



Neurotransmitter Switching in the Adult Brain Regulates Behavior Davide Dulcis *et al. Science* **340**, 449 (2013); DOI: 10.1126/science.1234152

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by clicking here.

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines here.

The following resources related to this article are available online at www.sciencemag.org (this information is current as of May 1, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

http://www.sciencemag.org/content/340/6131/449.full.html

Supporting Online Material can be found at: http://www.sciencemag.org/content/suppl/2013/04/24/340.6131.449.DC1.html

A list of selected additional articles on the Science Web sites **related to this article** can be found at: http://www.sciencemag.org/content/340/6131/449.full.html#related

This article **cites 40 articles**, 11 of which can be accessed free: http://www.sciencemag.org/content/340/6131/449.full.html#ref-list-1

This article has been **cited by** 1 articles hosted by HighWire Press; see: http://www.sciencemag.org/content/340/6131/449.full.html#related-urls

This article appears in the following **subject collections:** Engineering http://www.sciencemag.org/cgi/collection/engineering

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. Copyright 2013 by the American Association for the Advancement of Science; all rights reserved. The title *Science* is a registered trademark of AAAS.

SCIENCE VOL 340 26 APRIL 2013

Neurotransmitter Switching in the Adult Brain Regulates Behavior

Davide Dulcis,^{1,2}*† Pouya Jamshidi,¹†‡ Stefan Leutgeb,^{1,2} Nicholas C. Spitzer^{1,2}

Neurotransmitters have been thought to be fixed throughout life, but whether sensory stimuli alter behaviorally relevant transmitter expression in the mature brain is unknown. We found that populations of interneurons in the adult rat hypothalamus switched between dopamine and somatostatin expression in response to exposure to short- and long-day photoperiods. Changes in postsynaptic dopamine receptor expression matched changes in presynaptic dopamine, whereas somatostatin receptor expression remained constant. Pharmacological blockade or ablation of these dopaminergic neurons led to anxious and depressed behavior, phenocopying performance after exposure to the long-day photoperiod. Induction of newly dopaminergic neurons through exposure to the short-day photoperiod rescued the behavioral consequences of lesions. Natural stimulation of other sensory modalities may cause changes in transmitter expression that regulate different behaviors.

Δ

В

С

D

www.sciencemag.org

▼ ignal transmission in neuronal circuits uses specific neurotransmitters that bind to cognate receptors on other neurons. Genetic programs establish initial expression patterns of neurotransmitters in different classes of neurons (1-3), and activity-dependent neurotransmitter respecification modifies them during develop-

Fig. 1. Photoperiods regulate numbers of dopaminergic neurons in hypothalamic nuclei. (A) Exposure to 19L:5D or 5L:19D photoperiods for 1 week changes the number of tyrosine hydroxylase immunoreactive (TH) neurons in the PaVN relative to control (12L:12L) (left). Number of TH neurons in the LPO, PaVN and PeVN for each condition (right). n = 12 animals for each photoperiod. (B) Dopamine is colocalized with TH in the PaVN after exposure to each of the photoperiods. n = 5 animals for each photoperiod. (C) FFN511 is taken up in the PeVN and released by KCl after exposure to each of the photoperiods (left). Fluorescence measured in boxed regions (right). n = 6 animals for each photoperiod. (D) Circadian fluctuations do not account for changes produced by different photoperiods. Numbers of TH neurons for all three nuclei at all four times from 19L:5D and 5L:19D are significantly different from those from 12L:12D. n = 5 animals for each photoperiod. (E) The number of TH neurons in the nearby A13 nucleus is not significantly different after exposure to each of the photoperiods. *n* = 5 animals for each photoperiod. ***P* < 0.01. Scale bars, (A) 120 μm; (B) 100 μm; (C) 80 μm.

ment, either adding or switching transmitters (4-9). It is unknown, however, whether sensory stimuli promote transmitter switching in addition to other neuroplastic changes (10) in the adult brain. Alterations in photoperiod, circadian rhythm, and light exposure can each cause anxiogenic and depressive behavior in diurnal adult mammals (11, 12). We hypothesized that nocturnal mammals would respond to light manipulation in the opposite manner (13) and that these changes in behavior would be mediated by transmitter switching. To test this hypothesis, we exposed adult rats to different photoperiods and determined whether transmitter specification and behavior were affected.

RESEARCH ARTIC

Photoperiod-Dependence of Dopamine Expression

The number of dopaminergic neurons in hypothalamic nuclei innervated by the retino-hypothalamic projection via the suprachiasmatic nucleus (SCN) (fig. S1) (14, 15) changed with the light-dark (L-D) cycle to which animals were exposed. Animals were maintained in photoperiod chambers on long-day [19 hours light:5 hours dark (19L:5D)],

¹Neurobiology Section, Division of Biological Sciences and Center for Neural Circuits and Behavior, University of California, San Diego, La Jolla, CA 92093-0357, USA. ²Kavli Institute for Brain and Mind, University of California, San Diego, La Jolla, CA 92093-0357, USA.

*Corresponding author. E-mail: ddulcis@gmail.com †These authors contributed equally to this work. ‡Present address: School of Medicine, University of California, San Diego, La Jolla, CA 92093-0602, USA.



RESEARCH ARTICLE

short-day (5L:19D), or balanced-day (12L:12D; control) cycles for 1 week, and sections through the lateral preoptic area (LPO), paraventricular nucleus (PaVN), and periventricular nucleus (PeVN) were immunostained for tyrosine hydroxylase (TH), an enzyme in the dopamine synthetic pathway (16). The number of TH-immunoreactive (TH-IR) neurons decreased with long-day exposure and increased with short-day exposure in relation to control (Fig. 1A and fig. S2). TH and dopamine were colocalized in all three conditions (Fig. 1B). To assess the functional status of TH-IR neurons, we examined uptake and release of fluorescent false neurotransmitter 511 (FFN511) (17), which is taken up by vesicular monoamine transporter 2 (VMAT2). FFN511 generated fluorescent signals in hypothalamic slices from brains of animals exposed to each of the three photoperiods. Fluorescence decreased upon KCl depolarization (Fig. 1C).

Neurotransmitter respecification could be induced through changes in photoperiod or alteration of the circadian rhythm. To address the circadian contribution to changes in numbers of TH-IR neurons, we quantified their numbers at 6-hour intervals in animals exposed to each of the different photoperiods for 1 week (Fig. 1D). Although there is variation at some time points during each photoperiod (table S1), values for all three nuclei at all four times from 19L:5D and 5L:19D were significantly different from

Fig. 2. Photoperiods drive neurotransmitter switching between dopamine and somatostatin. (A) Exposure to 19L:5D or 5L:19D photoperiods changes the number of SST-IR neurons in the PaVN relative to control (12L:12D) (left). Number of SST neurons in the PaVN and PeVN for each photoperiod (right). n = 6 animals for each photoperiod. (**B** and **C**) SST and TH immunofluorescence in the (B) PaVN and (C) PeVN change after exposure to each of the photoperiods (left). TH/SST coexpression (right). n = 7 animals for each photoperiod. (**D**) The number of SST vesicles/neuron cell body in the PaVN follows photoperiod light cycle duration (left). n = 9 cells for each photoperiod. Scale bars, (A) 100 µm; (B) 20 µm; (C) 3 µm; (D) 20 µm. *P < 0.05. **P < 0.01. ***P < 0.01. those from 12L:12D photoperiod exposure (table S2). The effects of different photoperiods are thus unlikely to have arisen from measurements collected at nonequivalent phases of the circadian rhythm. Week-long exposure to each of the different photoperiods failed to produce changes in numbers of TH-IR neurons in an adjacent nucleus, A13 (Fig. 1E and fig. S2B), which does not receive retinal input via the SCN (*18*).

Transmitter Switching Between Dopamine and Somatostatin

Neurons in the PaVN and PeVN also express somatostatin (SST) (19, 20). The number of SST neurons showed an inverse relationship to the number of TH neurons with exposure to the three different photoperiods (Fig. 2A). This result could arise from changes in the numbers of neurons of each type or from transmitter switching within neurons. To determine whether these reciprocal changes result from adult neurogenesis (21, 22), we injected animals with BrdU on days 2 to 4 of the week of exposure. No significant BrdU labeling was detected in the LPO, PaVN, or PeVN (fig. S3A). To ascertain whether apoptosis causes decreased numbers of TH-IR neurons and SST-IR neurons after exposure to long- and short-day photoperiods, respectively, we subjected sections from animals after 1 week of exposure to each of the photoperiods to terminal deoxynucleotidyl

transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay. No significant differences in sporadic cell death were observed (fig. S3B). Photoperiod-dependent changes in the numbers of TH-IR neurons were reversible. When animals were exposed to 2 weeks of consecutive but opposite, short-, or long-day, photoperiods, TH expression did not differ from controls exposed to the 12L:12D photoperiod for 2 weeks (fig. S3C).

Are individual neurons reciprocally shifting transmitter expression between dopamine and SST? Double immunofluorescence revealed that different photoperiods changed the balance of dopamine and SST coexpression in neurons in the PaVN and PeVN. Long-day exposure reduced TH-IR and increased SST-IR, whereas short-day exposure increased TH-IR and decreased SST-IR (Fig. 2B). The 43% increase and 96% decrease in TH-IR/SST-IR coexpression after short- and long-day exposure, in contrast to the balanced photoperiod, suggest that TH-IR and SST-IR neurons are recruited from a reserve pool of cells (23) that are switching transmitters. Changes in TH and SST expression in cell bodies were also observed at high magnification in PaVN axons and en passant terminals in the periventricular region (Fig. 2C) and in PeVN cell bodies (fig. S4). The number of intracellular SST-IR storage vesicles depended on photoperiod light-cycle duration





(Fig. 2D). Decreased and increased numbers of TH-IR neuronal cell bodies resulting from exposure to long- and short-day photoperiods (Fig. 1A) were matched by corresponding increases and decreases in numbers of SST-IR neuronal cell bodies (Fig. 2A and fig. S5). Newly expressing TH-IR neurons induced through short-day photoperiod exposure coexpressed additional dopaminergic markers (*3*), VMAT2 (fig. S6), and the dopamine transporter, DAT (fig. S7).

To test the transcriptional dependence of transmitter respecification, we performed singlemolecule fluorescence in situ hybridization with probes for TH and SST. Photoperiod-dependent changes in the number of labeled neurons identified regulation at the transcriptional level (Fig. 3, A to I), which is consistent with increased SST transcripts in PeVN neurons after exposure to stressors (24). Simultaneous immunostaining showed that changes in numbers of TH (Fig. 3, B and G) and SST (Fig. 3, C and F) neurons paralleled changes in mRNA expression (Fig. 3, D, H, and I). These results demonstrate that transmitter respecification is not achieved by translation from preexisting transcripts and involves de novo induction of TH or SST mRNA. Not all neurons underwent this transmitter switch; after exposure to the long-day photoperiod, 25% of the number of 12L:12D TH-IR PaVN neurons (Fig. 1A) expressed TH immunoreactivity without detectable SST immunoreactivity (Fig. 2B). SST was not expressed in the LPO after exposure to any of the three photoperiods, and a transmitter switch partner for dopaminergic neurons in this nucleus remains to be determined.

Changes in Postsynaptic Receptor Populations

Are presynaptic changes in transmitter identity matched by changes in postsynaptic receptor populations? SST interneurons of the parvocellular PaVN and the anterior PeVN make synapses on corticotropin-releasing factor (CRF) neurons distributed along the third ventricle, as do dopaminergic interneurons (25, 26). To determine whether dopamine D2 receptor (D2R) and SSTR were coexpressed on periventricular CRF cells innervated by TH-IR neurons, we stained sections with antibodies for CRF, SST2/4R, and D2R (Fig. 4A) (27, 28). After exposure to the control 12L:12D photoperiod, the two classes of receptors appeared colocalized on CRF cells along the ependymal layer in the PeVN, which is consistent with presynaptic transmitter coexpression (Figs. 2 and 3). After the long-day photoperiod, colocalization of D2R and SST2/4R was rare because D2R expression was down-regulated in parallel with the decrease in number of dopaminergic neurons. In contrast, after short-day photoperiod exposure, colocalization of these receptors was abundant as the number of dopaminergic neurons expanded (Fig. 4, A to C, and fig. S5).

We hypothesized that levels of CRF in the cerebrospinal fluid (CSF) would decrease as a result of increased inhibition resulting from exposure to the short-day photoperiod that leads to





RESEARCH ARTICLE

coexpression of D2R with SST2/4R, if receptor activation is more substantial than the level of presynaptic SST. We envisaged that levels would increase with reduced D2R expression after the long-day photoperiod. Assays of CRF in the CSF and corticosterone in the plasma confirmed this to be the case (Fig. 4D). The number of CRFimmunoreactive neurons was also reduced after exposure to the short-day photoperiod (Fig. 4, A to C), reflecting low levels of CRF in the absence of apoptosis and consistent with reduced induction of CRF synthesis due to increased inhibition by both D2R and SST2/4R (*29, 30*). SST2/4R but not D2R expression was observed on CRF neurons in the PaVN.

Photoperiod-Dependence of Behaviors

To investigate behavioral consequences of these changes in transmitter status, we tested rats on the elevated plus maze (EPM) (31) and in the forced swim test (FST) (32, 33). Relative to controls, shortday exposure caused rats to spend more time exploring the open arm of the maze (movies S1 and S2) and spend a longer time swimming before becoming immobile (movies S3 and S4). Locomotor behavior and swimming ability appeared normal. Long-day exposure produced the opposite effects (Fig. 5A). The results of EPM testing during the middle of the light or dark phases after 24-hourcycle photoperiods were not different. EPM testing at the conclusion of treatment with the same 19L:5D, 12D:12L, or 5L:19D ratios of total light/dark exposure distributed across much shorter (72 min) cycles yielded indistinguishable results (fig. S8).

To determine whether the behavioral changes observed are specifically related to changes in the number of TH-IR neurons, we made stereotaxic injections of 6-hydroxydopamine (6-OHDA) adjacent to the PaVN (fig. S9, A and B) so as to focally ablate TH-IR neurons in animals maintained on a 12L:12D cycle. Artificial CSF (ACSF) was injected as a sham control. After ablation, we observed a 95% reduction in time spent exploring the open arm of the EPM and a 57% increase in duration of the immobility time in the FST (Fig. 5B). Infusion of animals on a 12L:12D cycle with dopamine receptor antagonists SCH23390 and sulpiride, which selectively block D1 and D2 dopamine receptors (34), affected these behaviors similarly to 19L:5D exposure (Fig. 5C). Neither 6-OHDA nor receptor antagonists affected locomotor activity or swimming performance. Post-test histology of 6-OHDA-injected animals revealed that the number of TH-IR neurons was decreased by more than 50% in all three of these hypothalamic dopaminergic nuclei (Fig. 5D). The total number of cells in the PaVN was reduced (fig. S9C), indicating loss of neurons after 6-OHDA treatment. However, counts of TH-IR neurons in A13 after 6-OHDA treatment were not affected, demonstrating that dopaminergic nuclei located as close as 200 µm were spared by focal toxin delivery (Fig. 5D). Sham operation and injection of ACSF had no effect on the number of TH-IR neurons.

Fig. 4. Dopamine and somatostatin receptor colocalization on CRF neurons. (A) Triple labeling of SST2/4R. CRF. and D2R in single sections after exposure to each photoperiod. (B and C) D2R and SST2/4R colocalization on CRF cells. Labeled cells were scored along the walls of the third ventricle (dotted lines), and the number was averaged for 10 30-µm sections of the rostral hypothalamus. For (B), n = 6 animals. (D) CRF levels in the CSF and corticosterone levels in the plasma after exposure to each photoperiod. *n* = 6 animals. ***P* < 0.01. Scale bars, (A) 120 µm; (C) 40 μm.

Role of Newly Dopaminergic Neurons in Stress Behaviors

We next determined whether photoperiod-dependent hypothalamic transmitter respecification reported here was likely to account for the observed changes in stress responses. We first ablated TH-IR neurons in these nuclei by means of local stereotaxic injection of 6-OHDA and then exposed animals to the long-day photoperiod, so as to further reduce their number, or to the shortday photoperiod, so as to recruit more neurons to become TH-IR (fig. S10). The behavioral results of focal ablation of TH-IR neurons were partially reversed by exposure to the short-day photoperiod, demonstrating behavioral rescue (Fig. 5E). Post-test histology demonstrated photoperioddependent reduction and induction of TH-IR neurons (Fig. 5F). The appearance of newly dopaminergic neurons is unlikely to have resulted from recruitment from a preexisting dopaminergic pool of neurons with undetectable levels of neurotransmitter because the inducible reserve pool was not ablated by 6-OHDA before exposure to the short-day photoperiod. FFN511 uptake and release in hypothalamic slices confirmed the functionality of newly dopaminergic neurons (fig. S11).

Because behavioral recovery could be achieved by a parallel mechanism not involving recruitment of newly TH-IR neurons in these nuclei, we determined whether it could be reversed by acute, local infusion of dopamine receptor antagonists. TH-IR neurons were again ablated with 6-OHDA, and animals were exposed to the short-day photoperiod for 1 week to recruit newly TH-IR neurons. ACSF or SCH23390 and sulpiride were stereotactically and acutely introduced through a cannula into the region in between the LPO and PaVN. Behavioral testing showed decreased time in the open arm of the EPM and increased immobility time in the FST in response to infusion



with antagonists as compared with infusion with ACSF (Fig. 5E).

Discussion

Our results demonstrate transmitter switching between dopamine and somatostatin in neurons in the adult rat brain, induced by exposure to shortand long-day photoperiods that mimic seasonal changes at high latitudes. The shifts in SST/dopamine expression are regulated at the transcriptional level, are matched by parallel changes in postsynaptic D2R/SST2/4R expression, and have pronounced effects on behavior. SST-IR/TH-IR local interneurons synapse on CRF-releasing cells, providing a mechanism by which the brain of nocturnal rats generates a stress response to a long-day photoperiod, contributing to depression (*35, 36*) and serving as functional integrators at the interface of sensory and neuroendocrine responses (*36–38*).

SST/dopamine interchange illustrates neuroplasticity in self-balancing networks (39) that tunes neural function and behavior to a dynamic environment. The molecular mechanism remains to be discovered, but AST-1 and Etv1 transcription factors bind to a specific dopamine motif in Caenorhabditis elegans and mouse to drive the expression of genes that encode components necessary for synthesis, packaging, and reuptake of dopamine (3, 40). Activity-dependent transmitter switching may serve functions similar to homeostatic synaptic scaling (41), changes in ion channel expression (42), and neuropeptide remodeling of sensory networks (43) and is an additional form of brain plasticity to add to modifications of synaptic strength, synapse number, and electrical excitability. Sensory stimulation driving transmitter switching in specific microcircuits affected in neurological or psychiatric disorders could contribute to noninvasive restoration of normal function in the mature nervous system.

RESEARCH ARTICLE



Fig. 5. Behavioral changes after lesions of dopaminergic neurons are rescued through photoperiod induction of newly dopaminergic neurons. (**A**) Baseline behavior on EPM and FST after exposure to 19L:5D, 12L:12D, or 5L:19D photoperiods for 1 week. (**B**) Effect of 6-OHDA ablation of DA neurons versus sham operation on behavior of animals exposed to 12L:12D for 1 week. (**C**) Behavior of 12L:12D animals without and with acute infusion of D1 and D2 receptor antagonists, SCH23390 and sulpiride. (**D**) Histology of tissue and stereology of TH-IR neurons after 6-OHDA, accompanying behavioral tests. Asterisks indicate injection sites. Scale bar, 150 µm. For (A) and (C), n = 5 animals; for (B) and (D) n = 10 animals. **P < 0.01 compared with 12L:12D control [(A) to (C)] and to sham (D). (**E**) Effect on behavior of 19L:5D or 5L:19D exposure for 1 week after 6-OHDA ablation of DA neurons in animals exposed to 12L:12D. The rescue achieved with 5L:19D exposure is selectively abolished by infusion of D1 and D2 receptor antagonists, SCH23390 and sulpiride. (**F**) Number of TH neurons after 6-OHDA ablation of LPO, PaVN, and PeVN in animals exposed to 12L:12D followed by 19L:5D, 12L:12D, or 5L:19D for 1 week. For (E) and (F), n = 5 and 4 animals, respectively. *P < 0.05. **P < 0.01 for (E) comparisons between adjacent groups and (F) comparisons with 6-OHDA [sham (12L:12D)].

References and Notes

- 1. S. Thor, J. B. Thomas, Neuron 18, 397 (1997).
- 2. A. Pierani et al., Neuron 29, 367 (2001).
- 3. N. Flames, O. Hobert, Nature 458, 885 (2009).
- 4. X. Gu, N. C. Spitzer, Nature 375, 784 (1995).
- 5. L. N. Borodinsky et al., Nature 429, 523 (2004).
- D. Dulcis, N. C. Spitzer, *Nature* 456, 195 (2008).
 Y. Lin *et al.*, *Nature* 455, 1198 (2008).
- 8. R. Pocock, O. Hobert, *Nat. Neurosci.* 13, 610 (2010).
- 9. T. D. Aumann *et al.*, *J. Neurochem.* **116**, 646
- (2011).
- 10. V. Krishnan, E. J. Nestler, Nature 455, 894 (2008).

- T. Ashkenazy, H. Einat, N. Kronfeld-Schor, Int. J. Neuropsychiatry 12, 83 (2009).
- 12. R. W. Lam et al., Am. J. Psychiatry 163, 805 (2006).
- K. Krivisky, T. Ashkenazy, N. Kronfeld-Schor, H. Einat, Neuropsychobiology 63, 191 (2011).
- R. M. Buijs, M. Markman, B. Nunes-Cardoso, Y. X. Hou, S. Shinn, J. Comp. Neurol. 335, 42 (1993).
- 15. S. Hattar et al., J. Comp. Neurol. 497, 326 (2006).
- 16. Materials and methods are available as supplementary materials on *Science* Online.
- 17. N. G. Gubernator et al., Science 324, 1441 (2009).
- A. Björklund, O. Lindvall, A. Nobin, Brain Res. 89, 29 (1975).
 L. Swanson, Organization of Mammalian Neuroendocrine System, Supplement 4: Handbook of Physiology, The Nervous System, Intrinsic Regulatory Systems of the Brain (Wiley, New York, 2011), pp. 317–343.
- 20. M. Sakanaka, S. Magari, N. Inoue, *Brain Res.* **516**, 313 (1990).
- 21. M. V. Kokoeva, H. L. Yin, J. S. Flier, Science 310, 679 (2005).
- 22. A. A. Pierce, A. W. Xu, J. Neurosci. 30, 723 (2010).
- 23. D. Dulcis, N. C. Spitzer, Dev. Neurobiol. 72, 465 (2012).
- 24. S. Arancibia et al., Exp. Brain Res. 134, 261 (2000).
- S. Cummings, R. Elde, J. Ells, A. Lindall, J. Neurosci. 3, 1355 (1983).
- 26. U. Kumar, J. Comp. Neurol. 504, 185 (2007).
- 27. Dopamine D2R and somatostatin SST1-5R are homologous and can assemble as heteromers (28), and SST-IR and TH-IR terminals colocalize with SST1-5R in the PeVN of the rat hypothalamus (26). Such arrangements could facilitate continuity of circuit function during transmitter switching.
- 28. M. Rocheville et al., Science 288, 154 (2000).
- 29. K. Kageyama, T. Suda, *Endocr. J.* 56, 335 (2009).
- F. D. Jeanneteau *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 1305 (2012).
- A. A. Walf, C. A. Frye, *Nat. Protoc.* 2, 322 (2007).
 R. D. Porsolt, M. Le Pichon, M. Jalfre, *Nature* 266, 730 (1977).
- B. Petit-Demouliere, F. Chenu, M. Bourin, *Psychopharmacology* (Berl.) 177, 245 (2005).
- 34. D. M. Eagle et al., J. Neurosci. 31, 7349 (2011).
- 35. C. B. Nemeroff et al., Science 226, 1342 (1984).
- 36. T. A. LeGates et al., Nature 491, 594 (2012).
- D. M. Simmons, L. W. Swanson, J. Comp. Neurol. 516, 423 (2009).
- J. D. Hahn, L. W. Swanson, Brain Res. Brain Res. Rev. 64, 14 (2010).
- T. P. Vogels, H. Sprekeler, F. Zenke, C. Clopath, W. Gerstner, *Science* 334, 1569 (2011).
- B. Tursun, T. Patel, P. Kratsios, O. Hobert, Science 331, 304 (2011).
- 41. G. W. Davis, Annu. Rev. Neurosci. 29, 307 (2006).
- E. Marder, Proc. Natl. Acad. Sci. U.S.A. 108 (suppl. 3), 15542 (2011).
- 43. C. I. Bargmann, Bioessays 34, 458 (2012).

Acknowledgments: We thank D. Berg, M. Gorman, and M. Scanziani for comments on the manuscript; H. Karten and A. Brzozowska-Prechtl for their expert advice on hypothalamic neuroanatomy and immunostaining protocols; Y. Zou and K. Onishi for technical help with FFN511 confocal imaging; and U. Kumar for a generous gift of SST receptor and CRF antibodies. We are grateful to M. I. Bharmal and M. Kucharski for stereological quantification and A. Guemez-Gamboa for the CRF-immunoassay. This work was supported by Ellison Medical Foundation grants AG-SS-2432-10 to N.C.S. and D.D., AG-NS-0724-10 to S.L., and a W. M. Keck Foundation grant to N.C.S. and D.D. We declare no conflict of interest. The data are included in the main article and in the supplementary materials.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6131/449/DC1 Materials and Methods Figs. S1 to S11 Tables S1 and S2 Movies S1 to S4

22 October 2012; accepted 12 February 2013 10.1126/science.1234152

Downloaded from www.sciencemag.org on May 1, 2013