Target-Specific Suppression of GABA Release from Parvalbumin Interneurons in the Basolateral Amygdala by Dopamine

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Dopamine (DA) in the basolateral amygdala (BLA) promotes fear learning by disinhibiting principal neurons (PNs) and enabling synaptic plasticity in their sensory inputs. While BLA interneurons (INs) are heterogeneous, it is unclear which interneuron subtypes decrease GABAergic input to PNs in the presence of DA. Here, using cell type-selective photostimulation by channelrhodopsin 2 in BLA slices from mouse brain, we examined the role of parvalbumin-positive INs (PV-INs), the major interneuronal subpopulation in BLA, in the disinhibitory effect of DA. We found that DA selectively suppressed GABAergic transmission from PV-INs to PNs by acting on presynaptic D₂ receptors, and this effect was mimicked by Rp-cAMP, an inhibitor of cAMP-dependent signaling. In contrast, DA did not alter GABA release from PV-INs to INs. Furthermore, neither suppressing cAMP-dependent signaling by Rp-cAMP nor enhancing it by forskolin altered GABA release from PV-INs to BLA INs. Overall, DA disinhibits BLA, at least in part, by suppressing GABA release from PV-INs in the target cell-specific manner that results from differential control of this release by cAMP-dependent signaling.

Introduction

The basolateral amygdala (BLA) is the major information entry site of the amygdala complex (LeDoux, 2000). It is regulated by dopamine (DA) released from dopaminergic axons originating from the midbrain areas (Asan, 1997). Stressful events enhance DA release in BLA (Harmer and Phillips, 1999; Inglis and Moghaddam, 1999; Suzuki et al., 2002), which facilitates formation of fear memories (Fadok et al., 2009), presumably by attenuating feedforward inhibition (FFI) of BLA principal neurons (PNs) and enabling LTP induction at sensory afferents (Rosenkranz and Grace, 2001; Bissière et al., 2003; Marowsky et al., 2005). However, very little is known about the identity of interneurons (INs) that mediate dopaminergic disinhibition. Yet this information is important for understanding the disinhibition mechanisms, because individual interneuronal subtypes control principal cell activity by releasing GABA onto BLA PNs in temporally and spatially distinct patterns (Freund and Katona, 2007; Bienvenu et al., 2012).

Local INs in BLA are heterogeneous; they differ in morphological, electrophysiological, and neurochemical characteristics (Sosulina et al., 2010; Spanpanato et al., 2011). Among them,

parvalbumin-positive INs (PV-INs) constitute ~40% and exert robust perisomatic inhibition of PNs (McDonald and Betette, 2001). PV-INs are also the major target of dopaminergic fibers in BLA (Pinard et al., 2008). Given these properties, we took advantage of a PV-IN-specific Cre line of transgenic mice (Hippenmeyer et al., 2005) and tested the role of PV-INs in dopaminergic disinhibition of BLA PNs using selective photoactivation of PV-INs expressing channelrhodopsin 2 (ChR2).

Materials and Methods

Stereotactic injections. Adeno-associated virus (AAV) pseudo-type 1 (titer of 10¹² particles per milliliter) containing Cre-activated *ChR2* gene was prepared by University of North Carolina Gene Therapy Vector Core (Chapel Hill, NC) using Addgene plasmids 20298 (pAAV-EF1a-double floxed-*hChR2(H134R)-EYFP-WPRE-HGHpA*) (Zhang et al., 2010) and 18917 (AAV-FLEX-*rev-ChR2-tdTomato*) (Aponte et al., 2011).

Three- to 4-week-old male *Cre-PV/GAD67-GFP* or *Cre-SOM/GAD67-GFP* double-positive transgenic mice (Tamamaki et al., 2003; Hippenmeyer et al., 2005; Taniguchi et al., 2011) on C57BL/6J background were injected bilaterally with 0.5 μ l of virus into BLA [coordinates from bregma (in mm): AP -1.7 ; ML ± 3.3 ; DV -3.8 from the brain surface] following the National Institute of Mental Health Animal Care and Use Committee-approved protocol. Experiments were performed 3–4 weeks after surgery.

Electrophysiology and photostimulation. Animals were anesthetized with isoflurane and decapitated. Brain was quickly removed, and coronal amygdala slices (300 μ m) were cut using DSK Microslicer (Ted Pella) in ice-cold partial sucrose artificial CSF (ACSF) containing the following (in mM): 80 NaCl, 3.5 KCl, 4.5 MgSO₄, 0.5 CaCl₂, 1.25 NaH₂PO₄, 23 NaHCO₃, 10 glucose, and 90 sucrose equilibrated with 95% O₂/5% CO₂. The slices were incubated in the same sucrose ACSF at room temperature for 1 h before recording, then transferred to a recording chamber superfused at 2 ml/min with ACSF, bubbled with 95% O₂/5% CO₂, containing

Received June 25, 2012; revised Aug. 28, 2012; accepted Aug. 31, 2012.

Author contributions: H.-Y.C., W.I., and A.M. designed research; H.-Y.C. and J.L. performed research; H.-Y.C. analyzed data; H.-Y.C., W.I., and A.M. wrote the paper.

This work was supported by the National Institute of Mental Health intramural research program. We thank Victor Luna for commenting on the manuscript.

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DOI:10.1523/JNEUROSCI.2997-12.2012

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Figure 1. Selective activation of GABAergic transmission between PV-INs and PNs in BLA by ChR2. **A**, Confocal images show ChR2-tdTomato fluorescence (red) and PV-immunofluorescence (blue). Note that all ChR2-expressing cells (arrows) express PV, but only $41 \pm 4\%$ of PV-INs ($n = 4$ mice) express ChR2. **B**, Left, Whole-cell recording from a ChR2-expressing neuron. Short pulses of photostimulation (Photo-stim) evoke membrane depolarization (red, 0.2 ms pulse) and action potential (black, 0.4 ms pulse). Right, Trains of action potentials induced by continuous photostimulation (red, firing frequency = 107 Hz) or by current injection (black, firing frequency = 70 Hz). **C**, Blockade of light-induced IPSCs by GABA_A receptor antagonist picrotoxin. **D**, Blockade of light-induced IPSCs by P/Q type Ca^{2+} channel blocker ω -agatoxin IVA. Left, IPSCs examples before (black) and after (red) ω -agatoxin IVA application. Right, Summary bar graph shows the blockade of light-induced IPSCs by ω -agatoxin IVA ($*p < 0.05$). **E**, Lack of DSI in light-induced IPSCs by a 6 s depolarization to 0 mV.

the following (in mM): 125 NaCl, 2.5 KCl, 1.0 MgSO_4 , 2.5 CaCl_2 , 1.25 NaH_2PO_4 , 23 NaHCO_3 , 10 glucose, and 0.05 Na-metabisulfite, an antioxidant to prevent oxidation of dopaminergic reagents. Temperature was $29 \pm 1^\circ\text{C}$. Whole-cell recordings were obtained with Multiclamp 700B amplifier and Digidata 1440A (Molecular Devices). Neurons were visualized under differential interference contrast optics and infrared and epifluorescent illumination with a CCD camera (Rolera-XR, QImaging), and recorded using 3–5 M Ω pipettes filled with Cs⁺-based internal solution containing the following (in mM): 130 Cs-methanesulfonate, 5 NaCl, 1 MgCl_2 , 0.2 EGTA, 10 HEPES, 2 ATP-Mg, 0.1 GTP-Na, and 5 QX314, pH 7.3, osmolality 285 mOsm. Depolarization-induced suppression of inhibition was tested using a high-Cl[−] internal solution containing the following (in mM): 100 Cs-methanesulfonate, 60 CsCl, 1 MgCl_2 , 0.2 EGTA, 10 HEPES, 2 ATP-Mg, 0.1 GTP-Na, and 5 QX314. Series resistance (R_s ; 10–20 M Ω) was monitored throughout the experiments. Data were not included if R_s changed >15% during recording. 470 nm light pulses were generated using a high-power LED (M470L2) and driver (Thorlabs), and were delivered through a 40 $\times$ objective lens (Carl Zeiss). The effect of DA was determined as the ratio between the mean IPSC amplitude during the last 2 min of DA application and the baseline.

All chemicals were obtained from Tocris Bioscience, except that Na-metabisulfite was from Sigma, ω -agatoxin IVA was from Bachem, and Rp-8-OH-cAMP was from Axon.

Fluorescence immunohistochemistry. To verify expression of ChR2 in PV-immunoreactive neurons 3 weeks after surgery, mice were intracardially perfused with 4% paraformaldehyde (PFA) dissolved in PBS. Brains were then postfixed in PFA overnight and sliced into 75 μm sections using a vibratome. Slices were incubated for 1 h in a blocking solution containing 1.5% normal goat serum in PBS with 0.2% Triton X-100 (PBS-T). Slices were then incubated overnight at 4°C with mouse anti-PV monoclonal antibody (1:1000) (Sigma). Sections were washed 3×15 min in PBS-T followed by 2–4 h incubation with Cy5 anti-mouse secondary antibody (1:400) (Jackson Laboratories) at room temperature. Confocal images were captured with Carl Zeiss LSM510 microscope.

Statistical analysis. Data are presented as mean $\pm$ SEM. Statistical comparisons were performed using a two-tailed paired t test unless stated otherwise, and differences were considered significant with $p < 0.05$.

Results

Selective ChR2-mediated photoactivation of PV-INs in BLA

BLA injection of PV-Cre transgenic mice with an AAV containing Cre-activated ChR2-tdTomato gene resulted in red fluorescence

in both soma and axonal arbors of infected neurons (Fig. 1A). Immunostaining confirmed that ChR2 was selectively expressed in PV-INs, as all ChR2-tdTomato neurons coexpressed parvalbumin, although not all PV-INs expressed ChR2-tdTomato (Fig. 1A). Short pulses of 470 nm light depolarized the infected cells and triggered single action potentials (Fig. 1B), whereas continuous photostimulation caused high-frequency firing (Fig. 1B) similar to the typical fast-spiking of PV-INs induced by prolonged current injection (Fig. 1B).

To confirm functional activation of synapses between PV-INs and PNs, we recorded from PNs held at the membrane potential of +10 mV. Short pulses of photostimulation (0.1–1 ms) evoked strong IPSCs, which were suppressed by the GABA_A receptor antagonist picrotoxin (100 μM) (from 1.0 ± 0.2 nA to 0.02 ± 0.01 nA, $n = 5$, $p < 0.01$) (Fig. 1C). It has been shown that GABA release from PV-INs is mediated by P/Q type Ca^{2+} channels (Wilson et al., 2001; Kruglikov and Rudy, 2008). Consistently, light-evoked IPSCs in BLA PNs were dramatically suppressed by specific P/Q type Ca^{2+} channel blocker ω -agatoxin IVA (0.5 μM) (from 1.5 ± 0.3 nA to 0.09 ± 0.03 nA, $n = 4$, $p < 0.05$) (Fig. 1D). Another typical property of the PV-IN–PN synapses is the lack of depolarization-induced suppression of inhibition (DSI) due to the absence of presynaptic cannabinoid receptor 1 (Wilson et al., 2001). As expected, light-induced IPSCs in BLA did not show any DSI ($96 \pm 3\%$ of baseline after 6 s depolarization to 0 mV, $p > 0.05$, $n = 5$) (Fig. 1E). Together, our data indicate that GABAergic synapses activated by ChR2 in PV-Cre transgenic mice originate from PV-INs.

DA suppresses GABAergic transmission from PV-INs to principal neurons, but not to interneurons

It has been reported that DA disinhibits BLA by suppressing inhibitory input to PNs while enhancing FFI to INs (Bissière et al., 2003). Given that PV-INs are the major group of BLA INs (McDonald and Mascagni, 2001), we tested a hypothesis that DA modulates the synaptic transmission from PV-INs to their postsynaptic targets in a cell type-specific manner.

To assess connectivity from PV-INs to INs, we expressed ChR2-tdTomato in the BLA of Cre-PV/GAD67-GFP mice, in

Figure 2. Selective modulation of GABAergic transmission from PV-INs to PNs by DA. **A**, Expression of ChR2 (left) in BLA of *Cre-PV/GAD67-GFP* mice (middle). **B**, Recording configuration. **C**, Top, example IPSCs evoked in a BLA pyramidal neuron by photostimulation (Photo-stim) before (black) and after (red) DA application. Bottom, Time course of the IPSC amplitude in the same cell. **D**, Top, Example IPSCs evoked in a BLA INs by photostimulation before (black) and after (red) DA application. Bottom, Time course of the IPSCs amplitude in the same cell. **E**, Summarized data for the effects of DA in BLA PNs and INs. **F**, DA effect on GABAergic neurotransmission from SOM-INs to PNs and INs. The red and blue traces indicate IPSCs after DA application. $^{**}p < 0.01$ when compared with baseline.

which interneurons can be identified by green fluorescence (Fig. 2A). First, we recorded from PNs and induced GABA release from PV-INs by photostimulation (Fig. 2B). Bath application of 50 μ M DA (which provided close to the maximal effect of DA: data not shown) effectively reduced the IPSCs amplitude in PNs to $63 \pm 4\%$ of baseline ($n = 11$, $p < 0.001$) (Fig. 2C,E). Thus, PV-INs are involved in dopaminergic disinhibition of BLA PNs. In contrast to its effect in PNs, DA did not reduce IPSC amplitude in INs ($101 \pm 3\%$ of baseline, $p > 0.05$, $n = 18$) (Fig. 2D,E), indicating that DA modulates GABAergic transmission

from PV-INs in a postsynaptic cell type-specific manner. However, when we stimulated somatostatin (SOM)-positive INs, DA suppressed IPSC amplitude in both PNs ($55 \pm 10\%$ of baseline, $n = 5$, $p < 0.01$) and INs ($51 \pm 7\%$ of baseline, $n = 5$, $p < 0.01$) (Fig. 2F), suggesting distinct effects of DA on GABA release from different subtypes of INs.

Presynaptic D₂ receptors mediate the inhibitory effect of DA in principal neurons

Dopaminergic projections from the mid-brain to BLA target both PNs (Muller et al., 2009) and PV-INs (Pinard et al., 2008); therefore, our next experiments were designed to determine the action locus of DA in PV-INs projections to PNs. First, we tested paired-pulse ratio (PPR) by two consecutive pulses of light at an interval of 100 ms, and this resulted in paired-pulse depression (Fig. 3A), which is typical for PV-IN synapses (Hefft and Jonas, 2005). Application of DA increased PPR from 0.78 ± 0.13 to 1.1 ± 0.16 ($n = 5$, $p < 0.05$) (Fig. 3A), suggesting involvement of presynaptic mechanisms in the inhibitory effects of DA. To examine possible postsynaptic effects of DA, we evoked GABA_A receptor currents by local puffing of GABA_A receptor agonist muscimol (50 μ M). However, DA did not change those currents ($95 \pm 7\%$ of baseline, $n = 7$, $p > 0.05$) (Fig. 3B), indicating that DA does not affect postsynaptic GABA_A receptors function. Thus, DA suppresses IPSCs in PNs by reducing presynaptic GABA release.

Next we tested the effect of DA on photoexcitability of PV-INs expressing ChR2 in current-clamp mode. DA significantly depolarized membrane potential from -63 ± 1.4 to -56 ± 1.0 mV ($n = 8$, $p < 0.01$) and reduced input resistance from 138 ± 11 to 112 ± 15 M Ω ($n = 8$, $p < 0.05$), but did not decrease the number of spikes evoked by 100 ms pulses of suprathreshold photostimulation (from 6.3 ± 1.5 to 6.6 ± 1.6 , $n = 8$, $p > 0.05$) (Fig. 3C), indicating that the effect of DA on GABA release to PNs did not result from changes in the somatic excitability of PV-INs.

We next examined the role of D₁ and D₂ receptors, which are both expressed in BLA (Pinto and Sesack, 2008), in the inhibitory effects of DA. DA inhibition was not affected by specific D₁ receptor antagonist SCH23390 (10 μ M) (DA $\times$ SCH23390 interaction: $F_{(1,15)} = 0.01$, $p > 0.05$, repeated-measures ANOVA). In the presence of SCH23390, DA inhibited IPSCs to $69 \pm 1\%$ of the pre-DA level ($p < 0.05$, $n = 6$). In contrast, IPSC inhibition by DA was abolished by the D₂ receptor antagonist raclopride (10 μ M) ($F_{(1,18)} = 7.6$, $p < 0.01$ for DA $\times$ raclopride interaction, repeated-measures ANOVA). In the presence of raclopride, DA

Figure 3. Presynaptic D_2 receptor mediates the inhibitory effects of DA in PV-IN projections to PNs. **A**, Top, Example traces of synaptic responses to paired-pulse stimulation before (black) and after (red) DA application. Bottom, Summary graph shows changes of paired-pulse ratio during DA application. **B**, Top, Example traces of muscimol-puffing-induced $GABA_A$ receptor current before (black) and after (red) DA application. Bottom, Summary graph shows muscimol-induced current during DA application. **C**, Top, Firing pattern of a PV-IN expressing ChR2 upon photostimulation (light) before and after DA application. Bottom, Effects of DA on membrane potential (MP; left), input resistance (R_{in} ; middle), and number of spikes (right) in PV-INs. **D**, Top, Example IPSC traces before and after DA application in the presence of raclopride (Rac), and the IPSC traces before and after application of quinpirole. Bottom, Summary bar graph shows inhibition of IPSC by DA, lack of such inhibition in the presence of raclopride, and inhibition by quinpirole. Each dot represents a single cell. ** $p < 0.01$, *** $p < 0.005$. n.s., Nonsignificant, when compared with baseline.

had a negligible inhibitory effect ($95 \pm 3\%$ of pre-DA level, $n = 9$, $p > 0.05$) (Fig. 3D). Consistently, specific D_2 receptor agonist quinpirole ($10 \mu M$) mimicked DA inhibition by suppressing IPSCs in PNs ($67 \pm 6\%$ of baseline, $n = 7$, $p < 0.01$) (Fig. 3D). Thus, DA inhibits GABA release from PV-INs to PNs mainly via presynaptic D_2 receptors.

cAMP-dependent signaling mediates the inhibitory effects of DA

To investigate the transduction mechanisms underlying dopaminergic inhibition in PNs, we evaluated the contribution of G-protein-gated inwardly rectifying K^+ (GIRK) channels and cAMP-dependent signaling cascade, both of which are regulated by D_2 receptor (Beaulieu and Gainetdinov, 2011). In the presence of the specific GIRK channel blocker tertiapin-Q (200 nM), DA inhibited IPSCs (from 1.5 ± 0.15 to 0.9 ± 0.07 nA, $n = 5$, $p < 0.05$) (Fig. 4A), suggesting that the GIRK channel is not involved in the DA-induced inhibition. In contrast, Rp-cAMP ($100 \mu M$), a nonhydrolyzable competitor of cAMP, abolished the inhibitory effect of DA (from 0.9 ± 0.1 to 0.89 ± 0.1 nA, $n = 6$, $p > 0.05$) (Fig. 4B). Moreover, H-89 ($10 \mu M$), a protein kinase A (PKA) inhibitor, also prevented DA inhibition of IPSCs in PNs (from 1.6 ± 0.2 nA to 1.6 ± 0.2 nA, $n = 5$, $p > 0.05$) (Fig. 4C), indicating that DA inhibition is due to the suppression of cAMP-PKA cascade upon D_2 receptor activation in PV-INs.

Gating of GABA release from PV-INs by cAMP

We next investigated the role of cAMP-dependent signaling in controlling GABA release from PV-INs. Direct suppression of

cAMP-dependent process by bath application of Rp-cAMP ($100 \mu M$) dramatically reduced IPSC amplitude in PNs (from 1.4 ± 0.2 to 0.9 ± 0.1 nA, $n = 6$, $p < 0.01$) (Fig. 4D), suggesting that GABA release from PV-INs to PNs is sustained by basal cAMP-dependent signaling. In contrast to its effect in PNs, Rp-cAMP did not alter IPSC amplitude in INs (from 0.7 ± 0.08 to 0.7 ± 0.1 nA, $n = 7$, $p > 0.05$) (Fig. 4E), suggesting that, at the basal state, cAMP-dependent signaling is not involved in GABA release from PV-INs to INs. Furthermore, bath application of forskolin ($50 \mu M$), an adenylate cyclase activator, did not change IPSC amplitudes in INs either (from 0.9 ± 0.1 to 1.0 ± 0.1 nA, $n = 8$, $p > 0.05$) (Fig. 4F), but increased the GABA release induced by local electrical stimulation (from 0.7 ± 0.2 to 1.0 ± 0.2 nA, $n = 8$, $p < 0.01$), suggesting that control of GABA release by cAMP-dependent signaling is selectively absent in the synapses from PV-INs to BLA INs. In these experiments, postsynaptic effects of Rp-cAMP were ruled out by intracellular application of a membrane-impermeable cAMP analog Rp-8-OH-cAMP (4 mM) through a recording pipette. The above results indicate that GABA release from PV-INs to BLA PNs and INs is differentially controlled by a cAMP-dependent process, which might be the major mechanism underlying the target-specific effect of DA in BLA.

Discussion

Here, we investigate a circuit mechanism of dopaminergic disinhibition in BLA, focusing on DA modulation of GABA release from local PV-INs. We find that DA suppresses GABA release from PV-INs in a target cell-specific manner—DA suppresses the release to PNs, but not to INs. This result indicates the PV-INs are

Figure 4. The role of cAMP-dependent signaling in target-specific inhibition of GABA release from PV-INs by DA. **A**, Specific GIRK channel inhibitor tertipin-Q did not interfere with DA inhibition of IPSCs in PNs. Left, Time course of IPSC amplitude from an example cell. Right, Summary graph showing the effect of DA after blockade of GIRK. Each symbol represents a single neuron. **B**, **C**, Inhibitory effect of DA was blocked by cAMP competitor Rp-cAMP (**B**) and by PKA inhibitor H-89 (**C**). **D**, **E**, Rp-cAMP alone reduced the IPSC amplitude in PNs (**D**) but not in INs (**E**). **F**, Adenylate cyclase activator forskolin did not affect GABA release from PV-INs to INs. * $p < 0.05$, ** $p < 0.01$.

one component of the amygdala disinhibition by DA, in which PV-INs decrease inhibition of PNs, but keep inhibition of interneurons intact. Finally, we show that cAMP-dependent signaling affects GABA release from the PV-IN terminals that target PNs, but not INs, which provides one mechanism for the target-specific action of DA. Given that distinct subtypes of INs differ in the firing patterns and in the subcellular domain of PNs that they target, it is expected that each IN subtype is specialized in controlling a particular aspect of PN activity. A recent study supports this notion by showing that distinct subtypes of BLA interneurons are differentially modulated by external stimuli (Bienvenu et al., 2012), which might indicate their differential involvement in BLA disinhibition. Therefore, to obtain a mechanistic understanding of BLA disinhibition, it is necessary to analyze DA effects on inhibitory synapses originating from defined IN classes. Previous studies documented that DA suppresses FFI in BLA PNs (Bissière et al., 2003; Marowsky et al., 2005). Here, we focused on a specific interneuronal subtype, the PV-INs, which we activate selectively as a group using an optogenetics technique. We find that DA suppresses GABA release from PV-INs to PNs. This observation is consistent with the notion that PV-INs contribute to FFI in PNs (Woodruff and Sah, 2007; Pape and Pare, 2010). Surprisingly, despite earlier reports of DA enhancing FFI in BLA INs (Bissière et al., 2003), we did not find any effect of DA on GABAergic transmission from PV-INs to BLA INs. Furthermore, DA suppresses GABAergic transmission from SOM-IN to INs, which argues that the enhancement of IPSC in INs by DA is mediated by an unknown group of INs that are PV-negative and SOM-negative cells. Altogether, the existing evidence indicates that DA effects are unique among different IN subtypes.

Accumulating data indicate that PV-INs are not a homogeneous class, they include basket cells providing perisomatic inhibition but showing no coupling with hippocampal theta, the axo-axonic cells activated by noxious stimuli, and the AStria-projecting neurons suppressed by noxious stimuli (Bienvenu et al., 2012). Since in our study IPSCs represent the cumulative activity of all PV-INs with ChR2, we do not rule out a possibility that a certain subgroup of PV-INs does not contribute to the observed effects of DA. None of our data rules out the contribution of other subtypes of INs in BLA disinhibition by DA, such as the paracapsular intercalated cells (Marowsky et al., 2005) or another unknown type of IN through which DA increases the inhibition of INs (Bissière et al., 2003). Nevertheless, thorough analyses of neuromodulation of inhibitory synapses from distinct IN subtypes using interneuron-specific Cre lines will help understand how different subclasses of interneurons interact to ultimately control BLA output.

The target cell-specific DA effect on GABA release from PV-INs is a plausible explanation of DA disinhibition of the amygdala; yet, it raises a mechanistic question—how it can be achieved. There are two potential mechanisms: (1) a specific subgroup of PV-INs with D_2 receptor sensitivity projects to PNs, but not to INs; and (2) presynaptic D_2 receptor differentially regulates GABA release from distinct terminals along the same axon of PV-INs, depending on the postsynaptic target. Importantly, our data suggest that synapses between PV-INs are not modulated by DA, because we did not see an effect of DA on IPSCs in *GAD67-GFP*-positive cells, a significant portion of which are PV-INs. Therefore, if the first mechanism was correct, the D_2 -sensitive PV-INs should not make any connections among themselves; however, the existence of such a special subtype of PV-INs is very unlikely, given the fact

that PV-INs are highly connected to one another (Muller et al., 2005; Woodruff and Sah, 2007). Moreover, it has been well documented that regulation of neurotransmitter release and synaptic plasticity can be compartmentalized at divergent release sites along a common axon (Markram et al., 1998; Scanziani et al., 1998), which is consistent with the second mechanism.

As for molecular and cellular mechanisms of the compartmentalization, the target cell-specific DA effect could be attributed to differential expression of D₂ receptors or distinct intracellular signaling at different presynaptic terminals. We explore the latter possibility by examining the signal transduction downstream D₂ receptor. Consistent with the earlier observations that basal cAMP/PKA signaling is involved in GABA release in the hippocampus and prefrontal cortex (Chevalleyre et al., 2007; Chiu et al., 2010), we find that GABA release from PV-INs to PNs in BLA is controlled by basal activity of cAMP-dependent signaling. However, GABA release from PV-INs to BLA INs is sensitive to neither suppression nor activation of cAMP signaling. Thus, cAMP signaling gates GABA release from PV-INs differently, depending on postsynaptic targets, and accounts for the target cell-specific DA effect in BLA.

While mechanisms that contribute to the gating of GABA release by cAMP remain unclear, exploring the downstream targets of cAMP-dependent cascade in PV-IN terminals projecting to PNs, such as voltage-gated Ca²⁺ channels and presynaptic active zone proteins, like RIM1 α (Chevalleyre et al., 2007), will help in understanding the principles underlying the fine-tuning of the amygdala interneuronal circuit by neuromodulators.

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