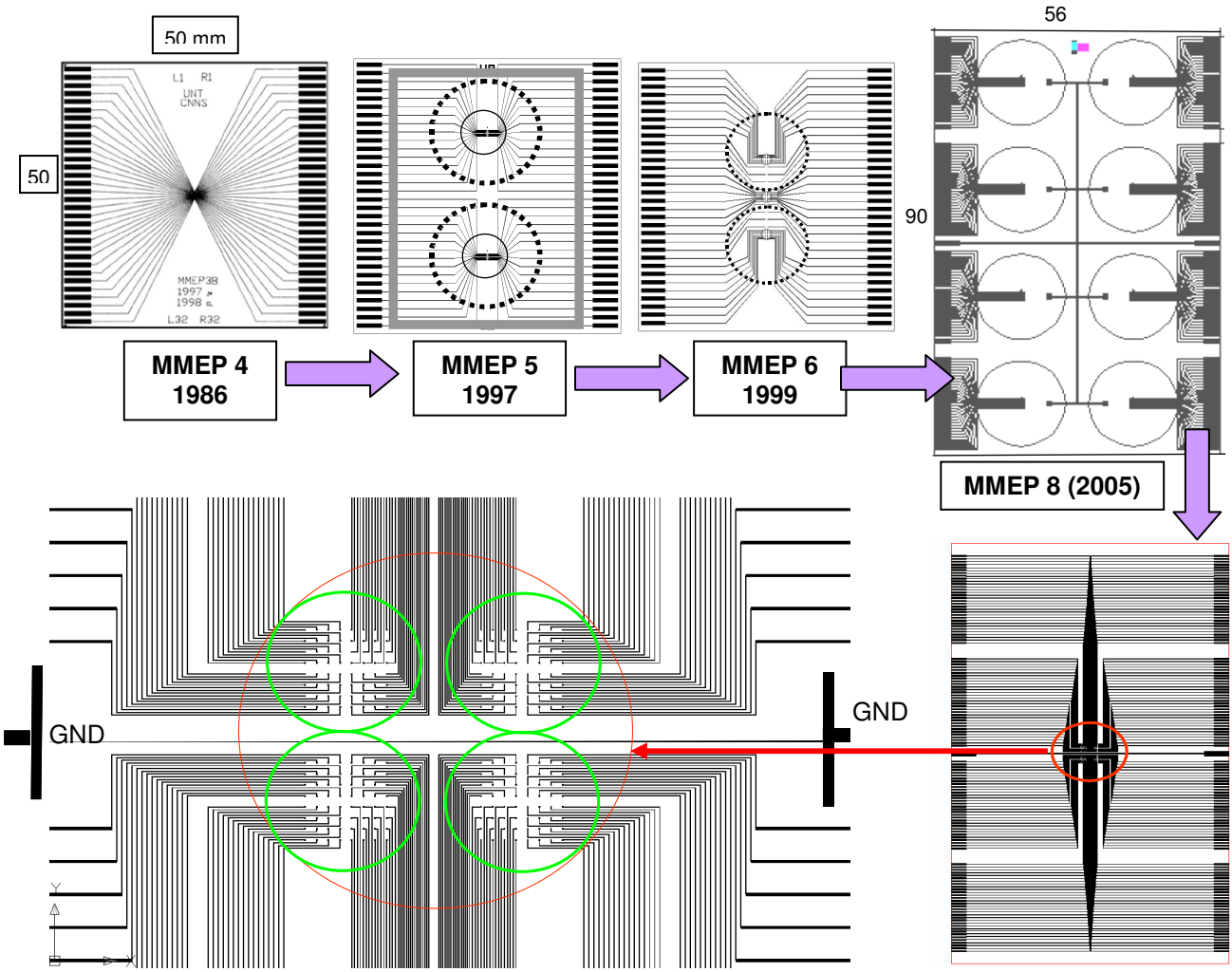
Evolution of microelectrode array plates fabricated at UNT

All plates consist of optically flat glass and transparent indium-tin oxide conductors. Plates are spin insulated with methyltrimethoxysilane resin and de-insulated at the tips of the conductors with single laser shots. Except for the masks, all fabrication is performed by students working with the Center.



Electrode spacing: 200 μm
Field separation: 2 mm

Edge contacts: 300 μm
Pitch 300 μm

MMEP 8.256 May 06

MMEP 8.256: Same plate as MMEP-8 but with all 256 electrodes located in a center area to record from one network. Amplifier contacts are also the same, allowing use of common electronic data acquisition equipment.

MMEP 4: 64 microelectrodes arranged in an 8 x 8 format with equal electrode separation of 150 um.
MMEP 5: 64 microelectrodes serving two separate recording matrices of 32 electrodes each.
MMEP 6: 64 microelectrodes arranged to form three recording areas with 16 electrodes each connected by two linear conduits each with 8 electrodes.
NOTE: MMEPs 4-6 have the same amplifier connections
MMEP 8: 8-network plate with 32 electrodes per recording matrix. Compatible with Plexon Inc. 256 preamplifier circuit board.

Summary of Methods and Examples of Results

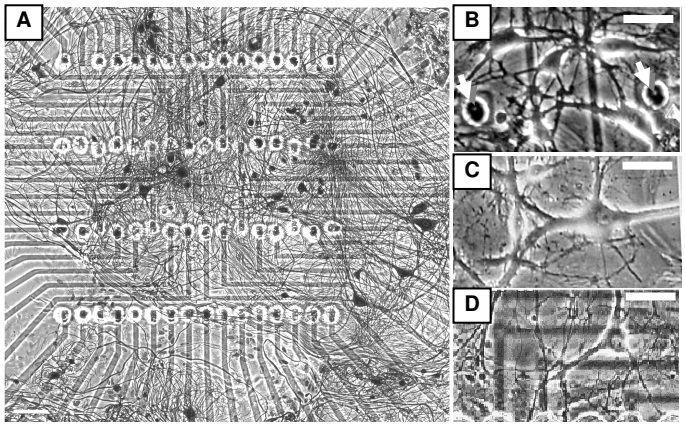


Figure 1. Examples of neuronal circuits on microelectrode arrays. Transparent conductors allow extensive optical access to the network morphology. (A) Neuronal network derived from murine spinal cord tissue (98 days *in vitro*), grown on the recording matrix of a 64-electrode array plate. Bodian stain. (B-D) Living neurons on MEAs Gold-plated exposed ITO conductors are shown by arrows in (B). The conductors are 8 µm wide. (all bars = 40 µm).

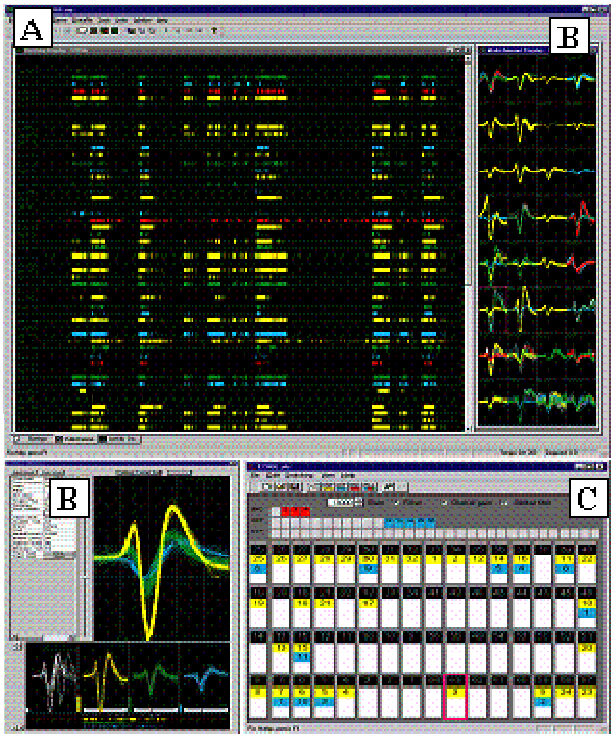
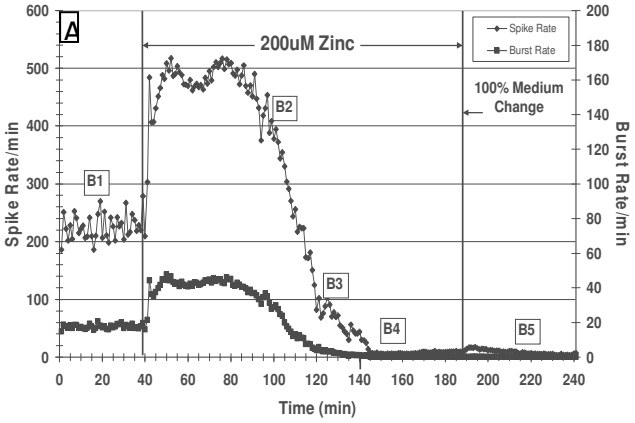


Figure 2. (Right) Electronic display of time stamp patterns (A), waveforms (B), and electrode layout (C). (Plexon Inc., Dallas).

EXAMPLE 1. Zinc Toxicity (Parviz, M. and Gross, G.W. (2007) Quantification of zinc toxicity using neuronal networks on microelectrode arrays. NeuroToxicology 28: 520-531.)



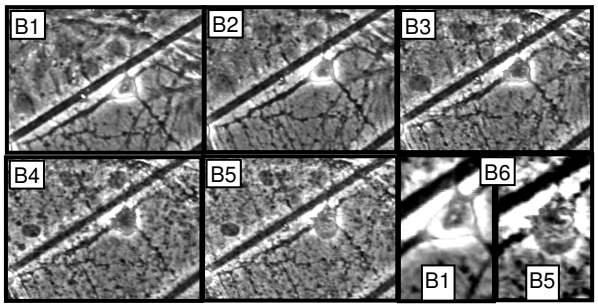
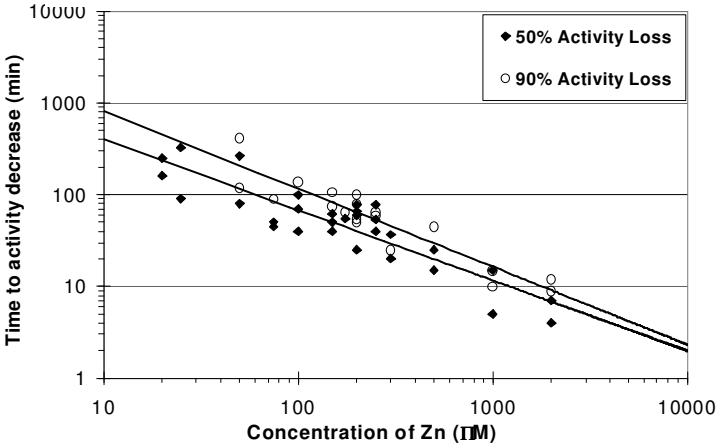
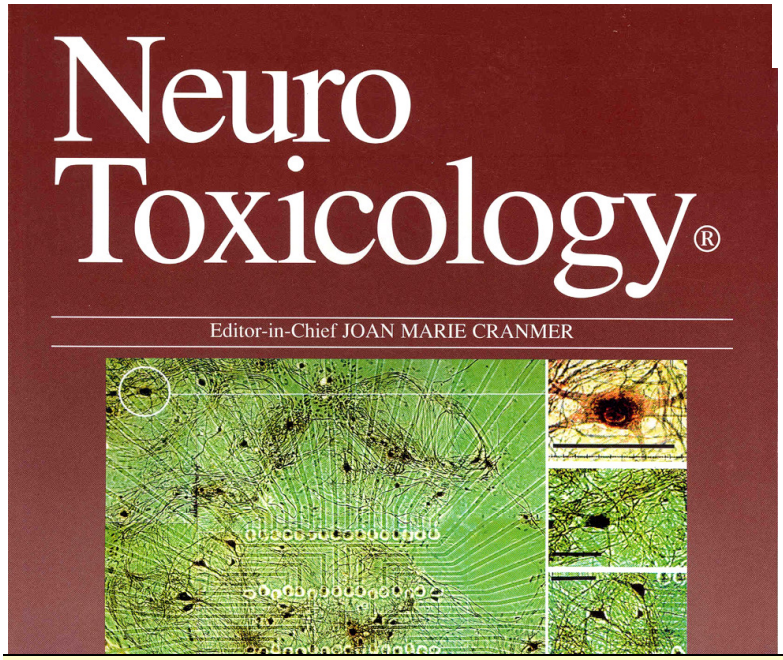


Figure 3. (A) Common network response to high concentrations of zinc. Each data point represents the average spike (left ordinate) and burst (right ordinate) rate in 1 min bins for all discriminated units. Addition of 200 µM zinc at 38 min results in an immediate excitatory period lasting 70 min followed by activity decay for about 30 min and complete, irreversible activity loss. (B1-B6) Consecutive pictures of the same neuron taken at approximately 30 min intervals. At 220 min (B6, right panel), the neuron is swollen and necrotic in appearance.

Figure 4 (below). Pooled data from spinal cord and frontal cortex cultures. A double log plot reveals linear functions for 50% and 90% activity decreases. All experiments (n=23) were conducted in serum-free and albumin-free medium. Activity losses were irreversible.





EXAMPLE 2. Screening Novel AChE Blockers

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.

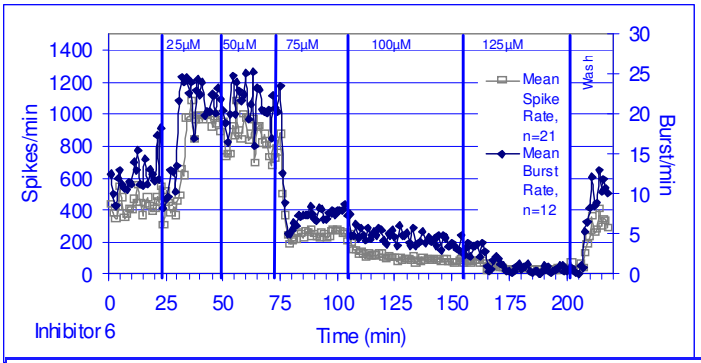
BIOCHEMICAL DATA CONFIRMED BINDING TO AChE.
However, two out of seven compounds were irreversible inhibitors of activity (**unexpected secondary binding**.)

Keefer, E.W., Norton, S.J., Boyle, N.A.J., Talesa, V., and Gross, G.W. (2001) Acute toxicity screening of novel AChE inhibitors using neuronal networks on microelectrode arrays. NeuroToxicology 22: 3-12.

NOVEL ACHE Blockers

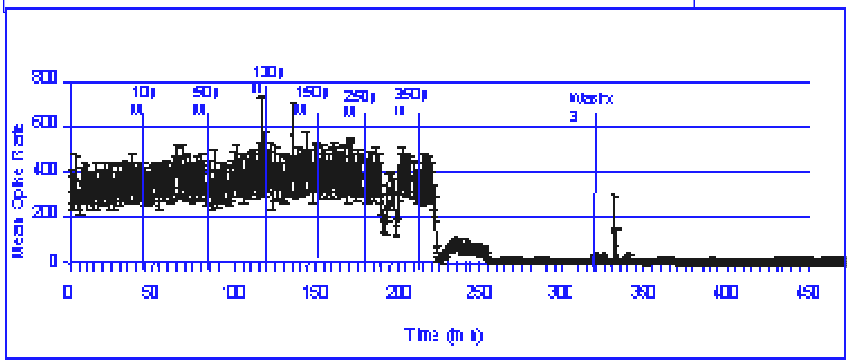
	AChE Inhibition. Const Ki (M)	Inhibit. Type	Excitat.	Inhibit.	Reversi-bility	No of experiments
Ch^+O-CO-S$(CH_2)_2$S-CO-OCh^+	$1.0x10^{-6}$	Mixed	10		R	2
Ch^+O-CO-S$(CH_2)_3$S-CO-OCh^+	$1.4x10^{-6}$	Mixed	50		R	3
Ch^+O-CO-S$(CH_2)_4$S-CO-OCh^+	$1.0x10^{-6}$	Mixed	10		R	2
Ch^+O-CO-S$(CH_2)_5$S-CO-OCh^+	$1.4x10^{-6}$	Mixed	NE	NE	--	2
Ch^+O-CO-S$(CH_2)_6$S-CO-OCh^+	$1.4x10^{-6}$	Mixed		350	I	2
$DMEA^+O$-CO-S$(CH_2)_4$S-CO-$DMEA^+$	$3.6x10^{-7}$	Mixed	25	125	R	3
$DMEA^+O$-CO-S$(CH_2)_6$S-CO-$DMEA^+$	$5.0x10^{-7}$	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.



$DMEA^+O$-CO-S$(CH_2)_4$S-CO-$DMEA^+$

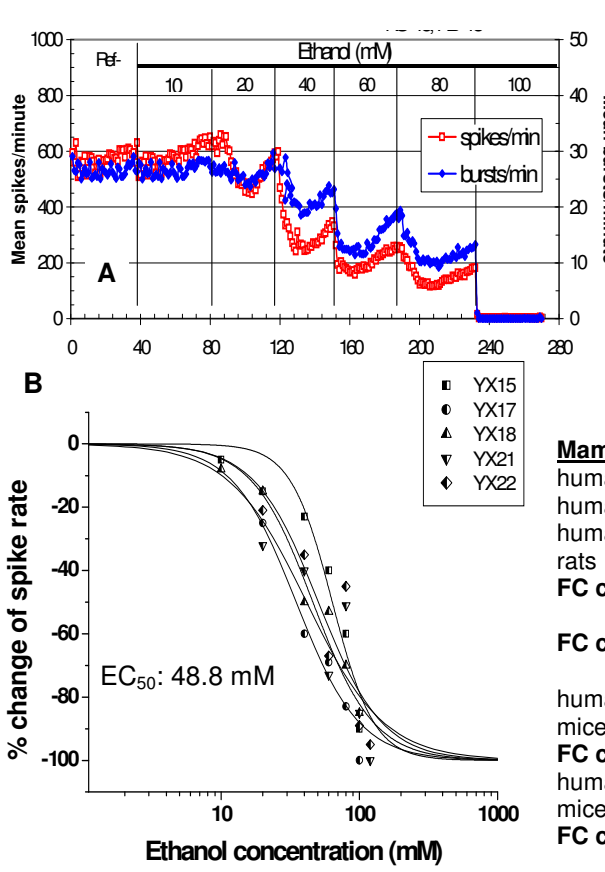
Figure 5. Desired spike and burst response profiles activity increases at defined concentrations with reversible toxicity at higher concentrations. Such drug efficacy range information can be obtained in a few hours and used to guide and minimized subsequent animal experiments.

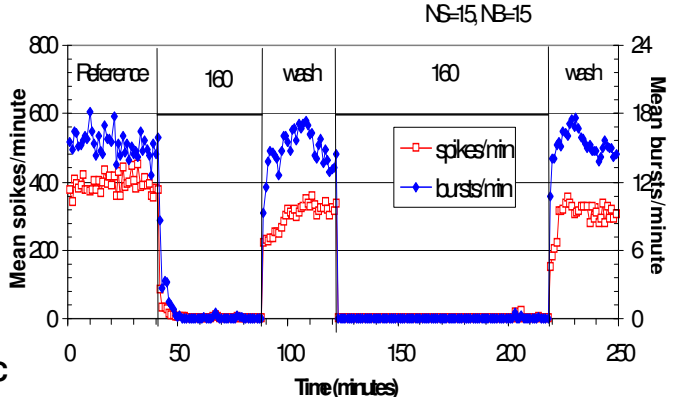


Ch^+O-CO-S$(CH_2)_6$S-CO-OCh^+

Figure 6. Unexpected irreversible toxic effect at 300 µM. Activity could not be recovered by three medium changes and during 24 hr monitoring.

EXAMPLE 3. Histiotypic Mammalian Network Responses to Ethanol and Responses to Botulinum Toxin A.





Comparison of ethanol effects on mammals and FC cultures

Mammal	Conc.	Effect
humans	5-15	slight impairment of attention and judgment
humans	10-22	impairment of speech and balance
humans	15-30	significant sedation
rats	20	sedation
FC cultures	10	first measurable change in activity pattern (slight) excitation
FC cultures	15	first overt change in spike and burst production (inhibition)
humans	30-55	mental confusion
mice	40	loss of righting reflex
FC cultures	48.8	EC_{50} for spike production
humans	100	coma and death
mice	120	coma, hypothermia
FC cultures	100-140	cessation of all spontaneous activity

(human and animal data from Charness et al., 1989; Little, 1991).
FC: frontal cortex cultures

Fig. 7. (A) Responses of networks to low and high concentrations of ethanol. 160 mM (60 mM past coma) for 2 hr exposures are still reversible. (B) Dose response curves from five networks. The mean EC_{50} is 48.8±5.4 mM. (C) Table comparing responses to alcohol for humans, mice, and mouse networks in vitro.

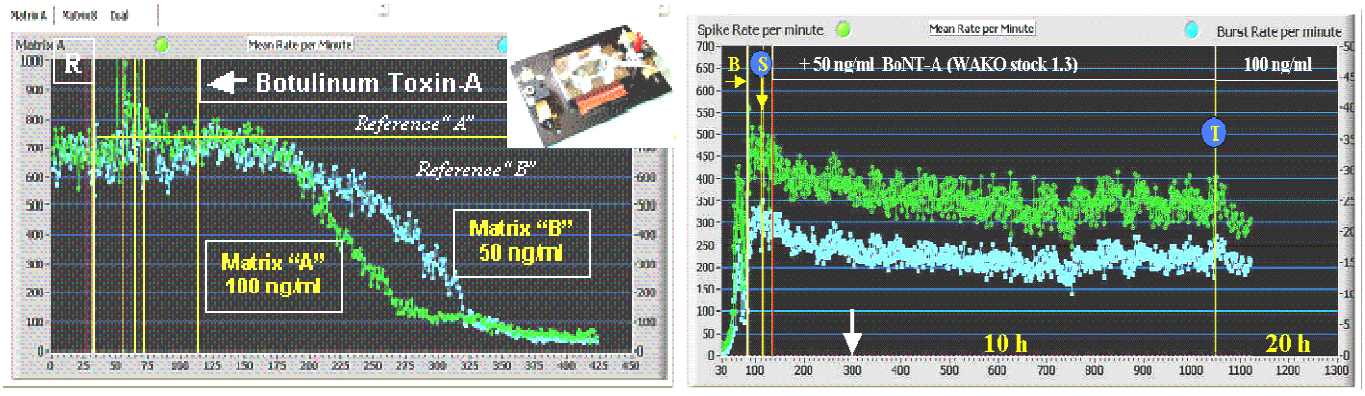


Fig. 8. Left Panel: Two-network module experiment showing normal responses to botulinum toxin A at 50 ng/ml and 100 ng/ml. Irreversible loss of activity occurs at approximately 3 hrs after application. Right Panel: Protection of exposed network by mouse antiserum. Activity is still strong at 20 h. Note: the two traces in the right panel are showing mean spike production (green) and mean burst production (blue) of a single network. The left panel shows mean spike production per minute for two independent networks. Paper in preparation.

EXAMPLE 4. Pharmacologically Induced Different Network States (Spike and Burst Patterns)

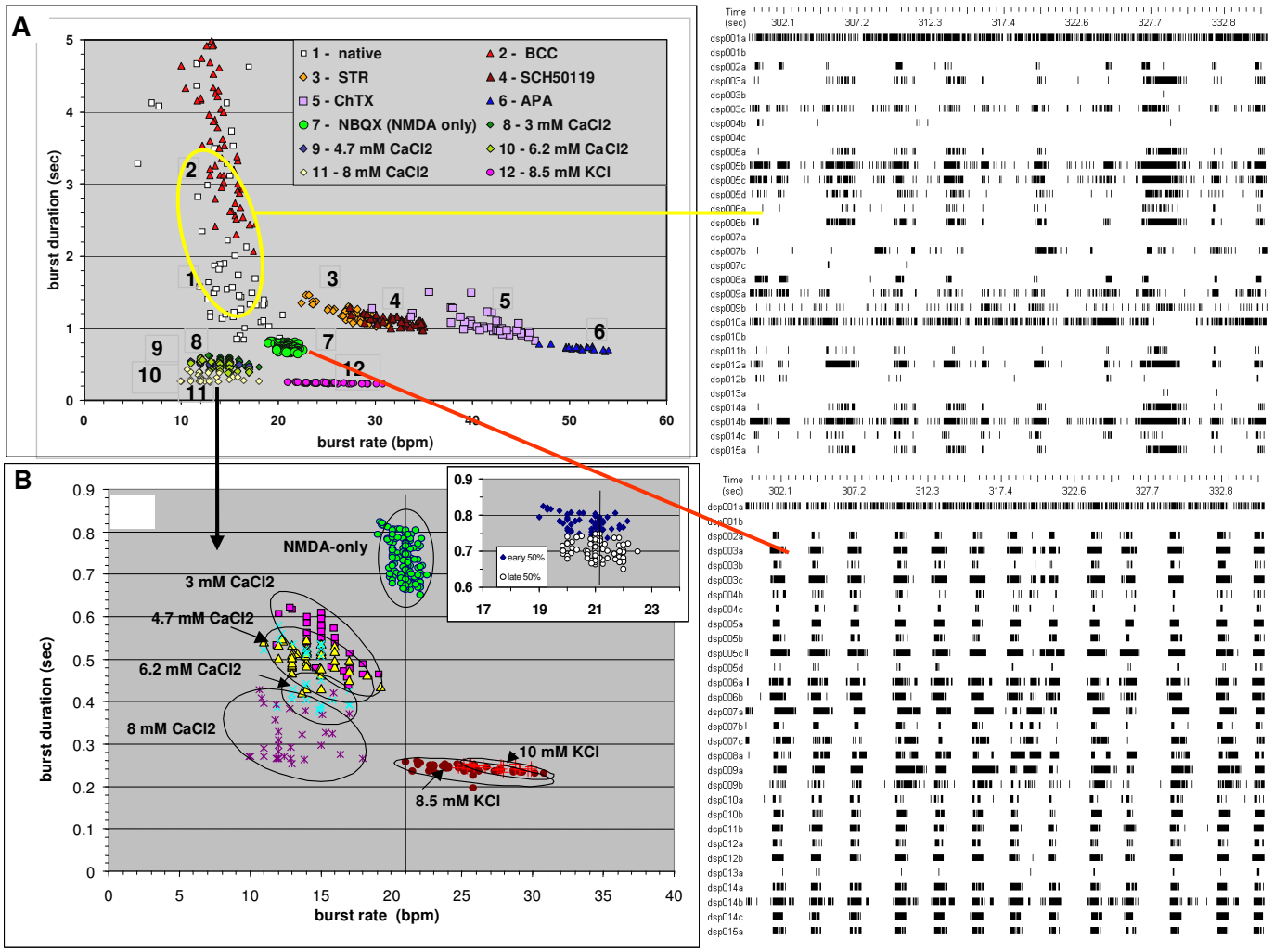


Fig. 9. Network exposure to 11 different pharmacological states results in reliable pattern changes expressed here as clusters of burst rate plotted against burst duration. The native raster plot and that for a much more organized burst oscillation state (induced by blocking all synapses except NMDA synapses) are shown in the panels to the right. State 11 at different Ca++ concentrations is expanded in B.

From Keefer, Grawowski and Gross (2001) J. Neurophysiol.

EXAMPLE 5. Quantitative Pharmacology: Determination of Dissociation Constants

Determination of Kd values for the GABAA receptor blockers bicuculline, gabazene, and trimethylol propanephosphate.

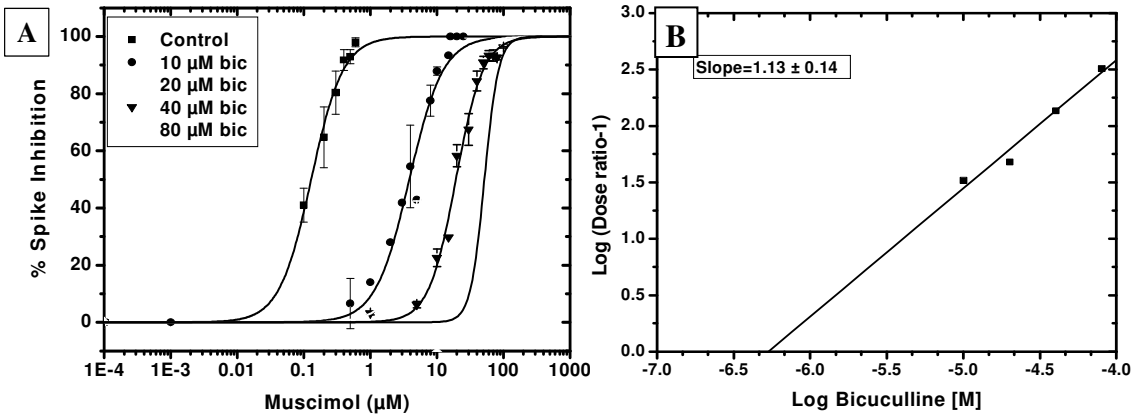


Figure 10. (A) Concentration response curves of the normalized data in absence and in the presence of 10, 20, 40, 80 µM of bicuculline. Vertical bar represented mean and SEM of spike activity change. (B): Schild plot of log (dose ratio-1) vs log bicuculline concentration (Molar) for the antagonism of muscimol. The fitted solid line is determined by a linear (least square) fit without slope constraint. PA2=6.3, Kd=0.52

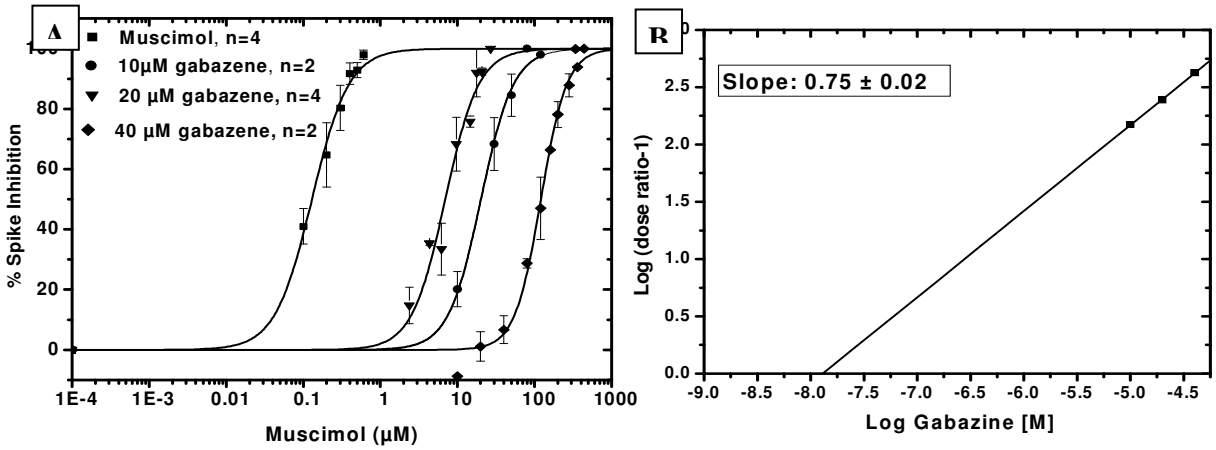
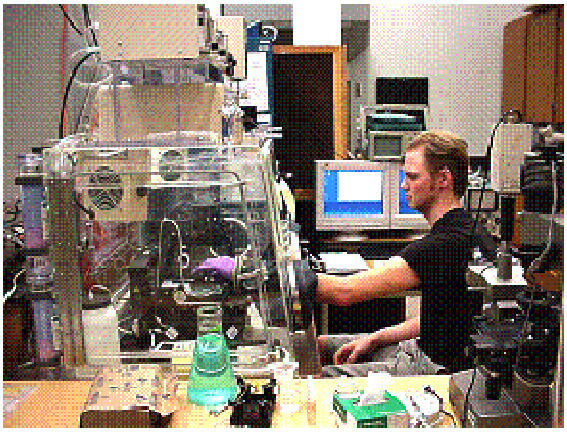
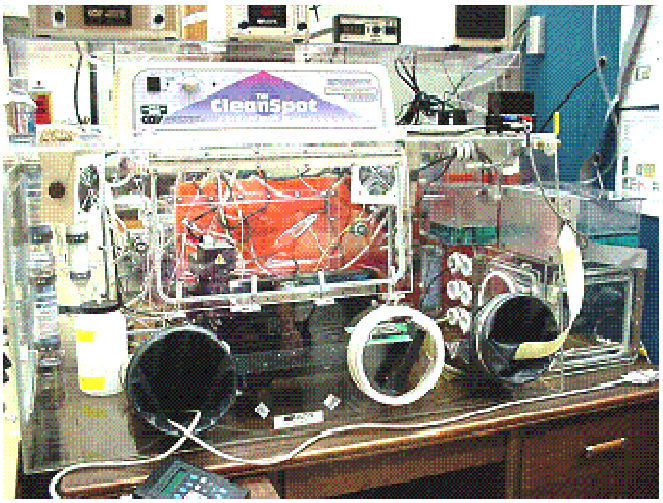


Figure 11. (A) Muscimol titration in the presence of 0, 10, 20, 40 µM of gabazine. Vertical bars represent mean and SEM of spike activity change. (B): Schild plot of log (dose ratio-1) vrs log gabazine concentration (M) for the antagonism of muscimol. Linear fit (least square) without slope constraint. PA2=7.9, Kd=0.015 µM

EXAMPLE 6. Robotic culture maintenance.





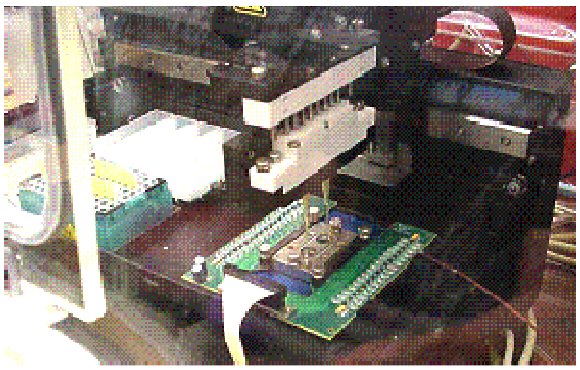


Fig. 12. Environmental chamber enclosing liquid handling robot. Chamber maintains sterility, 10% CO_2 for pH control , constant humidity, constant temperature. Oxygen levels can also be controlled.

Left: Preliminary tests with two-chamber module and 64 preamplifiers.

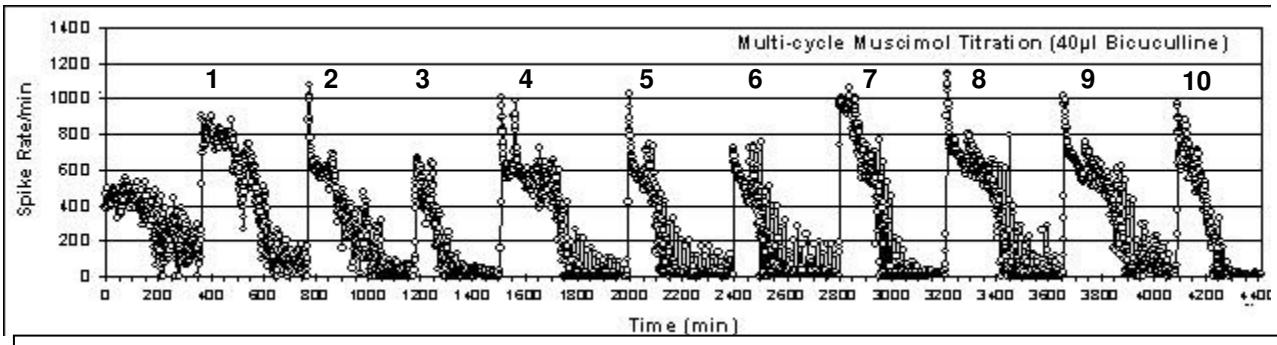
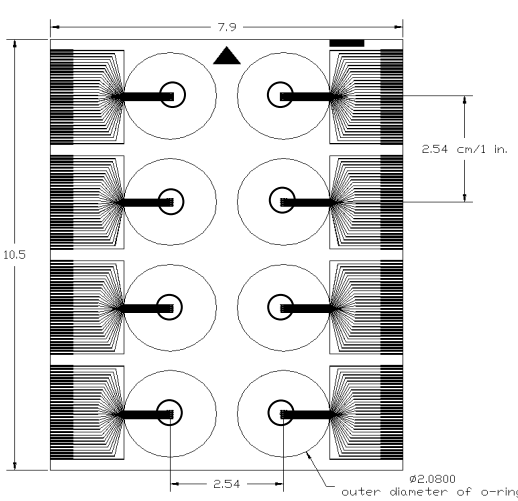


Fig. 13. Preliminary data from robotic pharmacological experiments. 72 hour robot manipulation of a single network in an environmental chamber. The panel shows 10 cycles of medium changes with 40 uM bicuculline and titration with muscimol. Program manipulations caused some response profile changes (e.g. cycle 3). System noise (N), is caused by stepping motors and some vibrations of amplifiers during robot movement. Improvements in programs, noise abatement, and mixing of test substance with medium should provide almost identical response profiles. All data points represent “minute means” (all discriminated, digitized units averaged in one minute bins). Bicuculline was added to wash medium pool before experiment.

EXAMPLE 7. 8-Network Array Design and First Recording



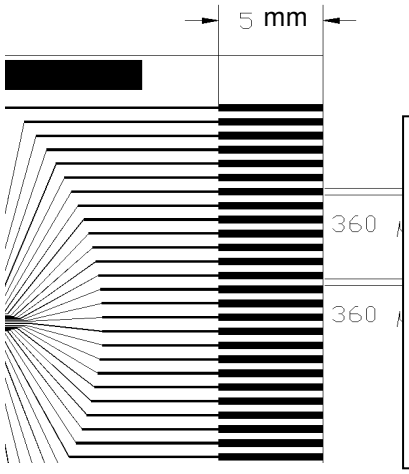
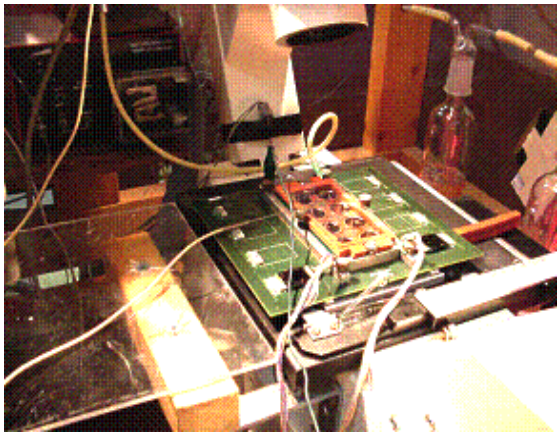
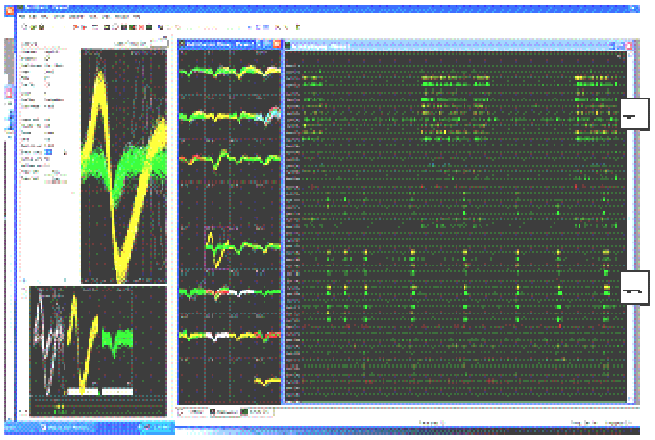
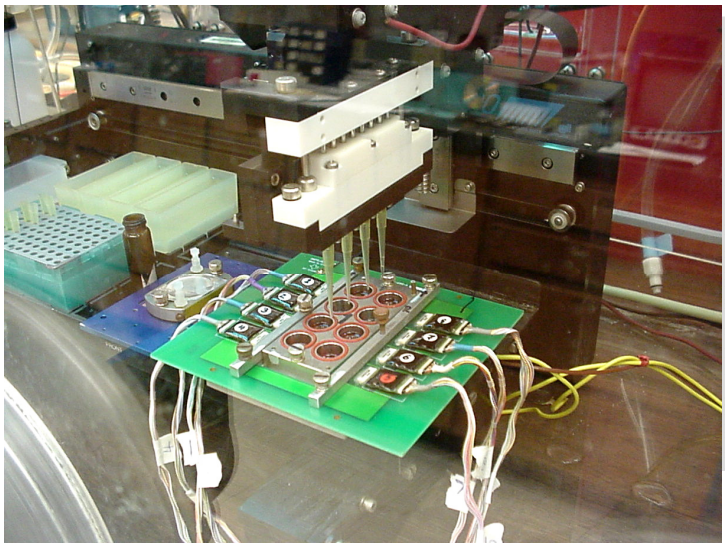


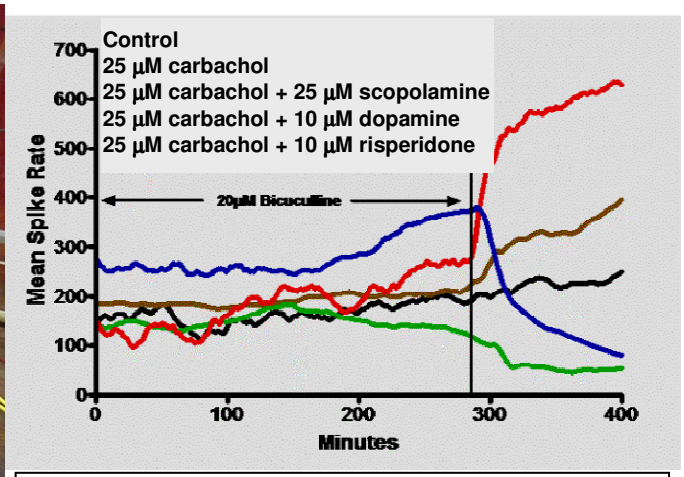
Fig. 14. Design of 8-network array plate (glass with transparent indium-tin oxide conductors) and first electrophysiological recording on a microscope stage (Dec 24, 2005). Note only two 32-channel preamplifiers were available.





First simultaneous multinetwork pharmacology experiment
(Dr. Edward Keefer, UT Southwestern Medical Center using CNNS facilities)





Left: 8-network assembly with 8 VLSI preamplifier modules for 32 channels each. Above. 5-network simultaneous recording