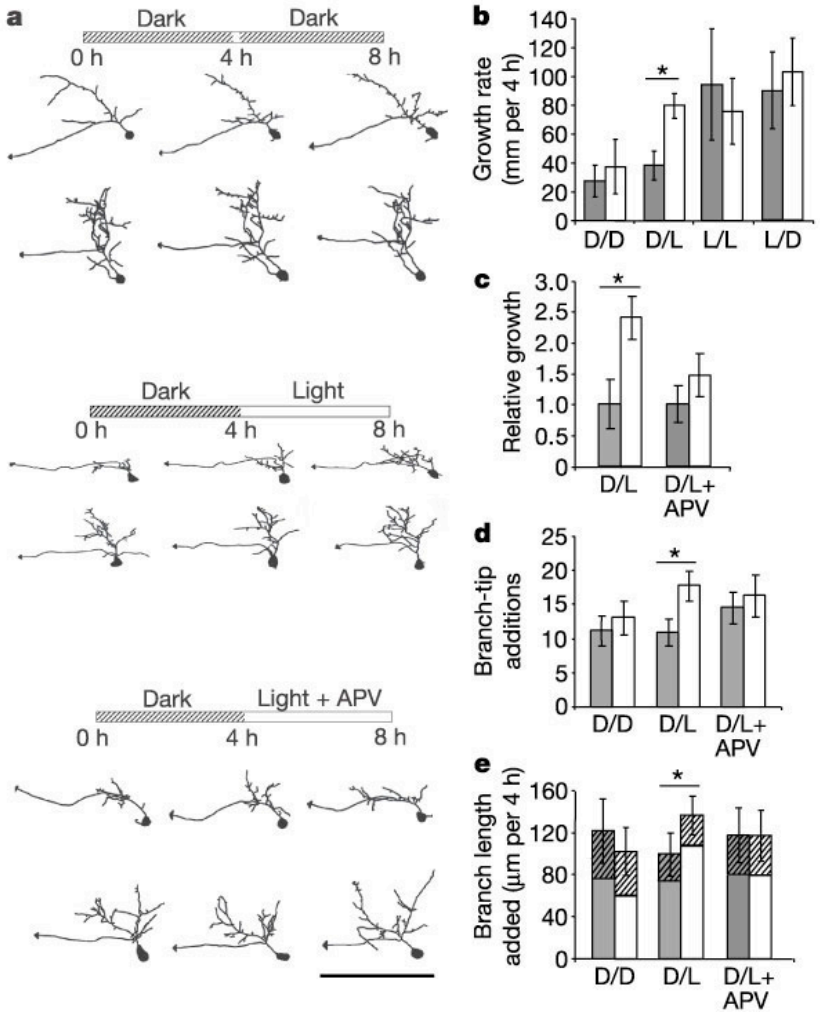
Bio 333 Spring 2012 Data Analysis Skills Test (1 of 3) Identifier: __ __ __ __

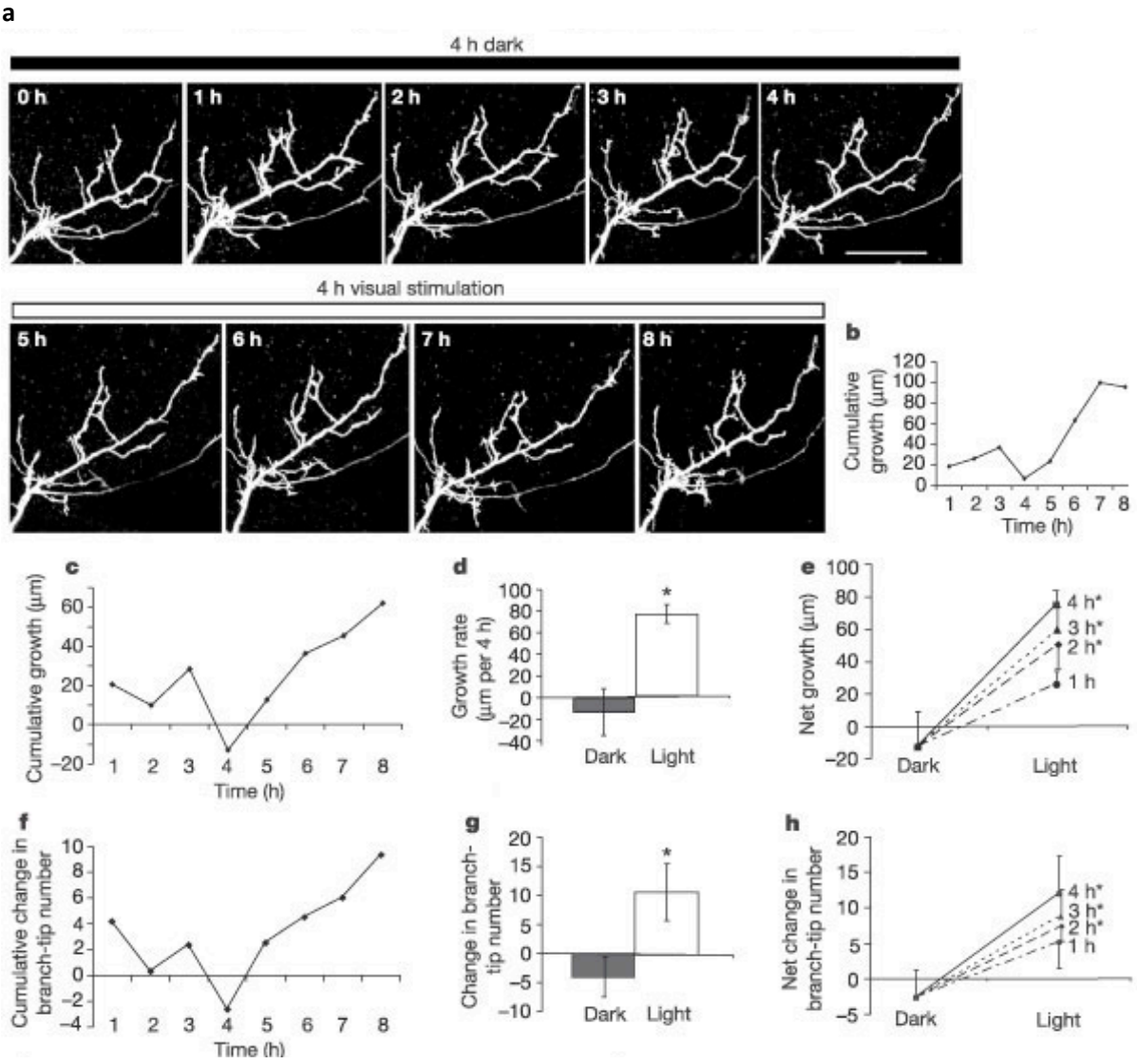
(Correct answers shown in bold)



(**a**) Sample drawings of neurons imaged at 4-hour intervals. Animals were placed in the dark for 4 h [dark] or exposed to light for 4 h [light] in the presence or absence of APV, a glutamate receptor inhibitor. Arrowheads identify axons. Scale bar, 100 µm. (**b**) Quantification of dendrite growth rates during 4 h in the dark [D] or with light stimulus [L]. (**c**) Dendrite growth rates normalized to growth rate in the 0–4 h period in the dark [D]. (**d**) Quantification of branch-tip additions. (**e**) Contribution of new branches [solid region] or branch extension [hatched region] to increased branch length. Asterisk, $P < 0.05$.

What conclusions can you draw from these data? [Circle all that apply]

A. Dendrite growth requires a light stimulus.
B. Glutamate receptors are required for light-dependent dendrite growth.
C. Glutamate receptors do not participate in light-independent dendrite growth.
D. **Light-stimulated dendrites grow at least twice as fast as dendrites kept in the dark**.
E. Dendrites exposed to an 8-hour light stimulus exhibit a reduced growth rate after 4 hours.
F. Greater than 50% of dendrite growth may be attributed to the extension of existing branches.
G. In a given four-hour period no dendrites exceeded 120 µm in length.
H. Light-stimulated dendrites maintain their growth rate following termination of the stimulus.



(**a**) Images of a neuron collected once an hour for 8 h. Scale bar, 50 µm. (**b**) Cumulative change in branch length for the neuron pictured. Average cumulative change in (**c**) branch length and (**f**) branch-tip number. *n* = 6. Average change in (**d**) branch length and (**g**) branch-tip number over the 4-h period in the dark [dark] or with visual stimulus [light]. Cumulative change in (**e**) branch length and **(h**) branch-tip number with each hour of visual stimulus [light] compared to 4 h without visual stimulus [dark]. Asterisk, $P < 0.05$.

What conclusions can you draw from these data? [Circle all that apply]

A. **Dendrites exhibited a net decrease in growth rate in the absence of light.**
B. Data presented in d and g were derived from the analysis of a single neuron.
C. Dendrites decreased in length and branch-tip number at hour 2 in the dark.
D. **More new branch tips are added in the first hour of light stimulation than in the third hour of light stimulation.**
E. Light stimulation prevents a neuron form losing any dendrite branches.
F. During light stimulation, most newly added branches exceed 50 µm in length.

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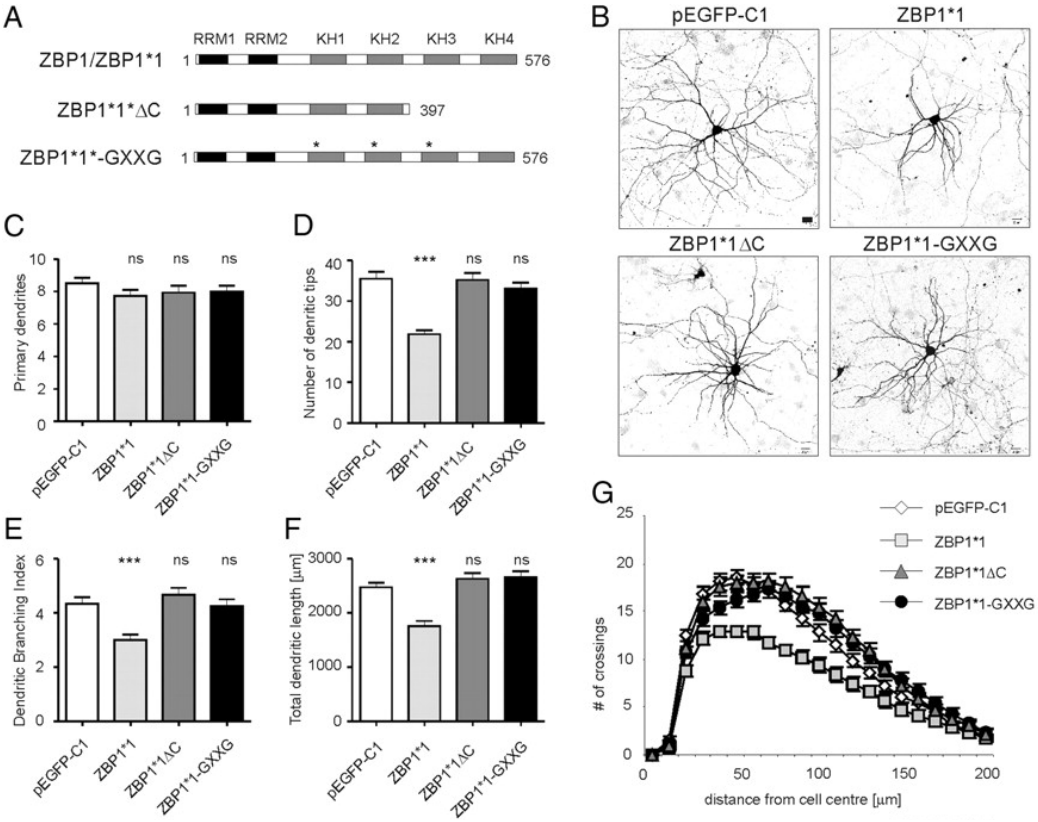


(**A**) Representations of full-length and mutant proteins overexpressed in these experiments. (**B**) Neurons transfected on DIV7 with pEGFP-C1 (empty vector), EGFP-ZBP1*1 (full length ZBP1), EGFP-ZBP1*1ΔC (truncated form), or EGFP-ZBP1*1-GXXG (contains three point mutations). (**C**) Number of primary dendrites, (**D**) total number of dendritic tips, (**E**) dendritic branching index, (**F**) total length of dendrites, and (**G**) Sholl analysis of neurons overexpressing the proteins described above. Scale bar, 20 μm.

(Note: Sholl analysis is done by drawing concentric circles over a neuron and counting the number of dendrites that cross each circle. More crossings = greater dendritic arbor complexity)

What conclusions can you draw from these data? (Circle all that apply)

A. Overexpressing full-length ZBP1 has no effect on total dendritic length.

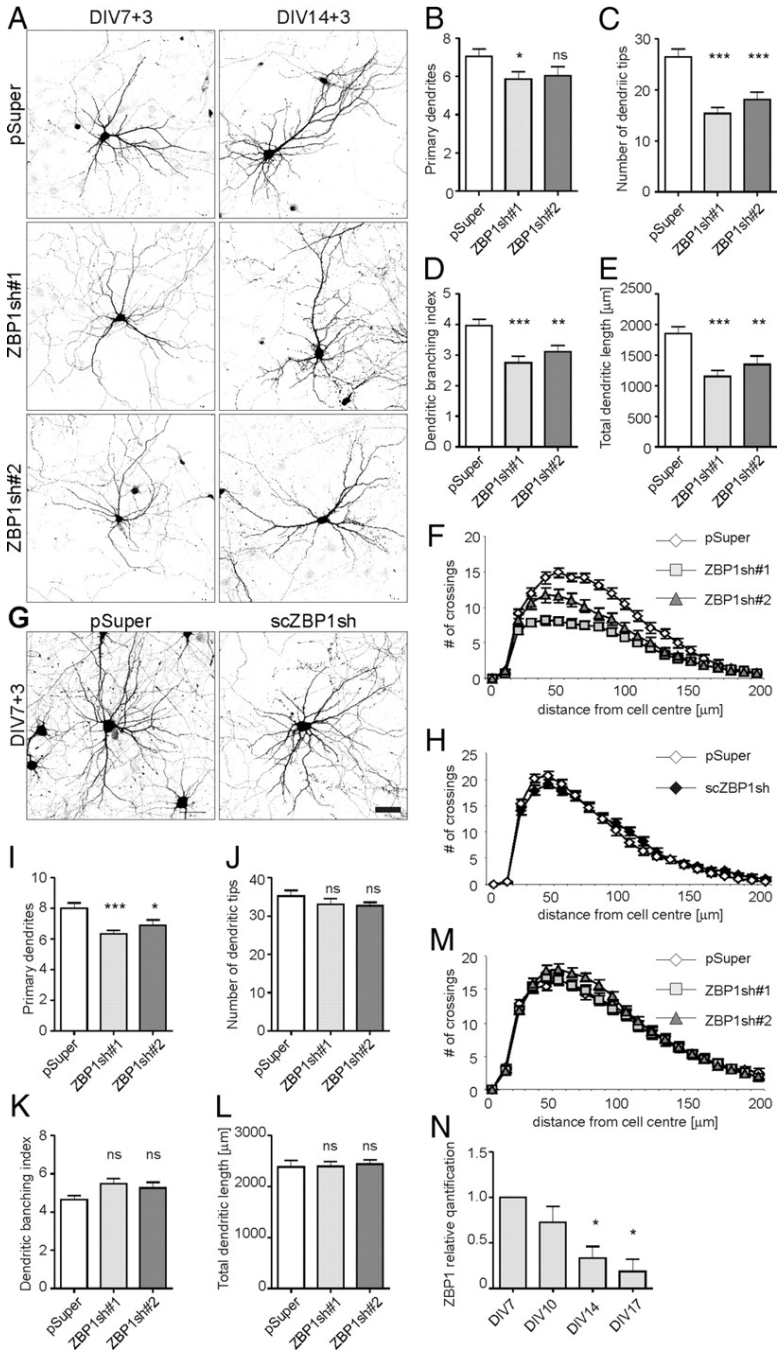
B. **Overexpressing full-length ZBP1 has no effect on primary dendrites.**

C. Truncating ZBP1 inhibits proper dendritic branching in neurons.

D. Overexpressing EGFP has a negative effect on dendritic arbor complexity.

E. **Point mutations in the KH domains reverse the effects of ZBP1 overexpression.**

F. Overexpressing ZBP1 reduces the number of dendritic tips by approximately 15%.



(**A**) Neurons transfected at 7 days in vitro (DIV7) or 14 days in vitro (DIV14) with empty vector (pSuper) or vectors containing shRNA to knock down ZBP1 (ZBP1sh#1 or ZBP1sh#2) and analyzed three days later. (**B**) Number of primary dendrites, (**C**) Number of dendritic tips, (**D**) dendritic branching index, (**E**) total dendritic length, and (**F**) Sholl analysis of neurons transfected on DIV7.

(**G**) Neurons transfected on DIV7 with pSuper or scrambled scZBP1sh. (**H**) Complexity of dendritic arbors of neurons transfected with pSuper or scZBP1sh.

(**I**) Mean number of primary dendrites, (**J**) mean total number of dendritic tips, (**K**) dendritic branching index, (**L**) total dendritic length, and (**M**) Sholl analysis of neurons transfected on DIV14.

(**N**) RT-PCR of ZBP1 expression in neurons. RNA was isolated from neurons on DIV7, DIV10, DIV14, and DIV17, and quantification was normalized to DIV7. Error bars = SEM. Scale bar, 50 µm.

What conclusions can you draw from these data? (Circle all that apply)

A. **ZBP1 is required for the addition of new dendritic tips at DIV7.**

B. ZBP1 is required for the addition of new dendritic tips at DIV14.

C. **Most primary dendrites are added to the cell body in the first 7 days of culture**.

D. Dendritic arbor complexity decreases in the presence of scrambled ZBP1 shRNA.

E. ZBP1 plays a critical role in increasing dendritic length at DIV14.

F. **ZBP1 expression decreases between DIV7 and DIV14.**

G. ZBP1 is not expressed prior to DIV7.

H. Knocking down ZBP1 increases dendritic branching at DIV14.

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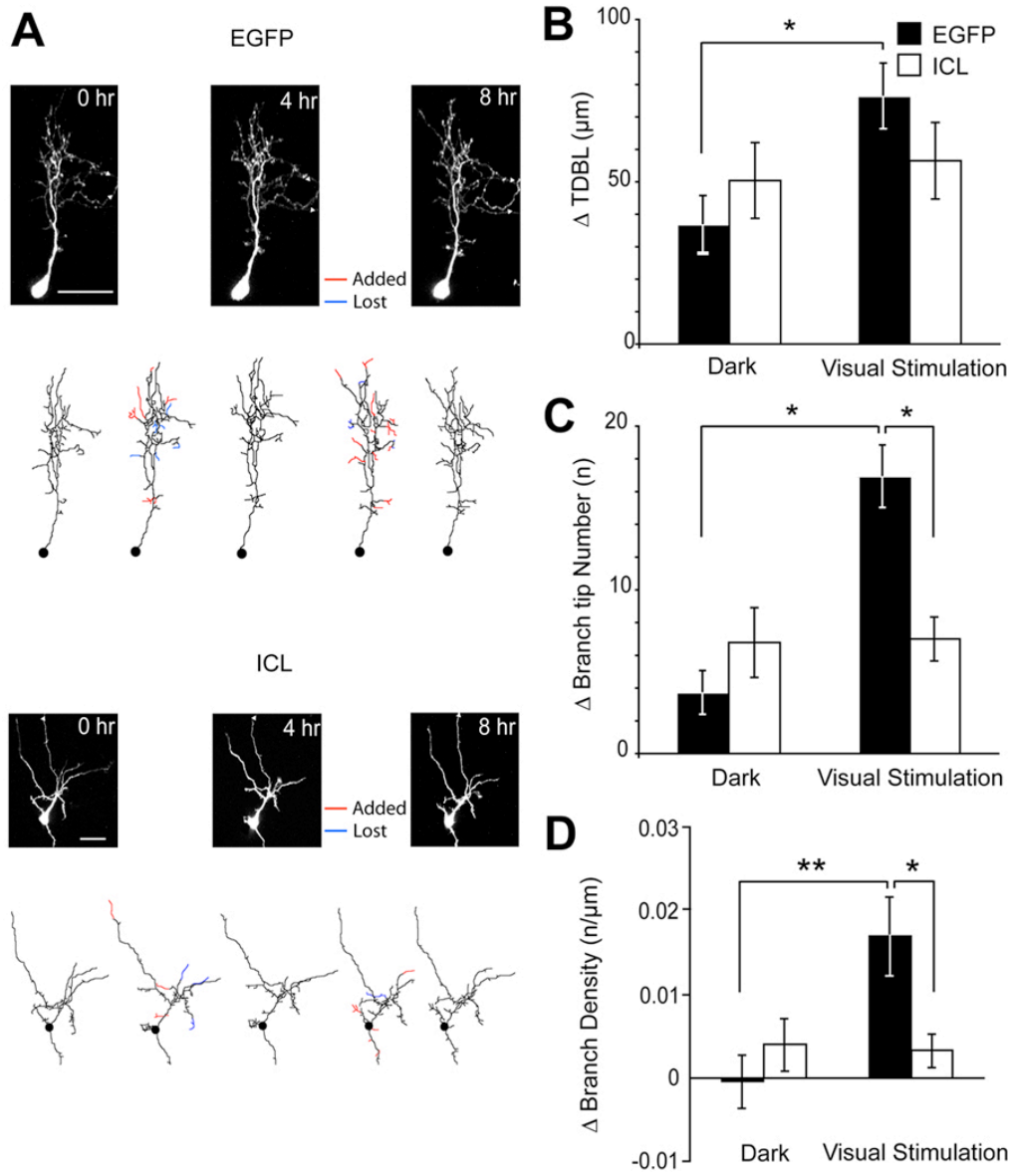


(**A**) Images and drawings of neurons expressing EGFP alone (top) or EGFP-ICL (bottom), which blocks GABA receptor signaling, at 0 hours, 4 hours and 8 hours. Color-coded drawings of the neurons are shown between 0 hr and 4 hr and between 4 hr and 8 hr. Newly added branches are shown in red and lost branches are shown in blue. Scale bar, 20μm. Net change in (**B**) total dendritic branch length (TDBL), (**C**) branch tip number and (**D**) branch density over 8 hours in the dark or with visual stimulation. $^{**}P < 0.01$; $^{*}P < 0.05$

What conclusion(s) can you draw from these data? (Circle all that apply)

A. **EGFP neurons exhibit an increase in branch tips in response to visual stimulation.**

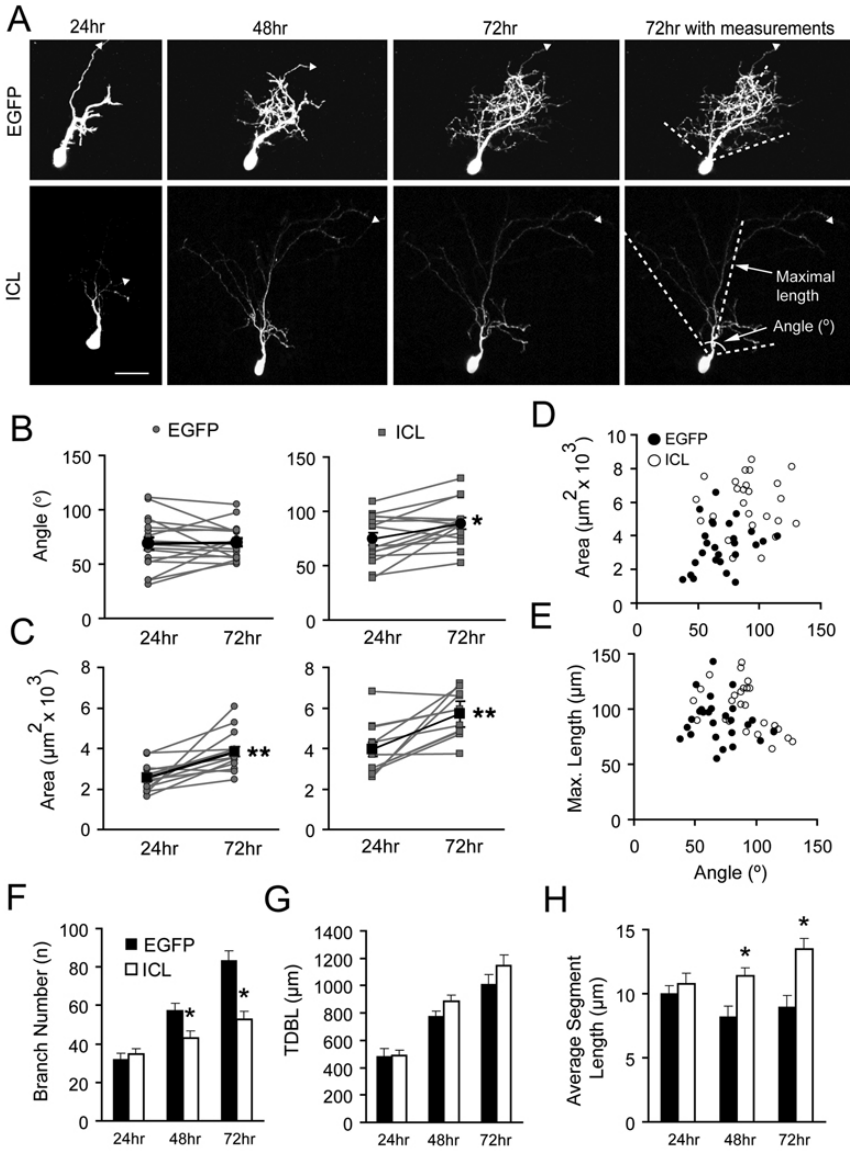
B. Neurons do not add new dendrite branch tips when GABA receptor signaling is blocked.

C. EGFP neurons exhibit a net increase in branch density with or without visual stimulation.

D. Neurons without GABA receptor signaling exhibit a significantly greater increase in branch length in the dark.

E. **In ICL-expressing neurons, visual stimulation does not trigger a significant increase in branch density.**

F. In EGFP neurons, most individual, newly added branches are greater than 20 μm in length.



(**A**) Representative neurons expressing EGFP alone (top) or EGFP-ICL (bottom), which blocks GABA receptor signaling, at 24 hr, 48 hr and 72 hr. Measurement of dendrite angle, maximal length and area are shown on 72 hr images of EGFP- and EGFP-ICL neurons. Scale bar, 20μm. (**B**) Dendritic angles and (**C**) dendritic areas for EGFP neurons (left) and EGFP-ICL neurons (right). (**D**) Plots of dendritic tree angle versus area and (**E**) dendritic tree angle versus maximal length at 72 hrs. (**F**) Branch numbers, (**G**) total dendritic branch length (TDBL), and (**H**) average segment length for EGFP- and EGFP-ICL neurons from 24 to 72 hours. **P*<0.05, ***P*<0.01.

What conclusion(s) can you draw from these data? (Circle all that apply)

A. On average, EGFP neurons exhibit a significant increase in dendritic angles between 24 and 72 hours.

B. Each ICL-expressing neuron exhibited an increase in dendritic area between 24 and 72 hours.

C**. ICL-expressing neurons with a wider dendritic angle tend to have a shorter maximal length.**

D. **Neurons without GABA receptor signaling add significantly fewer branches at 48 and 72 hours.**

E. **The average total dendritic branch length of EGFP neurons is about doubled between 24 and 72 hours.**

F. Neurons without GABA receptor signaling exhibit a significantly greater segment length at 24 hours.

G. GABA receptor signaling is required for neurons to increase their dendritic area between 24 and 72 hours.

H. Disrupting GABA receptor signaling affects the size of the neuronal cell body, or soma.