RESEARCH ARTICLE SUMMARY

NEURODEVELOPMENT

Convergence of adenosine and GABA signaling for synapse stabilization during development

Ferran Gomez-Castro†, Stefania Zappettini†, Jessica C. Pressey†, Carla G. Silva, Marion Russeau, Nicolas Gervasi, Marta Figueiredo, Claire Montmasson, Marianne Renner, Paula M. Canas, Francisco Q. Gonçalves, Sofia Alçada-Morais, Eszter Szabó, Ricardo J. Rodrigues, Paula Agostinho, Angelo R. Tomé, Ghislaine Caillol, Olivier Thoumine, Xavier Nicol, Christophe Leterrier, Rafael Lujan, Shiva K. Tyagarajan, Rodrigo A. Cunha, Monique Esclapez, Christophe Bernard*, Sabine Lévi*

INTRODUCTION: During development, brain circuits go through phases of synapse formation, stabilization, or elimination. γ-aminobutyric acid–mediated (GABAergic) synapse formation depends mainly on cell adhesion molecules, such as neuroligins and leucine-rich repeat transmembrane proteins, that interact with presynaptic neurexins and Slit- and Trk-like family proteins that bind to presynaptic protein tyrosine phosphatases. GABA and GABA type A ($GABA_A$) receptors are involved in an activity-dependent manner in the maturation and pruning of synapses. Adenosine triphosphate (ATP) and adenosine can be coreleased with GABA at synapses to be perceived by adenosine A_{2A} receptors. We tested the role of adenosine signaling in the stabilization and elimination of GABAergic synapses.

RATIONALE: A_{2A} receptors control migration speed, axonal elongation, and dendrite branching. Whether A_{2A} receptors control synapse formation, stabilization, or elimination in the brain is not known. In the adult brain, A_{2A} receptors are mostly expressed on presynaptic terminals, where they control the probability of synaptic vesicle release. The amount, location, and function of A_{2A} receptors at neural synapses during early brain development has been unclear.

RESULTS: During synaptogenesis in the developing mouse hippocampus, between postnatal days P5 and P16, the density of A_{2A} receptors increases transiently around the postsynaptic density. Activity-dependent release of its endogenous ligand, adenosine, increases as well. A_{2A} receptors control the fate of GABAergic synapses. Suppression of A_{2A} receptors, their pharmacological blockade, or the removal of adenosine results in the destabilization of pre- and postsynaptic sites in vivo, ex vivo, and in vitro. If A_{2A} receptors remain inactive for >20 min, synapse destabilization is irreversible. We found that A_{2A} receptor activation is necessary and sufficient for GABAergic synapse stabilization, whereas $GABA_A$ receptor activation is not necessary as long as A_{2A} receptors remain activated. We studied the molecular mechanism at play. A_{2A} receptor and $GABA_A$ receptor signaling pathways converge onto calcium-calmodulin–sensitive adenylyl cyclases to produce adenosine 3′,5′-monophosphate (cAMP). The resulting activation of protein kinase A leads to phosphorylation of the postsynaptic scaffolding molecule gephyrin at the protein kinase A–sensitive serine residue 303 site. Expression of the gephyrin mutant mimicking this phosphorylation state prevents synapse loss upon the removal of extracellular adenosine. Phosphorylated gephyrin can be coimmunoprecipitated with the postsynaptic transmembrane Slit- and Trk-like family protein 3 that binds in the synaptic cleft to presynaptic protein tyrosine phosphatase σ to organize inhibitory synapses. The contribution of Slit- and Trk-like family protein 3 in stabilizing GABAergic synapses through adenosine signaling is demonstrated with a short hairpin RNA (shRNA) approach or after the expression of a mutant. Finally, antagonizing A_{2A} receptors during synaptogenesis in vivo results in the loss of GABAergic synapses during development and cognitive deficits when animals reach adulthood.

CONCLUSION: A_{2A} receptors regulate the elimination of certain GABAergic synapses when they become inactive. A_{2A} receptors are poised to detect active presynaptic terminals and trigger synapse removal after a defined period of synaptic inactivity. ■

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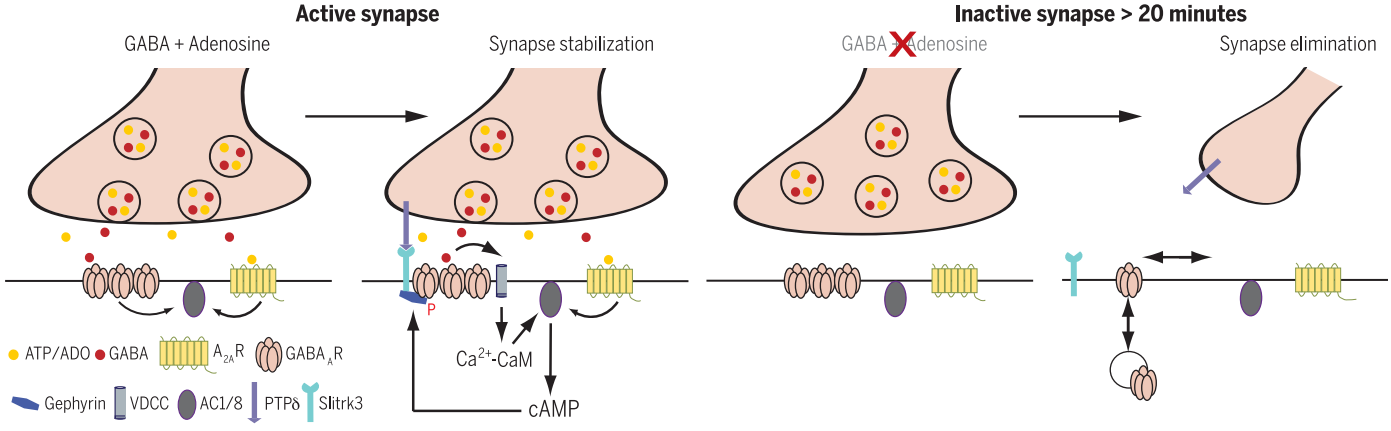

Adenosine signaling stabilizes nascent GABAergic synapses. (Left) Active synapse: Corelease of adenosine, ATP, and GABA activates A_{2A} receptors and $GABA_A$ receptors, whose signaling pathways converge on Ca^{2+}-calmodulin–dependent adenylyl cyclases and cAMP production, which in turn may stabilize the nascent synapse through recruitment of the Slitrk3-PTPσ transsynaptic organizers by gephyrin phosphorylated at a protein kinase A (PKA) site. (Right) Inactive synapse: In the absence of adenosine, ATP, and GABA release at inactive synapses, this pathway is not activated, and the synapse is eliminated. ADO, adenosine; A_{2A}R, adenosine type 2A receptor; $GABA_A$R, $GABA_A$ receptor; VDCC, voltage-dependent calcium channel; AC1 or AC8, adenylyl cyclase 1 or 8; PTPδ, protein tyrosine phosphatase δ; Slitrk3, Slit- and Trk-like family member 3; CaM, calmodulin.

RESEARCH ARTICLE

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Convergence of adenosine and GABA signaling for synapse stabilization during development

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During development, neural circuit formation requires the stabilization of active γ-aminobutyric acid–mediated (GABAergic) synapses and the elimination of inactive ones. Here, we demonstrate that, although the activation of postsynaptic GABA type A receptors ($GABA_AR$s) and adenosine A_{2A} receptors (A_{2A}Rs) stabilizes GABAergic synapses, only A_{2A}R activation is sufficient. Both $GABA_AR$- and A_{2A}R-dependent signaling pathways act synergistically to produce adenosine 3′,5′-monophosphate through the recruitment of the calcium–calmodulin–adenylyl cyclase pathway. Protein kinase A, thus activated, phosphorylates gephyrin on serine residue 303, which is required for $GABA_AR$ stabilization. Finally, the stabilization of pre- and postsynaptic GABAergic elements involves the interaction between gephyrin and the synaptogenic membrane protein Slitrk3. We propose that A_{2A}Rs act as detectors of active GABAergic synapses releasing GABA, adenosine triphosphate, and adenosine to regulate their fate toward stabilization or elimination.

During development, brain circuits go through different phases of synapse formation, stabilization, and elimination, which involve numerous molecular mechanisms, in particular at γ-aminobutyric acid–mediated (GABAergic) synapses. Synaptic cell adhesion molecules are essential for synapse formation and maturation, including neuroligins and leucine-rich repeat transmembrane proteins (LRRTMs) that interact with presynaptic neurexins, Slit- and Trk-like family proteins (Slitrks), which bind to presynaptic protein tyrosine phosphatases (PTPs), immunoglobulin superfamily proteins (IgSFs), cadherin family proteins, and transmembrane tyrosine kinase receptors (*1*–*4*). The γ-aminobutyric acid (GABA) neurotransmitter itself regulates the maturation and innervation patterns of GABAergic synapses as well as their elimination and pruning (*4*–*8*). GABA operates through the activation of GABA type A receptors ($GABA_AR$s) and the elevation in intraneuronal calcium Ca^{2+} levels after the activation of voltage-dependent Ca^{2+} channels (*9*, *10*). Additionally, the adenosine signaling pathway is also involved during development: Extracellular adenosine builds up with synaptic activity (*11*, *12*), and adenosine A_{2A} receptors (A_{2A}Rs) control the migration speed of GABAergic neurons (*13*), axonal elongation and dendritic branching (*14*), and synapse stabilization or elimination at the neuromuscular junction (*15*). Because GABA could be coreleased with adenosine triphosphate (ATP) and adenosine in a synaptic activity–dependent manner (*16*, *17*), we tested the role of adenosine signaling on GABAergic synaptogenesis in the brain.

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Results

A_{2A}Rs are transiently expressed at developing GABAergic synapses

In the adult hippocampus, there is a low density of A_{2A}Rs, which are essentially presynaptic (*18*). During the peak of synaptogenesis, between postnatal days P5 and P16, we found a transient increased density of A_{2A}Rs in mouse hippocampi, in particular in purified synaptic contacts (fig. S1). Electron microscopy showed their post- and perisynaptic localizations on symmetric, presumably GABAergic, synapses (fig. S1). We also confirmed the presence of A_{2A}Rs in primary cultures of hippocampal neurons. A_{2A}Rs were clustered at synapses containing glutamic acid decarboxylase 67 kD [(GAD67) a GABA synthesizing enzyme], and clustering increased during synaptogenesis between 7 and 14 days in vitro (DIV) (fig. S1). A_{2A}Rs were not present at all GABAergic synapses, but they accumulated at a subset (39.1 ± 3.7%, n = 34 cells, three independent experiments) of synapses. DNA points accumulation for imaging in nanoscale topography (DNA-PAINT) further confirmed that A_{2A}Rs were located near the GABAergic postsynapse identified by the presence of the scaffolding molecule gephyrin at DIV 10 (fig. S1). We then asked whether such enrichment of post- and perisynaptic A_{2A}Rs at GABAergic synapses was accompanied by an increase in activity-dependent release of their ligand adenosine.

ATP and adenosine release is increased during synaptogenesis

Adenosine can originate from its direct activity-dependent release by presynaptic terminals or neighboring glial cells and/or from the conversion of ATP released by neurons or glial cells through the ecto-5′-nucleotidase CD73 (*11*, *12*). The evoked release of adenosine was larger from P7 than P60 (adult) hippocampal synaptosomes (fig. S2), and blocking CD73 with adenosine 5′-(α,β-methylene)diphosphate (AMPCP) (100 μM) decreased extracellular adenosine by 25% (fig. S2). Accordingly, we found a large density of CD73 in synapses during the peak of synaptogenesis (fig. S2), in keeping with the tight association of the enzyme with A_{2A}Rs (*19*). Therefore, one fraction of extracellular adenosine comes from local extracellular ATP metabolism, and most of the adenosine is likely released as such through nonconcentrative nucleoside transporters upon its intracellular formation as a by-product of metabolic activity sustaining synaptic activity (*11*, *12*, *20*). The activity-dependent secretion of ATP and adenosine was also larger at P7 than at P60 (fig. S2). Thus, the activation of synaptic terminals at the early stage of synaptogenesis bolsters the release of adenosine (via yet-unclear mechanisms) and of vesicular ATP, which is efficiently converted into extracellular adenosine by CD73. Given the presence of A_{2A}Rs at inhibitory synapses and the activity-dependent production of its ligand adenosine during the period of synaptogenesis, we hypothesized a role for this pathway in GABAergic synapse formation and elimination. Because GABA controls the number of GABAergic synapses during development (*4*–*8*), we tested the relative contribution of $GABA_AR$ and A_{2A}R pathways.

A_{2A}Rs and $GABA_AR$s control GABAergic synapse fate

Incubating neurons with tetanus toxin (TeNT) (1 to 40 nM) in vitro, which abolishes vesicular release of neurotransmitters (*21*), resulted in

the disappearance of 43% of GABAergic synaptic terminals labeled for the vesicular GABA transporter (VGAT) and 62% of postsynaptic $GABA_AR$ γ2 subunit clusters, which indicates synapse dismantling (fig. S3). Synaptic loss was fully prevented by the activation of $A_{2A}Rs$ with CGS21680 (30 nM) or $GABA_ARs$ with muscimol (10 μM) for 30 min (fig. S3). The rescue of GABAergic synapses occurred within 30 min of $A_{2A}R$ or $GABA_AR$ activation, ruling out de novo synapse formation, which requires hours (*22*). Together, these results show that, in the absence of synaptic transmission, the activation of either $A_{2A}Rs$ or $GABA_ARs$ by their respective ligands is sufficient to maintain GABAergic synapses that would have otherwise disappeared. This led us to hypothesize that both adenosine and GABA signaling pathways play a role in activity-dependent synapse stabilization and that when presynaptic sites are inactive, such synapses are eliminated. Because the activation of either receptor could rescue GABAergic synapses to an extent similar to that observed when both receptors were simultaneously activated (fig. S3), we further hypothesized a convergence of both $A_{2A}R$ and $GABA_AR$ signaling pathways.

$A_{2A}Rs$ are sufficient to stabilize GABAergic synapses

To test the interdependence of the two $A_{2A}R$ and $GABA_AR$ pathways, we blocked one while activating the other, and we investigated the fate of GABAergic synapses in vitro. With specific short hairpin RNAs (shRNAs), we knocked down the $GABA_AR$ γ2 subunit—which leads to synaptic loss (*23*)—or $A_{2A}Rs$ (*24*), and we activated $A_{2A}Rs$ or $GABA_ARs$ with their selective agonists CGS21680 (30 nM) or muscimol (10 μM), respectively. Neurons transfected at DIV 6 to DIV 7 with shRNAs against $GABA_ARγ2$ (shγ2) or $A_{2A}Rs$ ($shA_{2A}R$) showed, respectively, a 51 and 43% reduction of GABAergic synapses (Fig. 1, A and B). Because <10 out of 100,000 cells were transfected and because we did not find any evidence of synapse destabilization in the nontransfected cells surrounding the transfected ones, we conclude that the destabilization of GABAergic synapses occurs as a result of the loss of postsynaptic $GABA_ARs$ or $A_{2A}Rs$. The activation of $A_{2A}Rs$ with CGS21680 rescued GABAergic synapses in neurons lacking $GABA_ARγ2$ (Fig. 1A). By contrast, activation of $GABA_ARs$ by muscimol failed to rescue GABAergic synapses in $shA_{2A}R$-expressing neurons (Fig. 1B). Thus, $A_{2A}R$ activation appears to be necessary and sufficient for GABAergic synapse stabilization, whereas $GABA_AR$ activation is not necessary as long as $A_{2A}Rs$ remain activated. According to this scheme, blocking $A_{2A}Rs$ should result in synapse loss.

$A_{2A}R$ blockade triggers GABAergic synapse loss

A 30-min application of the selective $A_{2A}R$ antagonist SCH58261 (100 nM) in vitro decreased the number of clusters of the presynaptic protein VGAT as well as postsynaptic $GABA_ARγ2$ and scaffolding protein gephyrin (fig. S4), demonstrating a destabilization of GABAergic synapses at both pre- and postsynaptic sites. Acute treatment with SCH58261 induced a loss of GABAergic synapses expressing the $GABA_AR$ γ2 subunit but also of synapses containing the α1 or α2 subunits (fig. S4), which suggests that several types of GABAergic synapses are controlled by $A_{2A}Rs$. Not all synapses disappeared, as ~30% of GABAergic synapses were destabilized (fig. S4), in keeping with the finding that not all GABAergic synapses are equipped with $A_{2A}Rs$ at any given developmental time (fig. S1). The fact that the loss of GABAergic synapses induced by $A_{2A}R$ blockade was similar to that found in $shA_{2A}R$-expressing neurons (Fig. 1B) suggests that synaptic loss was not a result of an indirect effect on network activity. In keeping with the reduced synaptic clustering of $GABA_ARγ2$, quantum dot–based single-particle tracking of $GABA_ARγ2$ in DIV 8 hippocampal

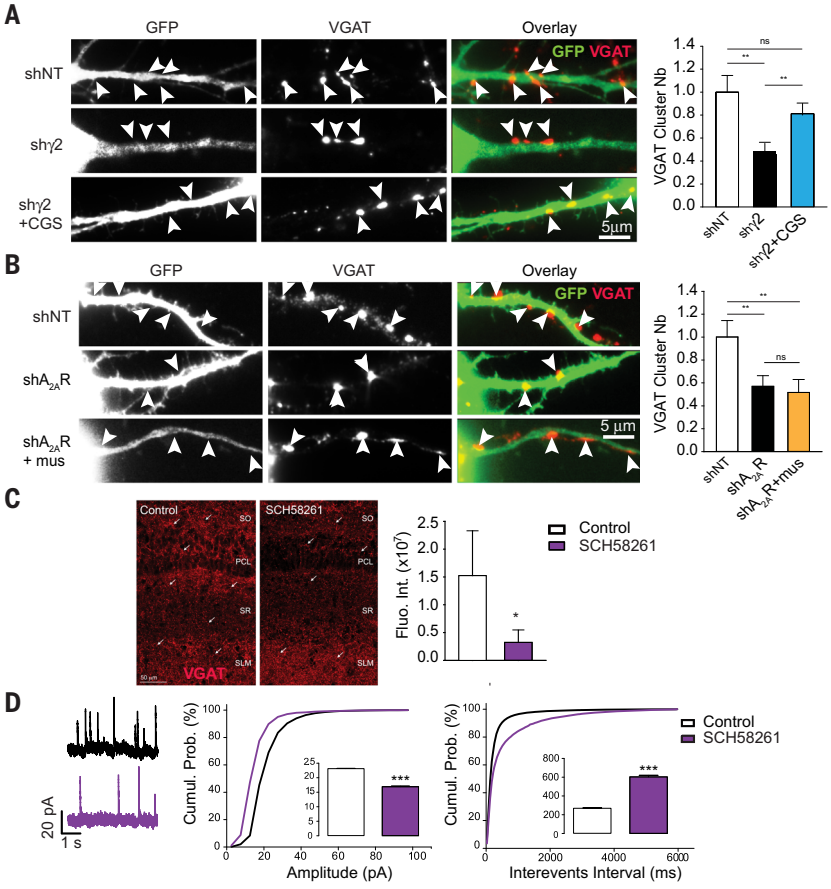

Fig. 1. $A_{2A}R$ activation stabilizes GABAergic synapses. (**A**) VGAT staining (left) and quantification (right) in DIV 10 to 11 neurons transfected with nontarget (shNT) or on-target $GABA_ARγ2$ (shγ2) shRNAs exposed or not exposed to CGS21680 (CGS) (30 nM) for 30 min. shNT, *n* = 60; shγ2, *n* = 52; shγ2 and CGS21680, *n* = 54; four cultures. The loss of GABAergic synapses upon suppression of $GABA_ARγ2$ was rescued by activation of $A_{2A}Rs$. Cluster Nb, cluster number. (**B**) VGAT staining (left) and quantification (right) in DIV 10 to 11 neurons transfected with nontarget (shNT) or on-target $A_{2A}R$ ($shA_{2A}R$) shRNAs exposed or not exposed to muscimol (mus) (10 μM) for 30 min. shNT, *n* = 44; $shA_{2A}R$, *n* = 47; $shA_{2A}R$ and muscimol, *n* = 36; four cultures. The loss of GABAergic synapses upon suppression of $A_{2A}R$ was not rescued by $GABA_AR$ activation. Scale bar, 5 μm. Arrowheads in (A) and (B) show examples of inhibitory synapses labeled for VGAT. (**C**) Acute application of SCH58261 (100 nM) for 30 min in hippocampal slices decreased the density of VGAT-immunoreactive terminals by 47% (eight slices, three P6 pups) compared with controls (six slices, three P6 pups). Fluo. Int., fluorescence intensity. (**D**) Application of SCH58261 (100 nM) for 30 min reduced the amplitude and frequency of $GABA_AR$-mediated mIPSCs in CA1 pyramidal cells (eight cells, eight slices, six P6 pups). (Left) Examples of mIPSCs recorded before and after application of SCH58261. (Insets) IPSC amplitude or interevent interval. Cumul. Prob., cumulative probability. Values were normalized to the control values [(A) and (B)]; histograms represent means and SEMs. Statistics were calculated using the Mann-Whitney test [(A), (B), and (C)] and the Kolmogorov-Smirnov test (D). ns, not significant; $*P \le 0.05$; $**P < 0.01$; $***P < 0.001$.

neurons revealed that the membrane diffusion of $GABA_A$Rs increased within 10 min after bath application of SCH58261 (100 nM) (fig. S5). Finally, this adenosine-dependent mechanism seems specific to A_{2A}Rs because blocking adenosine A_1 receptors had no effect on the density of GABAergic synapses (fig. S6). We then set out to confirm these results ex vivo.

Immunohistochemistry revealed that blocking A_{2A}Rs for 30 min with SCH58261 (100 nM) in P6 hippocampal slices triggered a 47% loss of VGAT-containing synaptic terminals (Fig. 1C). Single-cell recordings of miniature inhibitory postsynaptic currents (mIPSCs) in hippocampal CA1 pyramidal cells at P6 showed that a 30-min application of SCH58261 (100 nM) decreased the frequency of mIPSCs by ~40% (Fig. 1D). The amplitude of mIPSCs was decreased by ~30% (Fig. 1D). We found the same effect on mIPSCs recorded in CA1 interneurons (fig. S7) and in layer V pyramidal cells of the V1 visual cortex (fig. S7). Because the effects of SCH58261 on GABAergic currents could be an indirect consequence of the modulation of glutamatergic neurotransmission by A_{2A}Rs, we verified that the effect was still present in the presence of glutamate receptor blockers (fig. S8). Together, these results show that A_{2A}Rs control the stability of GABAergic synapses in different cell types and brain regions at early stages of development. We then assessed whether this role of A_{2A}Rs was developmentally regulated and studied the minimum time of A_{2A}R inactivity necessary to trigger synapse removal.

A_{2A}R-mediated stabilization of GABAergic synapses is time dependent

In keeping with the transient increased density of A_{2A}Rs during the first 2 postnatal weeks (fig. S1), we found that the control of GABAergic synapses by A_{2A}Rs ex vivo was restricted to the period of synaptogenesis (between P4 and P12), with a maximum effect at P6 (fig. S9). A morphological analysis performed in cultures confirmed that the action of A_{2A}Rs was also developmentally regulated (fig. S10).

We then assessed whether there is a specific time window beyond which A_{2A}R inactivity triggers an irreversible synapse destabilization. Varying the time of application of SCH58261, we found that the effect on mIPSCs was reversible if A_{2A}R blockade did not exceed 10 min (fig. S9). Beyond 20 min, the effect was irreversible (fig. S9). Similarly, there was no loss of GABAergic synapses 10 min after A_{2A}R blockade in cultures, whereas it occurred after 30 min of treatment (fig. S11). Thus, the A_{2A}R-dependent control of GABAergic synapse density is developmentally regulated, and a period of at least 20 min of inactivity of A_{2A}Rs is sufficient to trigger the removal of GABAergic synapses.

Together, our results show that A_{2A}Rs satisfy the three requirements of a system able to control the fate of synapses toward stabilization or elimination during development. It is time dependent, its activation maintains synapses, and the absence of activation results in synapse elimination. We then investigated the molecular mechanisms upstream and downstream of A_{2A}R activation.

Adenosine is required for synapse stabilization

Application of the CD73 inhibitor AMPCP (100 μM) together with adenosine deaminase (ADA) (4 to 20 U/mL), which hydrolyses adenosine into inosine, decreased mIPSC amplitude (30%) and frequency (74%) ex vivo (Fig. 2A). The same treatment reduced the time $GABA_A$Rγ2 spent at inhibitory synapses in vitro—an effect that was prevented by the direct activation of A_{2A}Rs with their agonist CGS21680 (fig. S5). This increased escape of $GABA_A$Rγ2 from inhibitory synapses upon the removal of ambient adenosine was accompanied by a rapid disappearance of active inhibitory synaptic boutons (labeled by preloading of a VGAT-oyster^{550} antibody into synaptic vesicles during multiple vesicular exocytosis-endocytosis cycles) and increased trafficking of vesicle packets (*22*) into axons (fig. S11), probably resulting from the destabilization of presynaptic active zones. This rapid dismantling of GABAergic pre- and postsynaptic compartments induced by the withdrawal of ambient adenosine with ADA and AMPCP resulted in a net loss of the number of GABAergic synapses that could be prevented by A_{2A}R activation in vitro (Fig. 2B).

To test whether glial cells are necessary to produce the adenosine required to activate A_{2A}Rs, we pretreated cultures with the gliotoxin cytosine arabinoside (araC), which resulted in the complete loss of glial fibrillary acidic protein (GFAP)–stained glial cells (fig. S12). The application of ADA and AMPCP still induced synapse loss, which was prevented by CGS21680 (fig. S12). This indicates that glial

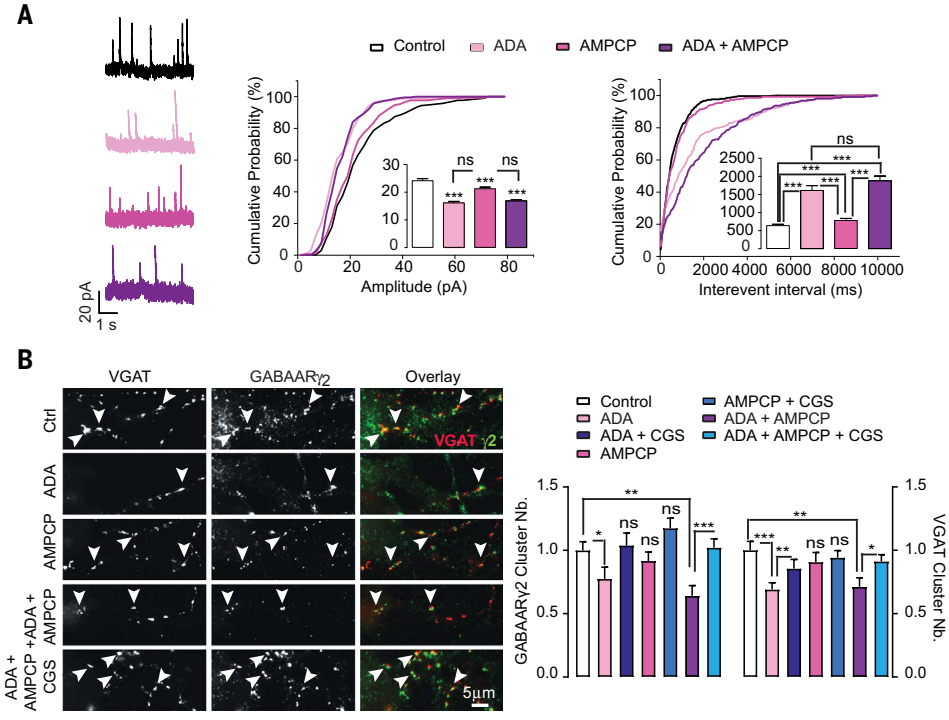

Fig. 2. Synapse stabilization requires extracellular adenosine production.
(**A**) Decreasing extracellular adenosine levels with a 30-min treatment of ADA (4 to 20 U/mL), AMPCP (100 μM), or ADA and AMPCP led to a decrease of mIPSCs' amplitude and frequency in CA1 pyramidal cells. *N* = 12 cells, 12 slices, 6 pups. (**B**) Immunostaining and quantification of VGAT and $GABA_A$Rγ2 in DIV 10 neurons in the absence or presence of the indicated drugs for 30 min. Arrowheads show examples of inhibitory synapses labeled for VGAT and $GABA_A$Rγ2. Scale bar, 5 μm. Decreasing extracellular adenosine levels lead to synapse destabilization, an effect prevented by the direct activation of A_{2A}Rs with 30 nM CGS21680. *N* = 26 to 41 cells, three cultures. Ctrl, control. Histograms represent means and SEMs. Statistics were calculated using the Kolmogorov-Smirnov test (A) and the Mann-Whitney test (B). ns, not significant; $*P \leq 0.05$; $**P < 0.01$; $***P < 0.001$.

cells are not the main source of adenosine and that neurons can produce adenosine (directly or indirectly as a by-product of its phosphorylated forms) in the absence of glial cells in a sufficient quantity to activate A_{2A}Rs.

Altogether, these results show that direct adenosine activation of A_{2A}Rs is necessary and sufficient to maintain synapse integrity. We then looked at the downstream effects of A_{2A}R activation, starting with the possible cooperativity between GABAergic and A_{2A}R signaling pathways, as suggested above (fig. S3).

A_{2A}Rs and GABA$_A$Rs converge to stabilize synapses

A_{2A}Rs stabilize GABAergic synapses via the AC-cAMP pathway

A_{2A}Rs are G protein–coupled receptors, and their prime transducing system is the activation of adenylyl cyclases (ACs), which generate adenosine 3′,5′-monophosphate (cAMP). If synapse stabilization is G protein–dependent, blocking G protein activity should destabilize synapses. Recording CA1 pyramidal cells with an intracellular solution containing guanosine diphosphate-β-S to block G protein activity directly led to a decrease of mIPSC frequency and amplitude ex vivo (~20 and 15%, respectively; fig. S13), reproducing the effect of A_{2A}R blockade.

We then tested the involvement of the AC-cAMP signaling cascade, which is downstream of A_{2A}R activation. Bath application of 3-isobutyl-1-methylxanthine (IBMX) (100 μM, a nonselective inhibitor of phosphodiesterases that metabolizes cAMP) and forskolin (10 μM, an activator of ACs) prevented the SCH58261-induced decrease of mIPSC frequency and amplitude in CA1 pyramidal cells ex vivo (fig. S13). Similarly, IBMX and forskolin prevented the loss of GABAergic synapses induced by SCH58261 in hippocampal neuron cultures (fig. S13). Therefore, the A_{2A}R-dependent control of GABAergic synapses occurs through the activation of the AC-cAMP signaling cascade.

A_{2A}Rs and GABA$_A$Rs elevate intracellular cAMP levels

At what level do the A_{2A}R and GABA$_A$R signaling pathways converge to regulate synaptogenesis? A_{2A}Rs and GABA$_A$Rs are linked to AC-cAMP and Ca^{2+} signaling, respectively. During development, the activation of GABA$_A$Rs leads to a rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (*9*, *10*). Because hippocampal neurons express type 1 and type 8 Ca^{2+}–calmodulin (CaM)–activated ACs (*25*), we hypothesized that GABA signaling converges on A_{2A}R signaling by tuning CaM-stimulated ACs and their downstream effectors.

Acute activation of GABA$_A$Rs with 10 μM muscimol significantly increased $[Ca^{2+}]_i$ in DIV 8 hippocampal neurons, whereas it was decreased in DIV 14 neurons (fig. S14), in keeping with the developmentally regulated effect of GABA$_A$Rs on $[Ca^{2+}]_i$. By contrast, an acute application of CGS21680 did not change $[Ca^{2+}]_i$ in DIV 8 or DIV 14 neurons (fig. S14). Buffering intracellular Ca^{2+} with BAPTA-AM (20 μM) lead to the destabilization of inhibitory synapses—an effect that could be prevented by A_{2A}R activation with CGS21680 (Fig. 3A). Thus, activation of A_{2A}Rs is sufficient to maintain synapse integrity in the absence of intracellular Ca^{2+} signaling.

Because GABA but not adenosine signaling can elevate $[Ca^{2+}]_i$ and because A_{2A}R-mediated activation of ACs can be boosted by CaM (*10*), we tested the convergence of the GABA$_A$R and

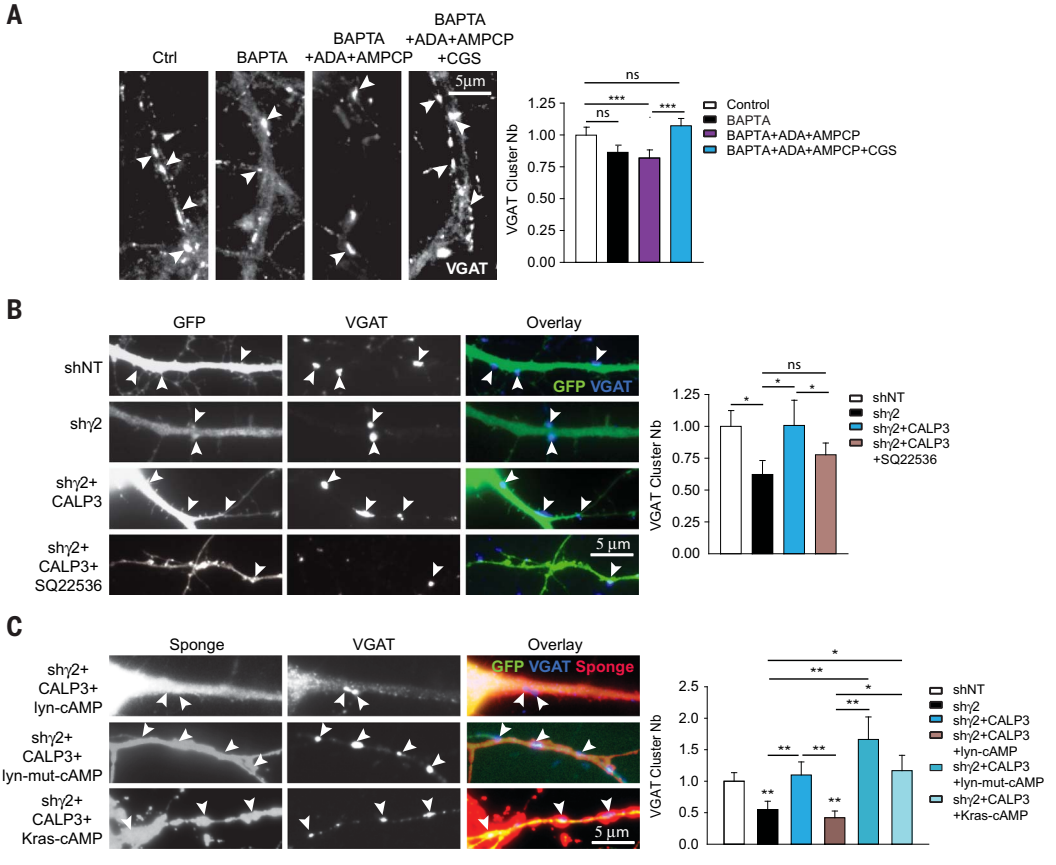

Fig. 3. GABAergic synapse stabilization by calmodulin-activated, calcium-sensitive ACs. (**A**) Immunostaining and quantification of VGAT in DIV 10 neurons in the absence or presence of the indicated drugs for 30 min. Scale bar, 5 μm. Decreasing intracellular calcium levels with BAPTA-AM (20 μM) in the absence or presence of ADA (4 to 20 U/mL) and AMPCP (100 μM) leads to synapse destabilization, an effect prevented by the direct activation of A_{2A}Rs with CGS21680 (30 nM). N = 35 to 38 cells, three cultures. (**B**) Calp3 (100 μM) activation of calmodulin for 30 min rescued the loss of GABAergic synapses in DIV 10 to 11 neurons expressing GABA$_A$Rγ2 shRNA (shγ2). The effect of calmodulin was blocked by an adenylyl cyclase inhibitor SQ22536 (20 μM). Scale bar, 5 μm. shNT, n = 38; shγ2, n = 42; shγ2 and CALP3, n = 43; shγ2 and CALP3 and SQ22536, n = 30; three cultures. (**C**) VGAT staining and quantification of DIV 10 to 11 neurons transfected with nontarget (shNT) or on-target GABA$_A$Rγ2 (shγ2) and/or the different sponges constructs (lyn-cAMP, lyn-mut-cAMP, and Kras-cAMP) exposed to CALP3 for 30 min. Scale bar, 5 μm. shNT, n = 40; shγ2, n = 37; shγ2 and CALP3, n = 40; shγ2 and CALP3 and lyn-cAMP sponge, n = 23; shγ2 and CALP3 and lyn-mut-cAMP sponge, n = 27; shγ2 and CALP3 and Kras-cAMP sponge, n = 31; four cultures. Arrowheads show examples of inhibitory synapses labeled for VGAT. In all graphs, histograms represent means and SEMs; values normalized to their respective controls. Statistics were calculated using the Mann-Whitney test. ns, not significant; *$P \leq 0.05$; **$P < 0.01$; ***$P < 0.001$.

$A_{2A}R$ pathways on cAMP. To address this question, we performed cAMP imaging in DIV 8 hippocampal neurons infected with recombinant Sindbis virus encoding EPAC-SH150, a sensor with increased sensitivity for cAMP (*26*). As expected, $A_{2A}R$ activation with CGS21680 increased intracellular cAMP levels (fig. S15). $GABA_AR$ activation with muscimol also increased cAMP levels within a similar range to that observed with CGS21680 (fig. S15). Application of both CGS21680 and muscimol strongly elevated intracellular cAMP levels (fig. S15), demonstrating that the activation of $GABA_ARs$ and $A_{2A}Rs$ converge with additive effects on cAMP production.

Stabilization of GABAergic synapses requires cAMP production

We then tested the implication of intracellular Ca^{2+} and CaM in GABAergic synapse stabilization. The loss of GABAergic synapses induced by the expression of shRNA against the $GABA_AR$ γ2 subunit (shγ2) in DIV 10 neurons was prevented by the activation of CaM with the cell-permeable activator CALP3 (100 μM) for 30 min (Fig. 3B). Thus, CaM is involved in the stabilization of GABAergic synapses. Application of the AC inhibitor SQ22536 (20 μM) prevented the CALP3-mediated rescue of GABAergic synapses (Fig. 3B), which demonstrates that CaM stabilizes synapses through the activation of ACs.

Hippocampal neurons express Ca^{2+}-dependent and -independent ACs targeted, respectively, to lipid raft and nonraft plasma membranes (*27*). To investigate the involvement of Ca^{2+}-dependent and -independent ACs, we used genetically encoded cAMP sponges targeted to (lyn-cAMP sponge) or outside (Kras-cAMP sponge) lipid rafts, which enabled the local perturbation of cAMP downstream signaling (*28*). A variant of lyn-cAMP sponge (lyn-mut-cAMP sponge) unable to bind and buffer cAMP was used as a control. In basal conditions, none of the cAMP sponges altered the density of GABAergic synapses (fig. S16). However, buffering cAMP near lipid rafts blocked the CALP3-mediated rescue of GABAergic synapses in neurons expressing shγ2 (Fig. 3C). This effect was specific to sponges targeted to lipid rafts because the density of synapses in neurons expressing lyn-mut-cAMP or Kras-cAMP sponges were not distinguishable from CALP3-treated neurons lacking $GABA_ARγ2$ (Fig. 3C). Thus, the convergence of $GABA_AR$ and $A_{2A}R$ pathways on the stabilization of GABAergic synapses relies on cAMP production by Ca^{2+}-dependent, CaM-activated ACs. We then investigated the mechanism downstream of cAMP production.

Adenosine stabilizes synapses through PKA-phosphoregulation of gephyrin

Protein kinase A (PKA) is a downstream effector of cAMP after $A_{2A}R$ activation. As expected, intracellular inhibition of PKA with the protein kinase inhibitor peptide (PKI) decreased mIPSC amplitude and frequency ex vivo (26 and 50%, respectively; fig. S17) to the same extent as that found with SCH58261 (100 nM). Because PKA-mediated phosphorylation of gephyrin is required for its postsynaptic stabilization and the anchoring of $GABA_ARs$ at synapses (*29*), we hypothesized that PKA activation via the $A_{2A}R$–AC-cAMP cascade ensures the phosphorylation of gephyrin required to maintain $GABA_ARs$ at the synapse.

Gephyrin is a direct substrate of PKA, so we tested whether gephyrin phosphorylation at the Ser^{303} PKA-sensitive site was increased after $A_{2A}R$ activation by CGS21680. For this, we used HEK293 cells and measured gephyrin phosphorylation using Ser^{303} site–specific gephyrin phospho-antibody (*30*). Removal of ambient adenosine with ADA and AMPCP and simultaneous activation of $A_{2A}Rs$ with CGS21680 significantly increased gephyrin phosphorylation at the Ser^{303} site (Fig. 4A). These effects of ADA and AMPCP or ADA, AMPCP, and CGS21680 were not observed in cells that expressed a gephyrin phospho-null mutant (gephyrin-S303A) (Fig. 4A). This demonstrates that gephyrin can be phosphorylated on its specific PKA site upon $A_{2A}R$ activation.

We then directly tested the contribution of PKA-dependent phosphorylation of gephyrin in synapse stabilization in vitro by expressing

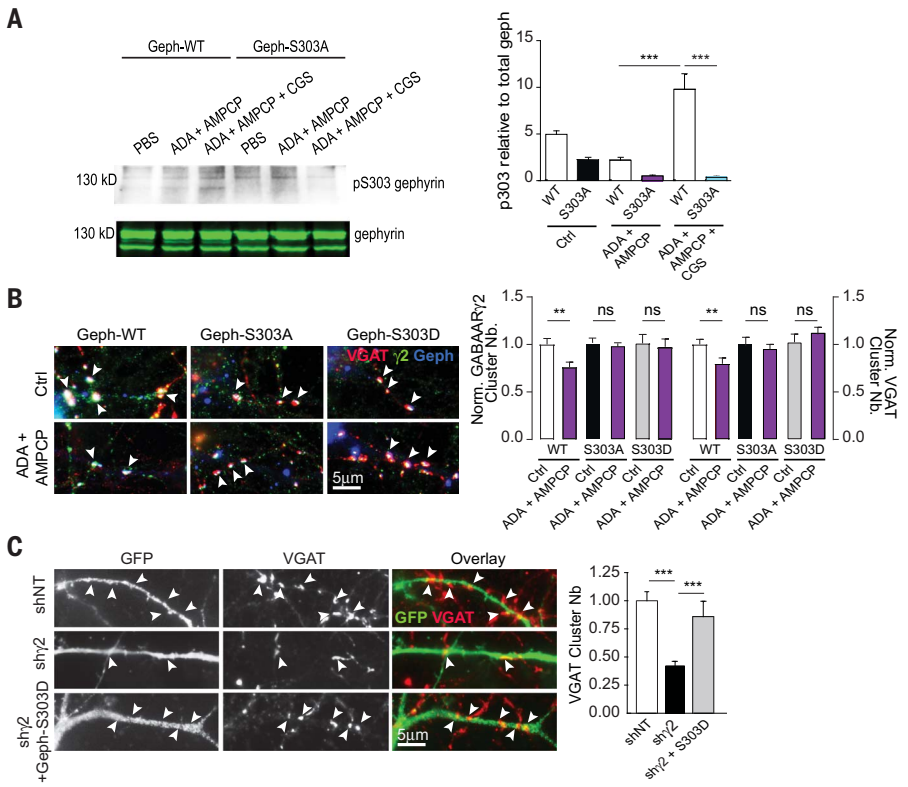

Fig. 4. GABAergic synapse stabilization requires phosphorylation of gephyrin on Ser^{303}. (**A**) Western blot and quantification of the gephyrin Ser^{303} phosphorylation in control and after 30-min exposure to ADA (4 to 20 U/mL) and AMPCP (100 μM) without or with CGS21680 (30 nM) in HEK293 cells transfected with gephyrin-WT or gephyrin-S303A constructs. There was significant increased phosphorylation of P-S303 of gephyrin-WT but not gephyrin-S303A upon acute $A_{2A}R$ activation with CGS21680. PBS, phosphate-buffered saline. (**B**) Immunostaining (left) and quantification (right) of $GABA_ARγ2$ and VGAT in the absence or presence of ADA and AMPCP in DIV 10 neurons transfected with 3′ untranslated region (3′UTR) gephyrin shRNA and eGFP-gephyrin-WT, S303A, or S303D constructs. Scale bar, 5 μm. Arrowheads show examples of inhibitory synapses triple-stained for VGAT, $GABA_ARγ2$, and gephyrin. WT, n = 53; WT and ADA and AMPCP, n = 49; S303A, n = 48; S303A and ADA and AMPCP, n = 53; S303D, n = 45; S303D and ADA and AMPCP, n = 47; four cultures. Gephyrin-S303A or S303D block the ADA and AMPCP effect. (**C**) VGAT staining (left) and quantification (right) of DIV 10 to 11 neurons transfected with nontarget (shNT) or on-target $GABA_ARγ2$ (shγ2) shRNAs with eGFP-gephyrin-WT or eGFP-gephyrin-S303D. Scale bar, 5 μm. Arrowheads show examples of inhibitory synapses labeled for VGAT. shNT, n = 35; shγ2, n = 49; shγ2 and geph-S303D, n = 60; three cultures. The loss of GABAergic synapses upon suppression of $GABA_ARγ2$ can be rescued upon overexpression of gephyrin-S303D. In all graphs, histograms represent means and SEMs; values were normalized to corresponding controls [(B) and (C)]. Statistics were calculated using the analysis of variance (ANOVA) test (A) and the Mann-Whitney test [(B) and (C)]. ns, not significant; $^{**}P < 0.01$; $^{***}P < 0.001$.

constructs harboring mutations of the PKA Ser^{303} site to aspartate (S303D) or alanine (S303A) to mimic the gephyrin phosphorylated and dephosphorylated states (*30*). Decreasing extracellular adenosine with AMPCP and ADA led to synapse destabilization in neurons expressing wild-type (WT) gephyrin (Fig. 4B), whereas the expression of the gephyrin-S303D or gephyrin-S303A mutants prevented synapse loss upon the removal of extracellular adenosine (Fig. 4B). Thus, the PKA Ser^{303} phosphosite of gephyrin is required for the $A_{2A}R$-mediated synapse stabilization. This effect was specific to this phospho site because decreasing extracellular adenosine led to synapse destabilization in neurons expressing the glycogen synthase kinase 3β (GSK3β) phospho-null gephyrin mutant (gephyrin-S270A) (fig. S18). We conclude that activation of $A_{2A}Rs$ leads to GABAergic synapse stabilization through CaM-AC-cAMP–PKA–mediated phosphorylation of gephyrin at position Ser^{303}. This mechanism accounts for the disappearance of the postsynaptic site when $A_{2A}Rs$ are not activated by adenosine for >10 min (fig. S11).

Gephyrin phosphorylated on Ser^{303} interacts with Slitrk3

How can we explain the concurrent loss of the presynaptic element? Overexpression of the PKA-phosphorylation site gephyrin-S303D mutant was sufficient to rescue the loss of VGAT-containing terminals on neurons transfected with shγ2 (Fig. 4C). This demonstrates that gephyrin is sufficient to stabilize synapses. We therefore hypothesized that gephyrin phosphorylated at Ser^{303} interacts with the postsynaptic neuroligin-2 (*31*, *32*) or Slitrk3 (*33*) transmembrane proteins that bind in the synaptic cleft to presynaptic neurexins or PTPδ to organize inhibitory synapses. The knockdown of postsynaptic neuroligin-2 with a specific shRNA or the antibody-induced blockade of presynaptic neurexin led, as expected (*34*–*36*), to a loss of GABAergic synapses, which could be rescued upon $A_{2A}R$ activation with CGS21680 (fig. S19). Thus, $A_{2A}R$-dependent synapse stabilization does not depend upon the neurexin–neuroligin-2 transsynaptic complex. By contrast, $GABA_AR$ activation did not rescue the synapse loss induced by the expression of a shRNA against neuroligin-2 (fig. S19).

$A_{2A}R$ or $GABA_AR$ activation did not prevent synaptic loss induced by a shRNA against postsynaptic Slitrk3 (*33*) (Fig. 5A), which supports the involvement of Slitrk3 in $A_{2A}R$- and $GABA_AR$-dependent synapse stabilization. We then overexpressed the Slitrk3-Y969A mutant because this site is involved in the formation of GABAergic synapses (*37*) (Fig. 5B). $A_{2A}R$ activation was not able to restore synapses in cells overexpressing Slitrk3-Y969A (Fig. 5B). Therefore, deletion of Slitrk3 or a single amino acid mutation of Slitrk3 was enough to prevent $A_{2A}R$-dependent stabilization of GABAergic synapses. Finally, using coimmunoprecipitation experiments, we found that gephyrin-WT and gephyrin-S303D interact directly with Slitrk3 (Fig. 5C). These results suggest that $A_{2A}R$ signaling stabilizes the pre- and postsynaptic GABAergic elements through PKA-dependent gephyrin phosphorylation and Slitrk3 recruitment.

Transient blockade of $A_{2A}Rs$ induces cognitive deficits

All previous experiments were performed in vitro and ex vivo to unravel the molecular mechanism. We next tested whether the role of $A_{2A}Rs$ could also be observed in vivo during the postnatal synaptogenesis period. We could not use $A_{2A}R$ knockout animals because a delayed migration of GABAergic neurons occurs in these animals (*13*), which would result in a decreased number of GABAergic synapses. This would prevent us from distinguishing between effects resulting from a delayed migration and those resulting from a direct effect on the stability of GABAergic synapses. We therefore injected shRNAs against $A_{2A}Rs$ in vivo in the hippocampus at P3. This led to the loss of GABAergic synapses evaluated at P16 (fig. S20), which confirmed the physiological role of $A_{2A}Rs$ in the stabilization of GABAergic synapses. Additionally, we treated pups during the peak of synaptogenesis between P3

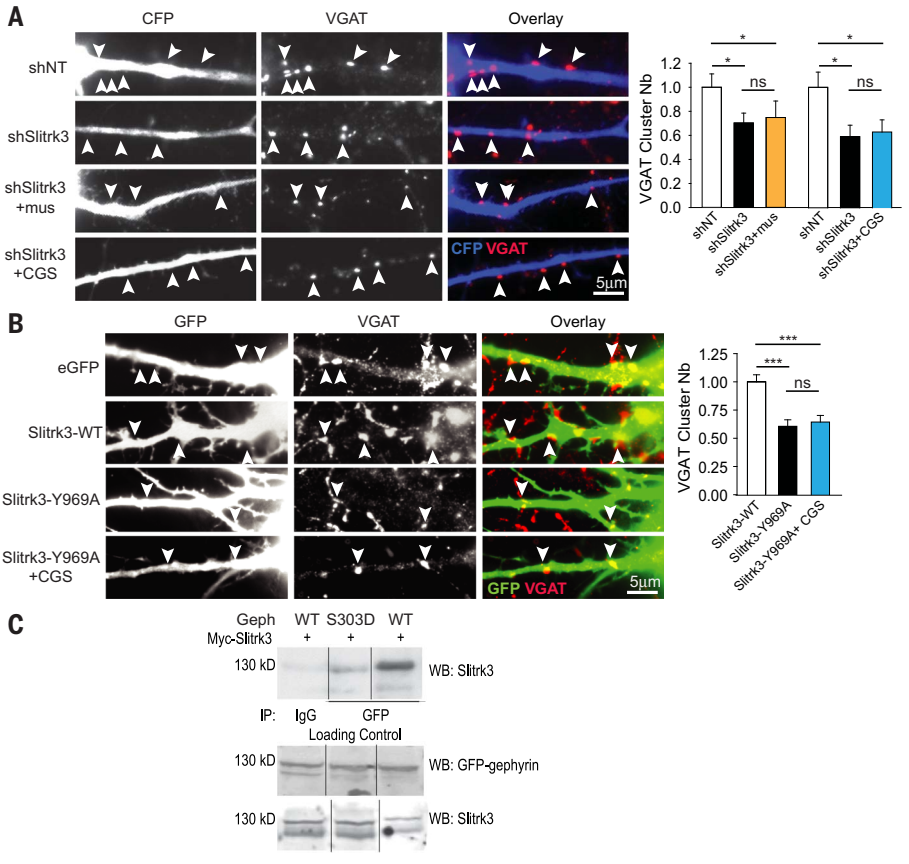

Fig. 5. $A_{2A}Rs$ stabilize GABAergic synapses through the synaptogenic organizer Slitrk3. (**A**) The loss of GABAergic synapses after Slitrk3 suppression cannot be rescued by $A_{2A}R$ or $GABA_AR$ activation. VGAT staining (left) and quantification (right) of DIV 10 to 11 neurons transfected with shMock or shSlitrk3 exposed or not exposed to muscimol (Mus) (10 µM) or CGS21680 (CGS) (30 nM) for 30 min. CFP, cyan fluorescent protein. Scale bar, 5 µm. Muscimol: shMock, *n* = 42; shSlitrk3, *n* = 34; shSlitrk3 and muscimol, *n* = 41; three cultures. CGS: shNT, *n* = 41; shSlitrk3, *n* = 37; shSlitrk3 and CGS, *n* = 36; three cultures. (**B**) The loss of GABAergic synapses after overexpression of the Slitrk3-Y969A mutant cannot be rescued by the activation of $A_{2A}Rs$. VGAT staining (left) and quantification (right) of DIV 10 to 11 neurons transfected with Slitrk3-WT or Slitrk3-Y969A exposed or not exposed to CGS21680 for 30 min. Scale bar, 5 µm. Muscimol: eGFP, *n* = 35; Slitrk3-WT, *n* = 38; Slitrk3-Y969A, *n* = 39; Slitrk3-Y969A and CGS, *n* = 41; two cultures. (**C**) Pull down of gephyrin-WT using the GFP tag followed by a blot for Slitrk3 showing gephyrin-Slitrk3 interaction in HEK293 cells. IP, immunoprecipitation; WB, Western blot; IgG, immunoglobulin G. In all images, arrowheads show examples of inhibitory synapses labeled for VGAT. In all graphs, histograms represent means and SEMs; values were normalized to corresponding controls. Statistics were calculated using the Mann-Whitney test [(A) and (B)]. ns, not significant; *$P < 0.05$; ***$P < 0.001$.

and P16 with SCH58261 (0.1 mg/kg). Although we cannot rule out nonspecific effects, the treatment produced a loss of GABAergic synapses at P16 in the hippocampus (fig. S20) similar to that found in vitro (fig. S4) and ex vivo (Fig. 1C). Given such synaptic loss, we predicted detrimental functional consequences, in particular for hippocampus-dependent spatial memory. We therefore tested P70 animals, which had been treated between P3 and P16 with SCH58261. As predicted, we found deficits in the novel object location task (fig. S20). By contrast, open field, anxiety, and novel object recognition tests were not modified (fig. S20). These results indicate that interfering with A_{2A}Rs in vivo during synaptogenesis has long-term deleterious effects on cognitive function.

Discussion

This A_{2A}R-dependent mechanism adds to other molecules and signaling pathways known to control hippocampal GABAergic synapses during development (*1*, *4*, *38*), including GABA (*4*–*8*) and $GABA_A$R-induced elevation of intraneuronal Ca^{2+} levels after the activation of voltage-dependent Ca^{2+} channels (*9*, *10*). We propose that this in turn activates Ca^{2+}-CaM–sensitive ACs, leading to a rise in intracellular cAMP in neurons. Thus, one outcome of $GABA_A$R activation in immature neurons is the elevation of intracellular cAMP levels. A possible explanation is that adenosine and ATP are coreleased with GABA (*16*, *17*) during development, which would provide A_{2A}Rs with direct information that the presynaptic terminal is active. Once activated, G protein–coupled A_{2A}Rs will trigger a rise in intracellular cAMP levels, leading in turn to PKA activation and stabilization of GABAergic synapses (fig. S21). Such A_{2A}R-mediated control of synapse selection seems to be autonomous because it does not require brain-derived neurotrophic factor (BDNF)–TrkB receptor signaling (fig. S22).

We found additive effects of GABA and adenosine pathways on cAMP levels. ACs can act as coincidence detectors, promoting cellular responses only when convergent regulatory signals occur close in time and space (*39*). Our results show that the $GABA_A$R and A_{2A}R systems are spatially close and operate within a similar time frame to control the fate of some GABAergic synapses. This effect of GABA-adenosine cosignaling at GABAergic synapses would occur only during a specific period of development because of the transient expression of A_{2A}Rs and because of the transient depolarizing action of $GABA_A$Rs and their ability to activate CaM-sensitive ACs.

The A_{2A}R mechanism we have described therefore provides a conceptual framework to understand how some synapses are stabilized or removed during development. During neural development, inactive synapses are eliminated. This requires the existence of a machinery that includes a detector of activity from the presynaptic terminal and a mechanism that removes the synapse when the detector is not activated. The adenosine-operated A_{2A}R can subserve all of these functions, in addition to the involvement of GABA itself. The post- and perisynaptic localizations of A_{2A}Rs can detect the activity-dependent release of adenosine and ATP (*20*, *40*, *41*), which mostly involves a direct release of adenosine as a signal proportional to the metabolic support of synaptic activity as well as a CD73-mediated extracellular formation of vesicular ATP–derived adenosine (*11*, *12*, *20*). The nonactivation of A_{2A}Rs will prevent the continuous phosphorylation of gephyrin, which is essential for synapse stabilization. Synapse removal requires a certain period of A_{2A}R inactivity (20 min), which permits the persistence of somewhat quiet but not silent synapses.

Caffeine, the most commonly consumed psychoactive drug in the world, including during pregnancy and lactation, is a natural antagonist of A_{2A}Rs. Exposure to caffeine during the perinatal period of synaptogenesis could trigger suppression of some synapses, with deleterious effects.

Materials and methods summary

Electrophysiology

mIPSCs were recorded in coronal CA1 hippocampal slices (350 μm) from male GIN-mice, as in (*42*).

Immunohistochemistry

For acute SCH58261 treatment, P6 slices were fixed with 4% paraformaldehyde (PFA), cryoprotected in 20% sucrose, frozen on dry ice, and incubated in rabbit anti-VGAT antibody (1:1000; SYSY). Images were acquired with a Zeiss LSM 510 microscope. For chronic SCH58261 treatment or shA_{2A}R expression, P16 brains were postfixed in 4% PFA, cryoprotected (30% sucrose), and cut in parasagittal free-floating sections (30 μm) (*43*) that were incubated in rabbit anti-VGAT (1:1000; from B. Gasnier). Z-stacks were acquired with a Leica SP5 confocal microscope and quantified using Imaris. For A_{2A}R detection, P3 to P12 coronal free-floating sections (100 μm) were incubated in goat anti-A_{2A}R antibody (1:200; Santa Cruz). Images were acquired with a Zeiss Z2 microscope. Ultrastructural analysis of A_{2A}R was performed using the pre-embedding immunogold method (*44*) with guinea pig anti-A_{2A}R (Frontier Institute, Japan). Images were acquired with a Jeol-1010 microscope.

Western blotting

A_{2A}R density (*18*, *24*) was studied with goat (Santa Cruz) or mouse (Millipore) anti-A_{2A}R antibodies. Validation of shA_{2A}Rs was done with rabbit anti-A_{2A}R (1:500; Alomone) antibodies in N2a cells infected with AAV2.1-U6-shNT-GFP or AAV2.1-U6-shA2AR-GFP. Gephyrin-Slitrk3 interaction was assessed with rabbit anti-Slitrk3 (1:1000; Sigma) antibodies in HEK293T cells transfected with pCMV3-Myc-Slitrk3, eGFP-gephyrin-WT (eGFP, enhanced GFP) (*45*), or eGFP-gephyrin-S303D (*30*). Gephyrin phosphorylation was analyzed with rabbit custom made anti–phosphogephyrin and mouse anti-gephyrin (3B11, 1:1000; SYSY) antibodies in HEK293T cells transfected with eGFP-gephyrin-WT or eGFP-gephyrin-S303A. A_{2A}R binding (*18*) was done with 2 nM3HSCH58261 using 140 to 210 mg of protein. Release of ATP and adenosine (*20*) from hippocampal synaptosomes was assessed using the luciferin-luciferase assay (ATP) and highperformance liquid chromatography (HPLC) (adenosine) upon K+- induced depolarization (*20*).

Behavioral testing

Open-field, novel object location (NOL) tasks, novel object recognition (NOR) tasks, and elevated plus maze were done as in (*46*) at P70 on WT males treated with saline or SCH58261 (0.1 mg/kg) between P3 and P16.

Neuronal culture

Cultures of hippocampal neurons were prepared as described in (*47*).

Immunocytochemistry was done using rabbit anti-VGAT (1:500; from B. Gasnier), mouse anti-gephyrin (mAb7a, 1:500; SYSY) and guinea pig anti-$GABA_A$Rγ2 (1:2000; from J. M. Fristchy), or mouse anti-VGAT (1:500; SYSY) and rabbit anti-$GABA_A$Rα1 or $GABA_A$Rα2 (1:500; SYSY), or mouse anti-GAD 67 (1:500; Chemicon) and rabbit anti-A_{2A}R (1:100; Alomone). Image acquisition and cluster analysis was performed as in (*47*).

Single-particle tracking of $GABA_A$Rγ2 was performed as described in (*47*, *48*).

DNA-PAINT was performed with rabbit anti-A_{2A}R (1:100; Alomone) and mouse anti-gephyrin (mAb7a, 1:500; SYSY) on an inverted Nikon Eclipse Ti microscope using 561- and 647-nm lasers.

Calcium imaging of AAV-GCaMP6-ruby–infected neurons was done on a Leica DMI4000 microscope (Yokogawa CS20 spinning Nipkow disk) with a 491-nm laser. Time-lapse images (0.33 Hz for 600 s) of stacks (~21 sections; step, 0.3 μm) were acquired.

cAMP imaging of EPAC-sh150 (*26*) was done on a two-photon Leica TCS/MP5 microscope with a Ti:sapphire laser (Coherent). Z-stacks (8 to 10 sections; step, 1 to 2 μm) were acquired every 15 s.

Video-microscopy of presynaptic terminals (*22*) stained with rabbit anti–VGAT-oyster^{550} (1:200; SYSY) was done using an Olympus IX71 microscope. Time-lapse images (one image every 5 min) were acquired.

A detailed materials and methods section can be found in the supplementary materials.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S22
MDAR Reproducibility Checklist

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Science

Convergence of adenosine and GABA signaling for synapse stabilization during development

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Synapse stabilization

Early in brain development, neurons connect to each other enthusiastically. With development, an overabundance of synapses is winnowed down to refine efficiently connected circuits. Inactive synapses are prime targets for elimination, whereas active synapses tend to be retained. Gomez-Castro *et al*. took a closer look at how those choices are made (see the Perspective by Blum and Lopes). When postsynaptic adenosine receptors are muted or do not find enough extracellular adenosine, synapses get eliminated. Neurotransmitter-dependent signaling pathways drive protein kinase A to phosphorylate the postsynaptic scaffolding molecule gephyrin. Together with a partner synaptogenic membrane protein, gephyrin is required for the stabilization of #-aminobutyric acid receptors. Adenosine receptors thus detect synaptic activity and in turn drive the stabilization of synapses that produce such activity. —PJH

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