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Study of Micro-Electrode Array for Neural Populations Stimulating and Recording

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Outline

1. Background and significances
2. Our researches
3. Summary

1. Backgound and significances

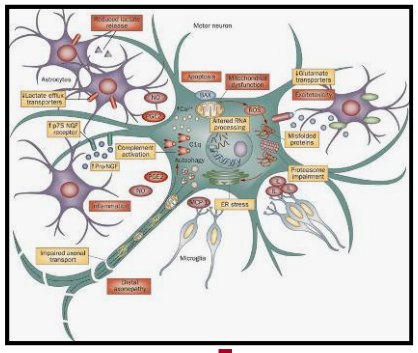
- **Common geriatric diseases:**
  - Alzheimer's disease (AD) → The etiology unclear, no specific therapy.
  - Parkinson's disease
  - Stroke
  - Heart disease
  - ……

  } Treat with drug

- **Brain research is full of challenge. It lays the foundation for:**
  - Explaining the brain mechanisms of human behavior;
  - Understanding the information coding mechanism of neural circuits and neural network system;
  - Clarifying the etiology and mechanism of neurological illnesses, exploring new method of treatment.
- **21 century is 'the century of brain':**

Related projects:
- USA: BRAIN Initiative
- EU : The Human Brain Project (HBP)
- Japan: The age of brain science
- China: The cellular and molecular basis of brain function

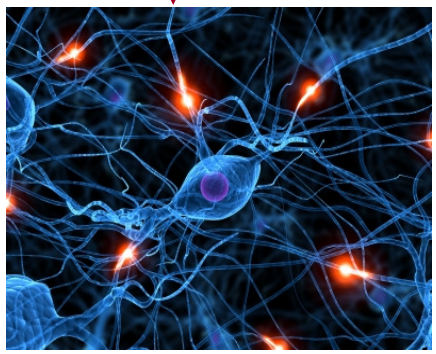
Research of brain science



Cellular and molecular levels

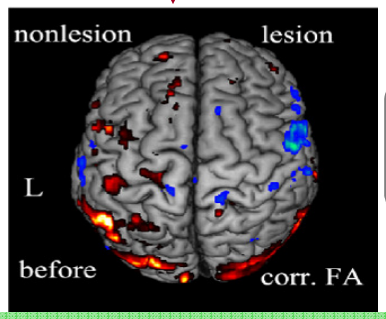
Biomics technologies

(gene/protein expression, cell morphology, synaptic length, etc)



Neural populations

Activity rule of the functional neural populations ?



Whole brain level

MRI

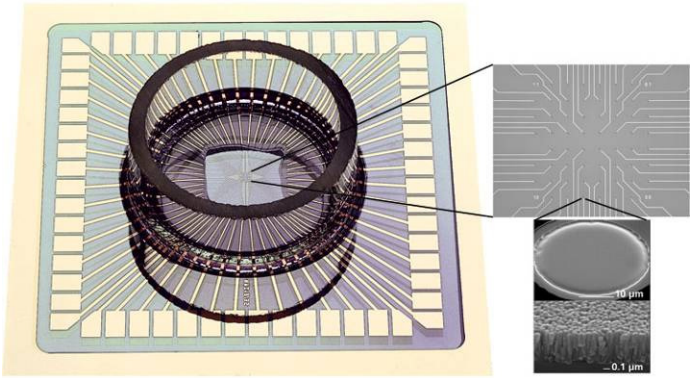
(The changes of blood oxygen saturation and blood flow in brain)

Why MEA and neurochip?

- **In order to understand the complex neural processing, high spatiotemporal resolution techniques to monitor the neuronal electrical activity are required.**
- **The development of MEA and neurochip provide powerful tools for investigating the electrical signal transmission and processing mechanism among neuron clusters in neural network, studying the function of the whole nervous system, thus overcome the "Great Gap" in brain research.**
- **With the invention of MEA in the early 1970s, related technologies have also been developed. MEA has been applied in :**
  - **Neuroscience**
  - **Drug screening**
  - **Pharmacology, toxicology**
  - **Etc.**

Micro-Electrode Arrays (MEA)

- Micro electrodes are arranged on the glass surface in lattice formation
- Tissue, cell or slice can be put in the recording chamber of the MEA directly and tightly. Extracellular field potential signals from 60 sites can be recorded simultanously.
- The electrodes are used to record and stimulate.
- MEA is suitable for studying the electrophysiology and ion channel characteristics of neural network, brain slices and myocardial cells, heart slices.



MEA plate

Advantage:

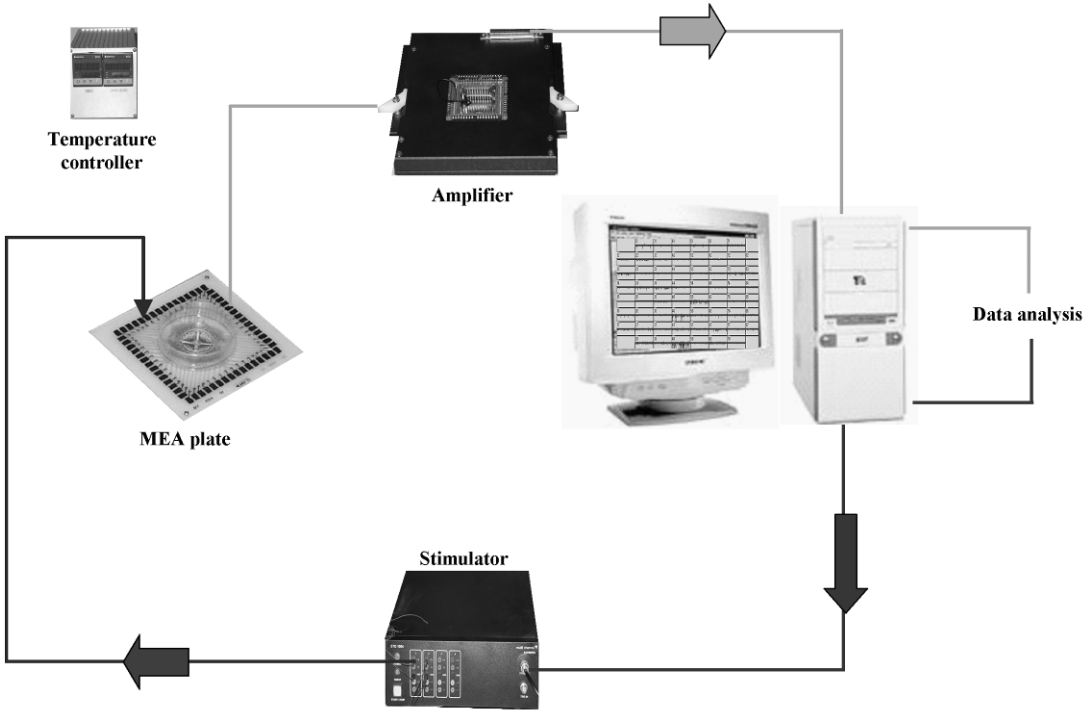
★ Noninvasive

★ Record and stimulation

★ Long-term recordings

★ Study in space and time

MEA system diagram



MEA system

Advantages:

★ Real-time display

★ Multi channel recording

★ Sample selectivity

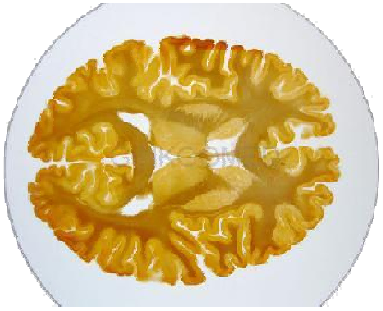
★ High resolution

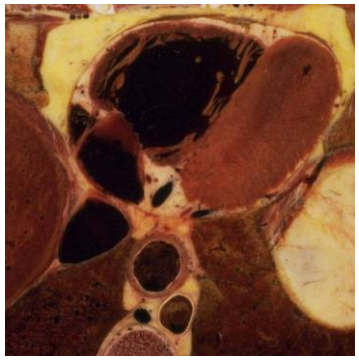
★ High Throughput

……

1) Application of MEA for studying brain and heart slices

Spontaneous electrical signal or induced signal can be detected from actue brain and heart slices placed on the MEA through MEA system. Then further research about neural system and autonomic nervous system could be carried out.





Main research directions:

- **Study of brain functions and dysfunction, cardiac diseases**
- **Study of neural signal transduction pathway**

2) Application of MEA on pharmacology, toxicology and drug screening

MEA is not only a special method to observe the activities of neural network, but also a drug screening method with the advantages of high-throughput, high sensitivity, stability and standardization.

MEA can help us solve many problems in central nervous system drugs' discovery, mainly including:

(1) Screen and optimize of lead drugs

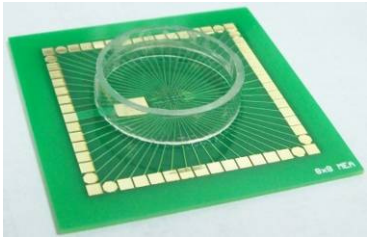
(2) Verify action mechanism of drugs

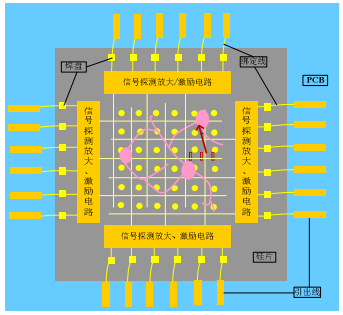
(3) Research neural properties of transgenic rodents

(4) Verify safety/toxicity of compounds

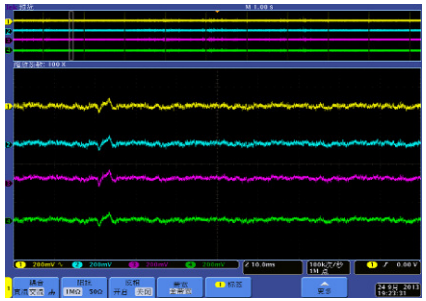
2. Our researches

1) Development of MEA and neurochip



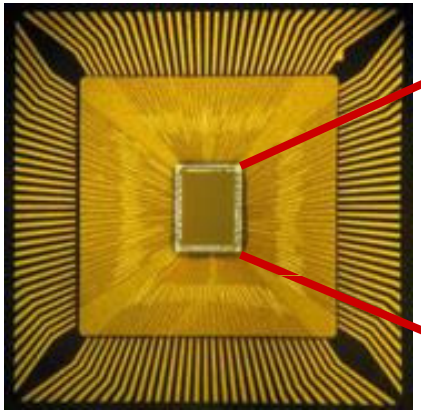


2) Stimulation and detection of neural signal based on MEA

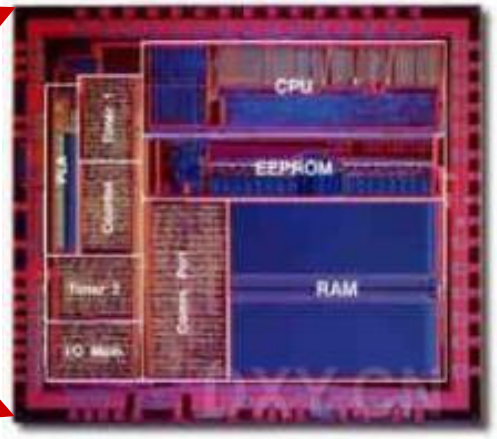


What is Neurochip?

Neurochip technology is developed on base of MEA technology, it combines the neurons or brain tissue and FET technology to study the activity of neurons and advanced function, for example, learning and memory of brain.



Neurochip



MEA

Advantages:

- **Overcome the interconnect limitations of commercial MEA, high-density sensor array could be realized.**
- **Enable the integration of active circuits on one chip, such as recording amplifiers and stimulating buffer arrays, which can ensure signal integrity.**

Design and Fabrication of a novel MEA and Neurochip using CMOS Technology:

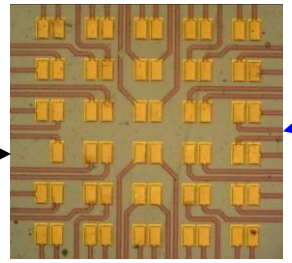
MEA in neurochip

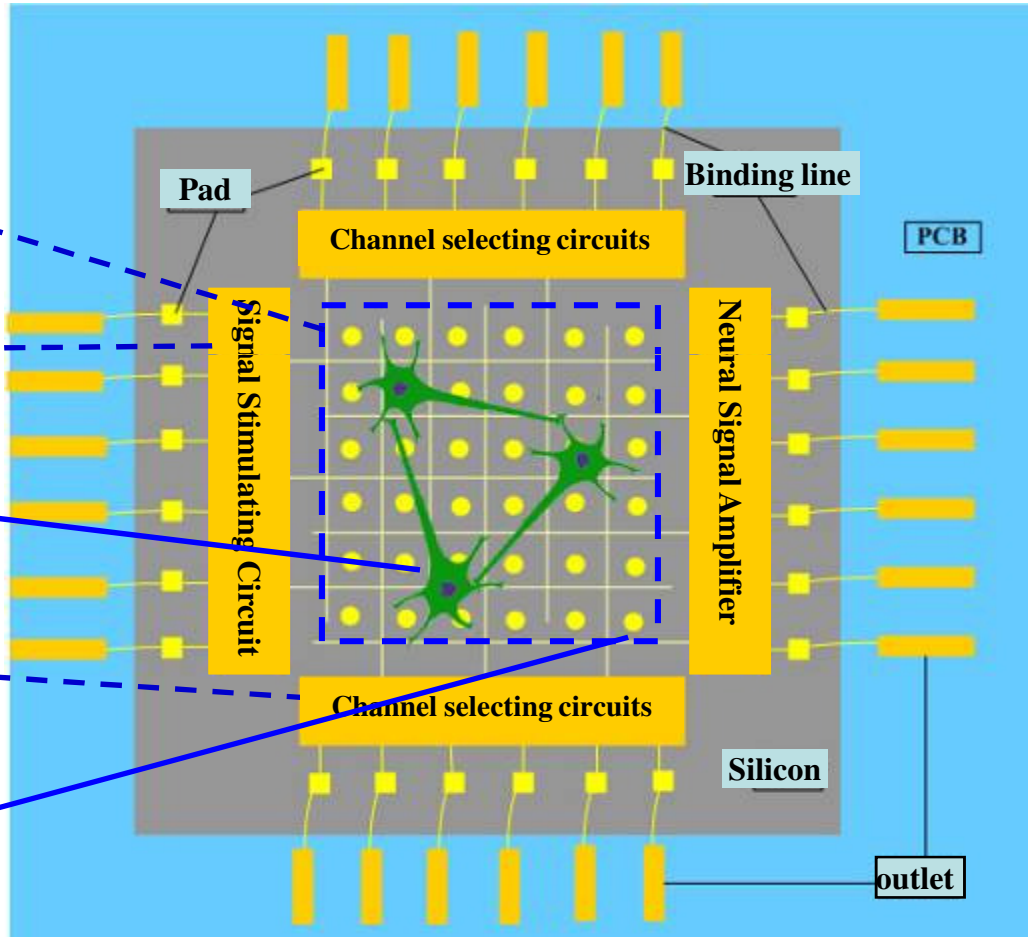
Neural Signal Amplifier and Stimulating Circuit

Neuron Body Size: 10~20μm

Channel Selecting Circuits

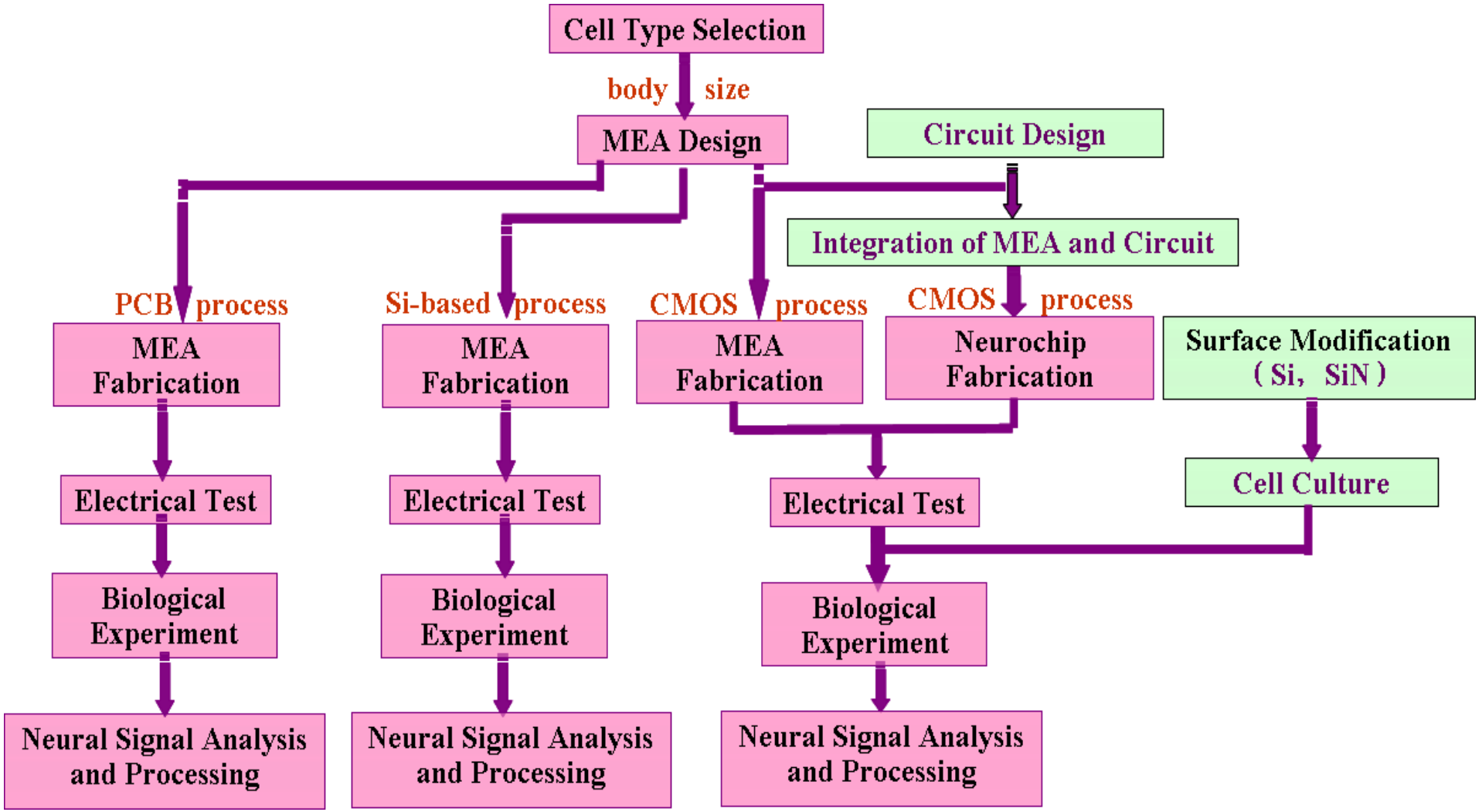
Micro-photo of gold electrode





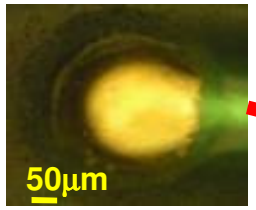
The Neurochip schematic diagram

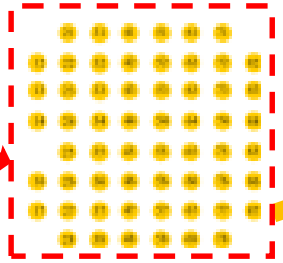
The realization route

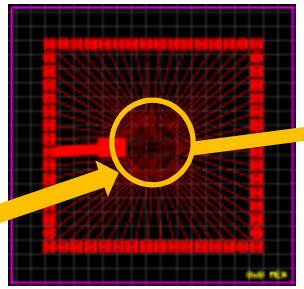


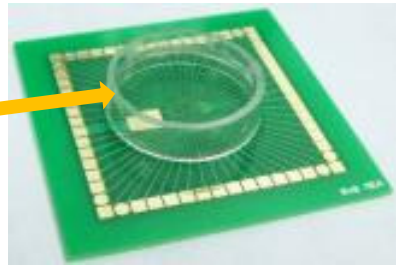
1) Development of MEA

(1) PCB MEA design



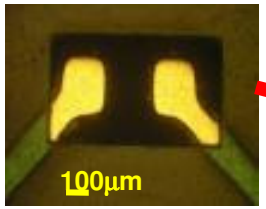


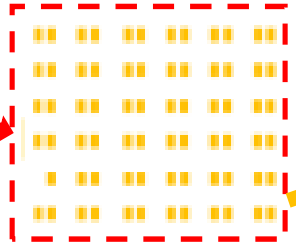


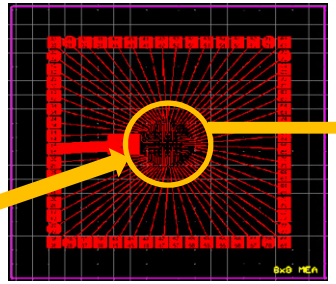


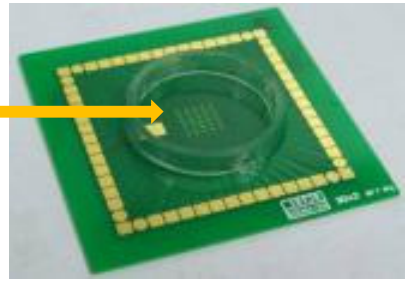
Working electrodes Layout of 60-sites MEA Final 60-sites MEA

60-sites MEA



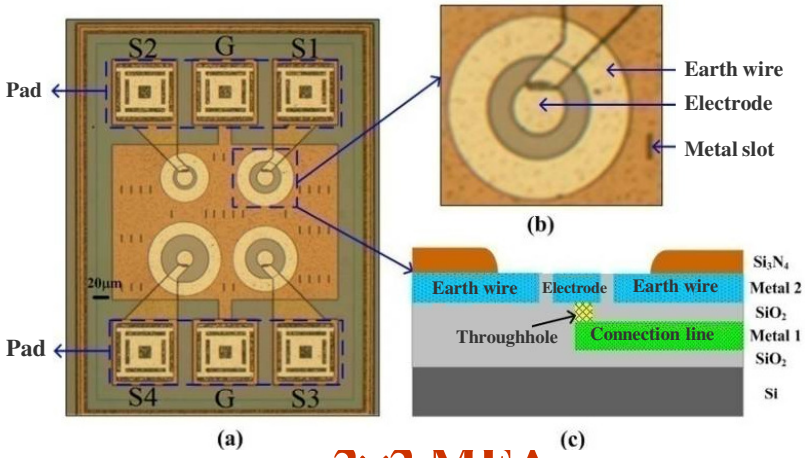




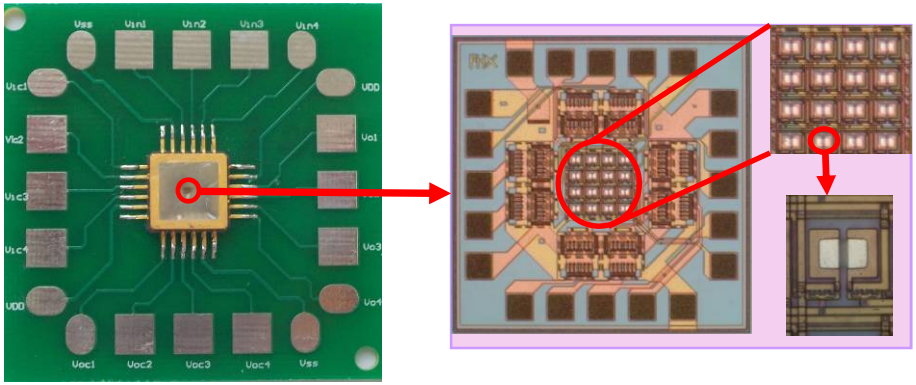


30 × 2 MEA

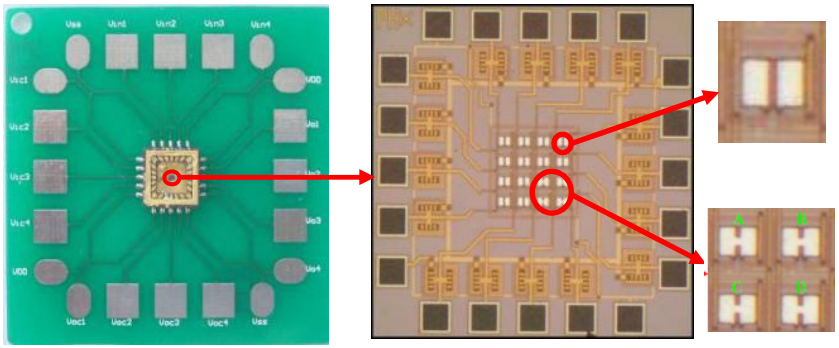
(2) CMOS MEA Design



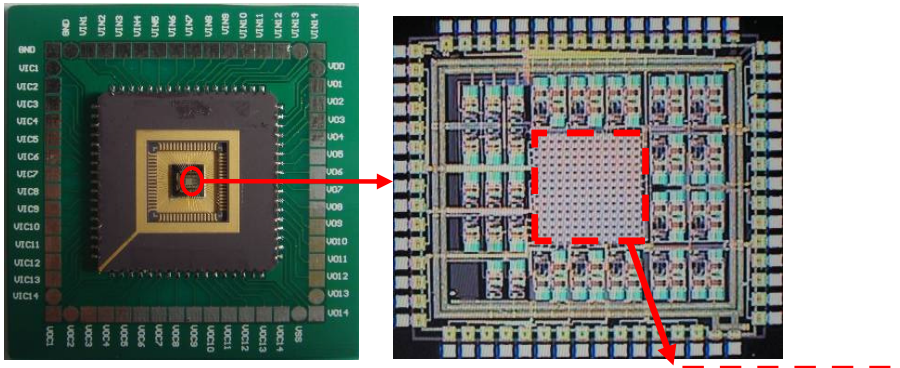
2×2 MEA



4×4 MEA

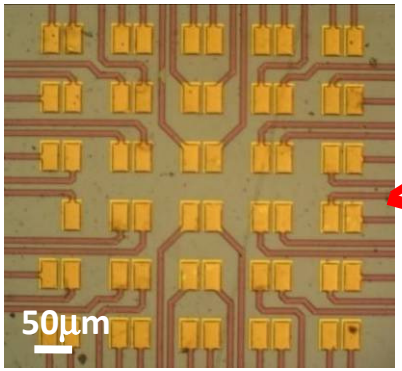


Improved 4×4 MEA

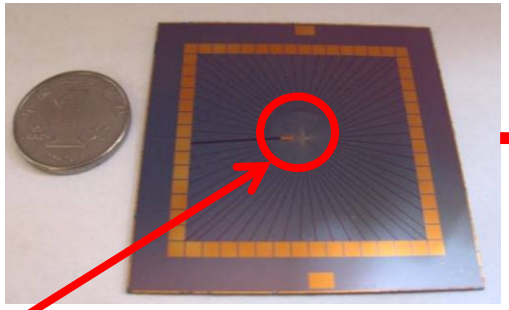


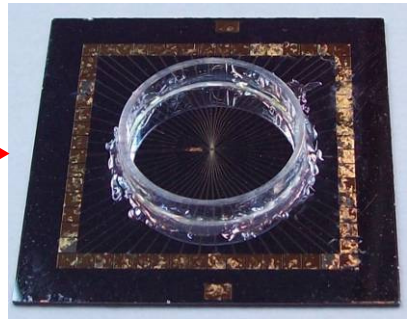
14×14 MEA

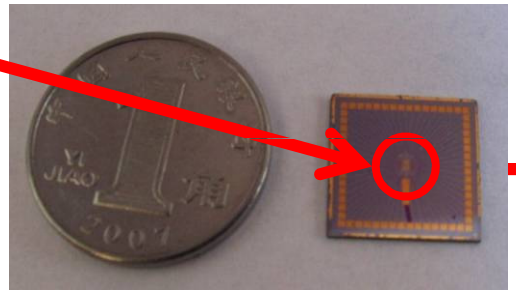
(3) Silicon-based MEA design

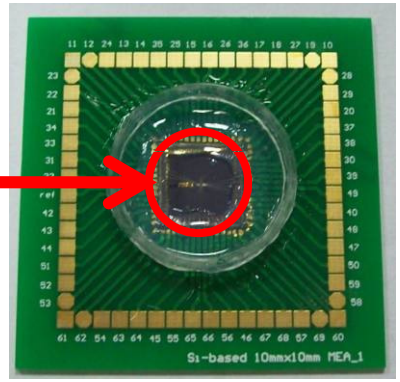


Micro-photo of gold electrode









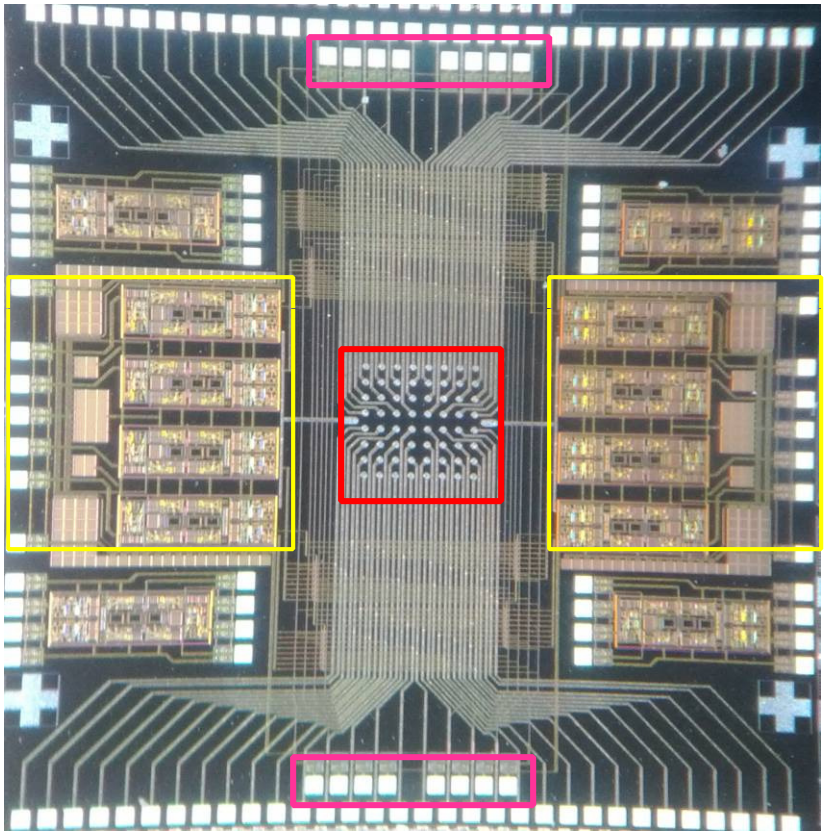
Photographs of assembled 30 ×2 MEA and local closed view

According to the size of silicon-based 30×2 MEA, the MEA is divided into two forms: culture chamber is directly adhered; or MEA is first bonded to the PCB board and then the culture chamber were adhered.

2) Development of neurochip

- **The recording channels consisting of pre-amplifier, the stimulation channels and shift register circuits are designed.**
- **The results from on-chip test demonstrates that the recording and stimulation channels meets the performance need of monitoring the activities of neurons.**
- **The function of choosing different electrodes is performed by the shift registers and the conversion of the parallel data from recording channels to serial data is processed by the multiplex circuits. All aforementioned modules fulfill the design requirements.**
- **Furthermore, an integration of micro-electrodes array and micro-electronic circuits are implemented. The measuring results shows that the integrated chip can record weak neural signals (μVs).**

The neurochip integrated of MEA and multi-recording channels



Die photo of the neurochip

Technology： 0.5mm DPDM CMOS

Size： 5mm × 5mm

① **In red box: 64×64 MEA;**

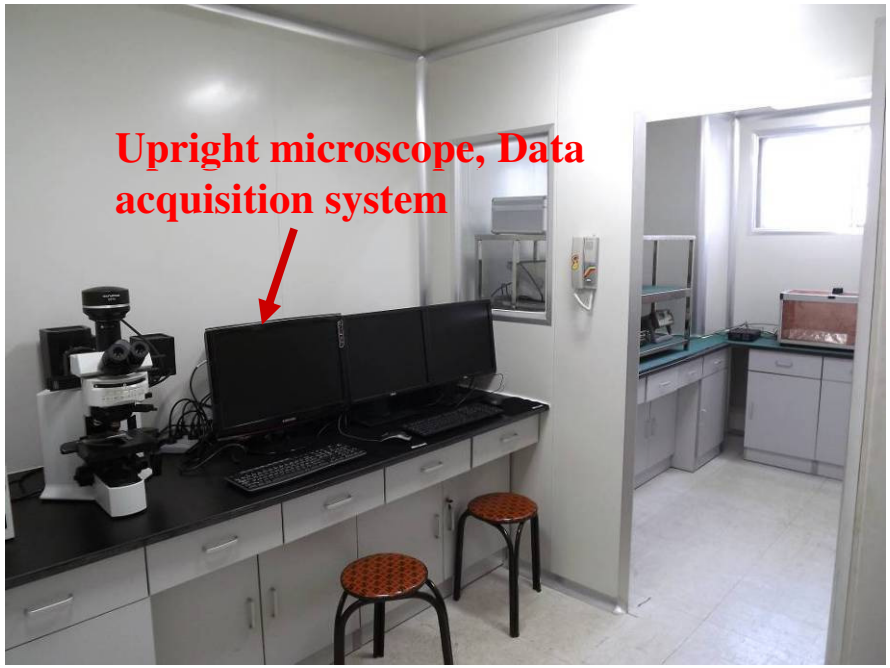
② **In yellow box: 8-recording channels (4 channels each side);**

③ **In pink box: control circuits for choosing different electrodes from the MEA (8 pads on upside for row choosing and 8 pads on bottom for column choosing).**

3) Stimulation and detection of neural signal based on MEA

(1) The experimental environment and MEA system

① Million-level laboratory



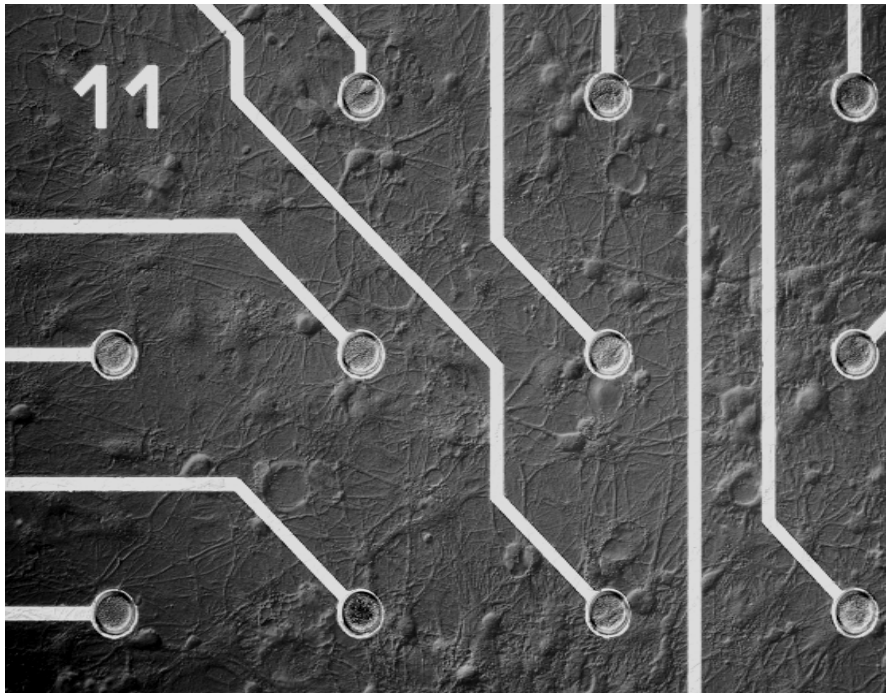
Outer room: Upright microscope, data acquisition system

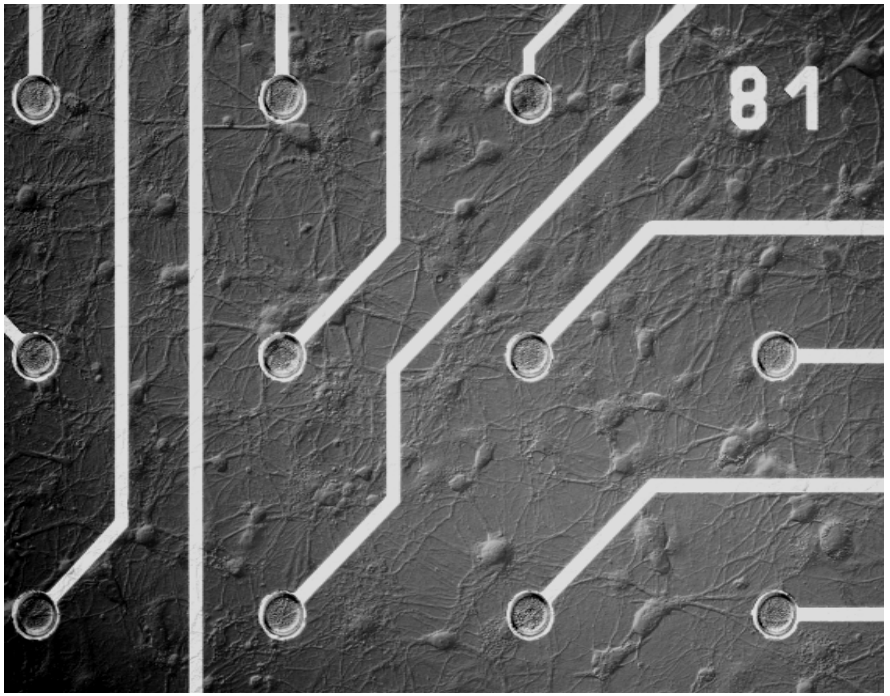
Inner room: Two detection systems

Inner room: Biology experiment equipment

(2) The response signal recording of hippocampal neurons cultured on MEA under the electrical signal stimulating

① Culture of hippocampus on MEA

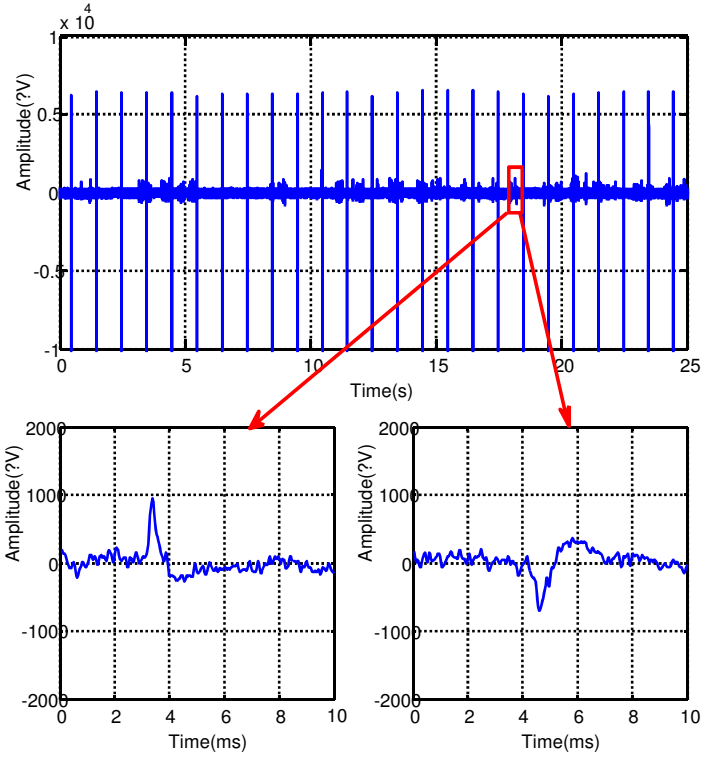




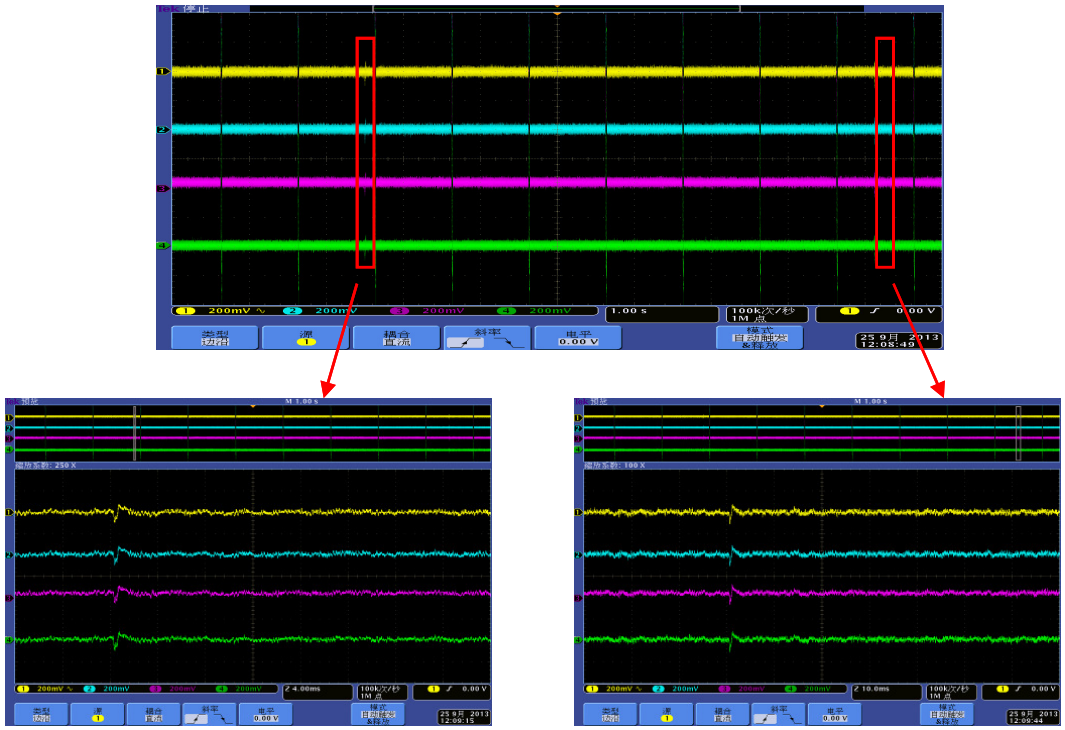
Photos of cultured hippocampal neurons on the glass-based MEA after 14 days.

- Hippocampal neurons grew well on the MEA and formed network.

② Single-channel electrical stimulation and multi-channel signal recording experiments of hippocampal neurons



The signal at stimulation of 55mV



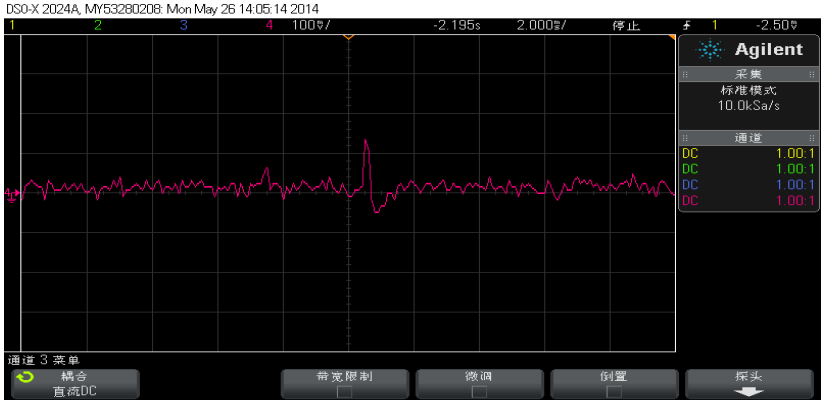
The signal at stimulation of 55mV on the oscilloscope

- The hippocampus signal could not be recorded until the amplitude of stimulating signal reached 55mV.

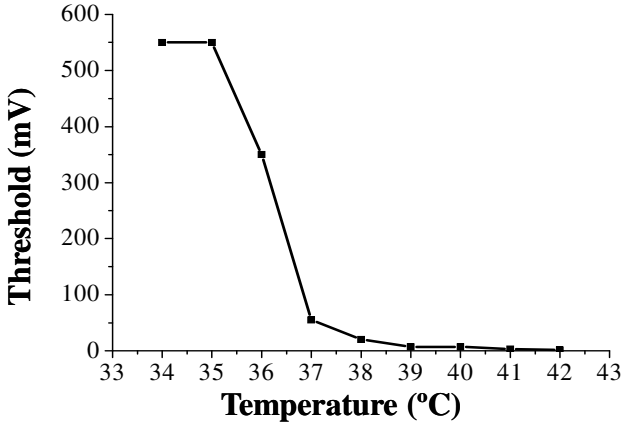
③Recording of hippocampal neurons cultured on MEA under electrical stimulation and different temperatures

Excitation thresholds of hippocampal neurons under different temperatures

mV	550	550	350	55	20	7	7	3	1	∞
ºC	34	35	36	37	38	39	40	41	42	43



The signal at stimulation of 100mV and 34℃



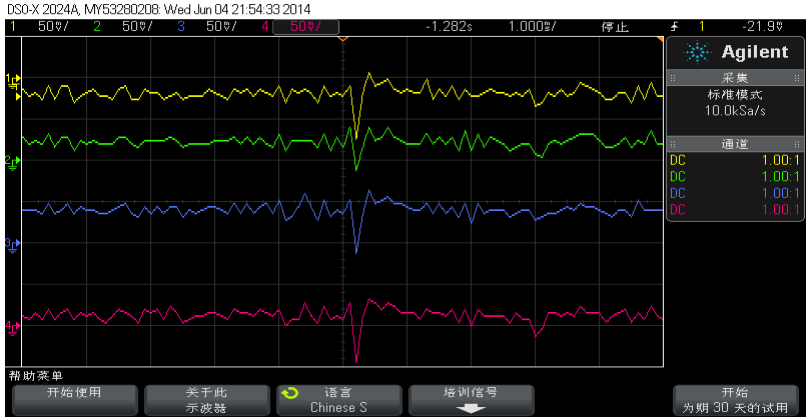
Relationship between the excitation threshold and temperature

- As temperatures increases, the excitation threshold decreases quickly. Cell apoptosis occurs rapidly at 43ºC, with 36ºC and 38ºC being significant turning points.

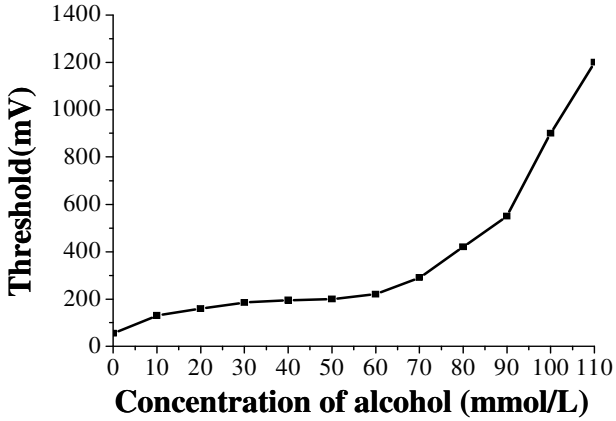
④Recording of hippocampal neurons cultured on MEA under electrical stimulation and influence of alcohol

Excitation threshold of hippocampal neurons under different alcohol concentrations

Alcohol concentration	0	10	20	30	40	50	60	70	80	90	100	110
Excitation threshold	55	130	160	185	195	200	220	290	420	550	900	1200



37°C, the signal at stimulation of 200mV and 50mM.



37°C, relationship between the excitation threshold and alcohol concentration

◆As alcohol concentration rises, the excitation threshold increases quickly. Cell apoptosis occurs rapidly at concentration of 110mM.

3. Summary

- The MEA which can record and stimulate, and a new neurochip design are implemented.
- Using the threshold of electrical signal to quantitative evaluate the influence of different stimulations on neural network are newly proposed.
- Electrical signals, temperature, alcohol and stimulating and signal recording experiments are performed. A series of useful results have been obtained for the quantitative evaluation of the electrophysiology activity of hippocampal neurons.
- Future work will study drug screening for Alzheimer's disease and other age-related neurological diseases

Thank you very much for your attention !

Acknowledgement

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