modally equipotential in the common ancestor) further evolved independently to subserve the species-specific repertoires that characterize human and chimpanzee communication and cognition.

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17. Eleven chimpanzee brains from the collection of Dr. Ralph Holloway and four from the Yerkes Primate Center (YN97-139, YN92-115, YN88-256, and YN77-117) were used (thanks to Daniel Anderson, Jeremy Dahl, and Harold McClure; and to James Rilling, Department of Anthropology, Emory University). The cortical surface area of the PT in these 15 brains was quantified as described below (*21*). Three brains from the Smithsonian Institution were also used (SI-292178, SI-225776, and SI-292176) (thanks to Dr. Richard W. Thorington Jr., Linda K. Gordon, and Jeremy Jacobs, Department of Vertebrate Zoology.) Two of us (P.J.G. and A.R.B.) first noted marked asymmetry of the PT and Heschl's gyrus in these three brains during a magnetic resonance imaging study. Because of the friable nature of these three brains, the SF could not be spread widely; thus, the lateral margin of the PT was quantified with calipers. Results showed that the PT was 51 ± 40% larger on the left than on the right (49, 12, and 91% greater on the left than on the right, respectively).
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19. The quantitative method employed here, which accounted for the 3D complexity of the PT unlike methods used previously, involved the following. (i) After wide spreading of the SF to obtain an unobstructed view of the PT, and with the use of an operating microscope at magnification ×4 to ×10, three of us (P.J.G., R.L.H., and D.C.B.) identified the suite of defined structures that demarcate the borders of the PT (*20*). In brief, these are as follows. Anterior: Heschl's sulcus, which forms the posterior border of Heschl's gyrus. In the event of a secondary posterior portion of Heschl's gyrus, the criterion used to include this within the PT was if it comprised a distinct retroinsular pedicle of origin. Lateral: superolateral margin of the superior temporal convolutions. Posterior: the termination of the horizontal portion of the SF. A distinct ridge, formed at the point of diversion of the horizontal and descending limbs of the SF was apparent (arrows in Fig. 1). Medial: the retroinsular point forming the apex of the approximately triangular PT. (ii) A sheet of thin black plastic was formed to fit precisely within the defined margins of the PT. The thin flexible plastic conformed to the 3D complexity of the PT and was able to be inserted within Heschl's sulcus even in cases where this structure was deeply invaginated (asterisk in Fig. 1). (iii) The black plastic templates were attached to white cards and scanned at 150 dots per inch into TIFF files. Their area was then determined in millimeters with the use of SigmaScanPro. Statistical analysis (Statistical Package for the Social Sciences) included analysis of variance and paired *t* tests.
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Stabilization of Dendritic Arbor Structure in Vivo by CaMKII

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Calcium-calmodulin–dependent protein kinase II (CaMKII) promotes the maturation of retinotectal glutamatergic synapses in *Xenopus*. Whether CaMKII activity also controls morphological maturation of optic tectal neurons was tested using in vivo time-lapse imaging of single neurons over periods of up to 5 days. Dendritic arbor elaboration slows with maturation, in correlation with the onset of CaMKII expression. Elevating CaMKII activity in young neurons by viral expression of constitutively active CaMKII slowed dendritic growth to a rate comparable to that of mature neurons. CaMKII overexpression stabilized dendritic structure in more mature neurons, whereas CaMKII inhibition increased their dendritic growth. Thus, endogenous CaMKII activity limits dendritic growth and stabilizes dendrites, and it may act as an activity-dependent mediator of neuronal maturation.

During brain development, neurons elaborate complex dendritic arbors. This process is controlled by mechanisms that promote and limit neuronal growth (*1*). Because neuronal activity and the resultant calcium influx can decrease neurite extension (*2*), activity may control dendritic growth by a calcium-mediated mechanism.

Calcium-sensitive enzymes such as CaMKII can influence both neuronal growth (*3*) and synaptic efficacy (*4*); however, it is not clear whether these effects are coordinated. Because CaMKII is concentrated in postsynaptic densities (*5*), with a wide range of substrates including transmitter receptors, channel proteins, and cytoskeletal proteins (*6*), it could transduce input activity into coordinated changes in both neuronal growth and synaptic strength. CaMKII expression and subcellular localization are developmentally regulated (*7*, *8*). Postsynaptic elevation of CaMKII activity influences development of presynaptic retinotectal axons (*9*) and maturation of retinotectal synaptic responses (*10*). These findings suggest that CaMKII may coordinate the development of synaptic physiology and neuronal morphology.

CaMKII immunoreactivity is distributed in a rostrocaudal gradient in the optic tectum (Fig. 1). A crescent-shaped proliferative zone in the caudomedial region of the optic tectum of *Xenopus laevis* tadpoles con-

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tinuously produces new cells, so that rostral and lateral tectal neurons are chronologically older and morphologically more complex than neurons in the caudal and medial tectum (*10, 11*). Single neurons at different positions along the rostrocaudal axis of the tectum were labeled with DiI and imaged in vivo (*12*). The tadpoles were then processed for CaMKII immunostaining (*13*). Simple neurons with total dendritic branch lengths (TDBL) less than ~300 μm have little or no detectable CaMKII immunoreactivity in their cell bodies. More mature neurons, with TDBL greater than ~300 μm, are located in the CaMKII-immunoreactive region of the tectum.

To examine the morphological development of tectal neurons in vivo, we labeled single cells at various positions along the rostrocaudal axis of the tectum with DiI. Confocal images through the complete structure of the individual neurons were collected over 3 to 5 days (Fig. 2). Neurons whose cell bodies are located in the caudal tectum have simple morphologies. While still close to the ventricular layer, cells extend a large growth cone that grows rapidly toward the lateral tectum, turns rostrally, and extends out of the tectum. Meanwhile, the few dendritic branches that are present are constantly rearranging, but there is no net increase in branch tip number or length. Over the next 2 days, the dendrites become more elaborate. Relatively few branches persist over 24 hours. Somata are displaced rostrally and laterally by cells more recently generated in the caudomedial proliferative zone. These more mature neurons continue to elaborate their dendrites, but at a slower rate (*14*). Growth rates gradually slow from ~175 μm/day in neurons with simple dendritic arbors to ~50 μm/day as neurons mature and develop a complex dendritic arbor. The decrease in growth rates correlates with the time when neurons express detectable amounts of somatic CaMKII. On the basis of the correspondence between CaMKII expression and morphological complexity (Fig. 1), we plotted the increase in TDBL for "simple" neurons (with branch lengths of <300 μm on the first day of imaging and relatively low CaMKII immunoreactivity) and for "complex" neurons (with branch lengths of >300 μm on the first day of imaging and relatively high CaMKII immunoreactivity). Simple neurons are in a phase of rapid growth. More mature neurons with complex dendritic arbors increase TDBL more slowly. Thus, more complex neurons may have a mechanism to limit the rate of dendritic growth.

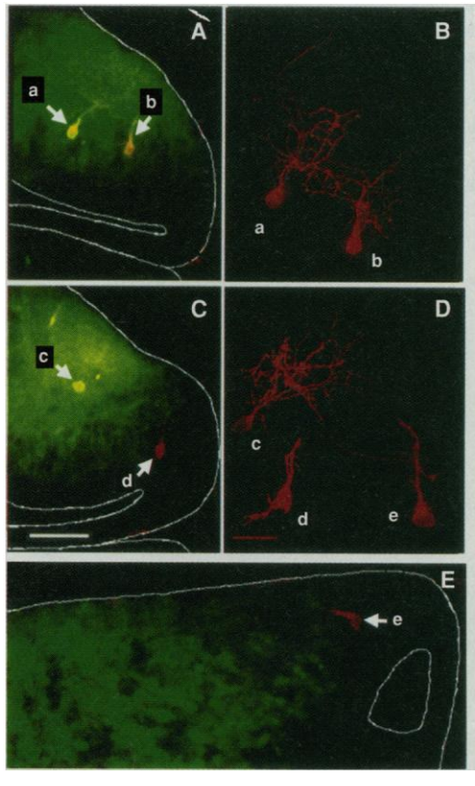
To test whether increased CaMKII activity has an impact on the rate of dendritic arbor development, we imaged single DiI-labeled neurons at various positions along the rostrocaudal axis of the tectum in vivo over 3 days, starting immediately before infection with a recombinant vaccinia virus (VV) expressing constitutively active CaMKII (CaMKII VV) or a control VV expressing β-galactosidase (β-Gal VV) (*15*). Neurons from CaMKII VV–infected animals (referred to as CaMKII neurons) were compared with uninfected controls and with neurons from animals infected with β-Gal VV (referred to as β-Gal neurons). β-Gal neurons were comparable to uninfected neurons in all parameters assayed. Neurons from all three groups grew at a comparable rate over the first day of imaging, but CaMKII neurons grew significantly slower over the next day and obtained a significantly smaller dendritic arbor (Table 1). This decreased growth rate in CaMKII neurons correlates with the time when virally expressed proteins can be detected (*16*) and indicates that increased CaMKII activity can slow dendritic growth rate. However, this protocol does not permit us to test the effect of elevated CaMKII activity on young neurons because of the delayed synthesis of virally expressed proteins. We therefore infected animals with VV and collected the first image of each series 2 days after virus injection.

CaMKII VV has no apparent effect on the rate of axon outgrowth, axon growth cone dynamics, or the initial formation of dendritic branches (Fig. 2B). Increasing CaMKII activity in simple neurons, before expression of detectable amounts of endogenous somatic CaMKII begins, slows the rate of dendritic arbor elaboration to a rate comparable to that seen in more mature normal neurons. CaMKII neurons from caudal tectum form simpler dendritic arbors than do uninfected or β-Gal neurons from caudal tectum, as also indicated by Sholl analysis (*17*). CaMKII neurons from rostral tectum are not as elaborate as

Table 1. Dendritic growth rate is decreased after CaMKII overexpression.

Treatment	Growth rate (μm/day)		TDBL (μm)
	Day 1–Day 2	Day 2–Day 3	Day 3
Control (n = 22)	172 ± 22	133 ± 31	532 ± 31
β-Gal (n = 33)	173 ± 22	125 ± 28	553 ± 52
CaMKII (n = 18)	180 ± 43	48 ± 11*	404 ± 31*

*$P < 0.01$.



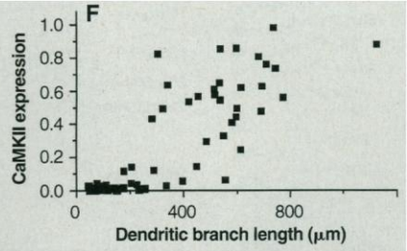


Fig. 1. Developmental regulation of CaMKII expression in tectal neurons. (**A** to **E**) Examples showing five DiI-labeled tectal neurons imaged in vivo [(B), cells a and b; (D), cells c, d, and e] and confocal images showing the positions of the DiI-labeled somata (A, C, and E) and CaMKII immunoreactivity. Complex neurons (a, b, and c) express CaMKII; simple neurons (d and e) are not immunoreactive for CaMKII. (A) and (C) are horizontal sections through tectum, with rostral up; (E) is a sagittal section with rostral to the left. Scale bars, 20 μm (A, C, and E), 40 μm (B and D). (**F**) CaMKII immunoreactivity increases as TDBL increases (n = 53).

rostral uninfected or β-Gal neurons at the first observation, 2 days after infection (see above). CaMKII neurons do add new branches and extend preexisting branches during the observation period. Increasing CaMKII activity in more mature neurons, which already express endogenous CaMKII, does not further decrease the rate of arbor elaboration below their already modest growth rate. Electrophysiological recordings indicate that these neurons have strong retinotectal synaptic transmission (*10*).

The arbor structure of CaMKII neurons appeared more stable over 24 hours than did arbors from control neurons (Fig. 2). To test whether CaMKII stabilizes the dendritic arbor, we took observations of uninfected and CaMKII VV–infected neurons at 2-hour intervals over 6 hours (*18*). Although there is little net change in TDBL or branch tip numbers in control neurons, branches are continually added and retracted from the dendritic arbor (Fig. 3). The majority of branches are observed only once and therefore have an average lifetime of less than 2 hours. Roughly twice as many dendritic branches as are initially present are added and retracted over 6 hours. In CaMKII neurons, these structural rearrangements are reduced by half. Branches seen at the first observation and those added during the 6-hour observation period in CaMKII neurons had longer lifetimes than in controls. Thus, increased CaMKII activity stabilizes the dendritic arbor by decreasing rates of branch additions and retractions (*19*). This likely accounts for the simpler arbor morphology seen in CaMKII neurons.

To test the role of endogenous CaMKII activity in dendritic arbor development, we examined the effect of the antagonist KN93 on dendritic growth. KN93 caused a significant increase in TDBL in more

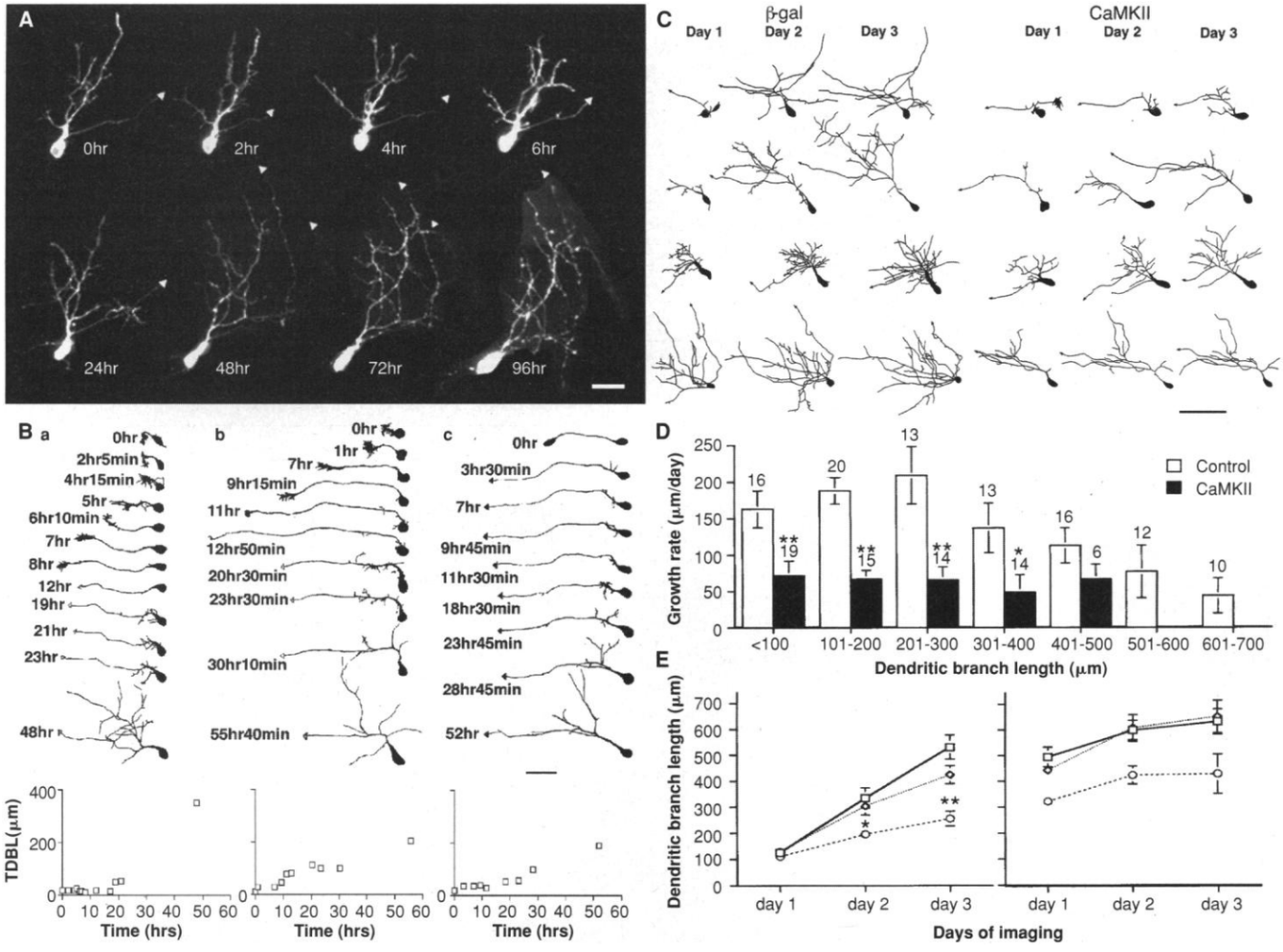


Fig. 2. Effect of CaMKII expression on development of the dendritic arbor. (**A**) Time-lapse in vivo images of a control DiI-labeled tectal neuron collected at the times indicated. The photomontage shows 3D reconstructions of the confocal optical sections. The arrowheads mark the efferent axons. The dendritic arbor was of intermediate complexity on the first day of imaging and became more elaborate over the next 5 days. Scale bar, 20 μm. (**B**) Drawings of short-interval observations from an control neuron (a) and two CaMKII neurons (b and c). Elapsed times since the first observation are next to each drawing. CaMKII VV does not change growth cone mobility or initial formation of dendritic branches. Increase in branch length is plotted below each neuron. Scale bar, 25 μm. (**C**) Neurons from β-Gal– and CaMKII VV–infected animals imaged at daily intervals. Neurons are arranged in order of increasing TDBL from top to bottom. Scale bar, 50 μm. (**D**) Daily growth rate plotted for control neurons (uninfected and β-Gal, pooled) and CaMKII neurons binned according to initial dendritic branch length. (**E**) Dendritic branch length for each day of imaging for simple (left) and complex (right) neurons from uninfected (squares), β-Gal–infected (diamonds), and CaMKII-infected (circles) animals. Simple CaMKII neurons, $n = 25$; uninfected, $n = 14$; β-Gal, $n = 19$. Complex CaMKII neurons, $n = 7$; uninfected, $n = 8$; β-Gal, $n = 12$ ($^*P < 0.01$, $^{**}P < 0.001$). CaMKII overexpression decreases the rapid rate of growth normally seen in simple neurons to the slower rate of more mature neurons. CaMKII overexpression in complex neurons does not further decrease their growth rate; however, dendritic structure is more stable.

complex neurons relative to controls, but no change in simple neurons was seen (Fig. 4). KN93 significantly increased the daily growth rate of neurons with an initial dendritic branch length greater than 300 μm (Fig. 4). This correlates roughly with the time of expression of CaMKII in tectal neurons.

Thus, tectal cell development has three phases. Stage 1 neurons undergo axonogenesis and extend few dendritic branches. They are not immunoreactive for CaMKII and may not respond to virally expressed CaMKII because they lack downstream signaling elements. Stage 2 neurons are morphologically simple and in a rapid growth phase. They have the cellular machinery necessary to carry out the CaMKII-dependent regulation of arbor elaboration, but do not normally have sufficient CaMKII activity. Stage 3 neurons, which express larger amounts of endogenous CaMKII, are morphologically more complex; relative to younger neurons, they have slower rates of dendritic arbor growth and their dendritic structure is more stable. Additional elevation of CaMKII in these neurons does not further slow dendritic growth. CaMKII expression increases the stability of dendritic branches in stage 2 and stage 3 neurons. Low CaMKII concentrations are permissive for rapid rates of dendritic arbor growth, as seen in younger neurons and in mature neurons exposed to KN93. Expression of endogenous CaMKII permits neurons to control the rate of dendritic arbor growth, possibly in response to afferent activity (*20*).

Glutamatergic synapses in vertebrates initially use the *N*-methyl-D-aspartate (NMDA) type of glutamate receptors and mature with the addition of an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) component (*10, 21*). Stable dendritic structure in tectal neurons correlates with mature synapses. Rapid growth rate and dynamic physical structure correlate with a preponderance of silent NMDA synapses. Stage 1 neurons from caudal tectum either have principally NMDA receptor–mediated retinotectal synaptic transmission (*10*) or have not yet received retinal inputs. Stage 2 neurons, which are rapidly growing, have retinotectal synaptic responses with relatively low AMPA/NMDA ratios, and about half of the synaptic responses are mediated solely by NMDA receptors. Stage 3 neurons, which exhibit CaMKII-dependent dendritic stabilization, have synaptic responses with high AMPA/NMDA ratios and relatively few silent NMDA synapses (*10*).

Thus, CaMKII plays a pivotal role in an activity-dependent mechanism that coordinates the development of neuronal structure and function. New retinal axonal branches (*22*) may form transient pure NMDA synapses with tectal neurons (*10*) that have no impact on the postsynaptic activity unless they are coactive with oth-

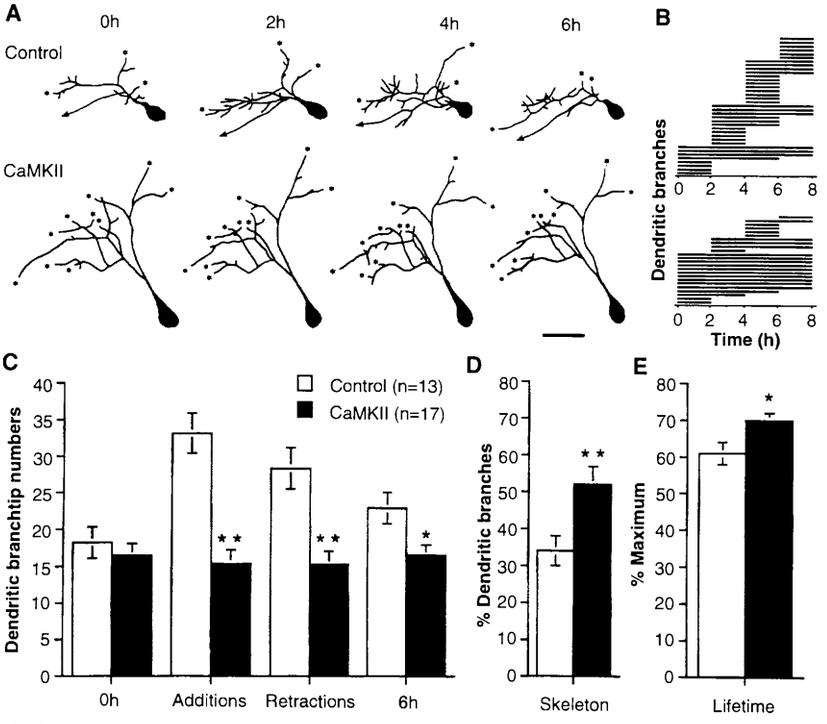


Fig. 3. CaMKII increases arbor stability. (**A**) Control and CaMKII VV–infected neurons imaged at 2-hour intervals over 6 hours. Branches marked by asterisks are those seen at the first observation that persist to the last observation. Stable branches are seen more often in the representative CaMKII neuron. Scale bar, 25 μm. (**B**) Graphic depiction of arbor dynamics in the control and CaMKII neurons shown in (A). Each bar represents a branch in the arbor. Bars are grouped along the *y* axis according to the time point when the corresponding branch was first seen, with those initiated early in the experiment at the lower left of the graph, and those initiated late at the upper right. The position of the bar along the *x* axis indicates the time point(s) during which the branch was observed. The length of each bar represents the lifetime of the branch. (**C**) Branch additions and retractions observed at 2-hour intervals over 6 hours (**$P < 0.001$, *$P < 0.05$). (**D**) The skeleton (the fraction of branches present in the first observation that persist through the 6-hour observation period) is significantly greater in CaMKII neurons than in control neurons (**$P < 0.01$). (**E**) Branch lifetime, an indicator of the stability of both new and initial branches in the arbor, is also significantly greater in CaMKII neurons than in controls (*$P < 0.05$). The neurons imaged for this experiment had initial branch lengths of about 200 μm (213 ± 18 μm for CaMKII neurons, $n = 17$; 205 ± 31 μm for control neurons, $n = 13$) and were located in the central tectal region. β-Gal neurons ($n = 5$), imaged at 2-hour intervals, are comparable to controls in all parameters tested.

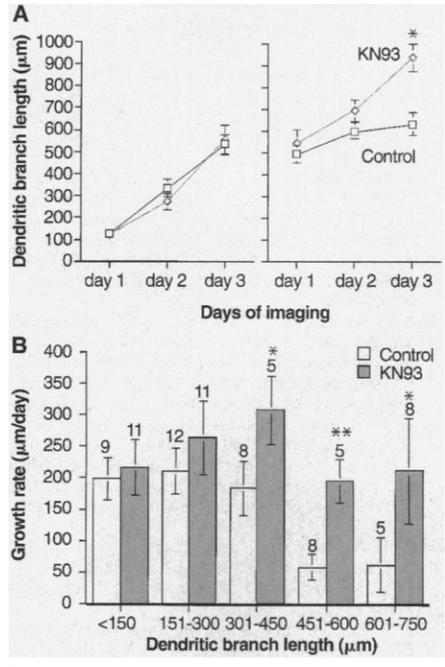


Fig. 4. KN93 increases dendritic growth in complex neurons. (**A**) Dendritic branch length for each day of imaging for simple (left) and complex (right) neurons treated with 1 to 2 μM KN93. The first image was collected immediately before exposure to KN93. Animals were maintained in KN93 in rearing solution over the next 2 days (*$P < 0.001$). (**B**) KN93 significantly increases growth rates only for neurons with initial branch lengths greater than 300 μm (*$P < 0.05$, **$P < 0.01$).

er activity in the tectal neuron. If these conditions are met, NMDA receptors will be active and result in calcium influx at that synaptic site (*23*). Elevated calcium would then activate CaMKII locally (*24*), reflecting the spatial and temporal patterns of afferent coactivity.

Elevated CaMKII activity would promote the maturation of the synapse through the addition of a functional AMPA component to synaptic responses (*10*, *25*) and would locally stabilize tectal cell dendrites (*26*). Other dendritic regions would not be affected by the local signal and could continue to elaborate. If synaptic inputs weaken (*27*) and fail to activate CaMKII, the low CaMKII activity would be permissive for increased local branch additions and retractions.

One potential function of afferent activity may be to consolidate the structure and function of developing neurons and their circuit properties, possibly through a calcium-sensitive mechanism. Here, we have shown that CaMKII is expressed at the correct time and place in developing neurons to play this role, and that increasing and decreasing CaMKII activity in developing tectal neurons has an impact on morphology and synaptic responses (*10*) of tectal neurons.

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12. Tectal cells in stage 46–48 [P. D. Nieuwkoop and J. Faber, *Normal Table of* Xenopus laevis (*Daudin*) (Elsevier–North Holland, Amsterdam, 1956)] albino *Xenopus laevis* tadpoles were labeled by DiI iontophoresis [1,1′-dioctadecyl-3,3,3′3′-tertmeththylindocarbocyanine perchlorate, or $DiIC_{18}$, Molecular Probes; 0.02% in absolute ethanol] using 1 to 10 nA positive current applied in three to five pulses of 200-ms duration. For use in combination with immunocytochemistry, chloromethylated DiI (Molecular Probes) was used. Dye injection, screening, and imaging were done while animals were anesthetized with 0.02% 3-aminobenzoic acid ethyl ester (MS222, Sigma) in Steinberg's solution. Single or well-isolated brightly labeled tectal cells were imaged with a Noran XL laser scanning confocal attachment mounted on an upright Nikon Optiphot, using a 40× Nikon lens (0.85 NA). Images were collected at optical steps of 1 to 4 μm in the *z* dimension; 8 to 16 frames were averaged for each optical section. Animals recovered from anesthetic between imaging sessions and were kept in a 23°C incubator with red illumination. Analysis was performed as described (*22*). Statistical significance was determined using a two-tailed *t* test. Only neurons with rostrally projecting axons were included in this study.
13. Animals were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer. CaMKII immunostaining was performed on 30- to 50-μm cryostat sections as follows: 1 hour preincubation in blocking solution containing 5% goat serum and 0.1% Triton X-100 in phosphate-buffered saline (PBS); 2 hours incubation in CaMKII antibody (Boehringer-Mannheim), diluted 1:100 in blocking solution; three times 15-min rinse in blocking solution; 1 hour incubation in Cy5-tagged goat antibody to mouse immunoglobulin G (Jackson) diluted 1:400 in blocking solution; rinse in PBS. The CaMKII antibody labels a single band of approximately 52 kD on a protein immunoblot, consistent with it recognizing the *Xenopus* homolog of the rat αCaMKII, against which the antibody was generated. Images of the sections were collected on the Noran Oz confocal equipped with a krypton-argon laser to assess whether the DiI-labeled neurons were immunoreactive for CaMKII. Cell morphology was reconstructed from the images collected in vivo, and branch length was determined as described above. For quantification of CaMKII immunoreactivity, mean CaMKII immunoreactivity intensity values for a 20-μm^2 box containing the DiI-labeled cell body were normalized to intensity values in the tectal neuropil, which were set at 100%. Background intensity values, measured from a 20-μm^2 box placed over the ventricular layer, were subtracted from both values before normalizing. CaMKII immunoreactivity intensity values were measured without knowledge of TDBL measurements.
14. Growth rates were calculated as the difference in TDBL between the two time points.
15. To increase CaMKII activity selectively in postsynaptic tectal neurons without directly altering CaMKII activity in the presynaptic retinal axons, we used a vaccinia virus expressing a constitutively active calcium-calmodulin–independent CaMKII (CaMKII VV) (*25*). The CaMKII is driven by a strong synthetic promoter. The same recombinant virus includes the reporter β-Gal driven by a weaker p7.5 promoter. Additional animals were infected with a β-Gal VV, in which lacZ is driven by the strong synthetic promoter. Purified CaMKII VV [10^8 plaque-forming units (pfu)] or β-Gal VV (10^8 pfu) was injected into the brain ventricle under MS222 anesthesia (*16*). Ventricular injection of vaccinia virus results in high expression of foreign protein in the majority of cells in the central nervous system within 2 days after injection (*16*). Infection with CaMKII VV increases the calcium-calmodulin–independent CaMKII activity in brain homogenates by about 50% over the calcium-calmodulin–independent kinase activity in uninfected or β-Gal–infected animals (*9*). In this and all following experiments, images were collected and analyzed blind to the treatment.
16. G.-Y. Wu, D.-J. Zou, T. Koothan, H. T. Cline, *Neuron* **14**, 681 (1995).
17. G.-Y. Wu and H. T. Cline, data not shown.
18. Observations of these neurons at 24- and 48-hour intervals showed that their daily rate of growth corresponded to those neurons imaged only at daily intervals (see Fig. 2). For analysis of branch dynamics in neurons imaged at 2-hour intervals, each branch tip was assigned a number. Drawings of subsequent images were superimposed on that from the previous time point, and the maintenance and retraction of each branch tip was noted, as was the addition of new branches.
19. Branch retractions may be preceded by synapse loss, as has been shown in the neuromuscular junction [R. J. Balice-Gordon and J. W. Lichtman, *Nature* **372**, 519 (1994); H. Colman, J. Nabekura, J. W. Lichtman, *Science* **275**, 356 (1997)]. Therefore, increasing CaMKII activity may decrease retractions by decreasing synapse loss. This is consistent with our electrophysiological experiments in which CaMKII increased the AMPA/NMDA ratio at retinotectal synapses (*10*). The cellular control of branch addition is not clear. CaMKII phosphorylates cytoskeletal proteins [H. Schulman, *Curr. Opin. Cell. Biol.* **5**, 247 (1993); J. M. Litersky *et al.*, *Biochem. J.* **316**, 655 (1996)], which may modify process extension or the initiation of new branches. It is also possible that the effect of CaMKII on decreasing branch initiations could be secondary to its effect of synaptic efficacy, as we suggested for branch retractions.
20. Because a single manipulation (expression of CaMKII) evoked three types of responses of tectal neurons at different stages of development, a potential reason for the diversity of responses to elevated Ca^{2+} or changes in CaMKII activity reported in the literature may be the spatial and temporal specificity of both the source and magnitude of Ca^{2+} influx, as well as the developmental regulation of Ca^{2+}-sensitive pathways that mediate neurite extension, retraction, or stabilization.
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26. One paradoxical observation is that CaMKII-expressing neurons and their presynaptic retinal axons both have a simpler morphologies, yet synaptic communication between pairs of retinal axons and tectal neurons is stronger than in control animals. One conclusion from these experiments is that robust synaptic physiology is not necessarily correlated with complex neuronal morphology. Other recent experiments have also found that synaptic efficacy can be divorced from morphological complexity [T. Tsujimoto, M. Umemiya, M. Kuno, *J. Neurosci.* **10**, 2059 (1990); Y. Zhong and J. Shanley, *ibid.* **15**, 6679 (1995); G. W. Davis, C. M. Schuster, C. S. Goodman, *Neuron* **17**, 669 (1996); B. A. Stewart, C. M. Schuster, C. S. Goodman, H. L. Atwood, *J. Neurosci.* **16**, 3877 (1996); C. M. Schuster, G. W. Davis, R. D. Fetter, C. S. Goodman, *Neuron* **17**, 655 (1996)].
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