Identification of an elaborate complex mediating postsynaptic inhibition

Akiyoshi Uezu,1 Daniel J. Kanak,1 Tyler W. A. Bradshaw,1 Erik J. Soderblom,1,2 Christina M. Catavero,1 Alain C. Burette,3,4 Richard J. Weinberg,3,4 Scott H. Soderling1,5

Inhibitory synapses dampen neuronal activity through postsynaptic hyperpolarization. The composition of the inhibitory postsynapse and the mechanistic basis of its regulation, however, remain poorly understood. We used an in vivo chemico-genetic proximity-labeling approach to discover inhibitory postsynaptic proteins. Quantitative mass spectrometry not only recapitulated known inhibitory postsynaptic proteins but also revealed a large network of new proteins, many of which are either implicated in neurodevelopmental disorders or are of unknown function. Clustered regularly interspaced short palindromic repeats (CRISPR) depletion of one of these previously uncharacterized proteins, InSyN1, led to decreased postsynaptic inhibitory sites, reduced the frequency of miniature inhibitory currents, and increased excitability in the hippocampus. Our findings uncover a rich and functionally diverse assemblage of previously unknown proteins that regulate postsynaptic inhibition and might contribute to developmental brain disorders.

Two anatomically distinct classes of synapses are present in the central nervous system: excitatory synapses, predominantly localized to postsynaptic spines, and inhibitory synapses, in which the postsynaptic is typically embedded in the soma and dendritic shaft (7). Purification and analysis of the protein complexes of the excitatory postsynapse have led to fundamental insights in neurobiology. These insights include how receptor trafficking, synaptic adhesion, cytoskeletal remodeling, and protein phosphorylation contribute to the synaptic plasticity underlying learning and memory (2,3). Moreover, genetic perturbations of excitatory postsynaptic proteins are strongly implicated in developmental brain disorders and psychiatric conditions (4,5).

In contrast, the biochemical purification and analysis of the inhibitory postsynaptic density (iPSD) has remained largely intractable. Accordingly, the molecular basis of postsynaptic inhibitory synapse regulation and its contribution to neurodevelopmental disorders is poorly understood. Recently, an affinity purification approach, BioID, has been developed that utilizes a promiscuous Escherichia coli biotinylation enzyme BirA (6) (here termed BirA, with G100 replacing Arg 118) fused to a bait protein expressed in cells (6). BirA-dependent covalent biotinylation occurs within 10 to 50 nm of the bait protein and allows for efficient isolation and analysis of proximal proteins by streptavidin-based affinity purification and mass spectrometry (MS) (7). Compared with affinity purification methods, the BioID reaction is executed in situ and thus enables the capture of protein complexes, including transient interactions and insoluble proteins from subcellular compartments refractory to biochemical isolation (8).

We adapted the proximity-dependent biotin identification (BioID) approach to enable in vivo BioID (iBioID) of synaptic complexes in mouse brain. We virally expressed inhibitory or excitatory neurons harboring BirA proteins and, with G100 replacing Arg 118, fused to a bait protein expressed in cells. Immunoprecipitation and mass spectrometry revealed a large number of previously unknown proteins, including a rich diversity of transmembrane and signaling proteins. These results provide a molecular perspective for the deeper understanding of synaptic physiology that was, until now, largely confined to the excitatory PSD.

In vivo capture of synaptic protein complexes

Gephyrin is the major scaffolding protein organizing the iPSD structure, interacting directly with L. Wittner, G. Ascoli, and D. Ropespry for sharing CA3 pyramidal neuron models. Supported by the Fond zur Förderung der Wissenschaftlichen Forschung (P 24599-B24 to P.J.), the European Union (European Research Council Advanced Grant 268548 to P.J.), and the Deutsche Forschungsgemeinschaft (DFG 3620/14-1 to M.F.). M.F. is Senior Research Professor of the Herfie Foundation. The authors declare no conflicts of interest. Original data and programs were stored in the scientific repository of the Institute of Science and Technology Austria and are available on request.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/353/6304/1123/suppl/DC1

Materials and Methods Figs. S1 to S11 Tables S1 to S4 References (48–68)

3 January 2016; accepted 15 July 2016

10.1126/science.aaf1836
glycine receptors (GlyRs) or γ-aminobutyric acid type A receptors (GABA_As) and other molecules such as neuriligin-2 (NL2) and collybistin (Arhe69) (9–11). To label proteins associated with synaptic proteins in the context of native tissue in vivo, we created adeno-associated viral (AAV) constructs for the expression of gephyrin- and the membrane-associated guanylate kinase, PSD protein 95 (PSD-95), fused with BirA, targeting the proteomes of inhibitory and excitatory postsynapses, respectively (Fig. 1A). To control for synapse specificity of biotinylation, we also expressed soluble BirA, which nonspecifically biotinylates proteins throughout the neuron. Each construct was validated by immunocytochemistry to determine its localization and synaptic site of biotinylation (Fig. 1B). To label synaptic structures in vivo, we developed a protocol that maximizes the number of synapses labeled by injection of AAV into the cortex of postnatal day 0 (P0) mouse pups, followed by 7 days of daily doses of exogenous biotin (Fig. 1C). Immunohistochemical analysis validates the approach for in vivo biotinylation (Fig. 1D), which yields punctate labeling of synaptic sites in brain tissue (Fig. 1E). Immune-electron microscopy verified that biotinylation occurred at symmetric (i.e., inhibitory) synapses for BirA-gephyrin, and at asymmetric (i.e., excitatory) postsynaptic sites for PSD-95–BirA (Fig. 1, F and G). Subcellular sites of background staining (perhaps due to overexpression or detection of endogenous biotinylated carboxyalkyls) were also noted to guide the final proteomic analysis (fig. S1A and table S1). Purification of biotinylated proteins from neurons expressing each BirA fusion protein and subsequent immunoblotting for known components of inhibitory (collybistin) and excitatory (NR2B and GluA1) synapses verified that each bait specifically labeled components of these synaptic sites, versus the nonspecific labeling of our negative control, soluble BirA (Fig. 1H).

**Discovery of the inhibitory postsynaptic proteome**

To pilot the identification of proteins labeled by iBioID, we prepared four cohorts each for BirA, PSD-95–BirA, and BirA-gephyrin using the labeling protocol outlined in Fig. 1C. Biotinylated proteins purified by streptavidin affinity purification protocol outlined in Fig. 1C. Biotinylated proteins purified by streptavidin affinity purification were identified by liquid chromatography–tandem mass spectrometry (LC-MS/MS). In total, 928 unique proteins were identified from 12 separate LC-MS/MS runs, with unique complements of proteins enriched over BirA in each synaptic fraction (fig. S1, B to E, and table S2). Analysis of the gephyrin-BirA fraction confirmed the presence of several proteins reported to reside at the iPSD (10, 12), including Arhe69, collybistin, VASP family proteins Mena and Evl, and IQSEC3, and two proteins of unknown function, which we termed “inhibitory synaptic proteins 1 and 2” or InSyn1 (UPF0583 protein Clisorf69 homolog) and InSyn2 (protein family 196a). This pilot study suggested that, although the BirA-gephyrin is highly specific, increased coverage of the iPSD would be desirable. We therefore expanded the analysis of the iPSD by performing the MS/MS analysis using more sensitive instrumentation and by including additional BirA-fusion proteins to further label the iPSD (collybistin-BirA and InSyn1-BirA) (fig. S1, F and G). InSyn1 was included because functional studies (described below) verified that it was an important component of the iPSD. Quantitative high-resolution LC-MS/MS was then performed for each sample (PSD data set: BirA versus PSD-95;
iPSD data set: BirA versus gephyrin, collybistin, InSyn1) in biological triplicates (seven to eight mice per biological fraction). For the excitatory PSD-95 data set, 18,207 peptides corresponding to 2533 unique proteins were quantified, whereas 17,024 peptides corresponding to 2183 proteins were quantified from the iPSD data sets. Proteins were considered enriched in the bait fraction if their average BirA-fusion protein amounts were at least two times the amount of BirA-alone fractions with $P < 0.05$. On the basis of these criteria, 121 excitatory PSD (ePSD) proteins (fig. S2) were specifically labeled by PSD-95–BirA (table S3). More than 95% of the ePSD proteins identified by iBioID were previously known ePSD components, as identified by traditional PSD biochemical fractionation and MS (116 of 121 proteins), demonstrating the specificity of the method. These proteins included glutamate receptors, scaffolding proteins, and signaling proteins of excitatory synaptic complexes. The iPSD data set identified a combined 181 proteins (table S4), including nearly all previously reported proteins of the iPSD (13); this data set suggested that coverage of the iPSD had approached saturation (fig. 2). We also identified a large number of proteins not previously known to reside at the iPSD,
including trafficking proteins, cytoskeletal regulatory proteins, integral membrane proteins, and several protein kinases and phosphatases (Fig. 2B). Many of these proteins (27/181) are encoded by genes implicated in either seizure susceptibility in humans or mice or other brain disorders such as intellectual disability (table S4). Comparison of iPSD and PSD-95 identified 17 proteins shared by the two data sets, 50% of which are signaling proteins (table S5).

**Candidate iPSD proteins colocalize and coimmunoprecipitate with gephyrin**

To validate our iBioID results, select iPSD proteins [InSyn1, InSyn2, IQSEC3, collybistin (Arhgeg9), and PX-RICS (Arhgap32)] were cloned and co-expressed in primary neuronal cells with GPHN, FingR-GFP, a recombinant protein sensor of endogenous gephyrin fused to green fluorescent protein (GFP) (14) (Fig. 3A). Collybistin served as a positive control, and PSD-95 colocalization served as a negative control. Quantification of colocalizing pixels (Fig. 3B) demonstrated that each iPSD protein extensively overlapped with endogenous gephyrin. Each candidate protein was also tested in coimmunoprecipitation experiments. Epitope-tagged iPSD proteins specifically coprecipitated with GFP-gephyrin, including InSyn1 and InSyn2 (Fig. 3, C to F).

**InSyn1 and InSyn2 are iPSD proteins functionally important for GABAergic inhibition**

InSyn1 is a previously uncharacterized protein that lacks protein domains of known function, but whose extensive iBioID interactions with core components of the iPSD (gephyrin, neurexin-2, collybistin, and GABAAR subunit δ-3) suggested a central role in GABAAR-dependent synaptic inhibition. To test whether InSyn1 is functionally important for synaptic inhibition, we used depletion of the endogenous protein mediated by single-cell clustered regularly interspaced short palindromic repeats (CRISPRs) (15) (Fig. 4A). GABAAR-mediated miniature inhibitory postsynaptic currents (mIPSCs) were recorded from CA1 pyramidal cells in hippocampal slices that were biolistically transfected with GFP, spCas9, and validated InSyn1 guide RNA (gRNA) (Fig. 4B). Untransfected (GFP-negative) cells located within a 400-µm radius from GFP-positive cells served as controls, and gRNA specificity was tested by reexpression of Cas9-resistant cDNAs (Fig. 4B). Inhibitory currents were confirmed to be GABAergic because they were reversibly abolished in the presence of bicuculline (1 µM) (fig. S3A). To verify the efficacy of the CRISPR strategy for functional testing of iPSD synaptic proteins, we targeted the obligatory γ2 subunit of the GABAAR and found a complete ablation of mIPSCs (fig. S3B). We also verified potential (LFP) recordings in acute slices from Cas9 knock-in (KI) mice infected with AAV:Cre/Insyn1 gRNA. Representative extent of AAV infection in hippocampus. (H) Representative LFP activities recorded in hippocampal area CA3 in the presence of 10 µM carbachol to model “awake state” gamma rhythm. Top trace, Pure 30 to 40 Hz gamma oscillation; middle trace, 3 to 5 Hz spike-wave discharges; bottom trace, ictal-like burst—the latter two indicative of hyperexcitable or epileptiform activity. (I) InSyn1 gRNA—expressing slices exhibit increased—epileptiform activity. (J) Averaged power spectra showing signal energy in the InSyn1 gRNA-expressing slices is increased in the 0 to 15 Hz frequency band and decreased in the 20 to 50 Hz frequency bands. *P < 0.05, **P < 0.01; ***P < 0.001; n.s., not statistically significant. Error bars ± SEM.
that biolistic transfection of GFP alone did not alter miPSC characteristics (fig. S3, C to E). Using sgRNA targeting InSyn1 with and without an InSyn1 rescue construct, we found a significant and reversible 69% increase in miPSC interevent intervals (IEI); the difference in amplitude was not statistically significant (Fig. 4, C and D). The effect of InSyn1 gRNA was specific to inhibitory currents, as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (AMPAR) currents remained unaltered. These findings suggest that InSyn1 plays a crucial role in the regulation of inhibitory currents in miPSCs.

**Fig. 5.** InSyn1 functionally associates with the dystrophin complex at the iPSD. (A) Network analysis of affinity-purified InSyn1-GFP proteome from mouse brain. Affinity purification–mass spectrometry (AP/MS) interaction. (B and C) Clustergram topologies of InSyn1-associated proteins in selected functional categories. (D) Colocalization of InSyn1 with α-dystroglycan (α-DG) is diminished after depleting InSyn1 with Cas9 conditional knock-in mouse hippocampal neurons infected with AAV:Cre/Insyn1 gRNA: EGFP, enhanced GFP. Scale bar, 10 µm. (E) InSyn1 is essential for GABAAR and α-DG cluster density. Hippocampal neurons from Cas9 KI mice were stained for GABAA subunit R2α and α-DG following infection with control AAV:Cre/(-)gRNA (top panel, control); AAV:Cre/Insyn1 gRNA (middle panel, knockdown), or AAV:Cre/Insyn1 gRNA, and transfected with InSyn1 gRNA–resistant plasmid (bottom panels, Rescue). (Insets) Higher magnification regions with open (colocalized puncta) and closed (noncolocalized puncta) arrows. (F to H) Quantification of α-DG and GABAAR2 puncta colocalization or density (n = 16 to 18). *P < 0.05, **P < 0.01, ***P < 0.001 one-way ANOVA followed by Tukey’s multiple comparisons test (F) to (H). Error bars ± SEM. Scale bar, 10 µm. (I) Loss of InSyn1 does not alter PSD-95 puncta density. Cas9 knock-in mouse hippocampal neurons infected with AAV:Cre/Insyn1 gRNA were stained with PSD-95 and quantified (n = 18 to 20 neurons); n.s., not significant, by two-tailed t test (I).
acid receptor (AMPA)-mediated miniature excitatory postsynaptic current (mEPSC) characteristics were unaltered (Fig. 5, E and F). To further test the predictive value of the iPSD data set, we performed analogous experiments targeting InSyn2. Depletion of InSyn2 resulted in a specific and reversible 62% increase of mIPSC I/EI (Fig. 5C).

Given the large effect of InSyn1 depletion on mIPSC frequency, we next asked whether the inhibitory deficits evident at the single-cell level manifest at the network level. We used a carbachol (CCH)-induced model of the “twinkle-strike” gamma rhythm, which is critically dependent on GABAergic inhibition (16). P0 Rosa26-LSL-Cas9 (17) pups were bilaterally infused with AAV-Cre/InSyn1-gRNA virus or AAV-Cre/(-)gRNA control virus in the hippocampus (Fig. 4G). In the majority of hippocampal slices prepared from control mice and kept vital in vitro, CCH induced a pure gamma rhythm with peak frequencies of ~30 to 40 Hz (Fig. 4H, top trace). In contrast, the majority of InSyn1 gRNA-infected slices exhibited a mixture of gamma oscillations with hyperexcitable events including interictal epileptiform discharges (IEDs) (Fig. 4H, middle trace) and prolonged (2- to 5-s) bursts resembling ictal discharges (Fig. 4H, lower trace). Overall, we found a fourfold increase in the proportion of slices with IEDs (Fig. 4I). Power spectral analysis revealed a 2.2-fold increase in low-frequency (0 to 15 Hz) power corresponding to epileptiform activity, and a 42% reduction in gamma-band (20 to 50 Hz) power (Fig. 4J). These effects likely reflect loss of GABAergic inhibition, as bath application of low concentrations of bicuculline (0.5-µM steps) abolished the gamma rhythm in control slices but had negligible effects in the absence of InSyn1.

**Discussion**

We report here the in vivo application of the BioID approach to analyze the local proteome of the postsynaptic compartment of inhibitory synapses by quantitative mass spectrometry. Comparing this approach with prior reports of the iPSD using affinity purification (19–21), BioID offers important advantages. Nearly all proteins previously reported to exist at the iPSD were identified. BioID also identified 140 proteins not previously associated with the iPSD, including a wide range of signaling- and actin-associated proteins, such as Trim3 (33–35), Stilkrk3 (36), and neuropilin (37, 38), as well as signaling- and actin-associated proteins, such as Trim3 (39), Enah (40), gephyrin (41), and dystrophin complex proteins (including alpha-1-syntrophin, dystrobrevin alpha, and dystrophin) (42).

**REFERENCES AND NOTES**

13. Known PSD proteins included eight GABA<sub>a</sub> receptor subunits, inhibitory transmembrane adhesion proteins (e.g., neurexin-2 (33–35), Stilkrk3 (36), and neuropilin (37, 38)), as well as signaling- and actin-associated proteins, such as Trim3 (39), Enah (40), gephyrin (41), and dystrophin complex proteins (including alpha-1-syntrophin, dystrobrevin alpha, and dystrophin) (42).
27. F. Niwa et al., PLOS ONE 7, e36348 (2012).
Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys

Peter Abbink,1,2 Rafael A. Larocca,1 Rafael A. De La Barrera,2 Christine A. Bricault,1 Edward T. Moseley,1 Michael Boyd,1 Marinela Kirilova,1 Zhenfeng Li,1 David Ng’ang’a,1 Ovini Nanayakkara,2 Ramya Nityanandam,2 Noe Mercado,1 Erica N. Borducchi,1 Richard G. Jarman,2 Kenneth H. Eckels,2 Nelson L. Michael,2 Brad Finneyfrock,4 Mark G. Lewis,4 Galit Alter,5 Kayvon Modjarrad,2,6 Katherine Molloy,1 Mayuri Shetty,1 George H. Neubauer,1 Kathryn E. Stephenson,1 Ovini Nanayakkara,1 Ramya Nityanandam,2 Noe B. Mercado,1 Erica N. Borducchi,1 Katherine Molloy,1 Mayuri Shetty,1 George H. Neubauer,1 Kathryn E. Stephenson,1 Jean Pierre S. Peron,2 Paolo M. de A. Zanotto,3 Johnathan Misamore,4 Brad Finneyfrock,4 Mark G. Lewis,4 Galit Alter,5 Kayvon Modjarrad,2,6 Richard G. Jarman,2 Kenneth H. Eckels,2 Nelson L. Michael,2 Stephen J. Thomas,2,† Dan H. Barouch,1,5,†,‡

Zika virus (ZIKV) is responsible for a major ongoing epidemic in the Americas and has been causally associated with fetal microcephaly. The development of a safe and effective ZIKV vaccine is therefore an urgent global health priority. Here we demonstrate that three different vaccine platforms protect against ZIKV challenge in rhesus monkeys. A purified inactivated virus vaccine induced ZIKV-specific neutralizing antibodies and completely protected monkeys against ZIKV strains from both Brazil and Puerto Rico. Purified immunoglobulin from vaccinated monkeys also conferred protective antibody responses in adoptive transfer studies. A plasmid DNA vaccine and a single-shot recombinant rhesus adenovirus serotype 52 vector vaccine, both expressing ZIKV premembrane and envelope, also elicited neutralizing antibodies and completely protected monkeys against ZIKV challenge. These data support the rapid clinical development of ZIKV vaccines for humans.

VACCINES

The explosive and unprecedented ZIKV outbreak in the Americas (1, 2) prompted the World Health Organization to declare this epidemic a public health emergency of international concern. ZIKV has been causally associated with fetal microcephaly, intrauterine growth retardation, and other congenital malformations in both humans (3–6) and mice (7–9), and it has also been linked with neurologic disorders such as Guillain-Barre syndrome (10). Several reports have shown that ZIKV can infect placental and fetal tissues, leading to prolonged viremia in pregnant women (11) and nonhuman primates (12). ZIKV also appears to target cortical neural progenitor cells (7–9, 13, 14), which likely contributes to neuropathology.

We recently reported the protective efficacy of two vaccines against ZIKV challenges in mice: a purified inactivated virus (PIV) vaccine from ZIKV strain PRVABC59 and a DNA vaccine expressing an optimized premembrane and envelope (prM-Env) immunogen from ZIKV strain BefH15744 (15). These studies used ZIKV challenge strains from Brazil (ZIKV-BR; Brazil/ZKV2015) (9) and Puerto Rico (ZIKV-PR; PRVABC59). ZIKV replication in mice was dependent on the mouse strain (15) and may be less extensive than in nonhuman primates (12). We therefore evaluated the immunogenicity and protective efficacy of inactivated virus, DNA-based, and vector-based vaccines against ZIKV challenge in rhesus monkeys.

ZIKV PIV vaccine study

We first immunized 16 rhesus monkeys by the subcutaneous route with 10^6 viral particles [vp; 10^6 plaque-forming units (PFU)] of ZIKV-PR or ZIKV-BR (n = 4 per group) (15). Viral loads after ZIKV challenge were quantitated by reverse transcript polymerase chain reaction (15), and viral infectivity was confirmed by growth in Vero cells. ZIKV-specific MN50 titers increased after challenge, particularly in the sham controls (fig. S5). Sham control monkeys exhibited 6 to 7 days of detectable viremia, with median peak viral loads of 5.82 log copies/ml (range, 5.21 to 6.29 log copies/ml; n = 8) on day 3 to 5 after challenge (Fig. 2A). Virus was also detected in the majority of sham control animals in urine and cerebrospinal fluid (CSF) on day 3, as well as in colorectal secretions and cervicovaginal secretions on day 7 (Fig. 2, B to E). In contrast, PIV-vaccinated monkeys showed complete protection against ZIKV challenge, as evidenced by no detectable virus (<100 copies/ml) in the blood, urine, CSF, colorectal secretions, or cervicovaginal secretions in any animal after challenge (n = 8; P = 0.0002, Fisher’s exact test comparing PIV-vaccinated animals with sham controls). We were unable to assess ZIKV in semen in the male animals in this study because of inadequate sample volumes. No major differences in plasma viral loads were observed between the sham controls that received ZIKV-PR or those that received ZIKV-PR (fig. S6).

Adoptive transfer studies

We next explored the mechanism of the observed protection through adoptive transfer studies.

Binding antibody titers correlated with neutralizing antibody titers in the PIV-vaccinated animals (P < 0.0001, coefficient of correlation R = 0.88, Spearman rank correlation test; fig. S3). Only minimal antibody-dependent cellular phagocytosis responses were observed. The majority of PIV-vaccinated monkeys (Fig. 1, C and D), but not sham control animals (fig. S4), also developed modest cellular immune responses, primarily to Env, as measured by interferon (IFN)-γ–enzyme-linked immunospot (ELISPOT) assays.

To assess the protective efficacy of the PIV vaccine against ZIKV challenge, we infected PIV-immunized and sham control monkeys by the subcutaneous route with 10^5 viral particles vp; 10^5 plaque-forming units (PFU) of ZIKV-PR or ZIKV-BR (n = 4 per group) (15). Viral loads after ZIKV challenge were quantitated by reverse transcript polymerase chain reaction (15), and viral infectivity was confirmed by growth in Vero cells. ZIKV-specific MN50 titers increased after challenge, particularly in the sham controls (fig. S5). Sham control monkeys exhibited 6 to 7 days of detectable viremia, with median peak viral loads of 5.82 log copies/ml (range, 5.21 to 6.29 log copies/ml; n = 8) on day 3 to 5 after challenge (Fig. 2A). Virus was also detected in the majority of sham control animals in urine and cerebrospinal fluid (CSF) on day 3, as well as in colorectal secretions and cervicovaginal secretions on day 7 (Fig. 2, B to E). In contrast, PIV-vaccinated monkeys showed complete protection against ZIKV challenge, as evidenced by no detectable virus (<100 copies/ml) in the blood, urine, CSF, colorectal secretions, or cervicovaginal secretions in any animal after challenge (n = 8; P = 0.0002, Fisher’s exact test comparing PIV-vaccinated animals with sham controls). We were unable to assess ZIKV in semen in the male animals in this study because of inadequate sample volumes. No major differences in plasma viral loads were observed between the sham controls that received ZIKV-PR or those that received ZIKV-PR (fig. S6).

Adoptive transfer studies

We next explored the mechanism of the observed protection through adoptive transfer studies.


ACKNOWLEDGMENTS

This work was supported by NIH grants MH104736 (S.H.S.) and NS09444 (R.I.M.). We thank J. Ding for animal perfusions, B. Carson for advice on image analysis, A. Swartz for cloning and K. Sakurai and J. Takatoh for advice on AAV production and injection protocols. Raw data relating to all mass spectrometry–based experiments can be viewed or downloaded from www.ChorusProject.org under the project title “Ueno_Soderling_RawData_July2016.”

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/353/6304/1123/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S5
Tables S1 to S6
References (45–61)
6 May 2016; accepted 25 July 2016
10.1126/science.aag0821

Downloaded from http://science.sciencemag.org/ on December 12, 2018
Identification of an elaborate complex mediating postsynaptic inhibition
Akiyoshi Uezu, Daniel J. Kanak, Tyler W. A. Bradshaw, Erik J. Soderblom, Christina M. Catavero, Alain C. Burette, Richard J. Weinberg and Scott H. Soderling

Science 353 (6304), 1123-1129.
DOI: 10.1126/science.aag0821