Neuron/Electronic Interfacing

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Content:

Contrasting neural and electronic systems
Connecting neural and electronic systems
In vitro approaches
Signals, applications and interpretation
Cultural and ethical issues

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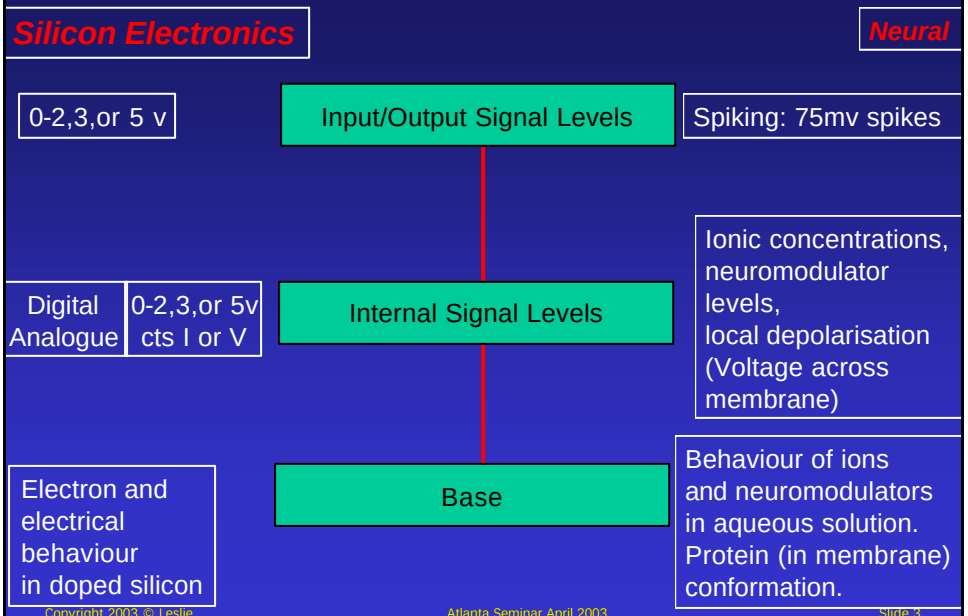
Neural and Electronic Systems

Electronic		*Neural*
Designed	Information Coding	Evolved
Logic levels (digital) Voltages/currents (analogue)	Signal Coding	Spike trains Chemical Signalling
0 to 3 volts (approx) (varies)	Signal Levels	-100 to +75mV Concentration levels and gradients
Transistors	Active Elements	Ionic Channels Membrane morphology and channel distribution
Electrons	Conduction Elements	Multiple species of ion Neuromodulators, Neurotransmitters

Silicon electronics and neural systems



Neurons and Electronics

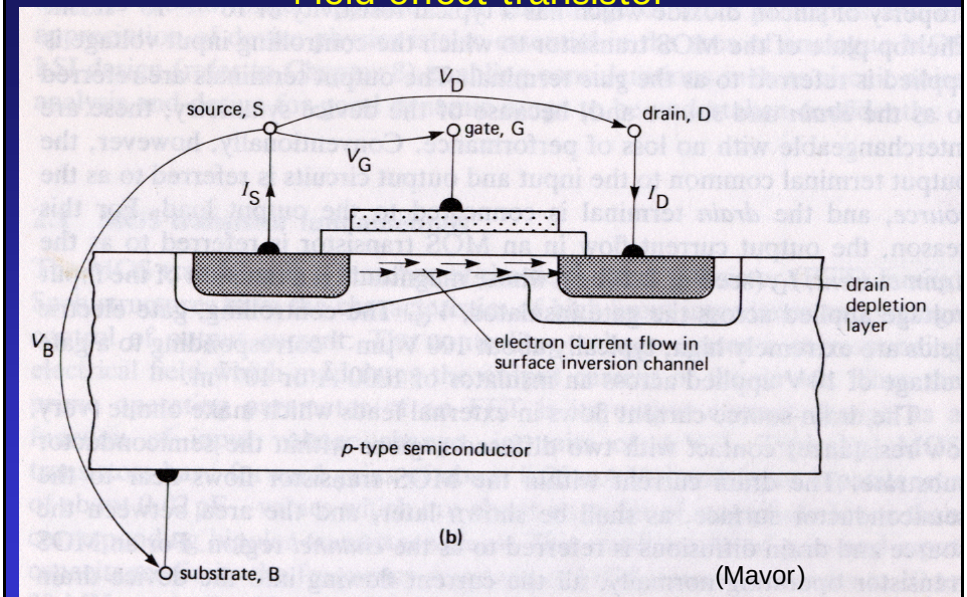
... or perhaps ionics and electronics

- Electronic systems work using potential differences mediated by the movement of electrons (electron currents)
- Semiconductor-based computers use a mixture of crystal-based electron energy (band) localisation and electrostatic effects.
- Neural systems utilise potential differences, mediated by the movement of a number of different species of ions
- Many mechanisms for modulating ion movement
  - numerous ion channel types
  - numerous modulating chemicals

Neural systems and electronics are tenuously connected by their use of electrical potentials

This can mislead us: even the mechanisms of conduction and of potential generation are different.

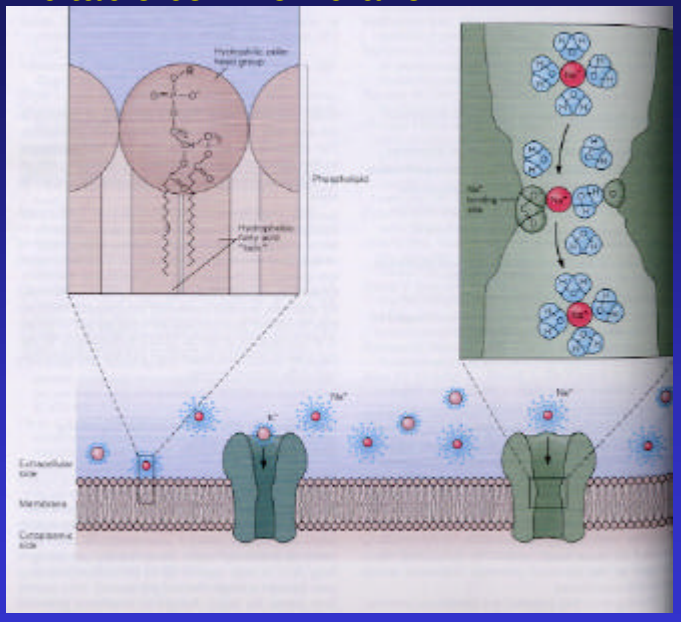
Field effect transistor



(Mavor)

Excitable cell membrane

Diagram of piece of cell membrane
Note that there are many types of charge carriers



(Kandel)

Mechanisms of conduction

- Electronic: movement/diffusion of electrons in electric field
- Neural:
  - Ionic diffusion due to concentration gradient (thermodynamic diffusion) and potential difference
  - Active ion transportation
    - ion pumps
    - selective ionic channels

Conduction modulation

- Electronic: modulating the electron banding in crystalline Silicon using dopants (static) and electric fields (dynamic)
- Neural:
  - Modulating ion channel proteins by altering potential difference across them, or using large ions (e.g. Mg++, NMDA channels)
  - Neuromodulators

Single form (or small number)

Multiple forms

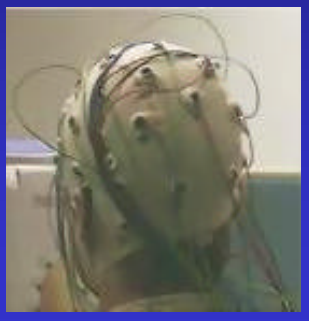
Differences at higher levels

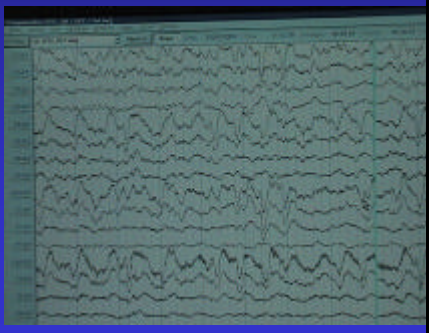
- Information Coding
- Little agreement about what spikes in neural tissue mean
  - sometimes clear: rate codes at neuromuscular junctions
- Rate codes, timing codes, synchronisation, synchronous oscillation, chains of spiking, ...
- Even with electronic systems, evolved codes are hard to comprehend
  - often impossible when number of neurons > 10
  - e.g. when GA's are used to evolve controllers
- ...and since brains are not defined precisely by genetics
  - (genetics defines structure, plasticity, etc., but not at an exact level)
- it may be that different brains code things differently

Connecting neural and electronic systems

- Electro-encephalography (EEG)
  - Earliest form of neural recording: Berger 1923.
  - relatively non-invasive
  - records only overall potentials generated by millions of neurons
  - does have the possibility of controlling electronic equipment (or biofeedback equipment)
    - but signal is very noisy and difficult to control/interpret reliably
    - see Brain/Computer Interface (BCI) competition 2003

Credits: University of Mannheim, University of Florida





Recording using metal electrodes

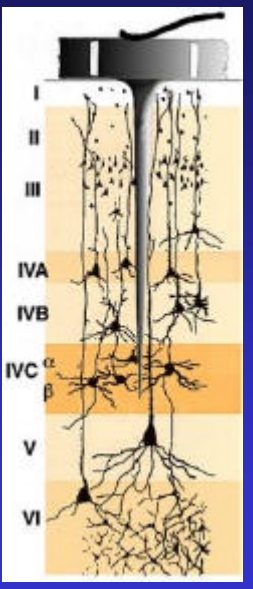
- Improving the localisation of the detection of signals entails placing the sensor and the neural system close together.
- Small metal electrodes (Platinum usually) are inserted into the neural tissue.
- These record field potentials.
  - again from many neurons, namely those near the electrode.
  - Not as many as EEG
- Technique is much more invasive than EEG

Single electrode

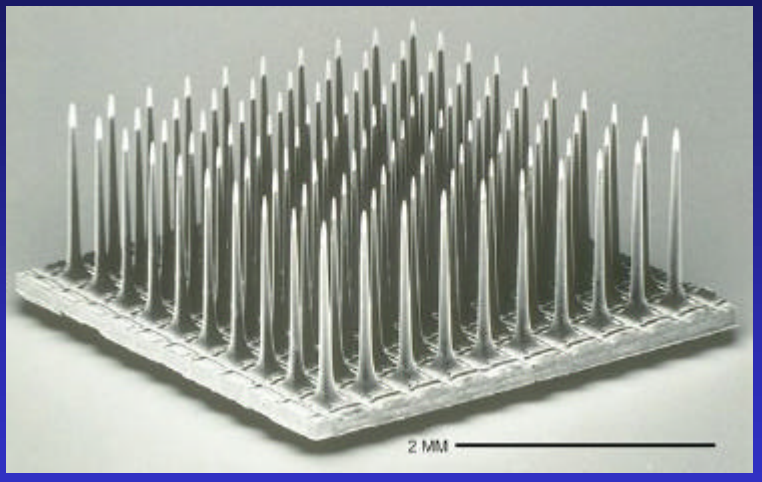
Single electrode in cortex

Note that cell density is much higher than shown!
Actual electrode in at tip of spike
30-100 microns in size

Picture from Bionic Technologies, LLC, Utah



Multiple electrode array



Electrode array is inserted into brain tissue.
Multiple signals are simultaneously recorded.
Picture from Bionic Technologies, LLC, Utah

Charge transfer to an electrode

- Voltage sensor or current sensor?
  - infinite impedance sensor detects potential at that point
  - finite impedance sensor detects charge carriers
    - different species of ions
- Actual charge transfer process can be complex
  - depends on ion species, electro -chemical processes at the electrode
- Interpreting the signal is also difficult
  - field potential
  - caused by a number of processes near the electrode
  - events at a number of nearby neurons
    - spikes are what we are interested in, but they are not the only events
- Spike sorting is the (difficult!) technique of finding the spikes (and their sources) from the electrode signal
    - see later

Patch clamping

From “Microelectrode Techniques” Standen, Gray, and Whitaker

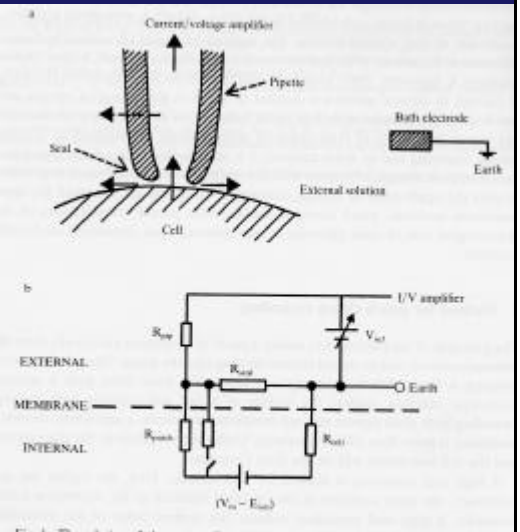


Fig. 1. The relation of pipette to cell and the equivalent electrical circuit during patch clamp recording. The opening of an ion channel is represented as the closing of the switch in Fig. 1b. The pipette resistance and seal resistance are in series between the amplifier and earth (the external solution) and the patch and cell resistances are in parallel with the seal resistance in this path.

Patch clamping

- Alternative technique for detecting neural signals.
- Micro-pipette is used to measure ionic flow across cell membrane, while holding membrane voltage constant
  - hence “clamp” in name
- Micro-pipette is usually boro-silicate glass
  - precise shape and chemical properties of the micro-pipette matter
- Aim is to get a good seal between the glass and the cell membrane
  - electrical resistance direct to the medium surrounding the cell is to be maximised

Patch clamping cont’d

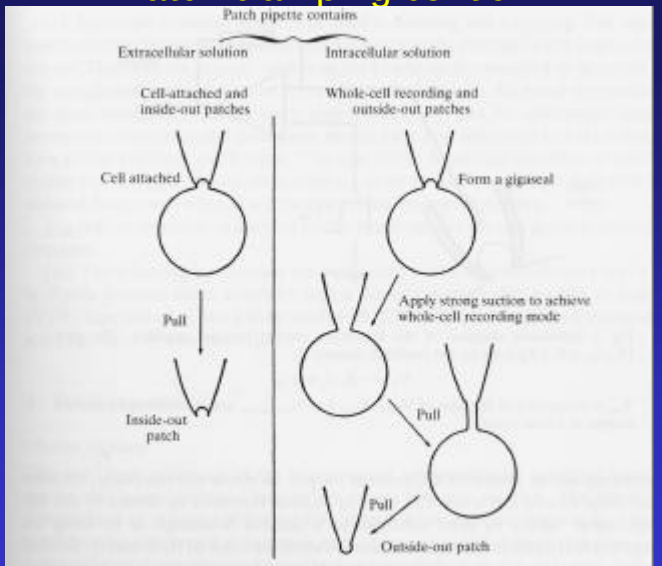


Fig. 2. Diagram illustrating the methods of making cell-attached and inside-out patches (left hand panel) and whole cell and outside-out patches.

In vitro vs. in vivo

- Instrumented neural systems may be
- in vivo
  - in the living animal
- in vitro
  - brain slice preparation
    - cells from a brain slice prepared and kept alive in a culture
  - cultured neurons
    - taken from embryonic or young animals, or immortalised cell lines

- In vivo systems are good for understanding overall brain systems, but
  - there are ethical problems
  - one does not know what other signals the neurons are receiving
- In vitro systems have fewer ethical problems, and the experimenter is much more in control.

In vitro systems

There are many difficulties with in vitro systems of neurons.

- Neurons are difficult to grow
  - they are relatively choosy cells
  - they need to grow in special serums
- Keeping them alive for long periods is difficult
  - usual cell culture problems
  - best to keep the cell culture chamber sealed
    - avoids evaporation, reduces contamination
- Instrumenting them is also difficult
  - electrodes can introduce contamination
  - difficult to keep chamber closed
- Real neurons have “helper”cells
  - glial cells frequently surround the neurons
  - appear to provide them with chemicals, and also to insulate them electrically
    - incompatible with instrumentation

Desiderata

What we would like...

1: Cell culture that lives indefinitely
2: Ability to record from identified cells at high SNR
3: Ability to stimulate identified cells

2 and 3 imply that we can actually identify neurons. Real neural systems are 3-dimensional and very dense.

Generally, one tries to grow neurons in a thin sheet, possibly with glial cells as well (for 1).
- Though this means capacitative coupling, and lower SNR

Approaches to neural culturing and recording

Essentially three different approaches appear to have been taken: many varieties of each approach

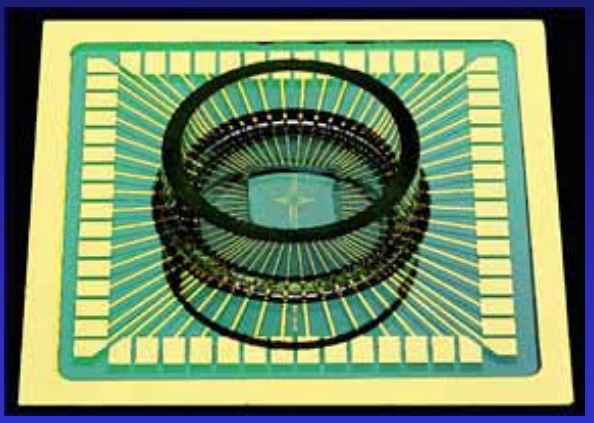
- Multielectrode array approach
  - Potter group, Univ. Freiburg and MCS/Ayanda (Reutlingen/Lausanne), EU VSamuel project, Plexon, Panasoni MED, Glasgow University + many others
  - neurons cultured on small dish, with multielectrode array at surface
- "Neural FET" approach
  - Fromherz group (MPI Biochemistry, Martinsried, Munich)
  - dissociated neurons, cultured on Si/SiO_2, neuron forming gate of FET
- Patch clamping approach
  - Aviva Biosciences (San Diego), Cytocentrics (Reutlingen), Edinburgh University
  - neurons cultured over (multiple) patch clamp electrodes

Multielectrode array approach

- Cells are cultured on top of a multielectrode array

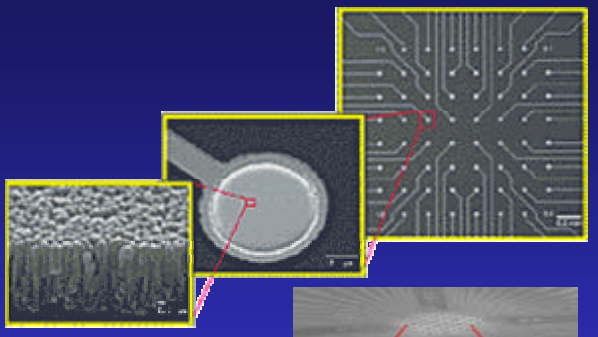
Multichannel Systems, Germany: this is inserted into a system which contains all the electronics.

Means that the cell culture (the “wet”) side can be processed (e.g. incubated) on its own.

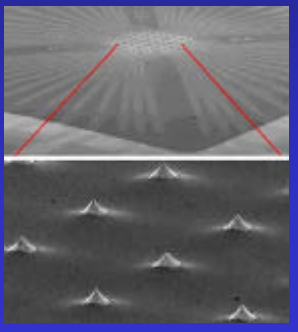


Multielectrode array approach (cont’d)

- Detail of the MCS array



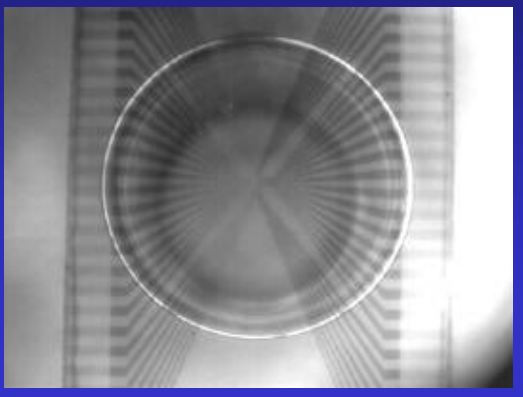
- Can also have 3-d electrodes
  - Ayanda



MEA approach cont'd

- Potter's group (Georgia, US) have a transparent MEA
  - uses indium tin oxide electrodes
- so they can identify what they are recording from

70 uM interelectrode spacing



(Courtesy of Steve Potter)

MEAs continued

- Electrodes may be either for recording or for stimulating

For recording

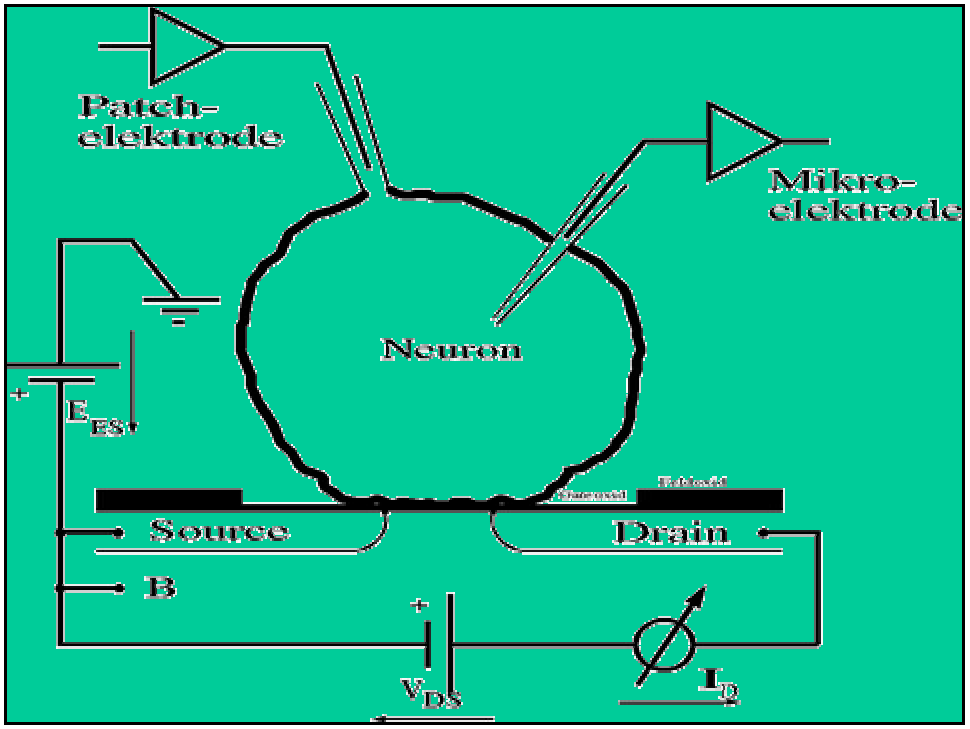
- field potentials are recorded
- capacitative connection
  - glial cells increase thickness of dielectric
- small signals
  - can cause noise problems

Stimulation can be tricky:

- there can be unwanted ionic effects
  - often a bi-phasic signal is used
- relatively high voltages may be required
  - connection is not direct
  - may interfere with nearby recording as well

“Neural FET”

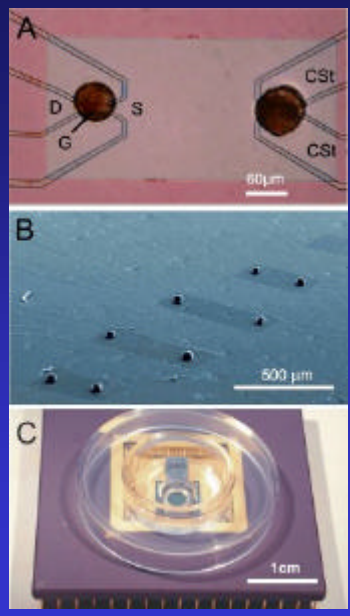
- Use neuron as gate of a de-metallised FET.
  - “direct” unidirectional amplification of voltage changes on neuron
  - large signal => low noise
- Also direct stimulation of neurons on silicon has been achieved.



Communication between Lymnea neurons

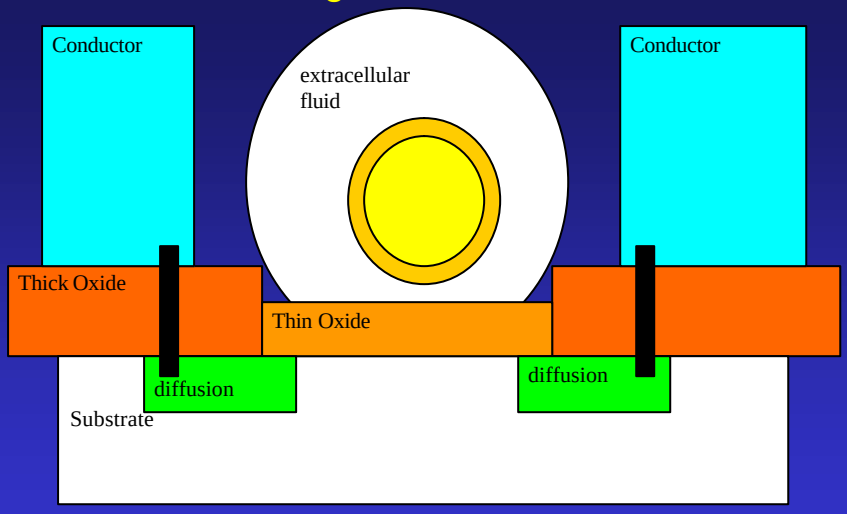
A: Two snail neurons: left one is acting as a neural FET.
Action potential is picked up, digitised, delayed, then applied capacitatively to other neuron, which then fires.

SEM showing 4 pairs of neurons

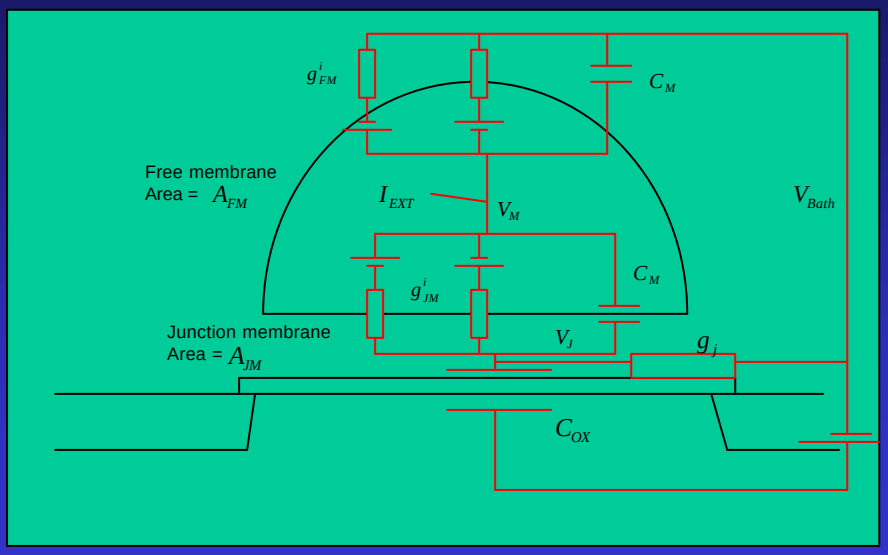


From Bonifazi & Fromherz, Advanced Materials, 14 (2002) 1190-1193

Slice through Fromherz transistor



Equivalent point circuit



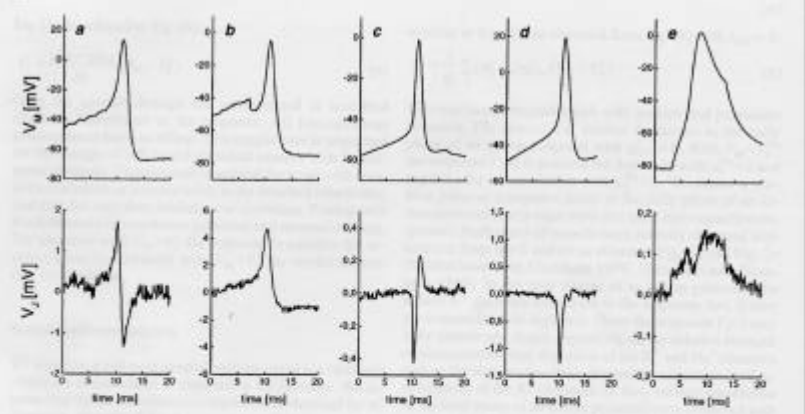


Fig. 2a–e Observations. *Upper row*: intracellular voltage $V_M(t)$ measured with a micropipette. *Lower row*: extracellular records $V_J(t)$ measured with a transistor. **a** Capacitive response (soma of leech neuron) (Jenkner and Fromherz 1997). Interpretation: low ionic conductance in the junction. **b** Ohmic response (soma of leech neuron) (Jenkner and Fromherz 1997). Interpretation: voltage-independent ionic conductance in the junction. **c** Anti-capacitive response (axon stump of leech neuron) (Schätzthauer and Fromherz 1998). Interpretation: accumulation of sodium and of potassium conductance in the junction. **d** Early anti-capacitive response (axon stump of leech neuron) (Schätzthauer and Fromherz 1998). Interpretation: accumulation of sodium conductance in the junction. **e** Early capacitive response with secondary positive response (rat neuron) (Vassanelli and Fromherz 1998). Interpretation: depletion of sodium conductance and accumulation of potassium conductance in the junction. (The two jumps at an interval of 10 ms mark the duration of the injection current)

the junction (with $g_{FM} \approx 0$). Then the response $V_J(t)$ in Eq. (3) appears as the difference of the ohmic current in the attached and of the voltage-gated currents in the free membrane. Eqs. (1) and (2) lead to Eqs. (4) and (5) with $I_{INJ} = 0$ for modest ohmic conductance: the free membrane creates an action potential $V_M(t)$ [Eq. (5)] which drives the junction [Eq. (4)]:

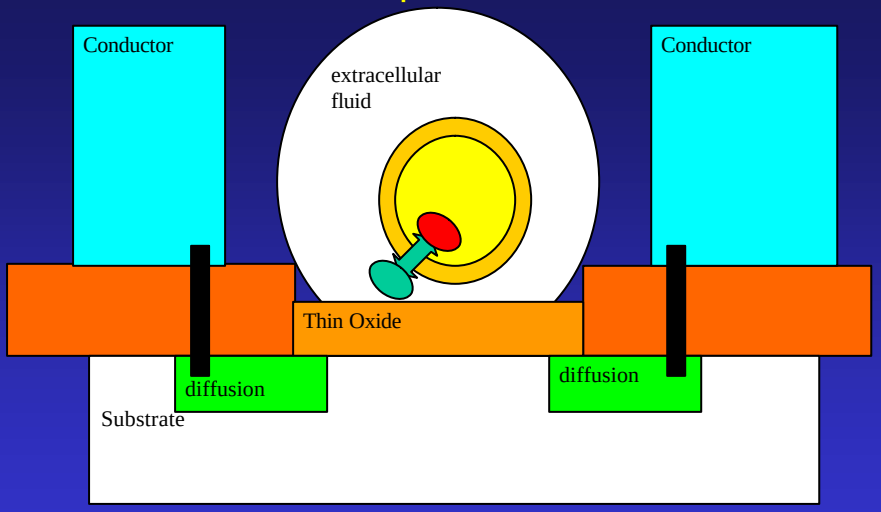
$$g_j V_J = g_{JM}(V_M - V_0) + c_M \frac{dV_M}{dt} \quad (4)$$

$$c_M \frac{dV_M}{dt} = -\sum_i g^i_{FM}(V_M - V^i_0) \quad (5)$$

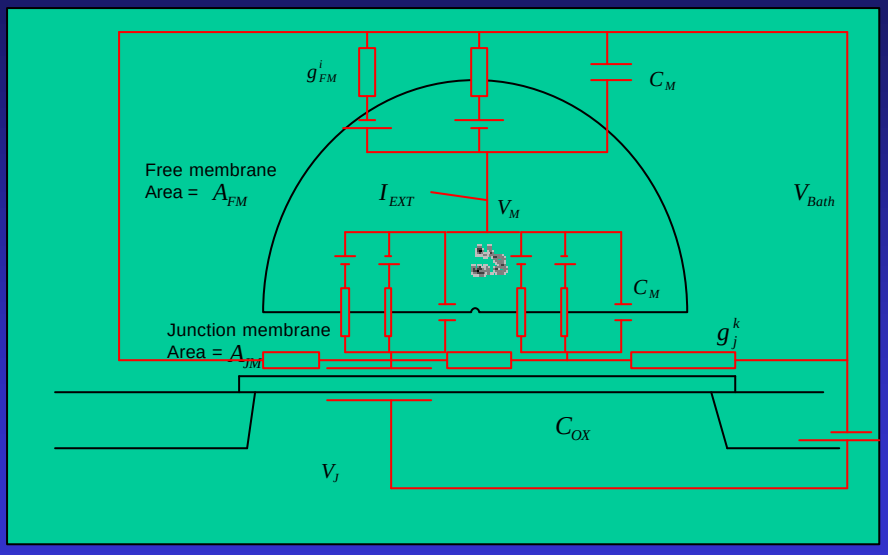
We may distinguish two limits with dominating voltage-

(from Fromherz P., "Extracellular recording with transistors and the distribution of ionic conduncances in a cell membrane", Eur Biophysics J. 1999, 28, 254-258 (page256))

Non-point model



Currents in a multi-point model



Neural FET Continued

- Can provide high SNR
  - but actual mode of signal detection is still unclear
- Directly detects depolarisation
  - highly localised
  - depends on actual ion channels in gate cleft
  - and on precise location of these channels
- Not easy to see how to use for stimulation of neurons

Patch clamping approach

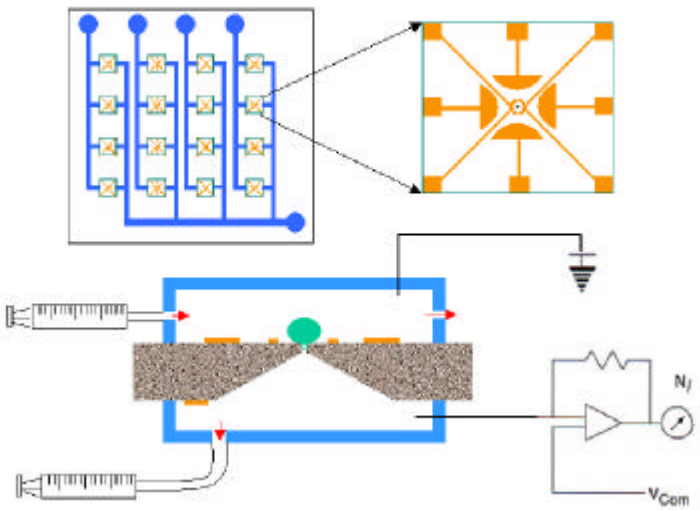
- A number of groups are trying to build multiple patch clamp systems
  - rather like multi-electrode arrays, but at an earlier stage
- Aim is to provide multiple
  - or possibly single, but automated
- patch clamp system for recording, and possibly stimulating, excitable cells

- Better noise figures than MEAs
- Precise recording of a single neuron
- Well understood mechanism of charge transfer
  - though what is actually recorded depends on ion channels in patch

Patch clamping approach (ctd)

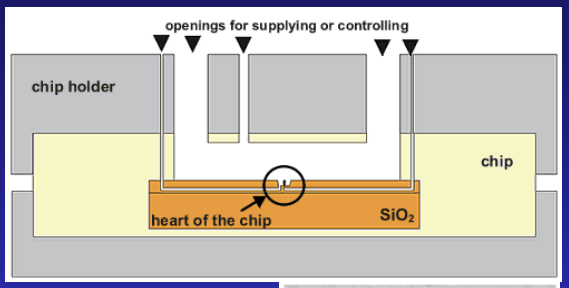
- Multiple patch clamping requires
  - accurately machined tiny holes
  - complex fluidics
- Automating patch clamping is useful not just for neural interfacing, also for drug testing etc.
- Several companies are interested:
- Aviva Biosciences (San Diego), Cytocentrics (Germany)

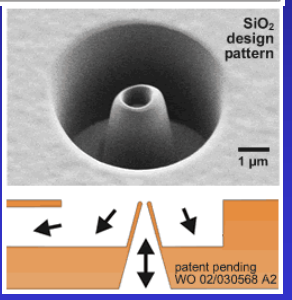
AVIVA's Microchip Approach



Cytocentrics approach

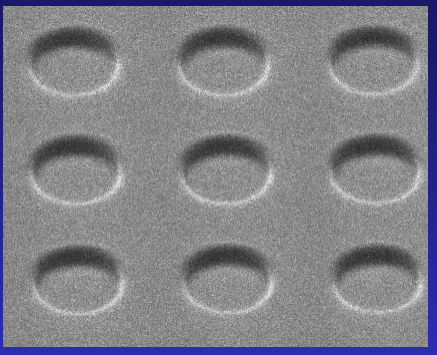
- Microfluidics used to transfer solution containing cells, to hold cell in place and to form patch clamp.





Edinburgh approach

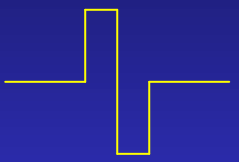
- The Edinburgh group is using healthy dissociated hippocampal neurons from 5-7 day old rats.
- Aim is to produce a 16 channel system, for use in a culture with few glial cells.



Circular holes 1-2um in diameter etched in a silicon nitride layer with chamfered edges; used as a micro-pipette.

New Ideas (1)

- Recording and stimulating from the same electrode
- MCS have developed the electronics
  - rapid switching between recording and stimulating modes
  - still issues due to chemical changes in medium due to stimulation voltages
  - minimised using biphasic stimulation
    - but still present



New ideas (2)

- Stimulate neurons using neurotransmitters
  - instead of electrically
- Why?
  - Real synapses use e.g. Glutamate for excitation, or Glycine for inhibition.
  - the electrical effects occurring postsynaptically are the results of neurotransmitters
- How? Two approaches:
  - Pisa group (De Rossi, Ahluwalia and Rocchia) are working on placing neurotransmitters in conductive polymers, and releasing them using voltage pulses
  - Stanford Opthalmology (Fishman and Blumenkranz) are developing a microfluidic neurotransmitter delivery device.

Neurotransmitters in conductive polymers

- Ions can be embedded in conductive polymers (CP)
- They can be released by applying a voltage to the CP
- Cover some electrodes in an MEA with CP, with neurotransmitter ions
  - glu, glycine, GABA,…
- Apply voltages to release neurotransmitter

- if neurotransmitter release is controllable, have a stimulation technique which is
  - local
  - excitatory or inhibitory, depending on ion embedded

Micro-fluidic neurotransmitter delivery

- Developed in context of retinal stimulation
- micro-aperture can release chosen neurotransmitter
- excitatory or inhibitory

- Can the delivery of the neurotransmitter be mad sufficiently localised?

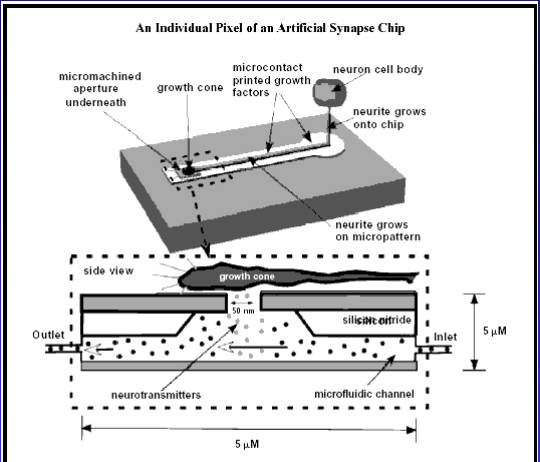


Figure 1: Shown is a schematic of the chip that has been stamped with the microcontact printing technique. Using an appropriate micropattern, the growth cone that grows off of the cell body is directed by a path of micropatterned growth factors to a nanoaperture (underneath the growth cone in the shaded region). The key hole-shaped light stripe is a micropattern of growth factors, cell adhesion molecules, and antibodies specific to cell surface proteins of the neurite. The pattern is made by aligning a microcontact printing stamp with the aperture on the substrate (shown in black).

Picking out the signals that matter: spikes

Neurons communicate using spikes

- Field potentials (from electrodes) need to be converted to spike times
  - yet each electrode may collect signals from multiple spiking neurons
  - and spikes may overlap
- Spike sorting may use
  - simple or multiple thresholding
  - characteristic spike shapes found using
    - PCA
    - Wavelet based transforms (NMT)
- Generally off-line
  - signal is analysed and characteristic spike shapes found
  - signal is returned into spike times using these shapes
- ... but some applications may require real-time response
  - simpler techniques or dedicated hardware

Picking out the signals that matter: sub-threshold

- Much processing takes place on the dendrites of neurons
- Not spiking, but linear and non-linear interactions on the dendrite itself
- Detectable either by
  - impaling dendrite on a micro-electrode or
  - patch clamping dendrite
- Both are difficult, and often incompatible with neural longevity.

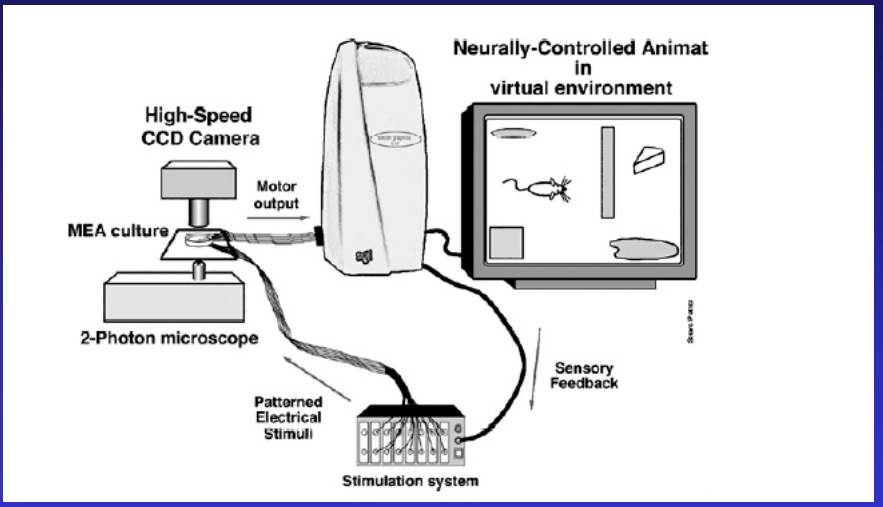
Applications 1: Neuropharmacology

- Examining the effect of different chemicals on a culture can allow assay of neuropharmacological preparations without using live animals
- Requires only that we can stimulate and record over a reasonable time period
- One of the major reasons for industrial interest in MEAs and cell cultures.

Understanding neuronal communication

- Stimulating and recording alone are not enough for higher-level applications
  - prosthetics, hybrid neuronal/computing machines
- Need to understand what is being transmitted between neurons
- This has been a major aim of neurophysiologists for decades!
  - Can in vitro systems help?
- Unlike real neural systems,
  - we can control the stimuli
  - we can record for longer
  - we can record simultaneously from more sites
- How can we go about this task?

Closing the loop



- Steve Potter's system: (SAB 2002)

Closing the loop: semantics

- How can we provide stimuli which deliver the information we want the neural culture to use?
- How can we interpret the spike trains produced by a neural culture?

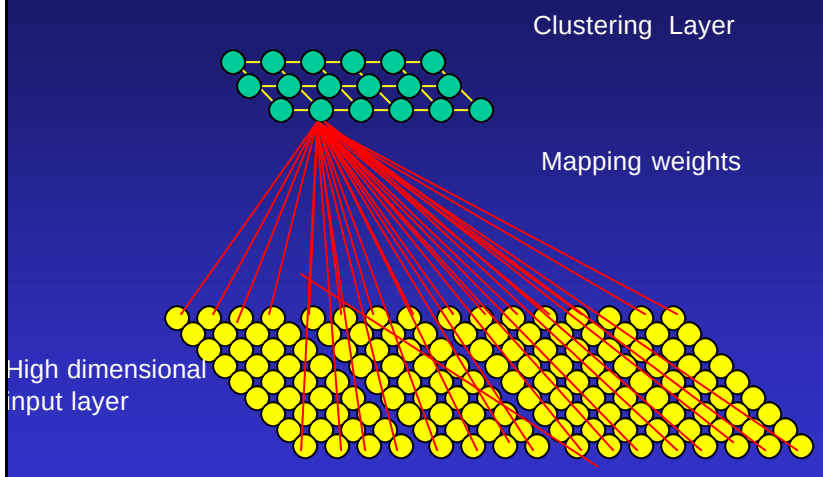
- We need to associate a semantics with the state of the neurons in the culture
  - or at least with those whose state we are measuring

- The semantics of an electronic system are implicit in its design
  - usually. One exception is genetic algorithm generated designs.

Inventing a semantics

- One possibility is to directly associate measurable states with particular outputs.
  - Like turn left, or go straight ahead, etc.
- Straightforward for a single neuron
- For multiple neurons, real system states are very complex
- We need to reduce the dimensionality of the signals in order to interpret them.
  - So we can use (e.g.) Kohonen Self-organised mapping to map the high dimensional space of measured states into a small number of clusters.
- Note that the final coding depends on the input to the network
  - hard not to imply decisions about the coding being used by the network

Kohonen network



Coding possibilities.

- **Windowed full state**
- For each neuron, create a vector V, v is 0 or 1 (1=spike) T_s is sampling rate, and j indexes the neurons

$$V_j(t) = (v_j^1, \ldots, v_j^{t/T_s})$$

- Window this vector, so that only some of the recent past is used
- Use the matrix M to train the Kohonen net

$$V_j^K(t) = (v_j^{(t/T_s - K)}, \ldots, v_j^{t/T_s})$$

$$M_{j,k}(t) = [V_j^K(t)]$$

- Problem: very high dimensional input space:
- dimension = N * K (no of neurons * window length)
  - also precise training data depends on T_s
- Alternative 1: use

$$V_j^K(t) = \sum_{i=t/T_s-K}^{t/T_s} v_j^i$$

Lower dimensionality (N) essentially spike rate in $K*T_s$ interval

Adaptation

- We expect the input-output characteristic of the system to alter
  - due to synaptic modification, in the light of the inputs the system receives, and the outputs it produces
  - could one keep the Kohonen network plastic, but with a reduced weight change rate
- If we invent a semantics, then use feedback from the controlled system to the culture, will it learn this semantics?
  - (learn, in that it will control appropriate behaviour in the target system)
  - Will it learn an arbitrary semantics, or do we need to choose a suitable one first?

Ethical and cultural issues

- Using long-lived neural cultures can have ethical implications.
  - Do cultured neurons have animal rights?
- Different cultures have different views of what to do with this sort of technology
- Pharmacological and medical applications aside,
  - USA: cyborg soldier
  - Europe: assistive technologies for the aged and disabled
  - Hybrid animal/machines systems for (otherwise) uninhabitable ecologies?
- The possibilities of replacing or augmenting sensory systems pose major ethical challenges
  - artificial/implanted retinae
  - artificial/implanted cochleae and brainstem implants
  - new/novel senses
  - when and on whom should they be used?

In conclusion

- There are many difficulties in interfacing neurons and electronics
- There are a number of competing techniques for achieving this
- Each has advantages and disadvantages:
  - electrodes: SNR, stimulation
  - Neural FET: stimulation, precise mode of operation
  - patch clamp: stimulation, workability over long periods
  - ...and the issue is not resolved: new advances change the balance
- The question of interpreting neural outputs, and coding neural inputs is not settled
  - and the techniques used often make assumptions about the neural coding
- and lastly: there are ethical implications which we are just starting to discuss.