Latrophilin 1 and its endogenous ligand Lasso/teneurin-2 form a high-affinity transsynaptic receptor pair with signaling capabilities

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Edited by Peter Scheiffele, Biozentrum, University of Basel, Basel, Switzerland, and accepted by the Editorial Board June 5, 2011 (received for review December 24, 2010)

Latrophilin 1 (LPH1), a neuronal receptor of α-latrotoxin, is implicated in neurotransmitter release and control of presynaptic Ca^{2+}. As an "adhesion G-protein-coupled receptor," LPH1 can convert cell surface interactions into intracellular signaling. To examine the physiological functions of LPH1, we used LPH1's extracellular domain to purify its endogenous ligand. A single protein of ∼275 kDa was isolated from rat brain and termed Lasso. Peptide sequencing and molecular cloning have shown that Lasso is a splice variant of teneurin-2, a brain-specific orphan cell surface receptor with a function in neuronal pathfinding and synaptogenesis. We show that LPH1 and Lasso interact strongly and specifically. They are always copurified from rat brain extracts. Coculturing cells expressing LPH1 with cells expressing Lasso leads to their mutual attraction and formation of multiple junctions to which both proteins are recruited. Cells expressing LPH1 form chimerical synapses with hippocampal neurons in cocultures; LPH1 and postsynaptic neuronal protein PSD-95 accumulate on opposite sides of these structures. Immunoblotting and immunoelectron microscopy of purified synapses and immunostaining of cultured hippocampal neurons show that LPH1 and Lasso are enriched in synapses; in both systems, LPH1 is presynaptic, whereas Lasso is postsynaptic. A C-terminal fragment of Lasso interacts with LPH1 and induces Ca^{2+} signals in presynaptic boutons of hippocampal neurons and in neuroblastoma cells expressing LPH1. Thus, LPH1 and Lasso can form transsynaptic complexes capable of inducing presynaptic Ca^{2+} signals, which might affect synaptic functions.

Latrophilin 1 (LPH1) is a neuronal G protein–coupled receptor (GPCR) that binds α-latrotoxin (α-LTX) (1–3), or its recombinant mutant LTX^{N4C} (4), and sends intracellular signals (5) leading to a massive increase in spontaneous and evoked exocytosis of neurotransmitters (6–8). The dramatic inhibition of the presynaptic action of α-LTX in the LPH1 knockout mouse (9) indicates that LPH1 is localized in presynaptic terminals and mediates most of the α-LTX effect.

LPH1 is a so-called "adhesion GPCR" (10, 11). Members of this family have large and divergent N-terminal ectodomains and conserved C-terminal domains containing seven transmembrane regions (TMRs) (12). After synthesis, adhesion GPCRs undergo autoproteolysis upstream of the first TMR (13, 14). The N-terminal fragment (NTF) is not normally released into the medium, but remains bound to the cell surface via the C-terminal fragment (CTF) (13) and/or an unknown hydrophobic anchor (5). On binding an agonist (e.g., LTX^{N4C}), the fragments of LPH1 reassemble, eliciting an intracellular Ca^{2+} signal via the CTF (5, 15).

The functions of LPH1 and its homologs (LPH2 and LPH3) (3) are unclear. In *Caenorhabditis elegans*, the LPH1 ortholog LAT-1 plays a role in embryo morphogenesis (16) and modulation of neurotransmitter release (17, 18). Null mutations in LAT-1 arrest worm development at the embryo stage (16). In rats, the LPH1 gene (*lphn1*) is up-regulated by antipsychotic drugs (19). In humans, deletions of a chromosome 19 fragment containing *lphn1* lead to brain malformations, mental retardation, and hyperactivity (20). Mutations in LPH3 are linked to attention deficit/hyperactivity disorder (21). However, the endogenous ligand of LPH1 has not yet been identified, hampering the elucidation of its physiological functions.

Teneurins (also called odd Oz, tenascin-m, neurestin, or DOC4) are large cell surface glycoproteins with a single TMR found in all animal species (22–24). Their ectodomains contain eight EGF-like repeats, two of which have unpaired cysteine residues that mediate covalent homodimerization of the molecule (25). The distal part of the C terminus, containing 6 NHL and 26 YD repeats, forms a 13-nm globule (25).

All four teneurins found in vertebrates are expressed specifically in the brain (25), although teneurin-3 and -4 also show low expression in other tissues (25–27). Teneurins play a role in development, neurite outgrowth, axon guidance, neuronal connectivity, and synaptogenesis (reviewed in refs. 28 and 29). In *C. elegans*, mutations in *ten-1* lead to aberrations in axon guidance and morphogenesis (30, 31). Knockout of Ten-m3 in mice impairs vision, consistent with abnormal patterning of visual pathways (32). In humans, ablation of the teneurin-1 gene and rearrangements of the teneurin-2 gene lead to mental retardation (33, 34). Teneurins are hypothesized to engage in homophilic interactions (25, 35), but attempts to isolate an endogenous ligand of teneurins have not been described.

In the present study, we used the NTF of LPH1 to isolate its natural ligand. Only one protein, Lasso (a splice variant of teneurin-2), has been found to bind LPH1. We demonstrate that the interaction of Lasso and LPH1 is specific, strong, and functional,

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Author contributions: K.E.V. and Y.A.U. designed research; J.-P.S., V.G.L., Y.S.E., N.V., P.G.H., O.B., M.A.R., A.Z., S.F., and K.E.V. performed research; A.G.T. and A.D. contributed new reagents/analytic tools; J.-P.S., Y.S.E., P.G.H., K.E.V., and Y.A.U. analyzed data; and J.-P.S. and Y.A.U. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. P.S. is a guest editor invited by the Editorial Board.

Data deposition: The sequences of the full-length Lasso and recombinant constructs used in this study are available at GenBank (accession nos. JF784339–JF784359).

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1019434108/-/DCSupplemental.

leading to intracellular signaling. LPH1 and Lasso interact across intercellular and synaptic junctions, suggesting that this trans-synaptic pair might be involved in synaptic functions.

Results

Isolation of the LPH1 Ligand. To create an affinity adsorbent, three soluble constructs based on the NTF of LPH1 were expressed in neuroblastoma (NB2a) cells (Fig. 1*A*): LPH-51, containing a V5 tag at the N terminus and the natural cleavage sequence at the C terminus; LPH-74, terminating exactly at the cleavage site; and LPH-62, with a V5 tag attached at the cleavage site but not cleaved in cells. α-LTX binding to these proteins demonstrated biological activity in only LPH-51 (Fig. 1*B*).

Purified LPH-51 was attached to an immobilized anti-V5 Ab and used for affinity chromatography of a detergent extract from rat brain. Bound proteins were desorbed using high pH, which also eluted LPH-51 but not the Ab. We used two control conditions: a V5 Ab/LPH-51 column not incubated with brain extract and a V5 Ab column (without LPH-51) incubated with the extract. A single brain protein of ~275 kDa was eluted from the test column, but not from the control columns (Fig. 1*C*). To explore the nature of this protein, we used two additional V5 Ab/LPH-51 columns, one incubated with a detergent-free extract from brain and the other incubated with a detergent extract from rat liver. The 275-kDa protein did not bind to control columns (Fig. 1*D*).

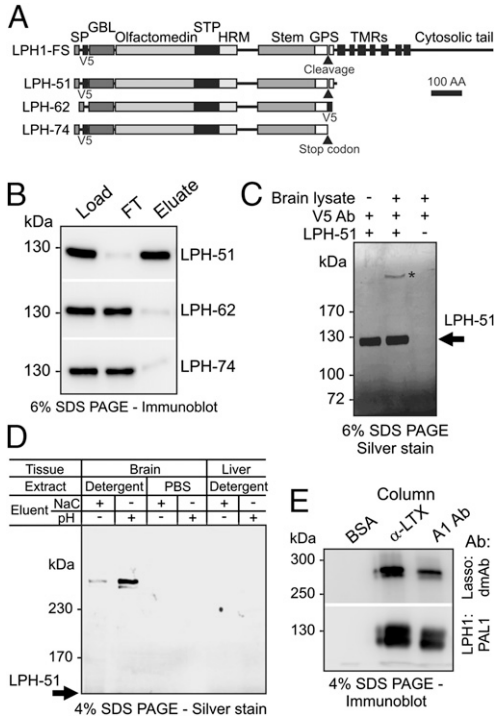

Fig. 1. Isolation of a LPH1 ligand. (*A*) LPH1-FS and variants of its soluble ectodomains. SP, signal peptide; GBL, galactose-binding lectin; STP, rich in serine, threonine, and proline; GPS, GPCR proteolysis site; V5, V5 epitope. (*B*) Only LPH-51 binds α-LTX with high affinity. Soluble constructs were expressed in NB2a cells, bound to α-LTX columns, eluted with SDS, and analyzed by immunoblotting with anti-V5 mAb ($n = 6$). (*C*) Lasso is only isolated on LPH-51. Detergent extract from rat brain was incubated with V5 Ab/LPH-51 or V5 Ab columns. A control V5 Ab/LPH-51 column was incubated with buffer only. The columns were washed and eluted with alkali. The asterisk denotes the protein (Lasso) isolated on the test column but not on control columns. (*D*) Lasso is a membrane-bound, brain-specific protein that binds LPH-51 tightly. Three V5 Ab/LPH-51 columns (as in *C*) were incubated with detergent extracts from rat brain or rat liver or with a buffer extract from rat brain, washed, and eluted with 1 M NaCl and then with alkali. (*E*) Lasso specifically copurifies with LPH1. Rat brain extract was incubated with an α-LTX column, an anti-LPH1 recombinant Ab (A1) attached to a V5 Ab column, or a BSA column. SDS eluates were analyzed by immunoblotting with the indicated Abs ($n = 3$).

Initial MS analysis failed to identify the LPH1-binding protein, but the affinity chromatography results (Fig. 1*D*) indicated that the isolated protein was brain-specific and membrane-bound. Thus, it was operationally termed Lasso (LPH1-associated synaptic surface organizer). For further analysis, we isolated a large amount of Lasso and elicited anti-Lasso Abs in mice.

These Abs (dmAbs) were used to test whether Lasso interacts with endogenous LPH1. We purified LPH1 from rat brain lysate by affinity chromatography on α-LTX or anti-LPH1 Ab and found that Lasso was isolated together with LPH1 in both cases, but not on a control column (Fig. 1*E*). Thus, in the brain, LPH1 and Lasso form strong complexes that survive stringent purification conditions.

We also used high-resolution tandem MS for de novo sequencing of Lasso and determined the sequences of nine tryptic peptides (Table S1). All of the peptides corresponded precisely to neurestin (26), also known as rat teneurin-2.

Based on the peptide sequences obtained, cDNA encoding full-size (FS) human Lasso was cloned (Fig. 2*A*). Lasso cDNA is 12,450 bp long and encodes a protein which has a calculated molecular mass of 292 kDa, no signal peptide, one predicted TMR, and is a splice variant of teneurin-2 (Fig. 2*A*).

Lasso/Teneurin-2 Is a Ligand of LPH1. To examine the interaction between Lasso and LPH1, we expressed Lasso as membrane-bound (Lasso-A) or secreted (Lasso-B to -H) proteins (Fig. 2*A*). These constructs were produced in stably transfected NB2a cells (Lasso-A to -F), transiently transfected HEK293 cells (Lasso-H), or bacteria (Lasso-G expressed as a GST fusion).

We tested the truncated soluble proteins for LPH1 binding by a pull-down assay (Fig. 2*B*). Lasso-B, -D, and -E strongly bound LPH-51, whereas Lasso-C and -F showed no appreciable binding (Fig. 2*B*), suggesting that LPH1 binding localized to Lasso's C-terminal globular domain. To confirm this, we tested Lasso-H in a cell-binding assay and found that it interacted specifically with LPH1-FS-expressing cells (Fig. S1 *C* and *G*).

We also prepared truncated constructs of LPH1 (Fig. 2*C*). Compared with LPH-51, these constructs displayed no affinity for Lasso (Fig. 2*D*). This lack of binding was not due to misfolding of the LPH1 mutants, because constructs LPH-97–99 strongly bound α-LTX (Fig. 2*E*), indicating that at least these proteins folded correctly. As all truncations in LPH1 led to a loss of binding to Lasso, but not to α-LTX, and considering the large size of the globular domain of Lasso, the latter likely binds LPH1 at multiple points, which cooperate to provide a high-affinity interaction.

Which of the three LPH homologs expressed in mammalian brain (3) binds Lasso specifically? We incubated Lasso-D with rat brain lysate and immunoprecipitated it. Analysis of the eluate with Abs against each LPH homolog (36) identified LPH1 as the main ligand of Lasso-D (Fig. 2*F*). Compared with the brain content of each LPH homolog, the binding was ranked as follows: LPH1, 100% ± 10%; LPH2, 9% ± 8%; LPH3, 0.5% ± 0.1% ($n = 3$).

The dissociation constant, K_d, of the Lasso–LPH1 complexes was found to be 1.7 ± 0.2 nM for Lasso-D (Fig. 2*G*).

Head domains of teneurin-1, -3, and -4 (similar to Lasso-H) (Fig. S1*A*) were also expressed in HEK293 cells (Fig. S1*H*) and tested for LPH1 binding; however, unlike Lasso-D and -H (Fig. S1 *B* and *C*), they demonstrated no interaction (Fig. S1 *D*–*G*). This agrees with the MS data showing that only teneurin-2 was purified from brain on the LPH1 column (Table S1).

Lasso and LPH1 Interact Across Artificial and Synaptic Junctions. We then verified that LPH1 and Lasso also interact on the cell surface. Lasso-D and LPH-51 were incubated with NB2a cells

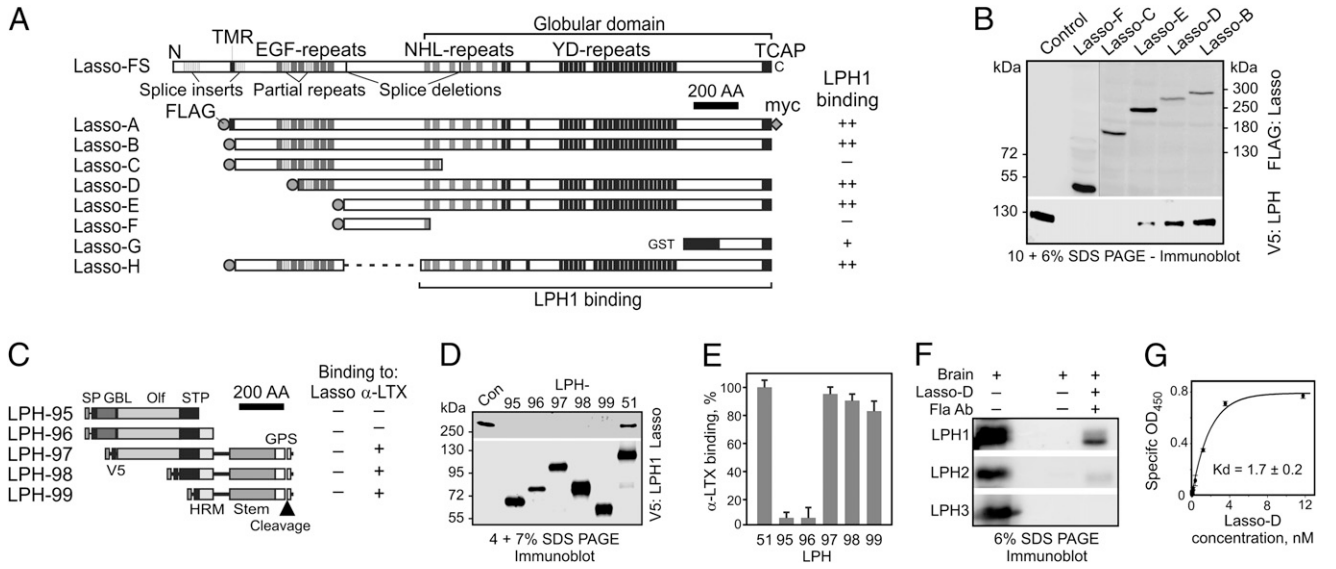

Fig. 2. Interaction of Lasso with LPH1. (*A*) Lasso (GenBank accession no. JF784339) and its recombinant constructs. Binding to LPH-51 is summarized on the right. (*B*) LPH1 binding localizes to the globular domain of Lasso. Soluble constructs (Lasso-B–Lasso-F) were expressed in NB2a cells, incubated with LPH-51, pulled down using FLAG Ab, and analyzed by immunoblotting with anti-V5 Ab. (*C*) Truncated NTFs of LPH1. Binding to Lasso-D and α-LTX is shown on the right ($n = 3$). (*D*) Truncated NTFs do not bind Lasso-D. Soluble LPH-95–LPH-99 were expressed in NB2a cells, incubated with Lasso-D, pulled down using anti-V5 Ab, and analyzed by immunoblotting with FLAG Ab ($n = 3$). (*E*) Some NTF constructs bind α-LTX. LPH-95–LPH-99 were attached to microtiter plates via V5 Ab, incubated with α-LTX and detected with anti-α-LTX Ab (mean ± SEM; $n = 3$). (*F*) Lasso binds LPH1, but not LPH2 or LPH3. Rat brain lysate was incubated with purified Lasso-D, precipitated with anti-FLAG Ab, and analyzed by immunoblotting with PAL1, PAL2, and PAL3 Abs (36) ($n = 2$). (*G*) Characterization of the Lasso–LPH1 interaction. LPH-51 was immobilized on microtiter plates via V5 Ab, incubated with different concentrations of Lasso-D, fixed, and stained with anti-FLAG Ab (mean ± SEM; $n = 3$).

expressing LPH1-FS and Lasso-A, respectively. Immunostaining showed that each soluble protein bound only to those cells that expressed its respective pair (Fig. 3 *A* and *B*).

To test whether Lasso and LPH1 also interact across intercellular junctions, we cocultured cells expressing LPH1-FS with cells expressing Lasso-A. As a control, Lasso-A cells were cocultured with cells expressing another adhesion GPCR, EMR2. We found that in LPH1/Lasso-A cocultures, the cells clearly preferred heterophilic interactions, i.e. LPH1 cells tended to aggregate with Lasso cells, forming multiple contacts (Fig. 3 *C* and *D* and Fig. S2*A*). The staining for each protein overlapped at cell contacts, to which both proteins were recruited (Fig. 3*D* and Fig. S2*A*). These effects were not observed in control cocultures (Fig. 3*C*). Quantification of colocalized immunostaining (Fig. 3*E*) showed that LPH1 and Lasso interacted specifically across intercellular junctions.

Similar to the unrelated receptor EMR2 (Fig. 3*C*), LPH2-FS, a LPH1 homolog that poorly binds Lasso, also failed to mediate cell aggregation, as demonstrated by the number and size of cell clusters formed (Fig. 3*F* and Fig. S2*B*). Thus, the attraction between LPH1 and Lasso cells is caused by a strong interaction between these proteins.

Both LPH1 and Lasso are found exclusively in the brain (Fig. 4 *A* and *B*). As this pair mediates cell–cell interactions in culture, could it participate in synaptic contacts? Fractionation of rat brain membranes revealed that both proteins were largely absent from the cell bodies (P1), but concentrated in isolated synapses and synaptic plasma membranes (Fig. S3*A*).

Immunostaining of rat hippocampal cultures (Fig. 4*C* and Fig. S3) revealed that endogenous LPH1 was absent from cell bodies, but present in axons (Fig. S3*B*), and enriched in growth cones and synapses, colocalizing well with synapsin and partially with PSD-95 (Fig. 4*C* and Fig. S3 *C–G*). Lasso was expressed mainly on dendrites (Fig. S3*H*), concentrating in dendritic spines and large patches on dendritic shafts (Fig. 4*C* and Fig. S3 *I–L*) and colocalizing partially with synapsin and strongly with PSD-95 (Fig. 4*C* and Fig. S3 *I–J*). Costaining for LPH1 and Lasso revealed puncta of distinct sizes (smaller for LPH1 and larger for Lasso) that often colocalized in shaft synapses (Fig. 4*C* and Fig. S3 *K* and *L*) and almost always in varicosities formed at neurite intersections (Fig. S3 *M* and *N*). The degree of LPH1 and Lasso colocalization with synaptic markers (Fig. 4*D*) appeared to be consistent with the two proteins residing on opposite sides of the synapse.

The synaptic localization of LPH1 and Lasso was confirmed by immunoelectron microscopy. To identify LPH1, synaptosomes were incubated with V5-tagged A1 Ab and gold-conjugated anti-V5 Ab (Fig. 4 *E* and *F* and Fig. S4). To localize Lasso, we incubated synaptosomes with LPH-51, then stained them with gold-conjugated anti-V5 Ab. This approach was tested on hippocampal neurons (Fig. S4*A*), before being applied in immunoelectron microscopy of synaptosomes (Fig. 4*G* and Fig. S4). The distribution of gold particles (Fig. 4*E*) showed that LPH1 was present on nerve terminals near synaptic clefts, but not on dendritic spines, whereas Lasso was widely present on dendritic spines and in the cleft.

To quantify the distribution of Lasso and LPH1 at synaptic junctions, we isolated rat brain synapses, dissociated them into pre- and postsynaptic junctional membranes (37), and analyzed by immunoblotting using voltage-gated K^+ channel (Kv1.2) and PSD-95 as pre- and postsynaptic markers, respectively. Further analysis of the membrane fractions (Fig. 4*H*) revealed that LPH1 was enriched in the presynaptic membranes, whereas Lasso was mostly postsynaptic (Fig. 4*I*). We verified our method using the known distribution of the NMDA-type glutamate receptor (Fig. 4*I*).

Taken together, these data indicate that LPH1 and Lasso are present in synaptic junctions and can form transsynaptic contacts.

LPH1 and Lasso in Chimerical Synapses. By forming transsynaptic pairs, LPH1 and Lasso could play a role in synaptogenesis. This prompted us to test this pair in artificial synapse formation. We cocultured primary hippocampal neurons with HEK293 cells expressing Lasso-A or LPH1-FS and immunostained these

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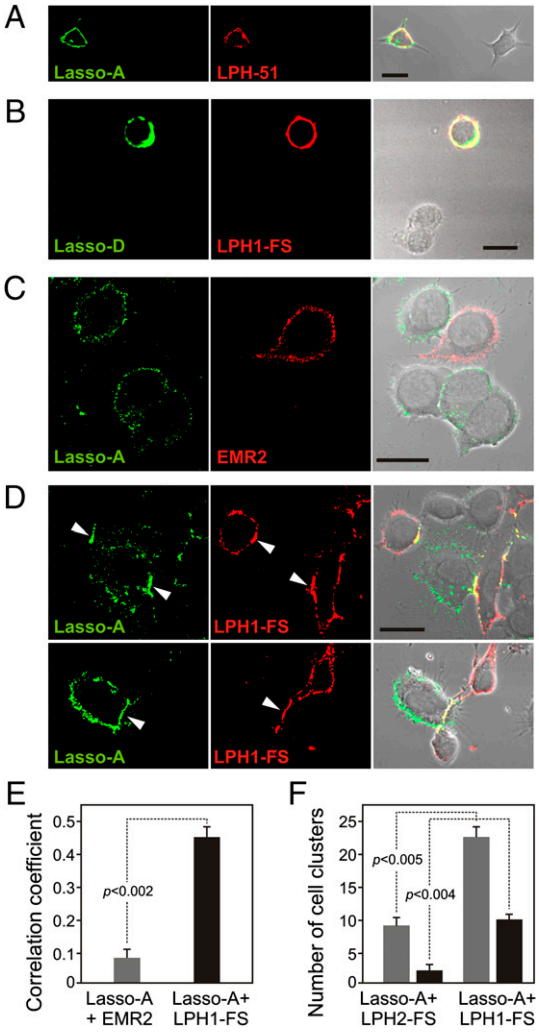

Fig. 3. Lasso and LPH1 interact on the cell surface and across intercellular junctions. (*A*) LPH-51 binds to cell-surface Lasso. NB2a cells expressing Lasso-A were incubated with LPH-51, fixed, and stained with V5 Ab (LPH-51) and with myc Ab (Lasso-A). (*B*) Soluble Lasso binds to cell-surface LPH1. Cells expressing LPH1-FS were incubated with Lasso-D, fixed, and stained with V5 Ab (LPH1-FS) and with FLAG Ab (Lasso-D). (*C* and *D*) Interaction between Lasso and LPH1 on opposite cells. NB2a cells expressing Lasso-A were cocultured with cells expressing a control GPCR (EMR2) (*C*) or LPH1-FS (*D*), fixed and stained for Lasso-A (green) and LPH-51 or EMR2 (red). Note that Lasso-A and LPH1-FS concentrate (arrowheads) and overlap (yellow color) at cell contacts. (Scale bars: 20 μm.) (*E*) Colocalization of Lasso-A with LPH1-FS (but not EMR2) at cell contacts (mean ± SEM; *t* test; $n = 10$). (*F*) Aggregation of Lasso-A–expressing cells with cells expressing LPH1-FS but not LPH2-FS. Gray bars, the number of cell clusters per field of view; black bars, the number of clusters > 100 μm (mean ± SEM; *t* test; $n = 5$).

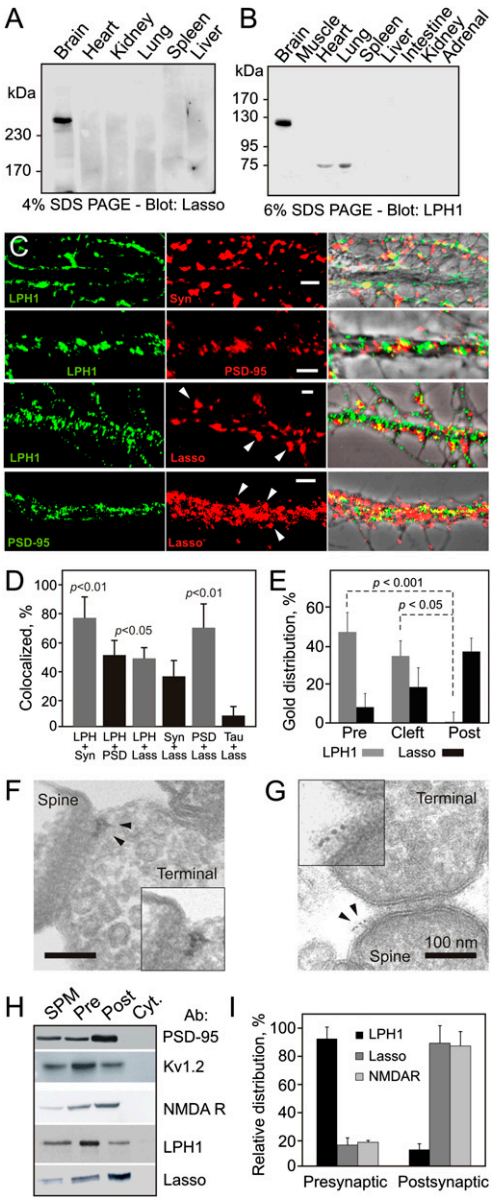

Fig. 4. Lasso and LPH1 are expressed on opposite membranes at synaptic junctions. (*A* and *B*) Lasso and LPH1 are brain-specific. Detergent extracts from rat tissues were analyzed by immunoblotting with dmAb or PAL1 Ab (typical results; $n = 3$–7). (*C*) Lasso and LPH1 are enriched in synapses. Hippocampal neurons (14 d in vitro) were immunostained for LPH1, synapsin, PSD-95, and Lasso, as indicated ($n = 5$–8 for each Ab). Arrowheads, dendritic spines. (Scale bars: 2 μm.) (*D*) Colocalization of LPH1 and Lasso with presynaptic and postsynaptic markers and the axonal protein tau in hippocampal cultures (images as in *C* and Fig. S3; the *t* tests compare each pair to tau+Lasso). (*E*) Quantitation of immunoelectron microscopy images (see *F* and *G* below and Fig. S4). The total number of gold particles in 517 micrographs was 1,241 for LPH1, 974 for control, and 558 for Lasso. (*F* and *G*) Immunoelectron microscopy of LPH1 (*F*) and Lasso (*G*) in isolated rat brain synapses. Synaptosomes were incubated with anti-LPH1 Ab A1 (*F*) or 100 nM LPH-51 (*G*), counterstained with anti-V5 Ab conjugated with 5 nm immunogold, processed, and imaged by electron microscopy. Synaptic terminals were identified as containing synaptic vesicles and an attached postsynaptic membrane with characteristic intracellular structures. (*H*) LPH1 is presynaptic and Lasso is postsynaptic in central synapses. Synaptic junction membranes were separated, enriched with presynaptic and postsynaptic membranes (37), and analyzed by immunoblotting with Abs against Lasso, LPH1, NMDAR, Kv1.2, and PSD-95. Note that Lasso distribution is similar to NMDAR but opposite of LPH1. (*I*) Quantitation of LPH1 and Lasso distribution (mean ± SEM; $n = 5$).

cocultures for Lasso or LPH1 and for the neuron-specific PSD-95 or synapsin 1. These experiments showed that LPH1-FS on the surface of HEK cells was recruited to contacts with passing dendrites, which respectively accumulated PSD-95 at these sites (Fig. 5*B*). By contrast, Lasso-A on HEK cells did not accumulate at artificial synapses marked by postsynaptic PSD-95 (Fig. 5*A*). Reciprocally, LPH1-FS did not colocalize with synapsin-positive axonal varicosities surrounding LPH1-FS–expressing cells (Fig. S5*B*), while Lasso-A did (Fig. S5*A*). In addition, Lasso-A induced strong axonal attraction (Fig. S5*A*), diverting axons from their original growth directions and causing them to envelop the Lasso-A+ cells. LPH1-FS expressed on HEK cells showed much lower axon-attracting activity and no ability to divert axonal growth

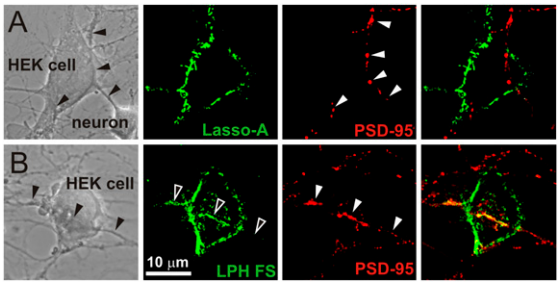

Fig. 5. LPH1 is recruited to artificial synapses. Cocultures of HEK293 cells expressing Lasso-A (*A*) or LPH1-FS (*B*) with primary hippocampal neurons were immunostained for respective tags and for the neuronal postsynaptic protein PSD-95. LPH1, but not Lasso, is recruited (empty arrowheads) to artificial junctions between HEK cells and *en passant* neuronal dendrites (filled arrowheads), denoted by PSD-95 ($n = 5$).

(Fig. S5*B*). These data indicate that while Lasso has a strong effect on axonal pathfinding and axon–target interaction, LPH1 is capable of inducing postsynaptic specializations.

C-Terminal Fragment of Lasso Induces LPH1 Signaling. What is the consequence of the LPH1–Lasso interaction? Hippocampal neurons and heterologous LPH1-expressing cells respond to LTX^{N4C} by increasing cytosolic Ca^{2+} (5, 6). To assess the ability of Lasso to stimulate similar signaling, we used Lasso-G (Fig. 2*A*), which was able to bind NB2a cells expressing LPH1-FS (Fig. S6*A*). The cells were loaded with the fluorescence Ca^{2+} indicator Fluo-4 and continuously imaged. When LTX^{N4C} (positive control) was added in the absence of Ca^{2+}, no signaling occurred, as expected from our previous experiments (5, 15); Lasso-G behaved similarly (Fig. S6*B*). However, when extracellular Ca^{2+} was added, the Ca^{2+} signal in the presence of LTX^{N4C} or Lasso-G was substantially stronger and longer-lasting than in negative control ($P = 0.001$) (Fig. S6 *B* and *C*). Thus, similar to LTX^{N4C}, the C-terminal fragment of Lasso can induce LPH1-mediated signaling to Ca^{2+} stores in LPH1-expressing cells.

We further tested the effect of Lasso-G on presynaptic Ca^{2+} dynamics in cultured hippocampal neurons. We simultaneously loaded a selected neuron with a fluorescent morphological tracer and Fluo-4 via a somatic patch-pipette in whole-cell mode (Fig. 6*A*; *SI Methods*). Using tracer fluorescence, we identified axons and presynaptic boutons (Fig. 6*B*) and measured presynaptic Ca^{2+} fluorescence before and after application of Lasso-G. Again, we found that, unlike the negative control, the C-terminal fragment of Lasso induced substantial increases in presynaptic Ca^{2+} concentration (Fig. 6 *C* and *D*).

Discussion

LPH1 has been implicated in multiple phenomena, including binding of α-LTX, release of neurotransmitters, intracellular signaling, neuronal morphogenesis, and mental conditions (reviewed in ref. 11). However, further studies of LPH1 require the identification of its endogenous ligand. Using LPH1-affinity chromatography, we have now isolated such a ligand. Lasso is a splice variant of teneurin-2. It interacts with LPH1 specifically and strongly (Fig. 2), but binds very weakly to LPH2 and does not bind to LPH3. Reciprocally, sequencing results (Table S1) and LPH1 binding (Fig. S1) suggest that LPH1 interacts with Lasso/teneurin-2 only, and not with teneurin-1, -3, or -4.

Both LPH1 and Lasso/teneurin-2 are highly abundant in the brain. Can they mediate neuronal cell interaction? Teneurin is proteolyzed between the TMR and EGF repeats (25, 38), and this could preclude its receptor activity. However, only a proportion of teneurin is cleaved, resulting in two bands on reducing SDS gels (Fig. 1 *C* and *D*). The fragment remains anchored on the cell surface (Figs. 3–5 and Figs. S1–S6), probably as part of the homodimer. This makes Lasso a bona fide cell-surface receptor.

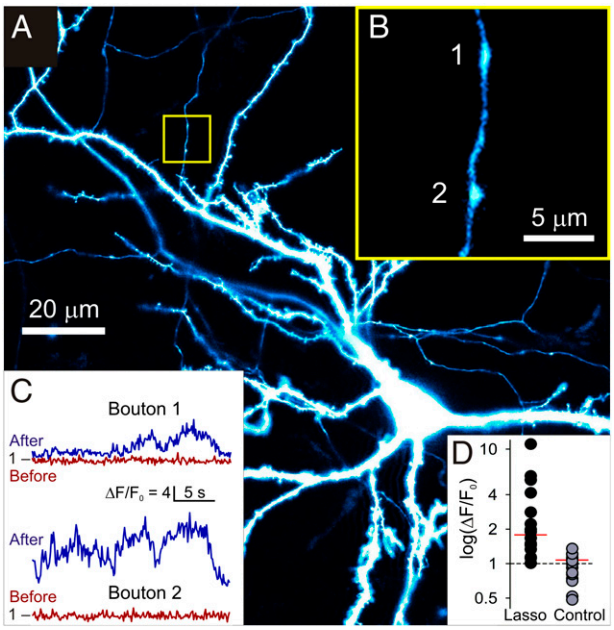

Fig. 6. C terminus of Lasso stimulates presynaptic Ca^{2+} signaling in cultured hippocampal neurons. (*A*) Fluorescence image of a typical hippocampal neuron loaded with the morphological tracer Alexa Fluor 568 and the Ca^{2+} dye Fluo-4 (only tracer fluorescence is shown). (*B*) A fragment of the axon containing two putative presynaptic boutons (magnified from the boxed area in *A*). (*C*) Normalized Ca^{2+} fluorescence responses in boutons 1 and 2, before (*red*) and 5 min after (*blue*) application of Lasso-G. (*D*) Relative change of Ca^{2+} fluorescence averaged over 15 min after application of Lasso-G (4 cells; 16 boutons) or in control conditions (6 cells; 22 boutons). Red lines represent median responses. Wilcoxon rank tests: control vs. 1, $P > 0.8$; Lasso-G vs. 1, $P < 0.001$; Lasso-G vs. control, $P < 0.002$.

Accordingly, Lasso and LPH1 mediate heterophilic cell-cell contacts between expressing cells in cocultures (Fig. 3) and formation of artificial synapses between fibroblasts and neurons (Fig. 5 and Fig. S5). Moreover, our data indicate that in cultured neurons and mature brain, LPH1 is localized in the presynaptic membranes, whereas Lasso is mostly postsynaptic (Fig. 4). Given the size of LPH1 (14 nm) and Lasso (~30 nm) (25, 39), their complex can span the synaptic cleft (20–30 nm), allowing this receptor pair to connect neurons at synapses.

The LPH1–Lasso interaction is not purely structural. LPH1 mediates signaling induced by LTX^{N4C} both in model cells expressing exogenous LPH1 (5) and in organotypic hippocampal cultures (6). This signaling requires the CTF and involves the activation of phospholipase C, production of inositol-trisphosphate, and release of stored Ca^{2+}. We demonstrate here that a soluble C-terminal fragment of Lasso also induces an increase in cytosolic Ca^{2+} in NB2a cells expressing LPH1 (Fig. S6*B*) and in hippocampal neurons (Fig. 6). Such a rise in cytosolic Ca^{2+} in neurons could modulate neurotransmitter release (6).

Interestingly, teneurin contains a sequence termed teneurin C-terminal–associated peptide (TCAP) that resembles corticotropin-releasing factor (Fig. 2*A*). It is hypothesized that TCAP is cleaved from teneurin and acts as a soluble ligand of unknown receptors (40). Synthetic TCAP regulates cAMP in immortalized neurons and, when injected cerebrally, affects behaviors related to stress and anxiety (40). However, there is no evidence that TCAP is released in vivo. Our work suggests an alternative possibility: TCAP, being part of Lasso, affects animal behavior by stimulating LPH1. This is supported by LPH being implicated in schizo-

phrenia (19), anxiety (offspring killing by LPH1$^{-/-}$ mice) (9), and attention deficit/hyperactivity disorder (21).

Our findings raise several interesting questions. Does Lasso send an intracellular signal in response to LPH1 binding? What are the relationships among the four teneurin homologs and the three LPH proteins found in most vertebrates? Can the LPH1–Lasso interaction affect synaptogenesis, neurotransmitter release, or synaptic plasticity? Answering these questions will provide important insights into the physiological functions of these two families of neuronal cell surface receptors.

Methods

Purification of Abs, isolation of Lasso, MS techniques, molecular cloning, protein binding analyses, cell culture and interactions assays, immunostaining procedures, immunoelectron microscopy and cytosolic Ca^{2+} measurements are described in detail in *SI Methods*.

ACKNOWLEDGMENTS. The work was supported by Wellcome Trust Project Grant WT083199MF (to Y.A.U.), Biotechnology and Biological Science Research Council Core Support Grant BBF0083091 and Integrative Systems Biology Grant BBC5196701 (to A.D.), and Medical Research Council Career Development Award G0600089 (to K.E.V.).

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