Astrocytic β_2-adrenergic receptors mediate hippocampal long-term memory consolidation

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Emotionally relevant experiences form strong and long-lasting memories by critically engaging the stress hormone/neurotransmitter noradrenaline, which mediates and modulates the consolidation of these memories. Noradrenaline acts through adrenergic receptors (ARs), of which β_2-adrenergic receptors (βARs) are of particular importance. The differential anatomical and cellular distribution of βAR subtypes in the brain suggests that they play distinct roles in memory processing, although much about their specific contributions and mechanisms of action remains to be understood. Here we show that astrocytic rather than neuronal β_2ARs in the hippocampus play a key role in the consolidation of a fear-based contextual memory. These hippocampal β_2ARs, but not β_1ARs, are coupled to the training-dependent release of lactate from astrocytes, which is necessary for long-term memory formation and for underlying molecular changes. This key metabolic role of astrocytic β_2ARs may represent a novel target mechanism for stress-related psychopathologies and neurodegeneration.

astrocyte | memory | hippocampus | β-adrenergic receptor | lactate

Emotional arousal and the stress hormone noradrenaline (NA) mediate and modulate memory consolidation, the process required to stabilize long-term memory (1). NA contributes to this effect through the activation of α- and β-adrenergic receptors (βARs) in brain regions such as the amygdala and the hippocampus, which are critical for encoding and consolidating memories (2). Of particular interest is the action of NA through βARs because blockers of these receptors, such as propranolol, have been reported to interfere with memory consolidation and strengthening (3–5) and have been suggested as potential therapeutics to treat anxiety disorders, including panic disorder (6) and posttraumatic stress disorder (7). All three subtypes of βARs—β_1, β_2, and β_3—are present in the central nervous system with distinct distributions (8): In the rat hippocampus, a region required for the consolidation of explicit/episodic memories, β_1ARs predominate, and their pattern of distribution is different from that of β_2ARs and β_3ARs (9–11), suggesting possible distinct roles for these receptor subtypes. Pharmacological investigations using agonists (e.g., NA itself) and antagonists (e.g., propranolol) that bind to both β_1ARs and β_2ARs have suggested βARs have a similar role in memory encoding, modulation, and retrieval in humans and rodents (3, 5, 12). Studies on animal models lacking β_1ARs or treated with selective β_1AR agonists or antagonists revealed the critical role of this adrenergic receptor subtype in both synaptic plasticity and memory formation, with particular emphasis on retrieval (12–14). Genetic deletion of β_2ARs results in impaired memory modulation by stress or corticosterone and impaired hippocampal plasticity, in agreement with a pharmacologically established role of β_2ARs in amygdala-dependent memory modulation and its effect on hippocampal function and in prefrontal cortex (2, 15, 16). However, little is known about the role of hippocampal β_2ARs in hippocampus-dependent long-term memory formation and processing.

In general, the functional contributions of βARs to memory processes have been thought to result mainly from their effect on neurons and therefore have been studied largely in neuronal/synaptic models (reviewed in ref. 17). However, in addition to being expressed in pre- and postsynaptic compartments of neurons, βARs are also found in other cell types, particularly in astrocytes (18–21). More specifically, it has been suggested that β_2ARs in the nervous system are expressed predominantly in glia (18, 21–23), whereas β_1ARs are found primarily in neurons, at synaptic junctions (18, 22, 23). This suggestion raises the question of which βAR subtype (β_1AR or β_2AR) and which βAR-expressing cells mediate hippocampal memory consolidation.

NA activates glycogenolysis and subsequent lactate release (24), a process known to occur mainly in astrocytes and not in neurons; in fact, glycogen is stored almost exclusively in astrocytes (25, 26). Glycogenolysis and its activation by NA are critical for memory consolidation in chicks (27). Furthermore, in the rat hippocampus, glycogenolysis, which results in lactate increase and astrocyte–neuron lactate transport, mediates memory consolidation (28, 29), in vivo hippocampal long-term potentiation, and the induction of learning-dependent molecular changes, including activity-regulated cytoskeletal protein (Arc) and phosphorylation of cAMP response element-binding protein at serine 133 (pCREB) and cofilin (p-cofilin) (29). Using the hippocampus-dependent task inhibitory avoidance (IA), here we asked which astrocytic and/or neuronal β_1ARs and/or β_2ARs in the hippocampus mediate memory consolidation.

Significance

Experiences are remembered long-term when these memories are formed in a state of arousal and heightened emotion. The arousal-induced release of noradrenaline is critical for modulating consolidation, the process that establishes long-term memory. Although the effects of pharmacological manipulation of adrenergic signaling on memory stability are already being investigated in the clinical setting, how adrenergic receptors mediate long-term memory consolidation remains unclear. This study reports a previously unidentified mechanism with important translational implications: The noradrenergic receptors that in the hippocampus mediate memory consolidation are β_2-adrenergic receptors (β_2ARs) expressed in astrocytes. These receptors are necessary for the learning-evoked release of lactate from astrocytes, which then is required to support the neuronal molecular changes essential for long-term memory formation.

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Results

βARs Mediate Memory Consolidation via Astrocytic Lactate. A bilateral injection of the β_1+β_2AR antagonist propranolol into the rat dorsal hippocampus (dHC) 15 min before training had no effect on short-term memory tested 1 h after training (Fig. 1*A*) but significantly impaired long-term memory tested 1 d and 7 d after training (Fig. 1*B*), suggesting that hippocampal β_1ARs and/or β_2ARs mediate IA memory consolidation. Given that NA promotes glycogenolysis and lactate release from astrocytes (30), which are necessary for memory consolidation (29), we tested whether the effect of propranolol on memory consolidation is linked to astrocytic lactate release. We found that coadministration of L-lactate with propranolol fully and persistently rescued memory impairment. In contrast, an equicaloric concentration of D-glucose had no effect. A threefold higher concentration of glucose rescued memory impairment at 1 d but not at 7 d after training ($P < 0.0001$; two-way repeated-measures ANOVA followed by Bonferroni's post hoc tests) (Fig. 1*B*). Together, these results indicate that the critical role of β_1ARs and/or β_2ARs in memory consolidation is likely dependent on lactate and hence on astrocytic mechanisms.

Propranolol injected bilaterally into the dHC 15 min before training also blocked the learning-dependent increase of molecular changes known to underlie synaptic plasticity and memory formation; these included the immediate early gene Arc 30 min after training (Fig. 1*C*), and phosphorylation of CREB (Fig. 1*D*) and of calcium calmodulin kinase II α (CaMKIIα) (Fig. 1*E*) 20 h after training, time points at which these molecular changes have been previously established as learning dependent (29, 31). Lactate coadministration rescued all these molecular impairments ($P < 0.01$; one-way ANOVA followed by Newman–Keuls post hoc test). The levels of total CREB and CaMKIIα remained unchanged ($P > 0.05$) (Fig. S1 and Table S3). Hence, βARs in the hippocampus engage astrocytic mechanisms to support memory formation.

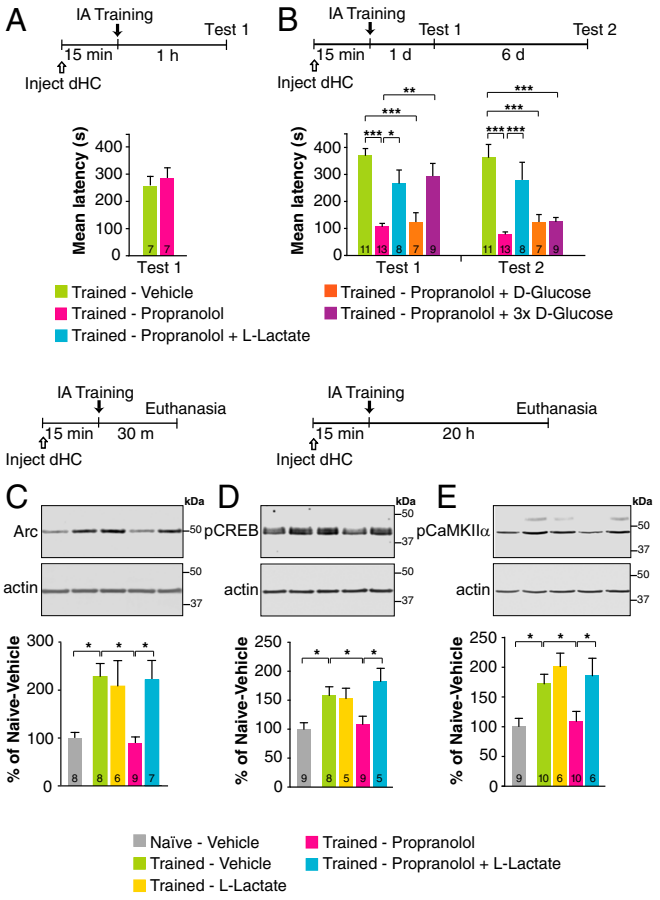

Fig. 1. L-lactate rescues the impairment of long-term IA memory and underlying molecular mechanisms produced by propranolol. Memory retention is expressed as mean latency ± SEM (in seconds). (*A*) Short-term memory tested 1 h after training following a bilateral dHC injection of propranolol given 15 min before training ($n = 7$). (*B*) Long-term memory tested 1 d after training (test 1) and 6 d later (test 2) following a bilateral dHC injection of vehicle or propranolol, in the presence or absence of L-lactate, or of an equicaloric concentration of D-glucose, or of a three times higher concentration (3×) of D-glucose, given 15 min before training ($n = 7$–13). (*C*–*E*) Densitometric Western blot analysis and representative images for Arc (*C*), pCREB (*D*), and pCaMKIIα (*E*) in dHC extracts from untrained (naive) rats injected with vehicle and rats injected 15 min before training with vehicle, L-lactate, propranolol, or propranolol + L-lactate and killed 30 min (for Arc) or 20 h (for pCREB and pCaMKIIα) after training. Data are expressed as mean protein percentage ± SEM of mean values (100%) in vehicle-injected naive rats. Each sample protein densitometric value was normalized to that of relative actin stained on the same blot ($n = 5$–10). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Numeric values and detailed statistical analyses are reported in Tables S1 and S2.

β_2ARs, but Not β_1ARs, Mediate the Learning-Dependent Lactate Increase and Long-Term Memory Formation. Next we sought to dissociate the roles of the βAR subtypes, β_1AR and β_2AR, in regulating training-induced astrocytic lactate release and memory consolidation. In agreement with our previous finding (29), we found that training leads to a significant increase in lactate levels in the dHC as measured by microdialysis; this increase lasted for more than 60 min and returned to baseline by ∼90 min. However, this lactate increase was completely abolished by a systemic (i.p.) injection of propranolol or of the β_2AR-selective antagonist ICI 118,551, but not the β_1AR-selective antagonist betaxolol, given 30 min before training ($P < 0.0001$; two-way ANOVA followed by Bonferroni post hoc tests) (Fig. 2*A*). Compared with vehicle-injected control rats, propranolol- and ICI 118,551-injected rats, but not betaxolol-injected rats, had significantly impaired long-term memory when tested 1 d after training ($P < 0.0001$; one-way ANOVA followed by Bonferroni post hoc tests) (Fig. 2*A*). The efficacy of betaxolol was confirmed in parallel by reproducing the previously established effect of this compound on retrieval of contextual fear conditioning (CFC) (Fig. S2 and Table S4) (12). In addition, as with systemic injections, bilateral injection of ICI 118,551 into the dHC 15 min before training impaired long-term memory when tested 1 d and 7 d after training, whereas injection of betaxolol had no effect ($P < 0.001$; two-way repeated-measures ANOVA followed by Bonferroni post hoc tests) (Fig. 2*B*). Following the 7-d test, rats received a reminder footshock (RS)—the unconditioned stimulus in a different context—and testing 1 d later showed that the memory deficit persisted, suggesting that the amnesia was likely caused by impaired consolidation rather than by inhibition of memory expression ($P < 0.01$; Student's *t* test) (Fig. 2*B*). Hence, both the IA-induced increase in hippocampal lactate and long-term memory consolidation require β_2ARs but not β_1ARs. We therefore focused our subsequent investigations on β_2ARs.

Astrocytic, Not Neuronal, Hippocampal β_2ARs Mediate Long-Term Memory Formation. The expression and distribution of β_2ARs on different cell types is not clear; although some studies suggest that β_2ARs are expressed mainly in glia (18, 21–23), others report that they are found on both astrocytes and neurons (20). To determine the selective functional contribution of astrocytic and/or neuronal β_2AR in long-term memory formation, we used cell-specific virus-mediated knockdown of the receptors. The astrocyte-specific promoter glial fibrillary acidic protein (short gfaABC1D) was packaged into adeno-associated virus 9 (AAV9), and the neuron-specific human synapsin (hSyn) promoter was packaged in AAVDJ; both were tagged with eGFP and injected into the dHC. Two weeks after injection, histochemical staining revealed that the expressed fluorescence of eGFP was distributed throughout the dHC (Fig. 3*A*). The AAV9-short gfaABC1D-eGFP expression was selectively astrocytic, as revealed by its colocalization with the endogenous astrocytic marker GFAP but not with the

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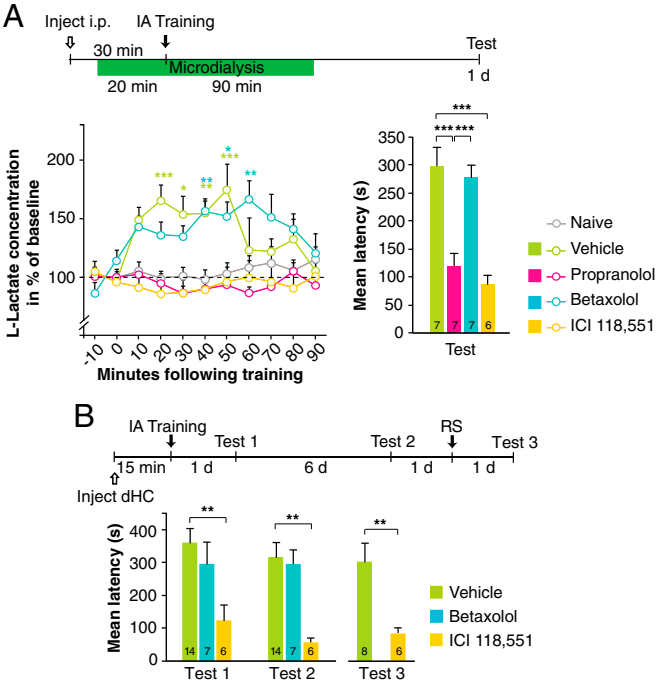

Fig. 2. Propranolol and ICI 118,551, but not betaxolol, block both training-evoked L-lactate increase in the dHC and long-term IA memory formation. (*A*, *Left*) The relative concentration of L-lactate in the dHC was measured by microdialysis in freely moving rats injected i.p. with vehicle, propranolol, betaxolol, or ICI 118,551 30 min before training. Untrained (naive) rats injected with vehicle were used as controls. Baseline data were collected for 20 min before training, and data collection continued for 90 min after training. Data are expressed as the percent of baseline L-lactate concentration ± SEM (mean of the first two samples set at 100%; n = 4–7). (*Right*) Long-term memory retention of the rats tested in microdialysis expressed as mean latency (in seconds) ± SEM (n = 6–7). (*B*) Long-term memory tested 1 d (test 1) and 7 d (test 2) after training and following an RS (test 3) in rats that received a bilateral dHC injection of vehicle, ICI 118,551, or betaxolol 15 min before training. Data are expressed as mean latency (in seconds) ± SEM (n = 6–14). $^{**}P < 0.01$; $^{***}P < 0.001$. Numeric values and detailed statistical analyses are reported in Tables S5, S6, and S7.

neuronal marker NeuN (Fig. 3 *B* and *C*) or with the microglial marker ionized calcium-binding adaptor molecule 1 (Iba1) (Fig. S3). Conversely, AAVDJ-hSyn-eGFP expression was found selectively in neurons and not astrocytes (Fig. 3 *D*–*F*).

To knock down β_2ARs, we used two methods: shRNA miR (Sh) or antisense (AS) sequences against β_2ARs. shRNA were designed by Vector Biolabs (*SI Materials and Methods*). The Sh and AS sequences were engineered into the astrocyte-specific virus, resulting in AAV9-short gfaABC1D-eGFP-β_2AR-Sh and AAV9-short gfaABC1D-β_2AR-AS (henceforth referred to as "gfa-Sh" and "gfa-AS," respectively) or into the neuron-specific virus, resulting in AAVDJ-hSyn-eGFP-β_2AR-Sh and AAVDJ-hSyn-β_2AR-AS (henceforth referred to as "hSyn-Sh" and "hSyn-AS," respectively). Relative scrambled (Scr) or sense (S) sequences were used as controls.

First, we measured the ability of the Sh and AS viruses to affect the selective expression of the β_2AR protein. Using β_1AR/β_2AR-knockout mouse brains (32) we used Western blot analyses and/or immunohistochemistry to conduct specificity testing of several available antibodies indicated to bind β_2ARs specifically, but we failed to identify any specific reactivity (Table S8). Thus, to quantify β_2AR and β_1AR protein levels, we used receptor autoradiography binding assays. As previously established (10), this method is based on the quantification of iodo-(-)-cyanopindolol ([^{125}I] CYP) binding in the presence of serotonergic inhibitors (SB 224289 and WAY 100,635) and of the β_1AR inhibitor CGP-20712A to assess the expression of β_2AR or in the presence of the β_2AR inhibitor ICI 118,551 to assess the expression of β_1AR. Using this method, we quantified the distribution of β_1AR and β_2AR binding and confirmed the previously described differential distributions of these receptors in the hippocampus (Fig. 4*A*) (10). We observed that β_2AR binding occurs in the lacunosum moleculare and the stratum oriens subregions (Fig. 4*B*), which are enriched in astrocytes rather than neurons (Fig. 4*C*), whereas β_1AR binding was diffused throughout the hippocampus (Fig. 4*B*).

Compared with relative Scr controls, the infection of both gfa-Sh and hSyn-Sh resulted in a significant decrease in β_2AR binding without affecting the binding levels of β_1AR ($P < 0.001$ Student's t test) (Fig. 5 *A* and *B*), providing evidence for the specific

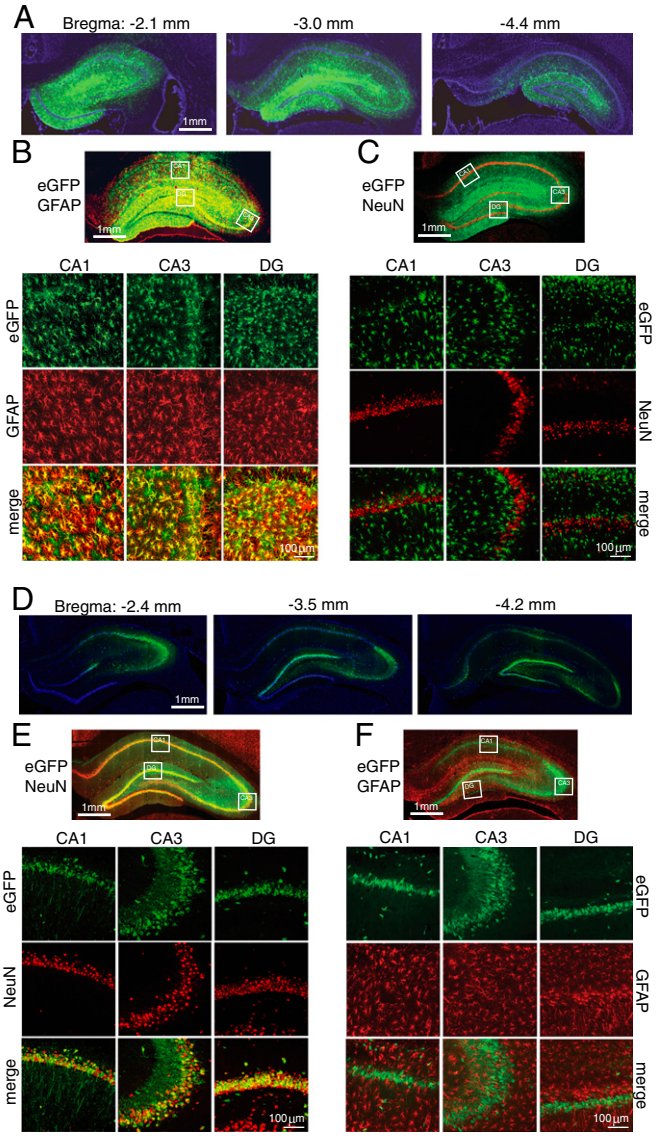

Fig. 3. AAV under the short gfaABC1D or the hSyn promoter selectively targets astrocytes or neurons, respectively. dHC infection of AAV9-short gfaABC1D-eGFP (*A*–*C*) or AAVDJ-hSyn-eGFP (*D*–*F*) 2 wk after bilateral injection (*A*, *D*). Shown are representative images of rostral-to-caudal brain sections [bregma −2.4 to −4.4 mm (45)]. All images of whole dHC are composite tile scans. In AAV9-short gfaABC1D-eGFP–infected cells, eGFP expression colocalizes with immunostaining of GFAP (*B*) but not NeuN (*C*) in subregions CA1 and CA3 and the dentate gyrus (DG). In AAVDJ-hSyn-eGFP–infected cells, eGFP expression colocalizes with NeuN (*E*) but not GFAP (*F*) in subregions CA1 and CA3 and the dentate gyrus.

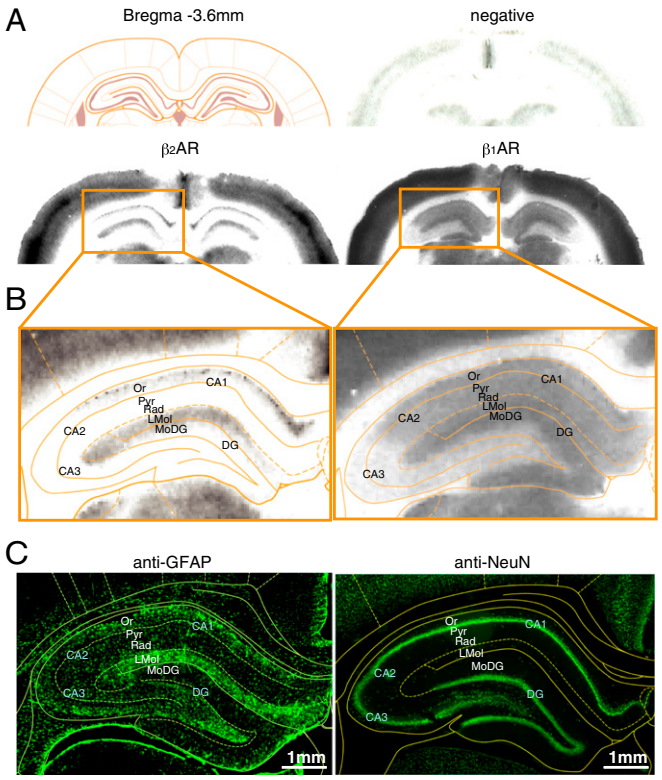

Fig. 4. β_2AR and β_1AR autoradiography. (*A*) Anatomical schematic representation of coronal brain sections at bregma −3.6 mm (45) relative to representative examples of receptor autoradiography: negative control and [^{125}I] CYP binding to β_2AR or β_1AR. (*B*) dHC autoradiography for β_2AR or β_1AR binding. (*C*) Composite tile scan images of immunostaining with anti-GFAP (astrocytes) or anti-NeuN (neurons) antibodies of a dHC section shown at the same bregma coordinates. DG, dentate gyrus; LMol, stratum lacunosum moleculare; MoDG, stratum moleculare of the dentate gyrus; Or, stratum oriens; Pyr, stratum pyramidale; Rad, stratum radiatum.

knockdown of β_2AR in astrocytes or neurons, respectively. Notably, the knockdown of the astrocyte-selective virus was more robust (about 50%) than that of the neuron-selective virus (about 20%), as is consistent with a more abundant β_2AR binding codistribution with astrocytes than with neurons and with previous literature suggesting higher expression of β_2AR in glia (18, 21–23).

Next we determined the effect of virus-mediated knockdown of β_2ARs on memory retention. gfa-Sh bilaterally injected into the dHC 2 wk before training had no effect on short-term memory tested 1 h after training (Fig. 5*C*) but impaired long-term memory 1 d and 7 d later ($P < 0.0001$; two-way repeated-measures ANOVA followed by Bonferroni post hoc tests) and following an RS ($P < 0.01$; one-way ANOVA followed by Bonferroni post hoc tests) (Fig. 5*D*). Lactate injected bilaterally into the dHC 15 min before training fully and persistently rescued the memory impairment (Fig. 5*D*). In contrast, 2 wk after hippocampal injection, hSyn-Sh elicited no change in long-term memory retention compared with Scr controls ($P > 0.05$; two-way repeated-measures ANOVA) (Fig. 5*E*). To rule out the contribution of behavioral ceiling retentions and/or suboptimal time of infection to the lack of an effect of neuronal β_2AR knockdown on memory, we also tested the effect of a longer, 4-wk infection of hSyn-Sh and of a weaker memory evoked by milder-intensity training (0.6 mA footshock) given either 2 or 4 wk after infection. Memory tested 1 d and 7 d after training showed no differences in any of these conditions ($P > 0.05$; two-way repeated-measures ANOVA) (Fig. S4 and Table S9), indicating that neuronal β_2ARs are not critical for IA memory consolidation.

Similar results were obtained using gfa-AS and hSyn-AS. Four weeks after infection, both gfa-AS and hSyn-AS significantly reduced β_2AR binding compared with S controls ($P < 0.05$; Student's *t* test), whereas β_1AR binding remained unchanged (Fig. 6 *A* and *B*). Gfa-AS had no effect on short-term memory (Fig. 6*C*) but significantly impaired long-term memory tested at 1 d and 7 d after training (Fig. 6*D*). The memory impairment was rescued by a bilateral injection of lactate into the dHC 15 min before training ($P < 0.0001$; two-way repeated measures ANOVA followed by Bonferroni post hoc tests) (Fig. 6*D*). In contrast, hSyn-AS had no effect on long-term memory ($P > 0.05$; one-way ANOVA) (Fig. 6*E*).

Together, these data show that β_2ARs expressed by hippocampal astrocytes rather than neurons are the critical effectors of the adrenergic-mediated effect on long-term memory formation. Specifically, astrocytic β_2ARs play a critical role in memory consolidation by activating lactate release that supports long-term memory formation and its underlying molecular changes.

Discussion

Using two distinct, virus-mediated, cell-specific knockdown approaches, we showed that hippocampal β_2ARs, but not β_1ARs, and specifically β_2ARs expressed on astrocytes but not on neurons, are required for IA memory consolidation. The critical action of the hippocampal β_2ARs is coupled to the training-evoked lactate release.

These results together with the previously established roles for β_1ARs in synaptic plasticity and CFC memory retrieval of both rats and mice (12, 14) show that β_1ARs and β_2ARs contribute differentially to hippocampal memory function and processes. We speculate that in the hippocampus β_1ARs may activate neuronal responses during retrieval and that β_2ARs activate astrocytic metabolic coupling to neurons to mediate memory consolidation. This differential role of β_1ARs and β_2ARs in memory processes seems to be part of a broader picture of distinct, and in some cases opposite, roles of the β_1ARs and β_2ARs in memory processes such as consolidation and retrieval which involve different brain regions in different memory systems (13, 33). Our findings of a cell-specific role of β_2ARs in hippocampal astrocytes may contribute to explaining controversial data on systemic or genetic ablation vs. intraregional inhibition of receptor subtypes (12, 15). Understanding these differential roles and contributions of glial vs. neuronal βARs, perhaps in different neuronal populations (excitatory vs. inhibitory neurons) as well as in different brain regions, is key to unraveling the mechanisms of NA-mediated responses. This understanding also should help the clinical use of βAR antagonists or agonists, and the understanding of the relative behavioral outcomes.

Similar to the results described in chicks (34), our data showed that astrocytic β_2ARs in the hippocampus play a critical role in memory consolidation. Although the abundance of βAR subtypes does not correlate in chick and mammalian brains (34), it seems that β_2ARs expressed by astrocytes have conserved roles in memory formation by controlling glycogenolysis (27). In addition, lactate production from aerobic glycolysis coupled to β_2AR stimulation does not seem to occur exclusively in the brain; in fact, it has also been described as occurring in muscle during shock states associated with a reduced or maintained blood flow (35).

Our results are consistent with several previous findings concerning functional regulation, mechanisms of action, and anatomical targeting of NA in the mammalian brain, where NA, produced by the locus coeruleus (LC), projects diffusely to a variety of brain areas, including the neocortex (36) and the hippocampus (37, 38). First, because NA is released in the extracellular space from junctional varicosities along its fibers, it can act on extrasynaptic receptors, which are localized on astrocytes in particular (39). Second, in the hippocampus the highest noradrenergic innervation is found in the dentate gyrus and in the stratum lacunosum moleculare, where we found an enrichment of β_2ARs (Fig. 4*B*). Third, one cellular consequence of NA release during LC firing in particular behavioral states or evoked by glutamatergic inputs is glycogenolysis (30). Fourth, NA signaling is necessary for long-term potentiation (17), memory consolidation (2), and

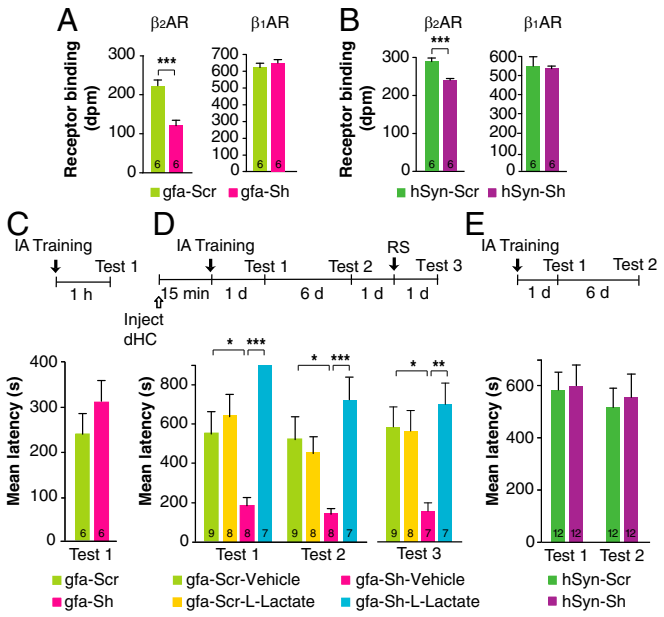

Fig. 5. ShRNA-mediated knockdown of β_2AR in astrocytes but not in neurons impairs IA long-term memory. This impairment is rescued by L-lactate. (*A* and *B*) β_2AR and β_1AR binding, expressed in disintegrations per minute (dpm), ($n = 6$) measured 2 wk after injection of gfa-Scr or gfa-Sh (*A*) or hSyn-Scr or hSyn-Sh (*B*). (*C*) Short-term memory tested 1 h after training and expressed as mean latency ± SEM (in seconds) in rats injected with either gfa-Scr or gfa-Sh 2 wk before training ($n = 6$). (*D*) Long-term memory tested 1 d (test 1) and 7 d (test 2) after training and after an RS (test 3), expressed as mean latency ± SEM (in seconds), in rats injected with either gfa-Scr or gfa-Sh 2 wk before training ($n = 7$–9) and with a bilateral dHC injection of vehicle or L-lactate administered 15 min before training. (*E*) Long-term memory tested 1 d (test 1) and 7 d (test 2) after training, expressed as mean latency ± SEM (in seconds), in rats injected with either hSyn-Scr or hSyn-Sh 2 wk before training ($n = 12$). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Numeric values and detailed statistical analyses are reported in Tables S10 and S11.

the induction of plasticity and memory genes such as Arc, pCREB, and pCaMKIIα in the hippocampus in vivo (Fig. 1).

Our findings that the training-dependent lactate increase in the hippocampus requires β_2ARs and not β_1ARs and that supplying lactate rescues the IA memory impairment caused by hippocampal astrocytic β_2AR knockdown strengthens the conclusion that astrocytically generated lactate is a key mediator of hippocampus-dependent memory formation under arousing conditions (29). Our results do not dissect whether this lactate originates solely from glycogenolysis or also from glutamate-mediated glycolysis, as proposed by the astrocytic–neuronal lactate shuttle hypothesis (40). As suggested by Hertz and Gibbs (41), the level of stress/arousal evoked by the task may dictate whether glycogenolysis and/or glycolysis are recruited to provide the lactate necessary for memory consolidation. It is possible that the relevance of the experience, and thus the experience-evoked stress level, determines whether astrocytic β_2ARs are recruited as essential mechanisms of memory consolidation.

Furthermore, similar to previous observations with the glycogenolysis inhibitor 1,4-dideoxy-1,4-imino-D-arabinitol hydrochloride (DAB) (29), a direct supply of glucose into the hippocampus was unable to replicate the effect of lactate, and only at higher concentrations were the memory impairments caused by β_2ARs disruption transiently rescued. Although further experiments are needed to dissect this issue, one possible explanation is that activity-dependent processes promote glucose entry into astrocytes rather than directly into neurons. Glucose then would be metabolized into lactate and finally transported from astrocytes to neurons (42). If this is the case, direct hippocampal delivery of lactate therefore might be more efficient.

Because the dysregulation of β_2ARs in astrocytes is associated with multiple sclerosis, Parkinson's disease, and Alzheimer's disease (43), we suggest that the disruption of the metabolic role of astrocytic β_2ARs in regions supporting cognitive functions may contribute to the pathological features of those diseases. Hence, targeting astrocytic β_2ARs mechanisms may help prevent or repair these disorders and/or their precipitation by stress.

Materials and Methods

All animal protocols complied with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (44) and were approved by the Icahn School of Medicine at Mount Sinai Institutional Animal Care and Use Committee or by the New York University Animal Welfare Committee. Adult male Long–Evans rats were implanted with cannulae and/or injected with AAV bilaterally into the dHC (from bregma: anteroposterior, −4 mm; mediolateral, ±2.6 mm; and dorsoventral, −3.5 mm) as described in ref. 31. IA training (0.9 mA for 2 s) and testing were performed as described in ref. 31. Drug injections (i.p.) of propranolol (Sigma-Aldrich), betaxolol (Tocris Bioscience), or ICI 118,551 (Sigma) were done at 10 mg/kg. Intra-dHC injections (1 μL per side) of propranolol (5 μg/μL), betaxolol (2.2 μg/μL), ICI 118,551 (5 μg/μL), L-lactate (100 mM), or D-glucose (50 mM or 150 mM, as indicated) were performed as described previously (29, 31). For Western blot analysis, dHC protein extracts were done as described in ref. 31; primary antibodies were rabbit anti-pCREB (1:2,000; Cell Signaling), rabbit anti-CREB (1:1,000; Millipore), rabbit anti-Arc (1:1,000; Synaptic Systems), rabbit anti-pCaMKII (1:5,000; Cell Signaling), and mouse anti-CaMKII (1:4,000; Millipore). Immunohistochemistry was performed using mouse anti-GFAP (1:400; Millipore),

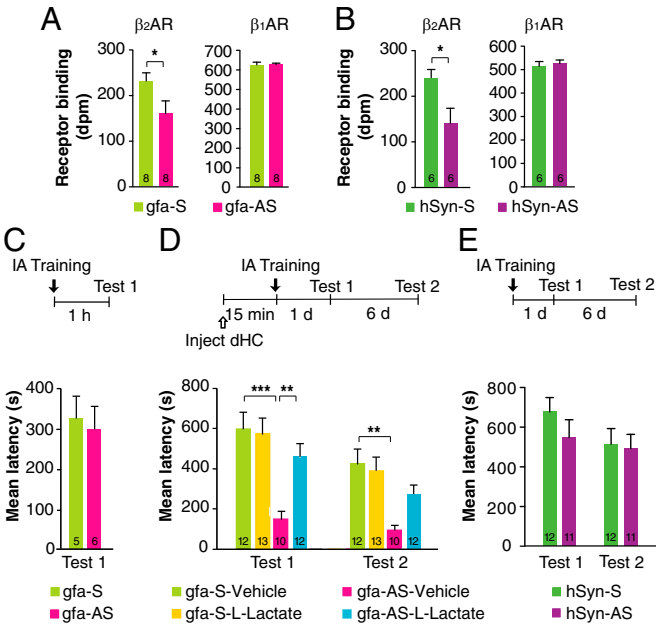

Fig. 6. Antisense-mediated knockdown of β_2AR in astrocytes but not in neurons impairs IA long-term memory. The impairment is rescued by L-lactate. (*A* and *B*) β_2AR and β_1AR binding expressed in disintegration per minute ($n = 6$) measured 4 wk after injection of gfa-S or gfa-AS (*A*) or hSyn-S or hSyn-AS (*B*). (*C*) Short-term memory tested 1 h after training and expressed as mean latency ± SEM (in seconds) of rats injected with either gfa-S or gfa-AS 4 wk before training ($n = 5$ or 6). (*D*) Long-term memory tested 1 d (test 1) and 7 d (test 2) after training, expressed as mean latency ± SEM (in seconds) of rats injected with either gfa-S or gfa-AS 4 wk before training ($n = 10$–13). A bilateral dHC injection of vehicle or L-lactate was administered 15 min before training. (*E*) Long-term memory tested 1 d (test 1) and 7 d (test 2) after training, expressed as mean latency ± SEM (in seconds) of rats injected with either hSyn-S or hSyn-AS 4 wk before training ($n = 11$ or 12). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Numeric values and detailed statistical analyses are reported in Tables S12 and S13.

mouse anti-NeuN (1:200; Millipore), or rabbit anti-Iba1 (1:400; Wako). Microdialysis samples, collected every 10 min, were analyzed using the Abcam Lactate Fluorescence Assay Kit as described in ref. 29. Receptor autoradiography was performed as described in ref. 10; [^{125}I] CYP (Perkin-Elmer) containing 1 μM SB 224289 (Tocris), 1 μM WAY 100635 (Tocris), and 100 nM CGP 20712 (Tocris) or 50 nM ICI 118,551 (Sigma) was used. Data were analyzed using one-way or two-way ANOVA or two-way repeated-measures ANOVA followed by Bonferroni or Newman–Keuls post hoc tests or Student's *t* test.

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