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Dopamine induces release of calcium from internal stores in layer II lateral entorhinal cortex fan cells.

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ABSTRACT

The entorhinal cortex plays an important role in temporal lobe processes including learning and memory, object recognition, and contextual information processing. The alteration of the strength of synaptic inputs to the lateral entorhinal cortex may therefore contribute substantially to sensory and mnemonic functions. The neuromodulatory transmitter dopamine exerts powerful effects on excitatory glutamatergic synaptic transmission in the entorhinal cortex. Interestingly, inputs from midbrain dopamine neurons appear to specifically target clusters of excitatory cells located in the superficial layers of the entorhinal cortex. We have previously demonstrated that dopamine facilitates synaptic transmission through the activation of D_1-like receptors. This facilitation of synaptic transmission is dependent on both activation of classical D_1-like-receptors, and upon activation of dopamine receptors linked to increases in phospholipase C, inositol triphosphate (IP_3), and intracellular calcium. In the present study we combined electrophysiological recordings of evoked excitatory postsynaptic currents with imaging of intracellular calcium using the fluorescent indicator fluo-4 to monitor calcium transients evoked by dopamine in electrophysiologically identified putative fan and pyramidal cells of the lateral entorhinal cortex. Bath application of dopamine (1 μM), or the phosphatidylinositol (PI)-linked D_1-like-receptor agonist SKF83959 (5 μM), induced reliable and reversible increases in fluo-4 fluorescence and excitatory postsynaptic currents in fan cells, but not in pyramidal cells. In contrast, application of the classical D_1-like-receptor agonist SKF38393 (10 μM) did not result in significant increases in fluorescence. Blocking release of calcium from internal stores by loading cells with the IP_3 receptor blocker heparin (1 mM) or the ryanodine receptor blocker dantrolene (20 μM) abolished both the calcium transients and the facilitation of evoked synaptic currents induced by dopamine. Dopamine also induced calcium transients in fan cells when calcium was excluded from the extracellular medium, further indicating that the calcium transients are linked to release from internal stores. These results indicate that following D_1-like-receptor binding, dopamine selectively induces transient elevations in intracellular calcium via activation of IP_3 and ryanodine receptors, and that these elevations are linked to the facilitation of synaptic responses in putative layer II entorhinal cortex fan cells.

1. Introduction

The entorhinal cortex is uniquely positioned as an interface between cortical sensory and associational areas, and the hippocampal formation [1], and previous reports have highlighted its significant role in numerous processes, including spatial navigation, sensory and object processing, learning, and mnemonic functions [2–6]. Glutamatergic inputs carrying sensory and associational information project from the neocortex to the superficial layers I-III of the entorhinal cortex [7]. In turn, principal neurons in these layers project to the dentate gyrus and hippocampal CA areas, which are critically involved in the encoding of long-term memories [8].

The activity of layer II and III entorhinal cells, which provide input to the hippocampal formation, is regulated by several neuromodulatory transmitters, including strong inputs from dopaminergic neurons [9–12]. Midbrain dopamine neurons are known to affect the salience of stimuli and to contribute to cortical and hippocampal mechanisms of learning and memory [13–15]. Interestingly, dopaminergic cells in the ventral tegmental area and substantia nigra project heavily, and preferentially, to the superficial layers of the lateral entorhinal cortex, where they directly target lateral entorhinal cortex cells [16,9]. We have previously found that low levels of dopamine enhance excitatory synaptic transmission post-synaptically via activation of D_1-like receptors [17,18]. Physiological release of dopamine in the lateral

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entorhinal cortex may therefore promote excitatory synaptic activation of entorhinal neurons and enhance transmission of reward-relevant and salient information within the hippocampal formation.

We have found that the dopaminergic facilitation of glutamate-mediated synaptic responses in layer II lateral entorhinal cortex neurons can occur via two intracellular signalling cascades that lead to rapid and reversible increases in glutamatergic AMPA-receptor-mediated currents [18,19]. The increase in AMPA-mediated currents can be blocked by inhibition of PKA [19], suggesting downstream effects of 'classical' adenylyl cyclase and protein kinase A (PKA)-linked D_1-like receptors which lead to inhibition of PP-1-dependent dephosphorylation of AMPA receptors [20–22]. In addition, we found that the facilitation induced by dopamine was mimicked by SKF83959 [19], an agonist of atypical phosphatidylinositol (PI)-linked D_1-like receptors which increase phospholipase C (PLC) activity, and thereby diacyglycerol (DAG) and IP_3 production [23,24], resulting in increased intracellular calcium. We also observed that during chelation of intracellular calcium using BAPTA, or while blocking calcium release from internal stores via either ryanodine or IP_3 receptors, the dopamine-induced facilitation of synaptic responses was prevented [19]. Previous reports suggest that activation of PI-linked D_1-like receptors can lead to enhancement of glutamatergic currents [25]. A dependency on intracellular calcium for the dopaminergic facilitation of synaptic transmission has also been reported in several other brain regions including the striatum [26], prefrontal cortex [27], and hippocampus [28]. In the entorhinal cortex, dopamine was found to facilitate AMPA currents via protein kinase A (PKA) and protein kinase C (PKC) [19], the latter of which can be activated by intracellular calcium and DAG. Upon activation, PKC can phosphorylate AMPA;1; receptor subunits at $Ser^{831/818}$, increasing channel conductance [29,30], thereby increasing synaptic currents. Together, these findings indicate that dopamine can facilitate AMPA-mediated synaptic transmission in layer II lateral entorhinal cortex neurons both via classical D_1-like receptors linked to adenylyl cyclase and by activation of PI-linked D_1-like receptors linked to intracellular calcium levels.

Although it is known that calcium signalling is required for the dopaminergic facilitation of AMPA currents in layer II lateral entorhinal cortex neurons [19], the time-course and size of the increases in calcium concentrations during dopamine application have not been determined. While dopamine was reported to modulate intracellular calcium levels by altering the activity of voltage-gated calcium channels in other brain regions [31,32], blocking L/N-type channels does not suppress the dopaminergic enhancement of synaptic transmission in the lateral entorhinal cortex [19]. However, the hippocampal and parahippocampal areas express an abundance of IP_3 receptors [33,34], and moderate levels of ryanodine receptors, with the highest density located within the superficial layers [35]. RyRs can be activated by caffeine and by increases in intracellular calcium mediated by IP_3 receptors, voltage-gated calcium channels, or NMDA glutamate receptors [36]. Previous electrophysiological evidence points to the requirement of activation of both IP_3R and RyR for the dopaminergic facilitation of AMPA currents [19], suggesting that the facilitation may require calcium-induced calcium release from RyR initiated by calcium release from IP_3 receptors [37]. However, imaging of intracellular calcium is required to monitor the amplitude and time-course of the associated changes in intracellular calcium concentration.

Here, we combined whole-cell patch-clamp recordings and fluorescence imaging of intracellular calcium using the indicator fluo-4 loaded into individual entorhinal cells to track changes in synaptic transmission and cytosolic calcium concentration during application of dopamine to layer II entorhinal principal cells. The classical D_1-receptor agonist SKF38393 and the PI-linked D_1-receptor agonist SKF83959 were used to assess effects of activating these pathways on cytosolic calcium concentration. The source of calcium transients was assessed by including either IP_3 or ryanodine receptor blockers in the intracellular recording solution, and by conducting recordings in the absence of external calcium. Dopamine consistently induces synaptic facilitation in layer II fan cells, while facilitation effects in pyramidal neurons are less reliable [18,19], and the current experiments allowed us to assess whether differences in the facilitation of excitatory postsynaptic currents (EPSCs) in electrophysiologically identified fan and pyramidal cells are linked to differences in calcium responses.

2. Materials and methods

2.1. In vitro slice preparation

Experiments were conducted following the guidelines of the Canadian Council on Animal Care. Horizontal brain slices were obtained from 34 male Long-Evans rats (Charles River) 4 to 9-week-old, for a total of 44 recorded cells. Each recorded cell was obtained from a different brain slice. Animals were anesthetised with isoflurane, decapitated, and brains were quickly extracted and submerged in 4°C high-sucrose artificial cerebrospinal fluid (ACSF) saturated with 95% O_2 and 5% CO_2 (pH ≈7.3) containing (in mM) 250 sucrose, 2 KCl, 1.25 NaH_2PO_4, 7 $MgCl_2$, 26 Na;1;HCO_3, 0.5 $CaCl_2$ and 10 dextrose. All drugs were obtained from Sigma–Aldrich unless indicated otherwise. Horizontal slices containing the entorhinal region were obtained using a vibratome (300 µm thickness; Leica VT1200, Leica Biosystems). Slices were allowed to recover for at least one hour at room temperature in ACSF containing (in mM) 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose (pH ≈7.4; 300–310 mOsm).

2.2. Electrophysiological recordings

During recordings, individual slices were held submerged using a nylon net, and were superfused with ACSF (2–4 ml/min) at ≈22 °C. Slices were visualized with an upright microscope (Leica, DM-LFS) using differential interference contrast optics and a 40x water-immersion objective. Layer II of the lateral entorhinal cortex was distinguished from layers I and III based on the characteristic presence of cell islands [38]. Borosilicate glass recording pipettes (1.0 mm OD; 3.2 to 4.8 MΩ) were filled with (in mM) 140 K-gluconate, 10 HEPES, 5 NaCl, 2 $MgCl_2$, 2 ATP-Tris, 0.4 GTP-Tris, and 0.15 fluo-4 pentapotassium salt (Thermo Fisher Scientific). A bipolar stimulating electrode made from two tungsten electrodes (˜1 MΩ, FHC) was placed in layer I of the lateral entorhinal cortex to evoke excitatory postsynaptic currents in layer II cells. Patch-clamp recordings were obtained using an Axopatch 200B amplifier, filtered at 10 kHz, and digitized at 20 kHz (Molecular Devices, Digidata 1322 A). Recordings using xestospongin C and Alexa 594 were conducted using an Olympus BX51WI microscope with a MultiClamp 700B amplifier (Molecular Devices), DSD2 (Andor), and Zyla 4.2 camera (Andor), courtesy of Dr. Hua Hu.

After obtaining tight seals (1–4 GΩ) and whole-cell configuration in voltage clamp mode, electrophysiological characteristics and firing properties of entorhinal neurons were characterized in current-clamp mode to distinguish between fan and pyramidal cells as previously described [39]. Membrane potential responses were recorded during 500 ms duration current-pulse injections ranging from -200 to +60 pA. Responses were analyzed using the Clampfit 8.2 software pac;1;kage (Molecular Devices). In comparison to pyramidal neurons, fan cells display clustering of action potential firing versus regular firing patterns, a marked sag in the voltage response to strong hyperpolarizing current steps. Similar to Tahvildari and Alonso [39], putatively identified fan cells and pyramidal cells recorded here differed in their firing patterns, the sag in voltage responses during negative current injection (1.17 ± .03 rectification ratio in fan versus 1.04 ± .03 in pyramidal cells; $t_{30} = 3.58$, $p < .001$), and cellular input resistance (91.8 ± 7.5 MΩ in fan vs. 50.9 ± 2.8 MΩ in pyramidal cells; $t_{31} = 3.50$, $p < .001$). Resting membrane potential, however, was more depolarized in fan cells than in pyramidal cells (-44.3 ± 2.3 mV in fan vs. -57.2 ± .8 mV in pyramidal cells; $t_{33} = 6.87$, $p < .001$).

Excitatory postsynaptic currents (EPSCs) were recorded close to resting potential (-60 mV) and were evoked by 0.1 ms-duration constant current pulses delivered using a stimulus timer and isolation unit (WPI, Models A300 and A360). Stimulus intensity (93 ± 13 μA) was adjusted to evoke responses of roughly 70% maximal amplitude. Cellular input resistance, access resistance, and capacitance were monitored using responses to -10 mV 50 ms voltage steps delivered following each evoked synaptic response. Recordings were discarded if values changed by > 15%. Synaptic responses were recorded once every 15 s, with 10 traces being obtained for averaging during each testing condition.

2.3. Fluorescence imaging

Prior to the start of fluorescence recordings, the Ca^{2+} indicator fluo-4 (excitation: 494 nM, emission: 516 nM) was allowed to diffuse into the cell from the recording electrode during a period of 20 min. Fluo-4 exhibits high fluorescence emission at low intracellular concentrations and has a high dynamic range, making it particularly suitable for assessing changes in cytosolic calcium concentrations during the application of dopamine. The timing of fluorescence illumination provided by a xenon lamp (Lambda LS, Sutter Instruments) was gated using the shutter of a Lambda 10-2 filter wheel and controller (Sutter Instruments), and a liquid light guide delivered light to the microscope containing a Fluor 488 filter cube (Chroma 49011, with ET480/40x excitation and ET535/50 m emission filters). Fluorescence images were acquired using a CCD camera (Sensicam QE, Optikon) controlled using Axon Imaging Workbench 4.0 software (Molecular Devices). Images were collected using 150 ms exposure times (8 bit/pixel, 8 × 12 pixel binning) once every 4 s.

2.4. Pharmacological testing

To ensure fluorescence stability, calcium fluorescence signals were monitored for a baseline period of 5 to 15 min in normal ACSF following electrophysiological characterization of cells. Following stable baselines, a 5 to 10 min bath application of either dopamine HCl (Sigma), the classical D_1-like receptor agonist SKF38393 (10 μM), or the PI-linked agonist SKF83959 (5 μM, Tocris Life Sciences) was used to characterize changes in cytosolic calcium induced by dopamine-receptor activation. Sodium metabisulfite (50 μM) was co-applied to inhibit oxidation of dopamine. Since 1 μM dopamine was previously reported to solely modulate AMPA-receptor-mediated currents in layer II of the lateral entorhinal cortex [18,19], GABA receptor blockers were not co-applied in the present study. A washout period of 10 to 20 min was recorded to assess the reversibility of drug effects. Separate tests of the effects of blocking release of calcium from intracellular stores during dopamine application were conducted by including the IP_3 receptor blockers heparin (1 mM) or xestospongin C (500 nM, Abcam) or the ryanodine receptor blocker dantrolene (20 μM) into the intracellular solution. To determine if calcium transients relied on the influx of extracellular calcium, we performed experiments in which dopamine was applied during constant perfusion of slices with a calcium-free ACSF containing EGTA (2 mM). Electrophysiological responses were recorded at the end of the drug-application period and at the end of the washout period, except when synaptic transmission was blocked during exclusion of extracellular calcium.

2.5. Data analysis

Fluorescence signals were quantified in each cell by determining the mean intensity within a manually-traced outline of the soma which excluded the recording electrode. Data were corrected for background fluorescence by subtracting the mean intensity of a remote region from the mean fluorescence recorded at the cell. Changes in fluorescence (ΔF) were then expressed as a percentage of the mean corrected fluorescence recorded during the baseline period. Strong depolarization of 5 to 10 s, which triggers calcium influx via activation of voltage-gated calcium channels, always resulted in much larger fluorescent signals than those induced by dopamine application (data not shown), indicating that the calcium transients recorded were well below saturation levels for the calcium indicator fluo-4. In addition, six cells were co-loaded with fluo-4 and the calcium-insensitive fluorophore Alexa 594 (50 μM; Thermo Fisher Scientific) to determine if changes in fluo-4 fluorescence induced by SKF83959 could be observed during stable fluorescence from a calcium-insensitive dye.

For statistical analysis, fluorescence values for each cell were averaged over the last 5 min of the baseline period, the last 30 s of the drug application period, and the last minute of the washout period. Differences between drug conditions for both fluorescence values and peak amplitudes of EPSCs were assessed using one-way repeated-measures ANOVAs, followed by posthoc Newman-Keuls (N-K) pairwise comparisons.

3. Results

3.1. Dopamine leads to increased calcium in lateral entorhinal cortex fan cells

Consistent with our previous findings that dopamine facilitates synaptic responses in layer II entorhinal neurons via a calcium-dependent mechanism [19], we found that bath application of 1 μM dopamine led to transient increases in somatic calcium fluorescence. Further, consistent with the more reliable facilitation of synaptic responses observed in fan cells versus pyramidal neurons [19], the calcium transients induced by dopamine were stronger and more reliably-induced in electrophysiologically identified fan cells, in comparison to pyramidal neurons (Fig. 1A). In fan cells, increases in fluorescence could be noticed within 2 min of dopamine application. The signals peaked within ≈5 min to 118.6 ± 3.8% of baseline levels ($n = 3$; $F_{(2,4)} = 9.6$, $p = .03$; Newman-Keuls $p = 0.028$), and reversed towards baseline levels during washout in ACSF (106.1 ± 3.0 %). In contrast, pyramidal cells did not show a significant change in calcium fluorescence in the presence of dopamine (102.7 ± 1.4% during dopamine and 98.1 ± 2.3% at washout; $n = 3$; $F_{(2,4)} = 3.1$, $p = .15$).

The increases in fluorescence were paired with increases in evoked excitatory postsynaptic currents (EPSCs). Fan cells, which showed elevated fluorescence during dopamine application, also reliably displayed a reversible increase in EPSCs ($F_{(2,4)} = 24.3$, $p = .006$; Fig. 1B). The peak amplitude of EPSCs was increased to 122.5 ± 8.3% of baseline during dopamine application ($p = .007$) but did not differ significantly from baseline during washout in normal ACSF ($p = .67$). The decay time constant (4.9 ± .7 vs 4.1 ± .2 ms, $t = .96$, $p = .44$) was not significantly altered by dopamine application in these cells. EPSC amplitudes in pyramidal cells did not increase significantly during dopamine application (106.6 ± 2.8% of baseline; $F_{(2,4)} = 1.13$, $p = .41$). Changes in EPSC amplitude were strongly correlated with changes in fluorescence among fan and pyramidal neurons ($r^2 = 0.95$, $p < 0.001$). We therefore conclude that dopamine has cell-type specific effects on both calcium fluorescence and facilitation of EPSCs in layer II entorhinal fan cells.

3.2. Activation of classical versus PI-linked D_1 receptors

The PI-linked D_1 receptor agonist SKF83959 induced increases in calcium-dependent fluorescence and EPSC amplitudes in fan cells that were similar to the increases observed following application of dopamine, without similar effects in pyramidal neurons (Fig. 2). Five min bath application of SKF83959 increased fluorescence signals in fan cells to 119.7 ± 3.3% of baseline levels ($n = 5$; $F_{(4,7)} = 20.7$, $p = .001$; N−K $p < .001$), and responses reversed to 106.6 ± 2.2% after washout in normal ACSF ($p = .011$). There was no significant change

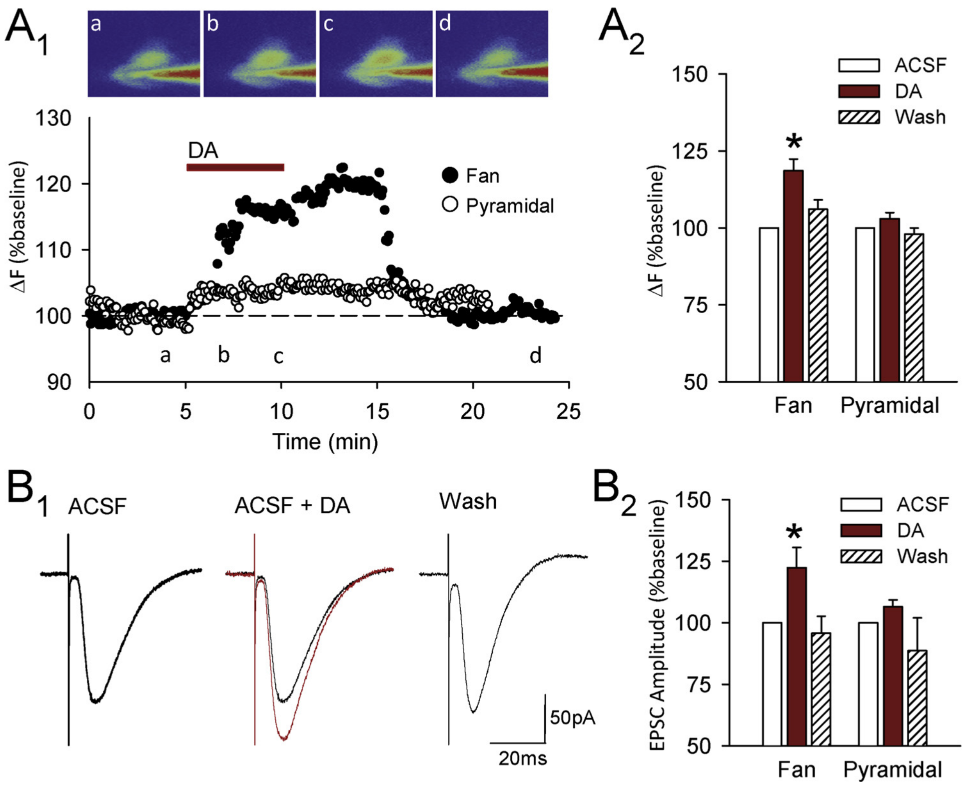

Fig. 1. Dopamine induces transient increases in somatic calcium and in excitatory postsynaptic currents (EPSCs) in fan cells in layer II of the lateral entorhinal cortex, but not in pyramidal cells of the same region. **A:** Changes in fluo-4 fluorescence induced by bath application of 1 µm dopamine (DA) are shown from a representative fan cell (closed circles; resting membrane potential -58 mV, input resistance 123 MΩ, 1.15 rectification ratio) and pyramidal neuron (open circles; resting membrane potential −55 mV, input resistance 52 MΩ, 0.99 rectification ratio) (A_1). The times at which the four sample images of calcium fluorescence in the fan cell were obtained are indicated by corresponding letters along the x-axis in the current, and subsequent, figures. The histogram in A_2 shows changes in mean fluorescence in the groups of fan and pyramidal cells tested. Bars indicate ± one SEM and * indicates $p < .05$ relative to baseline. **B:** Sample traces show averaged EPSCs recorded in a fan cell during the baseline period in normal ACSF, during bath-application of dopamine, and during the washout period (B_1). Dopamine significantly increased EPSC amplitudes in the group of fan cells, but not in pyramidal neurons (B_2).

in fluorescence, however, in pyramidal cells (107.6 ± 2.5% during SKF83959 and 100.6 ± 1.2% at washout; $n = 3$; $F_{(2,4)} = 4.9$, $p = .08$). In a subsequent group of fan cells loaded with both fluo-4 and Alexa 594, SKF83959 induced a significant increase in fluo-4 fluorescence expressed as a proportion of Alexa 594 fluorescence (1.7 ± 0.4 ratio during dopamine application versus baseline, $n = 3$, $t_2 = 4.59$, $p = .04$). In contrast, recordings from pyramidal cells loaded with fluo-4 and Alexa 594 showed no significant change (0.92 ± 0.1 ratio during dopamine application versus baseline, $n = 3$, $t_2 = 0.2$, $p = .86$).

Similar to our previous results [19], SKF83959 also induced reliable increases in EPSC amplitudes in fan cells (119.0 ± 4.0% of baseline; $F_{(4,7)} = 19.17, p < .001$; N−K $p = .002$ for baseline vs. SKF83959 and $p = .70$ for baseline vs washout). SKF83959 did not reliably increase EPSC amplitudes in pyramidal neurons (101.6 ± 4.7% of baseline;

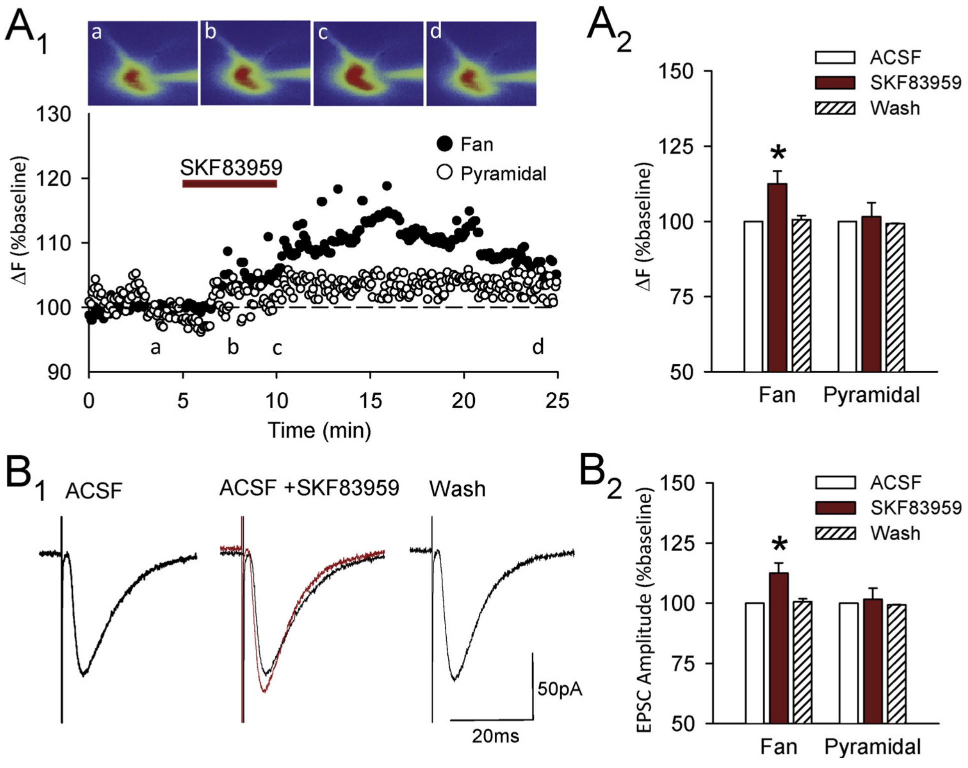

Fig. 2. Application of the PI-linked D_1 receptor agonist SKF83959 induces reversible elevations in cytosolic calcium and EPSC amplitudes similar to those observed during application of dopamine. **A:** SKF83959 (5 µM) induced a clear increase in fluo-4 fluorescence in a representative fan cell (closed circles; resting membrane potential -57.6 mV, input resistance 83 MΩ, 1.19 rectification ratio), but not in a pyramidal neuron (open circles; resting membrane potential −45 mV, input resistance 51 M Ω; 1.00 rectification ratio) (A_1). The histogram in A_2 shows changes in mean fluorescence in fan and pyramidal cells. **B:** Sample traces show averaged EPSCs recorded before, during, and following bath-application of SKF83959 in a fan cell (B_1), and the histogram indicates mean EPSC amplitudes among fan and pyramidal cells (B_2). (* indicates $p < .05$).

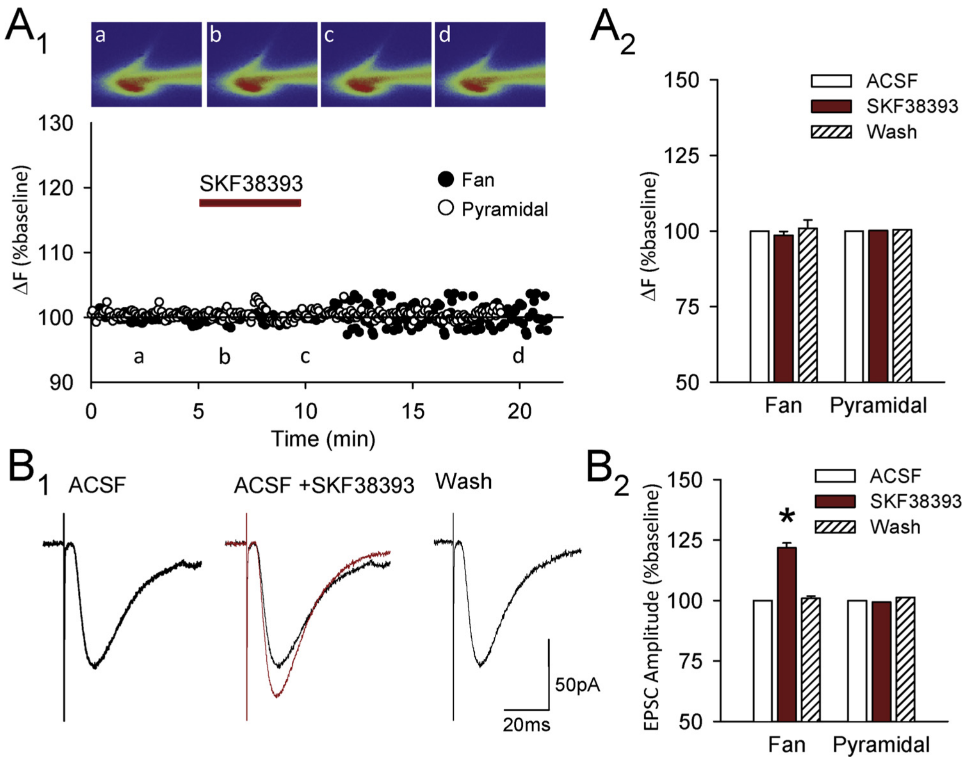

Fig. 3. The D_1-like receptor agonist SKF38393 linked to activation of PKA does not result in increased cytosolic calcium in either fan or pyramidal cells. **A:** Fluo-4 fluorescence remained stable in a representative fan cell (closed circles) and pyramidal cell (open circles) during application of SKF38893 (10 μM) (A_1). Mean fluorescence within fan and pyramidal cells was not significantly affected by SKF38893 (A_2). **B:** EPSC amplitudes were increased by SKF38393 in fan cells but not in the pyramidal neuron. Sample traces from a fan cell show averaged EPSCs recorded before, during, and after application of SKF38393 (B_1), and the histogram shows mean EPSC amplitudes in the groups of cells tested (B_2). (* indicates $p < .05$).

$F_{(2,4)} = 0.2$, $p = 0.82$), although one cell did show a moderate increase in EPSCs amplitude (109.8% of baseline) that was consistent with a small increase in fluorescence (109.3%). Overall, group changes in EPSC amplitudes induced by SKF83959 were strongly correlated with changes in fluo-4 fluorescence among fan and pyramidal neurons ($r^2 = 0.85$; $p = .001$).

Application of the classical D_1-like receptor agonist SKF38393 (10 μM) resulted in the facilitation of EPSC amplitudes in fan cells, without concurrent increases in calcium fluorescence (Fig. 3). Fluorescence remained stable at 99.0 ± 1.4% of baseline levels in fan cells ($n = 6$; $F_{(2,8)} = 0.46$, $p = .65$), while EPSC amplitudes were facilitated significantly to 121.8 ± 2.0% of baseline ($F_{(2,8)} = 101.8$, $p < .001$; N-K $p < .001$ for baseline vs. SKF38393, and $p = .80$ for baseline vs. washout). In the one pyramidal neuron tested, both fluorescence and EPSC amplitudes remained stable. These results indicate that activation of classical D_1-like receptors can induce a facilitation of EPSCs independent of substantial transients in calcium concentration.

Increases in PKA induced by D_1-like receptor activation can enhance activation of PLC [40]. To assess if this might play a role in calcium transients induced by dopamine receptors in layer II neurons, three fan cells were exposed to the classical D_1-like receptor agonist SKF38393 to enhance PKA activation, and the effect of 5 min subsequent co-application of PI-linked agonist SKF83959 on calcium signals was assessed (data not shown). SKF38393 alone did not significantly affect fluorescence (101.9 ± .1% of baseline, $p = .879$), and subsequent co-application of SKF83959 increased fluorescence to 115.5 ± 2.3% ($F_{(2,4)} = 49.08$, $p = .002$; N-K $p = .002$), which was similar to that observed during application of SKF83959 alone (see Fig. 2). Data from this small group of cells therefore suggest that increases in calcium induced by activation of PI-linked receptors are not substantially enhanced by concurrent activation of PKA via classical D_1-like receptors.

3.3. Increases in intracellular calcium depend on IP_3 receptors and RyRs

Our previous electrophysiological recordings demonstrated that blocking calcium release from internal stores via IP_3 receptors or RyR prevents the dopaminergic facilitation of EPSCs [19]. We found here that, in addition to preventing the facilitation of synaptic responses in fan cells, blocking IP_3 receptors prevents dopamine-dependent changes in calcium levels. In cells loaded with the IP_3 receptor blocker heparin (1 mM), fluorescence remained stable during dopamine application (102.0 ± 1.6% of baseline; $n = 3$ fan, $n = 1$ pyramidal, cells; $F_{(2,5)} = 1.65$, $p = .28$). Further, no significant increases in EPSC amplitudes was observed in these cells (102.0 ± 6.7% of baseline; Fig. 4 A,B). Since some studies indicate that heparin may have off target effects [41,42], we replicated these experiments with the specific IP_3 receptor blocker xestospongin C (XeC, 500 nM) [43,44]. Dopamine failed to increase calcium fluorescence in cells loaded with XeC (101.1 ± 1.07% of baseline), and there was a non-significant increase in fluorescence during washout to 112.7 ± 7.0% of baseline ($n = 3$; $F_{(2,4)} = 2.52$, $p = .20$; $t_2 = 1.65$, $p = .24$ for baseline versus wash; Fig. 4A).

Similarly, cells loaded with the ryanodine receptor blocker dantrolene (20 μM) did not show significant increases in either fluo-4 fluorescence (102.7 ± 1.9% of baseline during dopamine; $n = 4$ fan cells; $F_{(2,3)} = 1.05$, $p = .45$) or EPSC amplitudes (99.8% ± 3.7% of baseline; Fig. 4C,D). These data indicate that both the transient elevations in intracellular calcium and the facilitation of EPSCs induced by dopamine are dependent upon both IP_3R and RyR.

To determine if influx of extracellular calcium may contribute to dopamine-induced calcium transients, recordings were conducted after at least 10 min exposure to calcium-free ACSF containing the calcium chelator EGTA (Fig. 5). Application of dopamine significantly elevated fluorescence levels from baseline in fan cells (124.2 ± 3.9%; $n = 4$, $F_{(2,6)} = 38.54$, $p < .001$, N-K $p < .001$ for dopamine vs. baseline, and $p = .99$ for baseline vs. washout), while fluorescence in the pyramidal neurons tested remained stable ($n = 2$, 104.3 ± 4.4% of baseline in dopamine; $F_{(2,2)} = 1.17$, $p = .46$). Further, the magnitude of the calcium transients in fan cells was similar to that observed in normal ACSF, suggesting that intracellular calcium transients induced by dopamine do not depend on influx of extracellular calcium.

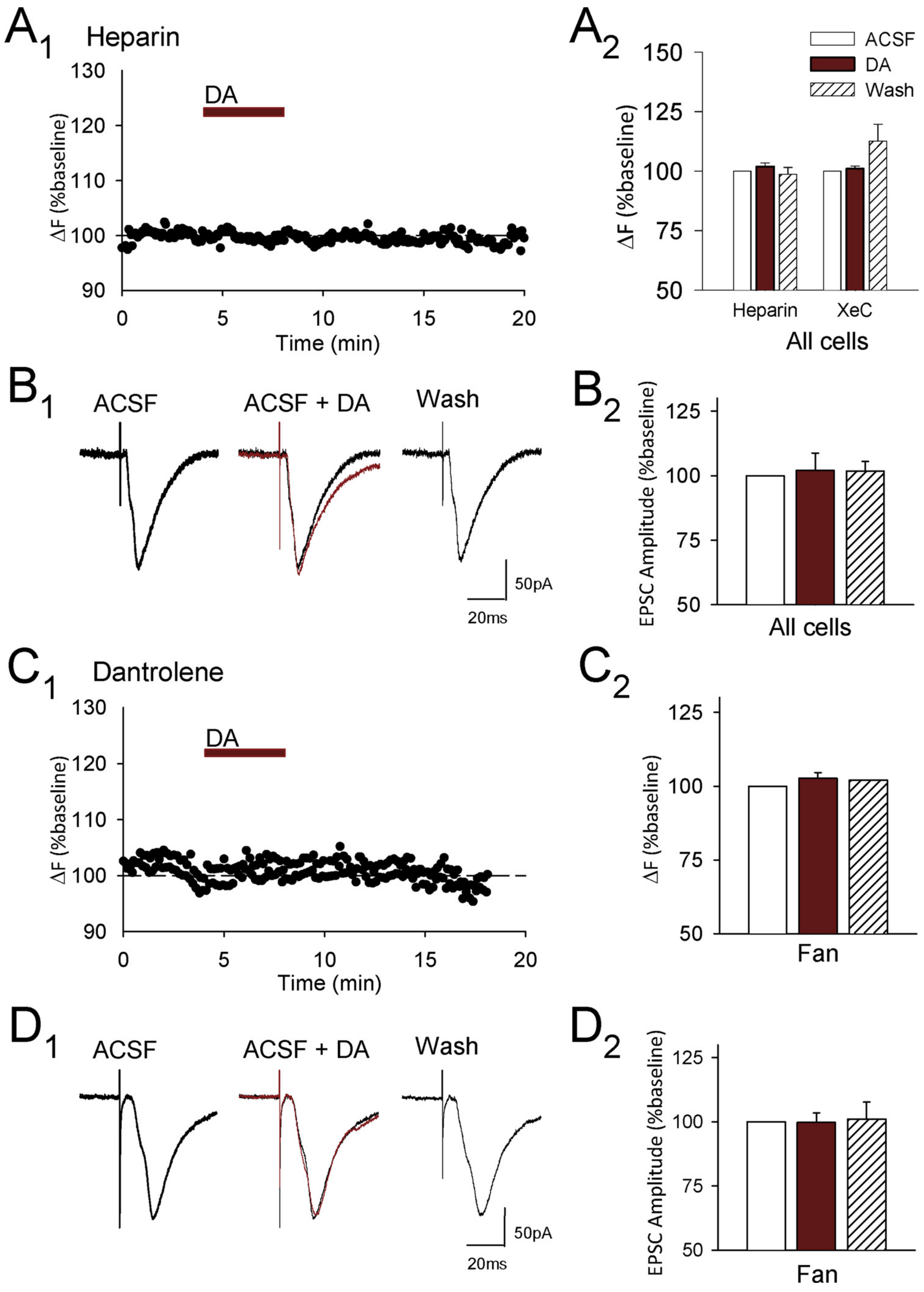

Fig. 4. Including IP_3 or ryanodine receptor blockers in the intracellular recording solution prevents increases in both calcium fluorescence and EPSC amplitudes induced by dopamine in fan cells. **A:** A representative fan cell loaded with the IP_3 receptor blocker heparin showed stable fluo-4 fluorescence during dopamine application (A_1). There were no significant changes in mean fluorescence in cells filled with either of the IP_3R blockers heparin (1 mM) or xestospongin C (500 nM) (A_2). **B:** Heparin also prevented increases in EPSC amplitudes in fan cells (B_1), and there were no significant changes in mean EPSC amplitudes in the fan cells or in the pyramidal cell tested (B_2). **C:** Blocking activation of ryanodine receptors with dantrolene (20 μM) prevented dopamine-induced increases in fluo-4 fluorescence in a representative fan cell (C_1), and there were no significant changes in fluorescence in the group of fan cells or the two pyramidal cells tested (C_2). **D:** Dantrolene also blocked dopamine-induced changes in EPSCs in fan cells.

4. Discussion

Application of either dopamine or the dopamine receptor agonist SKF83959, which is linked to increased PLC activity and IP_3 production [23], reliably led to transient increases in the concentration of intracellular calcium in electrophysiologically identified, putative layer II fan cells of the lateral entorhinal cortex. Dopamine-induced increases in intracellular calcium relied upon activation of both IP_3 and ryanodine receptors, and were not observed when calcium was excluded from the ACSF, indicating that dopamine activates intracellular signalling cascades leading to release of calcium from internal stores. Activation of IP_3 receptors alone, in the presence of the RyR blocker dantrolene, did not result in measurable increases in cytosolic calcium levels, suggesting that the dopamine-induced increases in somatic calcium are generated by an interaction between the activity of IP_3R and RyR, likely via calcium-induced-calcium-release in which an initial release of calcium via IP_3R is amplified by subsequent release via ryanodine receptor activation [see [45] for a review]. Our present results confirm that dopamine, and dopamine receptor agonists, induce a facilitation of EPSC amplitudes [19] that is dependent on calcium release from internal stores in fan, but not pyramidal, cells of the lateral entorhinal cortex.

The dopaminergic facilitation of synaptic responses in these entorhinal cells is mediated by postsynaptic mechanisms, rather than by changes in transmitter release. At a high concentration of 50 μM, dopamine has been shown to suppress synaptic responses and facilitate paired-pulse ratio in a manner dependent on D_2-like receptor activation [17]. However, lower doses of dopamine (1 μM) or SKF83959, were previously associated with a lack of change in paired-pulse ratio [18,19]. These findings suggest that lower doses of dopamine, such as those employed in the present study, do not substantially modulate presynaptic calcium transients or significantly affect other mechanisms

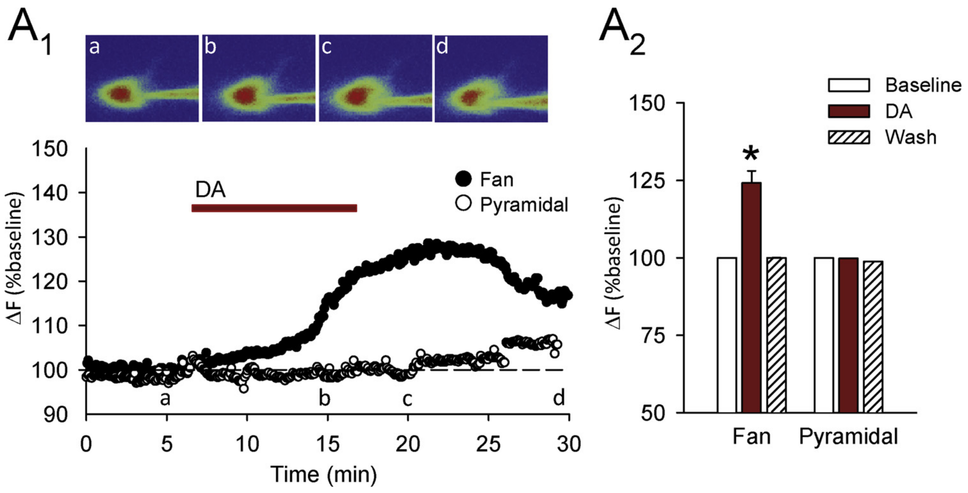

Fig. 5. Increases in intracellular calcium induced by dopamine in layer II entorhinal fan cells persist in the absence of extracellular calcium. **A:** Fluo-4 fluorescence was increased during 1 μm dopamine application in an individual fan cell (closed circles) but not in a pyramidal cell (open circles) in the absence of extracellular calcium (A_1). There was a significant increase in mean fluorescence in the group of fan cells tested but not in the two pyramidal cells tested (A_2) during application of 1 μm DA (A_3). (* indicates $p < .05$).

that regulate transmitter release in afferents to the entorhinal cortex. Previous results also demonstrate that this facilitation of synaptic responses is also blocked by U-73122 [19], a potent blocker of PLC, BAPTA, which buffers increases in postsynaptic calcium, and IP_3R or RyR blockers [19], further indicating that the facilitation is due to increased postsynaptic responsiveness, rather than presynaptic changes in transmitter release.

As opposed to results obtained in fan cells, dopamine and SKF83959 did not reliably induce increases in either intracellular calcium or EPSCs in pyramidal neurons. This differential effect of dopamine upon synaptic transmission in the two cell types is likely due to their different neurochemical profiles and functional properties. Whereas fan cells of the lateral entorhinal cortex express reelin, a signaling molecule involved in synaptic plasticity, pyramidal neurons preferentially express the calcium-binding protein calbindin, which may buffer intracellular calcium in these neurons, thereby providing a mechanism that can limit dopamine-induced increases in cytosolic calcium in pyramidal cells [46–48]. The differential modulation of synaptic transmission in fan and pyramidal cells is likely rooted in their different functions. Fan and pyramidal cells likely receive distinct patterns of synaptic inputs, as demonstrated by their dissimilar dendritic arbors [39], and their tendency to cluster together in cell type-specific clusters [49]. Further, fan and pyramidal cells have different axonal projections, with fan cells preferentially targeting the ipsilateral dentate gyrus, and pyramidal neurons targeting the granule cells of the olfactory bulb, the contralateral lateral entorhinal cortex, and, to a lesser degree, the piriform cortex [48]. The facilitation of synaptic responses in fan cells versus pyramidal neurons by dopamine may therefore selectively amplify the computational functions of fan cells during the processing of salient or reward-relevant stimuli, and enhance their contribution to cognitive and mnemonic processes within the hippocampal formation.

4.1. Dopamine receptors linked to phospholipase C activity

Dopamine and SKF83959 activate dopamine receptors linked to G-proteins that activate PLC, which results in increased intracellular calcium through the generation of IP_3 [23,50]. We have previously reported that dopamine-induced activation of PLC is required for the facilitation of synaptic responses in lateral entorhinal fan cells [19], and have shown evidence that this mechanism is due to binding to D_1-like receptors, as application of the D_1-like receptor blocker SCH23390 blocks the facilitation of synaptic responses induced by either dopamine [18] or SKF83959 [19]. Although it can be argued that increases in the classical cAMP-PKA pathway can lead to increased PLC activity via PKA-dependent phosphorylation of PLC [40,51], previous reports have demonstrated that D_1-like receptor activation can lead to potentiation of glutamatergic currents via mechanisms that are dependent on PKC [52,53], but not on PKA [54]. In line with this evidence, we report here that application of the conventional D_1-like agonist SKF38393, which leads to increases in the cAMP-PKA pathway, did not lead to significant increases in cytosolic calcium in entorhinal fan cells. Further, additional application of SKF38393 did not affect the magnitude of calcium transients induced by application of the PI-linked dopamine agonist SKF83959, suggesting that the effects of SKF83959 are unlikely to involve activation of classical D_1-like receptors and PKA.

In the striatum, increased PLC activity can be induced by D_1-D_2 receptor heteromers that are activated by SKF83959 [55,56]. However, D_1-D_2 receptor heteromers are unlikely to mediate the facilitation of EPSCs in entorhinal fan cells. Activation of D_1-D_2 receptor heteromers can be blocked by either D_1 or D_2 receptor antagonists [51], and we previously found that blocking D_2 receptors does not reduce the facilitation of synaptic responses induced by dopamine in entorhinal neurons [18]. In the lateral entorhinal cortex, therefore, dopamine is likely to lead to transient increases in intracellular calcium via atypical D_1-like receptors that activate PLC.

4.2. Role of calcium in the facilitation of synaptic responses

In the present study, dopamine and its agonist SKF83959 reliably and consistently induced elevations in cytosolic calcium in fan cells, which were paired to increases in synaptic responses. These findings are similar to other reports indicating that dopamine-induced facilitation of synaptic transmission requires calcium in the prefrontal cortex, striatum, and hippocampus [26–28,31]. In the entorhinal cortex, increases in the mixed EPSCs are due to increased AMPA receptor activation rather than increases in the NMDA component or changes in synaptic inhibition [18,19]. Increases in calcium may therefore lead to strengthening of EPSCs via activation of the calcium-dependent protein kinase PKC [19] which phosphorylates GluR1 AMPA-receptor subunits at Ser^{831} leading to increasing channel conductance [53,57,58]. The stability of the time constants of EPSCs following application of dopamine is consistent with this possible mechanism.

The conventional D_1-like receptor agonist SKF38393 caused an increase in the amplitude of synaptic responses as observed previously [19], but it did so without measurable increases in somatic fluo-4 fluorescence. Although measurable increases in somatic calcium were not observed here, it is possible that calcium may have been increased in dendritic regions, and this should be examined in future studies. Activation of conventional D_1-like receptors could contribute to increases in intracellular calcium permissive for the facilitation of EPSCs through PKA-dependent phosphorylation of IP_3R and/or RyR [59–62]. However, inhibition of either PKA or protein phosphatase 1 (PP-1) blocks the facilitation of synaptic responses induced by dopamine in fan cells [18], and the process through which classical D_1-like dopamine receptors facilitate synaptic responses in fan cells is likely to rely primarily upon PKA-dependent phosphorylation of either Inhibitor 1, or the 32 kDa dopamine- and cAMP-regulated phosphoprotein DARPP-32, which are both inhibitors of PP-1 [63,64]. PP-1 dephosphorylates $GluR_1$ AMPA receptor subunits [65,66], and the PKA-dependent inhibition of PP-1 likely results in the facilitation of EPSCs by increasing phosphorylation of AMPA-receptors.

4.3. Combined activation of IP_3 and ryanodine receptors

Use of either heparin or xestospongin C to block IP_3 receptors, or dantrolene to block RyR, eliminated the calcium transients recorded here, indicating that both IP_3R and RyR are required for dopamine-induced calcium transients in lateral entorhinal fan cells. Results obtained with heparin must be interpreted cautiously, as heparin can have side-effects including interfering with activation of some G-proteins [41,42]. However, results obtained with the more selective IP_3R blocker xestospongin C indicate a similar pattern, wherein dopamine-induced calcium transients are dependent on activation of IP_3 receptors [43,44]. The apparent dependence of calcium transients on both IP_3R and RyR suggest that the transients are mediated by IP_3-dependent calcium release that is amplified by RyR activation [37,67]. In addition, initial elevations in calcium may have also enhanced release via IP_3 receptors [68,69]. In the hippocampus, activation of PI-linked dopamine receptors results in the PLC- and IP_3-dependent release of calcium from internal stores that can support prolonged calcium levels, similar to those we observed during continued application of dopamine [70].

Our results are also similar to findings that activation of dopamine receptors can induce calcium release from internal stores in the hippocampus, via mechanisms dependent on both IP_3 and ryanodine receptors [37]. IP_3 and ryanodine receptors are expressed in overlapping populations of neurons, and their presence is widespread in both cortical and subcortical regions [e.g. 33, 35, 71]. Both the hippocampal and parahippocampal regions are rich in IP_3R and RyR [33,35,71,72], with both receptor subtypes being primarily present in dendritic shafts, but IP_3 receptors also distributed within synaptic terminals and the soma of neurons [33,73]. In the present study, we have used techniques for somatic calcium imaging, but it is possible that more localized changes in calcium in dendritic regions may occur during interactions between IP_3R and RyR [74]. Future studies should address how neuromodulators such as dopamine could alter calcium levels and synaptic integration and transmission in a more fine-tuned manner in different cellular subcompartments.

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References

[1] R.D. Burwell, The parahippocampal region: Corticocortical connectivity, Ann. N. Y. Acad. Sci. 911 (2000) 25–42.

[2] E.L. Hargreaves, G. Rao, J.J. Knierim, Major dissociation between medial and lateral entorhinal input to dorsal hippocampus, Science 308 (2005) 1792–1794.

[3] B.L. McNaughton, F.P. Battaglia, O. Jensen, E.I. Moser, M.B. Moser, Path integration and the neural basis of the 'cognitive map', Nat. Rev. Neurosci. 7 (2006) 663.

[4] M.M. Albasser, G.L. Poirier, J.P. Aggleton, Qualitatively different modes of perirhinal–hippocampal engagement when rats explore novel vs. familiar objects as revealed by c-Fos imaging, Euro J. Neurosci. 31 (2010) 134–147.

[5] E.L. Hargreaves, D. Yoganarasimha, J.J. Knierim, Cohesiveness of spatial and directional representations recorded from neural ensembles in the anterior thalamus, parasubiculum, medial entorhinal cortex, and hippocampus, Hippocampus 17 (2007) 826–841.

[6] M.P. Witter, E.I. Moser, Spatial representation and the architecture of the entorhinal cortex, Trends Neurosci. 29 (2006) 671–678.

[7] R.D. Burwell, D.G. Amaral, Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat, J. Comp. Neurol. 398 (1998) 179–205.

[8] M.P. Witter, H.J. Groenewegen, F.L. Da Silva, A.H.M. Lohman, Functional organization of the extrinsic and intrinsic circuitry of the parahippocampal region, Prog. Neurobiol. 33 (1989) 161–253.

[9] S. Mingote, N. Chuhma, S.V. Kusnoor, B. Field, A.Y. Deutch, S. Rayport, Functional connectome analysis of dopamine neuron glutamatergic connections in forebrain regions, J. Neurosci. 35 (2015) 16259–16271.

[10] S.J. Mitchell, J.N. Rawlins, O. Steward, D.S. Olton, Medial septal area lesions disrupt theta rhythm and cholinergic staining in medial entorhinal cortex and produce impaired radial arm maze behavior in rats, J. Neurosci. 2 (1982) 292–302.

[11] C. Köhler, V. Chan-Palay, L. Haglund, H. Stainbusch, Immunohistochemical localization of serotonin nerve terminals in the lateral entorhinal cortex of the rat: demonstration of two separate patterns of innervation from the midbrain raphe, Anat. Embryol. 160 (1980) 121–129.

[12] M. Palkovits, L. Záborszky, M.J. Brownstein, M.I. Fekete, J.P. Herman, B. Kanyicska, Distribution of norepinephrine and dopamine in cerebral cortical areas of the rat, Brain Res. Bull. 4 (1979) 593–601.

[13] S. Bandyopadhyay, C. Gonzalez-Islas, J.J. Hablitz, Dopamine enhances spatiotemporal spread of activity in rat prefrontal cortex, J. Neurophysiol. 93 (2005) 864–872.

[14] K.C. Berridge, The debate over dopamine's role in reward: the case for incentive salience, Psychopharmacology 191 (2007) 391–431.

[15] E.S. Bromberg-Martin, M. Matsumoto, O. Hikosaka, Dopamine in motivational control: rewarding, aversive, and alerting, Neuron 68 (2010) 815–834.

[16] A.M. Clark, S. Bouret, A.M. Young, B.J. Richmond, Intersection of reward and memory in monkey rhinal cortex, J. Neurosci. 32 (2012) 6869–6877.

[17] D.A. Caruana, C.A. Chapman, Dopaminergic suppression of synaptic transmission in the lateral entorhinal cortex, Neural Plast. 2008 (2008) 1–14.

[18] I. Glovaci, D.A. Caruana, C.A. Chapman, Dopaminergic enhancement of excitatory synaptic transmission in layer II entorhinal neurons is dependent on D_1-like receptor-mediated signaling, Neuroscience 258 (2014) 74–83.

[19] I. Glovaci, C.A. Chapman, Activation of phosphatidylinositol-linked dopamine receptors induces a facilitation of glutamate-mediated synaptic transmission in the lateral entorhinal cortex, PLoS One 10 (2015) e0131948.

[20] G.L. Snyder, A.A. Fienberg, R.L. Huganir, P. Greengard, A dopamine/D_1 receptor/protein kinase A/dopamine-and cAMP-regulated phosphoprotein (M r 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor, J. Neurosci. 18 (1998) 10297–10303.

[21] N. Kemp, Z.I. Bashir, Long-term depression: a cascade of induction and expression mechanisms, Prog. Neurobiol. 65 (2001) 339–365.

[22] R.C. Malenka, M.F. Bear, LTP and LTD: an embarrassment of riches, Neuron. 44 (2004) 5–21.

[23] L.Q. Jin, S. Goswami, G. Cai, X. Zhen, E. Friedman, SKF83959 selectively regulates phosphatidylinositol-linked D_1 dopamine receptors in rat brain, J. Neurochem. 85 (2003) 378–386.

[24] S. Panchalingam, A.S. Undie, SKF83959 exhibits biochemical agonism by stimulating [35S] GTPγS binding and phosphoinositide hydrolysis in rat and monkey brain, Neuropharmacology 40 (2001) 826–837.

[25] J. Liu, F. Wang, C. Huang, L.H. Long, et al., Activation of phosphatidylinositol-linked novel D_1 dopamine receptor contributes to the calcium mobilization in cultured rat prefrontal cortical astrocytes, Cell. Mol. Neurobiol. 29 (2009) 317–328.

[26] J. Flores-Hernandez, C. Cepeda, E. Hernandez-Echeagaray, et al., Dopamine enhancement of NMDA currents in dissociated medium-sized striatal neurons: Role of D_1 receptors and DARPP-32, J. Neurophysiol. 88 (2002) 3010–3020.

[27] K.Y. Tseng, P. O'Donnell, Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms, J. Neurosci. 24 (2004) 5131–5139.

[28] S.N. Yang, Sustained enhancement of AMPA receptor-and NMDA receptor-mediated currents induced by dopamine D_1/D_5 receptor activation in the hippocampus: an essential role of postsynaptic Ca^{2+}, Hippocampus 10 (2000) 57–63.

[29] A. Barria, V. Derkach, T. Soderling, Identification of the Ca2+/Calmodulin-dependent protein kinase II regulatory phosphorylation site in the α-Amino-3-hydroxyl-5-methyl4-isoxazole-propionate-type glutamate receptor, J. Biol. Chem. 272 (1997) 32727–32730.

[30] A.L. Mammen, K. Kameyama, K.W. Roche, R.L. Huganir, Phosphorylation of the α-amino-3-hydroxy-5-methylisoxazole4-propionic acid receptor GluR1 subunit by calcium/calmodulin-dependent kinase II, J. Biol. Chem. 272 (1997) 32528–32533.

[31] C. Cepeda, C.S. Colwell, J.N. Itri, S.H. Chandler, M.S. Levine, Dopaminergic modulation of NMDA-induced whole cell currents in neostriatal neurons in slices: Contribution of calcium conductances, J. Neurophysiol. 79 (1998) 82–94.

[32] S. Hernández-López, J. Bargas, D.J. Surmeier, A. Reyes, E. Galarraga, D_1 receptor activation enhances evoked discharge in neostriatal medium spiny neurons by modulating an L-type Ca^{2+} conductance, J. Neurosci. 17 (1997) 3334–3342.

[33] A.H. Sharp, P.S. McPherson, T.M. Dawson, C. Aoki, K.P. Campbell, S.H. Snyder, Differential immunohistochemical localization of inositol 1, 4, 5-trisphosphate-and ryanodine-sensitive Ca^{2+} release channels in rat brain, J. Neurosci. 13 (1993) 3051–3063.

[34] A. Evstratova, K. Tóth, Synaptically evoked Ca^{2+} release from intracellular stores is not influenced by vesicular zinc in CA3 hippocampal pyramidal neurones, J. Physiol. 589 (2011) 5677–5689.
[35] W. Zhao, N. Meiri, H. Xu, S. Cavallaro, A. Quattrone, L. Zhang, D.L. Alkon, Spatial learning induced changes in expression of the ryanodine type II receptor in the rat hippocampus, FASEB 14 (2000) 290–300.
[36] A.W. Trafford, P. Lipp, S.C. O'Neill, E. Niggli, D.A. Eisner, Propagating calcium waves initiated by local caffeine application in rat ventricular myocytes, J. Physiol. 489 (1995) 319–326.
[37] Y.-L. Wang, J.-G. Wang, F.-L. Guo, et al., Selective dopamine receptor 4 activation mediates the hippocampal neuronal calcium response via IP_3 and ryanodine receptors, Brain Res. 1 (2017) 1–5.
[38] D.G. Amaral, M.P. Witter, The three-dimensional organization of the hippocampal formation: a review of anatomical data, Neuroscience 31 (1989) 571–591.
[39] B. Tahvildari, A. Alonso, Morphological and electrophysiological properties of lateral entorhinal cortex layers II and III principal neurons, J. Comp. Neurol. 491 (2005) 123–140.
[40] P.Y. Yu, G.M. Eisner, I. Yamaguchi, M.M. Mouradian, R.A. Felder, P.A. Jose, Dopamine D_1 receptor regulation of phospholipase C isoform, J. Biol. Chem. 271 (1996) 19503–19508.
[41] L.L. Dasso, C.W. Taylor, Heparin and other polyanions uncouple alpha 1-adrenoceptors from G-proteins, Biochem. J. 280 (1991) 791–795.
[42] B.E. Ehrlich, E. Kaftan, S. Bezprozvannaya, I. Bezprozvanny, The pharmacology of intracellular Ca^{2+}-release channels, Trends Pharmacol. Sci. 15 (1994) 145–149.
[43] C.E. Young, C.R. Yang, Dopamine D1/D5 receptor modulates state-dependent switching of soma-dendritic Ca2+ potentials via differential protein kinase A and C activation in rat prefrontal cortical neurons, J. Neurosci. 24 (2004) 8–23.
[44] P. De Smet, J.B. Parys, G. Callewaert, A.F. Weidema, E. Hill, H. De Smedt, C. Erneux, V. Sorrentino, L. Missiaen, Xestospongin C is an equally potent inhibitor of the inositol 1,4,5-trisphosphate receptor and the endoplasmic-reticulum Ca(2+) pumps, Cell Calcium 26 (1999) 9–13.
[45] C.R. Rose, A. Konnerth, Stores not just for storage: intracellular calcium release and synaptic plasticity, Neuron 31 (2001) 519–522.
[46] Y. Fujimaru, T. Kosaka, The distribution of two calcium binding proteins, calbindin D-28K and parvalbumin, in the entorhinal cortex of the adult mouse, Neurosci. Res. 24 (1996) 329–343.
[47] E.S. Nilssen, G. Fjeld, M.P. Witter, Local connectivity and immunoreactivity of principal cells in layer II of lateral entorhinal cortex, Nordic Neurosci Abs. 1 (2015) 61.
[48] F.C. Leitner, S. Melzer, H. Lütcke, R. Pinna, P.H. Seeburg, F. Helmchen, H. Monyer, Spatially segregated feedforward and feedback neurons support differential odor processing in the lateral entorhinal cortex, Nat. Neurosci. 19 (2016) 935–944.
[49] A. Kobro-Flatmoen, M.P. Witter, Entorhinal Cell-specific Changes As an Initial Cause of Alzheimer's Disease, Trondheim: NTNU, (2017).
[50] N. Lezcano, C. Bergson, D_1/D_5 dopamine receptors stimulate intracellular calcium release in primary cultures of neocortical and hippocampal neurons, J. Neurophysiol. 87 (2002) 2167–2175.
[51] S.P. Lee, C.H. So, A.J. Rashid, et al., Dopamine D_1 and D_2 receptor Co-activation generates a novel phospholipase C-mediated calcium signal, J. Biol. Chem. 279 (2004) 35671–35678.
[52] K. Chergui, M.G. Lacey, Modulation by dopamine D_1-like receptors of synaptic transmission and NMDA receptors in rat nucleus accumbens is attenuated by the protein kinase C inhibitor Ro 32-0432, Neuropharmacology 38 (1999) 223–231.
[53] M.A. Jenkins, S.F. Traynelis, PKC phosphorylates $GluA_1$-Ser_{831} to enhance AMPA receptor conductance, Channels 6 (2012) 60–64.
[54] J. Harvey, M.G. Lacey, A postsynaptic interaction between dopamine D1 and NMDA receptors promotes presynaptic inhibition in the rat nucleus accumbens via adenosine release, J. Neurosci. 17 (1997) 5271–5280.
[55] A.J. Rashid, C.H. So, M.M. Kong, et al., D_1-D_2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of $G_{q/11}$ in the striatum, Proc. Natl. Acad. Sci. 104 (2007) 654–659.
[56] M.L. Perreault, M.Y. Shen, T. Fan, S.R. George, Regulation of c-fos expression by the dopamine D_1-D_2 receptor heteromer, Neuroscience 285 (2015) 194–203.
[57] H.K. Lee, K. Takamiya, J.S. Han, et al., Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory, Cell 112 (2003) 631–643.
[58] V. Derkach, A. Barria, T.R. Soderling, Ca^{2+}/calmodulin-kinase II enhances channel conductance of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors, Proc. Natl. Acad. Sci. 96 (1999) 3269–3274.
[59] M.D. Soulsby, R.J. Wojcikiewicz, The type III inositol 1, 4, 5-trisphosphate receptor is phosphorylated by cAMP-dependent protein kinase at three sites, Biochem. J. 392 (2005) 493–497.
[60] G. Meissner, Regulation of mammalian ryanodine receptors, Front Biosci. 7 (2002) d2072–d2080.
[61] S. Nakade, S.K. Rhee, H. Hamanaka, K. Mikoshiba, Cyclic AMP-dependent phosphorylation of an immunoaffinity-purified homotetrameric inositol 1, 4, 5-trisphosphate receptor (type I) increases Ca2+ flux in reconstituted lipid vesicles, J. Biol. Chem. 269 (1994) 6735–6742.
[62] S.K. Joseph, S.V. Ryan, Phosphorylation of the inositol trisphosphate receptor in isolated rat hepatocytes, J. Biol. Chem. 268 (1993) 23059–23065.
[63] M. Yger, J.A. Girault, DARPP-32, jack of all trades… master of which? Front. Behav. Neurosci. 5 (2011) 56–70.
[64] S. Shenolikar, A.C. Nairn, Protein phosphatases: recent progress, Adv. Second Messenger Phosphoprotein Res. 23 (1991) 1–121.
[65] Z. Yan, L. Hsieh–Wilson, J. Feng, et al., Protein phosphatase 1 modulation of neostriatal AMPA channels: Regulation by DARPP-32 and spinophilin, Nat. Neurosci. 2 (1999) 13–17.
[66] I. Song, R.L. Huganir, Regulation of AMPA receptors during synaptic plasticity, Trends Neurosci. 25 (2002) 578–588.
[67] A. Verkhratsky, A. Shmigol, Calcium-induced calcium release in neurones, Cell Calcium 19 (1996) 1–14.
[68] M.E. Larkum, S. Watanabe, T. Nakamura, N. Lasser-Ross, W.N. Ross, Synaptically activated Ca^{2+} waves in layer 2/3 and layer 5 rat neocortical pyramidal neurons, J. Physiol. 549 (2003) 471–488.
[69] T. Nakamura, J.G. Barbara, K. Nakamura, W.N. Ross, Synergistic release of Ca^{2+} from IP_3-sensitive stores evoked by synaptic activation of mGluRs paired with backpropagating action potentials, Neuron 24 (1999) 727–737.
[70] Y. Ming, H. Zhang, L. Long, F. Wang, J. Chen, X. Zhen, Modulation of Ca^{2+} signals by phosphatidylinositol-linked novel D_1 dopamine receptor in hippocampal neurons, J. Neurochem. 98 (2006) 1316–1323.
[71] R.A. Padua, T. Yamamoto, D. Fyda, M.A. Sawchuk, J.D. Geiger, J.I. Nagy, Autoradiographic analysis of [3H] ryanodine binding sites in rat brain: regional distribution and the effects of lesions on sites in the hippocampus, J. Chem. Neuroanat. 5 (1992) 63–73.
[72] A. Kapur, M. Yeckel, D. Johnston, Hippocampal mossy fiber activity evokes Ca^{2+} release in CA3 pyramidal neurons via a metabotropic glutamate receptor pathway, Neuroscience 107 (2001) 59–69.
[73] E. Vermassen, J.B. Parys, J.P. Mauger, Subcellular distribution of the inositol 1, 4, 5-trisphosphate receptors: functional relevance and molecular determinants, Biol. Cell 96 (2004) 3–17.
[74] M.J. Berridge, P. Lipp, M.D. Bootman, The versatility and universality of calcium signalling, Nat. Rev. Mol. Cell. Biol. 1 (2000) 11–21.