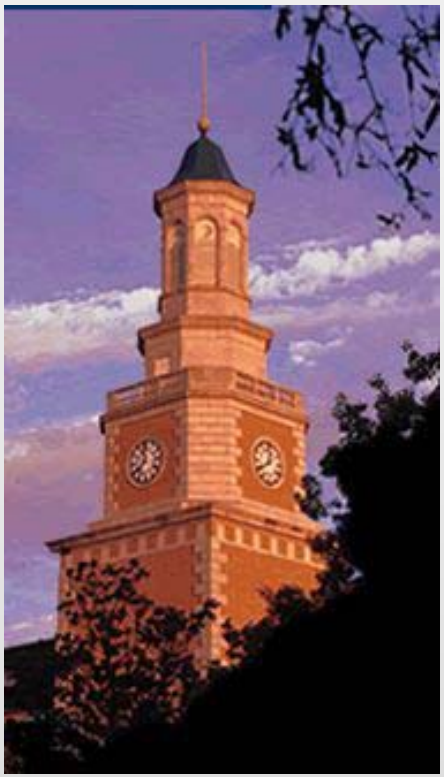
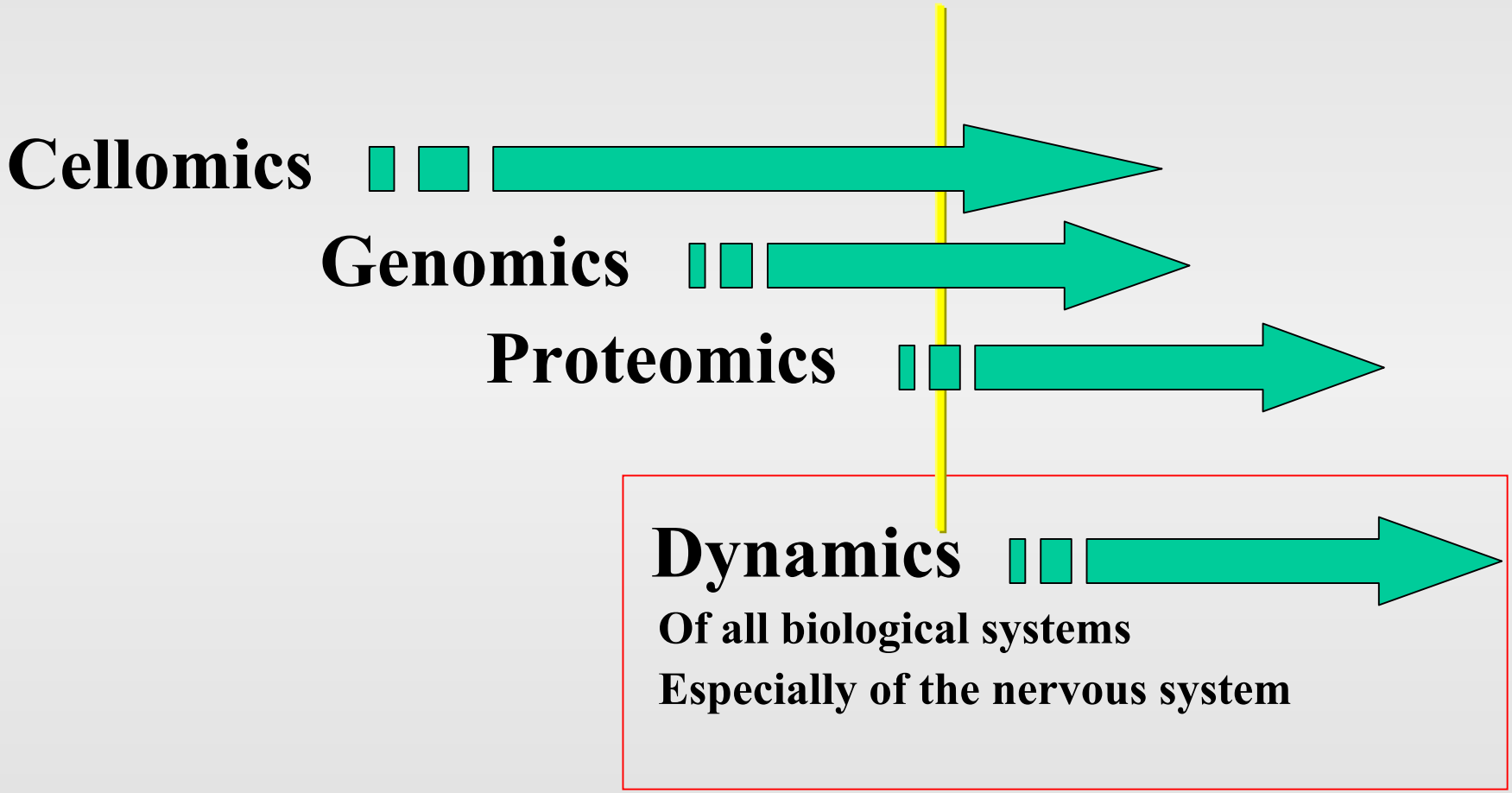
Brookhaven Biological Science Symposium

Neuronal Networks on Microelectrode Arrays: Applications to Pharmacology, Toxicology, and Biosensors.



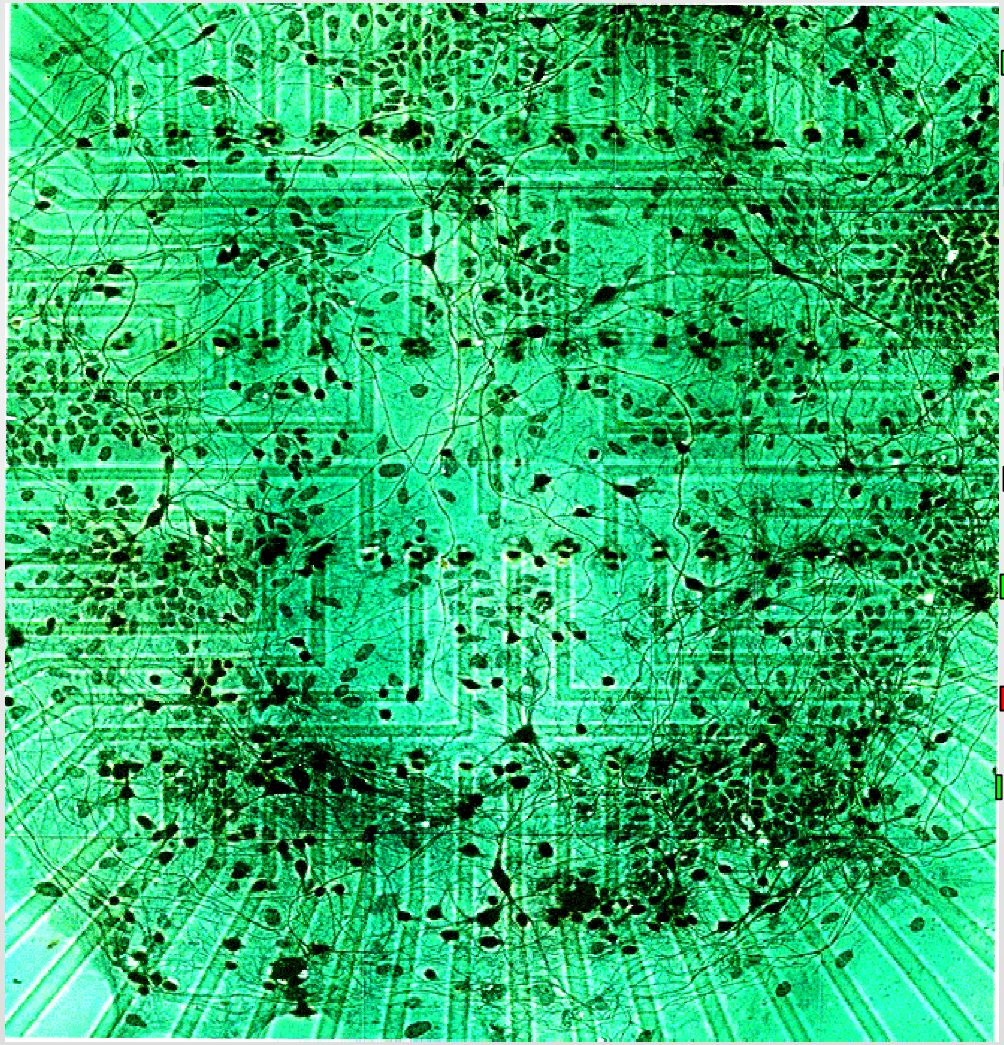
UNIVERSITY of NORTH TEXAS
Center for Network Neuroscience

Major Revolutions in the Life Sciences



Networks on Microelectrode Arrays:

Emerging *in vitro* platforms
with a wide impact spectrum and massive future potential.



- **Network Neurophysiology**
- **Chemical & Env. Toxicology**
- **Drug Development**
- **Pharmacology**
- **Trauma and Pathology**
- **Developmental Neurobiol.**
- **Tissue-Based Biosensors**
- **THEORY**

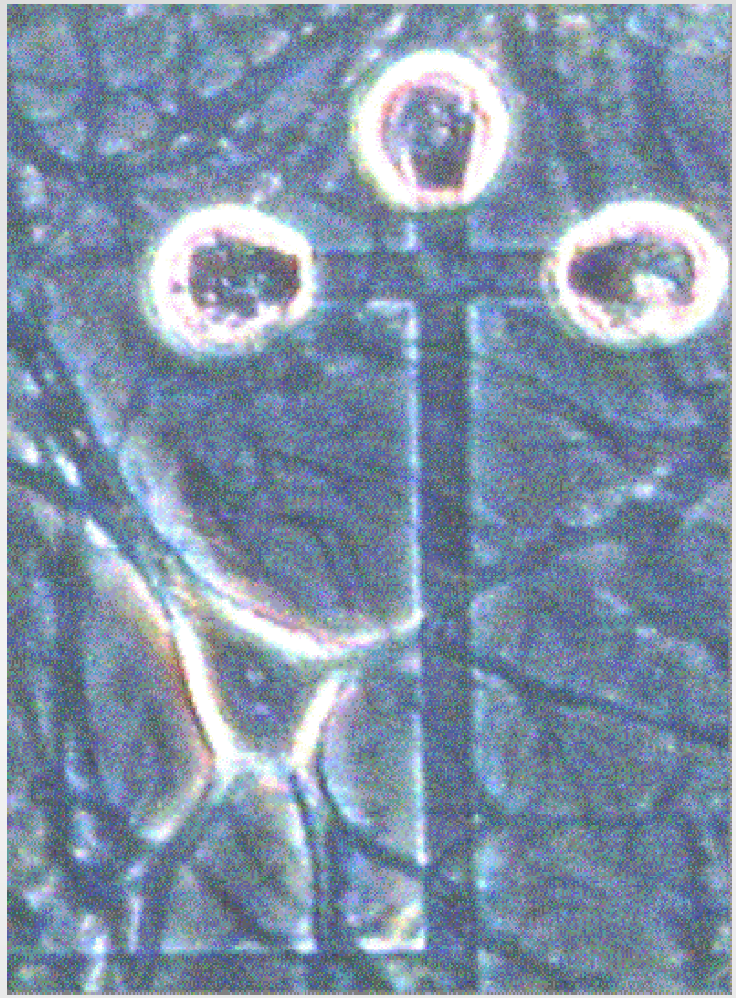
Self-organization; Pattern Generation; Pattern Processing; Pattern Storage; Feature Extraction; Plasticity; Anticipatory States; Information Processing

Neuronal Networks Growing on Microelectrode Arrays *in vitro* are Pharmacologically and Toxicologically HISTIOTYPIC

Networks are comprised of cells that report all metabolic poisons. But they also form dynamic pattern generation systems that respond to all compounds capable of changing the performance of the nervous system in the absence of cell death.

OUTLINE

1. GENERAL METHODS
   - 1.1 Cell Culture
   - 1.2 Microelectrode Arrays
   - 1.3 Multichannel Recording
2. NETWORK PHARMACOLOGY
   - 2.1 Ethanol
   - 2.2 Fluoxetine
   - 2.3 AChE blockers
3. NETWORK TOXICOLOGY
   - 3.1 Tetrodo Toxin
   - 3.2 Botulinum Toxin
4. BIOSENSORS for WATER MONITORING
   - 4.1 Detection, Classification, Identification, and Display
   - 4.2 Replacement Modules/Shipping
   - 4.3 Remaining Challenges



Cruciform microelectrode configuration for maximizing contact with active network components

METHODS

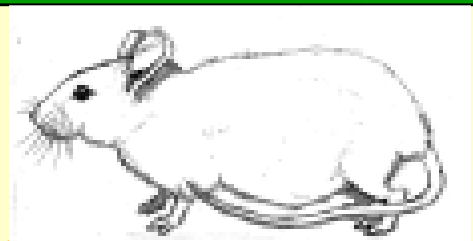
Nerve Cells in Culture

Microelectrode Arrays

Multichannel Recording

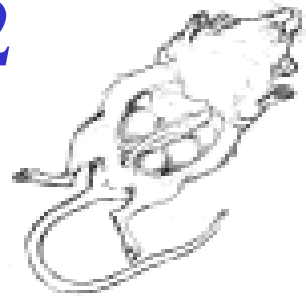
CNNS METHODS

1



1. Balb-C/ICR mice are mated for 24 hours, fourteen days before culturing.

2

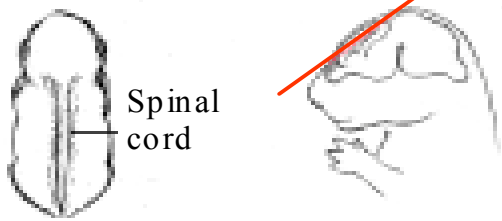


2. For each batch of cultures, a single pregnant female mouse is anesthetized, sacrificed by cervical dislocation, and dissected under sterile conditions to remove the uterus.

3

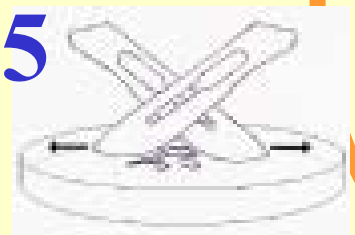
3. Ten to fourteen embryos are delivered from the uterus under a dissecting microscope in sterile D1SGH.

4



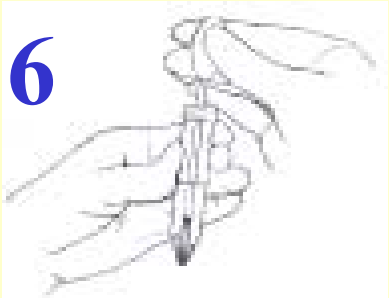
4. Each fetus is decapitated and has its spinal cord or frontal cortex (at red line) removed. The dorsal root ganglia and meninges are removed from the spinal cord, and only the strait portion of the spinal cord is used.

5

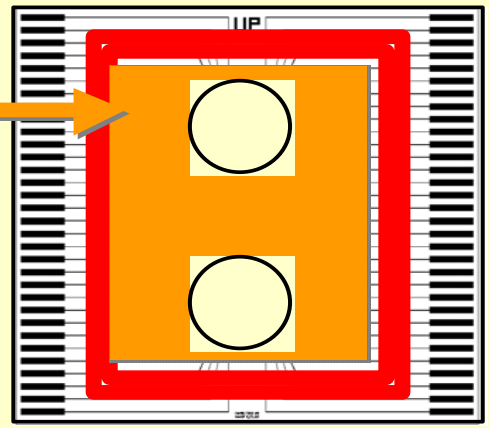


5. The D1SGH is aspirated and the tissue is minced with two sterile #20 scalpel blades.

6

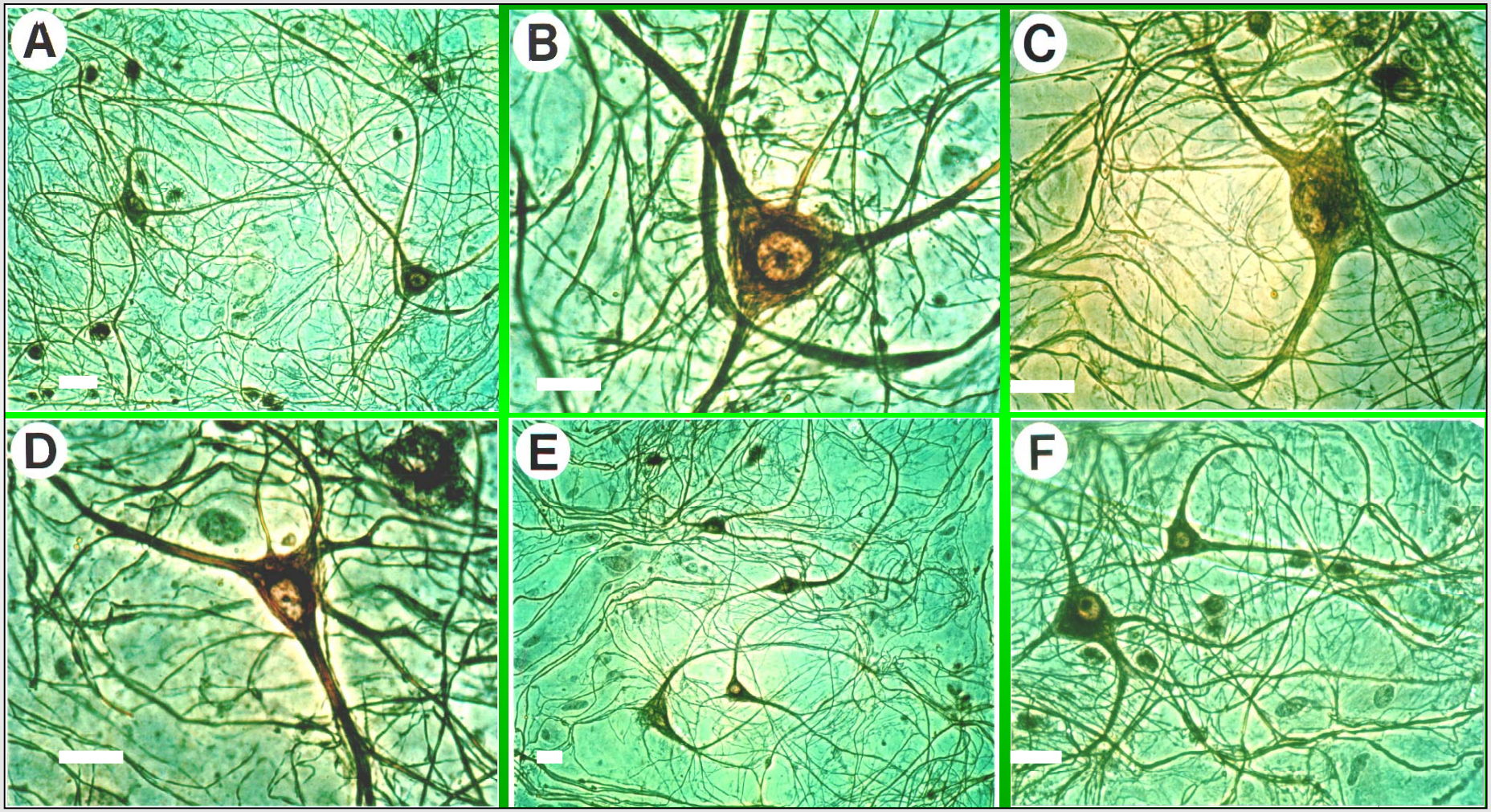


6. The spinal cord pellet was triturated in 5 ml MEM + 10% horse serum/10% fetal bovine serum (MEM 10/10). Minced frontal cortex was triturated in 5 ml DMEM + 5% horse serum/5% fetal bovine serum + B27 + 8 µg vitamin C/ml (DMEM 5/5).

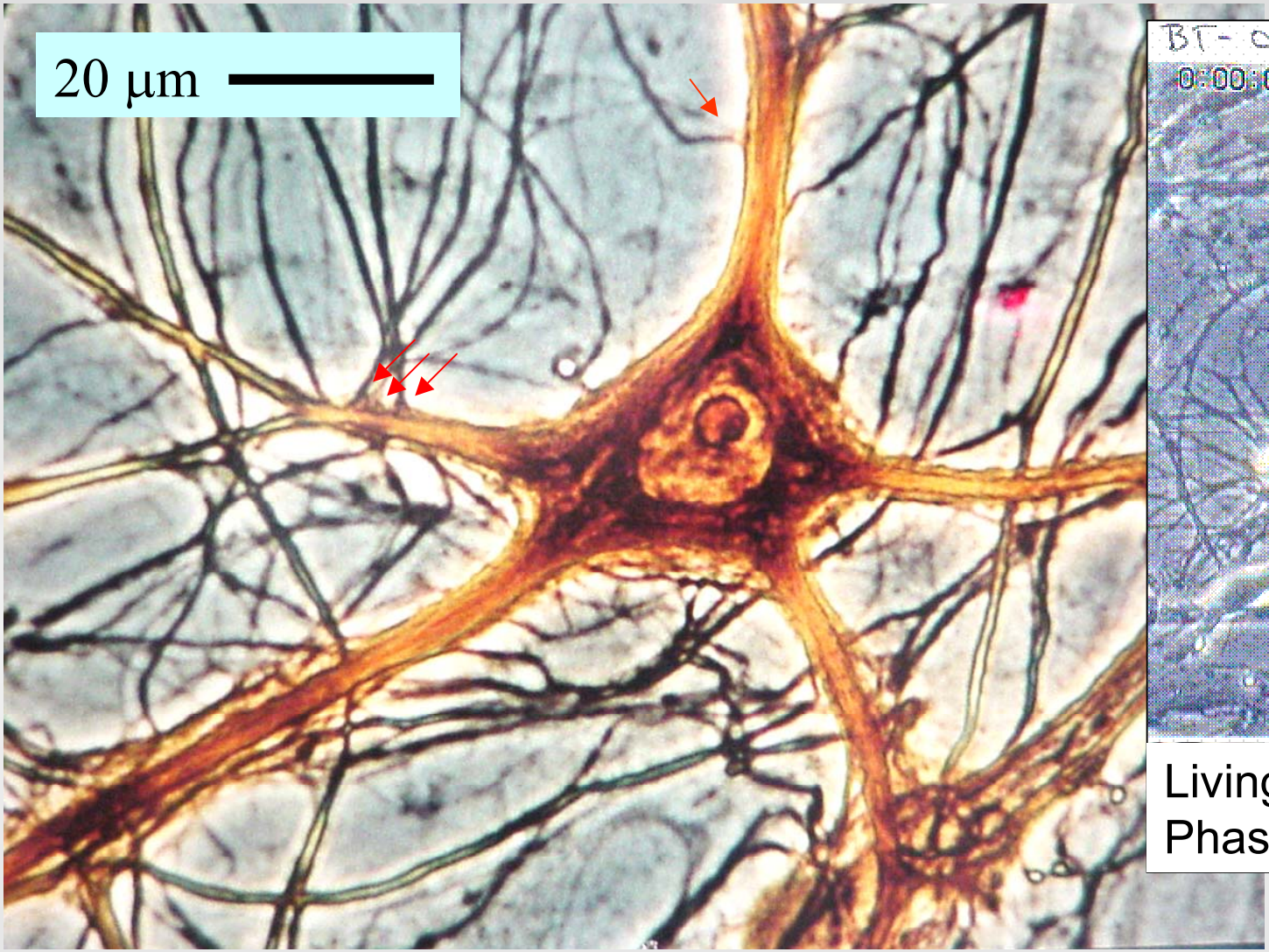


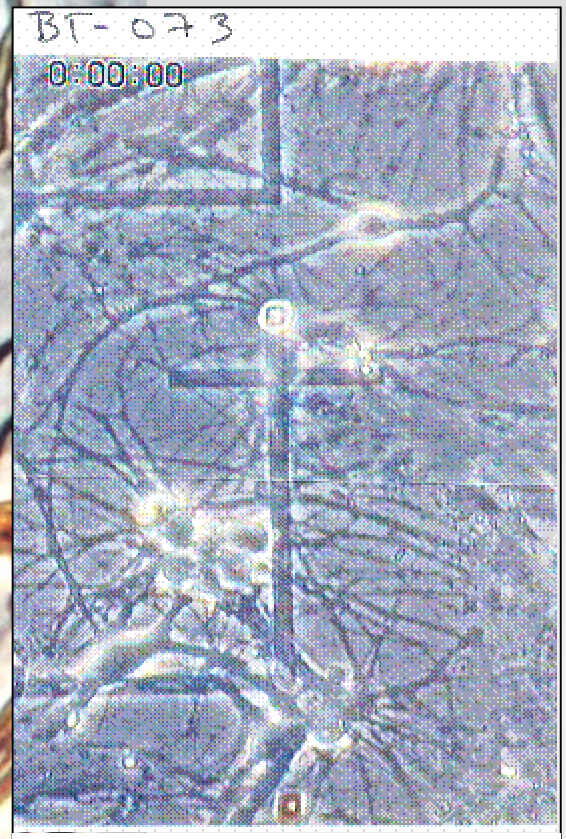
Very few modifications over method by Ransom et al. '77 (Phil Nelson, NIH)

Tissue Source: Murine dissociated Spinal Cord, Frontal Cortex, and Auditory Cortex cultures.



Spinal cord culture 138 days (4.6 months) after seeding. Loots modified Bodian stain. Bars: 20 μm. Useful lifetime > 6 months.



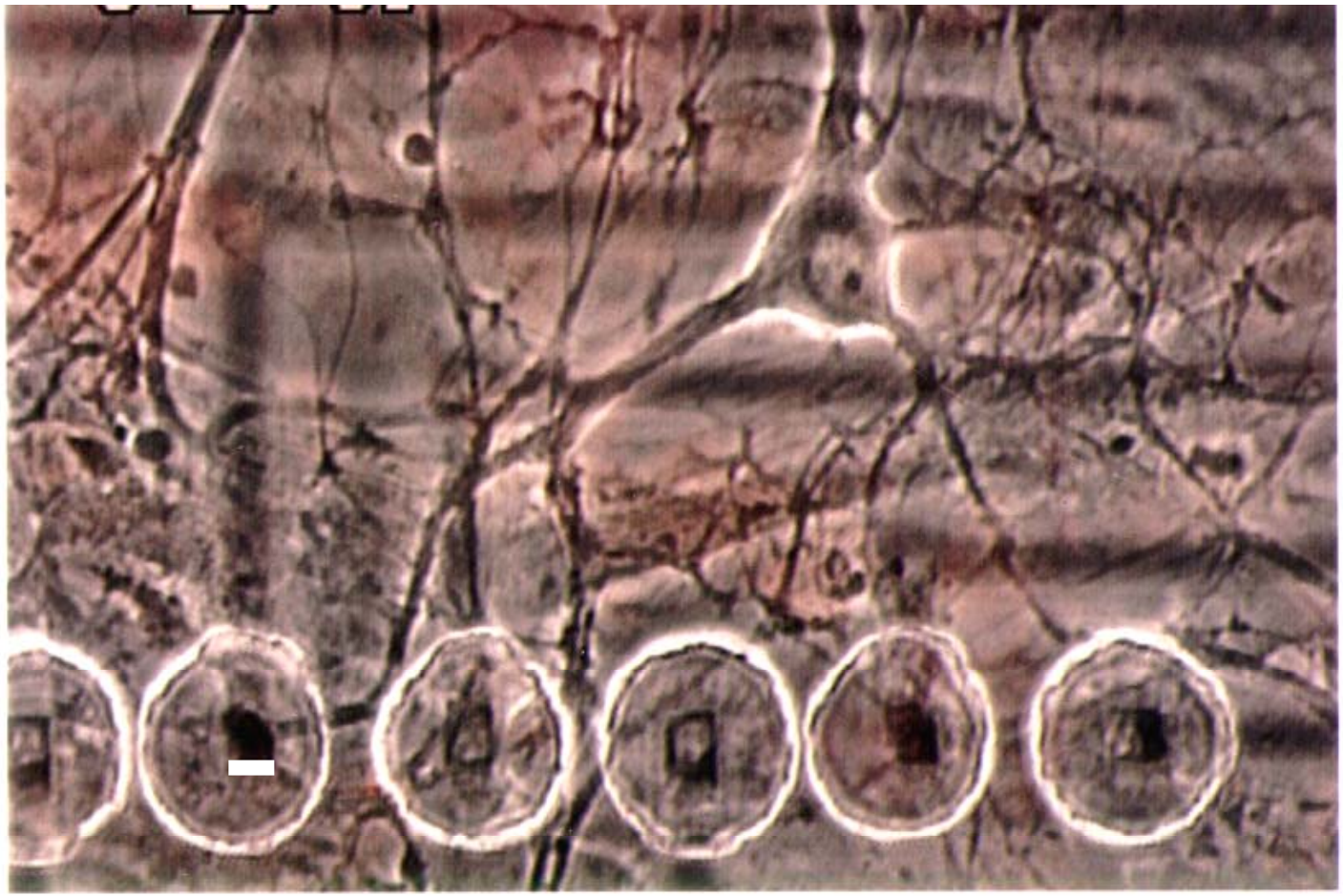


Living cells on MEA.
Phase Contrast

Murine spinal cord neuron after 3 months in culture.
Note numerous small synapses along dendrites.
Neurofilament antibody stain.

Center for Network Neuroscience
UNIVERSITY of NORTH TEXAS

Live Neurons on Electrode Array

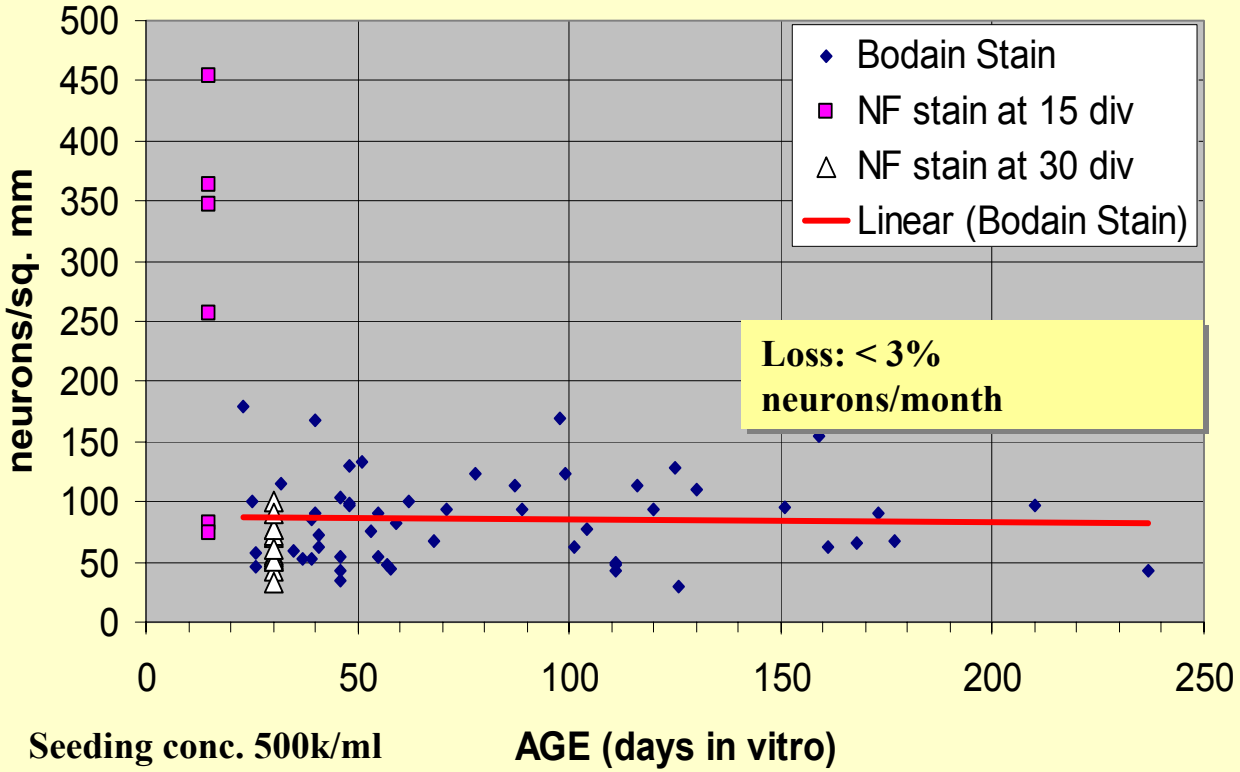


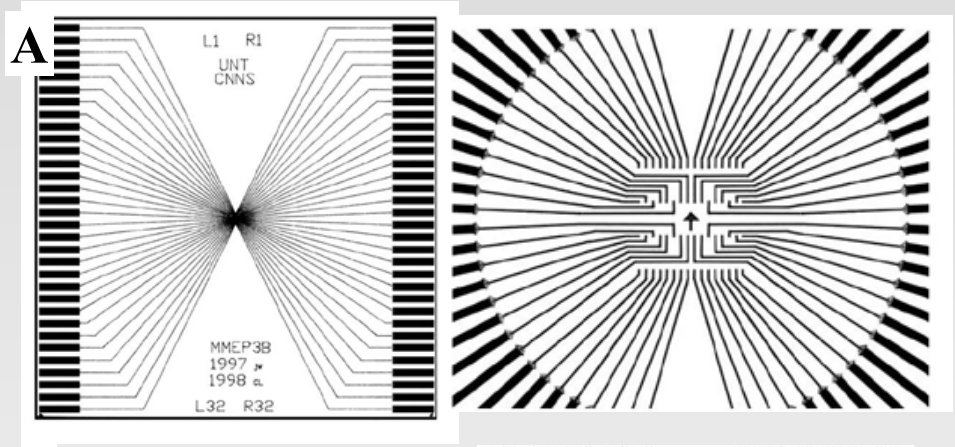
Bar = 8 μm

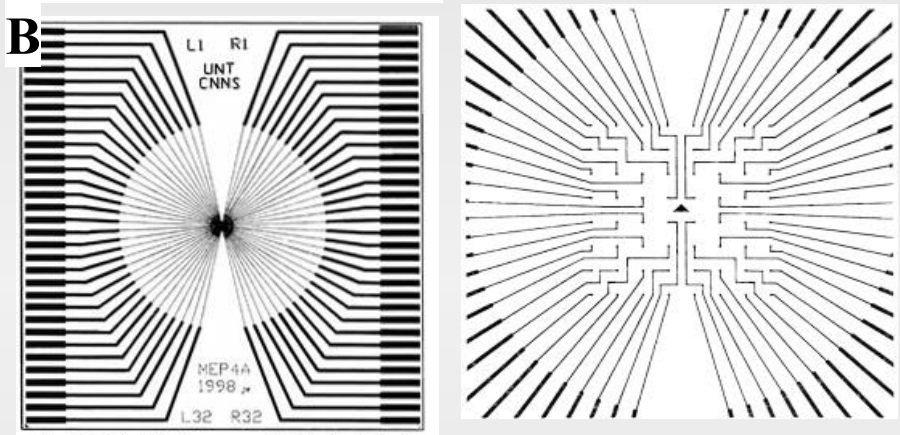
Living neuron as seen with phase contrast microscopy.

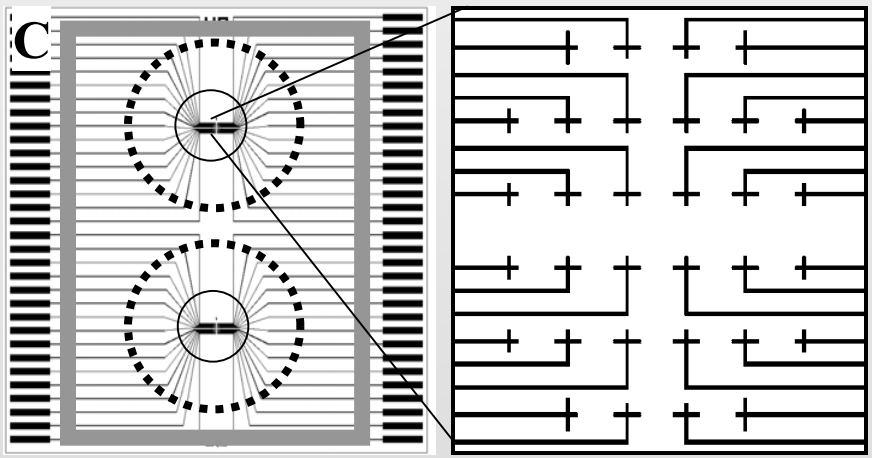
In low density cultures, much of the network morphology is visible, allowing optical monitoring of cell stress and death

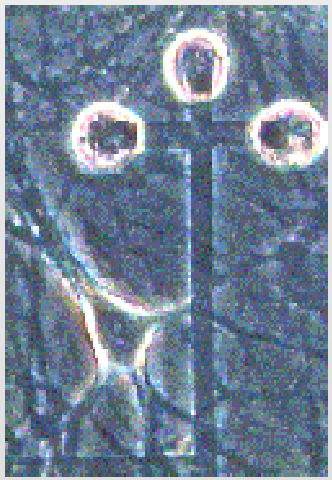
NEURONAL SURVIVAL in CULTURE

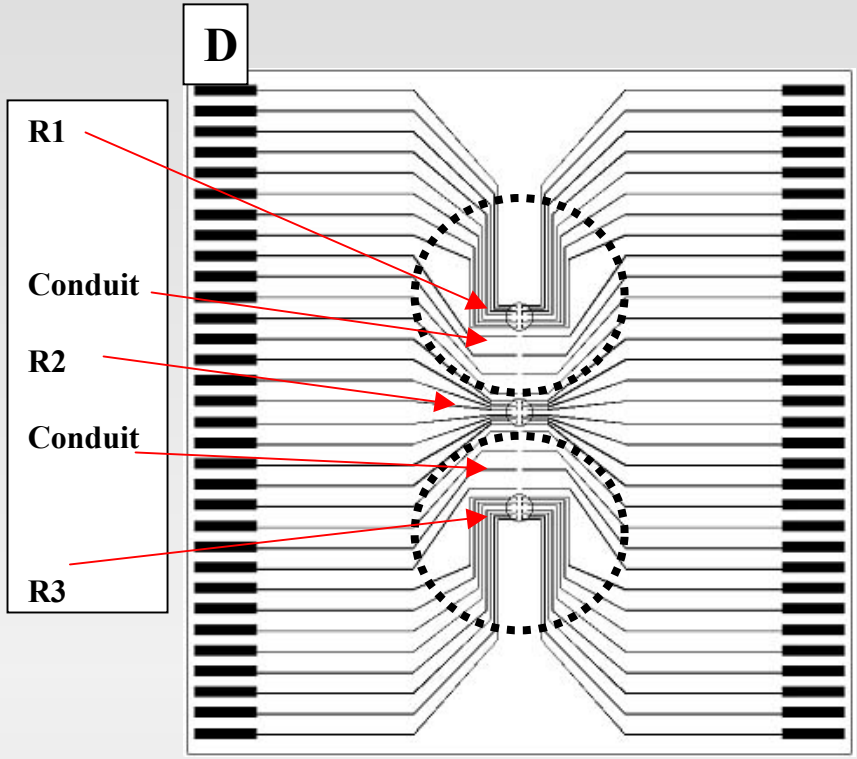






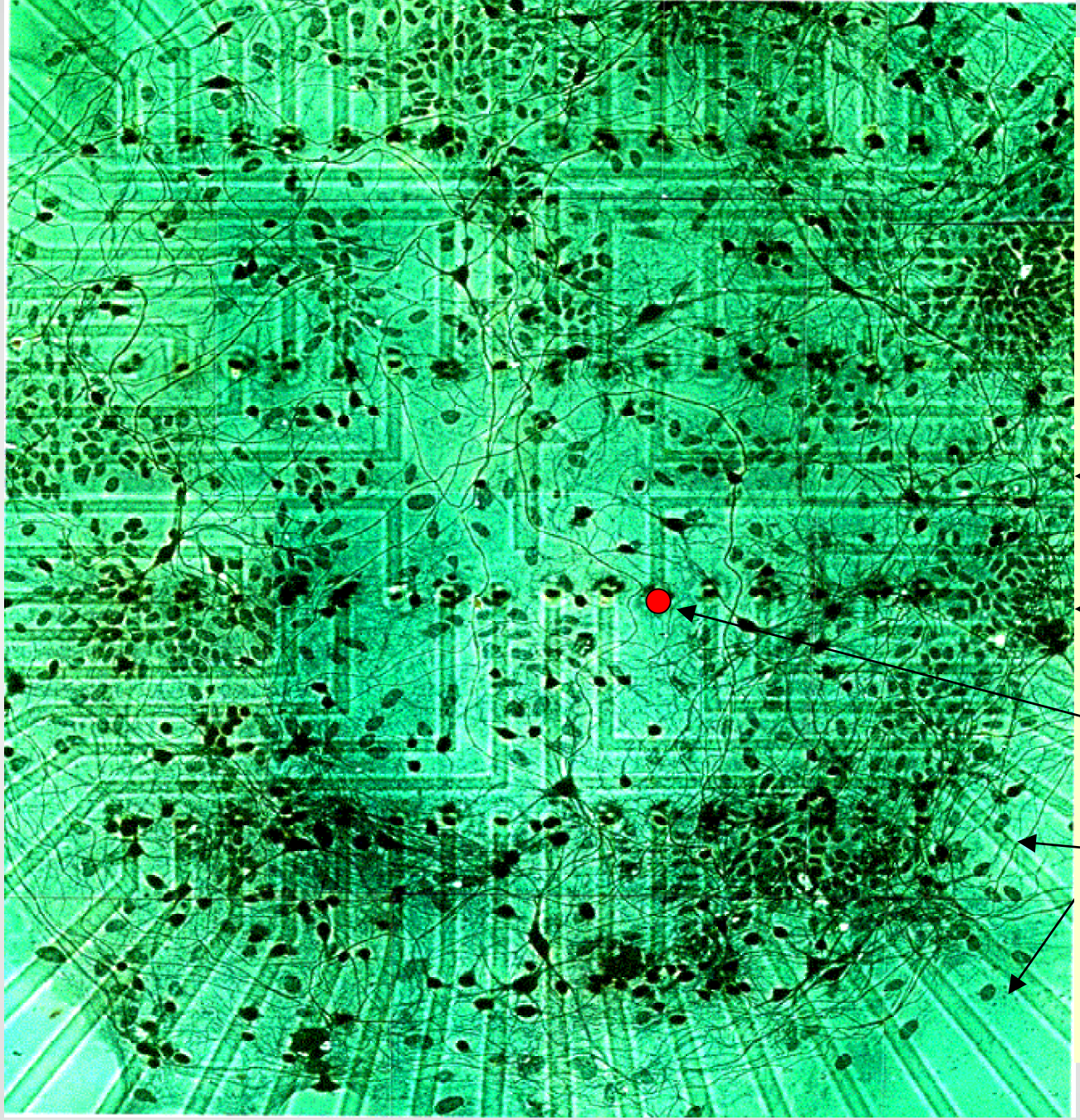






A. MMEP 3 (4x16)
B. MMEP 4 (8x8)
C. MMEP 5
(2 networks)

D. MMEP 6



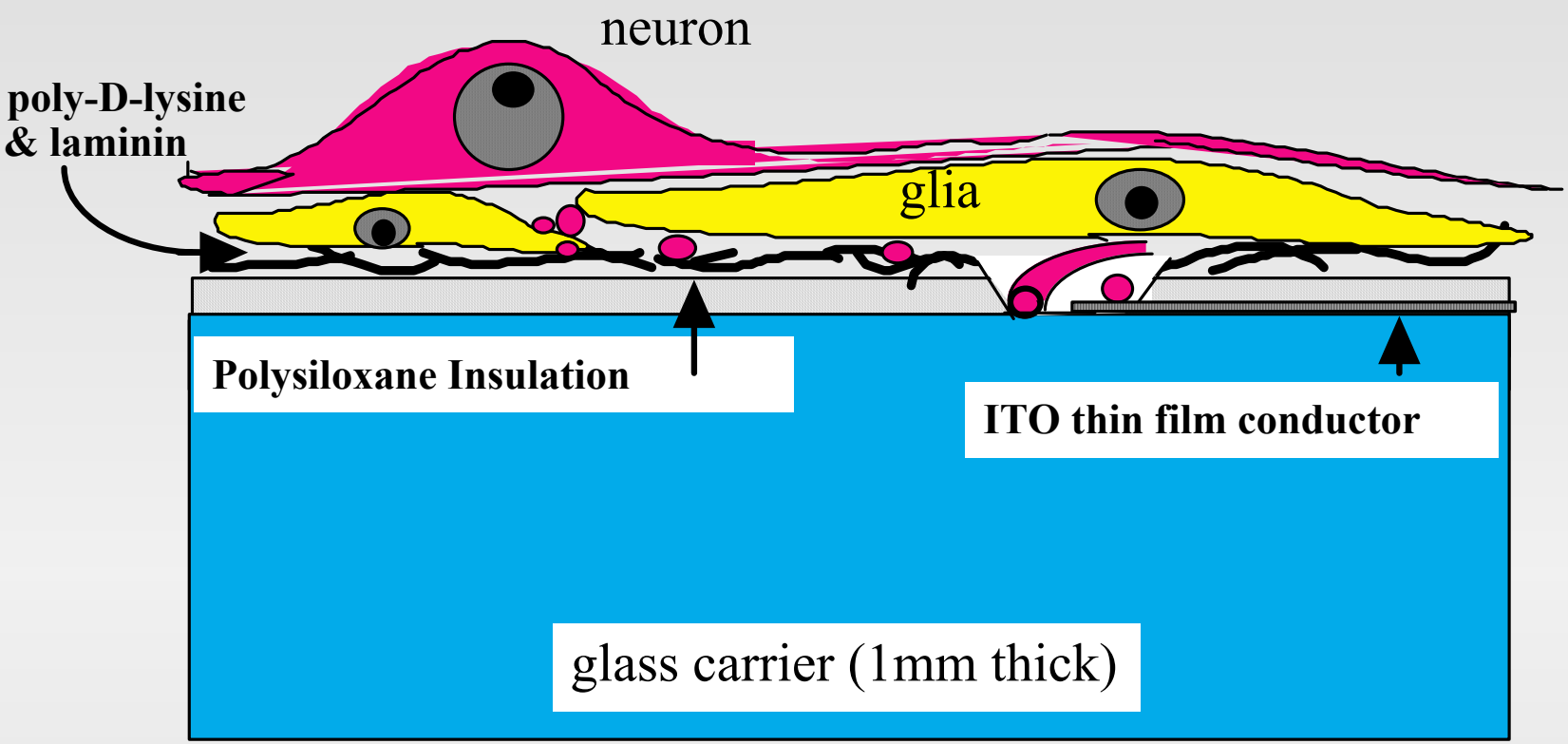
Low density culture on microelectrode array.

indium-tin oxide conductors (8-10 μm wide)

recording craters

glial nuclei

Glass substrates allow better microscopy and quality control

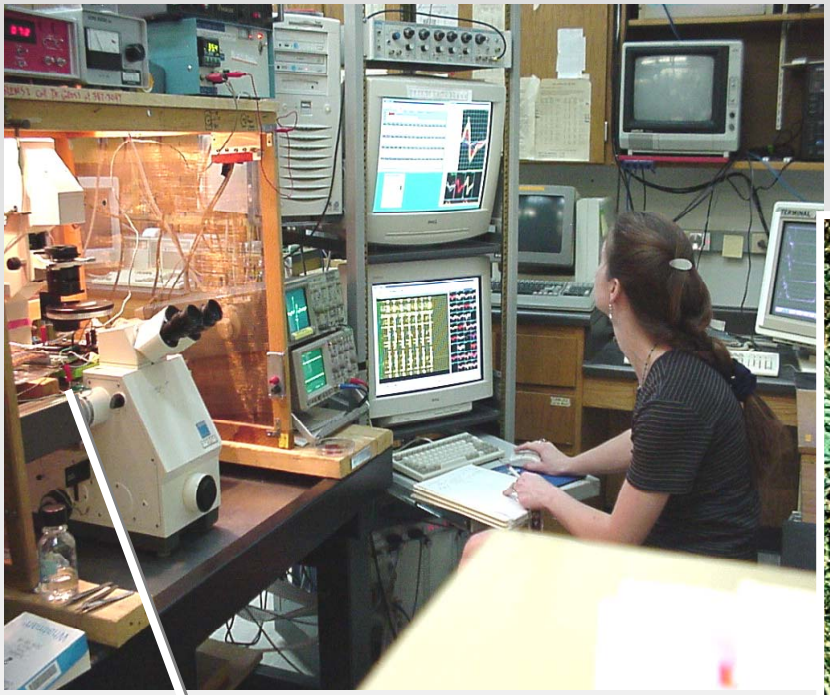


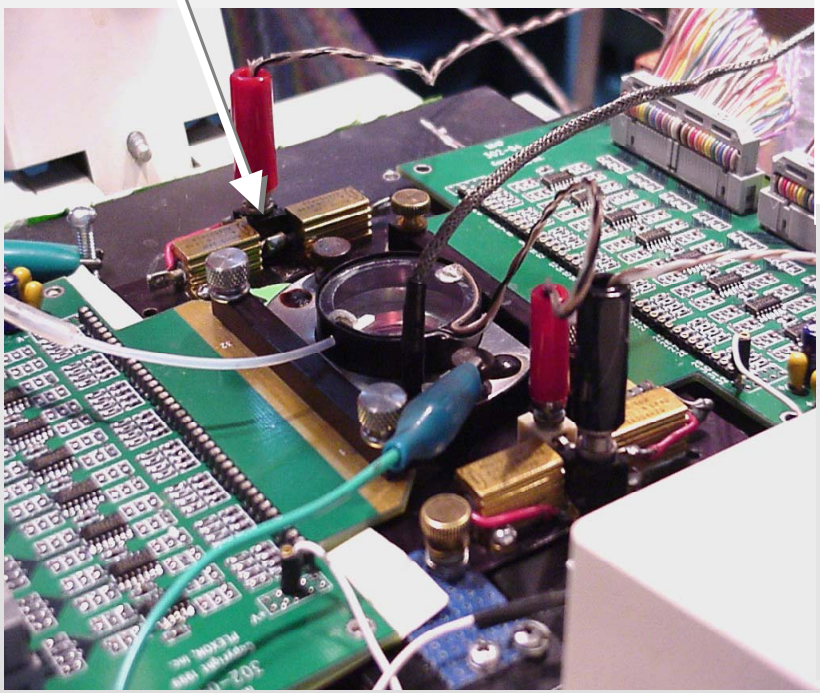
poly-D-lysine: 30kD to 70kD, 25 µg/ml, Boehringer-Mannheim

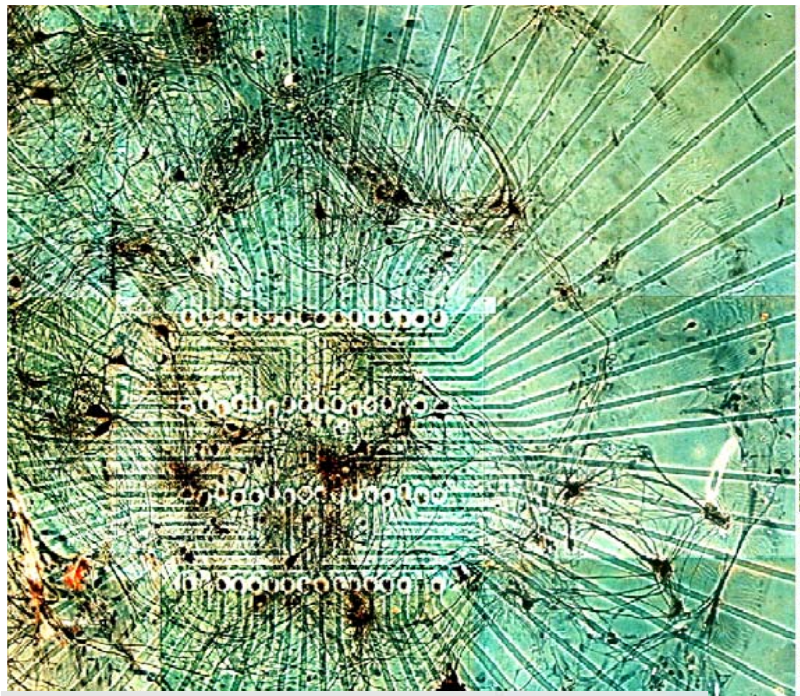
polysiloxane: Dow Corning DC 648; flamed to oxidize methyl groups to OH groups and other radicals.

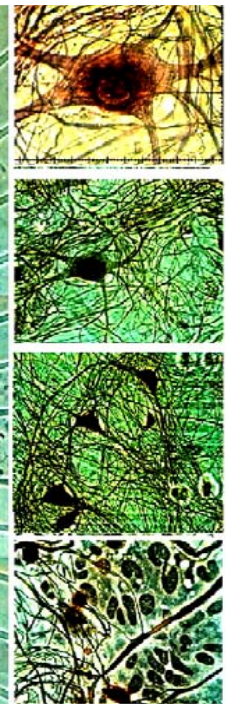
RECENT CHANGE: methylsilicone resin (PS233, Glassclad RC, United Chemical Technologies, Bristol, PA.).

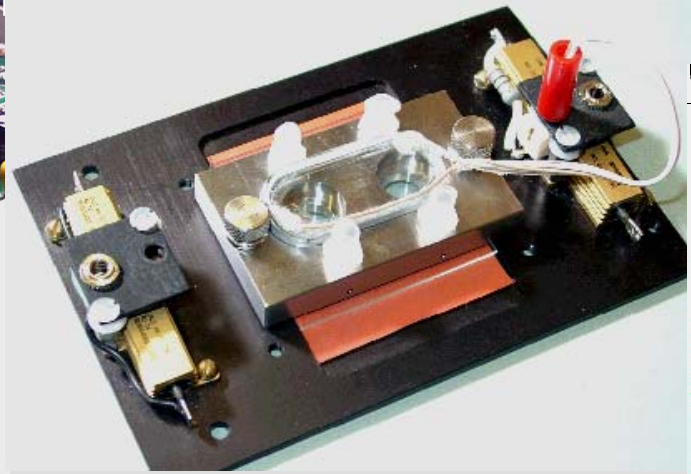
MULTICHANNEL WORKSTATION

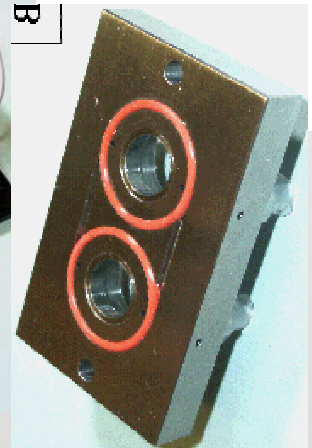








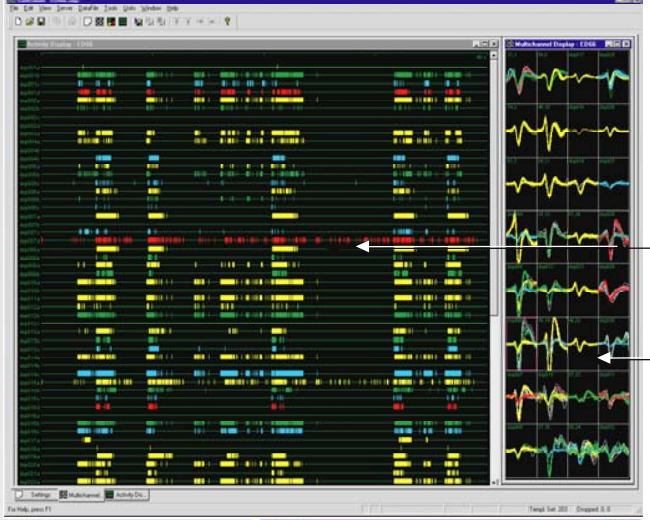




DATA ACQUISITION

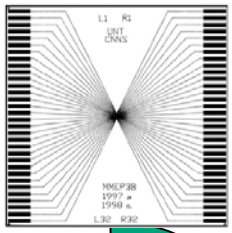
PLEXON DISPLAY

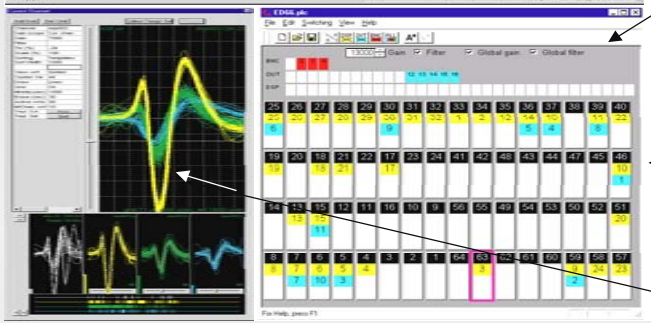
(Plexon , Inc., Dallas, TX)



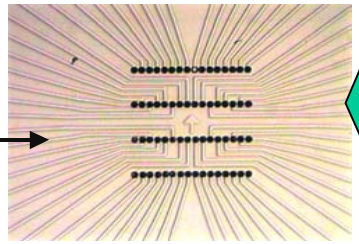
MULTI-UNIT ACTIVITY DISPLAY (40 s)

ACTION POTENTIAL SIGNATURES





Electrode selection client

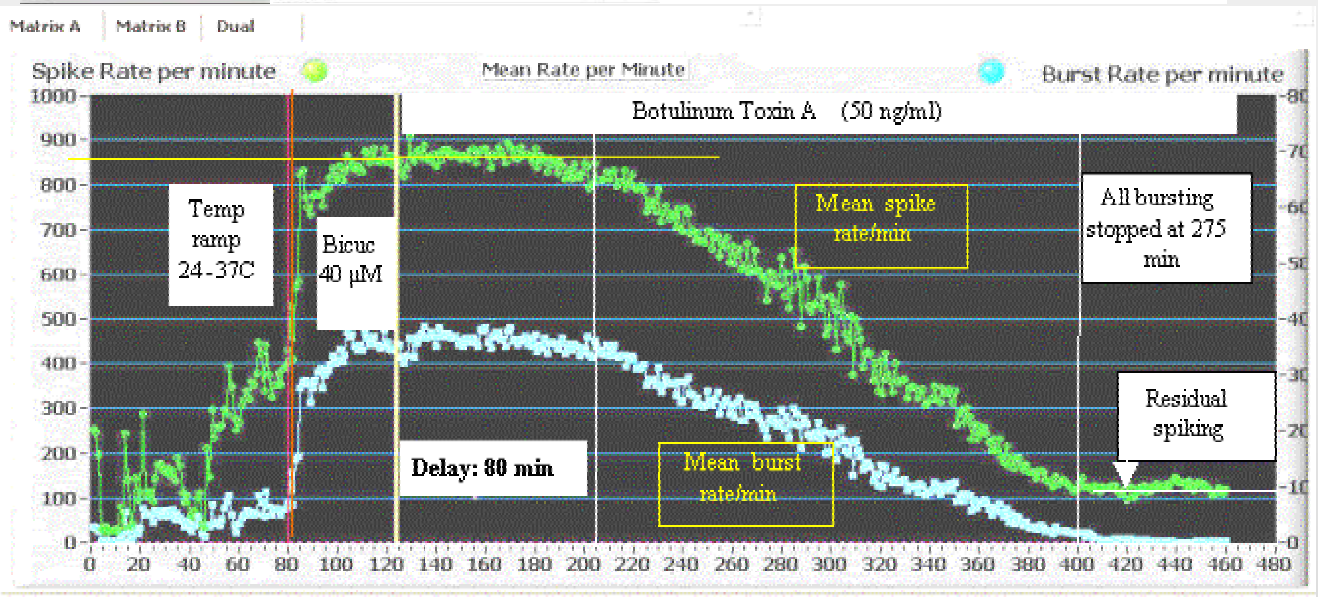


Current electrode window

ACTIVITY VARIABLES

- # Channels Active
- Spike Rate
- Spike Shape
- Burst Rate
  - Burst Duration
  - Burst Period
  - Burst Interval
  - Burst Amplitude (i)
  - Mean Spikes/Burst
- CV's for all variables
- Coordination

ALL VARIABLES
per channel,
network
or subset of channels



CNNS
NACTAN
program

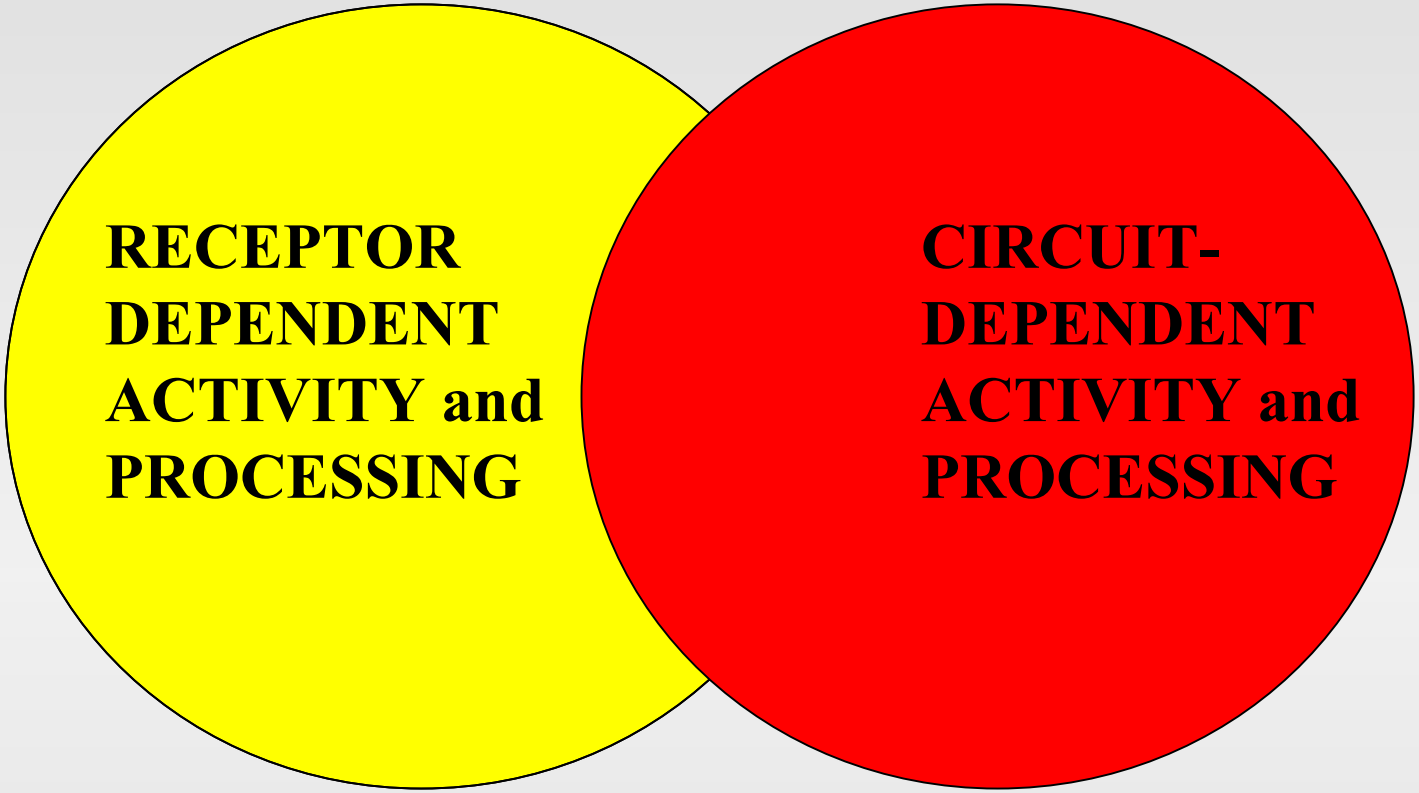
Center for Network Neuroscience
UNIVERSITY of NORTH TEXAS

Neuronal networks in culture are always spontaneously active

Changes in the spontaneous activity reflect physical and chemical changes in the environment!

Networks do NOT mimic the olfactory system but are physiological detectors of changes in CNS mechanisms and of CNS malfunction.

PRIMARY RESEARCH DOMAINS ADDRESSABLE IN CULTURE



Cultures are pharmacologically "histiotypic" and can be used effectively for biochemical and pharmacological experiments.

TOXICOLOGY/DRUGS/BIOSENSORS

Presently difficult in culture because exact circuits cannot be defined. Studies of coarse structure/function relationships are possible.

Summary

Nerve cell networks can be grown on microelectrode arrays and maintained viable for many months.

Cell-electrode coupling is stable and allows monitoring of many active units.

Recording hardware is now available (at reasonable prices) to support long-term, parallel, multichannel recording.

Software and display methods are evolving to provide adequate data processing.

"*Multichannel recording from nerve cell networks is like drinking from a fire-hose.*"

2. Network Pharmacology

Networks in cell culture are pharmacologically histiotypic (ie. like the parent tissue).

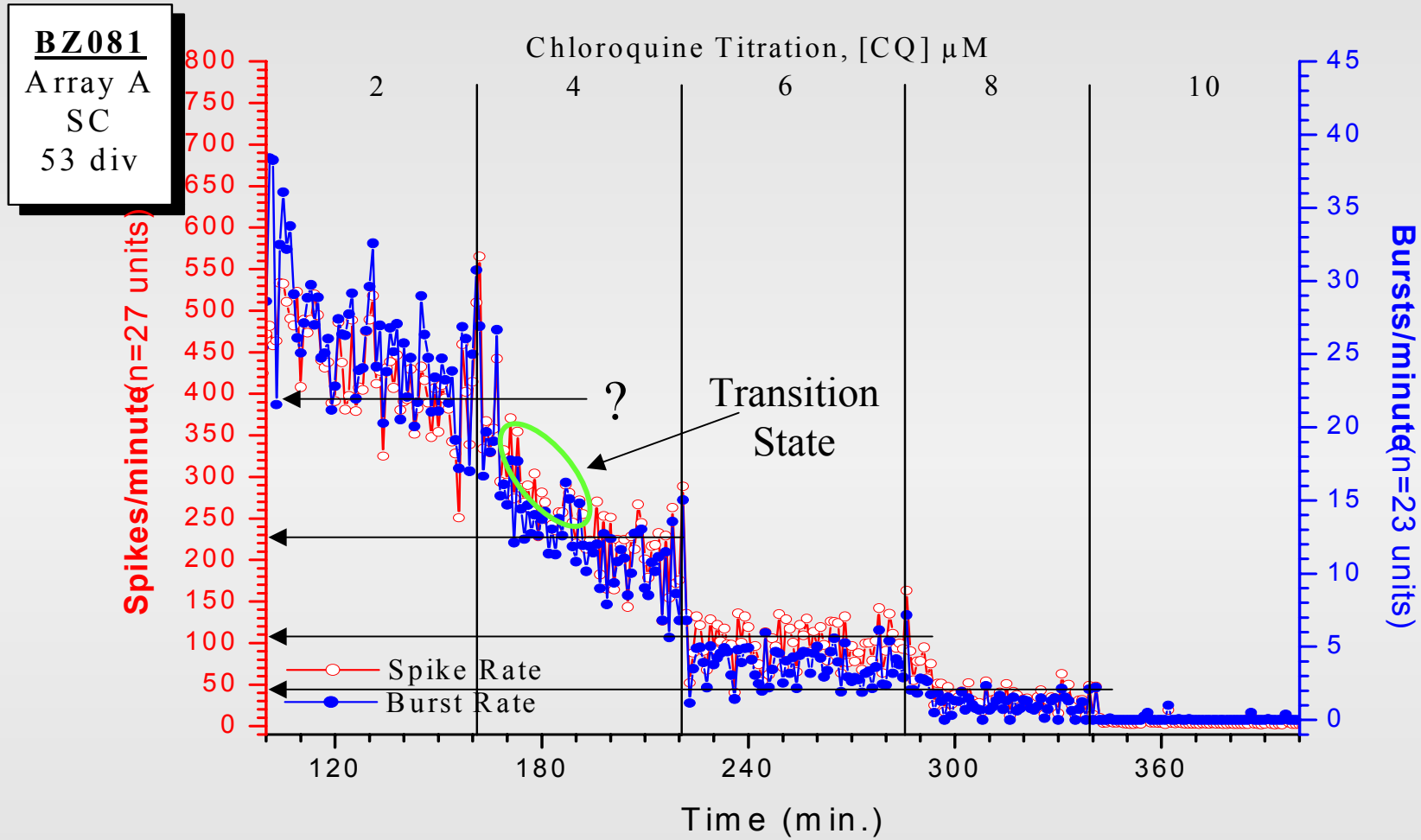
2.1 Dose-Response Curves

2.2 Network Responses to Ethanol

2.3 Network Responses to Fluoxetine (prozac)

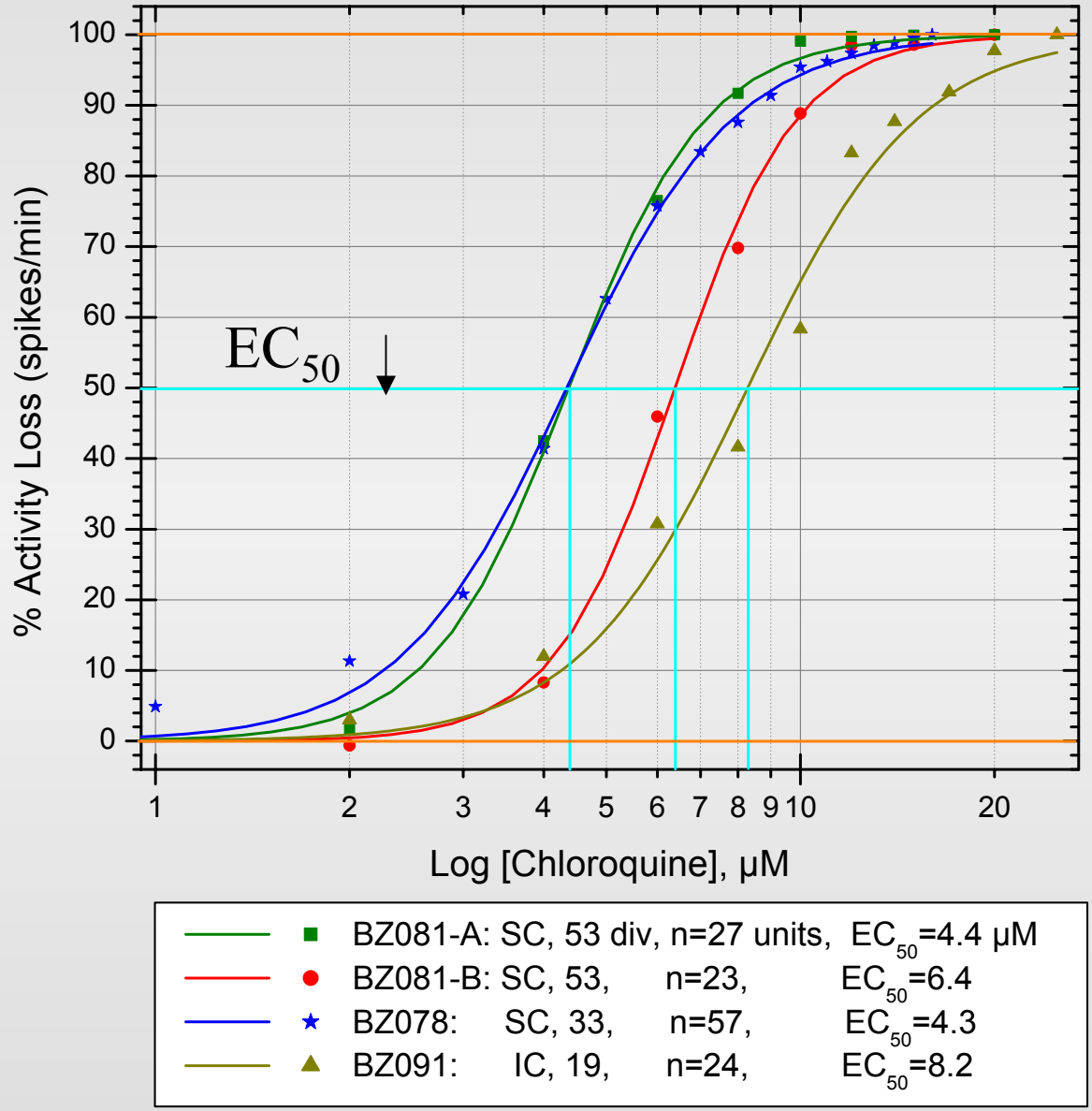
2.4 Unanticipated Poisons: when new molecules do not behave.

ANATOMY of a DOSE RESPONSE CURVE

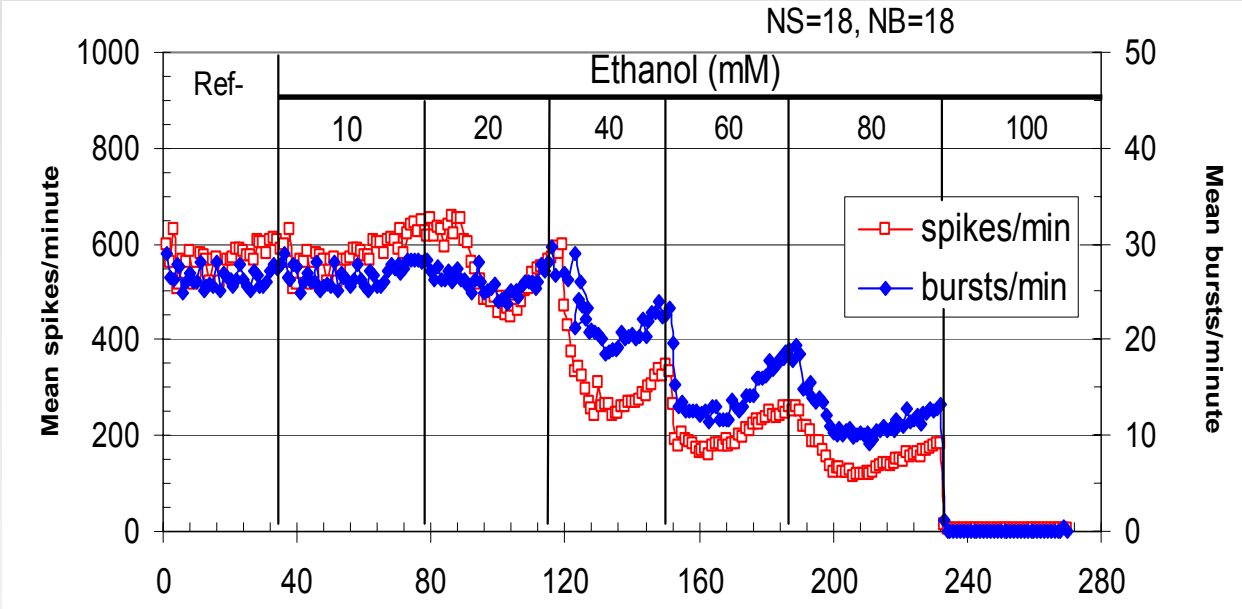


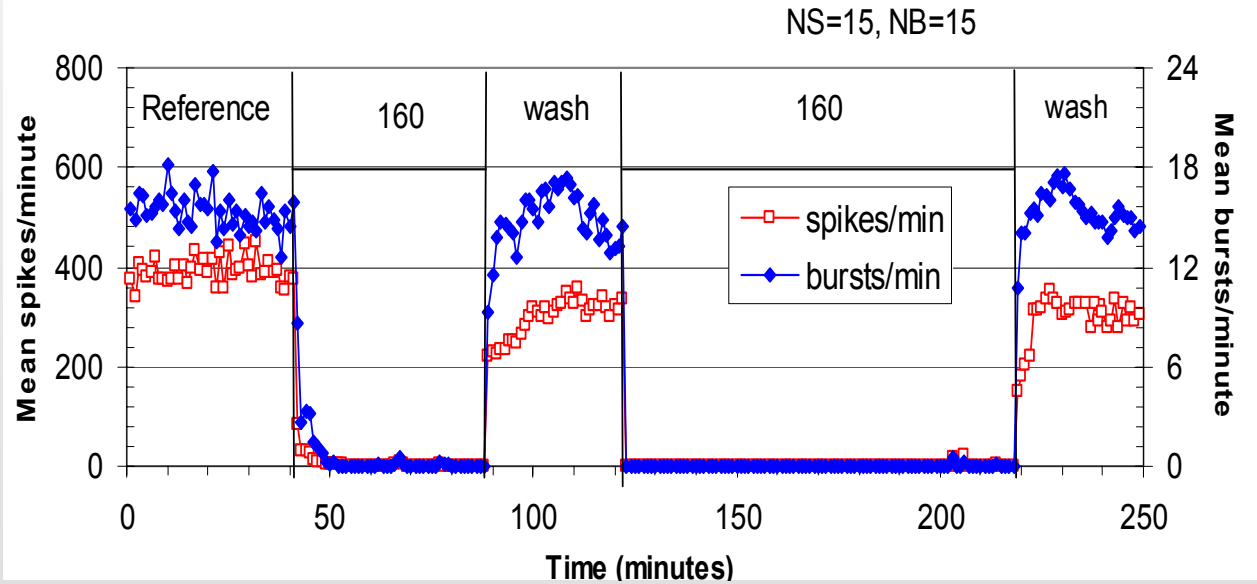
Dose-response curves require steady states (horizontal lines for spike data in red). Under 2 µM CQ, the steady state is not well defined. Transition states are ignored.

Chloroquine Concentration-Response Curves

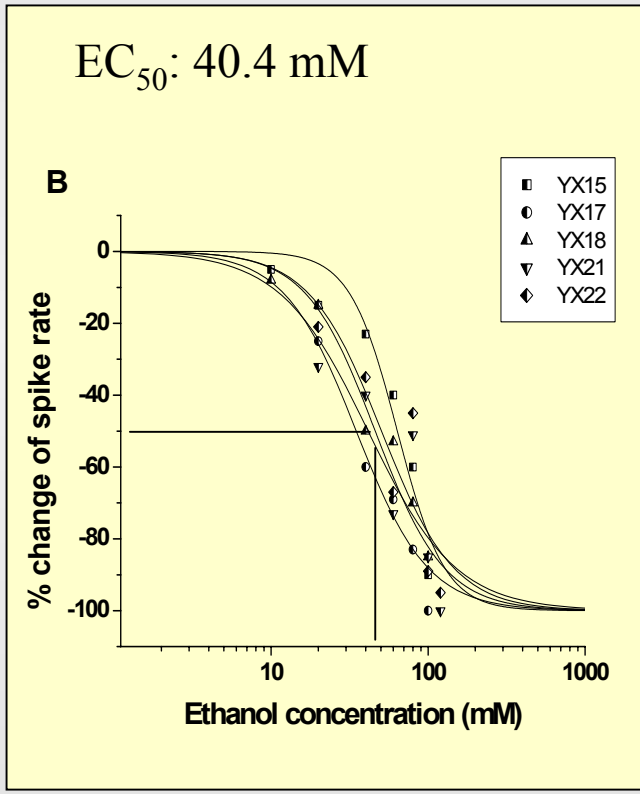


Typical response of frontal cortex culture to **ETHANOL**





System Validation Experiments



Yun Xia

MS Student

Comparison with *in vivo* Data

(Little, 1990)

Mammals	concentration(mM)	effects
Rats	20	sedation
Mice	40	a lack of righting reflex
Mice	122	sleep and hypothermia
Human	5-15	slight impaired attention, judgment and coordination
Human	30-55	total mental confusion
Human	above 100	Coma or death

Culture: 20 → 40.4 → 100- 140 mM

20 initial decrease

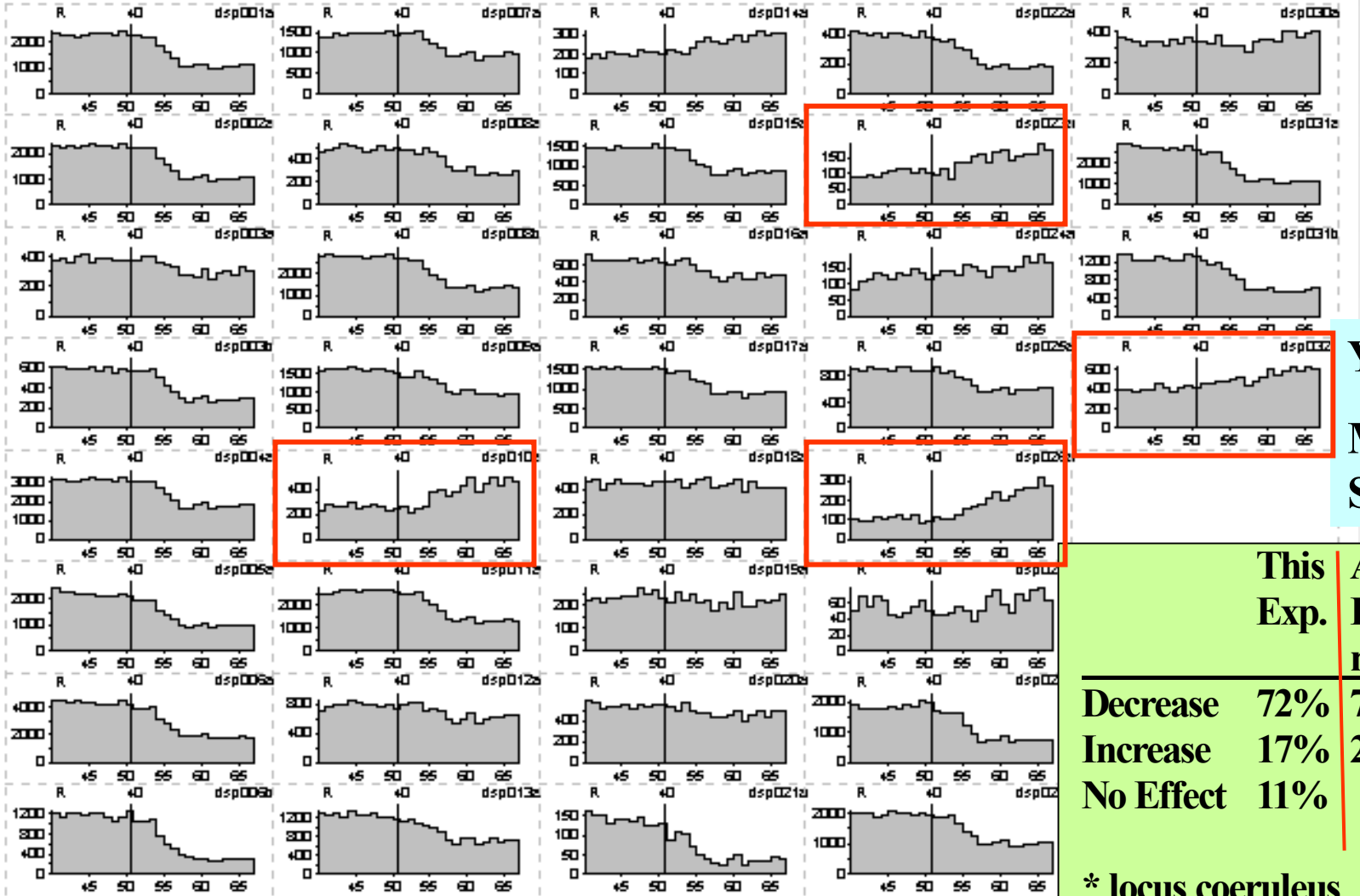
40.4 EC_{50}

100- 140 mM cessation of all activity

Based on spike and burst rates only.

Plexon/
NEX

Unit-specific effects in response to ethanol



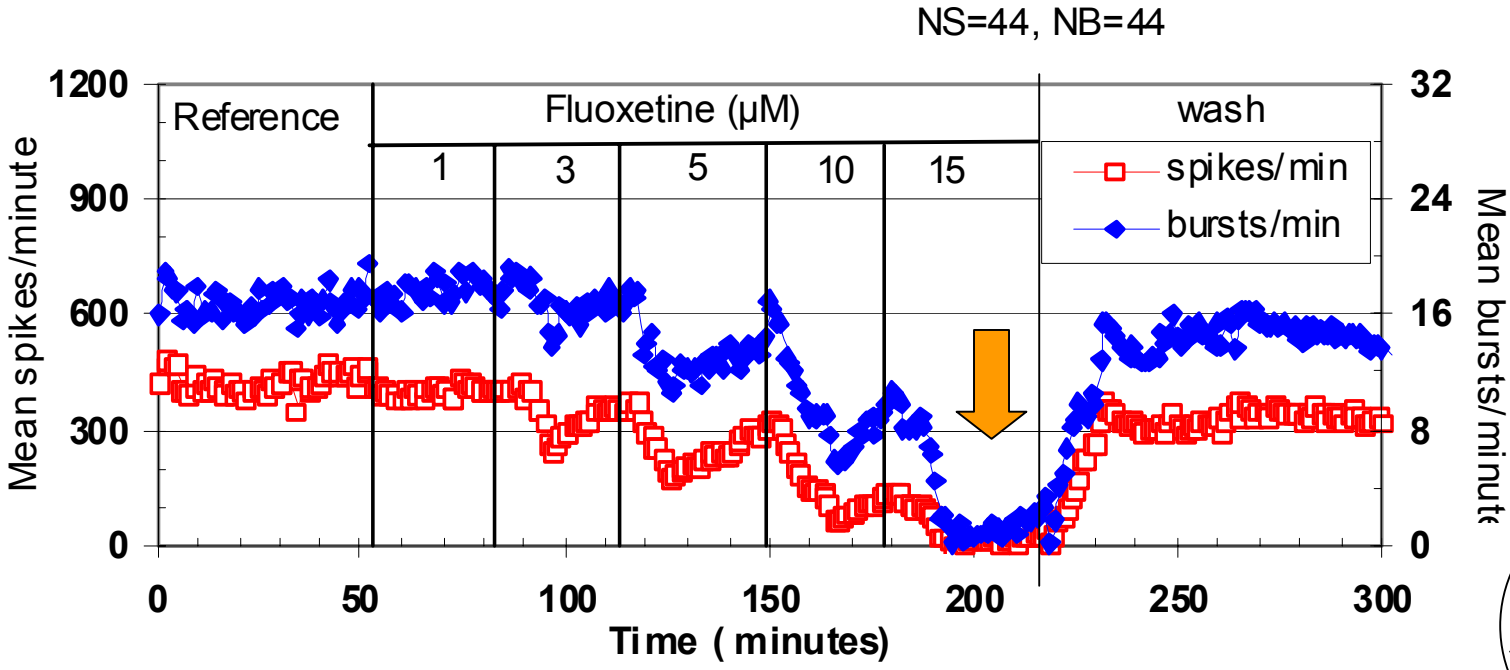
increases

Yun Xia

MS Student

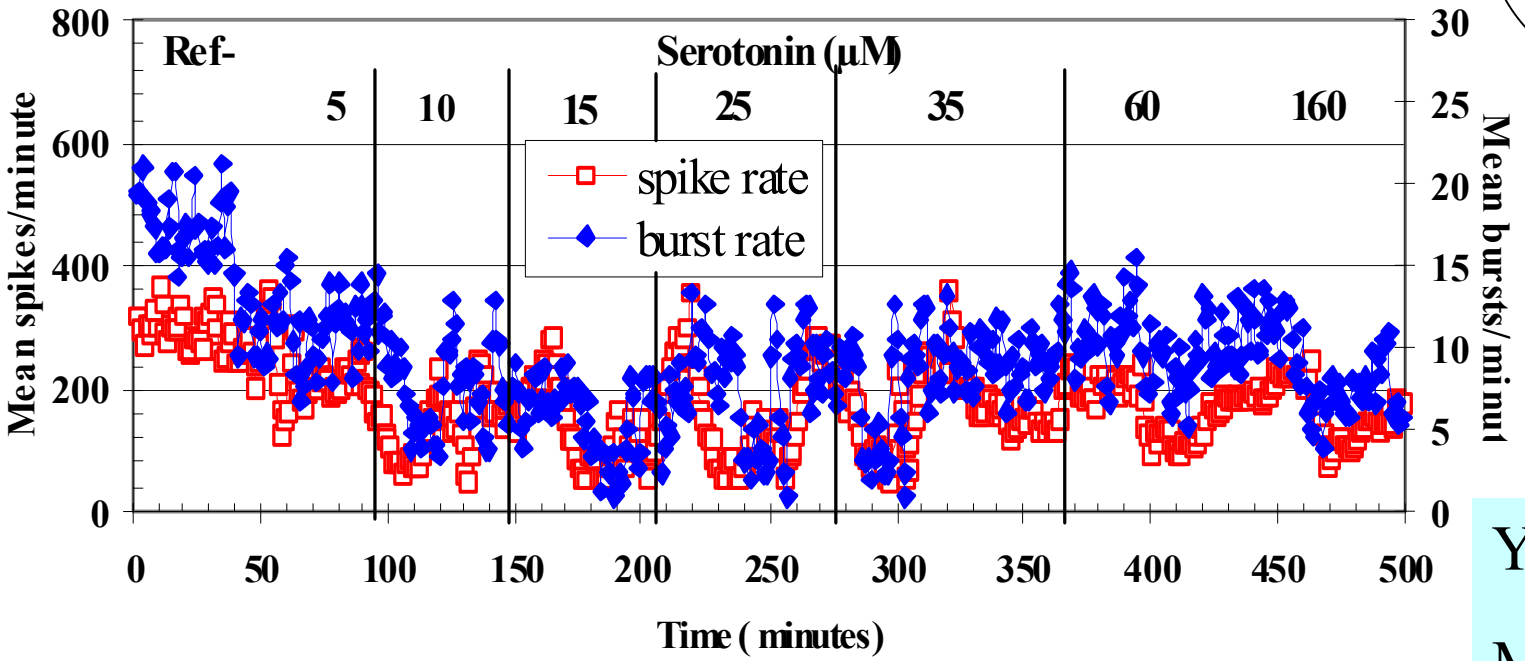
	This Exp.	All Exps n=14	Rats* (IP)
Decrease	72%	71%	62%
Increase	17%	20%	22%
No Effect	11%	9%	16%

* locus coeruleus (Pohorechy and Brick, 1977).



Prozac

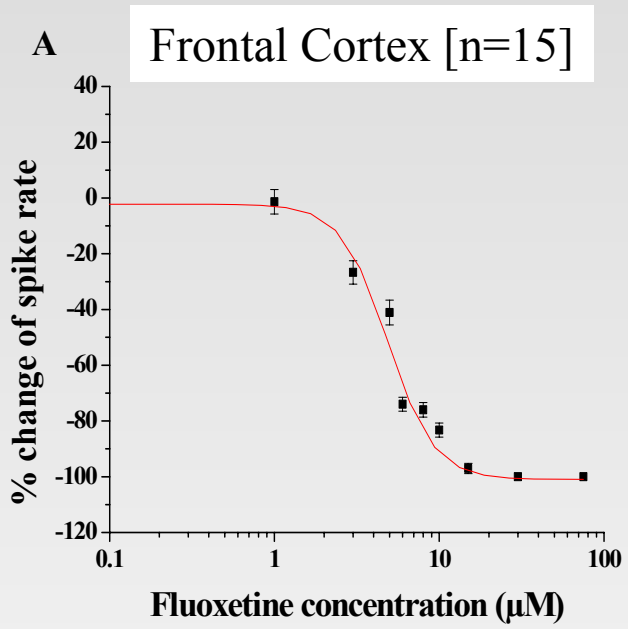
Secondary Effect of Prozac?

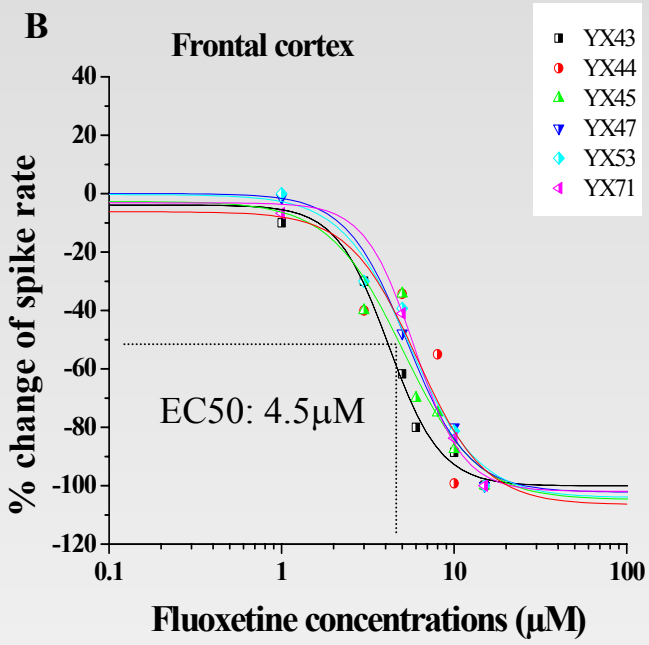


Serotonin

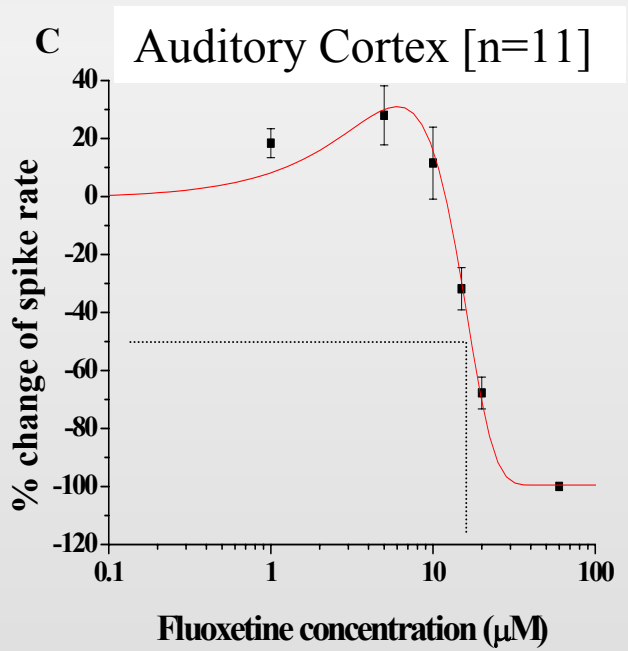
Yun Xia

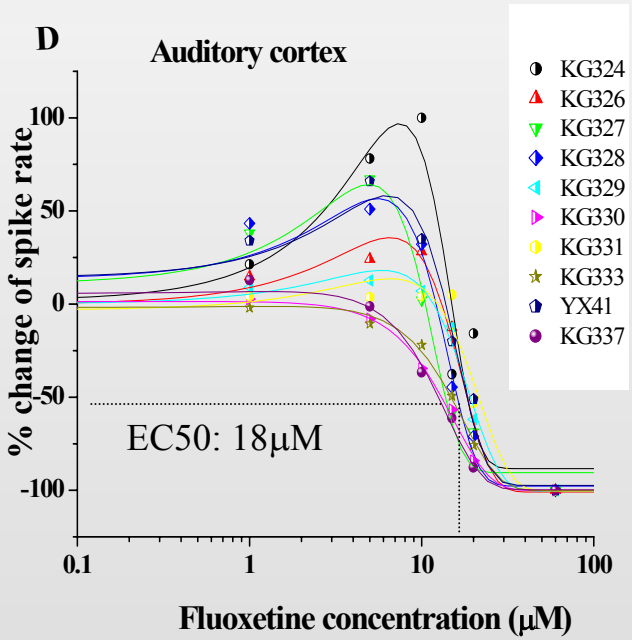
MS student



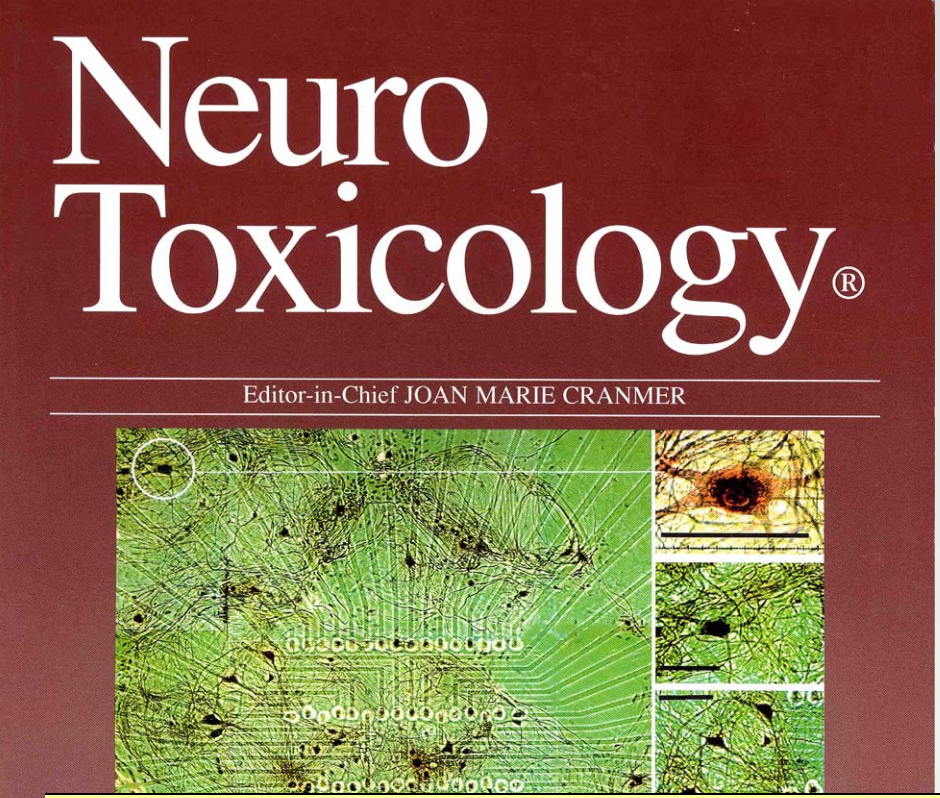


Concentration -Responses for Fluoxetine (Prozac)





Tissue Specificity: The frontal cortex responds differently than the auditory cortex.



SCREENING for SECONDARY BINDING

Screening of 7 newly synthesized weak AChE blockers for alleviation of Alzheimer's syndrome synthesized at the University of Perugia, Italy by Prof. Vincenzo Talesa.

BIOCHEMICAL DATA CONFIRMED BINDING TO AChE.

However, two out of seven compounds were irreversible inhibitors of activity (**unexpected secondary binding**.)

NOVEL ACHE Blockers

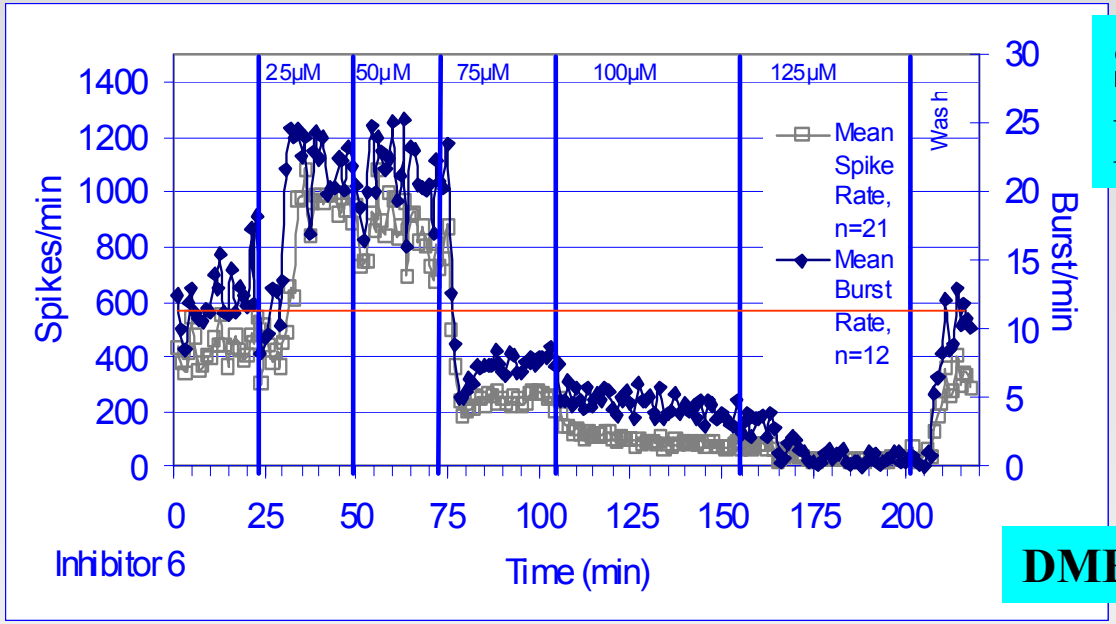
	AChE Inhibition. Const Ki (M)	Inhibit. Type	Excitat.	Inhibit.	Reversi-bility	No of experiments
$Ch^+O\text{-}CO\text{-}S(CH_2)_2S\text{-}CO\text{-}OCh^+$	$1.0x10^{-6}$	Mixed	10		R	2
$Ch^+O\text{-}CO\text{-}S(CH_2)_3S\text{-}CO\text{-}OCh^+$	$1.4x10^{-6}$	Mixed	50		R	3
$Ch^+O\text{-}CO\text{-}S(CH_2)_4S\text{-}CO\text{-}OCh^+$	$1.0x10^{-6}$	Mixed	10		R	2
$Ch^+O\text{-}CO\text{-}S(CH_2)_5S\text{-}CO\text{-}OCh^+$	$1.4x10^{-6}$	Mixed	NE	NE	--	2
$Ch^+O\text{-}CO\text{-}S(CH_2)_6S\text{-}CO\text{-}OCh^+$	$1.4x10^{-6}$	Mixed		350	I	2
$DMEA^+O\text{-}CO\text{-}S(CH_2)_4S\text{-}CO\text{-}DMEA^+$	$3.6x10^{-7}$	Mixed	25	125	R	3
$DMEA^+O\text{-}CO\text{-}S(CH_2)_6S\text{-}CO\text{-}DMEA^+$	$5.0x10^{-7}$	Mixed		200	I	2

NE: no effect; R: reversible inhibition (2 medium changes); I: irreversible inhibition (3 medium changes and 2 hr wait)

Ch^+O- represents choline residues, $DMEA^+$- represents N,N-dimethylethanolamine residues.

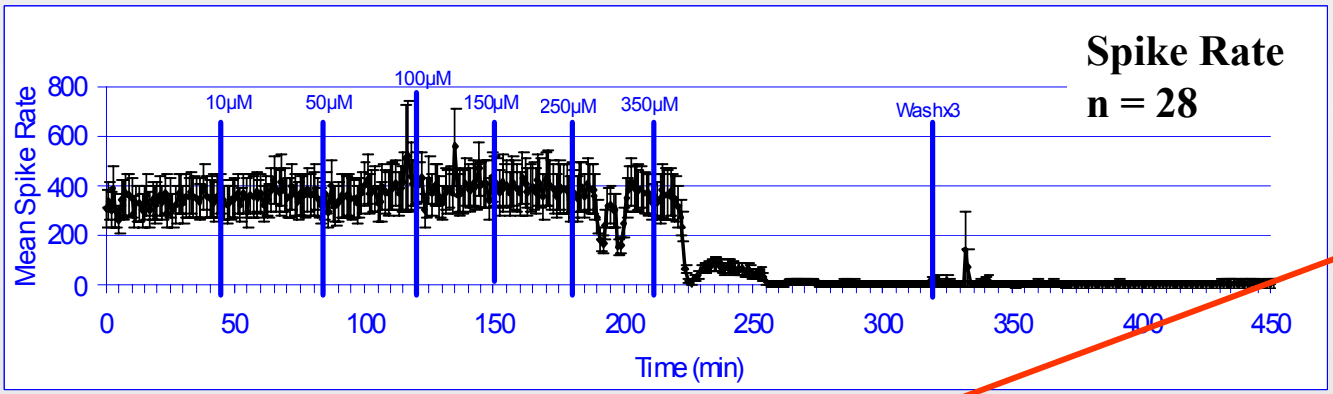
Paper published: Keefer et al, 2001

Spike and Burst Response Profiles for two Compounds

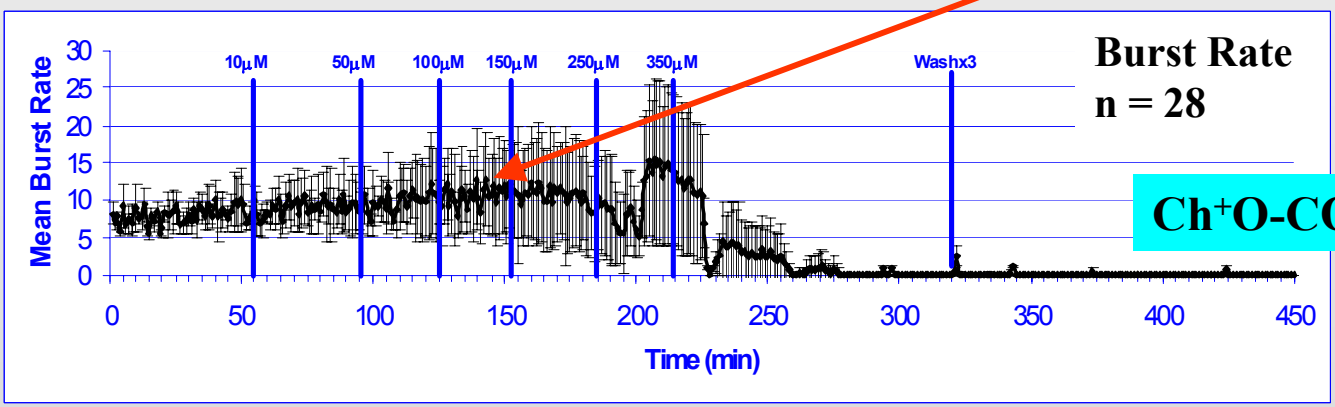


reversible

$DMEA^{+}O\text{-}CO\text{-}S(CH_2)_4S\text{-}CO\text{-}DMEA^{+}$



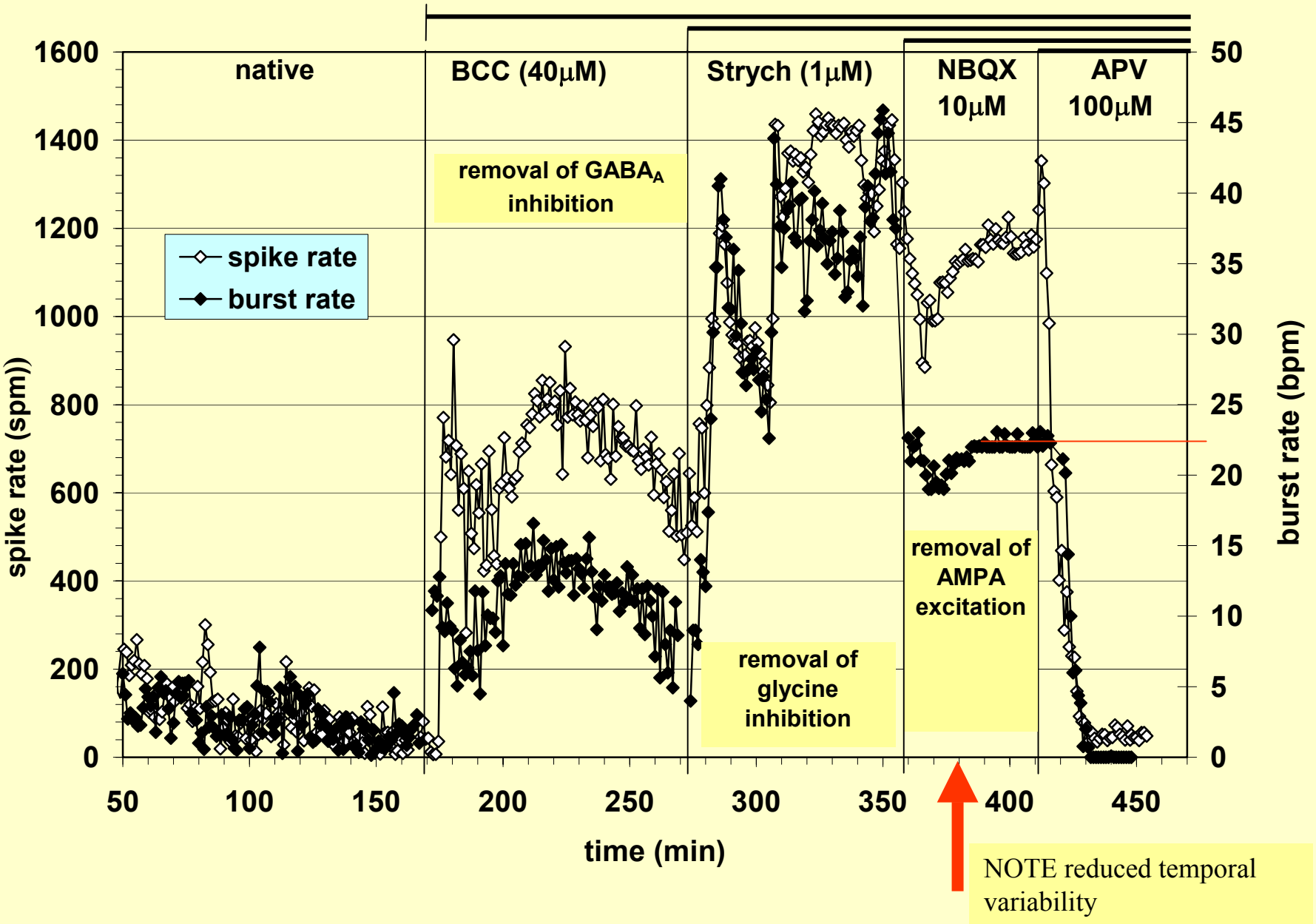
Increases in the magnitude of SD for burst rate reflect a loss of coordination among channels



irreversible

$Ch^{+}O\text{-}CO\text{-}S(CH_2)_6S\text{-}CO\text{-}OCh^{+}$

Blockage of major synaptic driving forces in spinal culture



Chemical Agents & CompoundsTested

98

TOXINS

botulinum toxin A	6
botulinum toxin B	1
tetanus toxin	8
tetrodotoxin	4

BACTERIAL AGENTS: *NONE*

VIRAL AGENTS

gp120 of HIV	4

CHOKING AGENTS

trichloroisocyanuric acid	34
sodium hypochloride	2

FUEL & COMPUSTION PRODUCTS

TMPP	14
JP-5 and JP-8	5
Toluene	

NERVE AGENTS
(nerve gas hydrolysis products)

methyl phosphonate	4
pinacolyl methylphosphonate	4
Isopropyl methyl phosphonate	2

TOXIC METALS

lead	15
mercury	18
trimethyl tin chloride	22

HERBICIDES/PESTICIDES

chlorpyrifos (Durspan)	5

Novel AChE blockers (7)

BIOREGULATORS (PHARMA COMPOUNDS):

Acetylcholine	8	Glutamate	7
Ammonia	4	Glutathione	5
AMPA	6	Glycine	12
Apamin	4	L-733,060	3
APV	12	MCPG	3
Ascorbic acid	5	Mg^{+2}	17
Atropine	3	NBQX	
Bicuculline	30	NMDA	15
Ca^{+2}		Norepinephrine	
Carbachol		Pralidoxime	1
Carbenoxolone	3	K^{+}	13
Charybdotoxin	3	SCH 50911	
Curare	5	Strychnine	33
Cyclothiazide	2	Verapamil	7
Diltiazem	6	ZD7288	3
Evan's Blue	2		
GABA	29		

PSYCHOGENIC/ HALLUCINATORY

anandamide	13
chloroquine	60
ethanol	23
chlorpromazine	2
fluoxetine (Prozac)	14
haloperidol	1
methanandamide	8
methysergide	3
quinine	18
risperidone	5

N-ACYL ETHANOLAMINES

palmitoyl (16:0)	**2**
oleoyl (18:1)	**2**
linoleyl (18:2)	**3**
linolenyl (18:3α)	**2**
linolenyl (18:3γ)	**3**
arachidonyl (20:4)	**2**
CB1 agonists: HU-210,	**5**
CB1 antagonist: SR141716A	**5**

MISCELLANEOUS COMPOUNDS

Propidium iodide	2
Bovine serum albumin (BSA)	5
Dimethyl sylfoxide (DMSO)	7
HEPES buffer	33
Penicillin	5
Anti-epileptic compounds (4)	

Summary

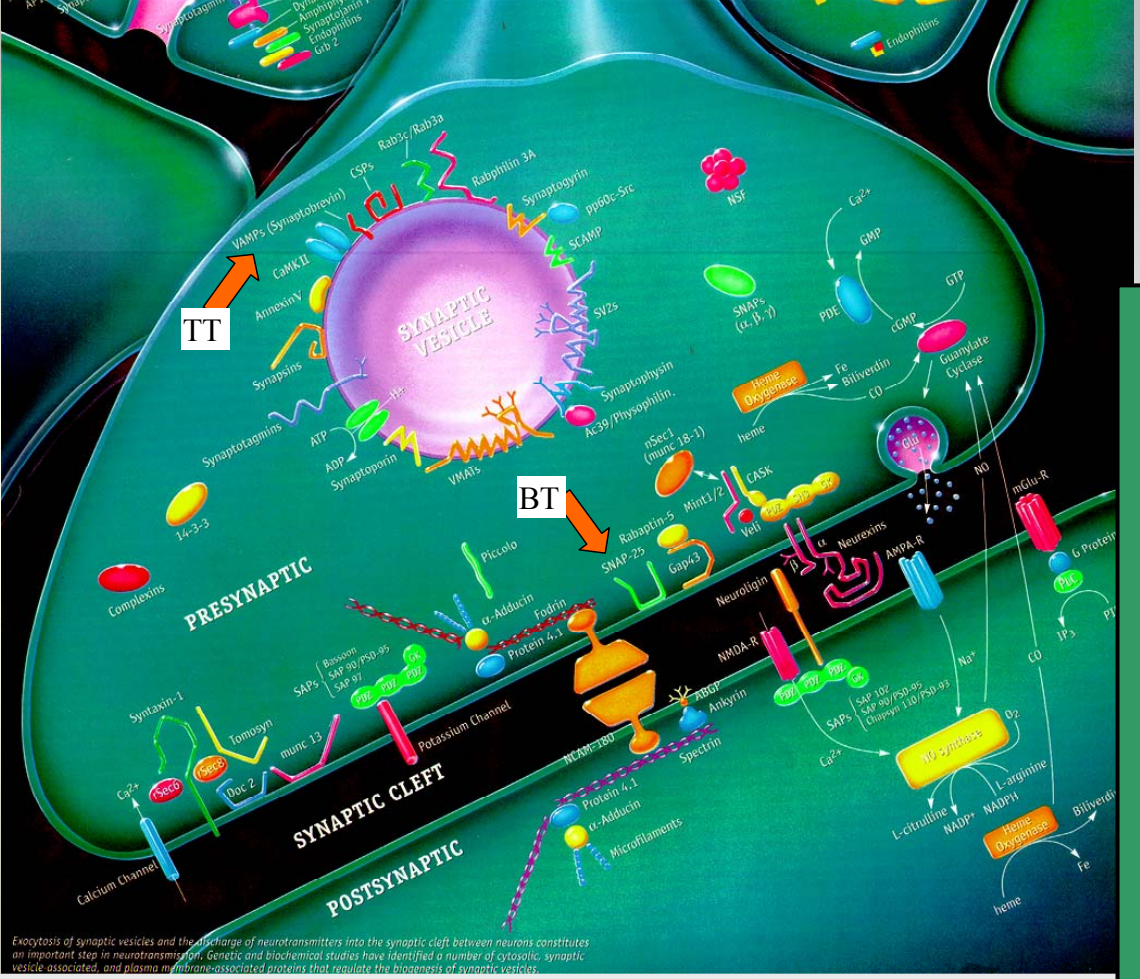
Nerve cell networks in culture are indeed “histiotypic” when we look at pharmacological data. They may not be histiotypic when we focus on ‘pattern processing”, which may be more circuit dependent.

Nerve cell networks *in vitro* also respond to toxins like the parent tissue.

There is “tissue specificity” in network responses (i.e. frontal cortex vs spinal cord).

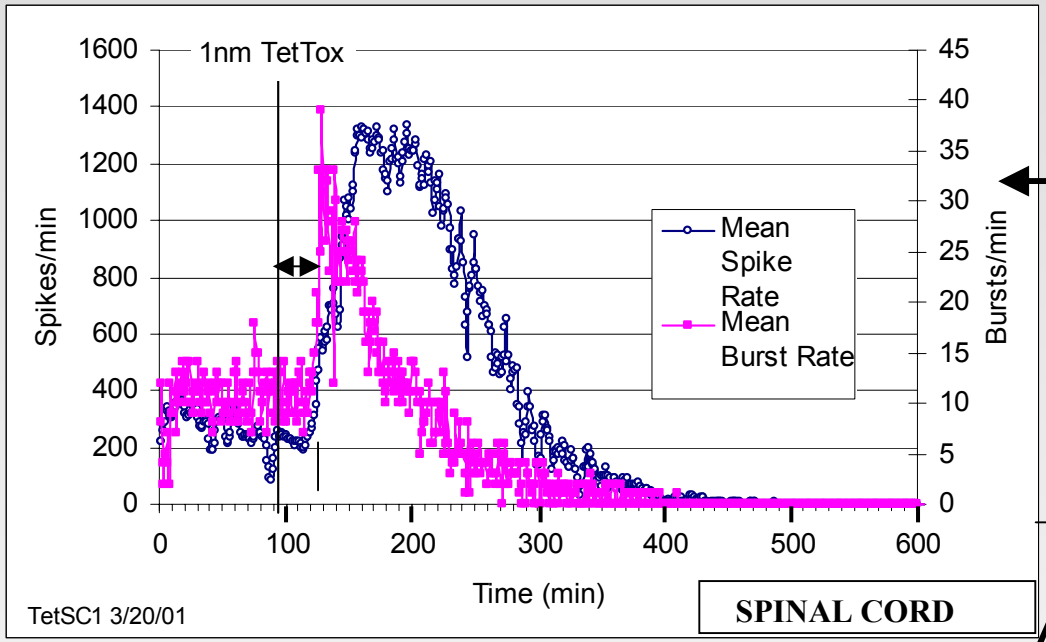
A primary tool for quantifying pharmacological responses is the “concentration-response curve” (dose-response curve) that provides EC_{50} data.

Network Toxicology



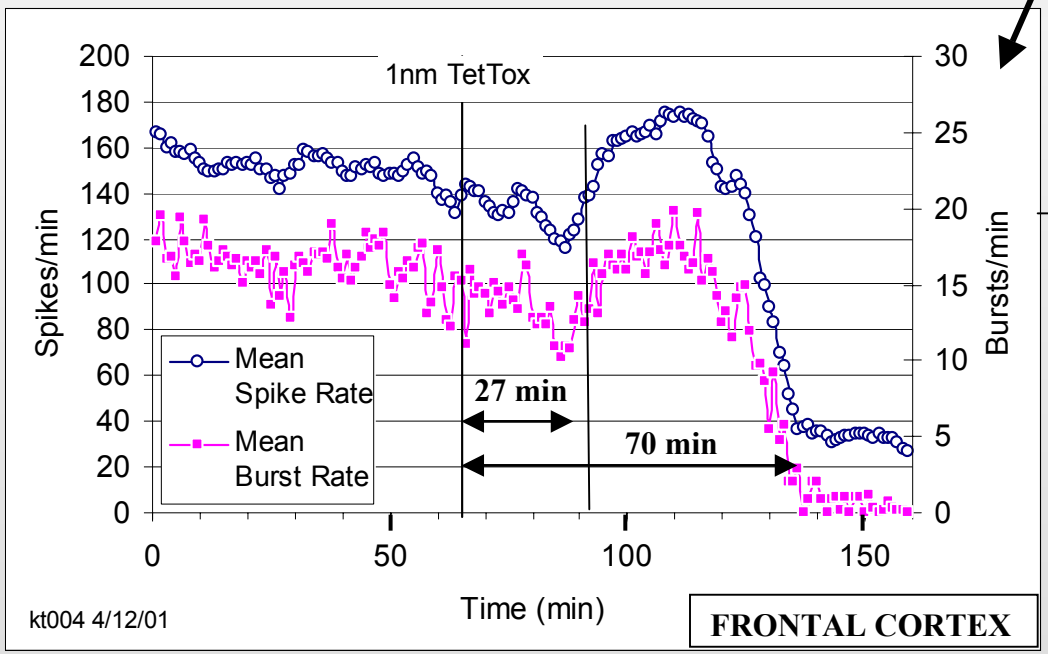
NETWORK RESPONSES to Tetanus Toxin and BOTULINUM TOXIN "A"

TETANUS TOXIN



SPINAL CORD: GABA and strychnine-sensitive (glycinergic) inhibition.

Strong excitation after 30 min delay followed by gradual decline to catastrophic failure in 200 min.



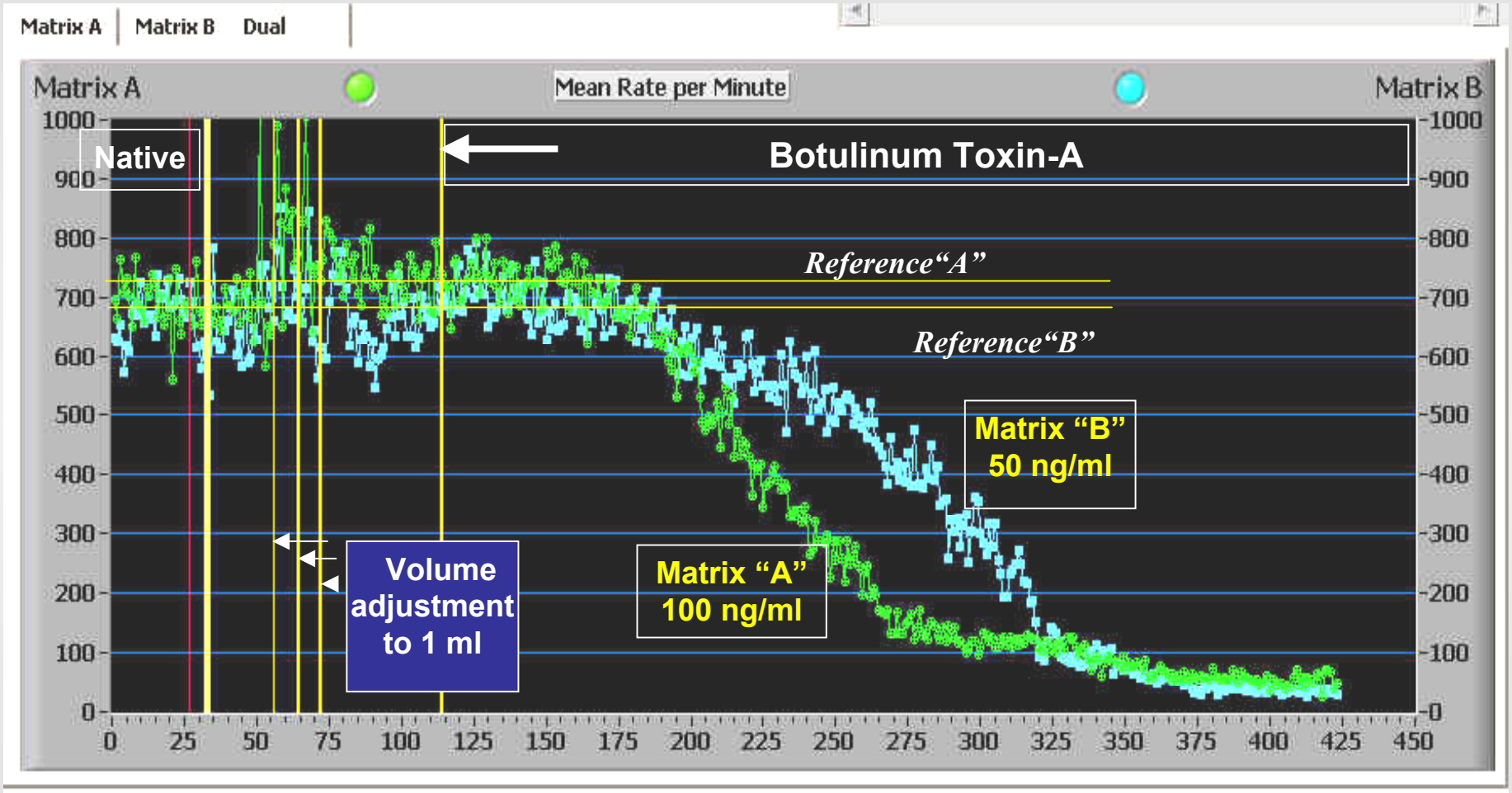
FRONTAL CORTEX: no glycinergic inhibition.

Weak excitation after 25 min followed by catastrophic network failure in 70 min.

MECHANISM: presynaptic inhibition of vesicle release with preference for glycinergic inhibitory neurons. (150kD peptide).

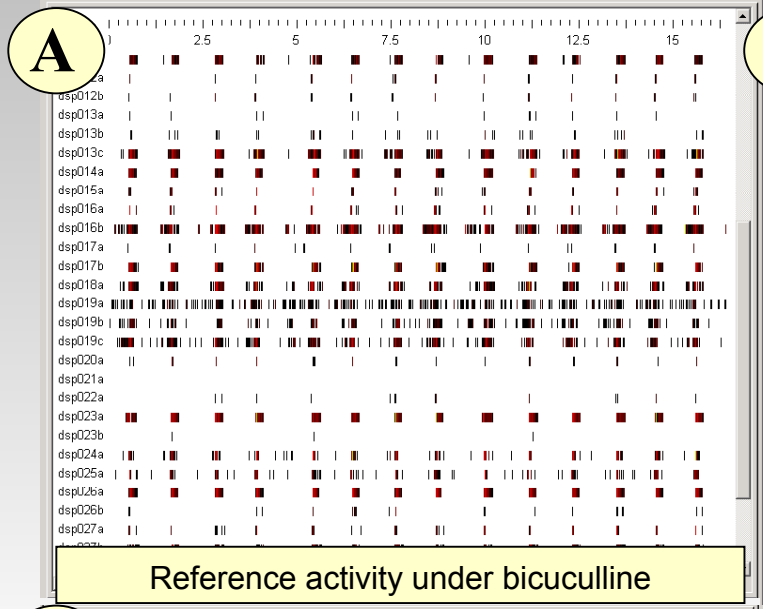
Low Conc.: inhibitory nerve endings
High Conc.: many (all?) nerve endings

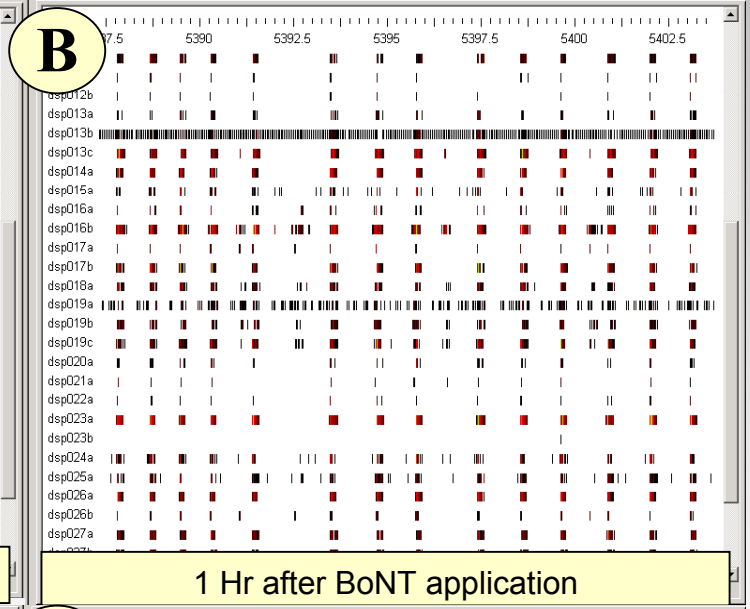
Botulinum Toxin-A

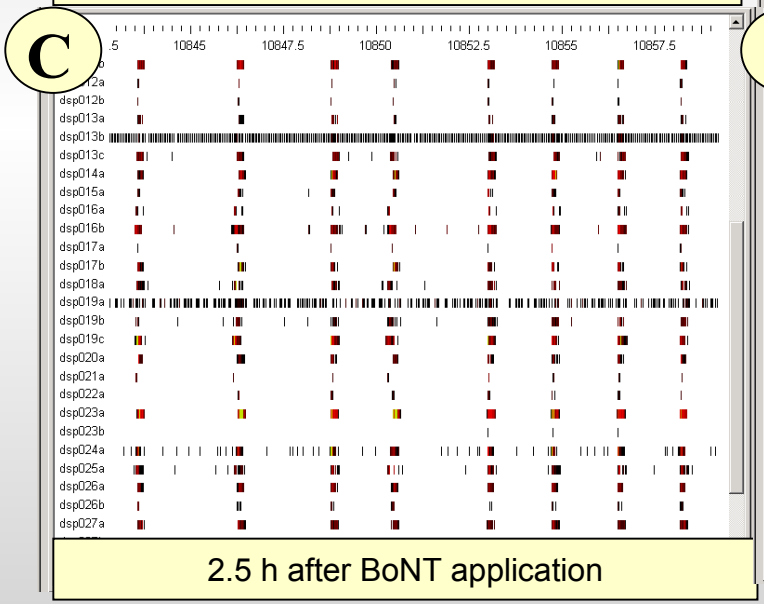


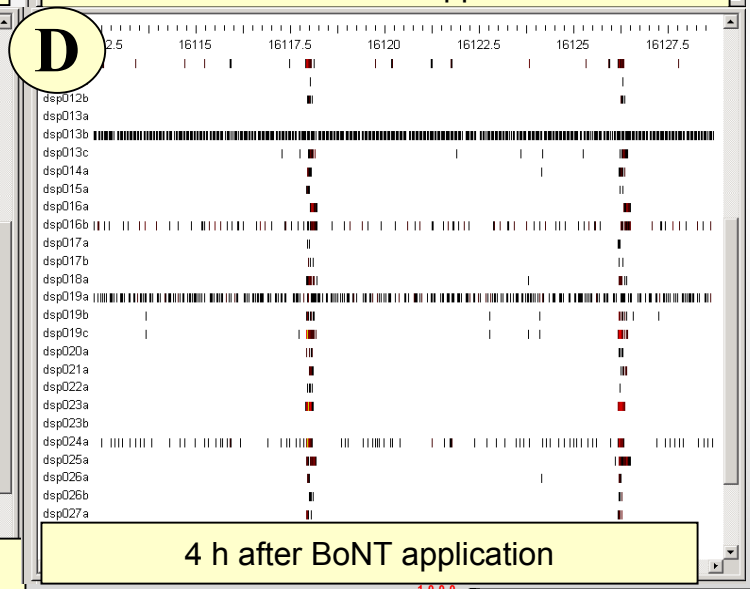
spike rate averaged across all channels each minute

Time (min)







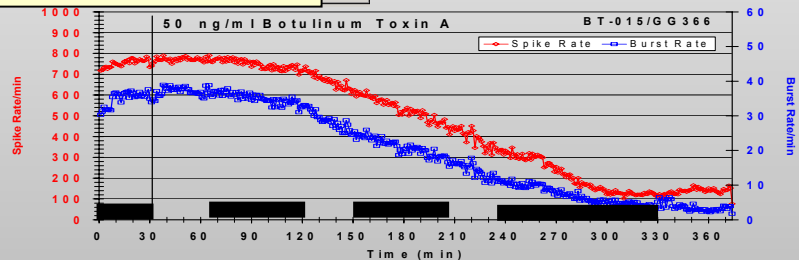


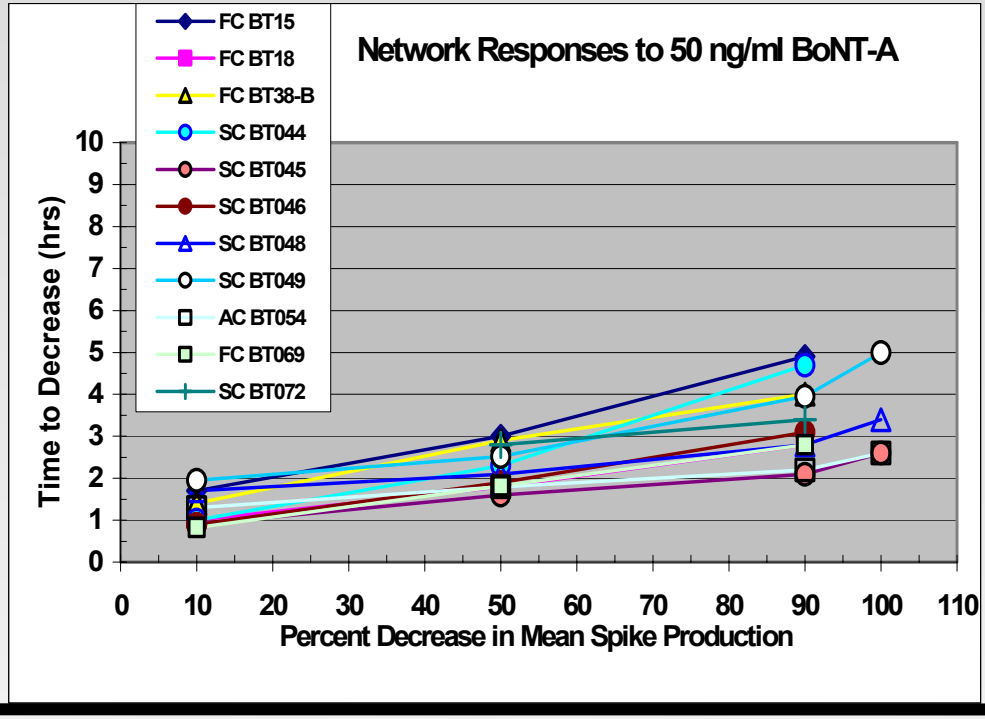
BT-015

Frontal Cortex

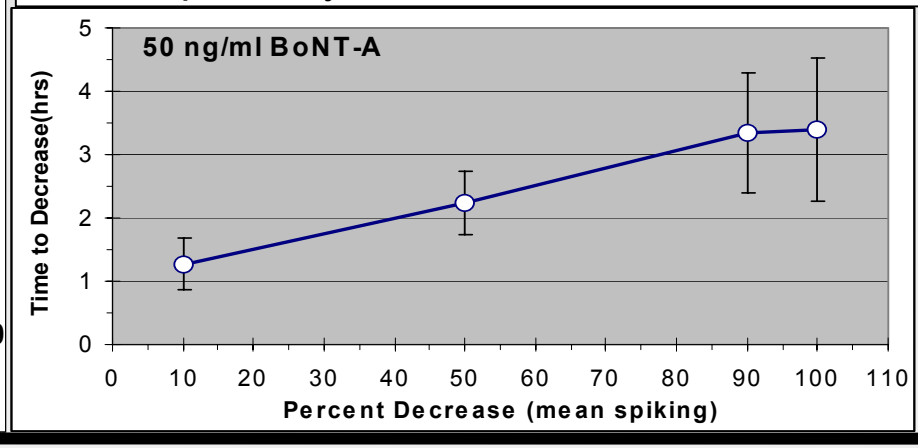
49 div

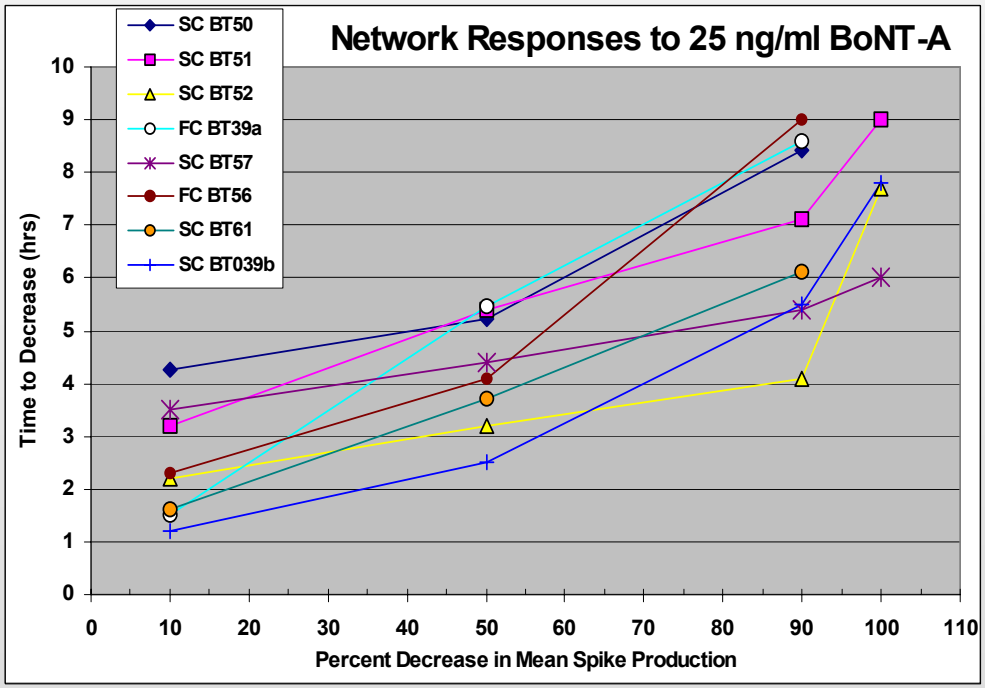
15 sec windows



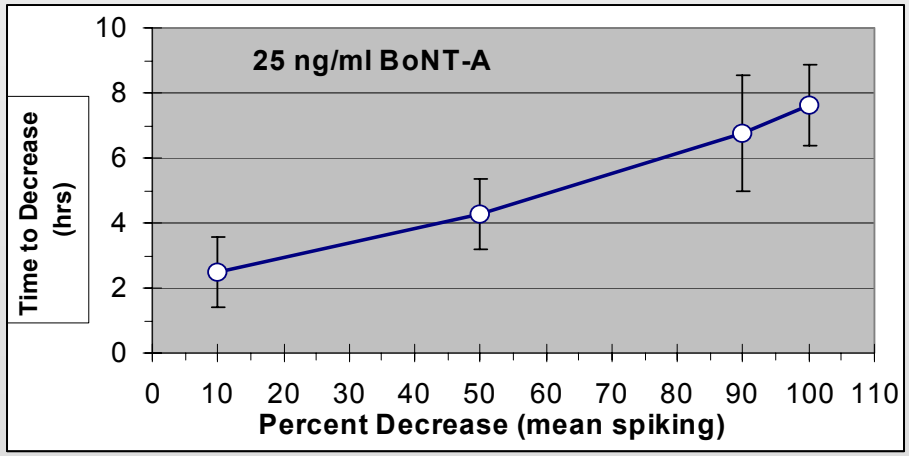


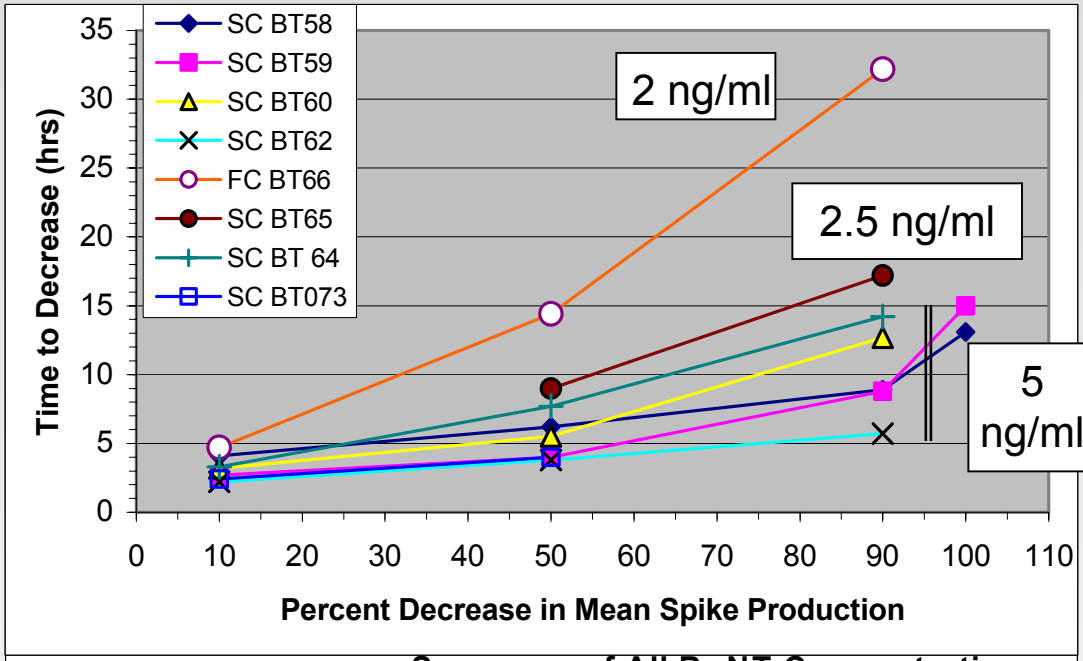
50 ng/ml (n=11).
Networks were derived from frontal cortex (FC), auditory cortex (AC), and spinal cord (SC) tissues There appears to be no overt tissue specificity.



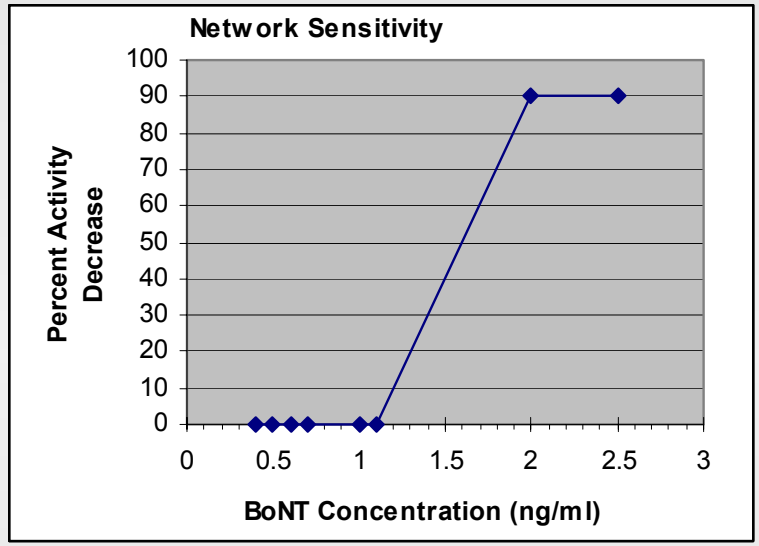


25 ng/ml BoNT-A (n=7).
Networks were derived from frontal cortex (FC) and spinal cord (SC) tissues.

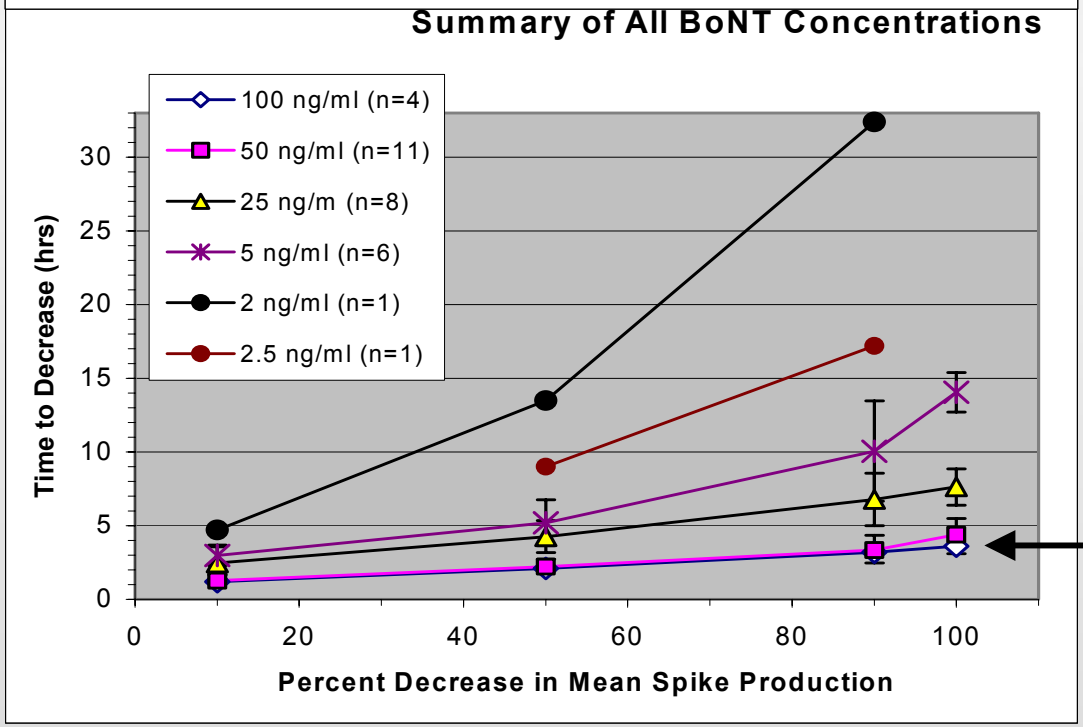




5 ng/ml (n=5) and 2.5 and 2 ng/ml.
At lower concentrations, delay times lengthen substantially.



Sensitivity: no responses below 2 ng/ml (~ 12 pico Molar).



Summary: all BoNT-A concentrations used. Note that saturation is achieved at 50 ng/ml.

Response Sensitivities to BoNT-A

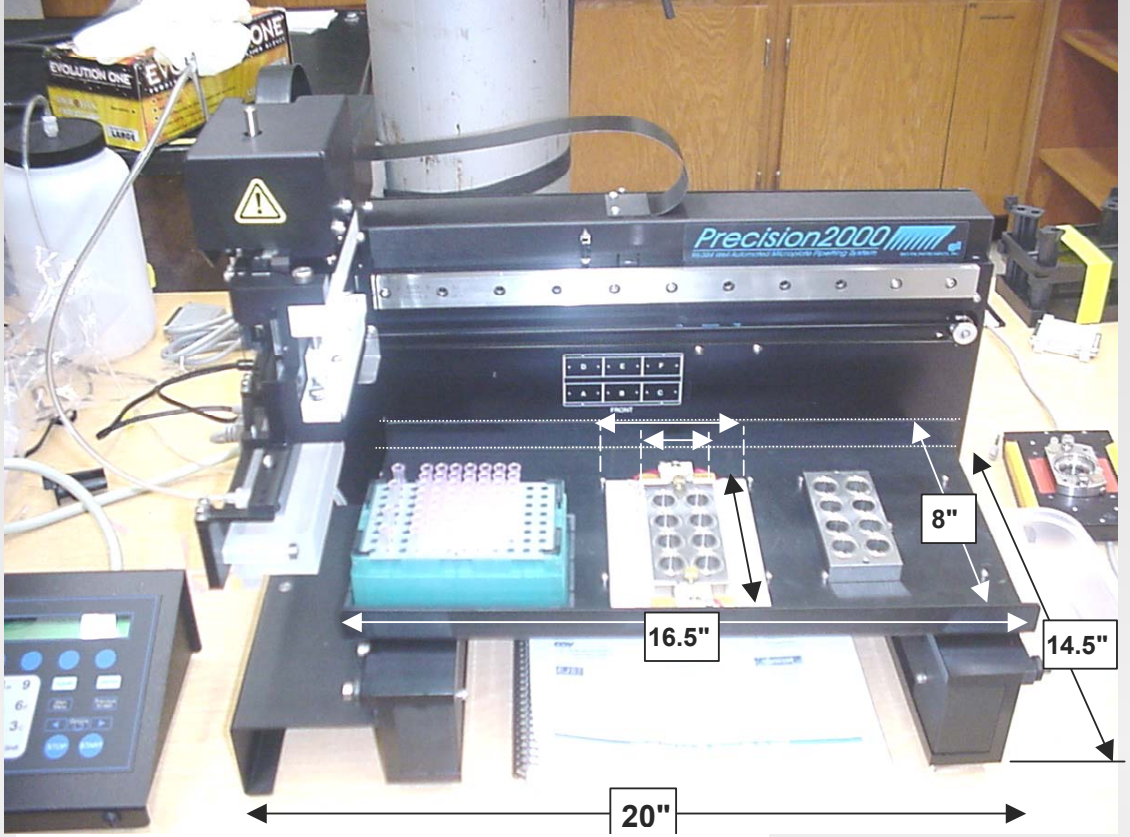
SYSTEM / ANIMAL	SENSITIVITIES		REFERENCE	
Synaptosomes (IC-50)	10 nM	1.5 ng/ml	Ashton and Dolly, 1988, 1991	
DRG in culture	50 pM	7.6 ng/ml	Welch et al, 2000	Subst. P release
Spinal cord cultures	0.4 pM	0.06 ng/ml	Keller et al, 1999	50% ↓ Snap 25
PC 12 cells (ACh release)	20 pM 50% reduction in 2 h	3 ng/ml	Ray, P., 1993	
Flow Cytom./Elisa Bot Fab5 antibody	detection limits 33 pM	 5 ng/ml	Emanuel et al, 2000	
Neuromuscular Preps	10 pM	1.5 ng/ml	Clark et al., 1987	
Mouse LD50	0.2 picoMoles (cystalline) ~ 20 pM	 3 ng/ml	Pearce et al., 1996	
Mammals	"fur ruffling" 1ng/kg	 1 pg/ml	USAMRIID Medical Management of Biological Casualties Handbook 4th Ed. February , 2001	
Radio-Immunoassay	6.6 pM	1 ng/ml	Ashton et al., 1985	
Hemagglutination	3.3-33 pM	0.5-5 ng/ml	Johnson, 1966, Sakaguchi et al, 1974	
Fluorescence	33 pM	5ng/ml	Ogert et al., 1992	
murine cultures: network activity	12 pM 330 pM*	2 ng/ml 50 ng/ml	this study: SC, FC, AC * saturation at 50 ng/ml	

FUTURE DIRECTIONS

Increase experimental efficiency
via parallel recording

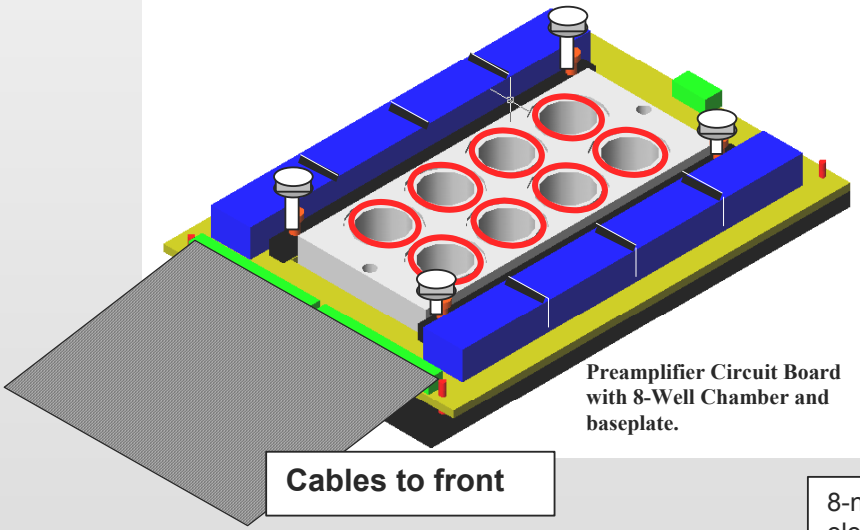
Bio Tek

Precision 2000 Liquid Handling Robot with 8-well Open Chambers

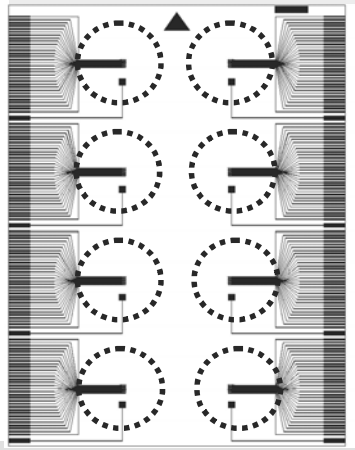


State of Texas Technology Development and Transfer Grant,

Jan. 04-Dec 05



Preamplifier Circuit Board with 8-Well Chamber and baseplate.



8-network microelectrode plate with 32 electrodes per network (256 channels)

Electrode plate design and proposed arrangement of amplifiers.

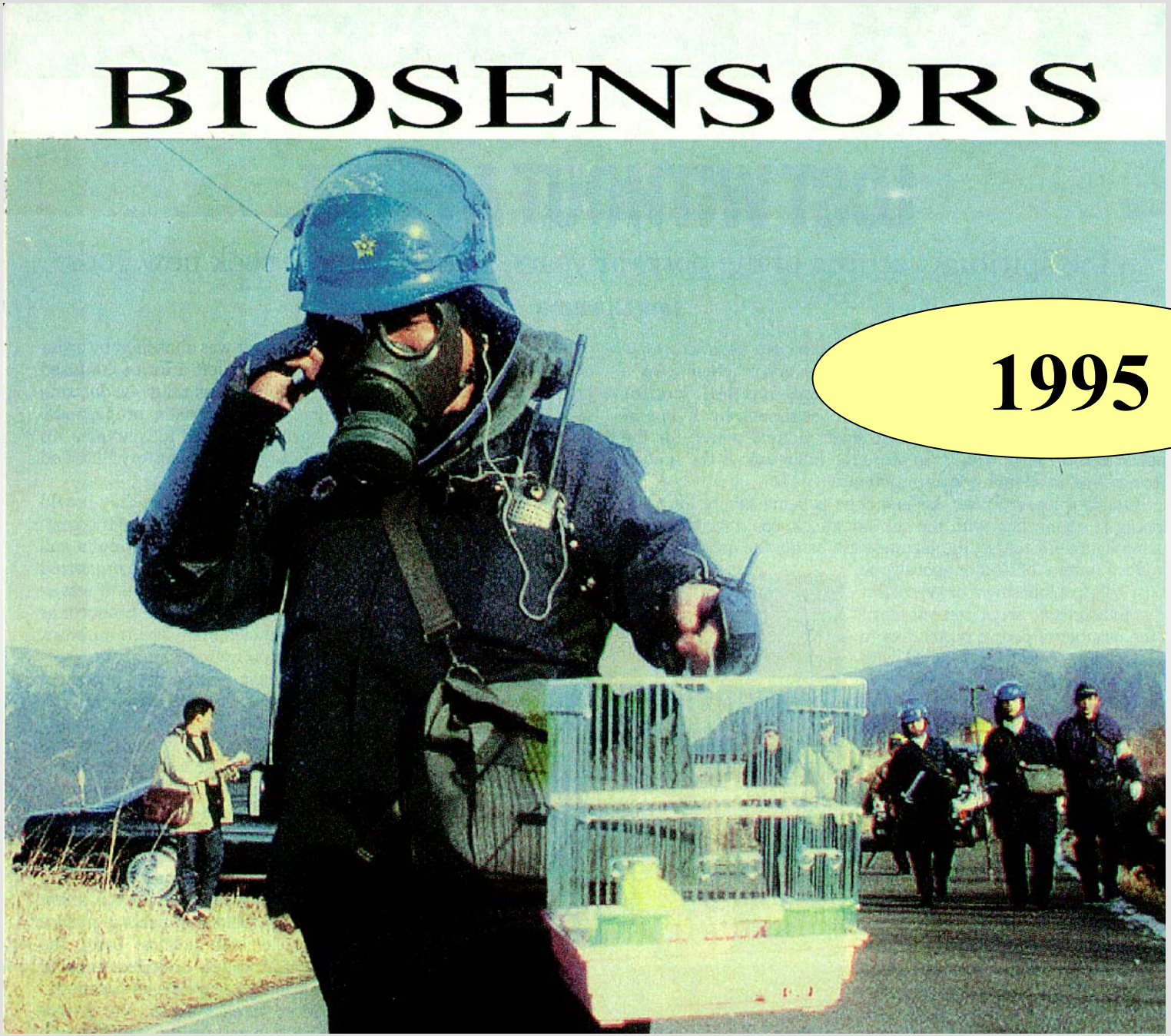
Neuronal Network Biosensors

Biosensors

1895

Photograph from the "Welsh Coal Mines" Collection from the National Museum of Wales.

BIOSENSORS



1995

MAJOR PROBLEM

We are unable to sense **unknown** molecules or engineered organisms!

All present sensor systems, no matter how sophisticated, must know the molecular signature of the threat.

We are defenseless against new chemical and biological agents!

There is a need for BROAD-BAND sensing systems that provide physiological responses and rapid suggestions for antidotes.

DARPA MISSION STATEMENT

December 1997 Workshop on Tissue-Based Biosensors

The ever-present threat of biological weapons and the operational dangers of their use in defense scenarios (against military or terrorist forces) require the development of new sensors capable of detecting and **functionally responding** to known and **unknown** potential biological and chemical agents.

The ability to create new biological threats, which have not been characterized and fingerprinted, dictates the need for research and development in new biosensors that result in a **physiological response** to a live agent with the ability to provide **functional signatures** of the threat.

New biological threats can appear in the form of novel chemical compounds, genetically engineered viruses or bacteria, and altered toxins. In such cases, present sensors would be rendered useless. Genetic fingerprints cannot predict a threat if the toxin is introduced into a formally innocuous organism. Antibody methods are outflanked by changes in toxin structure without loss of efficacy. Under these scenarios, only tissue based biosensors would provide the broad band protection needed.

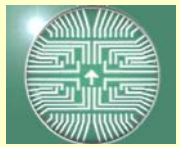
NEURONAL NETWORK BIOSENSORS

N N B S

Mammalian central nervous system tissue growing as mono-layers on arrays of substrate integrated electrodes *in vitro.*

The basic sensor function is represented by a change in the spontaneous activity of the network.

Characteristic changes in network activity are linked to specific classes of compounds. Activity changes are compound-specific and allow identification of mechanisms affected, neuroactive and toxic agents.



Center for Network Neuroscience
UNIVERSITY of NORTH TEXAS

UNT/ CNNS

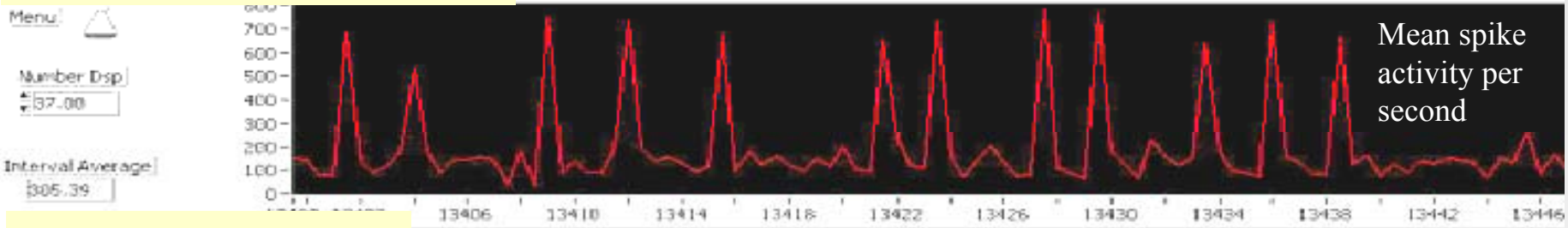
REALTIME DETECTION and CLASSIFICATION

mean network spike and burst rates in 1min bins.

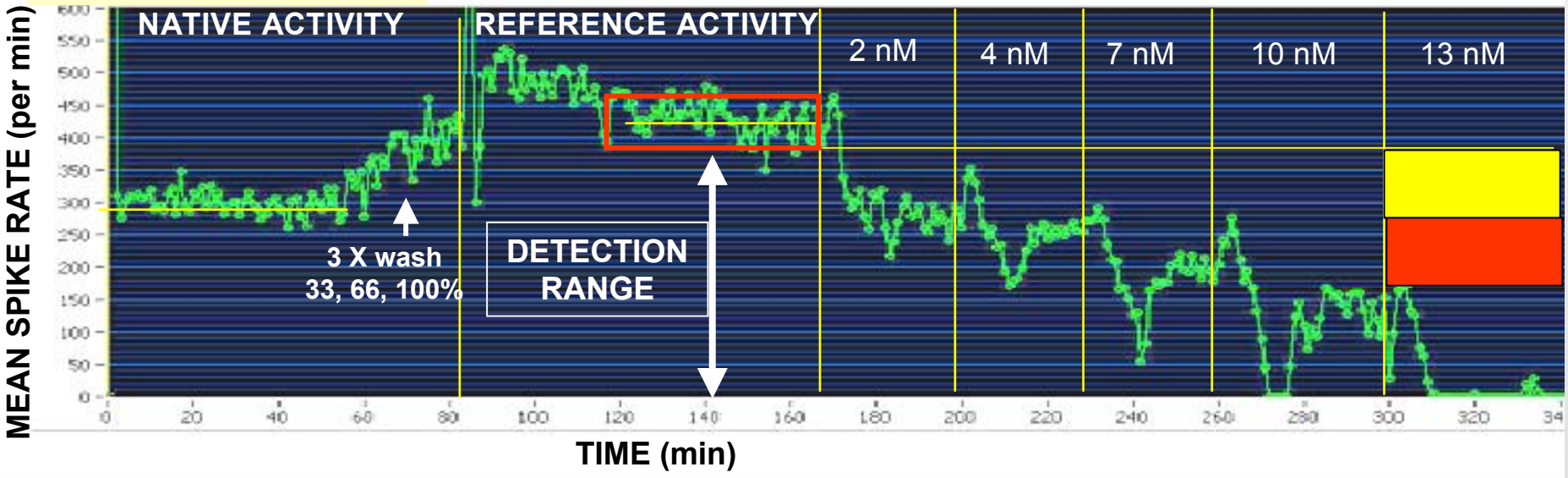
Data: Bret Zim; **Program:** Jacob Schwartz and Mario Jiminez

total spike production/sec

Network Response to TTX, mean of 37 identified units



mean spike rate/min



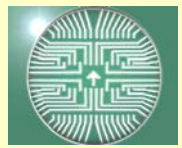
VERTICAL LINES: substance or medium additions (experimental episodes).
HORIZONTAL LINES: episode means for stationary activity; BOX: +/- 1 SD

WHAT CAN THE NNBS DETECT?

Any agent capable of interrupting central and peripheral nervous system functions in animals.

This detection is not limited to compounds that cause death, but also includes agents that generate epileptiform activity or cause mood- or major performance-altering responses, such as cannabimimetic compounds.

Some compounds are CNS-region-specific, future detectors may feature networks derived from different, selected CNS regions.



Center for Network Neuroscience
UNIVERSITY of NORTH TEXAS

WHAT CANNOT BE DETECTED BY NNBS?

LONG-TERM DEGENERATIVE DISEASES
multiple sclerosis
Guillain-Barre syndrome

SUBSTANCES THAT INTERFERE WITH THE CNS CIRCULATORY SYSTEM

SUBSTANCES THAT TARGET ONLY SPECIFIC ORGANS

HOWEVER
GENERAL TOXINS AFFECTING ENERGY METABOLISM WOULD BE RAPIDLY DETECTED BY REDUCTIONS IN SPIKE AMPLITUDES and PATTERN CHANGES.

RESPONSE QUANTIFICATION

1. Detection

z-score: $x_i - \mu/SD$

2. Classification

NEUROACTIVE / NEUROTOXIC

Inhibitory | Disinhibitory | Excitatory

METABOTOXIC

VARIABLES
burst rate,
spike rate

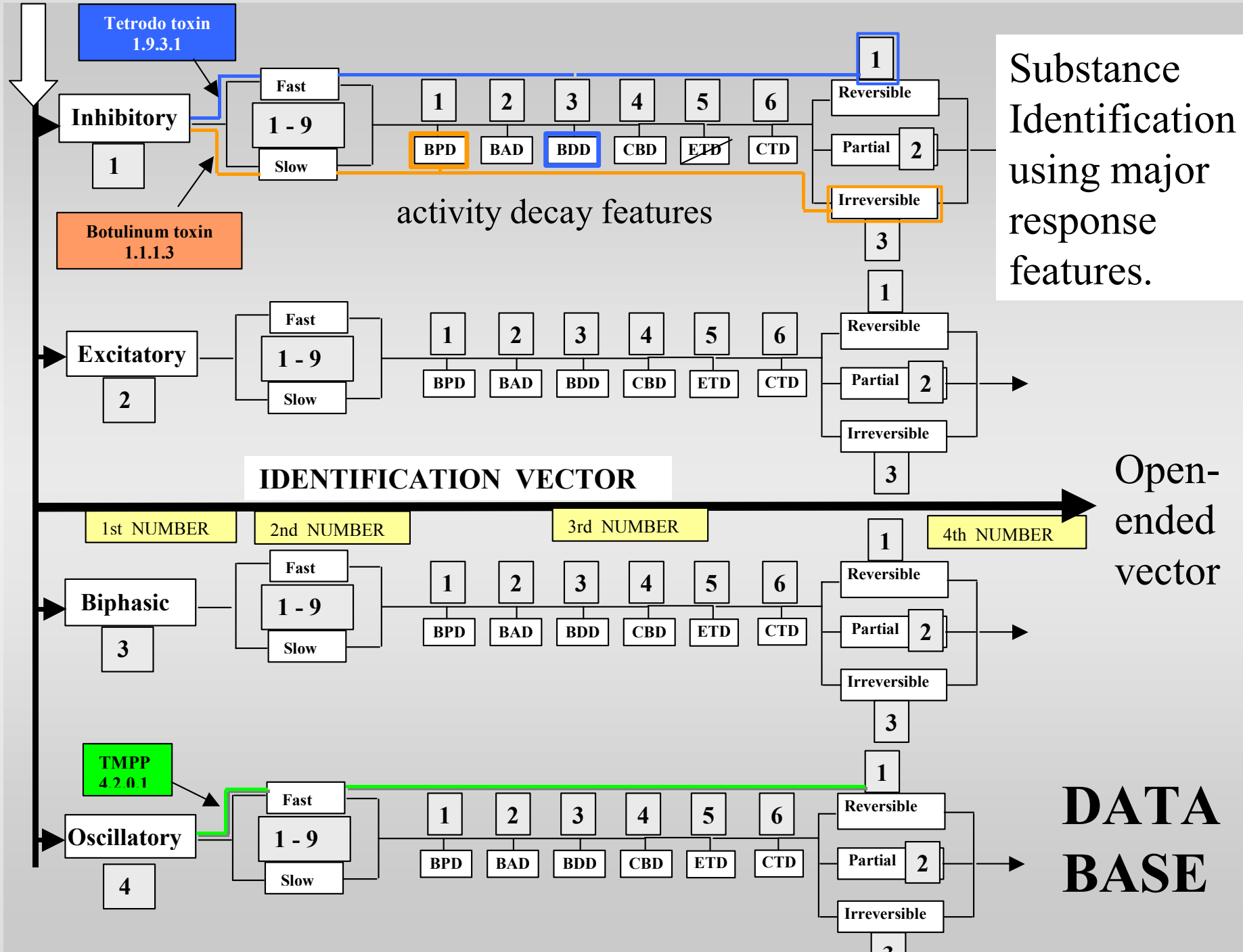
VARIABLES
burst duration & CVs,
spike freq. in bursts,
unit synchronization,
burst phase delays

VARIABLES
burst rate,
spike rate, CVs

VARIABLES
spike shape
(amplitude)

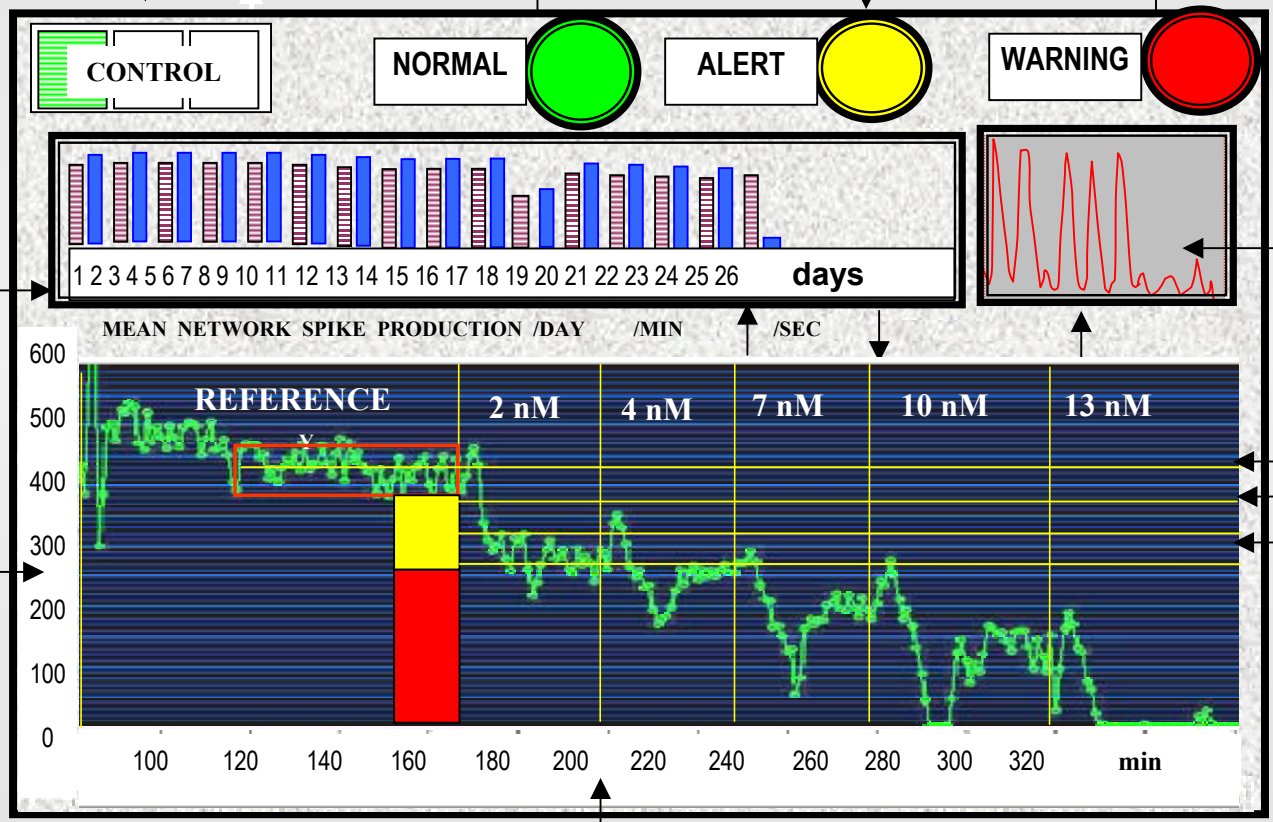
3. Identification (multivariate response profile)

concentration dependence, EC-50s, spontaneous recovery, pattern changes, characteristic delays to response, ...


Tetrodo toxin
1.9.3.1
Inhibitory
1
Botulinum toxin
1.1.1.3
Fast
1 - 9
Slow
1
2
3
4
5
6
BPD
BAD
BDD
CBD
ETD
CTD
Reversible
Partial
2
Irreversible
3
activity decay features
Substance Identification using major response features.
Excitatory
2
IDENTIFICATION VECTOR
1st NUMBER
2nd NUMBER
3rd NUMBER
4th NUMBER
Open-ended vector
Biphasic
3
TMPP
4.2.0.1
Oscillatory
4
DATA BASE

NEURONAL NETWORK BIOSENSOR FIELD DISPLAY

a recommendation



Control Network Status
green,
yellow,
red

SENSOR WARNING PANEL

2 Network History (days)

Immediate Network Activity (total APs per sec)

Mean network spikes /min

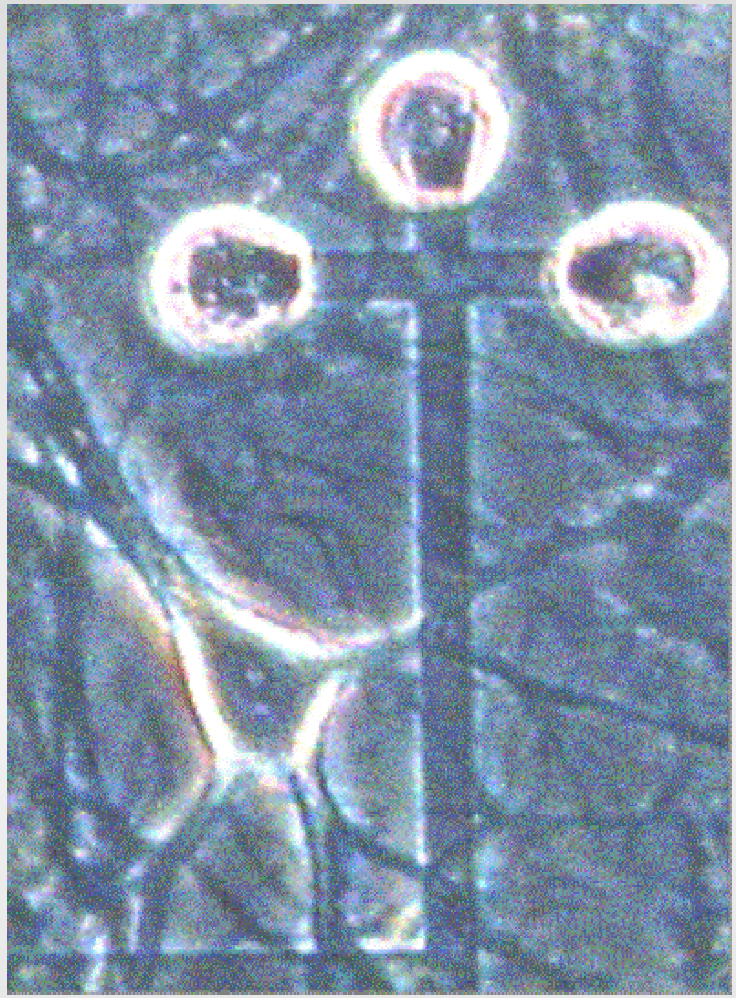
1 SD

SD's of Reference Mean

Real-Time Network Response Panel (minute bins)

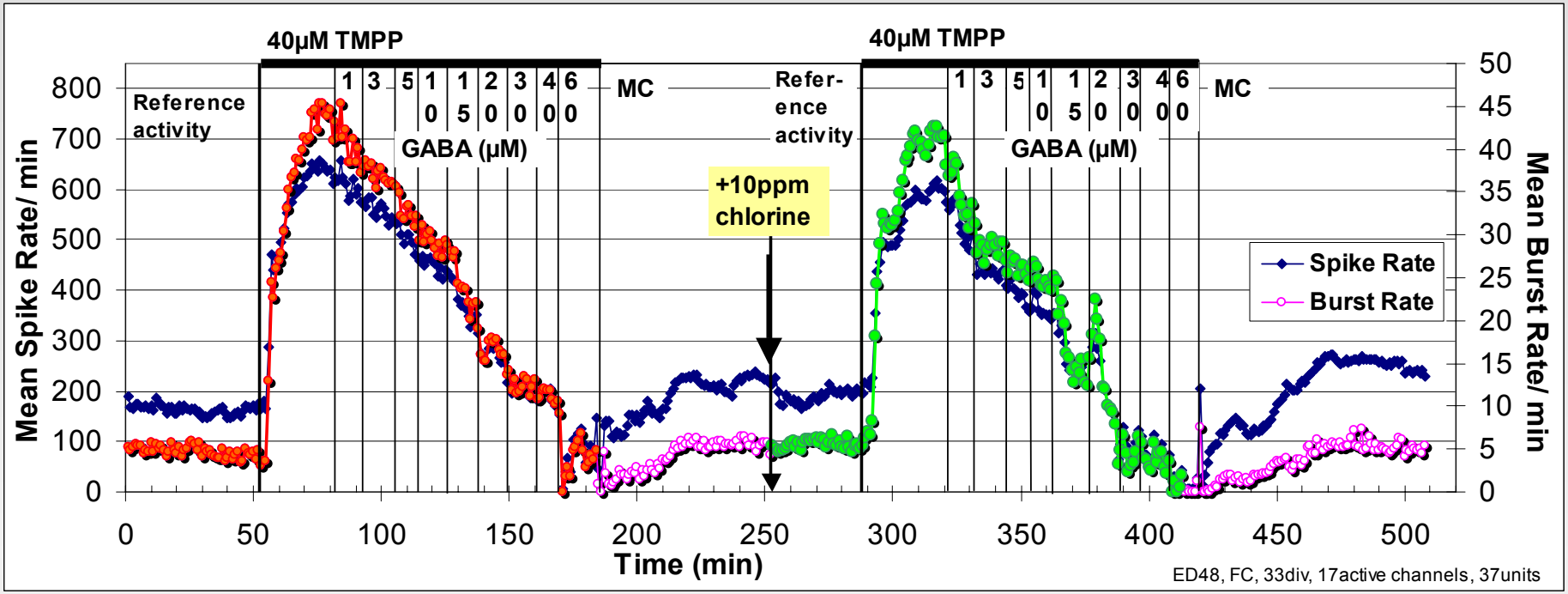
If the test water is chlorinated, will it not destroy the culture?

No. Cultures are protected by serum and amino acids up to 20 ppm (4 x chlorine in pool water)

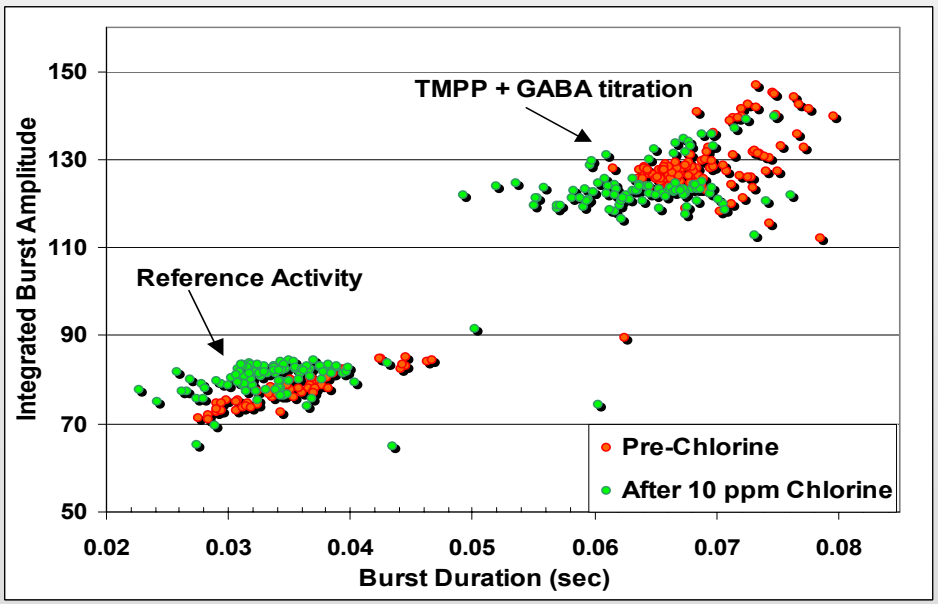


PHARMACOLOGICAL RESPONSES UNDER CHLORINE

A



B



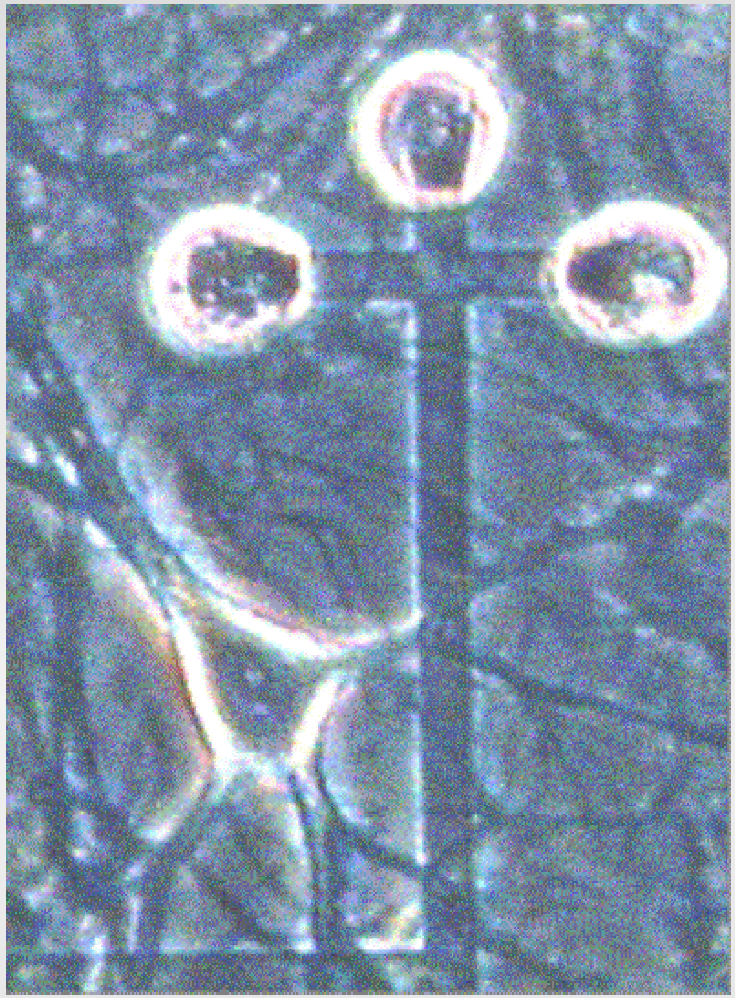
GABA titration in the presence of TMPP and chlorine.

40 µM TMPP elicits stereotypical increase in spike and burst rate, reaching a maximum 22 min after application.

Sequential GABA additions induce a stepwise reduction of activity with total cessation of at 60 µM. Following a medium change the same TMPP and GABA additions were performed in the presence of 10 ppm chlorine., are clustered and segregated by reference activity and TMPP (+GABA) to pharmacological agents are comparable in the absence and presence of 10 ppm chlorine.

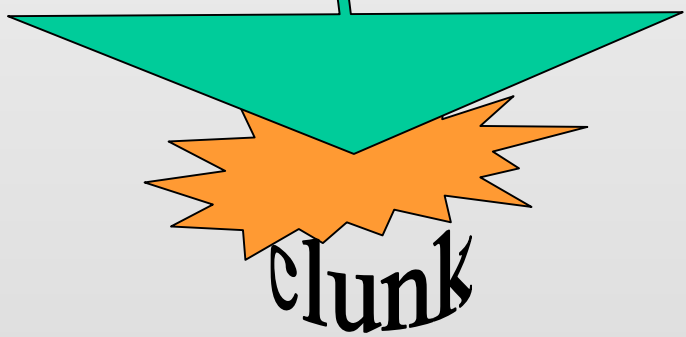
Networks cannot be frozen, so they cannot be shipped, and, therefore, replacement modules are not available!

Under the DARPA contract #2, network shipping in the living state was established in collaboration with the NRL and The Neuroscience Institute, LaJolla.

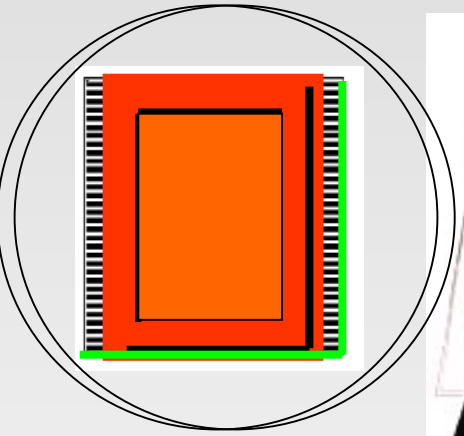


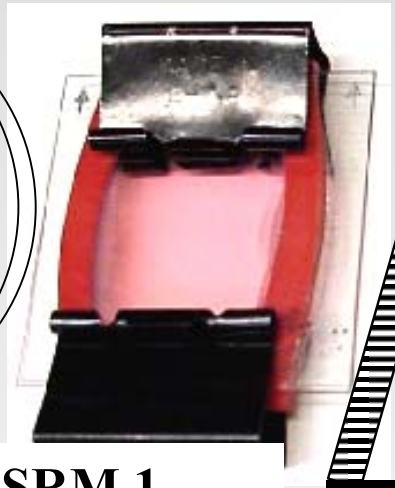
NETWORK SHIPPING PREREQUISITES

1. Glia to remove ammonia and for strong adhesion
2. Minimum cell mass to a maximum surface area: networks on MEAs withstand impact forces of 50g.
3. Temperature: keep between 15 and 40 deg C
4. Pressure: (?) cabin pressure
5. No bubbles in medium
6. Large medium volume (2-4 ml)?

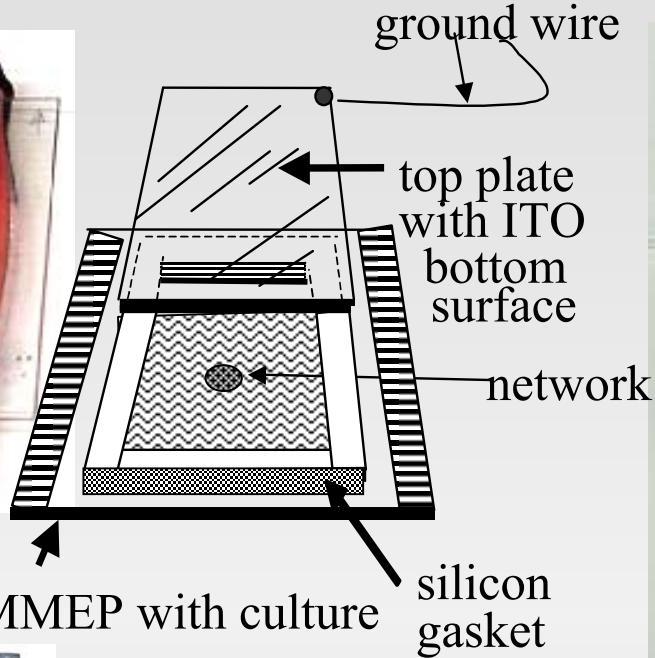


EVOLUTION of SHIPPING MODULES



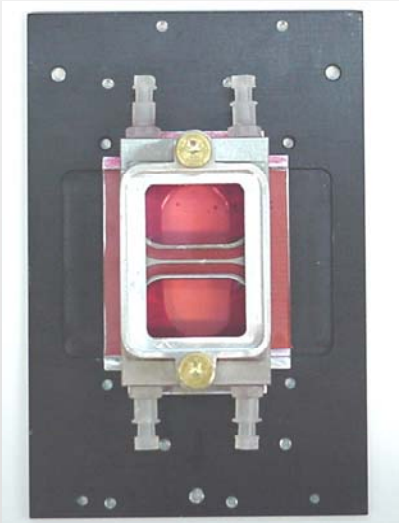


SRM 1
(one network)

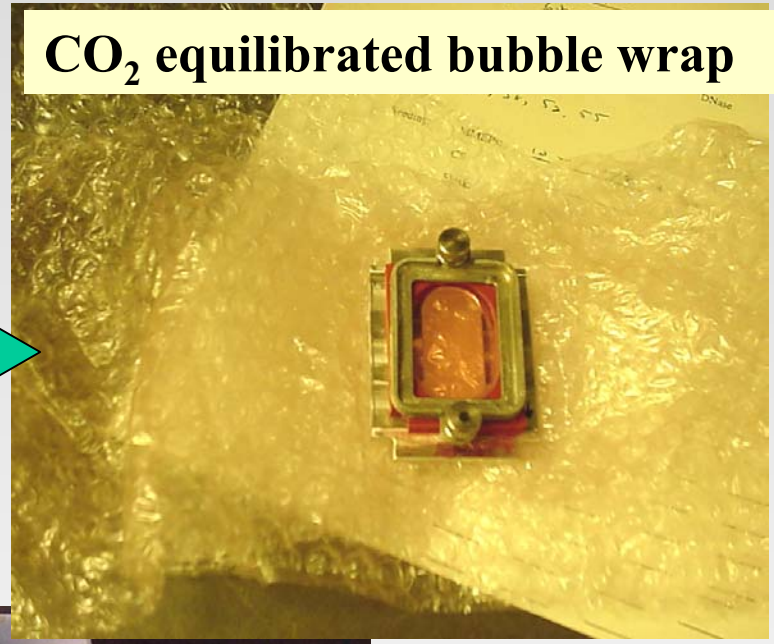


SRM 2
(one network)

SRM 3
two
networks

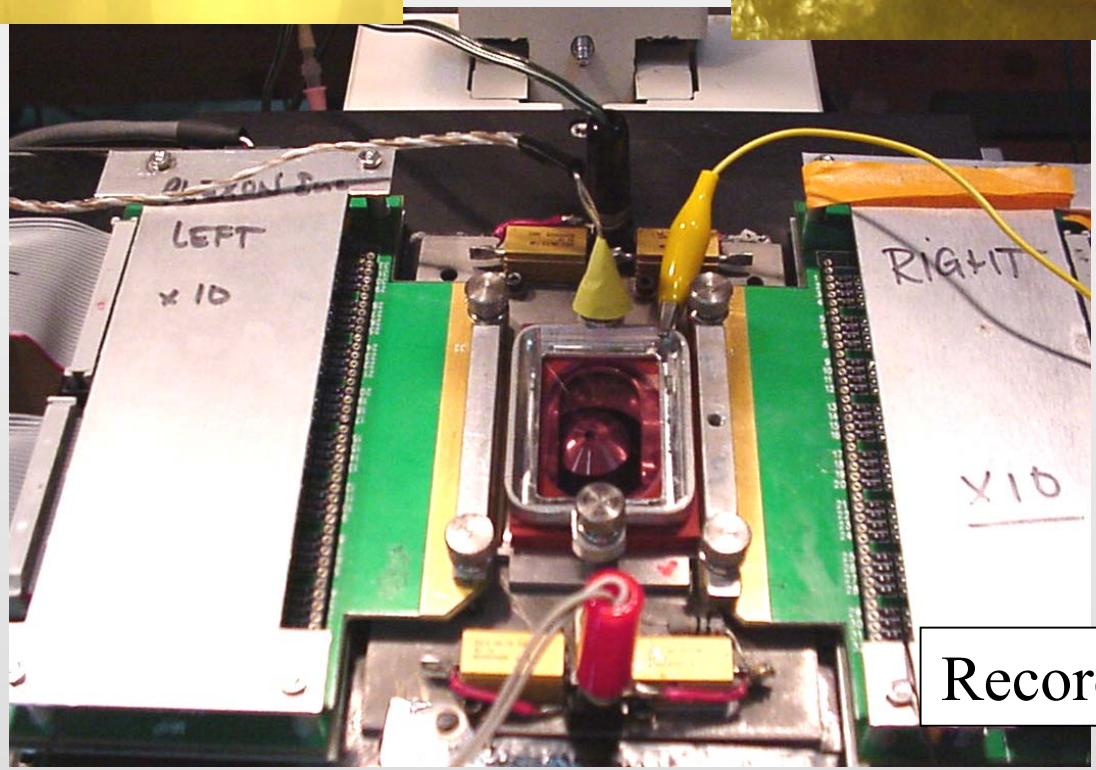


NRL implementation



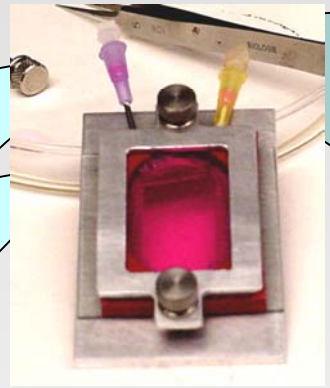
Ship in $3 Ball jar

Unwrap



Record

Network Shipping Experiments (April & May 2001)



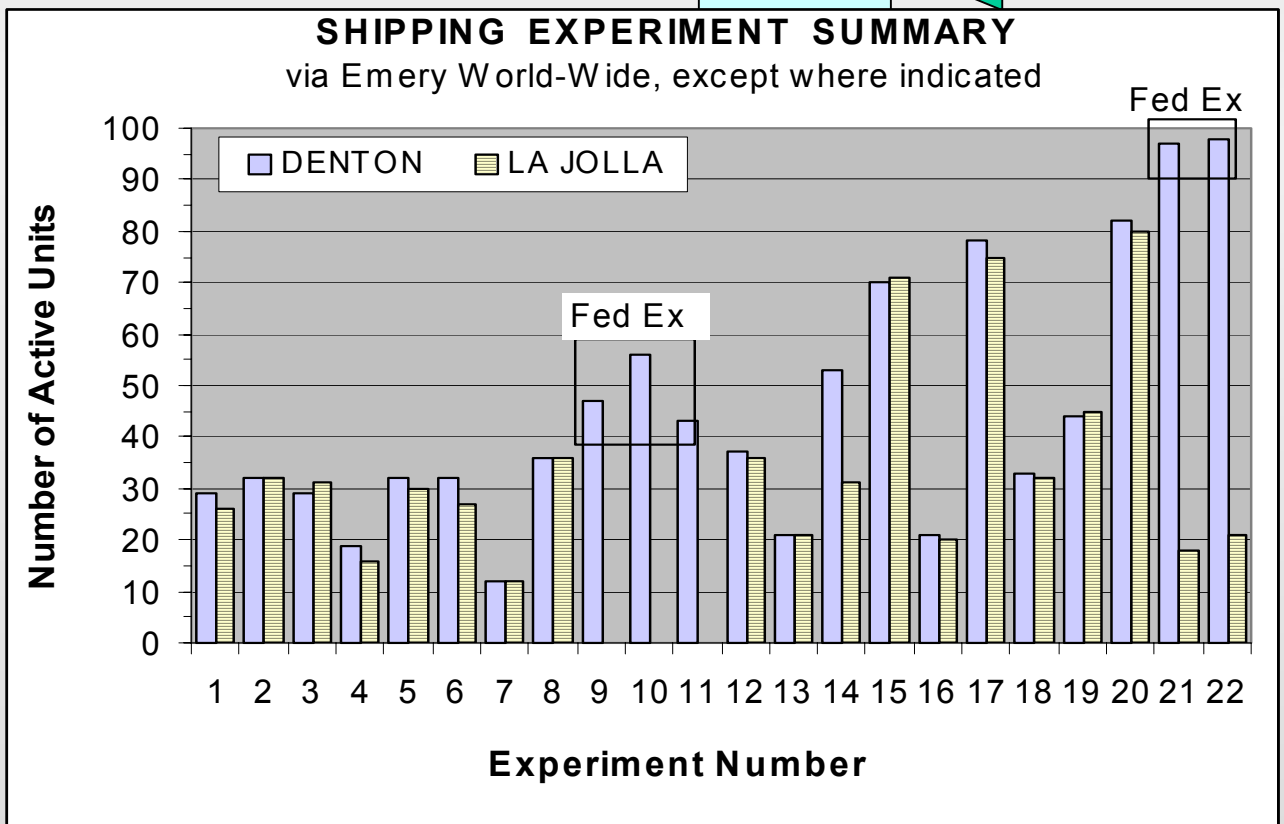
Network growth on MEAs
Screening of activity
Assembly of shipping modules

Denton

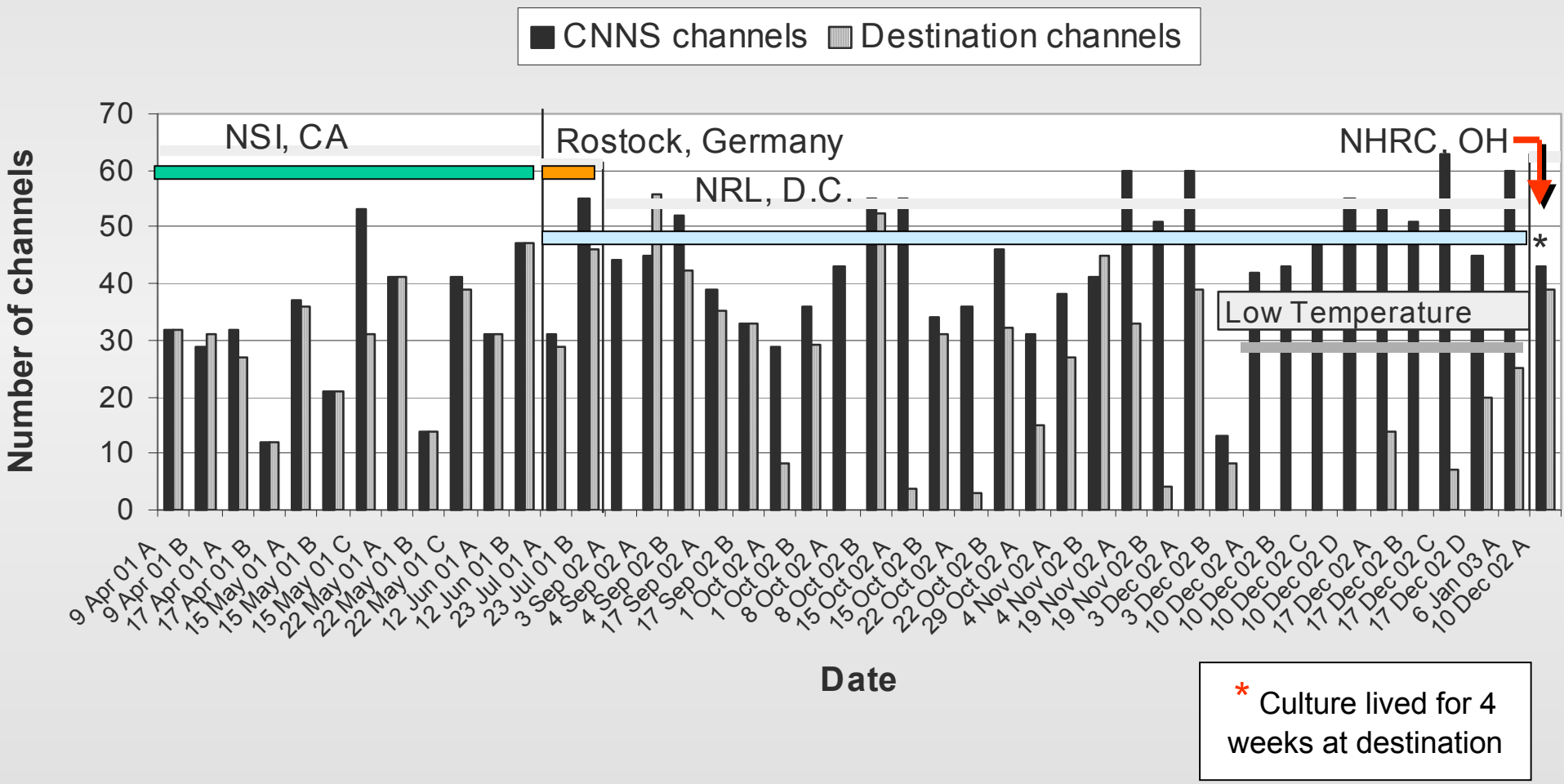
DFW

La Jolla, CA

Multichannel recording with Plexon MNAP system (Prof. Edelman's lab)



Network Shipping Summary (Frontal Cortex)



* Culture lived for 4 weeks at destination

SHIPPING EXPERIMENTS:
Exploring Limits of the Physical Environment

Acceleration

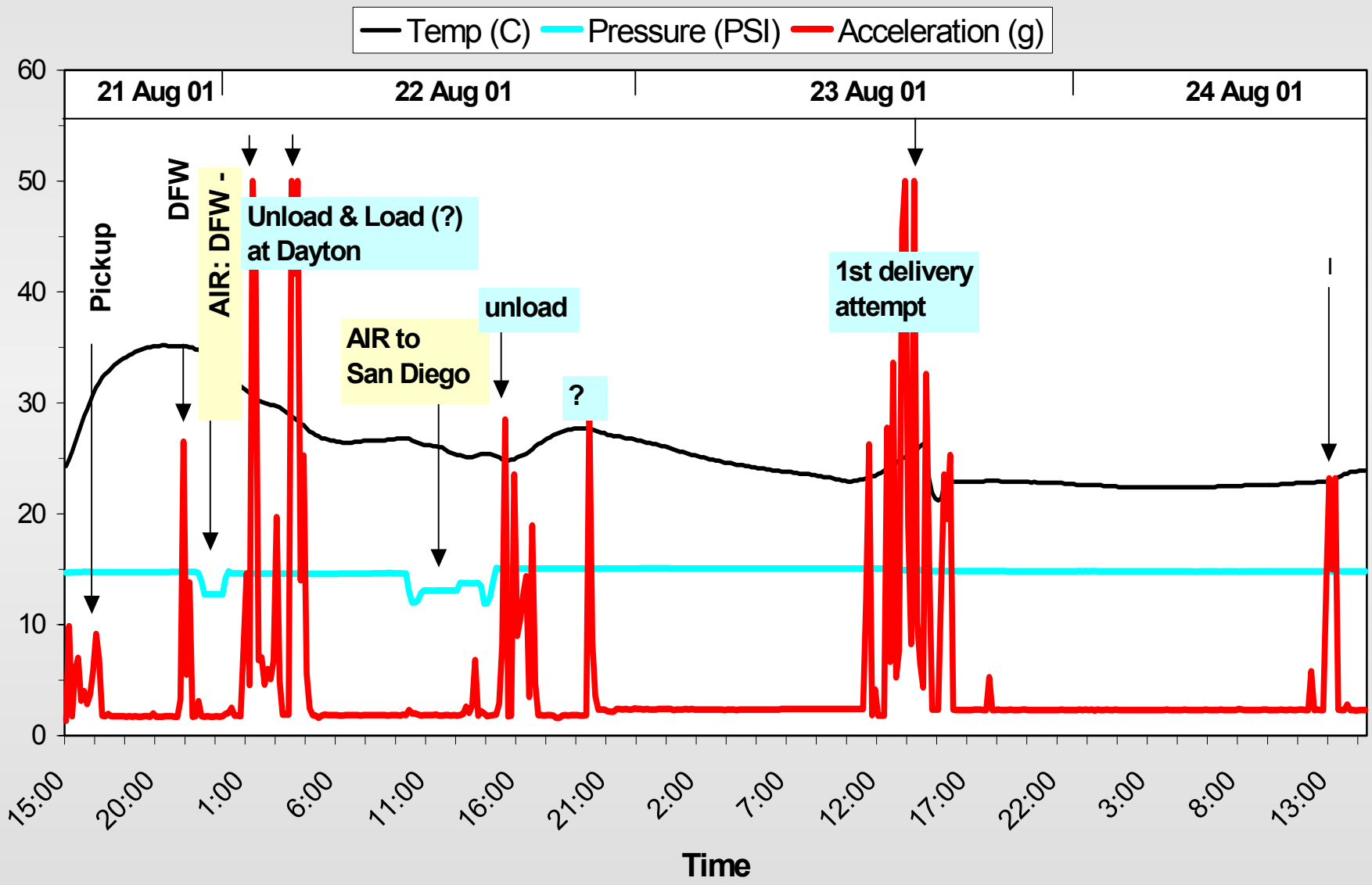
Shock

Temperature

Pressure

Humidity

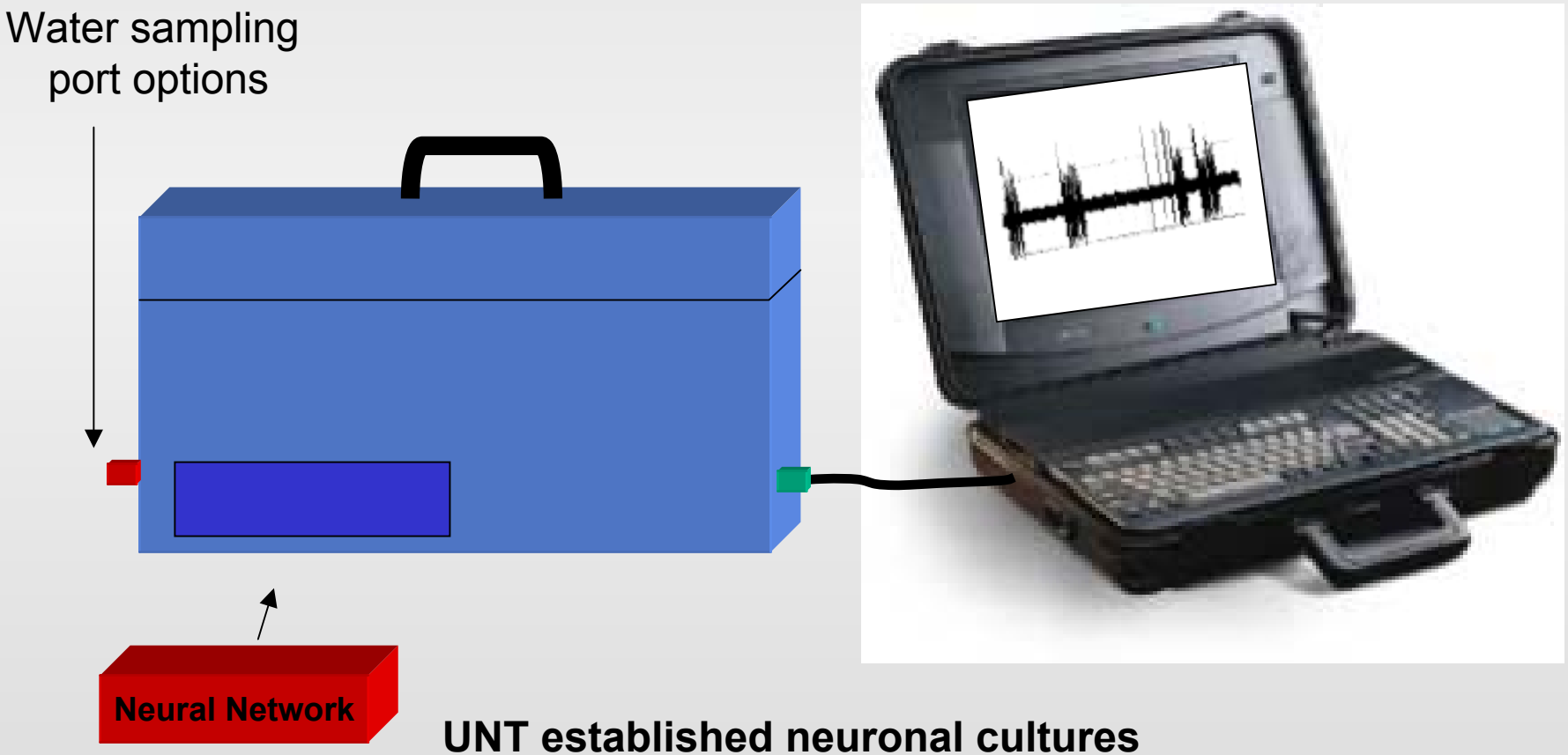
Recorder will be included in most test shipments


21 Aug 01 Shipment Recorder Data
Temp (C)
Pressure (PSI)
Acceleration (g)
21 Aug 01
22 Aug 01
23 Aug 01
24 Aug 01
Pickup
AIR: DFW - DFW
Unload & Load (?) at Dayton
AIR to San Diego
unload
?
1st delivery attempt
60
50
40
30
20
10
0
15:00
20:00
1:00
6:00
11:00
16:00
21:00
2:00
7:00
12:00
17:00
22:00
3:00
8:00
13:00
Time

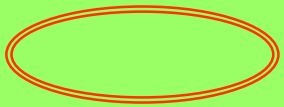
NRL

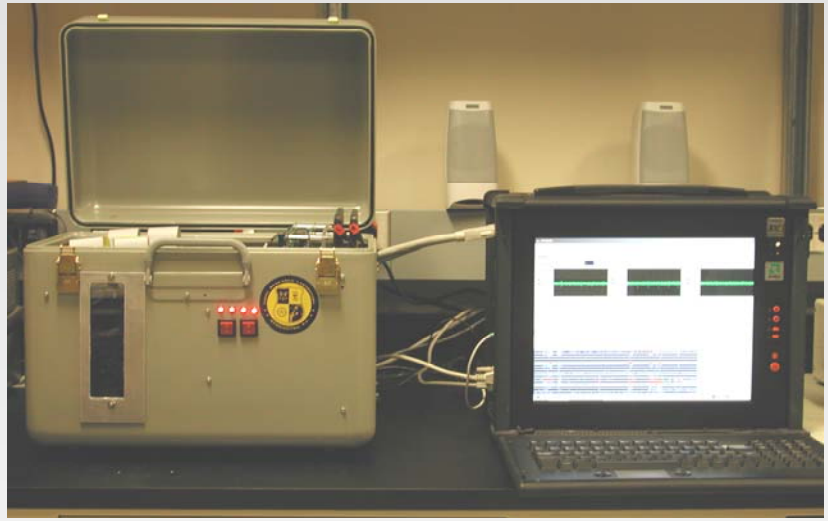
Project Vision

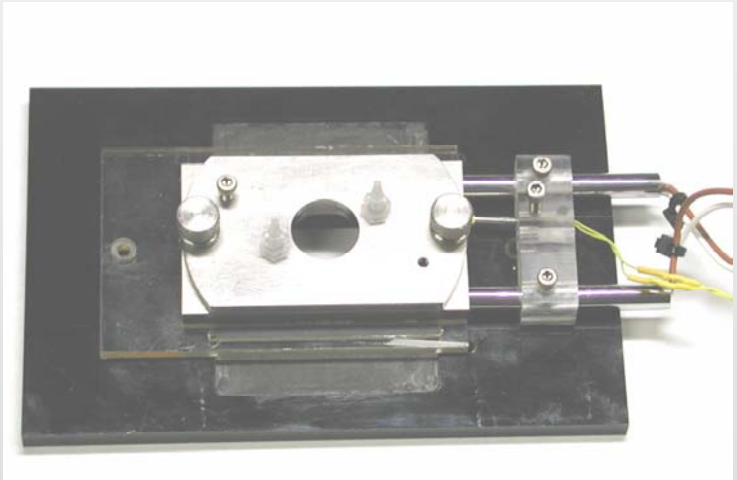
Deliver hardware & software that readily accommodates shipped neuronal networks cultured on a proven platform



UNT established neuronal cultures

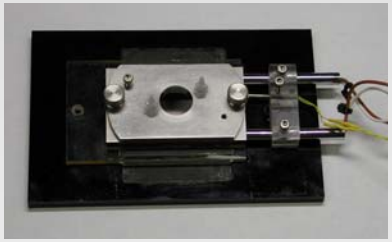






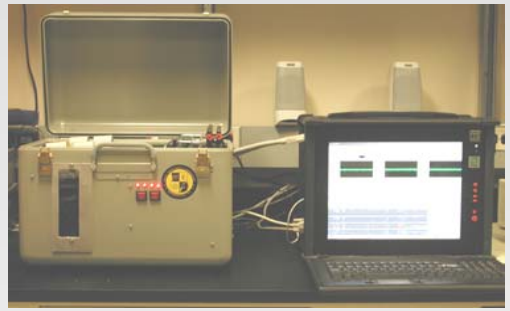
Use of Shipped Networks for Detection

Spinal Cord Network

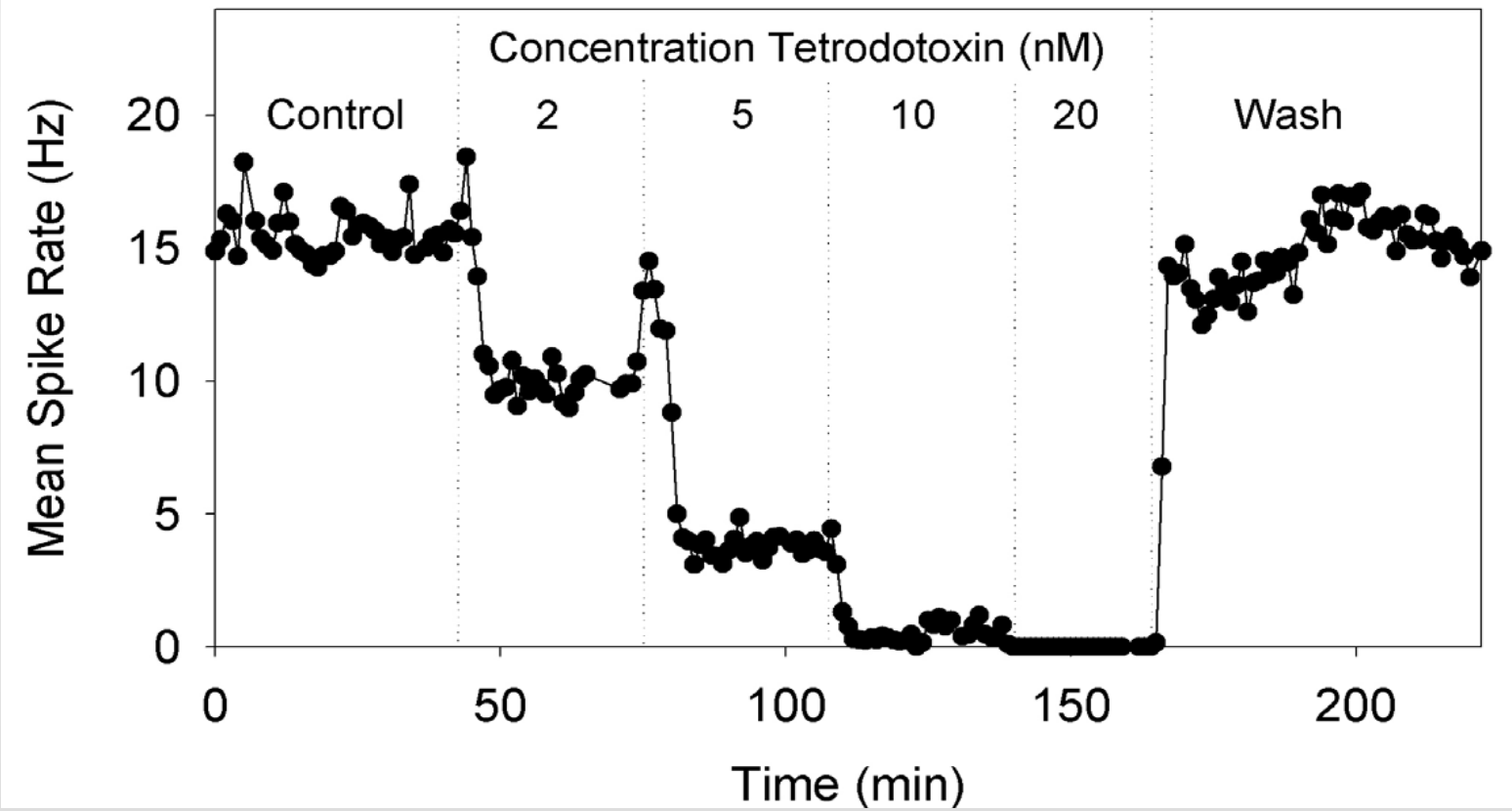


U North Texas

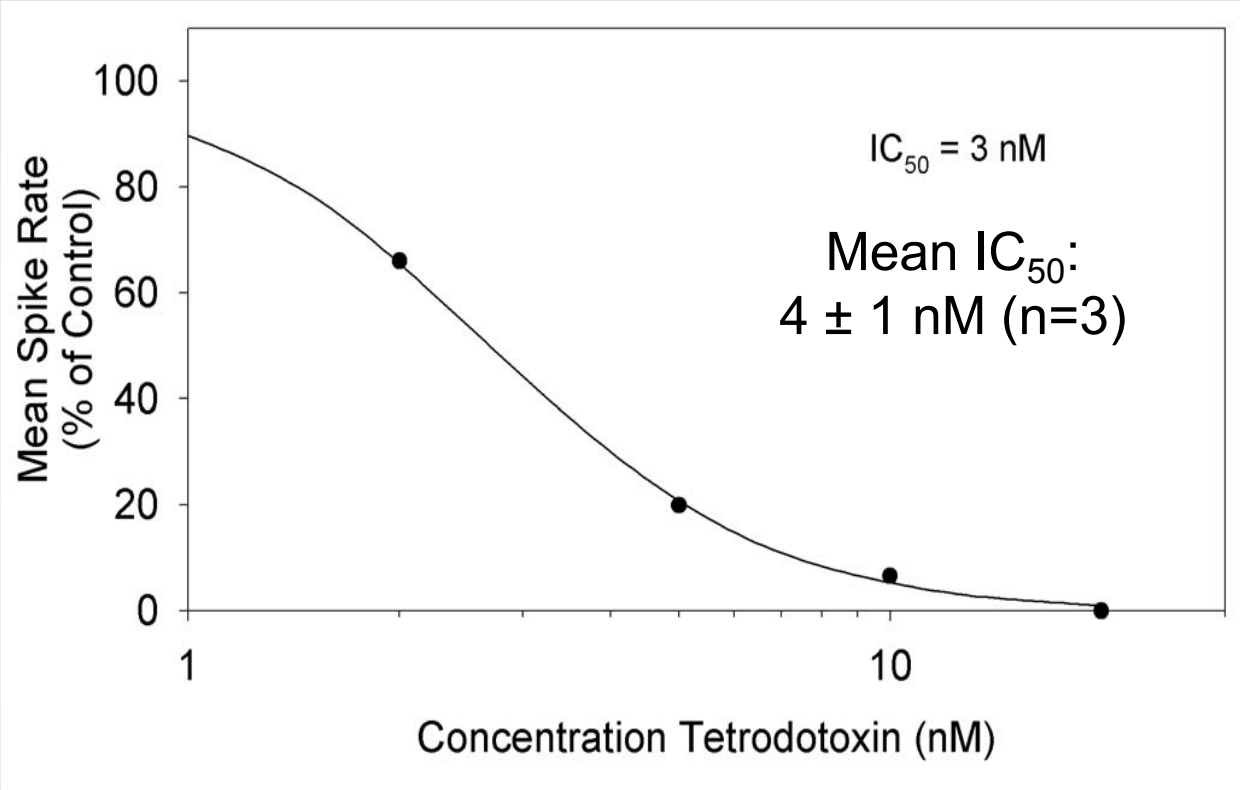
EMERY WORLDWIDE
A CNF COMPANY



Naval Research Laboratory



Quantifying Neurotoxin Effects



To reach oral LD_{50} for Tetrodotoxin. Assume:

300 μg/kg valid for 100 kg human

10 liters of water

Molecular Weight TTX=319 g/mol.

3 mg/l *m-mol/319 mg = 9.4 μM

- Shipping enables availability
- Neurotoxin detection at concentrations well below oral toxic levels

Development of Neuronal Network Biosensors (NNBS)
REMAINING CHALLENGES

Accomplishments

- **The general NNBS concept is viable**
- **NNBS are broad-band biosensors**
- **Networks in vitro are histiotypic**
- **Networks can be shipped.**
- **Extensive data on the pharmacological responses of networks, life support requirements, data management and display, have been amassed. (DARPA)**

NNBS response demonstrations have been scheduled and performed with shipped networks at distant locations such as the Naval Research Laboratory.

Validation and Publication

End-user confidence and interest must be earned

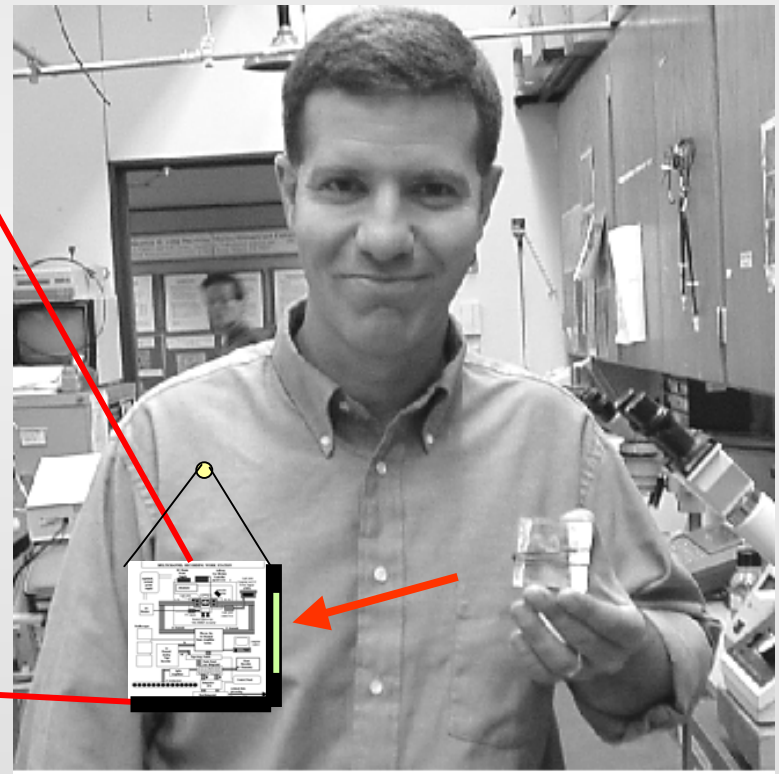
- Quantitative **comparisons** between in vitro and in vivo data (not limited to DOD toxins)
- Demonstration of **breadth of application**
- Demonstration of reliable response performance
- **Long-term survival and stability in flow systems**
- Demonstration of successful data management

Optimal Protocols for long-term sensor (1) survival, (2) reliability, and (3) multiple sample injection utility

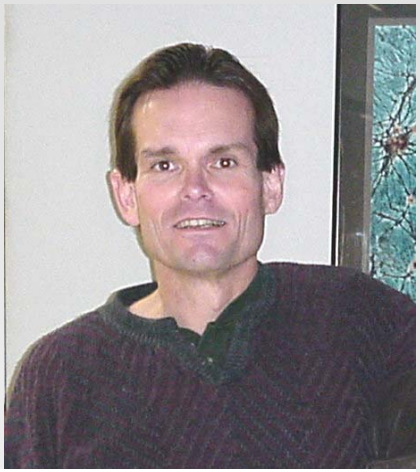
- Duplicate end-user scenarios: i.e. fresh water sample introduction every “n” hours every day.
- Gather data on long-term survival and activity stability.
- Provide information on expected module lifetime,
- Provide response failure test procedures to determine need for replacement.

Failure in the hands of end-users due to inadequate protocols based on limited experience must be avoided.

Miniaturization



CNNS Laboratory of Dr. Gross UNIVERSITY OF NORTH TEXAS



Edward Keefer
Now postdoc at NSI, CA

Alexandra Gramowski
Ph.D. Student, Univ. of Rostock

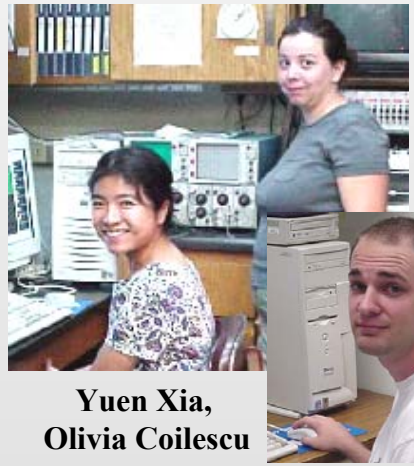
Anthony Curran
Cell Culture Technician

Todd Hall
Photolithography

Bret Zim
Ph.D. Student/MEA Fabrication

Vern Jones
Programmer

Emese Dian
Ph.D. Student



Yuen Xia,
Olivia Coilescu
new PhD students

Jason Brauner
new MS student

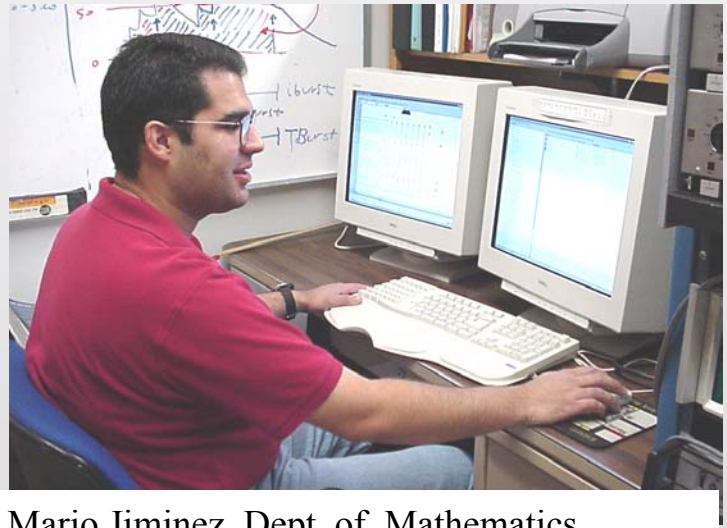
Raphael Toledo, undergraduate student assistant, cell culture

Erich K. Gross, #1 son (UT, Aerospace Engineering)

Dr. Simone Stuewe, Institute for Cell Technology, Univ. of Rostock

Tracy Howard, MEA Fabrication

Autocad designs of MEA's, chambers; equipment repair



Mario Jiminez, Dept. of Mathematics, programming

CNNS FACULTY MEMBERS

Kent Chapman, Assoc. Prof.,
Biology Dept. (Biochemistry)

Jannon Fuchs, Prof.,
Biology Dept.

Kamakshi Gopal, Assoc. Prof.
Dept. of Speech and Hearing Sciences.

Guenter Gross, Regents. Prof.,
Biology Dept. (Neuroscience)

Scott Norton, Prof.,
Biology Dept. (Biochemistry)

Ian Parberry (Computer Sci.)

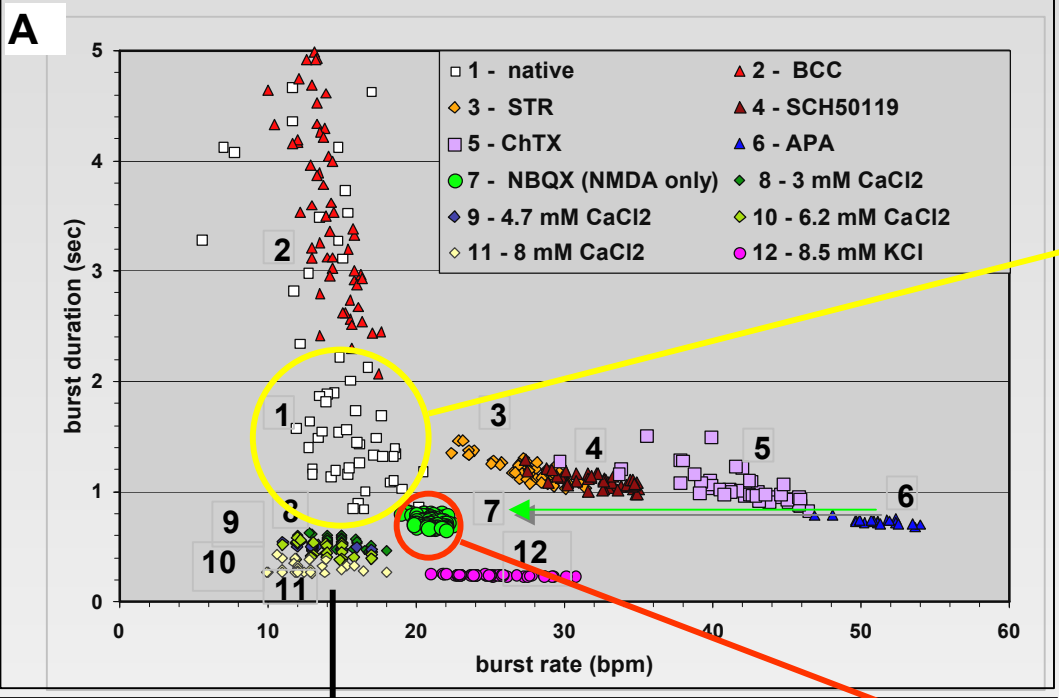
Jen Lucas (Ohio State School of Medicine)

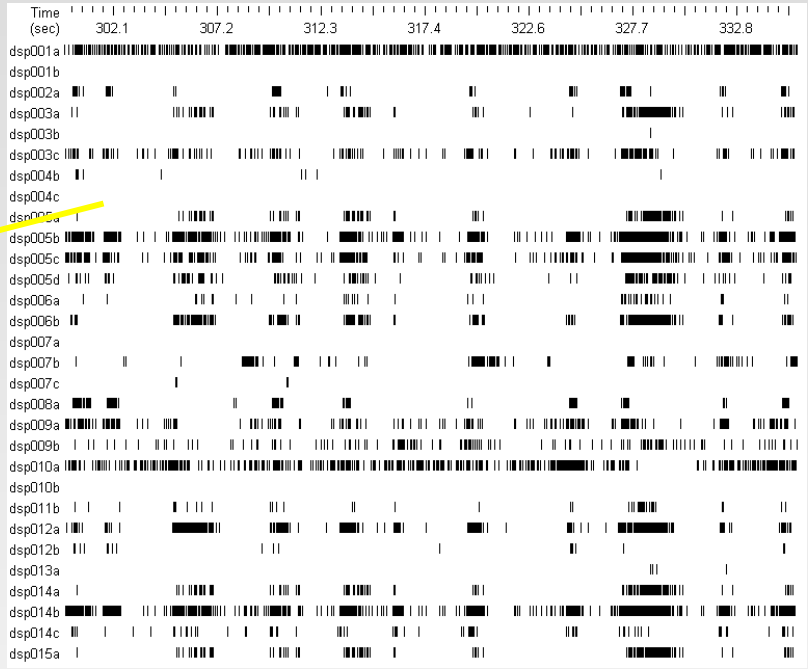
Lorn Howard (Electrical Engineering, SMU)

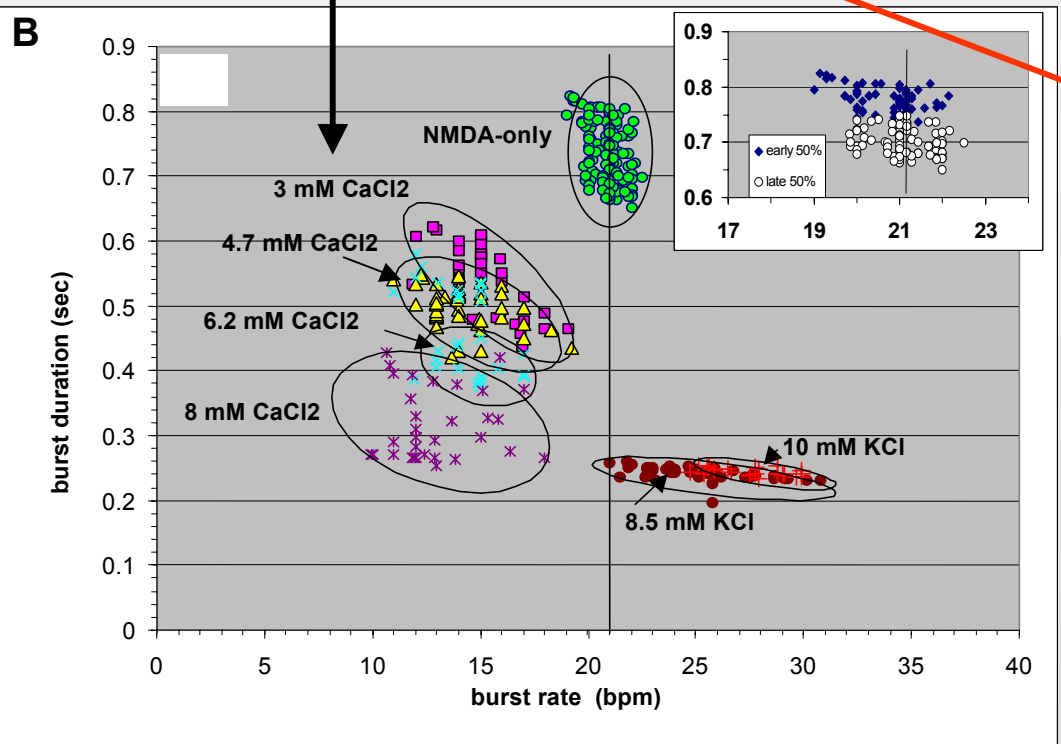
Joe Pancrazio, NRL, Washington, DC.

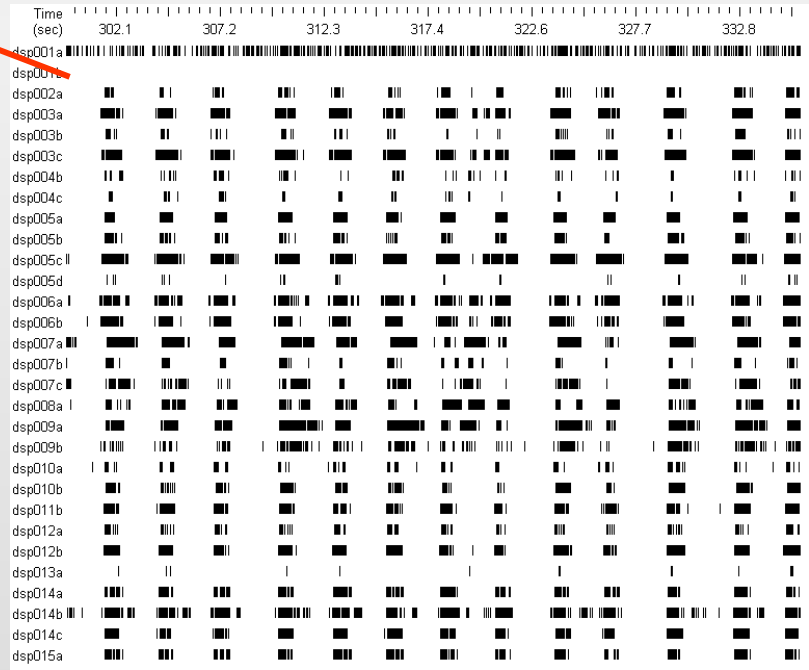
Plexon Inc., Dallas

Research International, Seattle





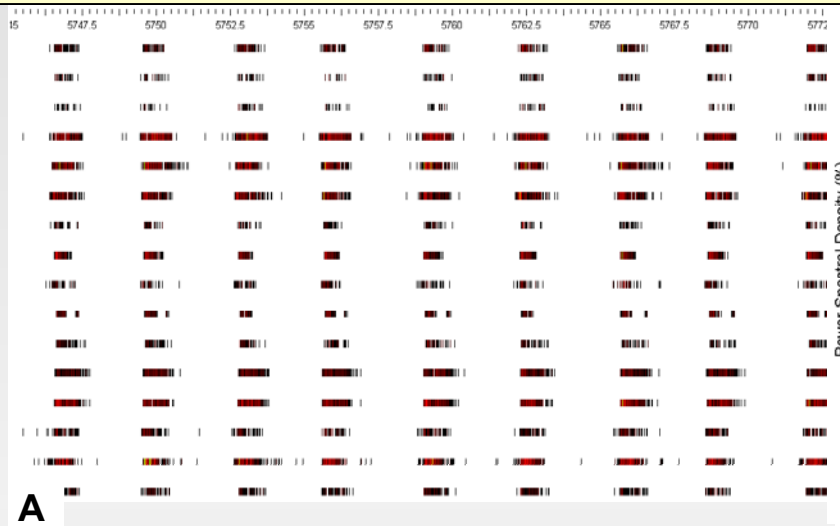


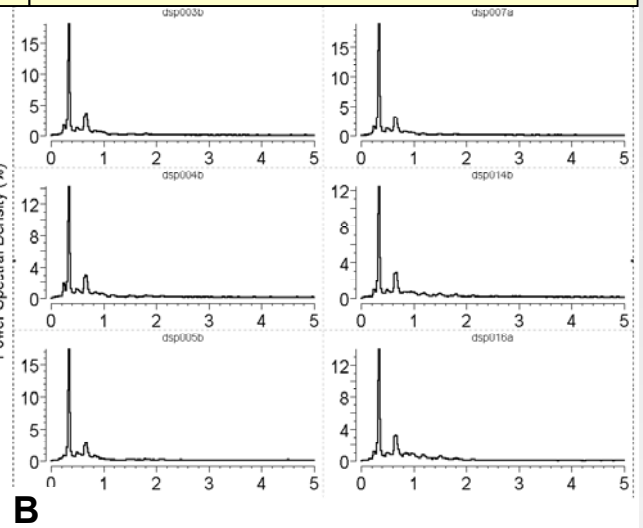


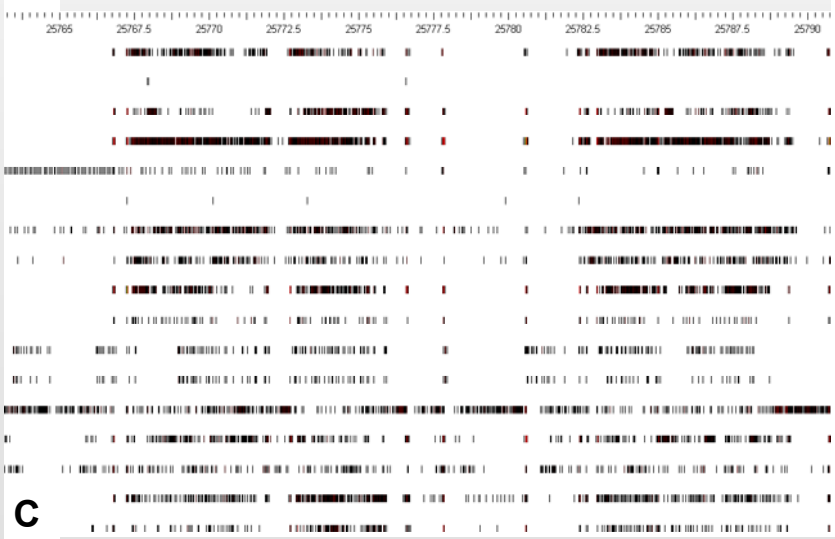
Keefer et al, 2001 J. Neurophysiol.

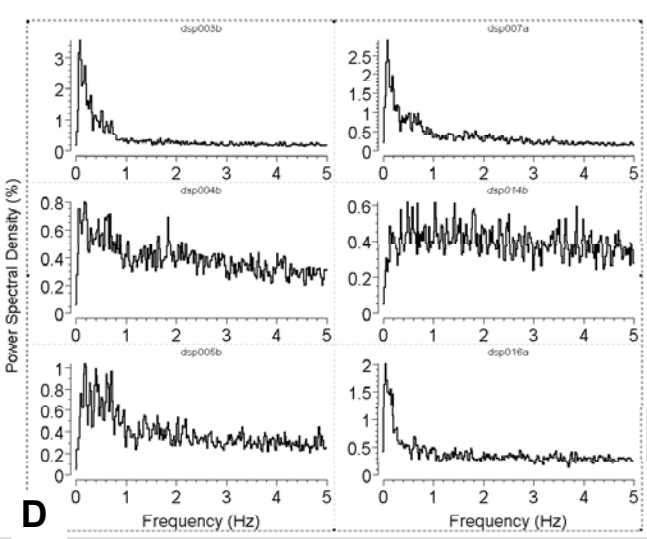
Control of Pattern Dynamics

RASTER DISPLAY of ACTION POTENTIALS	POWER SPECTRUM









NMDA-only State

(all other major synapses blocked by: **strychnine, bicuculline, SCH50119, NBQX.**

After addition of 10 mM NMDA to medium bath: pattern disruption via bypass of presynaptic mechanisms.

SUMMARY

1. Response Reliability: 100% at 2 ng/ml and above; 0% at 1 &1.5 ng/ml.

2. Time to 10%, 50%, and 90% Activity Reduction (mean±SD)

50%

100 ng/ml:	2.1 ± 0.2 hours	(n =7)
50 ng/ml:	2.2 ± 0.5 hours	(n = 11)
25 ng/ml:	4.2 ± 1.1 hours	(n = 8)
5 ng/ml:	5.2 ± 1.6 hours	(n = 5)

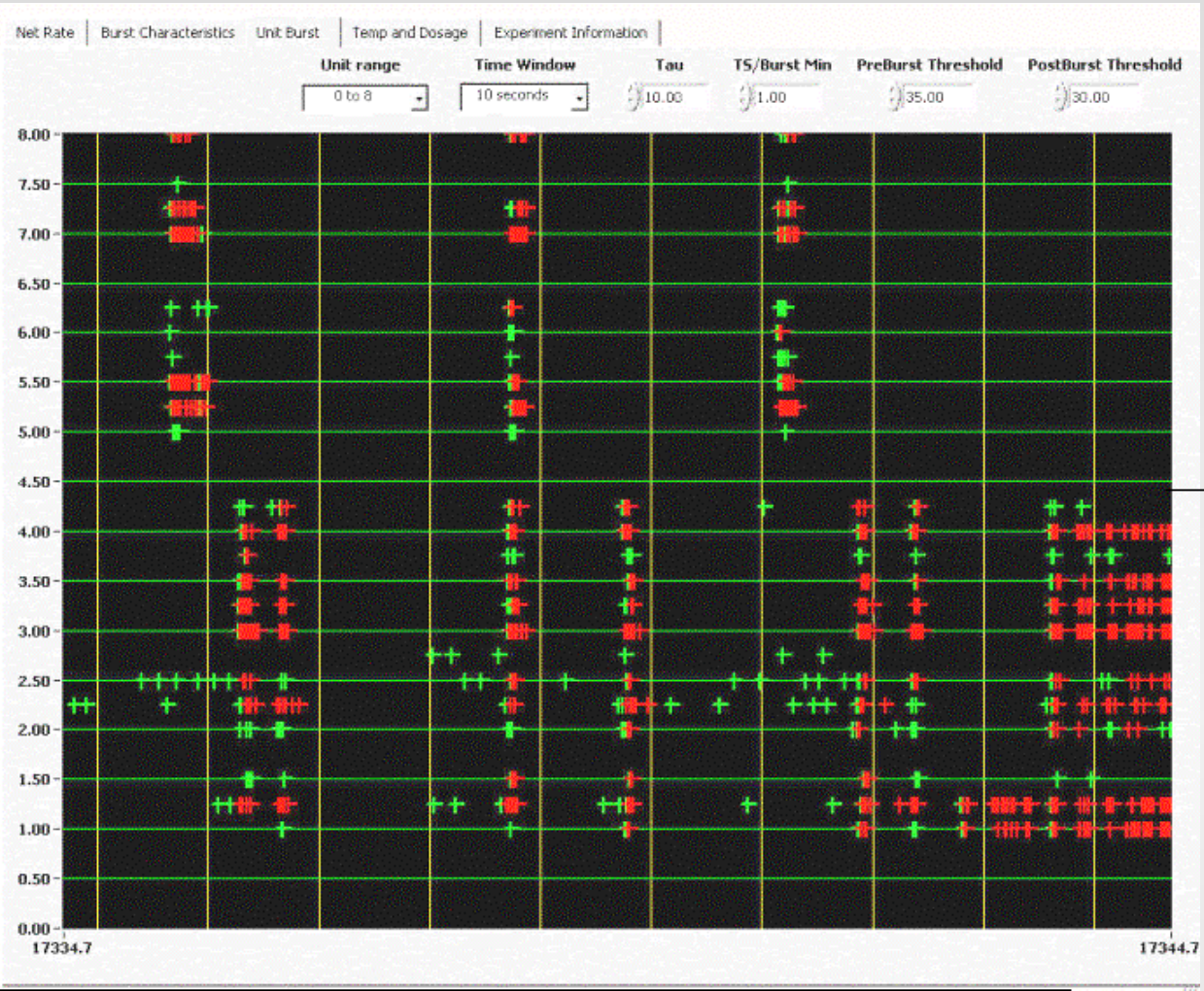
3. Subpopulation Responses (discriminated active units)
   - Global at 5 ng/ml and above
   - Heterogeneous at 2 ng/ml
   - Individual biphasic responses at all concentrations

4. Network Biphasic Reponses: 12% of all cultures.

5. Antisera Protection
   - Can be demonstrated by continued activity under BoNT

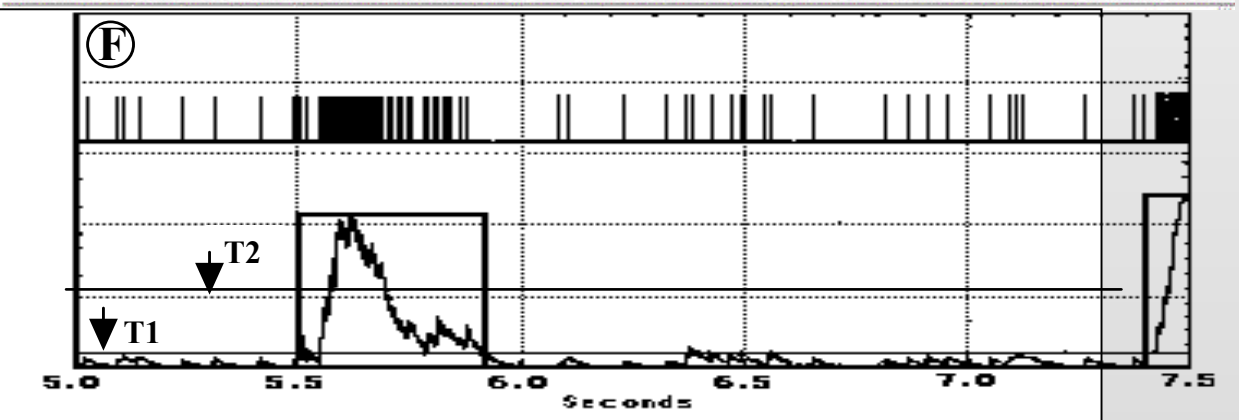
6. Network activity can be partially revived with 4-AP.

DISPLAY of BURST IDENTIFICATION USING NACTAN*



Matrix A

Matrix B



*CNNS program
by
Jacob Schwartz