Orchestrated experience-driven Arc responses are disrupted in a mouse model of Alzheimer’s disease

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Experience-induced expression of immediate-early gene Arc (also known as Arg3.1) is known to be important for consolidation of memory. Using in vivo longitudinal multiphoton imaging, we found orchestrated activity-dependent expression of Arc in the mouse extrastriate visual cortex in response to a structured visual stimulation. In wild-type mice, the amplitude of the Arc response in individual neurons strongly predicted the probability of reactivation by a subsequent presentation of the same stimulus. In a mouse model of Alzheimer’s disease, this association was markedly disrupted in the cortex, specifically near senile plaques. Neurons in the vicinity of plaques were less likely to respond, but, paradoxically, there were stronger responses in those few neurons around plaques that did respond. To the extent that the orchestrated pattern of Arc expression reflects nervous system responses to and physiological consolidation of behavioral experience, the disruption in Arc patterns reveals plaque-associated interference with neural network integration.

Over the last few years, a great deal of evidence has emerged that suggests a prominent role for Arc in activity-driven synaptic plasticity and long-term memory consolidation (recently reviewed in refs. 1, 2). Arc transcription in principal excitatory neurons is robustly upregulated in relevant cortical networks following sensory experience3–5. The product of this gene, Arc, is a postsynaptic protein that participates in dendritic actin remodeling6 and binds to endocytic machinery to mediate activity-dependent AMPA receptor (AMPAR) scaling7,8. Arc knockout mice exhibit a phenotype with impaired long-term learning and intact short-term memory9. Although Arc is clearly critical for long-term retention of behaviorally relevant experiences, the patterns of expression by which Arc activation codes for neural system savings are unknown. We characterized the patterns of Arc transcriptional response in visual association cortex to determine how exposure to a stereotyped visual stimulus shapes Arc responses in each individual neuron to a subsequent exposure to the same stimulus. To tackle this task, we used in vivo multiphoton imaging of Arc-dVenus reporter mice overexpressing a destabilized version of the yellow fluorescent protein (YFP) Venus under the control of 7.1 kb of the mouse Arc promoter10. As there is a greater than 100-fold enrichment of the reporter mRNA over endogenous Arc mRNA and comparable mRNA and protein clearance dynamics10, this reporter strain is well suited to study experience-induced plastic changes in the intact mouse brain using in vivo longitudinal microscopy. We found that repeated exposure of mice to the same stimulus led to an orchestrated pattern of re-activation of a subset of individual neurons and that the extent of neuronal activation predicted the extent of neuronal activation at the subsequent exposure to the same stimulus. This suggests that Arc encodes a behavioral stimulus through a complex pattern involving numerous neurons, representing in some sense neural system savings of an event.

We then used insight into normal patterns of Arc activation in the visual association cortex to examine whether or not deficits in neural system integration occur in a model of Alzheimer’s disease. Although the classical neuropathological view is that senile plaques, extracellular fibrillar deposits of amyloid-β (Aβ) often accompanied by structural changes that include dystrophic neurites and glial activation, are the key cortical lesion in the disease, whether plaques affect neural system function remains controversial. Recent data strongly implicate soluble, oligomeric forms of Aβ as being primarily important in the disruption of markers of neuronal function, including long-term potentiation and behavior11,12. In contrast with these extremes, based on high-resolution array tomography structural studies, we recently proposed a unified theory in which the region in the immediate vicinity of plaques (within ~50 µm) is occupied by high concentrations of synaptotoxic, soluble, oligomeric Aβ13. These models have different predictions regarding plaque’s relationships to neural system function: if plaques are a local structural lesion, neurons that are not directly affected by the plaque would function normally, if soluble, oligomeric Aβ were freely diffusible and independent of plaques, all of the neurons in a region that contains Aβ would be functionally impaired, and if oligomeric Aβ was present primarily in the immediate vicinity of plaques, neuronal function would be disproportionately affected in that small zone. Given that Arc responses to stimuli appear to occur in a stereotyped, complex pattern in which, normally, there is a specific and predictable pattern

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of Arc transcription, we examined whether plaques in a cortical field disrupt this complex pattern and, if so, examined whether the pattern of disruption is informative with regard to whether and how plaques impair information processing in the cortex. We found that the presence of amyloid plaques in a mouse model of Alzheimer’s disease, APPswe; PS1dE9 bi-transgenic mice14 (APP/PS1), altered both the likelihood and extent of reactivation of neurons in association cortex following re-exposure to visual stimuli, with predominant effects occurring in the immediate area around individual plaques. These results are consistent with a model in which plaque-induced functional alterations are secondary to distributed focal lesions consisting of the plaque and a surrounding halo, which lead to altered neuronal responsiveness to behaviorally relevant stimuli.

RESULTS

Orchestrated Arc::dVenus expression in the visual cortex
To study the characteristics of experience-dependent Arc mRNA expression, we employed transgenic mice expressing destabilized Venus (dVenus, which is rapidly degraded to allow imaging of responses over time) under the control of the Arc promoter10. This reporter faithfully represents activity-dependent Arc expression, as demonstrated by stimulus-induced mRNA and protein expression dynamics10 and immunohistochemical staining for dVenus and Arc proteins in the brain sections of Arc::dVenus mice (Supplementary Fig. 1). Mice were exposed to a visual stimulus similar to that used previously5,15,16. First, mice were housed in the dark for 60 h to allow for complete clearance of dVenus expressed in the visual cortex as a result of routine daytime experience. Following light deprivation, mice were placed into an illuminated glass cylinder with alternating vertical black and white stripes for 1 h and then transferred back into the dark home cage for 6 h until imaging (Fig. 1a).

The time point of 6 h was chosen because Arc-dVenus fluorescence in the visual association cortex reaches its maximum level ~6–8 h after the end of 1 h of exposure to light (Supplementary Fig. 2 and ref. 10). When the mice were killed 6 h after this stimulation and brains processed postmortem for immunohistochemical staining for dVenus protein (Fig. 1b), we observed that this stimulation procedure led to robust expression of Arc-dVenus in a subset of neurons in the visual cortex, whereas almost no Arc-dVenus–positive neurons were detected in the same cortical areas following the initial light-deprivation period (Fig. 1b). Particularly, visual stimulation for 1 h in the striped cylinder resulted in Arc-dVenus expression primarily in the layer II/III neurons of the medial areas of extrastriate visual cortex, whereas the number of Arc-dVenus–positive neurons in the primary visual area was limited (Fig. 1b and Supplementary Fig. 3a).

Continuous unstructured light stimulation for 7 h resulted in more Arc-dVenus expression in the primary visual area and less in the medial extrastriate cortex (Supplementary Fig. 3b).

Arc-dVenus expression 6 h after stimulation could also be observed in vivo through a cranial window implanted over the visual cortex using multiphoton microscopy (Fig. 1c) and quantified in large neuronal populations (Fig. 2). Relative spatial positions of Arc-dVenus–positive neuronal cell bodies and methoxy-XO4–positive amyloid plaques in the visual cortex were obtained from in vivo multiphoton image stacks (Figs. 2f and 3a) and using postmortem stereological quantification of immunostained brain slices (Fig. 3b–d). Arc-dVenus expression levels were sufficient for direct high-resolution visualization of neurites of activated neurons on multiphoton images obtained in vivo (Fig. 4a) and using postmortem confocal microscopy of immunostained tissues (Fig. 4b,c). We observe that a discrete subset of neurons in the layer II/III...
of extrastriate visual cortex was activated in response to the structured visual stimulus, as seen postmortem (21.6 ± 1.6% of NeuN-positive neurons (mean ± s.d.); Fig. 3b). To test the hypothesis that Arc expression reflects the neural network’s response to experience, we returned mice to the light-proof enclosure at the end of the imaging session and repeated the stimulation and imaging procedure (Figs. 1d, 5 and 6). Overall, the number of neurons expressing Arc-dVenus (activated neurons) in the second trial was lower than that in the first trial (87.5 ± 8.4% of trial 1 (mean ± s.d.); Fig. 5b), which is consistent with previous findings. We found that, with the second presentation of the identical stimulus, some of the same neurons were reactivated (50.3 ± 3.2% of trial 1; Fig. 5c) and some new neurons were activated (40.9 ± 4.3% of trial 2; Fig. 5d). Notably, the level of Arc-dVenus expression in trial 1 was a significant predictor of neuron reactivation in trial 2. The reactivation probability was approximately 30% for the neurons with lowest detectable Arc-dVenus expression levels and reached 90% for neurons with the highest expression levels (Fig. 6d). When fitted with a generalized estimating equations logistic regression model with accounting for the data clustering in mouse, the Arc-dVenus fluorescence intensity in trial 1 was strongly associated with reactivation probability (P < 0.0001). Moreover, the extent of activation was similar in the two trials with no change (on average) in the intensity of each neuron (Fig. 6b). These data indicate that high levels of Arc expression alter the synaptic properties of neurons to increase their chances of reactivation to subsequent presentations of the same stimulus.

**Arc mRNA expression is locally altered by amyloid plaques**

To test the hypothesis that Alzheimer-like senile plaque pathology impairs the Arc response to physiologically relevant stimuli, we crossed Arc::dVenus mice with APP/PS1 transgenic mice (Arc::dVenus × APP/PS1). As the first readout, we quantified experience-driven Arc promoter activity, as represented by the mean fluorescence intensity of Arc-dVenus–positive neurons. After the first imaging session of mice with plaques (6-7 months of age), we observed a bimodal distribution of expression levels, with Arc::dVenus × APP/PS1 mice having a larger proportion of neurons in the second, brighter modality (Fig. 2c,e). This effect contributed to the significant increase of the median of the intensity distribution in Arc::dVenus × APP/PS1 mice compared with their Arc::dVenus littermates (P < 0.01; Fig. 2d). We did not observe a significant difference (P = 0.13) between dVenus intensity distributions in 3–4-month-old (pre-plaque age for APP/PS1 mice) Arc::dVenus × APP/PS1 mice compared with non-APP/PS1 controls (Fig. 2b,d).

To estimate the potential effects of the inflammatory response to the cranial window implantation on the neuronal activation patterns, we killed Arc::dVenus × APP/PS1 and control Arc::dVenus mice 3 weeks after the window implantation and analyzed their brains postmortem using immunohistochemical staining for the microglia and macrophage marker Iba1 (Supplementary Fig. 4a) and the activated astrocyte marker GFAP (Supplementary Fig. 4b). Although Arc::dVenus × APP/PS1 mice clearly had activated microglia and astrocytes associated with amyloid plaques, we detected no overt cortical gliosis linked to the presence of a cranial window in either genotype.

To determine the spatial relationship between Arc-dVenus–expressing neurons and amyloid plaques, we identified the three-dimensional coordinates of the centers of mass of all detected neurons and methoxy-X04–positive plaques in the Arc::dVenus × APP/PS1 data sets. We observed higher Arc-dVenus expression levels in neurons closer to an amyloid plaque (Fig. 2f), indicating that the

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**Figure 2** Effect of amyloid plaques on stimulus-induced Arc-dVenus expression levels. (a) Example of maximum-intensity projection of the in vivo imaging data set used for quantification. Blue represents amyloid plaques visualized with methoxy-X04 and green represents Arc-dVenus fluorescence. Scale bar represents 100 µm. (b) Frequency distribution histograms of in vivo activity-induced Arc-dVenus expression levels in neurons of young pre-plaque Arc::dVenus × APP/PS1 mice (n = 4 mice, 1,590 neurons; bottom) and control littermates (n = 4 mice, 1,322 neurons; top). AU, arbitrary units. (c) Frequency distribution histograms of in vivo activity-induced Arc-dVenus expression levels in neurons of aged plaque-bearing Arc::dVenus × APP/PS1 mice (n = 8 mice, 3,410 neurons; bottom) and control littermates (n = 8 mice, 2,753 neurons; top). Dashed lines in b and c indicate threshold Arc-dVenus expression level separating 5% of brightest neurons in control mice. (d) Comparison of distributions of Arc-dVenus expression levels from b and c. Distribution medians with interquartile ranges are plotted. **P < 0.01, n.s., not significant, P = 0.13. (e) Mean (across mice) percentage of neurons with stimulus-induced Arc-dVenus expression levels above the threshold. Error bars represent s.e.m. *P < 0.05, n.s., not significant, P = 0.09. (f) Stimulus-induced Arc-dVenus expression levels in Arc::dVenus × APP/PS1 mice were elevated in the vicinity of amyloid plaques. Neurons were binned on the basis of their distance to the closest amyloid plaque and the medians of Arc-dVenus expression levels are shown with interquartile ranges (n = 8 Arc::dVenus × APP/PS1 mice, 3,250 neurons). ***P < 0.001.
amyloid plaques locally increase experience-dependent Arc promoter activity. We next sought to determine whether the presence of an amyloid plaque affects the number of Arc-dVenus–positive neurons in its direct vicinity after stimulation. We found that the number of activated neurons was reduced in the 20–50-µm vicinity of a methoxy-XO4–positive amyloid plaque compared with the same distance from randomly placed virtual plaques introduced into neuronal coordinate data sets of Arc-dVenus × APP/PS1 mice and control littermates (Fig. 3a). At the same time, the number of Arc-dVenus–expressing neurons in the 50–80-µm area was not affected (Fig. 3a).

As the APP/PS1 model of Alzheimer’s disease is known to have no significant cortical neuronal loss at this age17, this finding indicates that the amyloid plaques locally reduce the ability of neurons in close proximity to express Arc mRNA in response to a stimulus. However, neuronal loss has been reported in the local environment of plaques in a model of Alzheimer’s disease17; we therefore performed stereological quantification postmortem of neuronal density and the percentage of Arc neurons activated in mice killed 6 h after the end of visual stimulation. Brain sections were immunostained for GFP to visualize Arc-dVenus and for the pan-neuronal marker NeuN (Fig. 3d). We determined the proportion of Arc-dVenus–positive neurons in layer II/III of medial extrastriate visual cortex in the areas immediately adjacent to the plaques and those far away from the plaque. The proportion of NeuN-positive neurons with Arc-dVenus expression in control brain was 21.6 ± 1.6%. In Arc-dVenus × APP/PS1 brain, 24.3 ± 2.06% of neurons far from plaques expressed Arc-dVenus, whereas only 10.7 ± 0.67% of neurons expressed Arc near plaques (P < 0.01; Fig. 3b). In the same 20–50-µm region around a plaque in which we observed a decrease in Arc activation, we did not observe a significant neuronal loss (P = 0.90), as evidenced by maintenance of NeuN density (Fig. 3c.d). The only detectable loss of NeuN-positive cells was in the immediate vicinity of plaques (5–10 µm from the plaque edge, data not shown).

We observed another effect of plaques on Arc activation in local neurites. Dystrophic swelling of neurites is commonly present next to amyloid plaques both in the brains of individuals with Alzheimer’s disease18 and amyloid plaque-bearing mouse models, including APP/PS119, but their functional role in the disease pathogenesis is unknown. Abnormally swollen plaque-associated axons and dendrites represent only a small fraction of total neuropil, but when neurons of plaque-bearing mice are transgenically modified to express a fluorescent protein, either by crossing with a reporter strain or by using a gene delivery vector, the dystrophies belonging to the modified neurites accumulate the fluorescent protein and can be easily visualized20,21. In Arc-dVenus × APP/PS1 mice, we did not observe a single dVenus-filled neuritic dystrophy (over 300 total methoxy-XO4–positive amyloid plaques imaged in 16 mice), whereas the YFP-positive dystrophies were present in APP/PS1 mice crossed with YFP (Thy1::YFP × APP/PS1 strain), as expected20,22, as well as in APP/PS1 mice expressing GFP in the layer II/III of the visual cortex after stereotactic injection of AAV2/1–CBA-GFP virus (Fig. 4a). Given that Thy1-YFP, CBA-GFP, and Arc-dVenus were all expressed in cortical pyramidal neurons, which usually have abundant dystrophic neurites, this would imply that the neurites with neuritic dystrophies were not able to activate Arc promoter in response to the relevant stimulus. To confirm that dystrophic neurites are present around amyloid plaques in Arc-dVenus × APP/PS1 brains, we performed immunohistochemical analysis of brain sections of Arc-dVenus × APP/PS1 mice killed 6 h after the end of stimulation. Indeed, axonal dystrophies visualized with SMI-312 antibody (Fig. 4b) and dendritic dystrophies visualized with SMI-32 (Fig. 4c) were present around plaques in Arc-dVenus mice, but they did not contain dVenus, despite activation of nearby neurites in response to stimulation. These data indicate that amyloid plaques are associated with the opposing effects of reducing the proportions of neurons that express Arc in response to a stimulus in the vicinity of plaques and eliminating the ability of neurons with...
plaque-associated dystrophies in their neurites to respond to stimulation, whereas the few neurons near plaques that do respond appear to be hyperactive, with an increased level of Arc expression.

**Coordinated Arc expression is disrupted in APP/PS1 mice**

To assess the effect of Aβ pathology on Arc response to repeated presentation of the same stimulus, which the mice should have a memory of, we monitored activation status and Arc-dVenus expression levels in cortical neurons in two consecutive stimulation trials (Figs. 1d and 5a). The number of activated neurons in the second trial in Arc::dVenus × APP/PS1 mice was similar to that observed in control mice, which decreased in comparison with the first trial, as observed in control littermates (68.4 ± 5.3% of trial 1 (mean ± s.d.; Fig. 5b). The percentage of trial 1 neurons that were reactivated in the trial 2 (45.0 ± 3.0% of trial 1) and the percentage of newly activated neurons in trial 2 (33.6 ± 3.1% of trial 2) were not significantly affected by APP/PS1 genotype either (Fig. 5c,d). At the same time the proportion of neurons that had a higher level of Arc-dVenus expression in trial 2 was smaller in Arc::dVenus × APP/PS1 mice than in the control littermates (APP/PS1, 35.1 ± 3.4% of trial 1; control, 47.7 ± 2.6% in controls; Fig. 5e). Notably, the APP/PS1 genotype had a significant effect only on Arc-dVenus expression levels (measured in trial 1) in the neuronal population that was activated in both trials (P < 0.001; Fig. 5f), and not in populations that were activated only in trial 1 or only in trial 2 (P = 0.13 and 0.15; Fig. 5g,h).

To better determine the differences in the patterns of Arc-dVenus expression changes, we created a spaghetti plot in which each line connecting every neuron’s intensities in trials 1 and 2 was color-coded according to the absolute change of intensity (Fig. 6a). In Arc::dVenus × APP/PS1 mice, a large population of neurons with increased by amyloid plaque pathology in neurons that were activated in both trials (f), but not in neurons that were activated only in trial 1 (g) or only in trial 2 (h). *P < 0.05, **P < 0.001, n.s., not significant, P > 0.05. In b–h, n = 6 Arc::dVenus mice and 7 Arc::dVenus × APP/PS1 mice.

**Figure 4** Neurons with dystrophic neurites do not express Arc-dVenus in response to a stimulus. (a) Maximum intensity projections of an in vivo image stack from a Thy1::YFP × APP/PS1 transgenic mouse, an APP/PS1 mouse injected with GFP AAV2 virus and an Arc::dVenus × APP/PS1 mouse 6 h after visual stimulation. Blue indicates amyloid plaques visualized with methoxy-X04 and green indicates either YFP, GFP or dVenus fluorescence. Arrowheads point to YFP- and GFP-filled neuritic dystrophies characteristic of the APP/PS1 model. Scale bar represents 30 μm. (b,c) Postmortem immunostaining of Arc::dVenus × APP/PS1 mouse brain tissue acquired 6 h after visual stimulation for Arc-dVenus (with antibody to GFP, green channel) showed no colocalization of experience-dependent dVenus expression with either axonal (SMI-312, b) or dendritic (SMI-32, c) neurofilament staining (red channel) that typically accumulates in dystrophic axons or dendrites (arrowheads) around amyloid plaques (methoxy-X04, blue channel). Scale bar represents 30 μm.

**Figure 5** Arc-dVenus expression in repetitive stimulation procedure. (a) Repeated visual stimulation procedure. Following the completion of the first imaging session (trial 1), mice were returned to the light-proof, dark enclosure for an additional 60 h until trial 2 stimulation and imaging. (b) The total number of neurons expressing Arc-dVenus in the second trial was lower than in the first trial, which was not affected by the APP/PS1 genotype (repeated-measures two-way ANOVA: effect of trial P < 0.01, effect of genotype P = 0.39). (c–e) The proportions of neurons activated in both trials (c) and neurons only activated in the second trial (d) were not changed in Arc::dVenus × APP/PS1 mice, but the number of neurons that responded in both trials and had higher Arc-dVenus expression levels in the second trial was significantly decreased in Arc::dVenus × APP/PS1 mice (e). Data in b–e are presented as means ± s.e.m. (f–h) Differential comparison of distributions of Arc-dVenus expression levels in different population of neurons; data are presented as medians with interquartile ranges. Stimulus-induced Arc-dVenus expression levels were specifically increased by amyloid plaque pathology in neurons that were activated in both trials (f), but not in neurons that were activated only in trial 1 (g) or only in trial 2 (h). *P < 0.05, **P < 0.001, n.s., not significant, P > 0.05. In b–h, n = 6 Arc::dVenus mice and 7 Arc::dVenus × APP/PS1 mice.
Figure 6 Stimulus-specific Arc-dVenus expression is affected by amyloid plaque pathology. (a) Change of levels of visual stimulus-induced Arc-dVenus expression in two consecutive trials. Each line connects expression levels for a single neuron on trial 1 and trial 2 and is color-coded on the basis of absolute intensity changes. In control mice (left), populations of neurons experiencing both maximal changes of expression levels between two trials were balanced, whereas, in Arc::dVenus × APP/PS1 mice (right), a large population of neurons hyperactive in trial 1 reactivated in trial 2 with minimal expression levels. (b) This effect contributed to the significant negative shift of median expression levels in Arc::dVenus × APP/PS1 mice (bottom), a distribution that is relatively symmetrical in control littermates (top). (c) Distributions of Arc-dVenus expression level changes normalized to trial 1 expression confirmed this negative shift in intensity of reactivated neurons in Arc::dVenus × APP/PS1 mice. (d) Probability of activation of a neuron in trial 2 as a function of Arc-dVenus expression level in trial 1 was reduced in Arc::dVenus × APP/PS1 mice. Solid lines represent logistic regression model-based probabilities for Arc::dVenus × APP/PS1 mice and control littermates. Data are presented as mean ± s.e.m. **P < 0.01. (e,f) Comparison of medians of distributions presented in b (e) and c (f). Data in e and f are presented as medians with interquartile ranges. *P < 0.05. In a–f, n = 6 Arc::dVenus mice and 7 Arc::dVenus × APP/PS1 mice.

abnormally high levels of expression in trial 1 was reactivated in trial 2 with much lower expression levels, whereas there were very few neurons that experienced the opposite change of activity, from low to high. In the control littermates, these two populations were balanced and the distribution of Arc-dVenus intensity change from trial 1 to trial 2 appeared symmetrical, with the median around 0 arbitrary units (Fig. 6b,e). The distribution of the intensity change in Arc::dVenus × APP/PS1 mice was skewed to the left, indicating that the majority of reactivated neurons had lower expression levels in trial 2 (Fig. 6b,e). After normalization to the trial 1 expression levels, relative intensity change in Arc::dVenus × APP/PS1 mice was significantly shifted to the negative side (P < 0.05; Fig. 6c,f).

The association of Arc expression level in trial 1 with reactivation probability in trial 2 observed in control mice was significantly reduced in Arc::dVenus × APP/PS1 mice (P < 0.01). In Arc::dVenus × APP/PS1 mice, the log odds of reactivation were decreased by 0.59 compared with controls after adjustment for trial 1 intensity (P < 0.01; Fig. 6d). This loss of correlation between the level of activation at first presentation and reactivation at the second presentation could represent a failure of the APP/PS1 brain to change synaptic properties in response to Arc activation, thereby contributing to disrupted memory consolidation.

DISCUSSION
We used a mouse model expressing dVenus under the control of the Arc promoter and longitudinal multiphoton imaging in living mice to study the activity-dependent expression of Arc in visual circuits in response to physiologically relevant, complex stimulation. We found that, in control brains, there was robust activation of Arc promoter in neurons of medial extrastriate visual cortex in response to a structured visual stimulation and that the probability of neuronal activation was tightly correlated with the Arc expression level following previous exposure to the same stimulus. The characteristic patterns of Arc responses were found to be altered by cerebral amyloid plaque deposition. In contrast with wild-type animals, in the visual cortex of amyloid plaque-bearing Alzheimer’s disease model mice we found that the proportion of Arc-responsive neurons was strongly decreased in the closest vicinity of plaques, a small population of cortical neurons expressed Arc mRNA in response to a sensory experience at abnormally high levels, especially in the same small region surrounding plaques, neurons with plaque-associated neuritic dystrophies did not express Arc mRNA in response to a stimulus, and the correlation between the level of Arc activation at the first stimulus and the probability of reactivation with a subsequent presentation was weakened by amyloid plaque pathology. Together, these data support a model in which plaques disrupt the normal integrative functions of the cortex in their immediate microenvironment.

Arc has been strongly implicated in memory consolidation and synaptic change9,23,24. Coordinated levels of Arc expression are extremely important for correct execution of the synaptic plasticity underlying the encoding of long-term changes, via mechanisms that include regulation of AMPAR endocytosis8 and promotion of F-actin polymerization6. Thus, mice with Arc knockout exhibit impaired long-term synaptic consolidation in various behavioral tasks9,24, and Arc overexpression leads to a decrease in surface AMPARs8 and reduced AMPAR-mediated synaptic transmission20. Our data showing a positive correlation between neuronal activation in response to a stimulus and levels of Arc expression following subsequent exposure to a similar stimulus suggest that one role of Arc in memory consolidation is to strengthen responses to stimuli in cells with high levels of Arc after the first stimulus presentation. This highlights the importance of precise regulation of Arc promoter activity for normal neurophysiology.

Our findings are consistent with the well-established association of Aβ deposition and soluble Aβ with memory and plasticity deficits, synapse loss, and neuritic structural changes (reviewed in ref. 26).
Our results indicate that abnormal hyper- and hypo-active behavior of viable neurons in Alzheimer’s disease brains extends to higher order functions, such as the long-lasting consolidation of synaptic connections mediated by Arc, and that these effects are predominantly found in the immediate vicinity of plaques. These findings highlight the same microanatomical areas that we have previously demonstrated using structural tools to contain high concentrations of oligomeric Aβ. Our results are also broadly consistent with observations using calcium imaging in living mice that showed clusters of neurons and neurites with calcium overload in anesthetized mouse cortex in close proximity of amyloid plaques, extending those observations to the level of neural circuit integration in awake, behaving animals.

Our findings also shed light on the controversy as to whether Arc is up- or downregulated in the brain in Alzheimer models and in Alzheimer’s disease itself. For example, Alzheimer’s disease-related pathology is linked with an increase in Arc expression in six studies, and no effect in three studies (Supplementary Table 1). These discrepancies could be a result of artifacts of in vitro systems or the static nature of postmortem experiments that do not allow analysis of Arc dynamics or of Arc in the context of specific behavioral stimuli. With our longitudinal imaging in vivo, we observe that amyloid plaques acted locally to abnormally increase Arc promoter activity in the responsive neurons, but the proportion of activated neurons in the direct vicinity of plaques was decreased. Moreover, neurons that had amyloid plaque–associated dystrophic neurites were not able to express Arc mRNA, a fact that suggests that an amyloid plaque can affect functional properties of neurons with somata located relatively far away. One might argue that the observed plaque-associated functional impairments affect only a small proportion of neurons. In fact, when we take into account the extent of amyloid deposition in the brains of Alzheimer’s disease patients, a 50-μm vicinity of Aβ plaque containing functionally defective neurons would represent at least 70% of the total affected cortical volume (conservative estimate on the basis of temporal lobe data from ref. 47).

Recent evidence suggests that cognitive deficits in early Alzheimer’s disease are a result of large-scale neuronal network dysfunction, although the extent to which this is mediated by Aβ plaques as opposed to associated tangles, neuronal loss or even soluble forms of amyloid is uncertain (for example, reviewed in ref. 48). Patients with Alzheimer’s disease exhibit regional hypometabolism and disrupted whole-brain activation patterns, whereas pre-symptomatic Alzheimer’s disease patients characterized by amyloid deposits noted on neuroimaging, exhibit abnormal functional hyperactivity during memory encoding tasks. Overall, our results in a mouse model that develops senile plaques (but no neurofibrillary tangles or overt neuronal loss) in the cortex suggest that amyloid deposits and surrounding soluble Aβ introduce complex aberrations into the coordinated execution of activity-dependent transcriptional programs in excitatory neurons, which we hypothesize at least partially underlie the abnormalities in neural system function noted even before clinical symptoms in Alzheimer’s disease.

METHODS
Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
N.R., T.L.-S., and B.T.H. designed the experiments, analyzed the data and wrote the paper. N.R. and J.M.H. performed the experiments. M.E. and S.Y. generated ArcδVenus mice. R.A.B. contributed with statistical analysis of the data.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ONLINE METHODS

Mice and surgery. Arc-dVenus mice overexpressing destabilized Venus under the control of 7.1 kb of the mouse Arc promoter10 (C57BL/6 background) were crossed with APPswp PS1ΔE9 mice14. Arc:dVenus-positive noncarriers of the APP/PS1 transgene were used as controls. Mice had cranial windows implanted as described previously22 over the right visual cortical area at least 3 weeks before imaging to allow for recovery from surgical trauma. Anesthesia used for cranial window implantation was induced with 4% isoflurane (vol/vol) in balanced oxygen and maintained at the level of 1.2–1.6% during the surgery. Body temperature was maintained at 37 °C.

Brain tissue from Thy1::YFP × APP/PS1 mice was acquired from a previous study22. Briefly, Thy1::YFP × APP/PS1 mice were perfused with 4% paraformaldehyde (vol/vol) and 0.1% glutaraldehyde (vol/vol) fixative and 50-μm coronal sections were obtained through the brain. All mice were 6–7 months old, except for the pre-plaque Arc:dVenus × APP/PS1 cohort and their control littermates, which were 3–3.5 months old. All animal experimentation was performed in conformance with institutional and US National Institutes of Health guidelines and approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee.

To express GFP in the layer II/III neurons of the visual cortex, we stereotactically injected APP/PS1 mice with AAV2/1–CBA-GFP virus (MassGeneral Vector core) during window implantation. We allowed 3 weeks for transgene expression and recovery from the surgical trauma before imaging.

Visual stimulation and in vivo multiphoton imaging. Prior to each visual stimulation, mice were given an intraperitoneal injection of the amyloid plaque-labeling agent methoxy-XO4 (5 mg per kg of body weight)20 and placed for 60 h in their home cages and into the dark, light-proof ventilated cardboard enclosures accommodating one cage each. At the end of the light-deprivation period, mice were transferred to a glass cylinder (30 cm tall, 20 cm wide) with alternating vertical black and white stripes (2 cm wide) applied to the wall. The cylinder was illuminated from the outside, yielding illumination of approximately 200 lx inside the cylinder. Following visual stimulation, mice were transferred back to their home cages and placed into dark, light-proof enclosures for 6 h until imaging. Anesthesia was induced inside the light-proof enclosure with 4% isoflurane, and mice were transferred to custom-made imaging stage and anesthesia maintained at the level of ~1.5%. Texas Red–conjugated dextran (MW 70,000 Da, 12.5 mg ml in sterile phosphate-buffered saline (PBS), Molecular Probes) was injected intravenously to provide fluorescent angiogram used to locate the same brain region in consecutive imaging sessions and as a reference fluorophore for the control of cranial window quality. Imaging was performed with an Olympus Fluoview 1000 MPE system coupled to an Olympus BX61W1 upright microscope with a XPLN 25× water-immersion objective (NA = 1.05). Excitation light was produced by a mode-locked titanium/sapphire MaiTai laser (Spectra-Physics) tuned to 860 nm with the output power set to 100 mW. Emitted light was collected in three channels: 460–500 nm (amyloid-core) channel using the 3D Stitching plug-in51. Background was subtracted and median filter applied. For quantification of Arc:dVenus expression levels, stitched stacks were z-projected and the mean fluorescent signal in each individually detected cell body was measured. To determine the three-dimensional coordinates of amyloid plaques and neuronal cell bodies, three-dimensional stacks were segmented using 3D Object Counter plug-in. When counting the number of Arc:dVenus-positive neurons at different distances from plaques, we excluded from analysis real and virtual plaques that had no neurons in a 100-μm radius (this criterion eliminated roughly 40% of real and virtual plaques), as the Arc:dVenus-positive neurons were not distributed evenly across each data set, unlike the methoxy-XO4–positive amyloid plaques.

Postmortem tissue analysis. To obtain tissue for immunohistochemistry, we transcardially perfused mice with ice-cold PBS followed by 4% paraformaldehyde in PBS. Brains were incubated in fixative at 4 °C for an additional 48 h. We cut 50-μm free-floating sections on a Microm HM400 microtome. For primary antibodies, we used antibody to chicken GFP (1:500, cat. no. GFP-1020, Aves Labs), mouse SMI-312 (1:500, cat. no. SMI-312R, Covance), mouse SMI-32 (1:500, cat. no. SMI-32R, Covance), mouse NeuN (1:500, cat. no. MAB377, Millipore), mouse Arc (1:1000, cat. no. sc-17839, Santa Cruz) and rabbit Iba1 (1:500, cat. no. 019-19741, Wako). For secondary antibodies, we used AlexaFluor 488–conjugated goat antibody to chicken (1:500, cat. no. A-11039, Molecular Probes), Cy3-conjugated goat antibody to mouse (1:500, cat. no. 115-165-144, Jackson ImmunoResearch) and goat antibody to rabbit (1:500, cat. no. 111-165-144, Jackson ImmunoResearch). The high-resolution images for Figure 4 were acquired on a Zeiss LSM 510 META confocal microscope equipped with two-photon Coherent Chameleon laser and aligned to the z projections. The overview low-resolution images for Figure 1 were acquired on Zeiss Axios Observer.Z1 fluorescent microscope and aligned to Allen Brain reference atlas (brain-map.org). Immunohistochemical images for Supplementary Figures 1 and 4 were acquired on Zeiss Axios Observer.Z1. Stereological counting of Arc::dVenus- and NeuN-positive neurons was performed on Olympus CAST system in neuronal layer II/III of medial extrastriate visual cortex of mice that had not undergone window implantation and in vivo imaging. To define a region as ‘near plaque’, the edge of stereological counting frame (65 × 65 μm) was placed at a distance of 10 μm from a plaque’s center. Regions defined as ‘far from plaque’ were selected randomly with the condition that no plaque was present within at least 50 μm from the edge of the counting frame.

Statistical analysis. The normality of all data sets was tested using Kolmogorov-Smirnov method. Multiple comparisons of non-normal data was performed using Kruskal-Wallis method with Dunn’s post-test for pair-wise comparison. Multiple comparisons of normal data was performed using one-way or repeated-measures two-way (when indicated) ANOVA with Bonferroni post-test for pair-wise comparison of mean values across mice. Simple comparison of two sets of normal data across mice was performed using unpaired Student’s t test. Mann-Whitney test was used to compare two sets of non-normal data.
Non-normal distributions of fluorescence intensity levels in Arc::dVenus neurons were compared using Wilcoxon rank-sum test with correction for clustering of values within individual mice. The association of Arc::dVenus intensity with reactivation probability (Fig. 6d) was tested by fitting a generalized estimating equations logistic regression model using an independent working correlation matrix (accounting for the data clustering in mouse), implemented in SAS version 9.3. All reported P values are two-tailed.

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