequency (left) and poststimulus potential (right) comparison with two-way repeated-measures ANOVA followed by Tukey’s post hoc test. The first two columns show measurements without Rp-cAMPS from previous experiments. C, Representative traces of the effect of Rp-cAMPS on the forskolin-mediated inhibition of the cholinergic persistent firing. D, Poststimulus frequency (left) and poststimulus potential (right) comparison with two-way repeated-measures ANOVA followed by Tukey’s post hoc test.
the blockade of HCN channels (Zhang et al., 2013). This mechanism might, however, not be involved in the suppression of persistent firing in our study because the sag potential, which indicates the amount of HCN current, was rather reduced by NA, suggesting a reduction, if any, of HCN current. In addition, a receptor agonist clonidine did not reduce the sag potential in our study. This could be because (1) the cAMP levels were already low because of Cch, and a2 activation could not further reduce the cAMP level; (2) modulation of HCN channels by cAMP is pronounced in young but is weak in adult animals in the hippocampus (Surges et al., 2006); and (3) the a2 receptor does not modulate the HCN channels because co-localization of the a2 receptor with the HCN channels, which is reported in the PFC (Wang et al., 2007), does not occur in the hippocampal CA1 pyramidal cells.

In addition, we cannot completely exclude the possibility that mGluR is involved in the suppression of persistent firing by NA. Similarly to Cch, mGluRs activate the Gq (group 1) and Gi (groups 2 and 3) pathways. When Cch is not present, NA-induced mGluR activation could play a role in modulating persistent firing as was the case in Zhang et al. (2013). However, in the presence of Cch as in our study, G1 and Gq are already highly activated, and an additional activation of the same G1 and Gq through mGluRs, if exists, could be marginal. This point has been tested by Zhang et al. (2013) by applying the mGluR blocker MPEP on persistent firing induced by Cch, and they showed no change of persistent firing, indicating that the role of mGluR in persistent firing is limited when Cch is present. In contrast, when mGluRs are the main activator of the Gq and Gi pathways, NA may have a similar suppressive effect on persistent firing in the hippocampus. This view is supported by the suppression of mGluR-induced “plateau potential” and persistent firing by cAMP and PKA in the hippocampus and PFC (Sidiropoulou et al., 2009; El-Hassar et al., 2011).

Figure 5. Roles of NA receptor subtypes in suppression of persistent firing. A, Representative traces of the effect of clonidine on the cholinergic persistent firing. B, Poststimulus frequency (left) and poststimulus potential (right) in Cch (gray) and Cch and clonidine (white). C, Representative traces of the effect of β2 (inhibited by ICI) versus β1 and β3 adrenergic receptors (activated by isoproterenol in the presence of ICI) on the cholinergic persistent firing. D, Poststimulus frequency (left) and poststimulus potential (right) in a cocktail of Cch and ICI (gray) and in a cocktail of Cch, ICI and ISO (white). E, Representative traces of the effect of β-receptor stimulation by isoproterenol. F, Poststimulus frequency (left) and poststimulus potential (right) in (gray) and Cch and ISO (white).

Suppressed persistent firing as a possible cellular mechanism of impaired working memory in high NA levels

Roles of cholinergic receptor activation on persistent firing supported by intrinsic cellular mechanisms have been studied intensively in vitro (for review, see Major and Tank, 2004; Yoshida et al., 2012). Similarly to these prior studies, persistent firing was observed under cholinergic receptor activation in our experiment in hippocampal pyramidal CA1 cells in mice. This is in line with the supportive role of cholinergic activation on working memory (Kaneko and Thompson, 1997; Weiss et al., 2000). In addition, we have shown that cholinergically-induced persistent firing is suppressed by NA. In line with these
results are the detrimental effects of high concentrations of NA on working memory (Arnsten, 2009; Roozendaal and McGaugh, 2011). Together, these observations indicate that both supportive (cholinergic) and detrimental (NA) modulations on working memory are in agreement with the cellular level modulation of PF, which is a possible cellular correlate of working memory.

Interestingly, persistent firing in our study was suppressed by β1/3 receptors activation (Fig. 5D), while the use of general β receptor agonist did not alter the response (Fig. 5E), suggesting that receptor subtypes have different effects on persistent firing. Traditionally, it has been believed that the actions of different β receptors were identical, all activating the Gs cascade. However, recent studies have suggested that β2 receptors might act through different mechanisms than β1, possibly by activating the Gi cascade in addition to the Gs cascade (Hall, 2004; Schutsky et al., 2011a,b). Since Gi cascade counteracts the action of Gs cascade by decreasing cAMP levels, unclear effect of the general β receptor agonist (Fig. 5E) could have been resulted from this action of β2 receptors. Interestingly, the role of β receptors in working memory was tested using generic agonists or antagonists, resulting in no apparent effect on this cognitive function (Arnsten et al., 1999). On the other hand, the specific activation of β1 receptors has been shown to impair working memory, while the activation of β2 has improved it (Ramos et al., 2005, 2008). Therefore, modulations of persistent firing and working memory agree with each other at the level of receptor subtypes in this case.

Our data using Rp-cAMPS and forskolin further suggested that the suppression of persistent firing was through cAMP upregulation, which can be induced by the activation of the Gs cascade. Interestingly, multiple studies have indicated that increased cAMP levels impair working memory, while a reduction of cAMP levels improves it (Taylor et al., 1999; Ramos et al., 2003; Runyan et al., 2005). Moreover, increased cAMP reduced persistent firing in vivo during working memory tasks (Vijayraghavan et al., 2007; Wang et al., 2007). It is relevant to note that Rp-cAMPS has been tested in vivo in monkeys during an oculomotor delayed response working memory task (Vijayraghavan et al., 2007). In this study, Rp-cAMPS rescued D1 receptor mediated reduction of neural firing while an application of Rp-cAMPS alone increased the neuronal firing (Vijayraghavan et al., 2007). The rescue effect of Rp-cAMPS is in line with the suppressive roles of cAMP on persistent firing observed in our study. In addition, increased neural activity by the application of Rp-cAMPS in vivo is in line with the increased depolarization during persistent firing we observed in our study (Fig. 4B). In summary, Gs-mediated cAMP upregulation seems to be detrimental for both working memory and persistent firing, while Gd-mediated cAMP downregulation seems to support both working memory and persistent firing.

Supportive role of moderate levels of NA on working memory

In contrast to the detrimental effect of high levels of NA, it has been proposed that moderate concentrations of NA aid working memory through the α2 receptors because of relatively high affinity of NA to the α2 receptors (Arnsten and Li, 2005). In fact, working memory is facilitated by the α2 receptor activation in several studies (Järkäla et al., 1999; Li et al., 1999; Birnbaum et al., 2000; Ma et al., 2003; Arnsten and Li, 2005). In the present study, we tested two doses of NA (5 and 10 μM); however, we did not observe facilitative effect of NA on persistent firing either with or without cholinergic agonist Cch. The lack of facilitative effect of NA on persistent firing could be because the two concentrations tested were both high doses equivalent to situations of stress, where β1/3 receptors were active in addition to the α2 receptors, masking the positive effect of the α2 receptors. However, our experiment with the α2 receptor agonist indicates that there is no room for facilitation of persistent firing through α2 receptor activation at least in the presence of cholinergic agonist Cch. The lack of facilitative effect of NA on working memory could be because the two concentrations tested were both high doses equivalent to situations of stress, where β1/3 receptors were active in addition to the α2 receptors, masking the positive effect of the α2 receptors. However, our experiment with the α2 receptor agonist indicates that there is no room for facilitation of persistent firing through α2 receptor activation at least in the presence of cholinergic agonist Cch (Arnsten and Li, 2005). In the present study, we tested two doses of NA (5 and 10 μM); however, we did not observe facilitative effect of NA on persistent firing either with or without cholinergic agonist Cch. The lack of facilitative effect of NA on persistent firing could be because the two concentrations tested were both high doses equivalent to situations of stress, where β1/3 receptors were active in addition to the α2 receptors, masking the positive effect of the α2 receptors. However, our experiment with the α2 receptor agonist indicates that there is no room for facilitation of persistent firing through α2 receptor activation at least in the presence of cholinergic agonist Cch. The lack of facilitative effect of NA on working memory could be because the two concentrations tested were both high doses equivalent to situations of stress, where β1/3 receptors were active in addition to the α2 receptors, masking the positive effect of the α2 receptors. However, our experiment with the α2 receptor agonist indicates that there is no room for facilitation of persistent firing through α2 receptor activation at least in the presence of cholinergic agonist Cch.
and traumatic brain injury in addition to high stress conditions (Amstren, 2011; Wang et al., 2011; Kobori et al., 2015). In support of this view, knocking down the disrupted in schizophrenia 1 (DISC1) protein (a protein that participates in cAMP catalombism) results in a decrease of the TRPC current in rat PFC neurons (El-Hassar et al., 2014). In addition, an infusion of Rp-cAMPs in traumatic brain injury or aged rats with working memory deficits, improves the performance on those tasks (Ramos et al., 2003; Kobori et al., 2015). Therefore, the mechanism of suppression of intrinsic persistent firing we present here might be relevant to working memory impairment in different conditions such as aging, traumatic brain injury and schizophrenia as well.

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