Developmental regulation of Medium Spiny Neuron dendritic arborization

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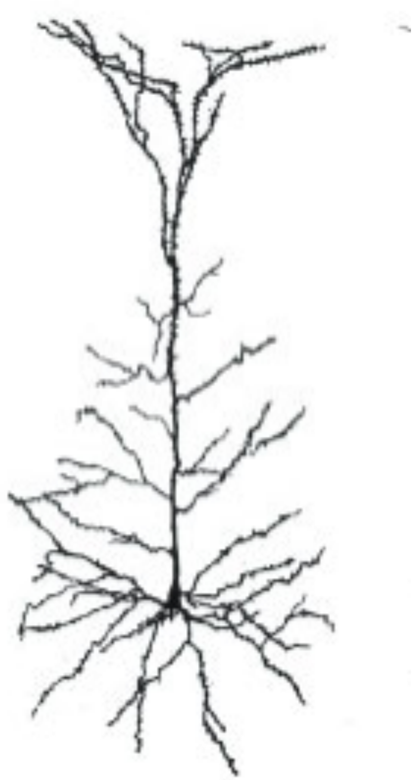
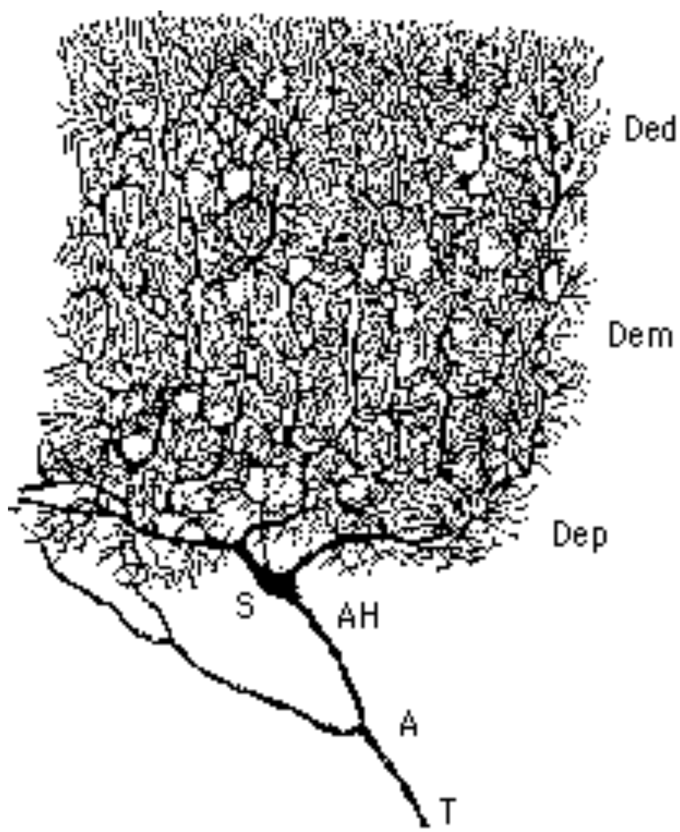
Department of Neuroscience

Diversity in dendritic arbors

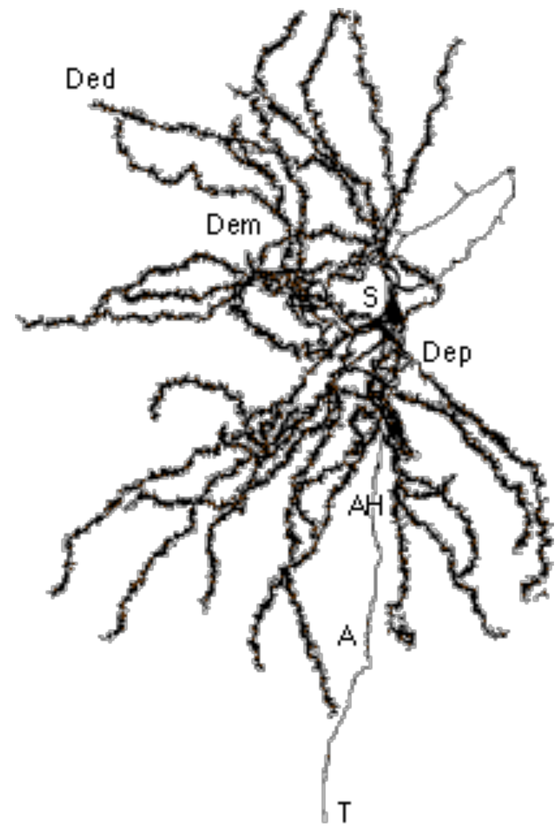
Pyramidal



Purkinje



Medium Spiny



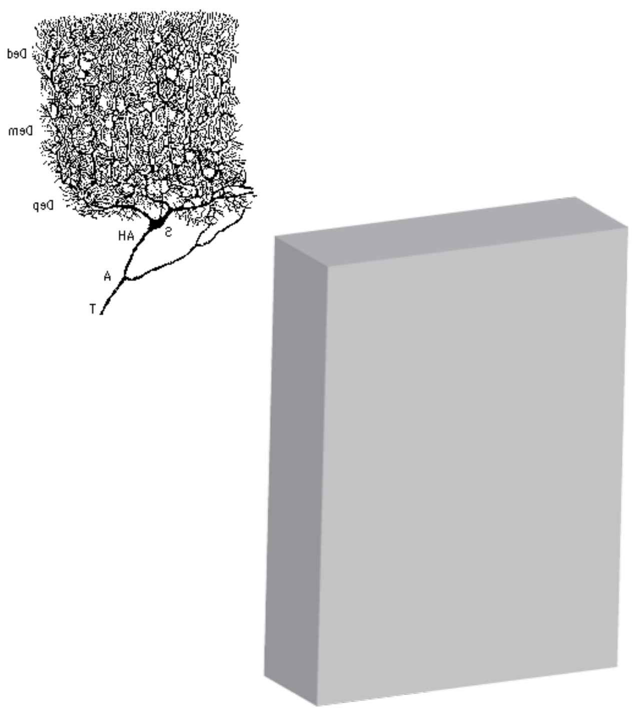
http://youtu.be/_tQPCa6wX84

http://youtu.be/Q4uh2r1djWw

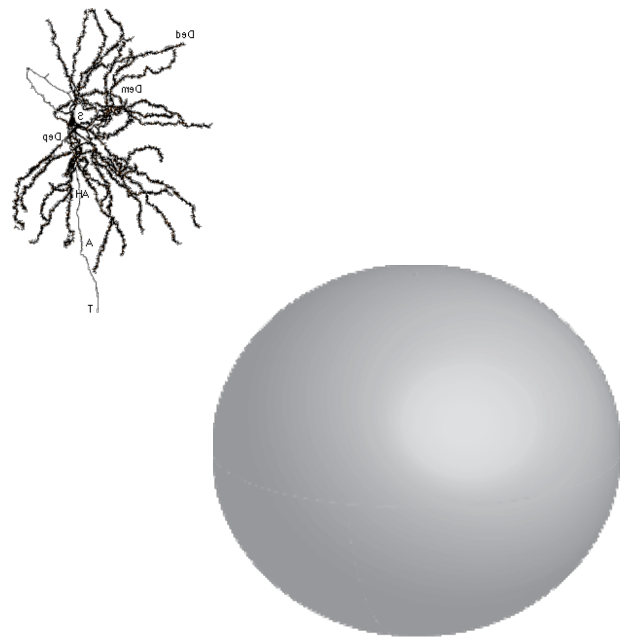
Diversity in dendritic arbors

Pyramidal

Purkinje



Medium Spiny

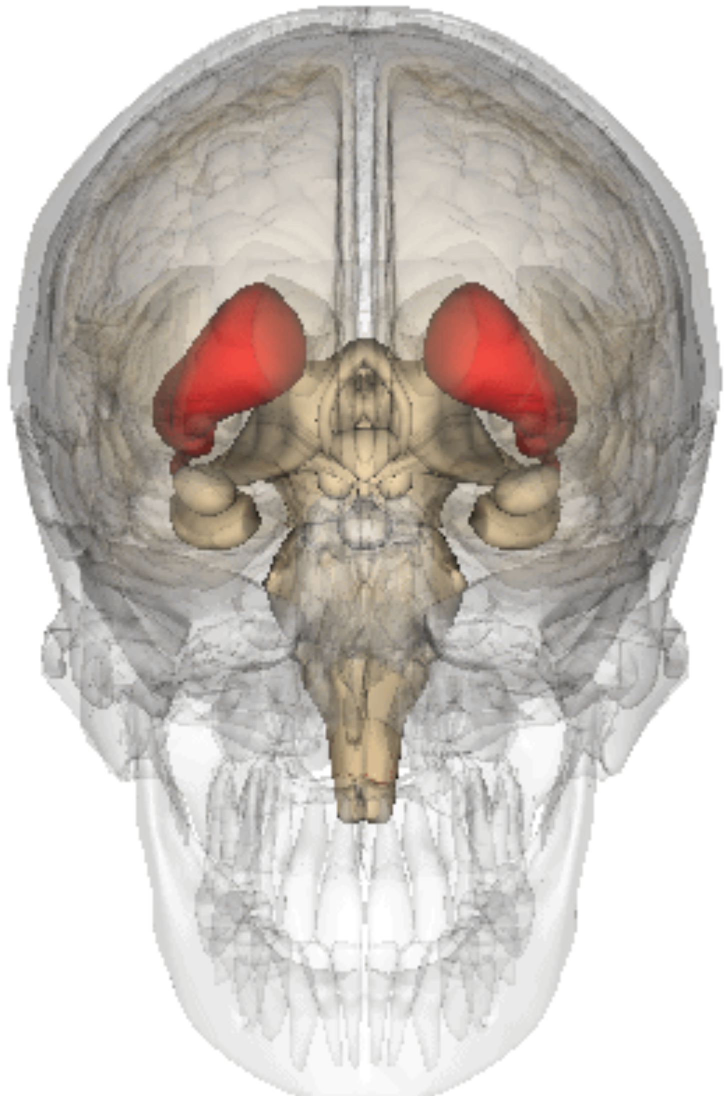


- Why do different types of neurons have such different dendritic arbors???

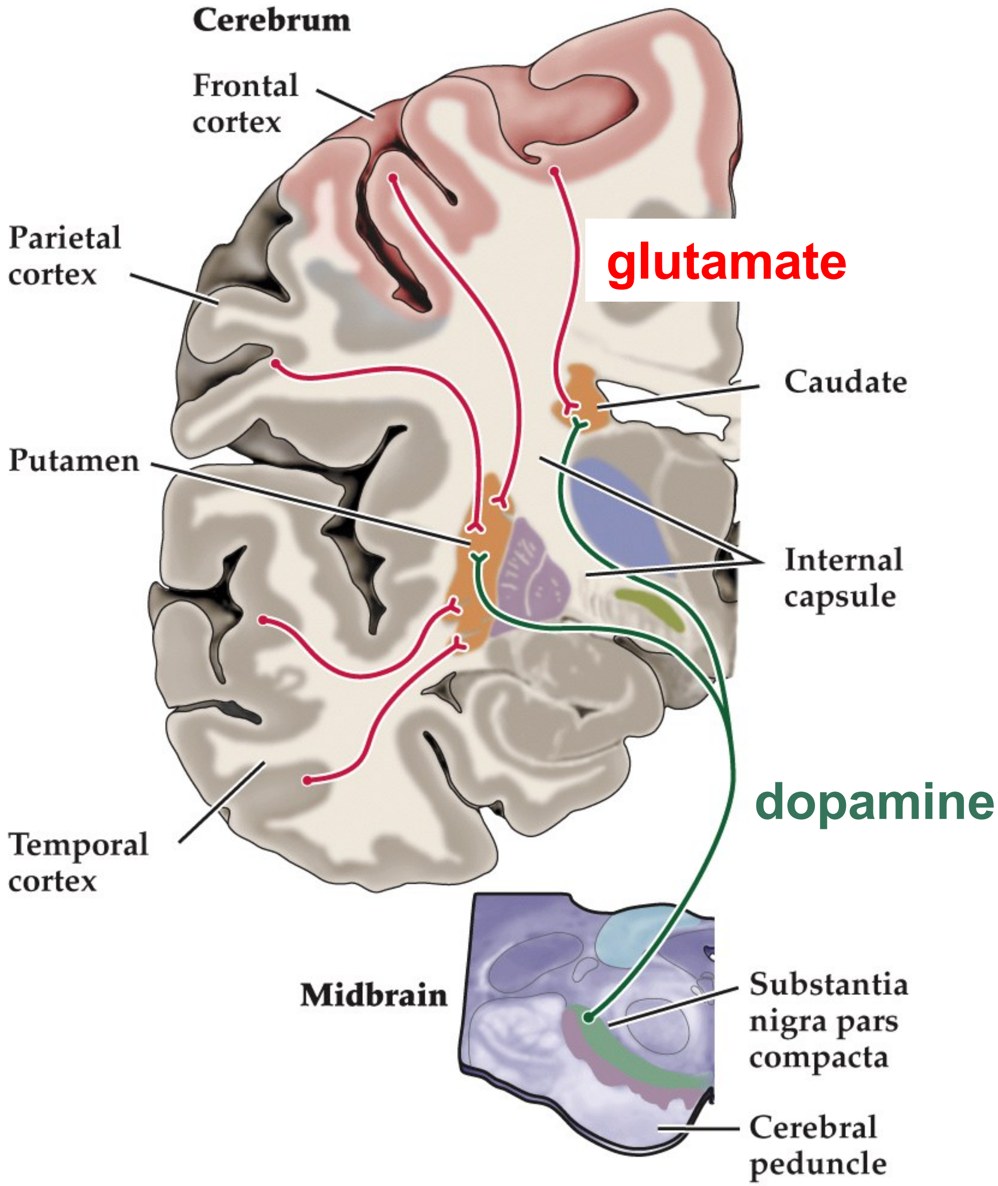
- How does a neuron “know” what it’s shape should be?

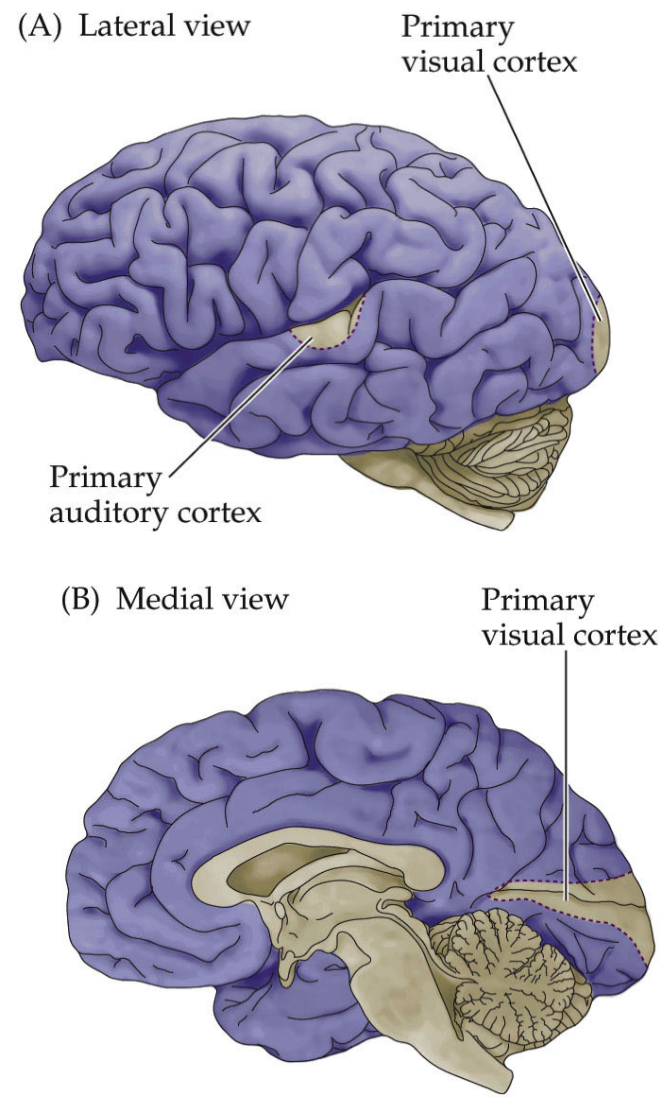
- How is this affected by the environment?

The Striatum



The striatum (caudate + putamen) receives input from the SNc and almost all cortical areas



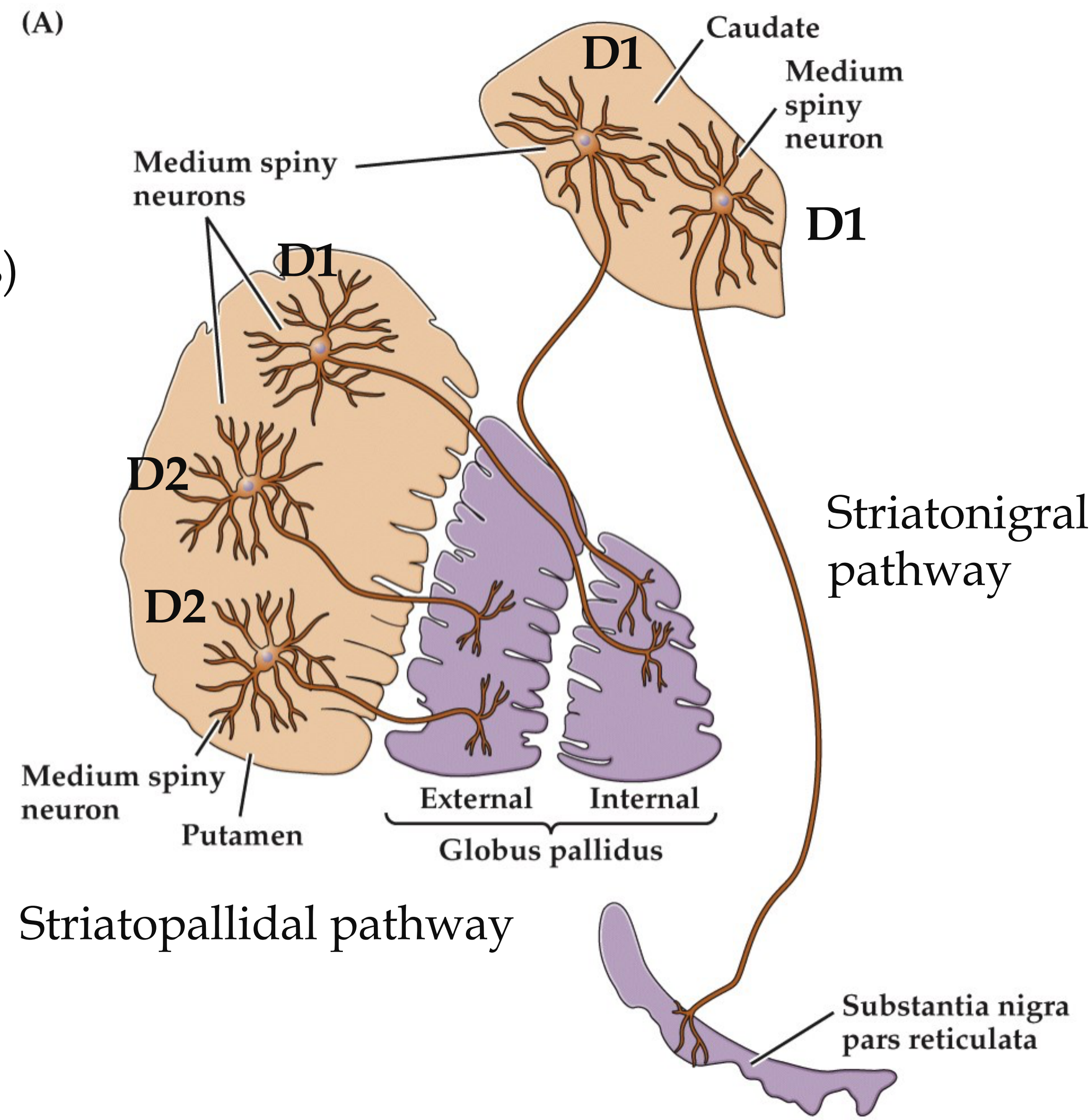


Nuclei of the basal ganglia

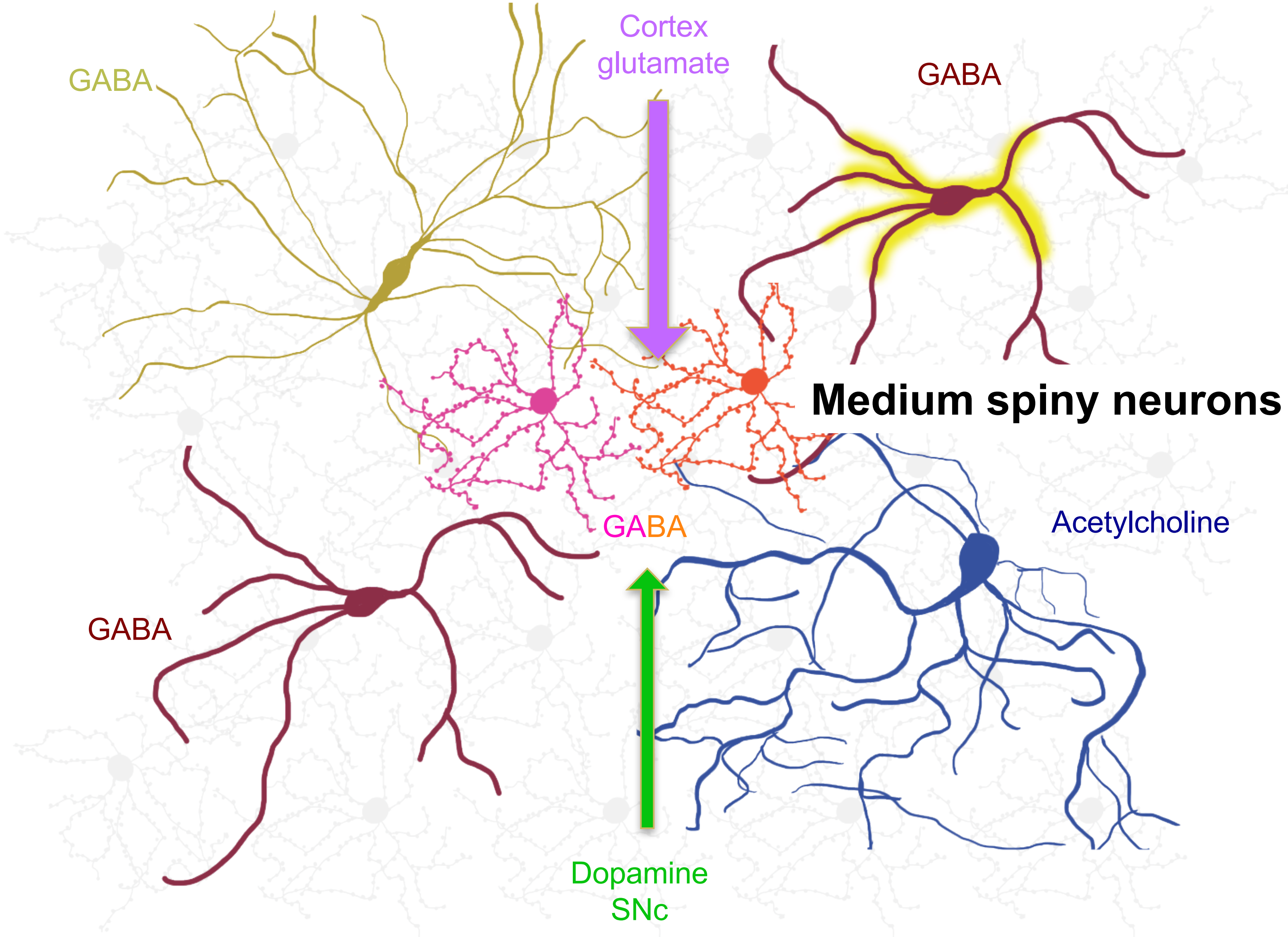
Caudate & Putamen

Medium spiny neurons (MSNs)
- GABAergic
- dopamine receptors
  - D1 coupled to Gs
  - D2 coupled to Gi
- Low basal activity

Globus pallidus

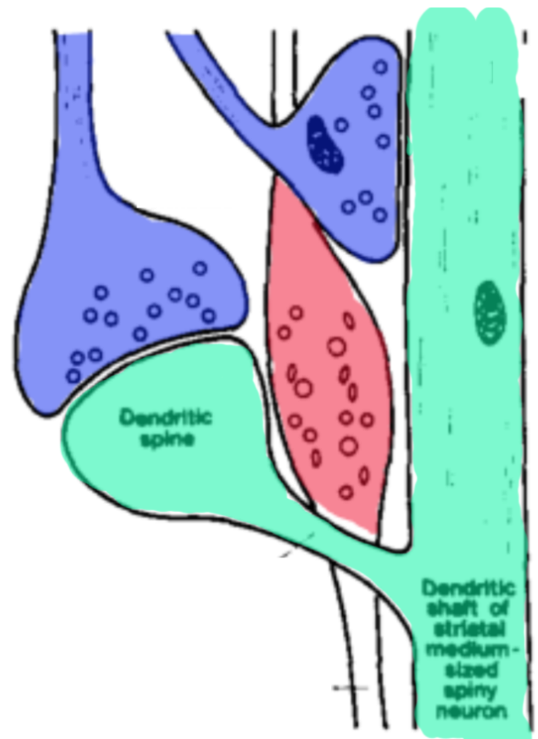


Cell types in the Striatum



Medium spiny neurons (MSNs)

- >90% of neurons in striatum are MSNs
- Dendritic spine heads are the major site of excitatory synapses
- Dendritic spine necks are the site of dopamine synapses
- Integrate glutamate and dopamine inputs
- Release GABA (inhibitory signal)
- Modulate movement
- Play a major role in motivation and addiction
- One of the first cell types affected in Huntington's disease
- Aberrant function in Parkinson's Disease due to death of dopamine expressing neurons
- Many *in vivo* models for MSN plasticity



Kotter Prog Neurobiol 1994

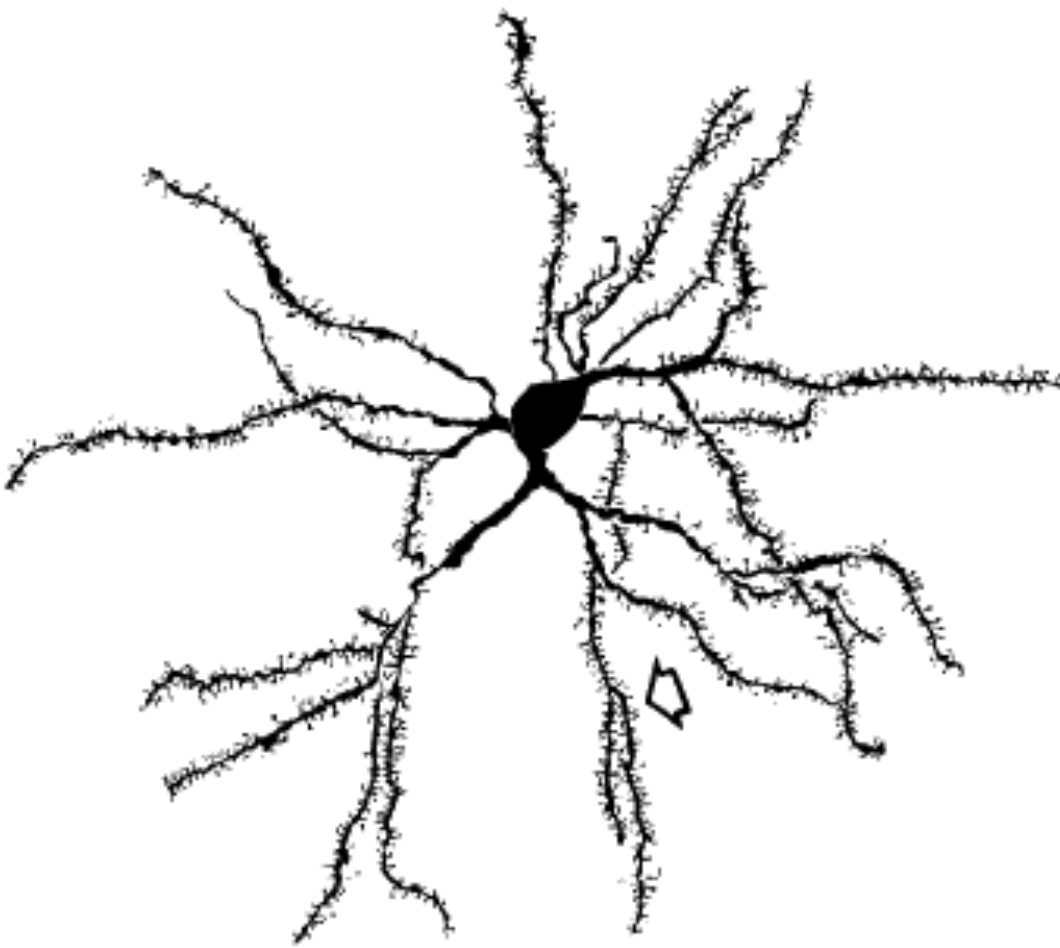
MSN
glutamate synapse
dopamine synapse

What are the molecular mechanisms regulating MSN development and plasticity?

→ need an *in vitro* model

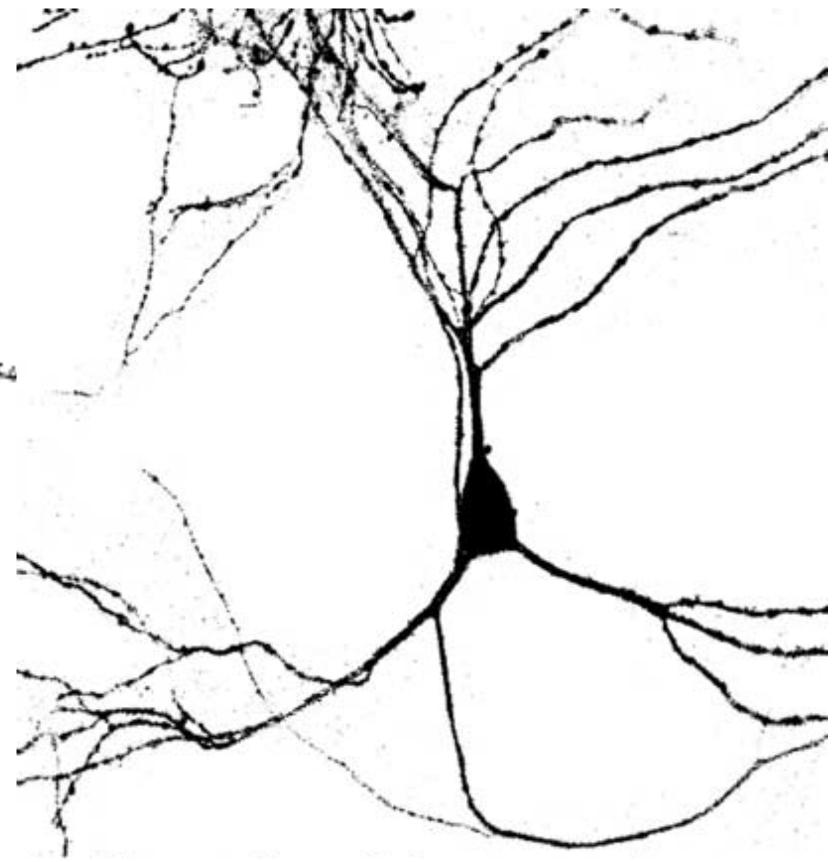
Medium Spiny Neurons

In Vivo



J Comp Neurology 1989

In Vitro



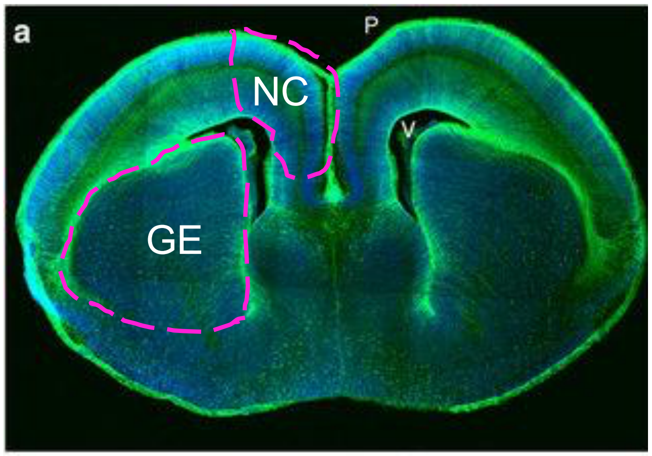
Segal et al. (2003) Eur. J. Neurosci 17: 2537

European Journal of Neuroscience, Vol. 17, pp. 2573–2585, 2003

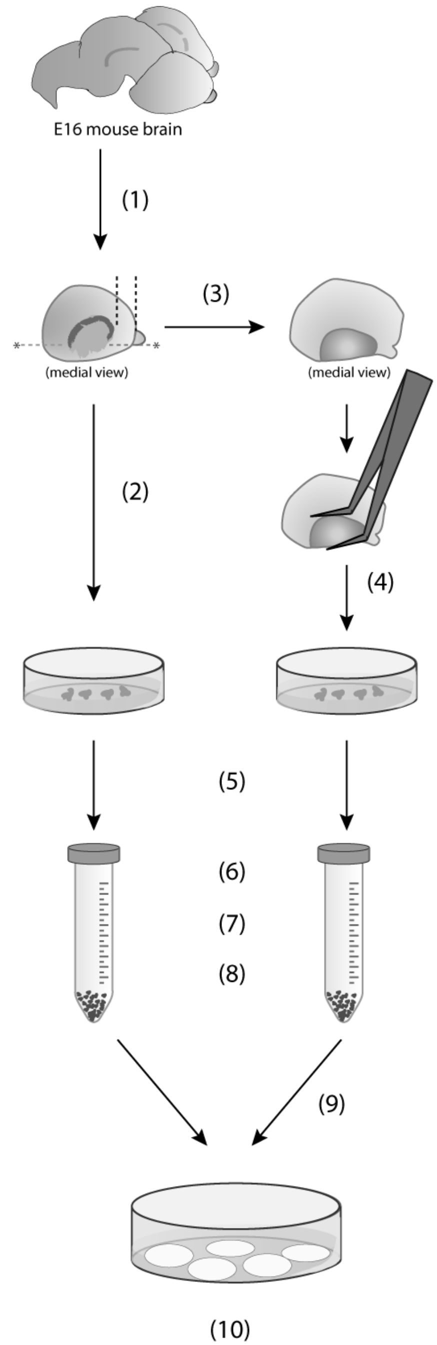
Formation of dendritic spines in cultured striatal neurons depends on excitatory afferent activity

Menahem Segal, Varda Greenberger and Eduard Korkotian
Department of Neurobiology, The Weizmann Institute, Rehovot 76100, Israel

Our co-culture method



- Start with E15 neocortex & ganglionic eminence
- Dissect
- Dissociate
- Co-plate 3 parts cortex:2 parts GE
- Goals:
    - in vivo-like morphology
    - reproducibility



(1) Dissect cerebral hemispheres & remove meninges

(2) Use angled forceps to snip cortex at rostral and caudal ends of the hippocampus (dashed gray lines with *), then cut region of prefrontal cortex (dashed black lines) & transfer to petri dish #1 containing CMF-HBSS

(3) Use angled forceps to flatten the cortex and expose the striatum

(4) Use angled forceps to "scoop out" the striatum & transfer to petri dish #2 containing CMF-HBSS

(5) Chop tissues with sterile razor blades, then transfer to 15 ml test tubes

(6) Digest with 0.25% tryspin-EDTA 30 min. 37˚C

(7) Centrifuge 5 min @ 1,000 rpm

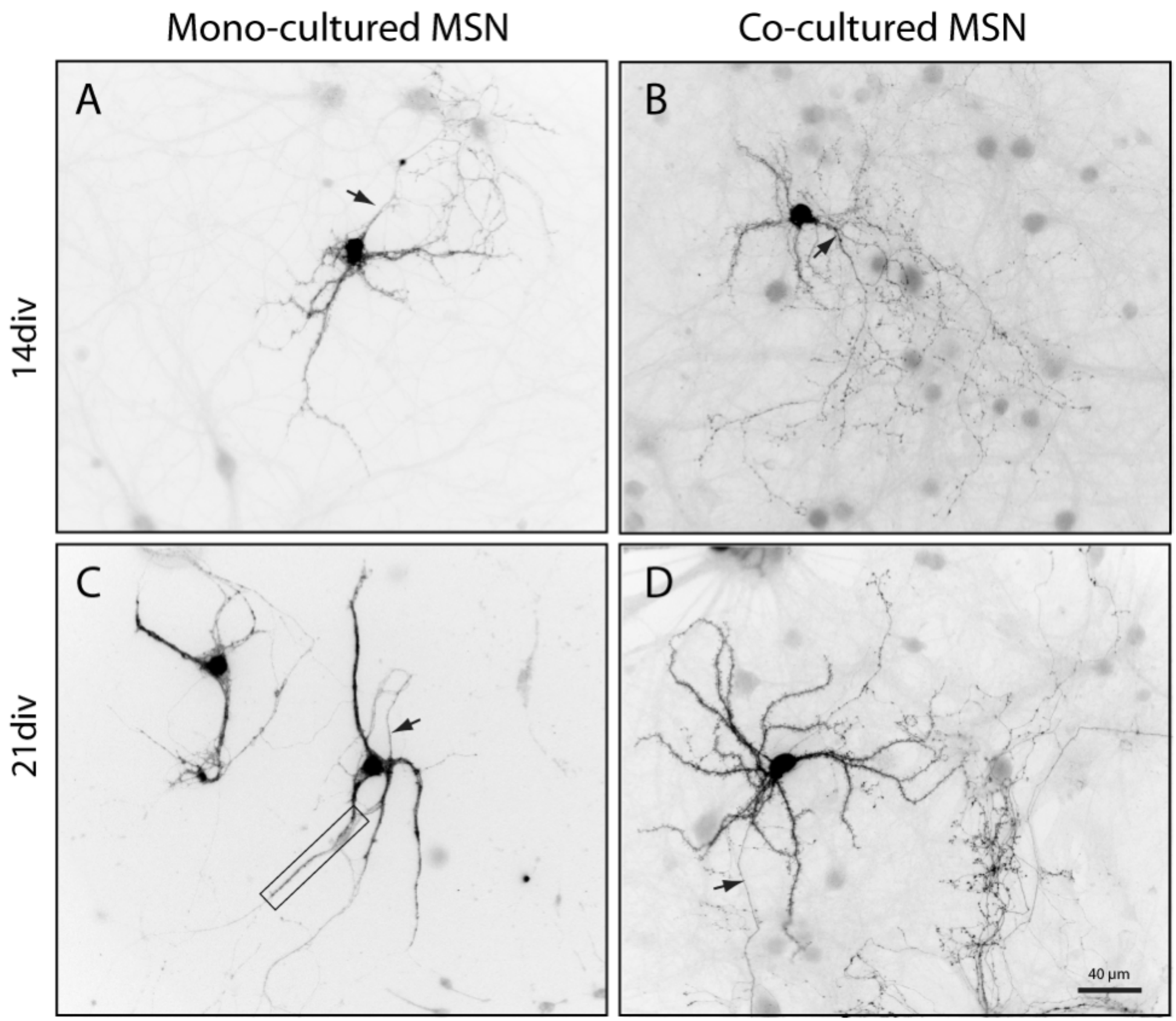
(8) Resuspend in neuronal plating media & dissociate with fire polished pipette

(9) Count cells & plate onto coverslips @ ~200 cells/mm^2 in a ratio of 3 parts cortex : 2 parts striatum

(10) After cells adhere (1-3 hr), change to glia-conditioned media (GCM). Feed weekly by changing 50% of media for fresh GCM.

Penrod et al. (2011) J. Neurosci. Methods

MSNs grown in co-culture have more complex morphologies



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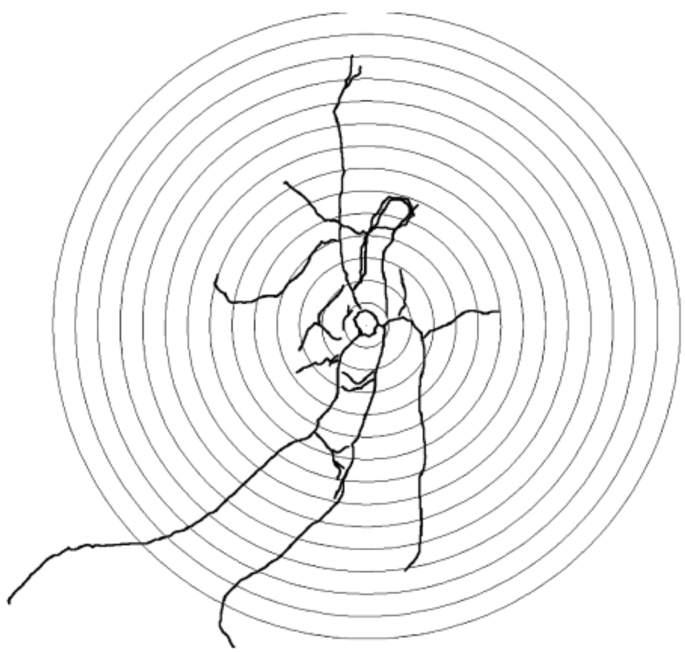
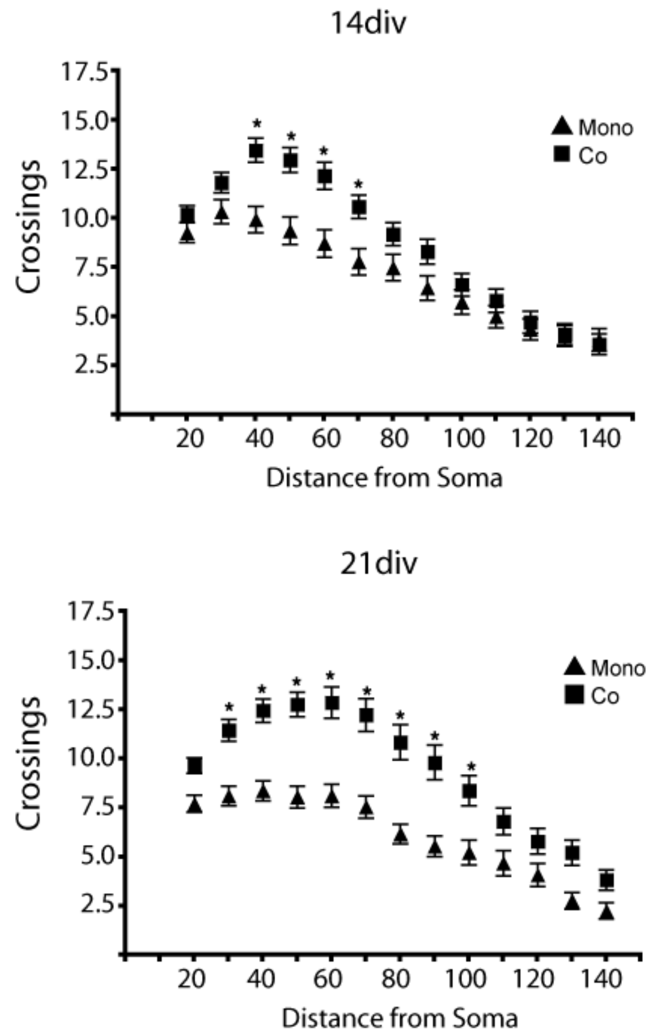
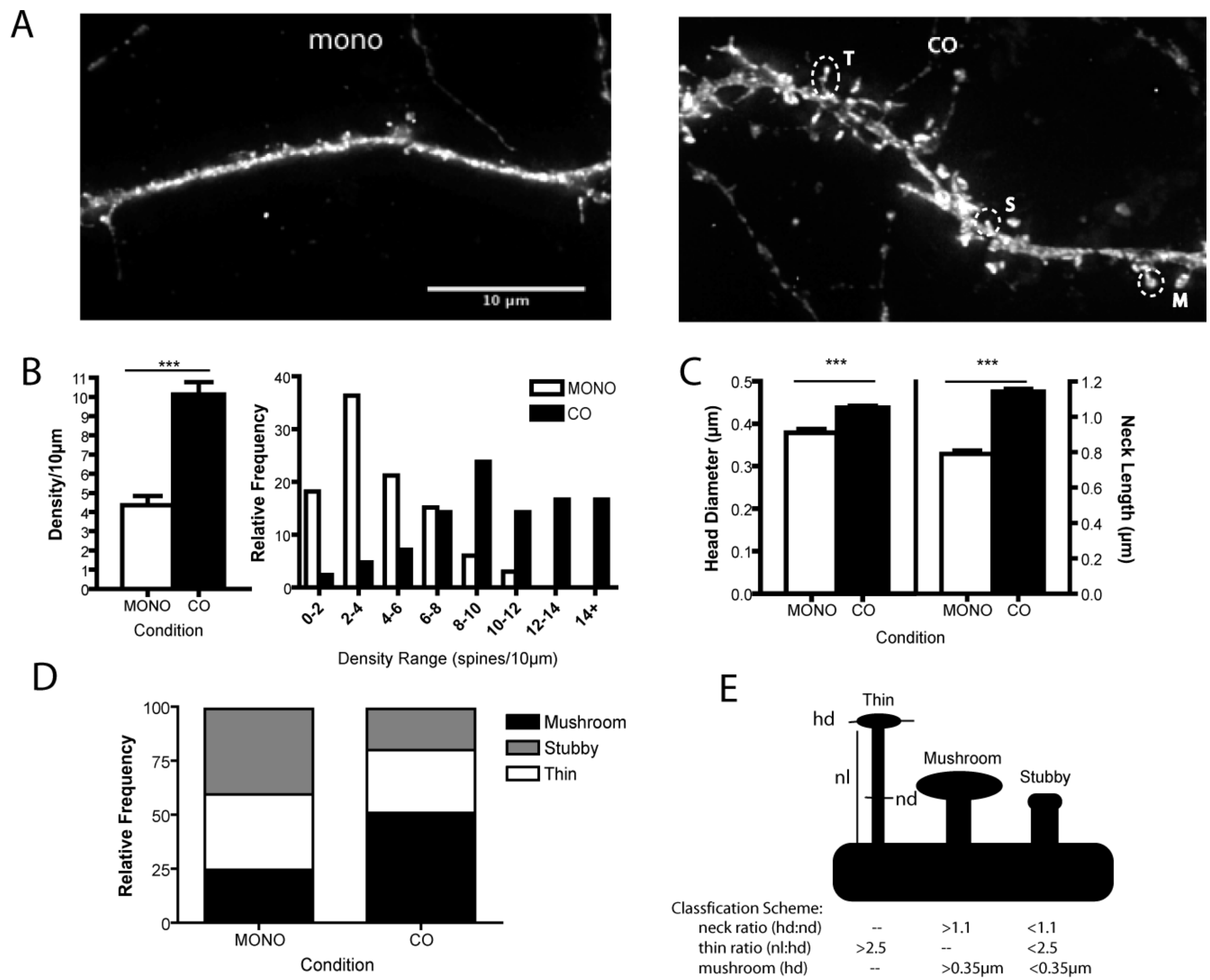


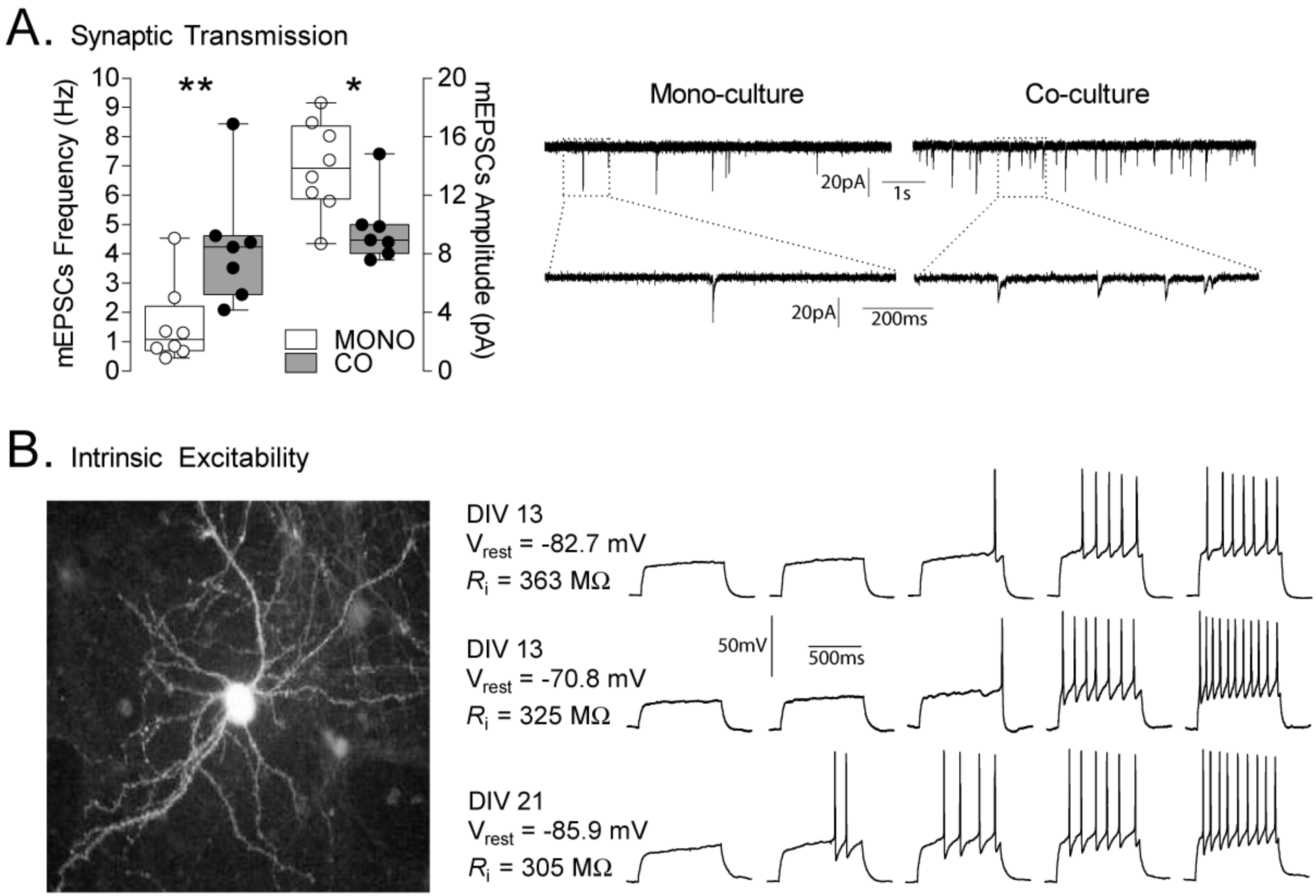
Sholl analysis



Co-culture yields MSNs with a high density of "mature" dendritic spines

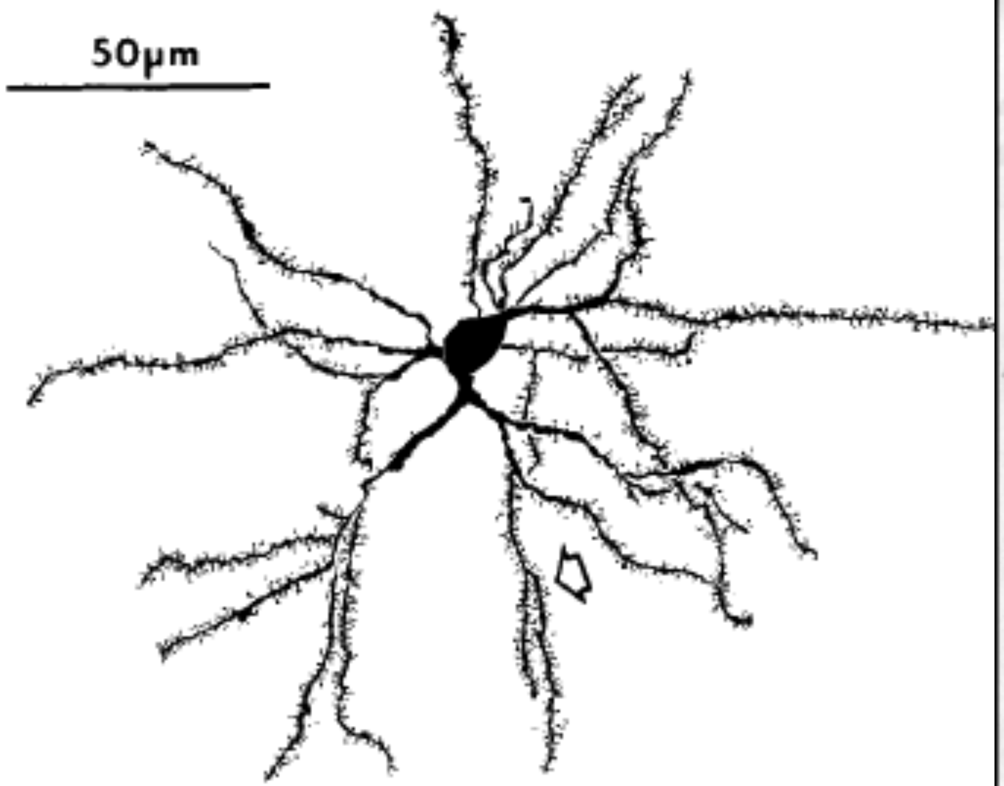


Electrophysiological properties of MSNs in co-culture

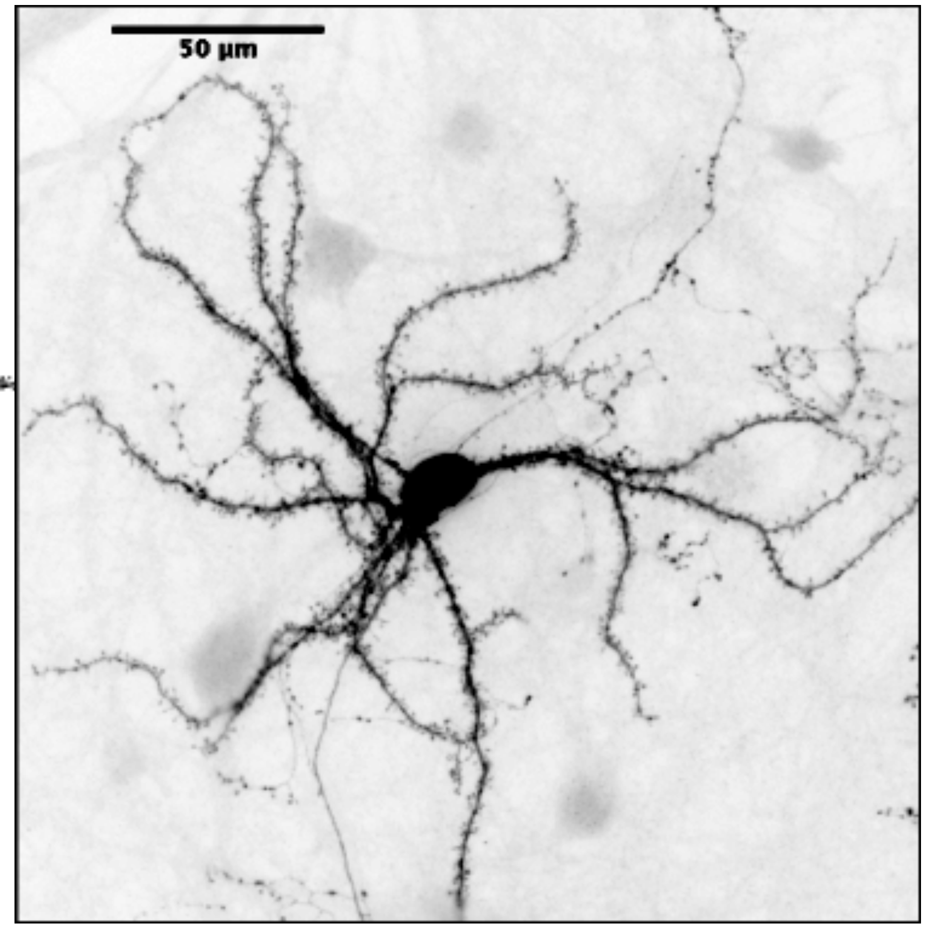


Medium Spiny Neurons

In Vivo

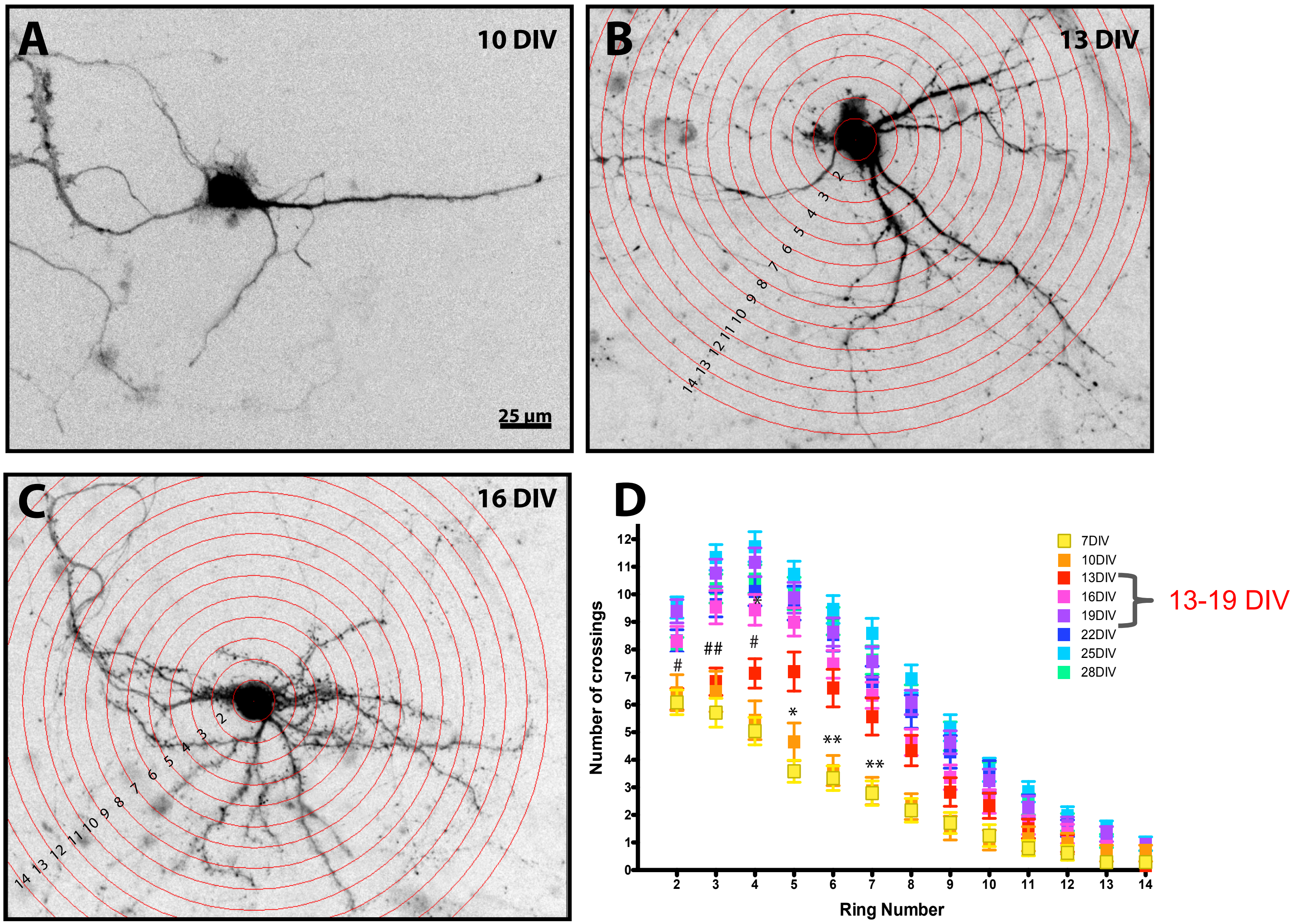


In Vitro



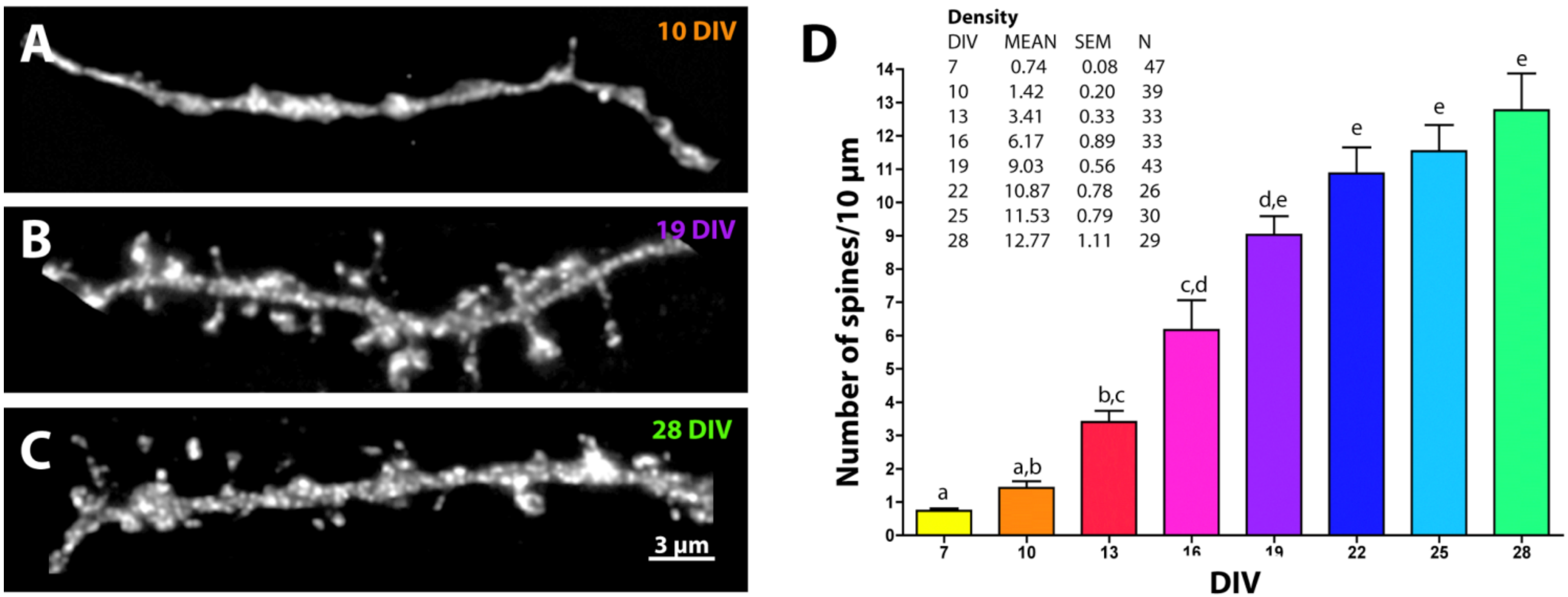
What next?

MSNs dendrite complexity increases during development



Penrod et al. 2015

MSNs dendritic spine density increases during development



DIV	MEAN	SEM	N
7	0.74	0.08	47
10	1.42	0.20	39
13	3.41	0.33	33
16	6.17	0.89	33
19	9.03	0.56	43
22	10.87	0.78	26
25	11.53	0.79	30
28	12.77	1.11	29

Penrod et al. 2015

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- Integrate glutamate and dopamine in same spine
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- Play a major role in motivation and addiction
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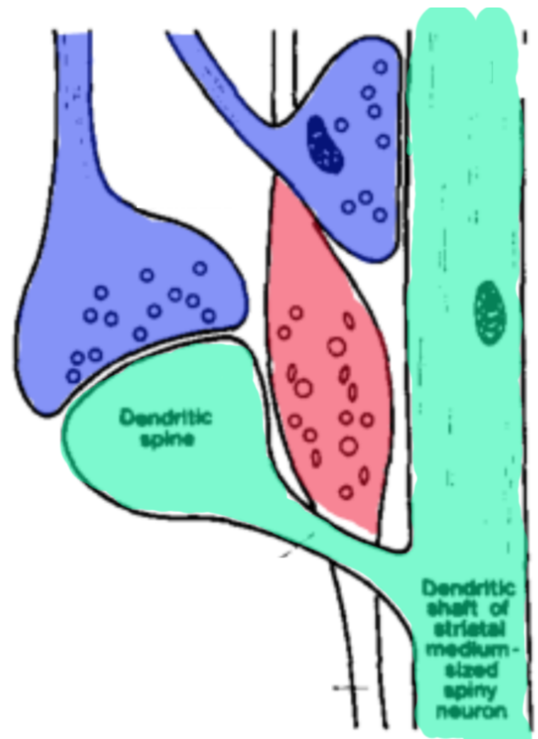


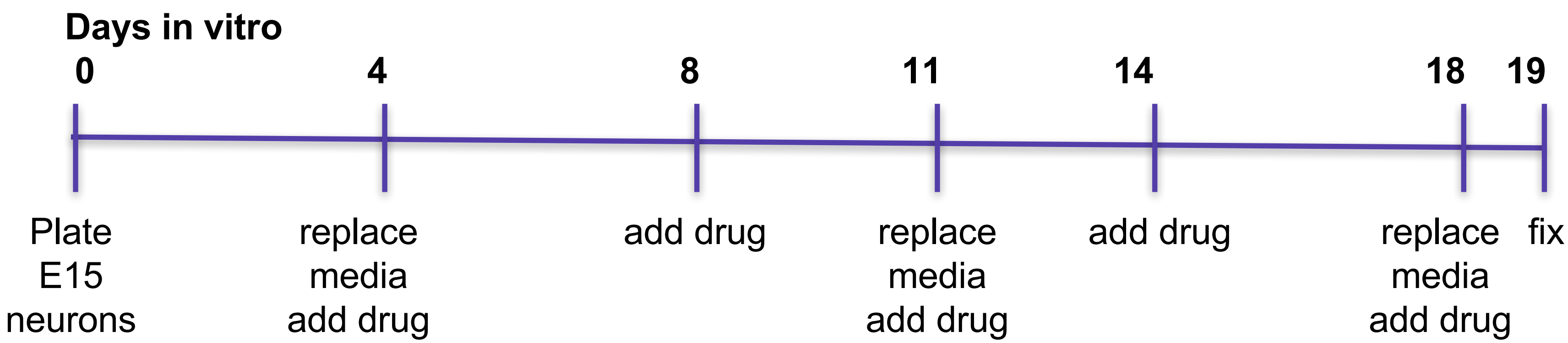
Kotter Prog Neurobiol 1994

MSN
glutamate synapse
dopamine synapse

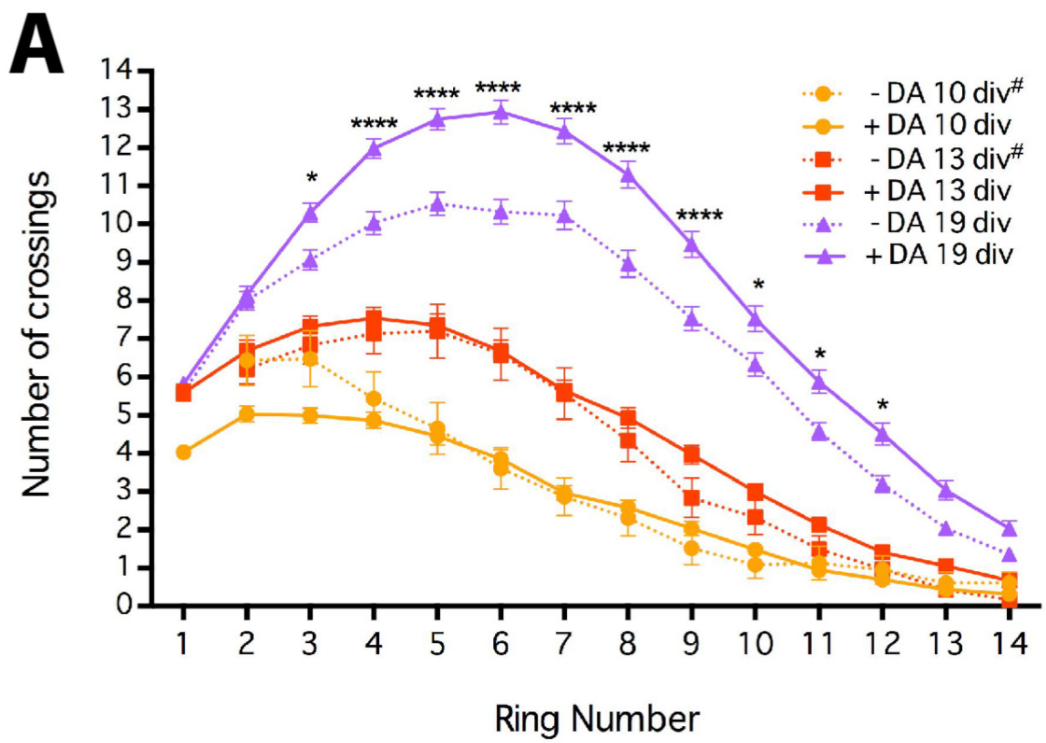
Loss of dopamine leads to the death or mature MSNs,

but does dopamine play a role in development?

Experimental time line



Effect of dopamine on the time course of dendritic development

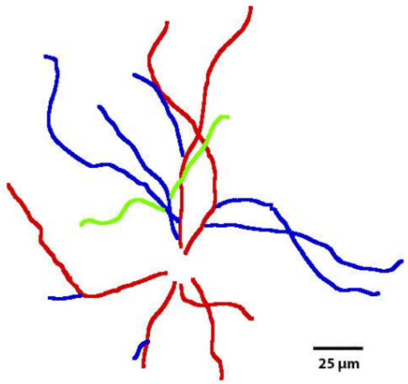


Conclusion: dopamine enhances arborization, but not the initial formation of MSN dendrites

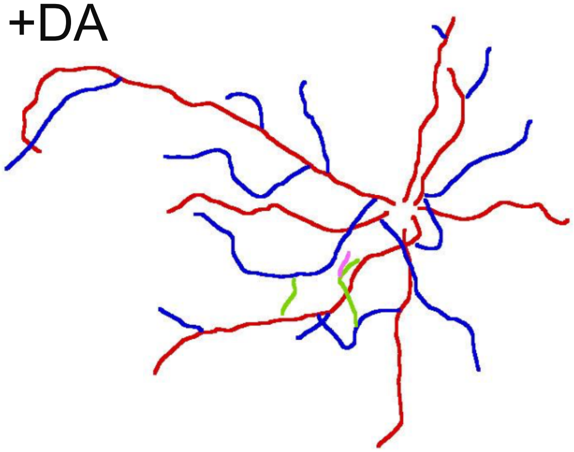
Penrod et al. 2015

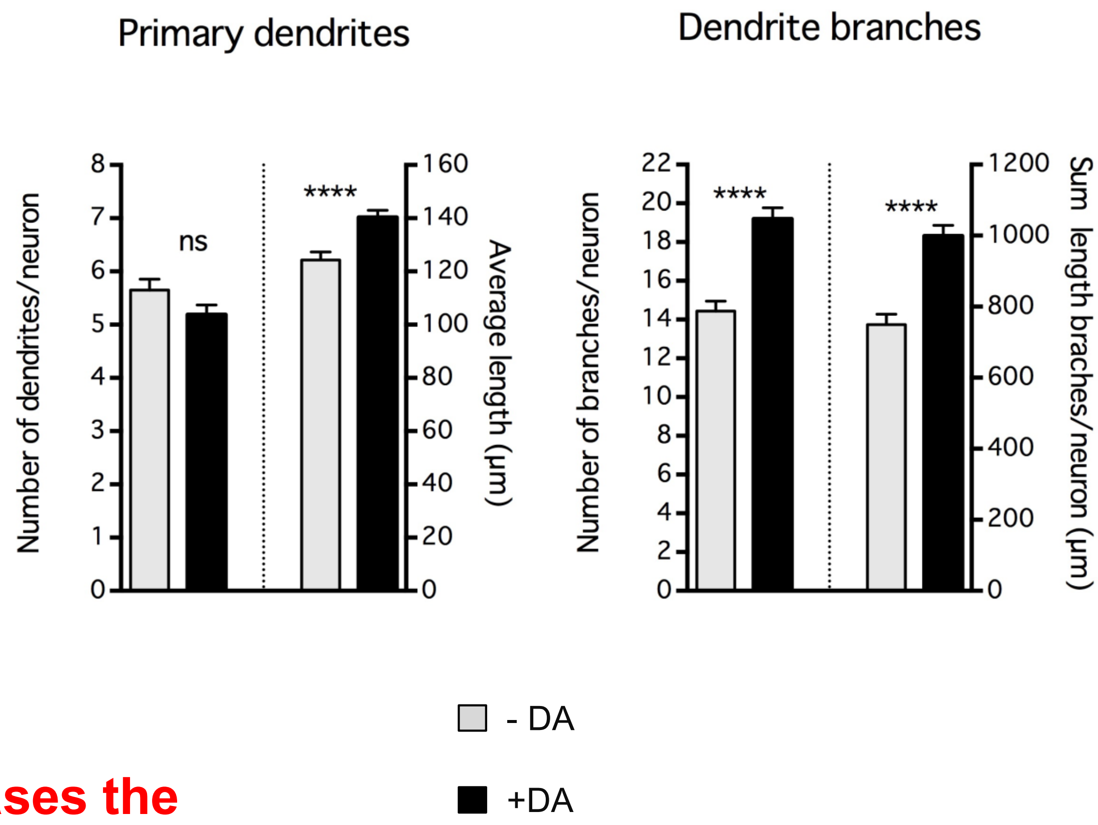
Effect of dopamine on development of dendrites

- DA



+DA





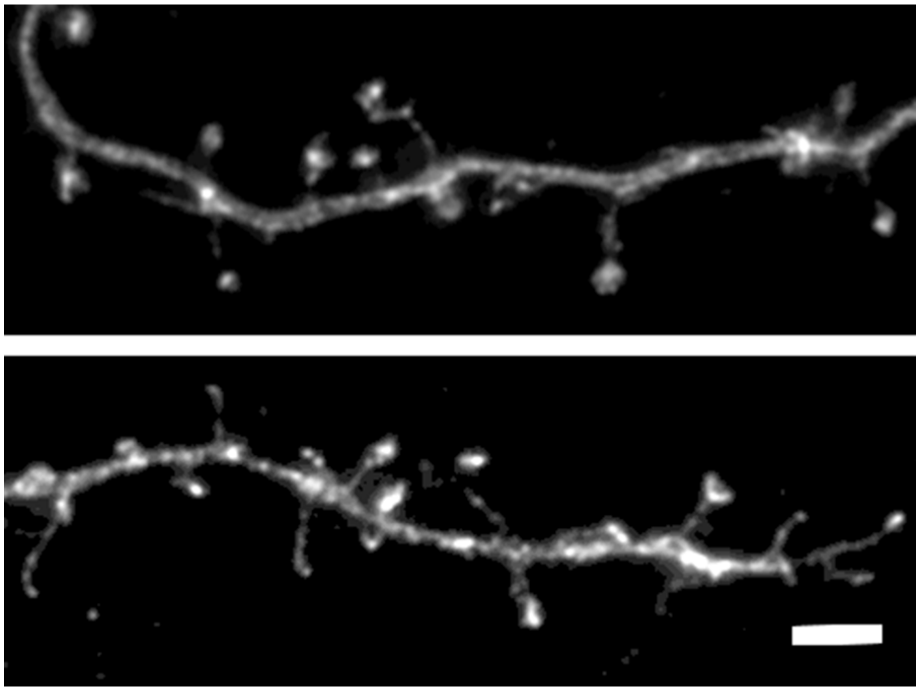
Conclusion: Dopamine increases the length and number of dendrite branches

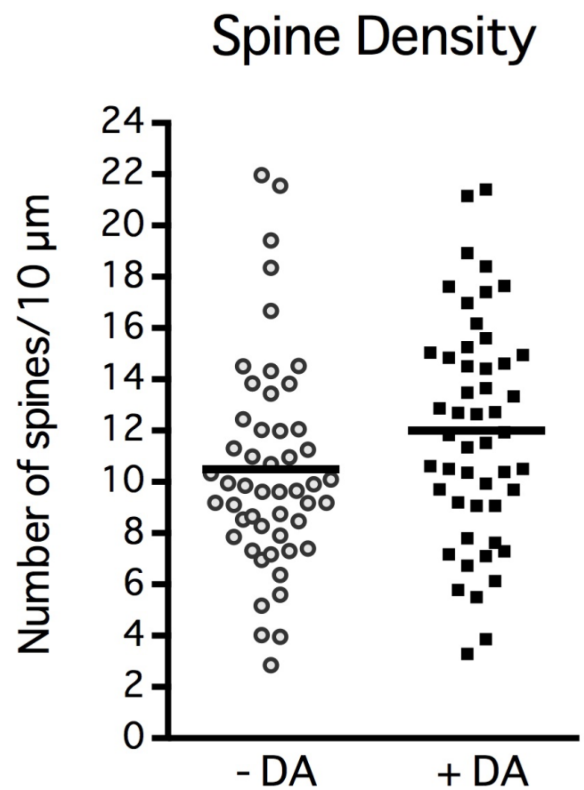
Penrod et al. 2015

Effect of dopamine on development of dendritic spines

- DA

+DA

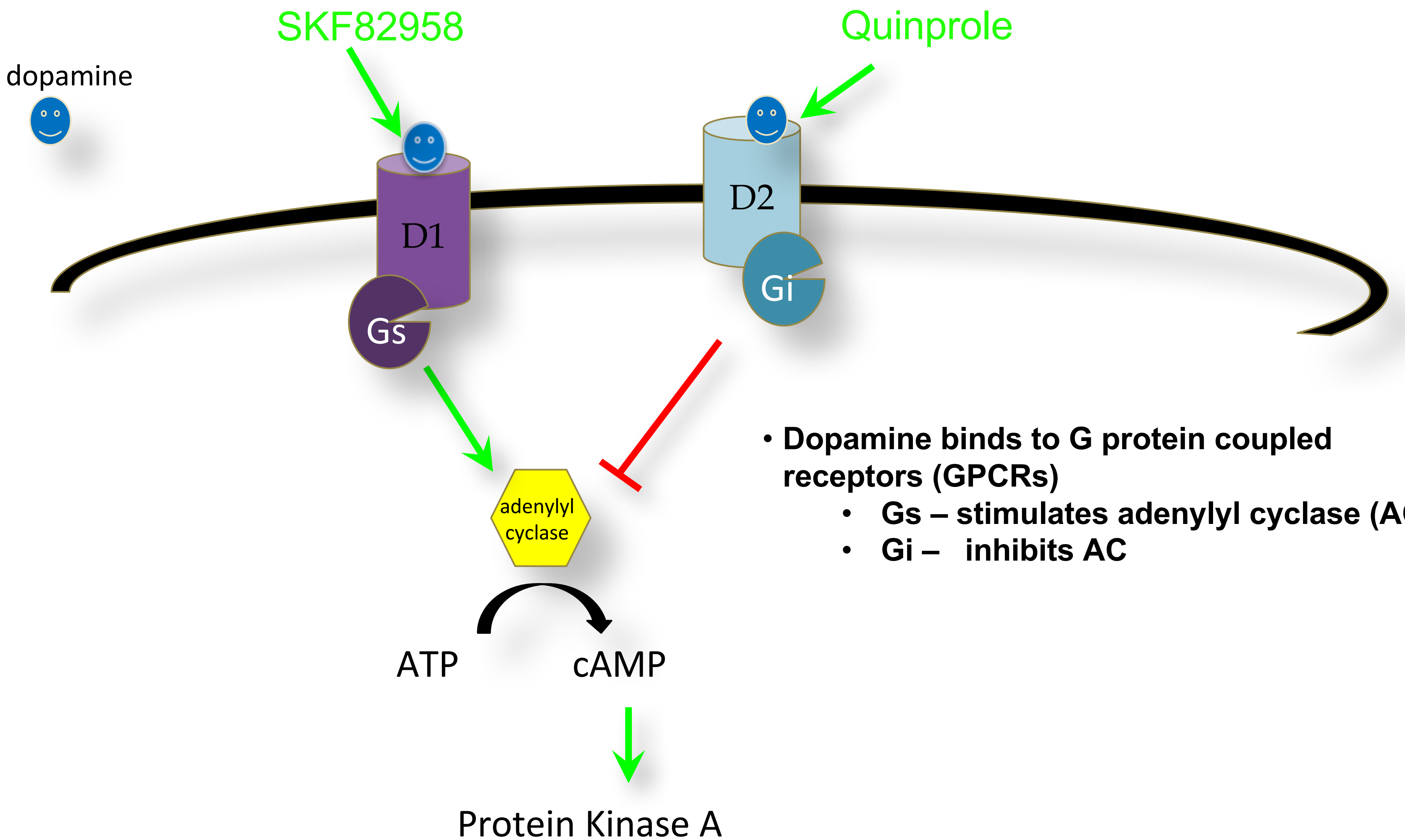




Conclusion: Dopamine increases the number of dendritic spines

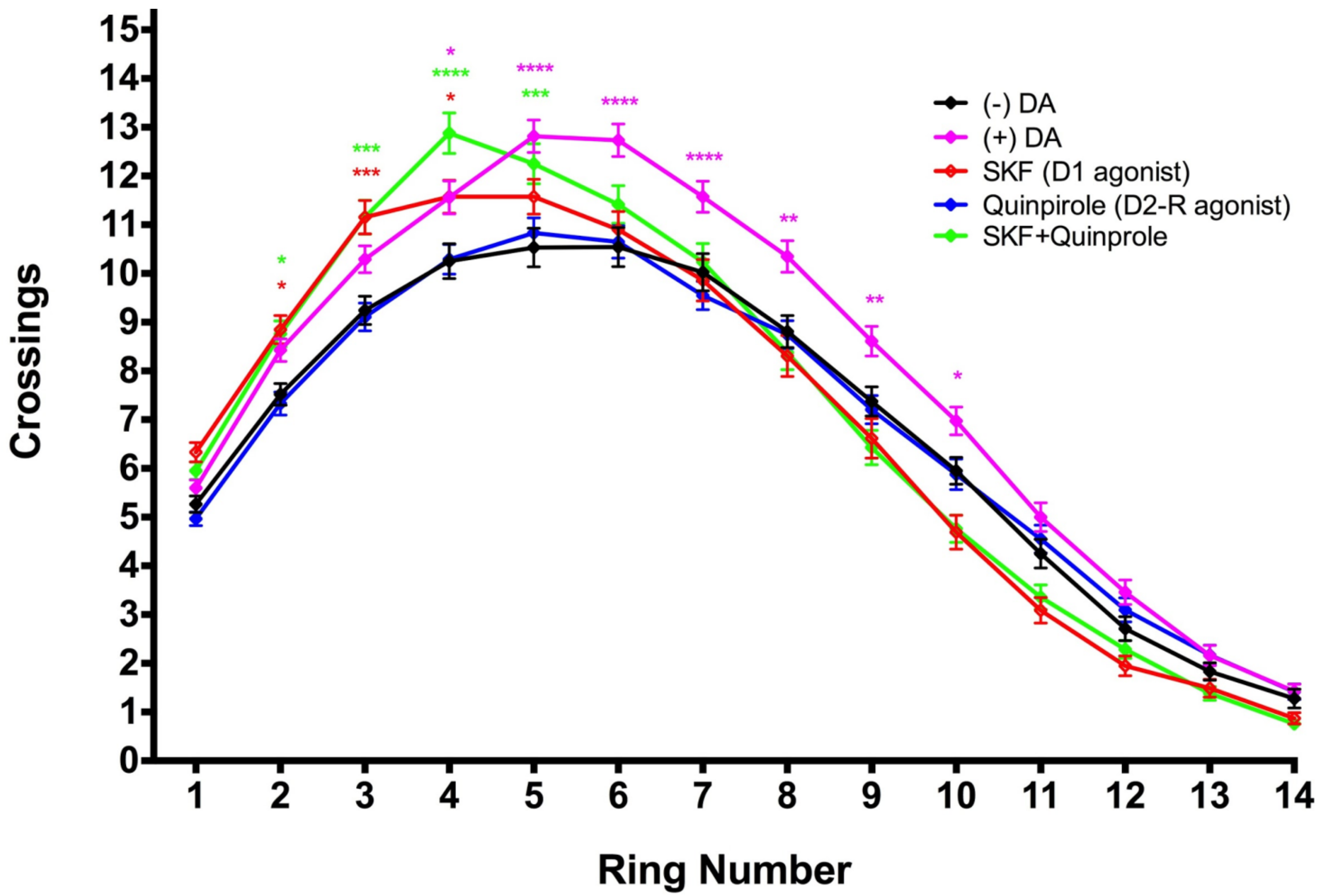
Penrod et al. 2015

Typical dopamine signaling pathways: which are important for dendrite development?



- **Dopamine binds to G protein coupled receptors (GPCRs)**
  - **Gs – stimulates adenylyl cyclase (AC)**
  - **Gi – inhibits AC**

D1 and D2 Receptor agonists cannot replicate the effects of dopamine



Conclusion: This is not a "typical" dopamine signaling pathway

Possible explanations for the atypical nature of the dopamine signal

- The signal does not involve the D1 or D2 receptors
  - *Always possible, could be D3, D4, or D5*

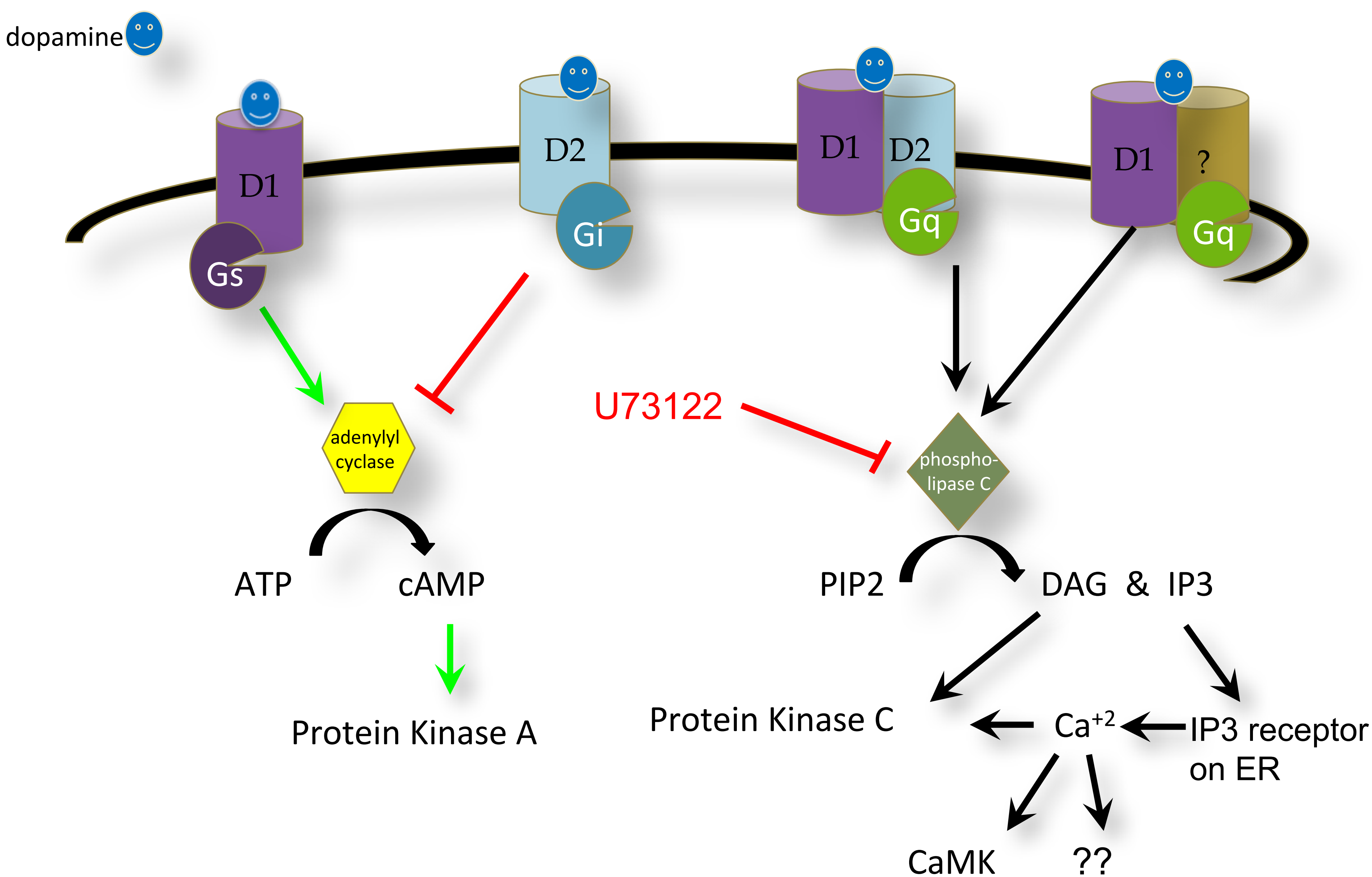
- The agonists are too unstable to be effective over the long time course of the experiment
  - *Unlikely, agonists are more stable*

- The agonists are stable, but their signal is down regulated over the long time periods
  - *Possible…*

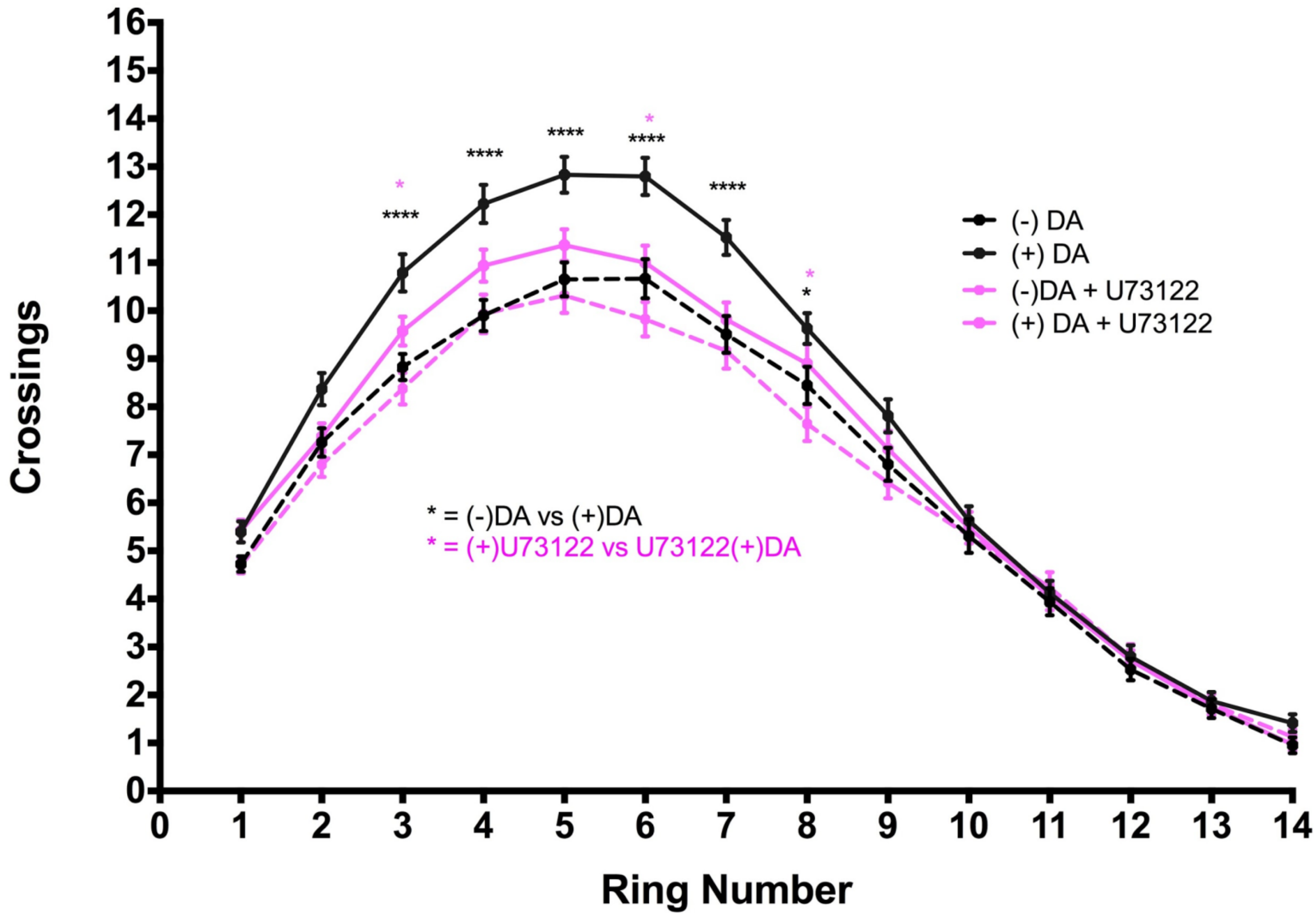
- The agonists do not bind the receptors in the same way as dopamine
  - *Definitely true (they are D1 or D2 selective, dopamine is not)*

- One of dopamine receptors may be hetero-dimerizing with another type of receptor and this dimer is not stimulated by the agonists
  - *There is precedence for this…*

Atypical dopamine signaling pathways

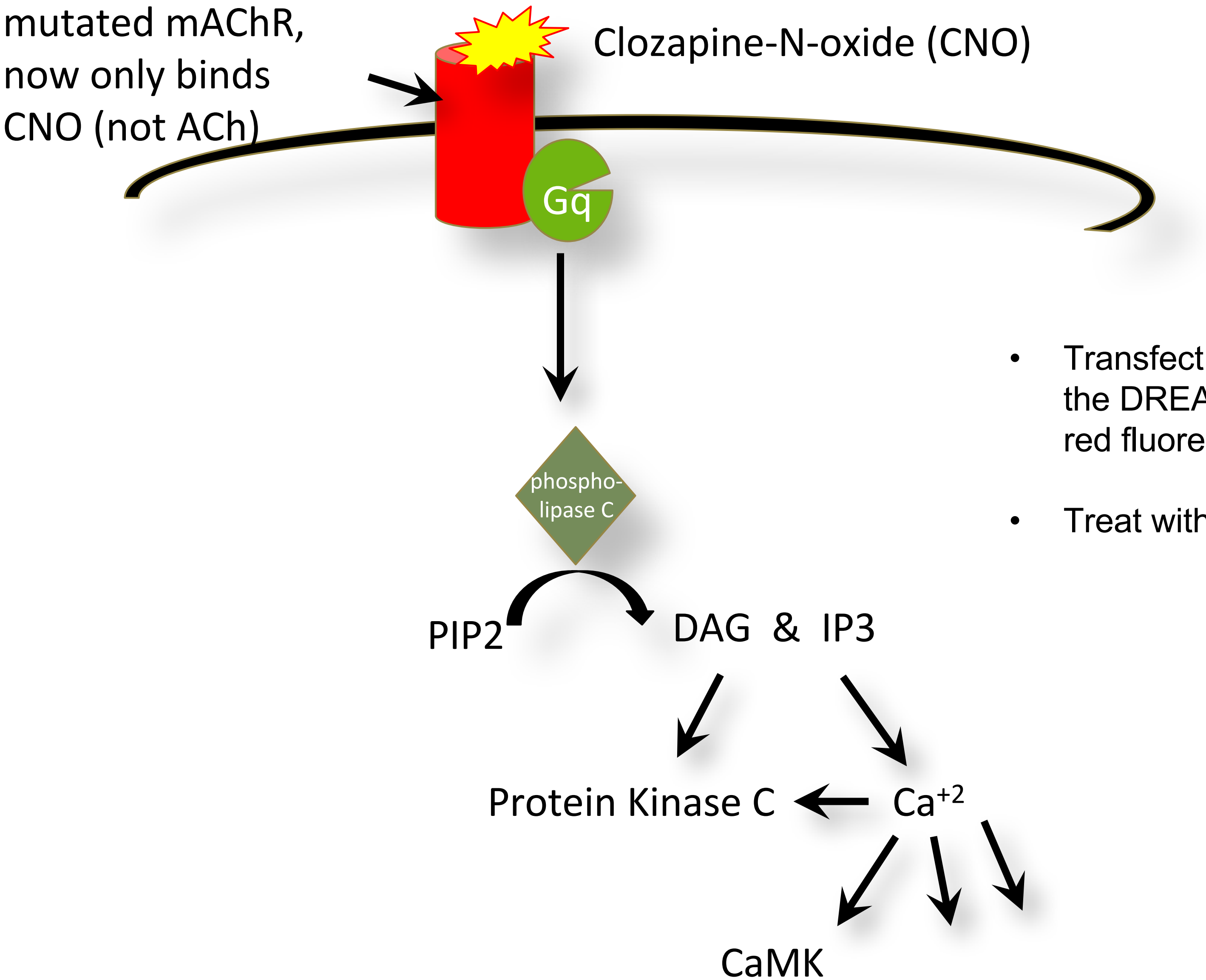


A PLC antagonist blocks the effects of dopamine



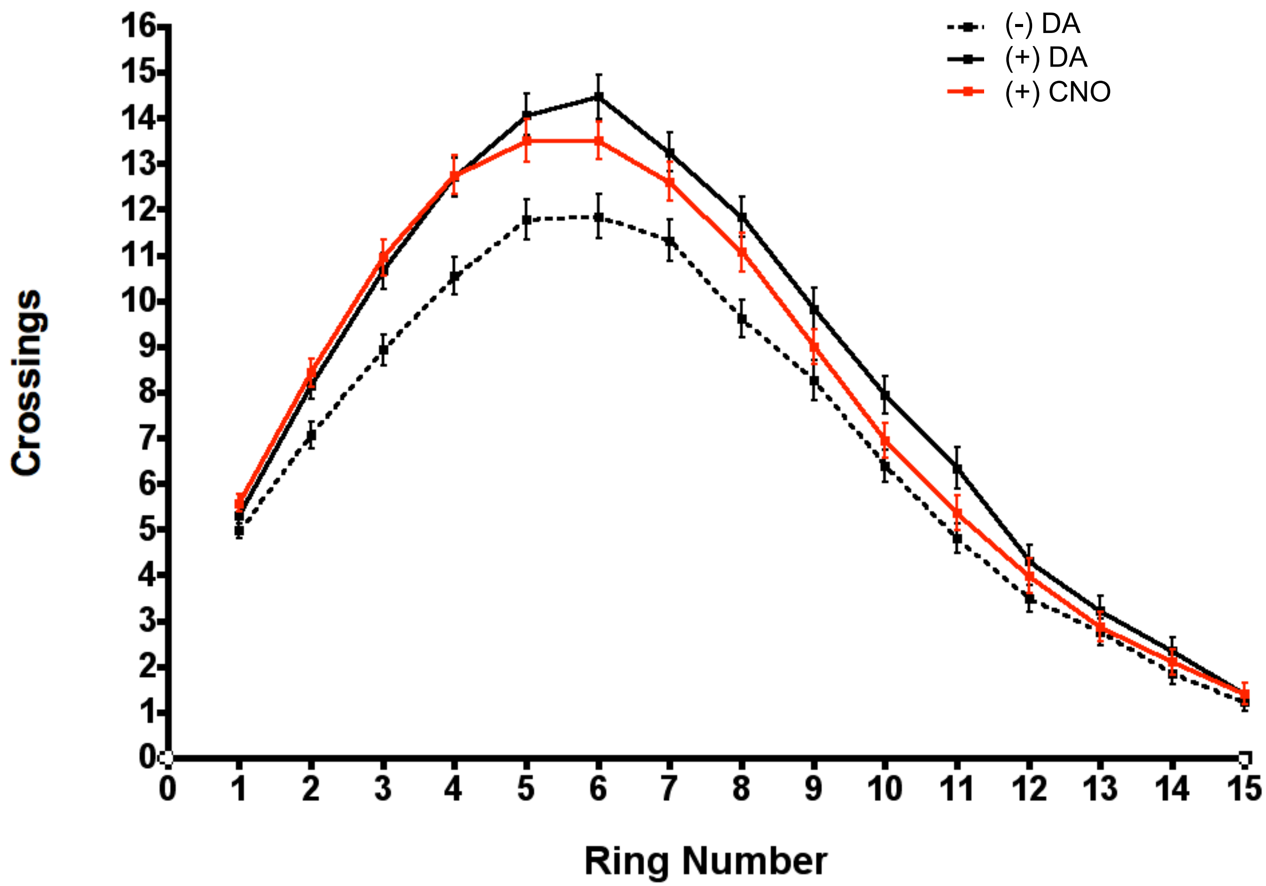
Conclusion: PLC signaling is required downstream of dopamine

Designer Receptor Exclusively Activated by Designer Drugs



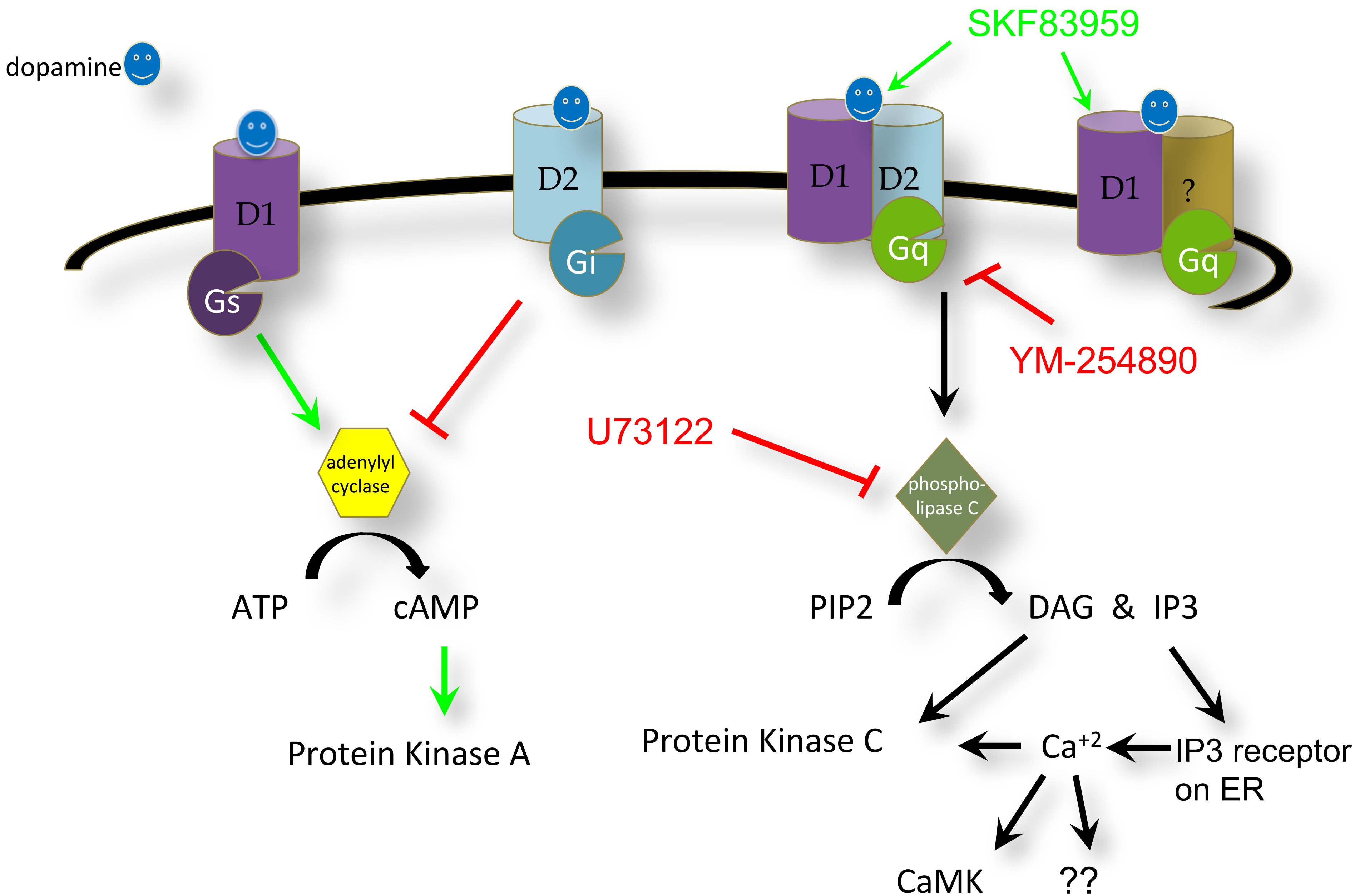
- Transfect plasmid that expresses the DREADD receptor fused with red fluorescent protein
- Treat with CNO (12.5 nM)

Chemogenetic stimulation of the Gq pathway increases MSN dendritic arborization



Conclusion: Gq signaling can mimic the effects of dopamine

Additional experiments "in the works"...



Conclusions:

- Dopamine is not required for MSN development, but dopamine….

- enhances dendritic arborization by increasing the number and length of dendrite branches

- increases the number of dendritic spines

- appears to act via an “atypical” mechanism that requires PLC activation and is mimicked by Gq signaling