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Neural Network Tissue Engineering

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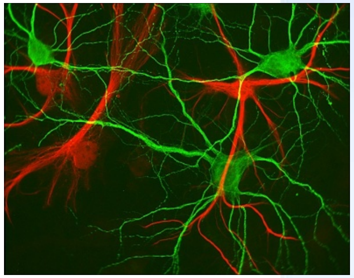

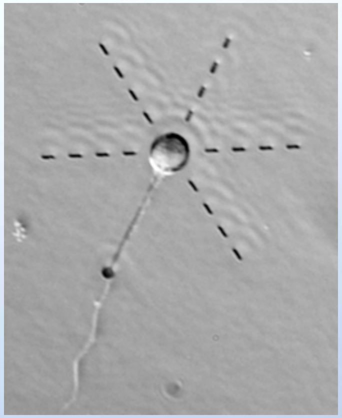

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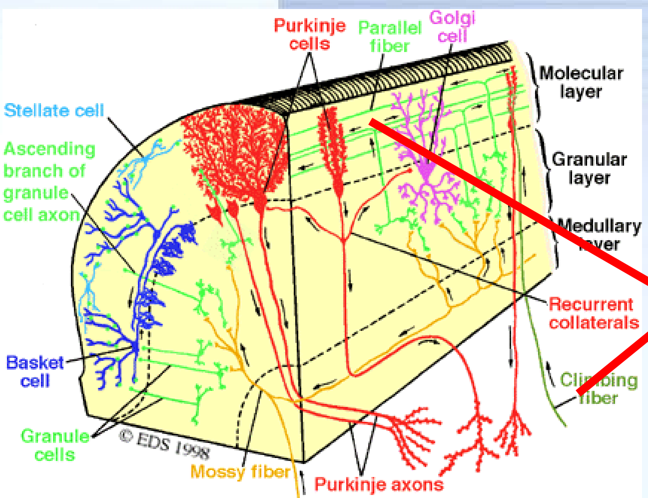
Outline

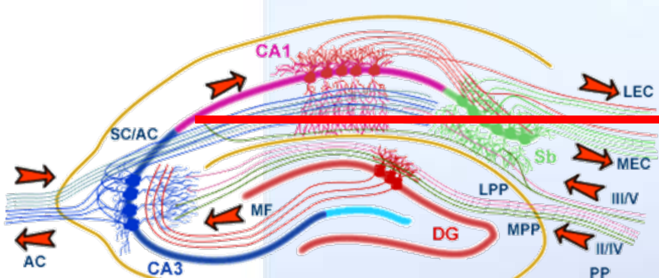
- **Problem: Neural engineering**
- Approach & Results
  - Network architecture
  - Neuron polarization
  - Microfluidic compartmentalization
- Engineered networks & Human decision-making
- Conclusions & future Work

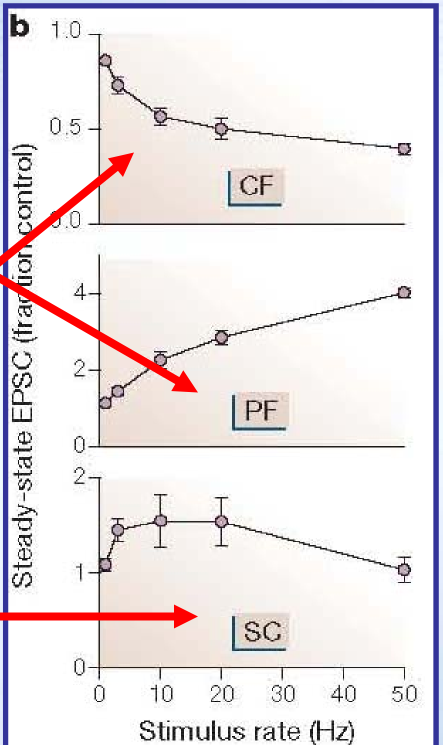
Problem

• *In vivo* neural networks have 1) structure and 2) function. What functional capabilities are afforded by nanostructures (molecules) vs. micro/meso-structures (network architecture)?

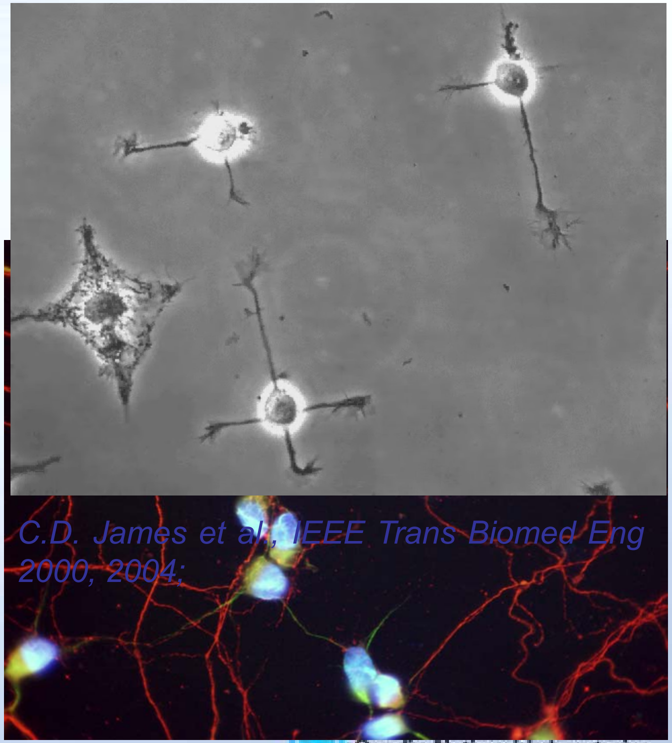
• Networks will be engineered and interrogated; experimental data will then be used to train a computational model to predict network functionality.







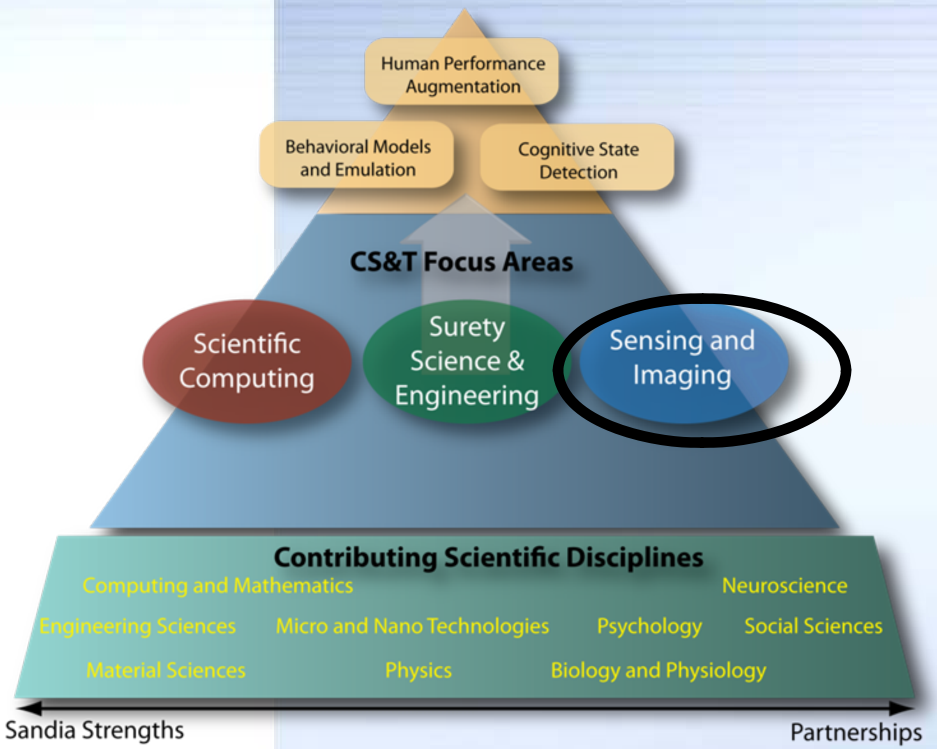
Abbot, Regehr 2004



C.D. James et al., IEEE Trans Biomed Eng 2000, 2004;

Relevance & Significance

Our objective is to develop methods for engineering living neural networks to interrogate the role of network architecture in neural function, with an ultimate objective in understanding human decision-making.



- The technology will provide an *in-vitro* test-bed for therapeutic interventions (chemical, electrical):

-physical and psychological defects (TBI, PTSD, confusion, etc.)

- DOD applications in enhancing human performance, augmented memory, learning, and visual perception for the war-fighter.

- optimized network architectures

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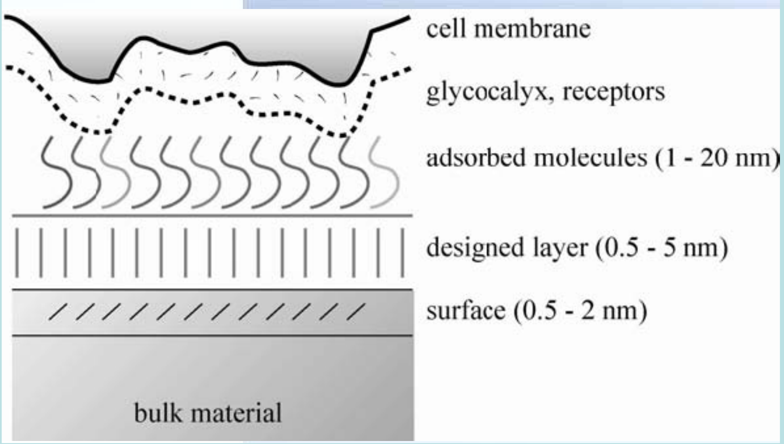
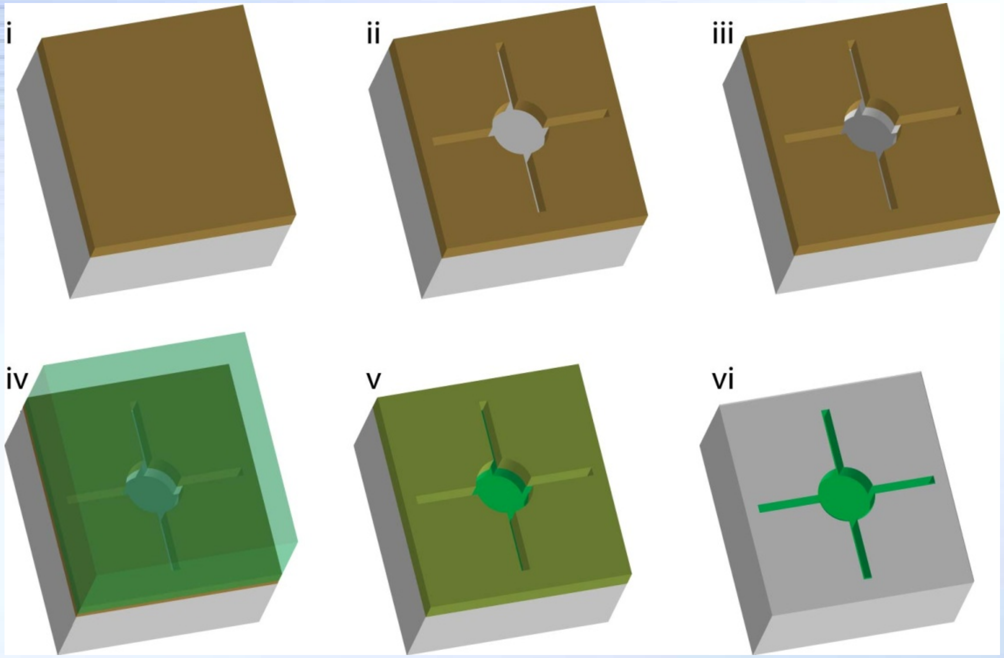
Approach

• Surface chemistry and surface topography can be utilized to influence cell adherence and outgrowth. 3D guidance cues may improve cell network stability.

The cell-substrate is a complex and dynamic environment.

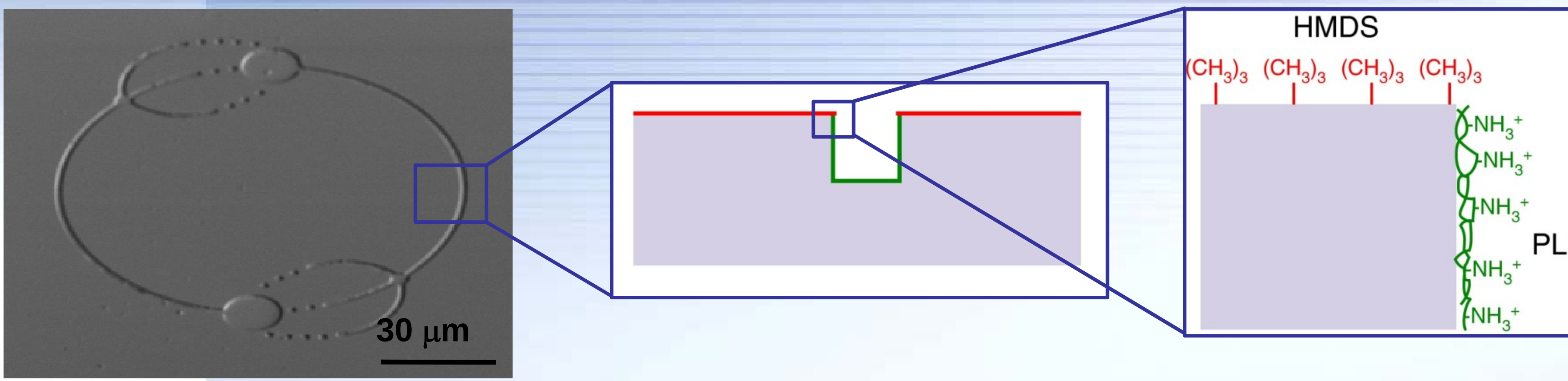


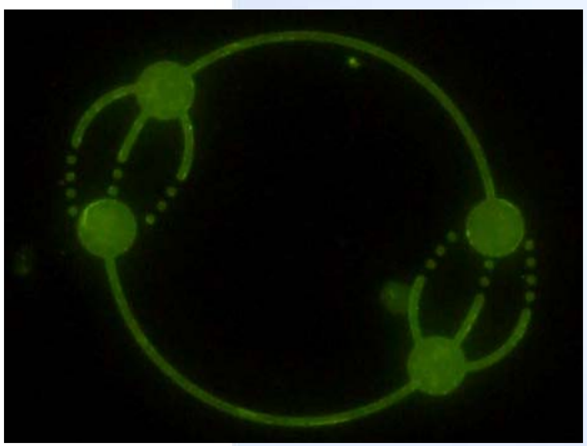


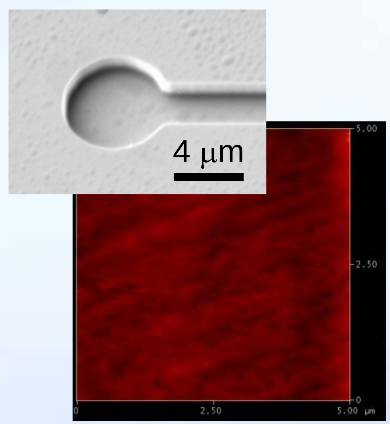
Dual guidance cues: CHF_3/O_2 reactive ion etching of fused silica for defining topographical cues; lift-off patterning of poly-L-lysine (PLL) for chemical cues
Bifunctional surface chemistry: cell-adherent PLL and cell-repellent HMDS
Biologically-active proteins: A.A. Oliva, et al., Neurochem. Res. 2003, 28, 1639.

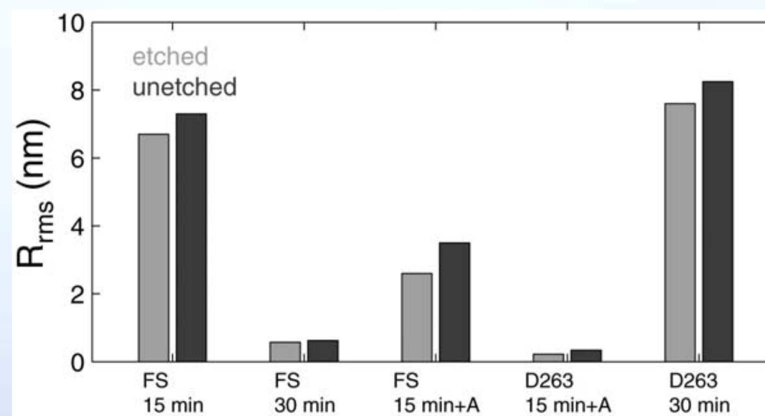
Results: Topographical & chemical cues

• Adhesive molecules (PLL, PEI, SAMs) and repellent molecules (Teflon-based, HMDS, PEO, PEG)







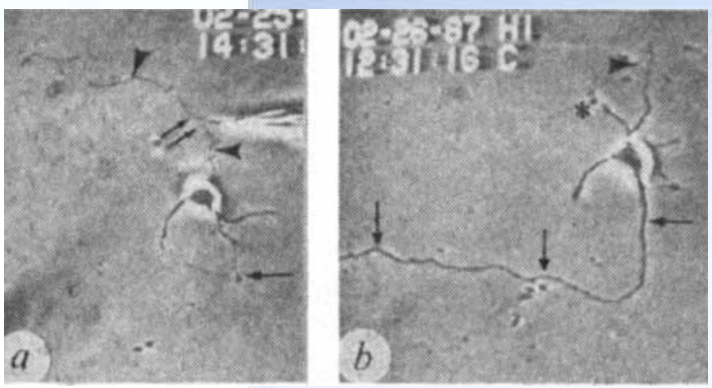


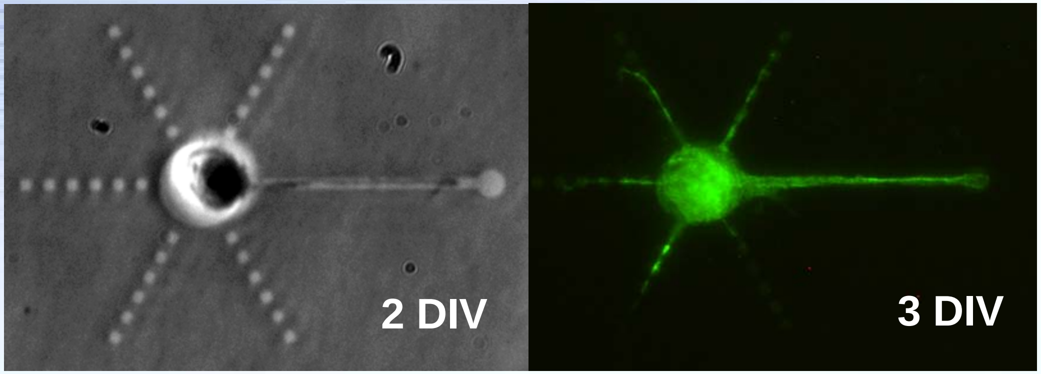
PLL drives the adsorption of negatively charged species, HMDS drives the adsorption of hydrophobic species

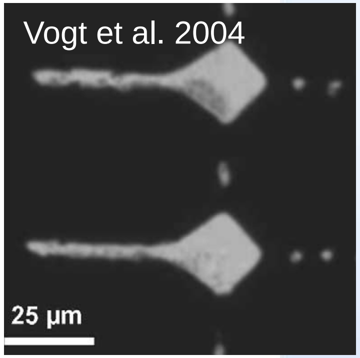
Control of neuron polarity using geometrical cues

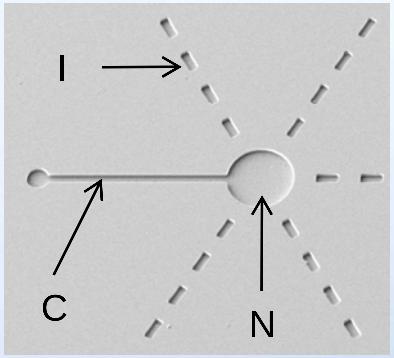
• Cell attachment, axon growth, and dendrite growth are promoted differentially with simple changes in the geometry of the guidance cues

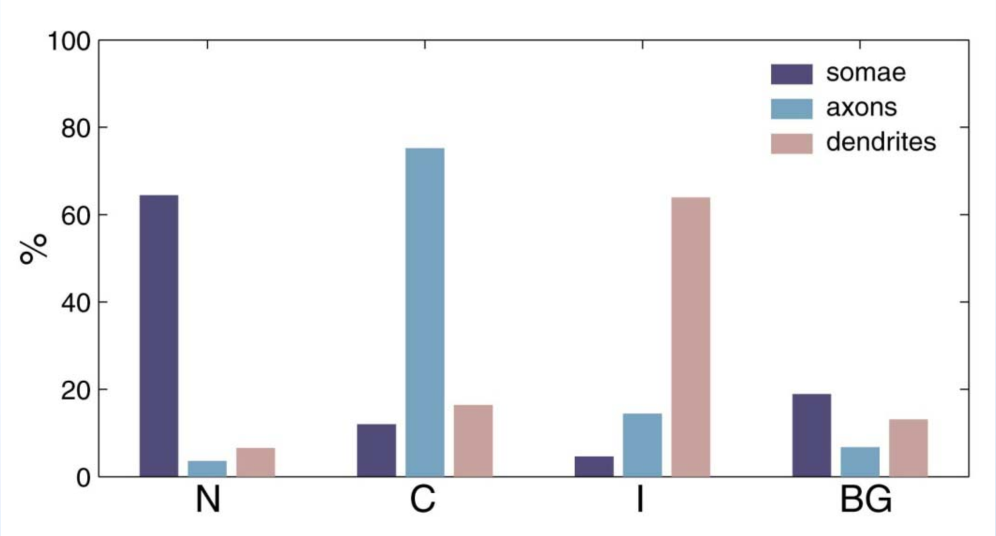
Dotti and Banker 1987 found that the first neurite to grow the longest developed into an axon.







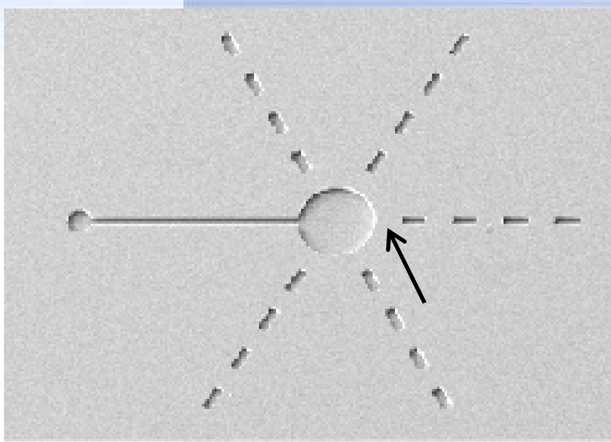


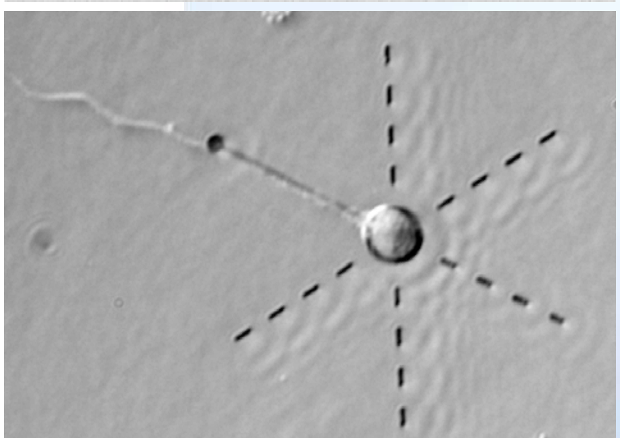


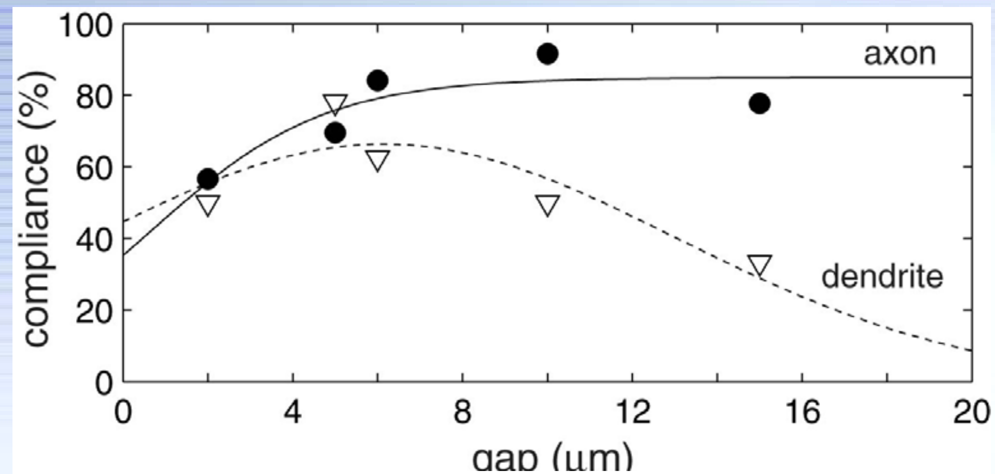
Effects of guidance cue geometry on neuron polarization

Guidance cue characteristics for robust control of neuron polarization

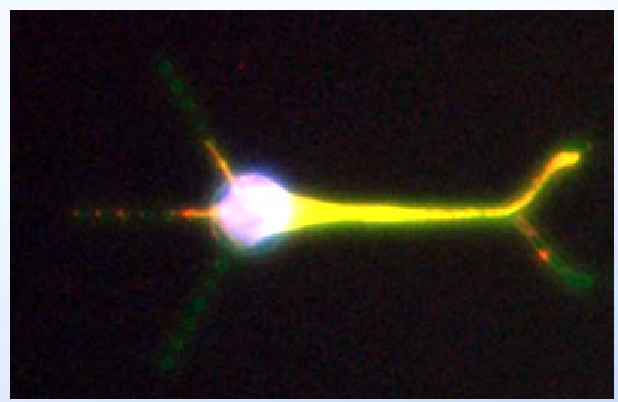
- distance between the cell body node and interrupted cues for dendrite guidance
- distance between interrupted cues

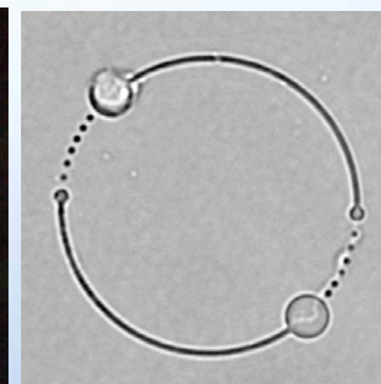






Geometry optimized for 6 μm to promote efficient axon and dendrite development



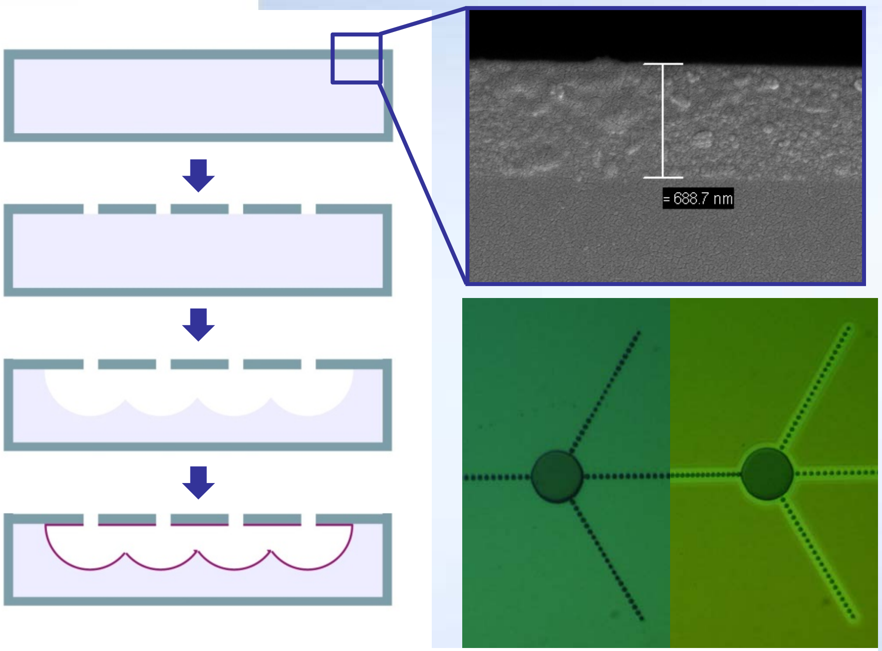


Additional geometries for 1) neurite branching and 2) synaptic connections between neurons

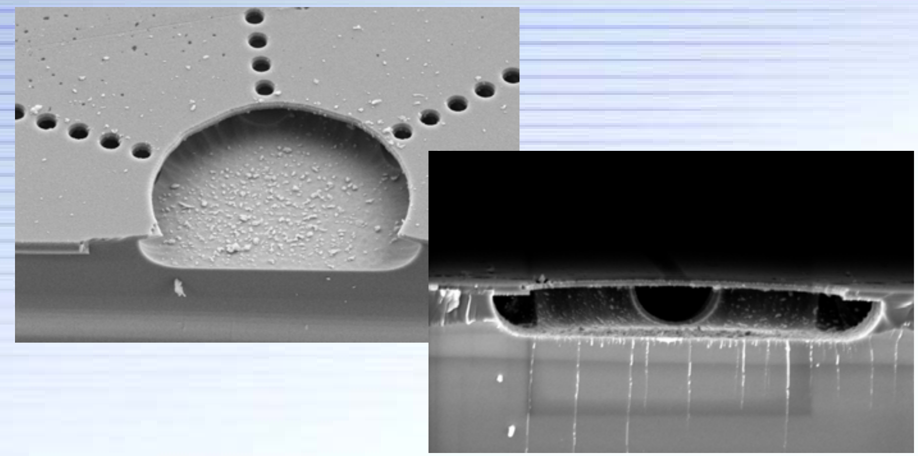
Buried microfluidic channels for dual cell guidance cue structures

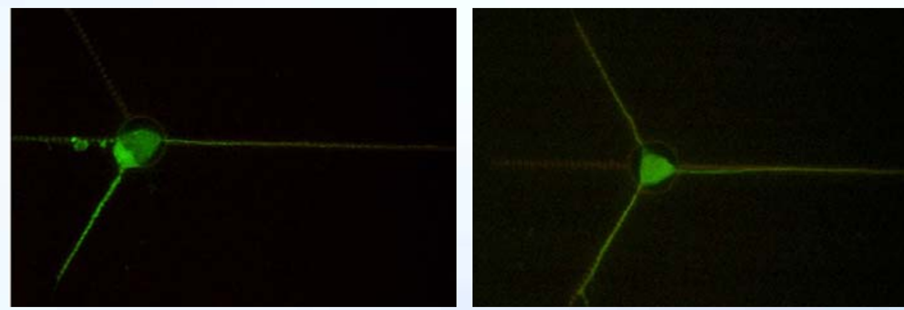
Encapsulated microfluidic channels with a selectively adsorbed chemical cue

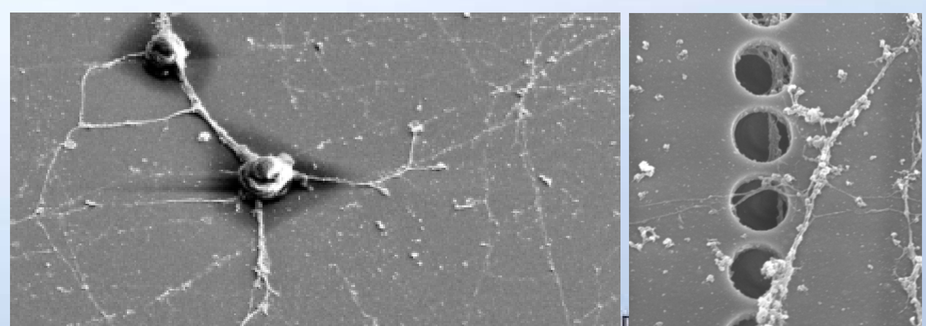
- proximity of individual features for continuous fluidic structures
- irrigation pores for perfusion of media, removal of waste by products



Substrate processing: thin-film masking and HF undercut

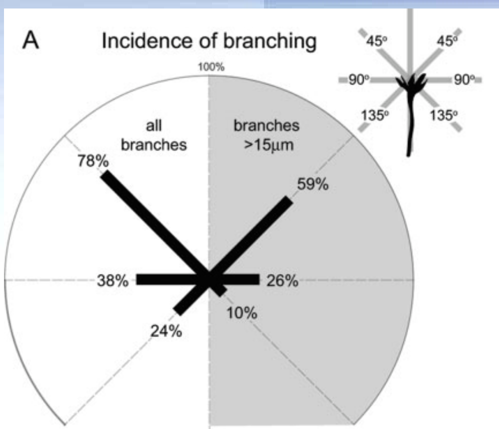


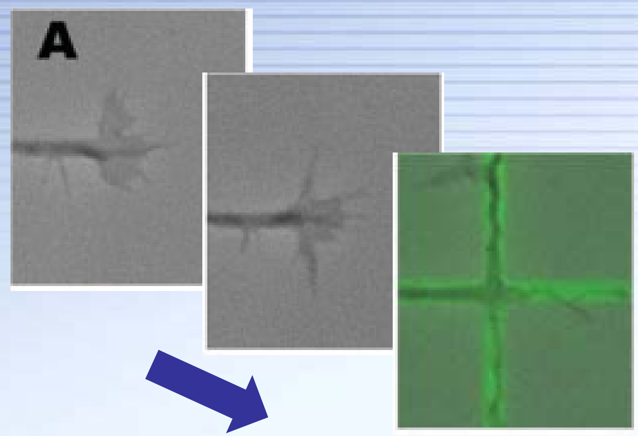


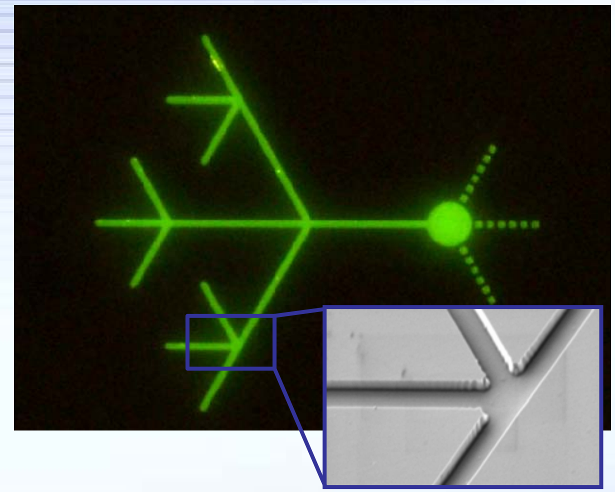


Network engineering: synaptic connections

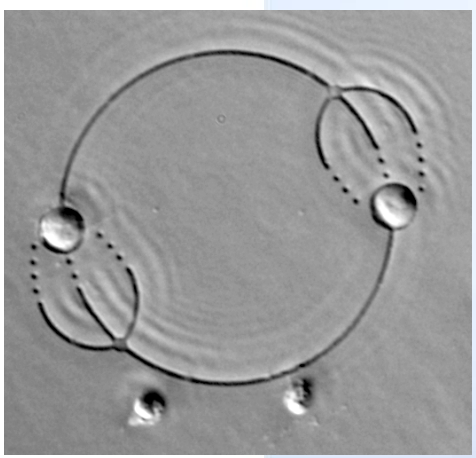
Network engineering requires 1) cell placement, 2) neurite outgrowth, 3) neurite polarization, 4) neurite branching, and 5) neurite-neurite contact

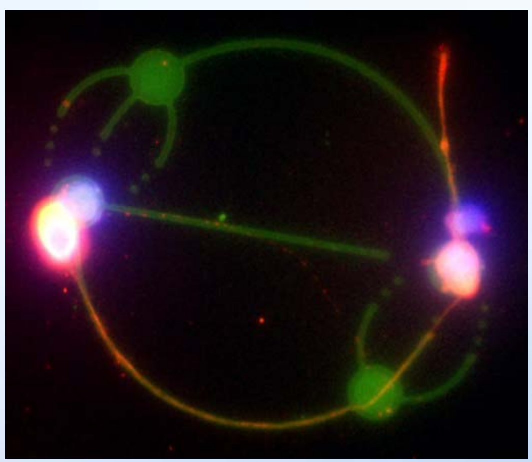


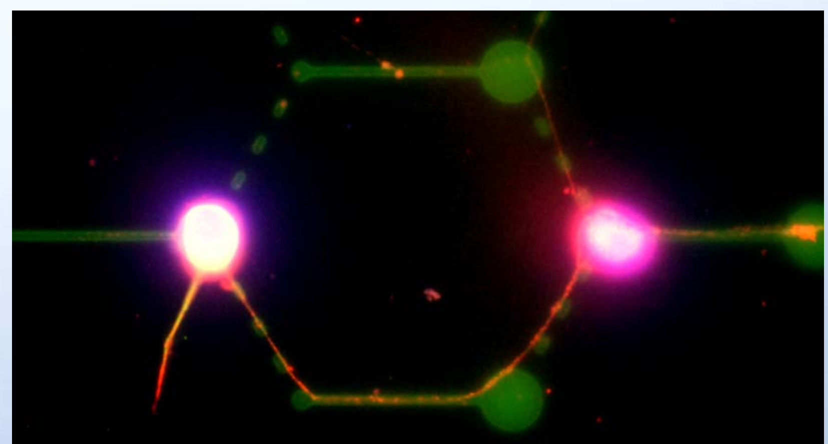




G.S. Withers, C.D. James, et al., J Neurobio 2006, 66, 1183

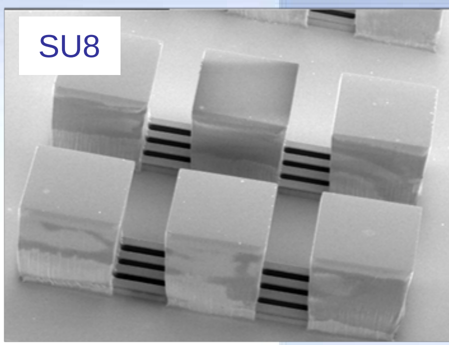


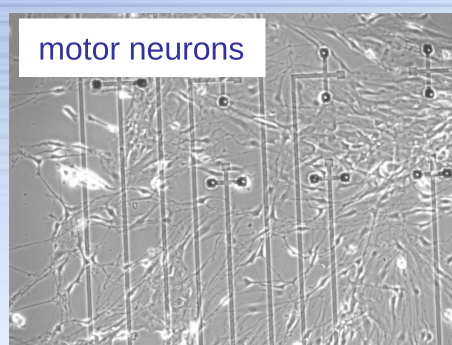


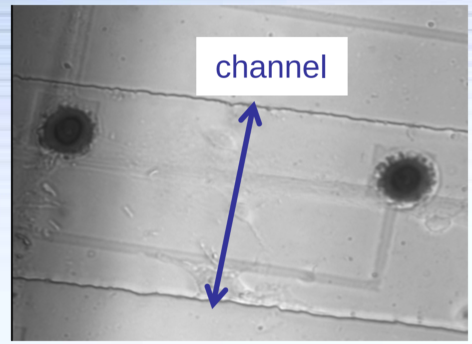


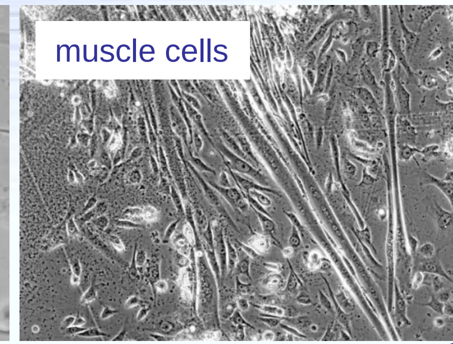
Heterogeneous network engineering using μfluidic compartmentalization

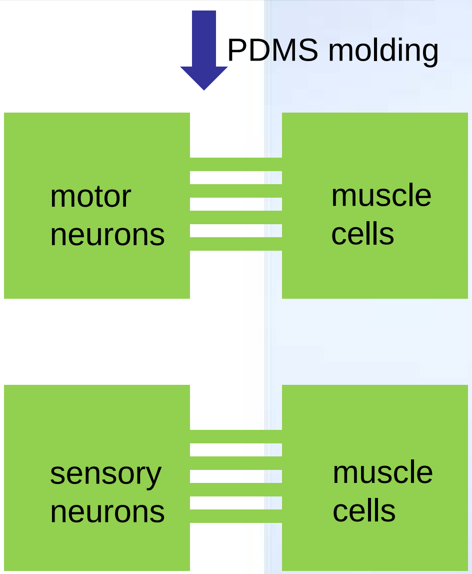
- Tissue requires compartmentalization of multiple cell types for proper function
- Spinal cord networks: examine whether the intrinsic neural activity of spinal cord neurons is modified by target innervation (muscle cells) or sensory input (sensory neurons)

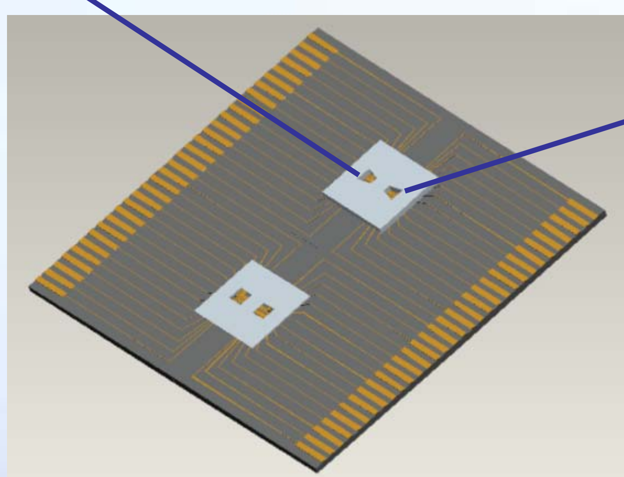


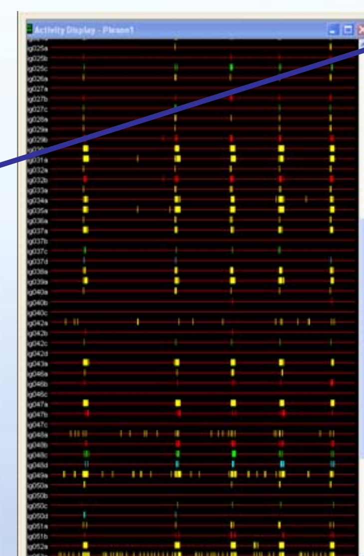


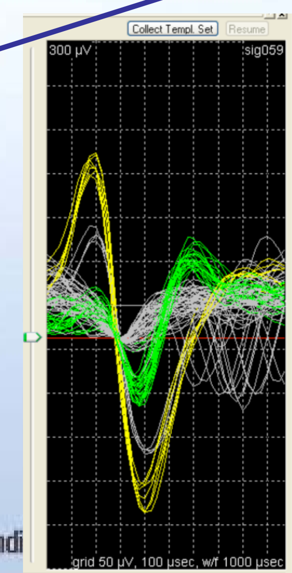












M. Romero-Ortega, UTA

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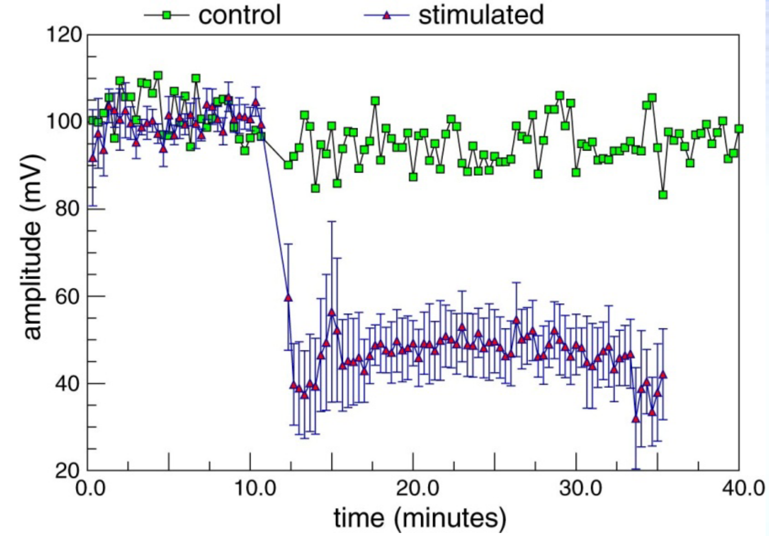
Engineered networks & Human decision-making

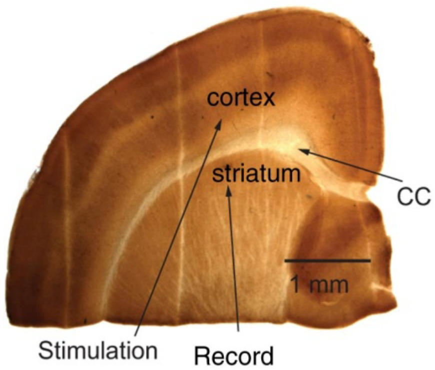
• Decision-making is a complex process involving the integration of many inputs (sensory, reward, cognitive, motor, etc.)

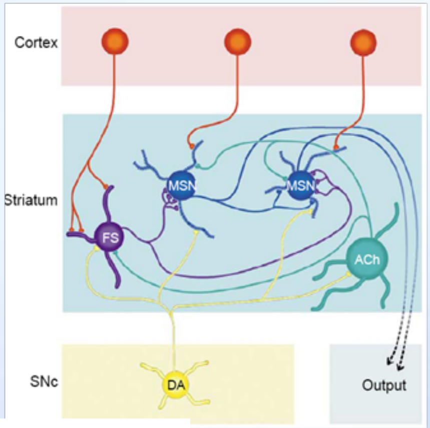
• **Corticostriatal networks** are involved in decision-making and exhibit mechanisms of synaptic plasticity (persistent changes in synaptic strength) – metric for information processing

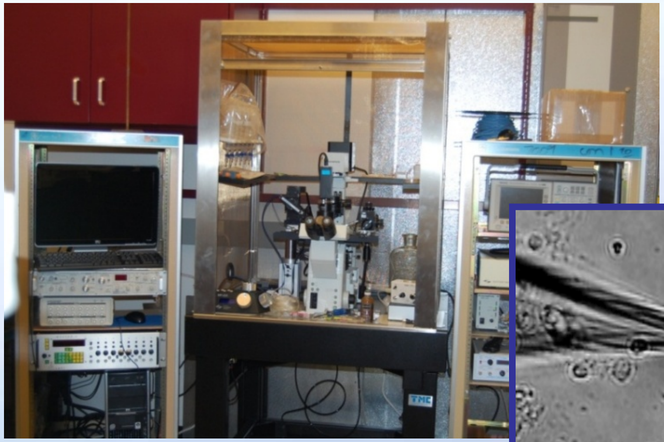
• **Measure plasticity in engineered networks and examine the phenomena as a function of the network architecture**



Plasticity in brain slices – high *f* stimulation in the **cortex** depresses activity in the **striatum**







M. Baca with D. Partridge & M. Wilson (UNM)

Prediction of network behavior using computational modeling

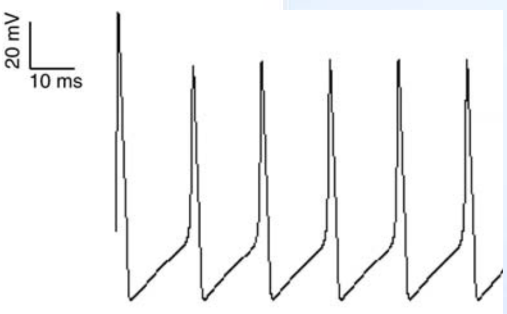
• Implemented basic Hodgkin-Huxley dynamics in Xyce; Xyce will scale to larger networks, and interfaces directly to Dakota for optimization/tuning

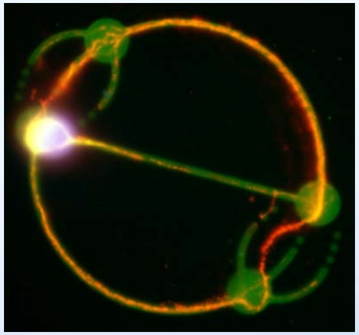
• Synaptic connections: kinetic model release and uptake with non-linear gating functions for signal transmission; receptors (NMDA, AMPA, Ach, Da, Glu)

HH Model: $$C_m \frac{dV_m}{dt} = I_{stim} - \sum_{ion} g_{ion}\left(V_m - E_{ion}\right)$$

Conductances	Soma	Dendrites	Axon
Na (pS/μm^2)	200	200	400
K_A (pS/μm^2)	250	250(1+d/100)	50
K_{dr} (pS/μm^2)	100	100	100
I_h (pS/μm^2)	0.5	0.5(1+3d/100)	0

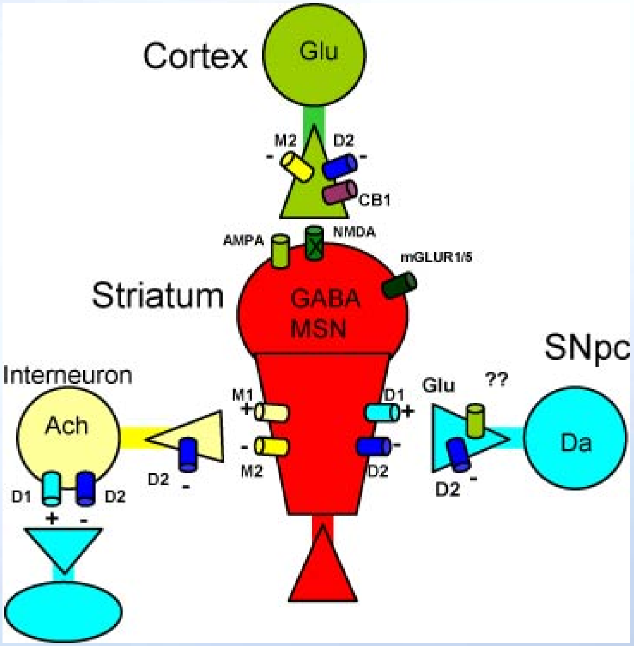
Single neuron circuit simulation:





Autapse – neuron connected to itself; oscillatory spiking after input stimulus

Corticostriatal networks are involved in ***decision-making***:



…utilize modeling to identify crucial players in observed phenomena

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Conclusions & Future Work

- Current neural engineering technologies have shown promise in cell placement, cell polarization, and cell morphology
- Significant challenges remain in controlling synaptic connections and in robust maintenance of network architecture
  - Explore biologically-active proteins
  - 3D guidance cues
- DARPA ReFiTS seedling project: Restoring Function Through Structure Program being formulated by DARPA PM LCDR Joseph Cohn with Chris Forsythe
  - Develop a program on high-resolution engineering synaptic connections between heterogeneous cell populations; hippocampal networks
- Two technical advances filed; manuscripts in preparation on topographical and chemical guidance cues, electrophysiological analysis of small engineered networks

Acknowledgments

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- James Forsythe;
- Mario Romero-Ortega (U. Texas Arlington)
- Donald Partridge and Michael Wilson (UNM)

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