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Activation of Epac2 improves A β -induced impairment of memory retrieval in an acute model of Alzheimer's disease

Tong Zhang^{a,b}, Yuequ Zhang^c, Pascal Chameau^e, Tingting Chen^c, Alejandro Marmolejo-Garza^c, Wanda Douwenga^b, Amalia M. Dolga^b, Helmut W. Kessels^e, Martina Schmidt^{c,d}, Ulrich L.M. Eisel^{b,*}

^a Department of Biopharmaceuticals and Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics, School of Pharmacy, Tianjin Medical University, Tianjin, China

^b Department of Molecular Neurobiology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, the Netherlands

^c Department of Molecular Pharmacology, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, the Netherlands

^d Groningen Research Institute for Asthma and COPD, GRIAC, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

^e Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, the Netherlands

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ABSTRACT

Impaired memory retrieval is one of the cognitive markers in the early stage of Alzheimer's Disease (AD). Previous studies report that exchange protein directly activated by cAMP 2 (Epac2) plays a specific and time-limited role in promoting memory retrieval. In this study, we investigated the effect of a novel Epac2 activator, S220, on neuronal and synaptic activities, and memory impairment in an acute AD mouse model. S220 treatment increased the firing rate of action potential and intracellular calcium in primary neuronal cultures. Moreover, S220 treatment increased synaptic currents in CA1 neurons. In the acute AD mouse model, intra-hippocampal injection of amyloid- β (A β) oligomers impaired memory performance. Notably, administering S220 20 min before retention of contextual fear conditioning recovered the A β -induced memory impairment, suggesting an enhancing effect on memory retrieval. Collectively, our data demonstrate that the novel Epac2 activator S220 promotes synaptic communication and neuronal firing, and thereby improves A β -induced memory impairment via enhancing memory retrieval, indicating the role of Epac2 as a potential treatment target for AD.

1. Introduction

Impaired episodic memory appears to be one of the earliest cognitive symptoms in Alzheimer's disease (AD) and closely correlated with amyloid beta (A β) accumulation (Villeneuve et al., 2014; Bischof et al., 2016; Burnham et al., 2016). Some studies suggested that formation of episodic memory was impaired in the early stage of AD (Kaushik et al., 2022; Lopez-Perez et al., 2023), whereas other studies observed declined memory retrieval in the early AD (Tromp et al., 2015). In transgenic AD mouse models, memory retrieval, rather than memory formation, appears to be primarily impaired, since optogenetic activation of the hippocampal memory engram restores the expression of memory responses (Roy et al., 2016; Etter et al., 2019). Therefore, in addition to direct clearance of A β pathology, enhancing memory retrieval may provide a novel therapeutic strategy to improve A β -induced memory deficits in the

early stage of AD.

A signaling protein that has been implicated in promoting memory retrieval is the exchange protein directly activated by cAMP (Epac) (Ostroveanu et al., 2010; Ouyang et al., 2008), a cAMP-regulated guanine exchange factor for the small G proteins (Gloerich and Bos, 2010). Our group previously reported that pharmacological activation of Epac enhanced hippocampus-dependent memory retrieval rather than the acquisition or consolidation of memories in mice (Ostroveanu et al., 2010). Activation of Epac by cAMP appears to affect synaptic plasticity. Epac activation can increase basal synaptic transmission pre-synaptically via increasing release probability (Gekel and Neher, 2008; Fernandes et al., 2015) and/or post-synaptically by increasing AMPA-receptor (AMPA) currents (Gutierrez-Castellanos et al., 2017). In addition, Epac activation facilitates maintenance of long-term potentiation (LTP) (Gelinas et al., 2008; Maity et al., 2020; Brandwein

* Corresponding author. P.O. Box 11103, NL-9700 CC, Groningen, the Netherlands.

E-mail address: u.l.m.eisel@rug.nl (U.L.M. Eisel).

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and Nguyen, 2019). Reversely, the enhancement of LTP by norepinephrine (NE) through a rise in cAMP upon activation of β -adrenergic receptors was blocked by Epac inhibition (Maity et al., 2020). This NE-driven synaptic plasticity through activation of β -adrenergic receptors is considered as a cellular event closely related to memory retrieval (Murchison et al., 2004). In line with this, hippocampal

activation of Epac and protein kinase A (PKA), two main cAMP effectors, synergically facilitated memory retrieval in NE/epinephrine (NE/E) deficient mice (Ouyang et al., 2008).

Epac has two isoforms: Epac1 and Epac2. Epac1 is ubiquitously expressed in all tissues, while Epac2 is specially expressed in central nervous system (CNS) and thought to be the real player in mediating

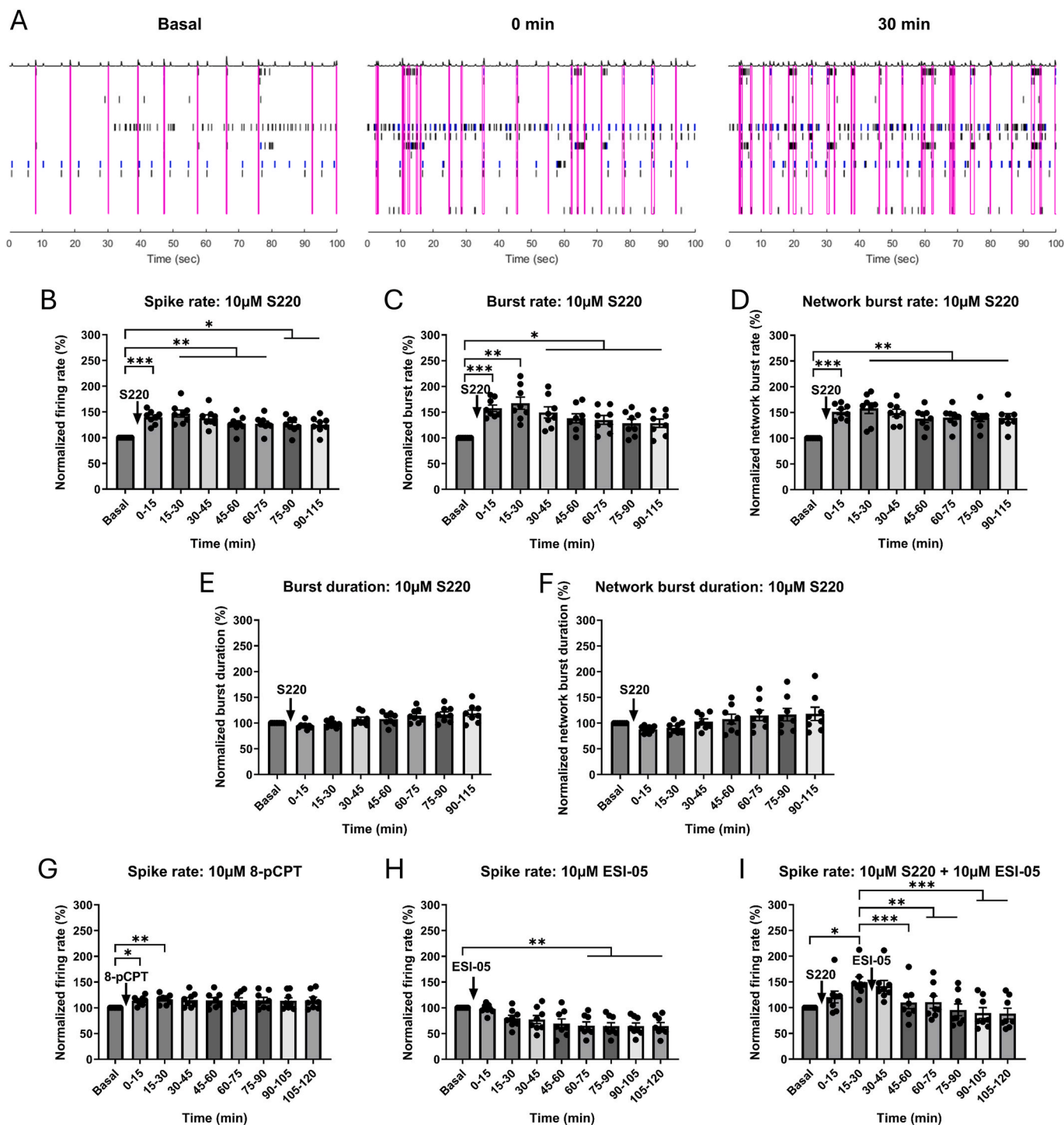


Fig. 1. Epac2 activator S220 increases frequency of action potential. (A) Representative raster plots before (left), immediately after (middle) and 30 min after (right) S220 treatment. Black lines: spikes; Blue lines: bursts; Purple lines: network bursts. (B–D) Application of 10 μ M S220 increased spike rates, burst rates and network burst rates ($n = 8$, one-way ANOVA). (E–F) Application of 10 μ M S220 did not affect burst duration nor network burst duration ($n = 8$, one-way ANOVA). (G) Application of 10 μ M 8-pCPT increased spike rates ($n = 8$, one-way ANOVA). (H) Application of 10 μ M ESI-05 decreased spike rates ($n = 8$, one-way ANOVA). (I) Application of 10 μ M ESI-05 reversed the increased spike rates induced by 10 μ M S220 ($n = 8$, one-way ANOVA). Data are expressed as mean \pm SEM. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

synaptic plasticity and memory (Schmidt et al., 2013). Previous studies used an Epac activator 8-pCPT, which exhibits higher selectivity for Epac1 over Epac2 (Courilleau et al., 2013; Reverte-Salisa et al., 2024), due to the lack of specific Epac2 modulators (Zhang et al., 2023a). However, Epac2 knockout models have shown that it is Epac2 that triggers synaptic potentiation in hippocampal neurons upon a rise in cAMP (Fernandes et al., 2015). Furthermore, the use of combination of 8-pCPT and Epac2 siRNA specifically impaired memory retrieval, suggesting it is Epac2, rather than Epac1, promotes a specific enhancement in memory retrieval (Ostroveanu et al., 2010). In AD, hippocampal expression of Epac2 protein was decreased in postmortem patients and J20 mice, while Epac1 was not altered (Zhang et al., 2023b). However, it is unknown whether the downregulation of Epac2 is responsible for the A β -mediated deficit in memory retrieval.

We hypothesize that Epac2 activation may counteract the loss of expression and/or function of Epac2 and its related proteins, thereby improving cognitive deficits in the early stage of AD (Zhang et al., 2023a). To be able to manipulate memory retrieval pharmacologically, a drug is needed that selectively activates Epac2. Using a structure-guided approach, a novel potent activator of Epac2 was developed, named S220 (Schwede et al., 2015). S220 has a 10 times higher affinity for Epac2 than cAMP ($AC_{50} = 0.1 \mu\text{M}$, $k_{\text{max}} = 7.7$) with very little or almost no effect on Epac1 or PKA (Schwede et al., 2015). In this study, we tested the effects of S220 on neuronal firing and synaptic function, and further investigated its effect on the A β -impaired memory in an acute AD mouse model.

2. Results

2.1. Epac2 activator S220 increases frequency of action potential in primary neuronal cultures

To assess the effect of the Epac2 specific activator S220 on action potential (AP) firing in a population of neurons, a microelectrode array (MEA) was used on two-week-old primary cortical cultures. Application of $10 \mu\text{M}$ S220 to these neuronal cultures induced an increase in firing rates ($F(2.257, 15.80) = 26.99$, $DF = 7$) (Fig. 1A and B). In addition, we also observed that compared to the control group, neurons produced more burst of spikes (burst rates) ($F(1.784, 12.49) = 24.89$, $DF = 7$) (Fig. 1C) and an increased incidence of synchronous spiking of neurons (network burst rates) ($F(2.218, 15.53) = 20.37$, $DF = 7$) (Fig. 1D), without affecting burst duration ($F(1.659, 11.62) = 10.44$, $DF = 7$) and network burst duration ($F(1.174, 8.219) = 4.624$, $DF = 7$) (Fig. 1E and F). This S220-induced increase in firing peaked at 30 min and was maintained for at least 1 h (Fig. 1B and D). In contrast, application of $10 \mu\text{M}$ 8-pCPT, a less potent activator for Epac2 ($AC_{50} = 3.5 \mu\text{M}$, $k_{\text{max}} = 0.8$) (Schwede et al., 2015), led to a small increase by $\sim 20\%$ compared to the basal condition ($F(1.679, 11.76) = 3.412$, $DF = 8$) (Fig. 1G). Treatment with $10 \mu\text{M}$ ESI-05, a specific Epac2 inhibitor, exhibited a delayed effect 15 min after application and significantly reduced the firing rate to $\sim 70\%$ of the basal level ($F(1.470, 10.29) = 20.89$, $DF = 8$), suggesting a basal Epac2 activity (Fig. 1H). Furthermore, treatment with $10 \mu\text{M}$ ESI-05 30 min after application of $10 \mu\text{M}$ S220 significantly reversed the S220-induced increase to baseline levels ($F(2.148, 15.04) = 17.74$, $DF = 8$) (Fig. 1D). In addition, we note that $10 \mu\text{M}$ S220 did not activate PKA via measuring pan-PKA substrates (Fig. S1A), suggesting S220 selectively activated Epac2 in these experiments. These results together indicate a specific role of Epac2 in promoting AP firing in primary neuronal cultures.

2.2. Epac2 activator S220 increases intracellular calcium in primary neuronal cultures

To visualize the effect of S220 on neuronal activity in a population of primary cortical neurons (PCNs), imaging of intracellular calcium ($[Ca^{2+}]_i$) by Fluo-4 AM staining (Koshiya and Smith, 1999) was

compared with the recording of MEA. After a 2-min baseline measurement, treatment with $10 \mu\text{M}$ S220 led to a continuous increase of $[Ca^{2+}]_i$ in the PCN population which lasted for a period of 28 min (Fig. 2A). Furthermore, this S220 treatment resulted in a significant elevation of $[Ca^{2+}]_i$ in PCNs compared to the basal condition observed at 2 min ($T = 22.20$, $DF = 10$) (Fig. 2B).

2.3. Epac2 activator S220 promotes synaptic basal neurotransmission

To examine the effect of S220 on synaptic currents, we performed whole-cell recording in cornu ammonis 1 (CA1) neurons from acutely isolated brain slices of C57BL/6J mice by measuring miniature excitatory post-synaptic currents (mEPSCs). CA1 neurons exposure to $50 \mu\text{M}$ S220 showed a significantly increased frequency of mEPSC events ($p = 0.0016$, Fig. 3A and B). This result indicates that activation of Epac2 leads to synaptic potentiation in CA1 neurons. S220 application also resulted in a higher mEPSC amplitude on average, although did not reach statistical significance ($p = 0.087$, Fig. 3A and C). In addition, the distribution of mEPSC amplitudes was not significantly changed by S220 ($p = 0.38$), suggesting that the effect of Epac2 activation was similar for both small and large synapses.

2.4. Epac2 activator S220 improves A β -induced memory deficits

To establish an *in vivo* model for studying acute effect of A β oligomers on memory, a double guided canulae was implanted towards the hippocampal CA1 area of C57BL/6J mice (Fig. 4A). The implantation of canulae did not affect the performance of mice in the EPM or in the Y-maze, indicating that the surgical procedures did not perturb anxiety or working memory respectively (Figs. S2A and B). The location of the canulae was confirmed by follow-up DAPI staining (Fig. S2C). As a pilot experiment to test at which concentration intrahippocampal (IH) injection of A β oligomers induce acute memory deficits, 15 pmol or 30 pmol of human A β_{42} oligomers, or PBS as a control, was administered 1 h before exposing the mice to a contextual fear conditioning (CFC) paradigm (Fig. 4B). Based on this experiment, we chose IH injection of 30 pmol of A β oligomers as the dose for examining their effects on Epac2 ($F(2, 18) = 2.712$, $DF = 2$). According to the previous study that 300 ng per brain of 8-pCPT enhanced the memory retrieval (Ostroveanu et al., 2010), we opted to administer an equivalent dose of 300 ng per brain for S220. Furthermore, immunohistochemistry was used to detect whether Epac2 expression was affected by A β oligomers and/or S220 in the hippocampus. A β oligomers resulted in decreased expression of Epac2 in the pyramidal cell layers of CA1 and granule cell layers of dentate gyrus (DG), compared with the PBS group (Fig. S3A-B and D), but not affected the Epac2 expression in the pyramidal cell layers of CA3 areas (Fig. S3C). In contrast, S220 did not alter Epac2 expression levels, not by itself and not when S220 was administered 24 h following A β application (Figs. S3A-D).

We next assessed the capacity of Epac2 activation on fear responses triggered by contextual fear conditioning (CFC). To investigate the effect of specific activator S220 on memory acquisition, consolidation and retrieval in A β -treated mice, a single dose of S220 (300 ng) was administered at 3 distinct time points: 20 min before training, immediately after training or 20 min before retention of CFC (Fig. 4A). When S220 was administered 20 min prior to training, the A β -induced memory deficits were not improved compared with the A β alone group ($F(3, 28) = 4.457$, $DF = 3$) (Fig. 4C). Similarly, the S220 injection immediately after training did not rescue the A β -induced memory deficits ($F(3, 28) = 6.768$, $DF = 3$) (Fig. 4D). In contrast, administering S220 20 min before retention significantly reversed the A β -induced memory impairment ($F(3, 30) = 7.753$, $DF = 3$) (Fig. 4E). Taken together, these data suggest that hippocampal Epac2 activation improves the A β -induced impairment in fear contextual memory via enhancing memory retrieval.

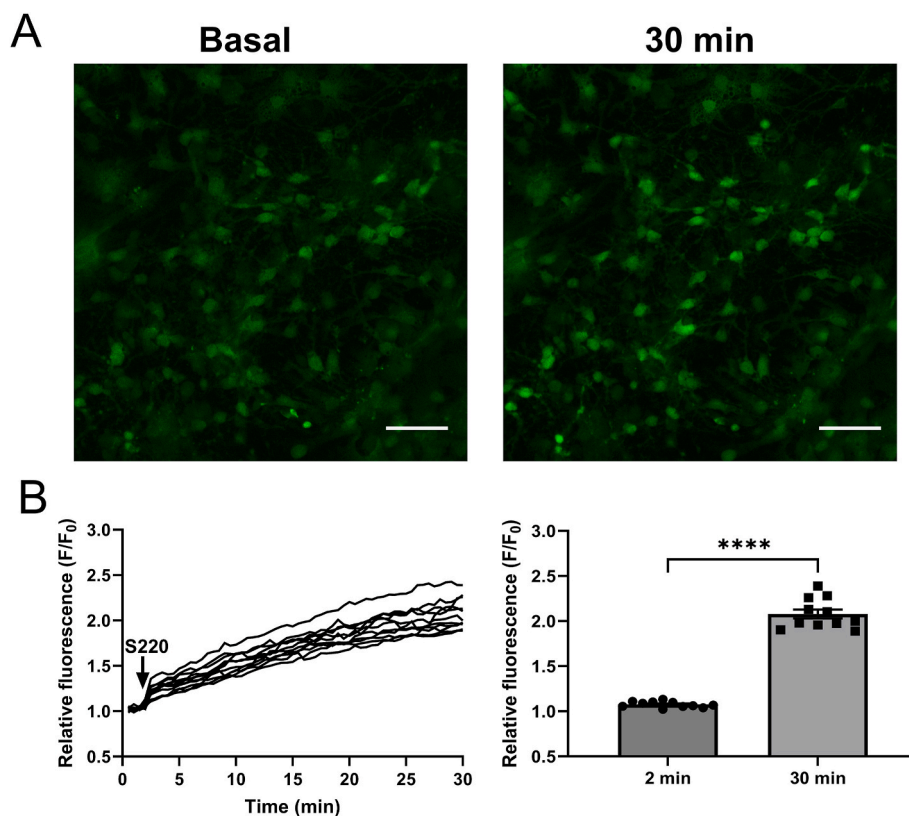


Fig. 2. Epac2 activator S220 increases intracellular calcium ($[Ca^{2+}]_i$) in primary neuronal cultures. (A) Representative images of S220 application in primary neuronal cultures. Scale bar, 50 μ m. (B) Application of S220 led to a continuous elevation in $[Ca^{2+}]_i$ (left) and a significant increase of $[Ca^{2+}]_i$ (right) in primary neurons ($n = 11$, paired T-test). Each graph showed at least 10 representative changes of $[Ca^{2+}]_i$ within single cells. Data are expressed as mean \pm SEM. **** $p \leq 0.0001$.

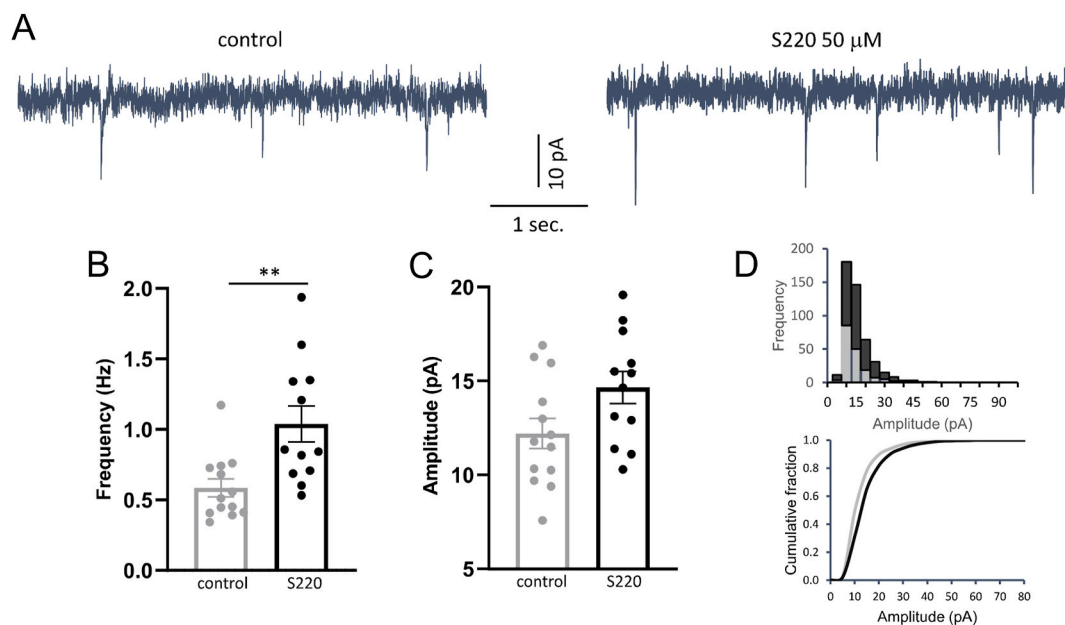


Fig. 3. Epac2 activator S220 promotes synaptic basal neurotransmission in CA1 neurons. (A) Typical examples of mEPSC recordings in control conditions (left) and in the presence of 50 μ M S220 (right). (B) Average absolute amplitude of mEPSCs in control conditions (12.21 ± 0.79 pA, $n = 13$) and in the presence of S220 (14.65 ± 0.85 pA, $n = 12$) (Control, $n = 13$; S220 group, $n = 12$, Mann-Whitney test). (C) Average absolute amplitude of mEPSCs in control conditions (12.21 ± 0.79 pA, $n = 13$) and in the presence of S220 (14.65 ± 0.85 pA, $n = 12$) (Control, $n = 13$; S220 group, $n = 12$, Mann-Whitney test). (D) Histogram distribution (top) and cumulative distribution (bottom) of absolute amplitude of mEPSCs in control conditions (light grey) and in the presence of S220 (dark grey) (Kolmogorov-Smirnov test $p = 0.38$). Data are expressed as mean \pm SEM. ** $p \leq 0.01$.

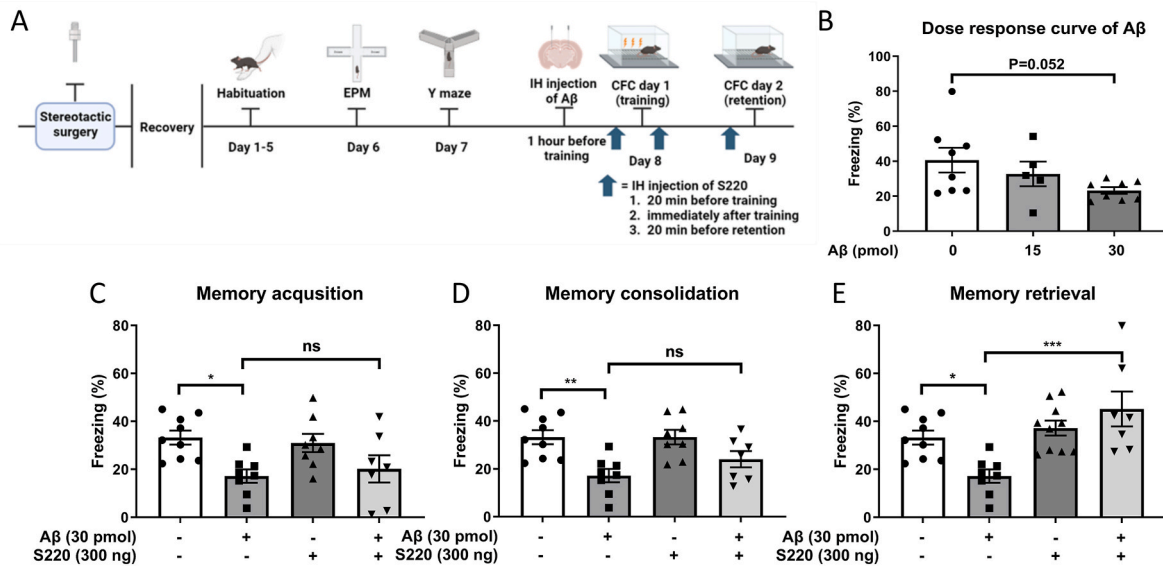


Fig. 4. Epac2 activator S220 improves the A β -induced memory deficits in an *in vivo* AD model. (A) A schematic timeline for animal experiments. (B) Dose response of A β -induced memory impairment. The mice were respectively injected with PBS (n = 8), 15 pmol of A β (n = 5) or 30 pmol of A β (n = 8, one-way ANOVA). (C–E) S220 application during memory retrieval (PBS + PBS, n = 9; A β +PBS, n = 8; PBS + S220, n = 10; A β +S220, n = 7; one-way ANOVA), rather than memory acquisition (PBS + PBS, n = 9; A β +PBS, n = 8; PBS + S220, n = 8; A β +S220, n = 7; one-way ANOVA) or consolidation (PBS + PBS, n = 9; A β +PBS, n = 8; PBS + S220, n = 8; A β +S220, n = 7; one-way ANOVA) improved the A β -induced memory impairment. Data are expressed as mean \pm SEM. *p \leq 0.05, **p \leq 0.01, ***p \leq 0.001.

3. Discussion

In the present study, we demonstrate that specific activation of Epac2 by S220 promotes neuronal/synaptic activities and enhances impaired memory retrieval in an acute AD model. A recent study reveals that the memory deficits in the early stage of AD primarily result from impaired retrieval (Roy et al., 2016), resolving earlier controversies over which memory stage was affected (Kaushik et al., 2022; Lopez-Perez et al., 2023; Tromp et al., 2015). In our *in vivo* AD model, we have shown that specific activation of Epac2 can rescue fear memory deficits. However, Epac2 activation only restores the A β -induced fear memory responses when applied immediately before re-exposure to the fear context, suggesting functional memory acquisition and consolidation, but impaired memory retrieval. Therefore, this *in vivo* AD model with the impaired memory retrieval is reflective of the early stage of AD pathology.

Studies by Roy et al. and Etter et al. have demonstrated that optogenetic activation of hippocampal engram cells can restore the impaired memory retrieval in AD (Roy et al., 2016; Etter et al., 2019). To our knowledge, our study is the first to employ pharmacological modulation to rescue impaired retrieval in AD. Our previous study reported that the enhanced memory retrieval induced by the Epac activator 8-pCPT was reversed by intrahippocampal injection of Epac2 siRNA, suggesting that specific Epac2 activation in the hippocampus improves memory retrieval (Ostroveanu et al., 2010). In line with previous studies (Ostroveanu et al., 2010; Ouyang et al., 2008), our result firstly reveals that specific activation of Epac2 by S220 enhances memory retrieval, rather than memory acquisition or consolidation, thereby counterbalancing the A β -induced memory deficit. Additionally, in contrast to 8-pCPT in previous researches (Zhang et al., 2023a), we use the specific Epac2 activator S220, with higher selectivity, potency and efficacy (Schwede et al., 2015), which achieves a more pronounced enhancement of memory retrieval. Therefore, our results not only reveal solid evidence for the specific role of Epac2 in memory retrieval, but also indicate S220 as a potential candidate for therapeutic intervention in patients in the early stage of AD.

APs are essential cellular events underlying memory retrieval. A previous study reported that application of 8-pCPT altered the AP associated sodium current in PCNs (Gekel and Neher, 2008). In line with

this study, our MEA results show that 8-pCPT induces an approximately 20% increase in AP rates in PCNs, while the same concentration of S220 results in higher AP rates. Notably, we observe the AP rates reach plateau effect at approximately 30 min after S220 application, which aligns with the timing of the enhanced memory retrieval in the *in vivo* AD model. The S220-induced increase in AP further promotes burst rates and network burst rates, potentially enhancing synaptic plasticity and neuronal synchronization (Bean, 2007; Zeldenrust et al., 2018). Conversely, Epac2 inhibition by ESI-05 decreases the AP rates below the basal condition, indicating the role of Epac2 in basal neuronal activity. The increased AP by S220 and subsequent decreased AP by ESI-05 strongly suggests the effect of S220 is Epac2 specific.

Intracellular calcium is implicated in neuronal activity and plasticity (Grienberger and Konnerth, 2012). Studies reported that Epac regulated Ca²⁺ influx in several tissues, such as cardiac myocytes (Ruiz-Hurtado et al., 2013) and pancreatic β -cells (Kang et al., 2003). In this study, we observe S220 treatment induces a continuous increase in [Ca²⁺]_i in PCNs, suggesting increased neuronal activity. Intriguingly, the [Ca²⁺]_i pattern induced by S220 differs from that induced by glutamate alone (Dolga et al., 2011), suggesting a different mechanism from pre-synaptic neurotransmitter release. This S220-induced increase in [Ca²⁺]_i suggests the postsynaptic calcium channels involved in synaptic plasticity, such as NMDARs and calcium permeable AMPARs (CP-AMPA) (Grienberger and Konnerth, 2012), which needs further investigation.

mEPSC is a key parameter in synaptic plasticity, reflecting the postsynaptic response to neurotransmitter release. In line with previous studies (Gekel and Neher, 2008), we found that Epac2 activation primarily affects mEPSC frequency with an increasing trend on average mEPSC amplitude. This result may indicate that Epac2 activation predominantly has a pre-synaptic effect on excitatory synapses, where it enhances the probability of vesicle release (Gekel and Neher, 2008; Fernandes et al., 2015). However, we cannot exclude the possibility that Epac2 activation also has a post-synaptic effect. Previous studies have shown that Epac can activate the channels of GluA3-containing AMPA-receptors at synapses, and this GluA3-dependent form of post-synaptic plasticity is predominantly expressed by an increase in mEPSC frequency and to a lesser extent mEPSC amplitude (Gutierrez-Castellanos et al., 2017; Reinders et al., 2016). Notably, A β oligomers can weaken synaptic transmission by perturbing pre-synaptic

vesicle release (Barthet and Mulle, 2020), and by removing GluA3-containing from the post-synaptic membrane (Reinders et al., 2016; Alfonso et al., 2014). We therefore propose that Epac2 activation could potentiate both pre- and post-synaptic mechanisms, thereby restoring both pre- and post-synaptic mechanisms that are perturbed by A β oligomers.

In addition, we found that 14-days intraperitoneal (IP) administration of S220 could not improve the memory deficits in 7–8 month old J20 mice (Fig. S4E). Preliminary high-performance liquid chromatography (HPLC) data indicate that S220 could be detected in very low concentrations (~0.2–0.3 μ M) in lyophilized brain tissues of mice sacrificed 3 days after 14-day IP administration of S220 (unpublished data), suggesting S220 is likely to cross blood brain barrier. However, it is not clear it is sufficient compensating memory deficits in J20 mice. A potential explanation could also be that memory deficits in J20 mice is not only due to impaired memory retrieval but also to disturbances in memory acquisition and/or consolidation.

In conclusion, we reveal that the activation of Epac2 by S220 promotes electrophysiological and neuronal activities including action potential, intracellular calcium level and mEPSCs, thereby contributing to memory function. Furthermore, our data provides strong evidence that specific activation of Epac2 is effective in ameliorating the acute memory impairment induced by A β . Compared to previous studies that have focused on optogenetic intervention, our study pioneers a pharmacological strategy and provides a translational basis on targeting memory retrieval for alleviating the early cognitive symptoms in AD. This study suggests that Epac2 could represent a promising therapeutic target for impaired memory retrieval in the early stage of AD or diseases where memory deficits are related to memory retrieval.

4. Experimental procedures

4.1. Primary cortical neuron preparation

Primary cortical neurons (PCNs) were generated from E14 embryos of C57BL/6J mice. The isolation was performed as previously described (Granic et al., 2010; Zhang et al., 2024; Marmolejo-Garza et al., 2023; Chen et al., 2023). Briefly, the cortices were carefully separated from meninges, followed by mechanical dissociation. The PCNs were seeded on plates coated by 0.5 % polyethylenimine (PEI) and cultured in Neurobasal medium (Gibco, #2508186) supplemented with 2 % B27 supplement (Gibco, #17504044), 2 mM L-glutamine (Gibco, #25030149) and penicillin-streptomycin (1 ml in 500 ml culture medium, 10,000 U/ml, Gibco, #15070063). After 7- or 14-days culture, the neuronal cultures were ready to use.

4.2. Microelectrode array

PCNs were seeded in the medium with 10 μ g/ml of laminin (Sigma, #3400-010-02) on 48-well Microelectrode array (MEA) plates (Axion Biosystems, M768-tMEA-48W) that were coated with polyethylenimine (PEI). Two weeks old PCNs without CAR treatment were used to perform MEA (Zhang et al., 2024). MEA was recorded by a Maestro multi-well MEA recorder (Axion Biosystems) and Axion AxIS Software. The detection of bursts was defined as minimum 5 spikes on an electrode, each separated by an inter-spike interval of less than 0.1 s (Chiappalone et al., 2005). The detection of network bursts was defined as a collection of at least 10 spikes across all electrodes in the well, each separated by an inter-spike interval of less than 0.1 s with at least 25 % of electrodes participating in the burst (Bakkum et al., 2013).

4.3. Fluo-4 AM staining

One week old PCNs were seeded in 8-well ibidi plates and used to perform Fluo-4 staining. The PCNs were washed by 100ul of HBSS (Gibco, #14065056), followed by incubation with 100 μ l of 3 μ M Fluo-4

AM (reconstituted in DMSO with 0.01 % Pluronic F-127, Invitrogen, #F14201) for 20 min. Subsequently, Fluo-4 AM was removed and PCNs were loaded with 270 μ l of HBSS. During live imaging, compounds were added after recording of the basal condition for 2 min. Images were taken by Nikon ECLIPSE Ti2 microscope with NIS elements AR software at 20x magnification. For analysis, as previously described (Ohata et al., 2001), the intensity of Fluo-4 of soma was quantified. Subsequently, the intensity was subtracted by background fluorescence, followed by being divided by the average of resting fluorescence intensity (F/F_0) before compound application.

4.4. Electrophysiology

During recording slices were kept submerged at 28–30 °C and were continuously superfused with either ACSF (control conditions) or ACSF+ 50 μ M S220 (Epac2 activator). Glass recording pipettes were pulled from borosilicate glass (Science Products) and had a resistance of 2–3 M Ω when filled with intracellular pipette solution (in mM: 115 CsMeSO₃, 20 CsCl, 10 HEPES, 2.5 MgCl₂, 4 Na₂-ATP, 0.4 Na-GTP, 10 Na-Phosphocreatine and 0.6 EGTA pH = 7.2). Whole-cell voltage clamp recordings were performed on the soma of CA1 pyramidal neurons of the hippocampus. Signals were acquired using a MultiClamp 700B amplifier controlled by Pclamp software (Molecular Devices). Signals were low-pass filtered at 4 kHz and sampled at 10 kHz. Series resistance ranged from 6 to 15 M Ω and was compensated to ~55 %. Data analysis was performed with Clampfit (Pclamp software, Molecular Devices) using the template search function. Slices were incubated in ACSF at room temperature in the presence of 50 μ M S220 at least 1 h prior to electrophysiological recordings and recordings were performed in the continuous presence of 50 μ M S220. Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, La Jolla). The mEPSC average amplitude and average frequency were analyzed using the non-parametric Mann-Whitney test and the cumulative fraction distribution was analyzed using the Kolmogorov-Smirnov test. Statistical significance was set as follows * $p < 0.05$; ** $p < 0.01$. Data are represented as mean \pm SEM.

4.5. Animals

Four-six months old male C57BL/6J mice were used in this study. The mice had ad libitum access to food and water and were maintained on a 12-h dark/light cycle. In this study, the use of animals was approved by ethical committee of university of Groningen (AVD10500202010985).

4.6. Stereotactic surgery and injection

A double guide canulae (C235, Plastics One) was implanted under 1–1.5 % isoflurane anesthesia, according to the following hippocampal coordinates: AP-1.5 mm, bilateral 1 mm, depth 2 mm (Franklin and Paxinos, 1997). The dummy canulae was projected 0.4 mm below canulae pedestal to avoid contacting brain surface. After surgery, the mice were individually housed and allowed to recover for 7–12 days. The placement of canulae was confirmed by DAPI staining. During bilateral intrahippocampal injection, compounds were manually injected at a rate of 0.1 μ l/min via a Hamilton syringe to reach final volume of 0.3 μ l per side, followed by 30 s waiting before removing injector canulae. The injection of A β or its vehicle PBS was performed 24 h before CFC. Subsequently, the injection of S220 or its vehicle PBS was performed 20 min before CFC training, immediately after CFC training and 20 min before CFC retention, to investigate memory acquisition, consolidation and retrieval. Regarding the second injection in the control group (PBS + PBS), three mice received PBS before acquisition, three before consolidation, and three before retrieval. Occasionally, the guide canulae was found to be blocked during injection, which was excluded from the dataset.

4.7. Contextual fear conditioning

Contextual fear conditioning (CFC) was used to assess associative memory related to fear after drug treatment. The procedures were performed as before (Ostroveanu et al., 2010; Granic et al., 2010). Briefly, the mice were placed in a chamber with a stainless-steel grid floor and constant light. During CFC training, the mice were exposed to a contextual environment for 180 s, followed by 2 s electrical footstock (0.7 mA, constant current). The mice were kept in the chamber for 30 s and subsequently removed to avoid aversive association from handling. During retention, the mice were placed in the same chamber for 180 s without footshock. The freezing, defined as complete lack of movement except for heartbeat and respiration, was measured via EthoVision XT software, and the freezing percentage was calculated by the duration of freezing divided by 180 s (Pham et al., 2009).

4.8. Statistical analysis

Two-tailed Student's T test or one-way ANOVA followed by Tukey's post-hoc analysis was used to assess statistical significance. In addition, the mEPSC average amplitude and average frequency were analyzed using the non-parametric Mann-Whitney test and the cumulative fraction distribution was analyzed using the Kolmogorov-Smirnov test. Statistical significance was defined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ or not significance (ns). All data were expressed as mean \pm SEM. GraphPad Prism software was used to analyze the data.

CRedit authorship contribution statement

Tong Zhang: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Yuequ Zhang:** Methodology, Investigation, Formal analysis. **Pascal Chameau:** Methodology, Investigation, Formal analysis. **Tingting Chen:** Methodology, Investigation. **Alejandro Marmolejo-Garza:** Methodology, Investigation. **Wanda Douwenga:** Methodology, Investigation. **Amalia M. Dolga:** Writing – review & editing, Resources, Conceptualization. **Helmut W. Kessels:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Martina Schmidt:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Ulrich L.M. Eisel:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.neuropharm.2025.110468>.

Data availability

No data was used for the research described in the article.

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Glossary

- AD:** Alzheimer's Disease
AMPA: AMPA-receptor
AP: action potential
A β : amyloid- β
[Ca²⁺]_i: intracellular calcium
CNS: central nervous system
CFC: contextual fear conditioning
Epac2: exchange protein directly activated by cAMP 2
IH: intrahippocampal
LTP: long-term potentiation
MEA: microelectrode array
mEPSCs: miniature excitatory post-synaptic currents
NE: norepinephrine
PCN: primary cortical neuron
PKA: protein kinase A