Advanced Research on the cerebellum(STNS603)
Cerebellar function: Integrate motor command and sensory information - - coordinate movement, motor learning and timing

Cerebellum function------motor learning

----non motor function-Affection, emotion, cognitive

In vivo , intracellular recording

AMPA receptor desensitization (wedge recording)-------LTD

LTD (Long-Term Depression)-------Professor Masao Ito (Sakurai, Tongroach)

Study metabotropic glutamate receptor (slice)

Long-Term Depression

VOR, HOKR….LTD

Classical conditioning eye blink
Professor Masao Ito

1. Found LTD, (Long-Term Depression), Sakurai, Tongroch, 1980
2. Founder of Brain Science Institute (in RIKEN), RIKEN (3….50 teams)
3. Support young scientists (neuroscience), all over the world
4. Expert both in experiments and administration (surgery, operated animalas)
Electrophysiologists-- numbers decline
- progress in Thai

Nature work of researchers
- challenge
- be patient
- effort
- time consume
- do not give up
- honest

Key success: Love to do (any career), never give up, be patient

Reward: Beautiful responses, cells, see things that other can not see, discover, happiness
Long-Term-Depression (LTD)

-synaptic plasticity

-occurred at the synapses between parallel fibers (PF) and Purkinje cells after conditioning with conjunctive stimulation (PF and CF, climbing fibers), the response of PF is reduced for more than 1 hr, but the response of climbing fibers do not change= LTD

Purkinje cells receive 2 excitatory inputs-PF (from granule cells)

-CF (climbing fibers, from Inferior olive)

Professor Masao Ito discovered LTD in cerebellum

(Sakurai, Tongroch)
• In vivo-----Intracellular recording from Vestibular nucleus (rat)…After deafferentation of CF with 3-acetylpyridine

• In vitro (Cerebellar slices)------Wedge recording recording
  - Intracellular recording
  - Whole cell clamp recording
In vivo

Long-Term effects of 3-Acetylpyridine induced destruction of cerebellar climbing fibers on Purkinje cell inhibition of Vestibulospinal tract cells of rat
Purkinje cell
lobule II-III
(anterior lobe)

VN

Record IPSPs

P-cells output to Deep cerebellar Nuclei

Inferior olive

3-acetylpyridine

Fanardjian VV et al, 1980

stimulating
• In vivo

• Climbing fibers deafferentiation by 3-acetylpyridine
• Test every 10 day interval

• Methods:
  Stimulating electrodes (PT-IR, bipolar electrodes)
  Stimulating Purkinje cells at ant. lobe (lobule II-III)
  Stimulating at C1 for anti-dromic stimulation

• Vestibular neurons… Identify IPSPs from P-cell by hyper- or depolarized-current
Control
10-20 days
30-60 days
70-101 days
110-160 days
Fig. 7A–E. Latency of the IPSPs in control rats. Ordinates, number of cells. Abscissae, latency of IPSPs in ms. A Control. B 10th–20th days. C 30th–60th days. D 70th–101st days. E 110th–160th days. Shaded parts of B are for rats on days 10 and 11. Arrows with open heads mark median values and those with closed heads mean values.
Fig. 9A–D. Threshold for evoking the IPSPs. Traces are IPSPs recorded from a VST cell. Figures indicate intensities of stimuli applied to the deep interior of the cerebellar anterior lobe. Triangles mark the moments of onset of the IPSPs. A–D Ordinates, numbers of VST cells. Abscissae, threshold currents. A Control. B 10th–40th days. C 50th–101st days. D 110th–160th days.
• Check position of recording - DC current 0.5mA
• Mark stimulation position – DC current 0.5mA
• Check effect of 3-AP by staining the cells with cresyl violet
Summary

After 3-AP treated (rats)

• 1. Occurrence of IPSPs: reduced
• 2. Latency of IPSPs: increased
• 3. Amplitude of IPSPs: reduced
• 4. Threshold of stimulation: increased
• (Long-term effects of 3-AP on electrical property of P-cells)
• Preamplifier (hand made)---------injection current
• Karachot. L, Ito. M and Kanai. T
Wedge Recording (TTX, post synaptic response)
Wedge recording

- Desensitization of AMPA receptors (glutamate receptors)
- Ionotropic glutamate receptors (AMPA receptors, NMDA receptors) and metabotropic glutamate receptor (mgluR)
- Messengers involved in desensitization of AMPA receptors in Purkinje cells
Parallel fiber

Climbing fiber

Ito 2002
AMPA and metabotropic receptors
AMPA

A B D E F

t-ACPD
Aspartate

AMPA
• Sodium Nitroprusside, 8-BrcGMP /… L-NMMA
• Specific PKG inhibitor ….KT5283
• Non specific…K252a
• PTX(Pertussis toxin)… G protein inhibitor
• HB(hemoglobin….absorb NO
• BAPTA-AM
Inhibitors block desensitization

QA 4 mins
BAPTA-AM

SNP

8-Br-cGMP

AMPA 4 mins
Ito.M and Karachot.L
• Protocol to induce long-term desensitization in AMPA receptors in cerebellar slices:

• 8Br-cGMP 15 min and AMPA 10 uM 4 min.

  Phosphorylation of AMPA receptors ...(confirm by Protein kinases and phosphatase inhibitors:

• Phorbol esters (PKC), okadaic acid and calyculin

  A(phosphatase inhibitors) induced long-term desensitization(similar to above)

• Mechanism of long-term desensitization of AMPA receptors due to

  phosphorylation of AMPA receptors
Long-Term desensitization of AMPA receptors

(Phosphorylation of AMPA receptors)

LTD...Long-Term Depression
desensitization AMPA receptors (test)
8-Br-cGMP(injection) Hartell 1994......LTD
Protocol for inducing long-term desensitization of AMPA receptors:

• 8-Br-cGMP 15 min, AMPA 10uM 4 min
• Induced long-term desensitization of AMPA receptor
• Also induce expression of immediate early junB

• We measured JunB by using anti-JunB antibody(c-fos)
K. Nakazawa, L. Karachot, Y. Nakabeppe, and T. Yamamori
Parameters of LTD induction by using intradendritic recording
Long-Term Depression (LTD) in cerebellum

- LTD is thought as neuronal mechanism of learning and memory in cerebellum (motor learning).
- Purkinje cells receive 2 inputs (PF and CF, inferior olive) LTD is plasticity at synapse between PF and Purkinje cell after conditioning with conjunctive stimulation of PF and CF...
  response of PF is reduced..(LTD)
- The synaptic weight (PF-P-cell) is reduced (>1h) after conditioning (conj).....LTD
- Long-Term desensitization of AMPA receptors: Long-Term Depression in cerebellum
• PF stimulate at 100 um from pia surface
• CF stimulate at white matter
• Record intradendritic at the middle of dendrite
Fig. 1. LTD induced with 4 Hz conjunction. Sample records obtained by intradendritic recording from a Purkinje cell by averaging five sweeps repeated at 0.2 Hz. (A) PP-evoked EPSPs. Control (cont.). Others were taken at the time indicated (in min) after the onset of 4 Hz conjunction for 75 s (300 pulses) at 0 ms CF-PF intervals. (B) The early parts of the records for the control and at 30 and 60 min are superimposed. A, point of divergence of the superimposed traces, which indicates the onset of PF-EPSPs. Vertical lines between which the EPSP slopes were measured are at 0.3 and 1.5 ms after the triangle. (C) CF-induced responses before conjunction; (D) 65 min after conjunction onset.
Fig. 3. Dependence of LTD induction on the frequency of conjunctive CF-PF stimulation. $D_m$, average amount of depression of PF-evoked EPSPs. $P_{25\%}$, probability of obtaining depression greater than 25%. Both $D_m$ and $P_{25\%}$ values were measured 40 min after the onset of conjunction. While the stimulus frequency was varied, the number of stimuli was kept at 300 and the CF-PF interval at zero. Vertical bars, S.E. Numbers attached to columns indicate the number of Purkinje cells tested.
Fig. 4. Dependence of LTD induction on the number of conjunctive stimuli in a trial. Stimulus frequency was 1 Hz and CF-PF interval zero in all cases. $D_m$ and $P_{25\%}$ as in Fig. 3.

L.Karachot, R.T.Kado, M.Ito
• Protocol to induce long-term depression

  –PF, CF at 1 Hz, 300 pulses simultaneously
  –Test

LTD = synaptic plasticity at synapse between PF and Purkinje cell
Protein synthesis and LTD

• Used LTD induced protocol to study LTD ….Rapidly turned over protein requires for LTD induction

• Local protein synthesis in dendrites:
• Ribosomes, polyribosomes, ER, Golgi complex (LTP, mRNA, certain proteins)
FIG. 3. LTD in PCs applied with 5-min anisomycin pulses. Illustration similar to that in Fig. 2 but with varied intervals between conjunction (indicated by shaded columns) and 5-min pulse applications of anisomycin (indicated by horizontal thick bars): 0 min (A), -10 min (B), -30 min (C), 5 min (D), 10 min (E), and 15 min (F). Dotted curves represent the regression line for the 15 control PCs shown in Fig. 2.
FIG. 5. Critical time window for blocking LTD with cycloheximide.
FIG. 6. Effect of a mRNA cap analogue on LTD. Data obtained with whole cell configuration current-clamp recording from PCs. Illustration similar to that in Fig. 2. ○, control cases of conjunctive stimulation. ●, with infusion of 7-methyl guanosine 5' triphosphate (m7GpppG). Conjunctive stimulation started 17–25 min after the onset of giga sealing.
Karachot, L, Shirai, S, Vigot, R, Yamamori, T and Ito, M
Summary: Rapidly turned over protein involved in LTD induction (what protein(s), further study is necessary)
LTD-functional roles:

- adaptation in motor learning
- eye blink conditioning
- adaptation in posture and locomotion
- motor coordination
Metabotropic glutamate receptor

-NBQX 10μM, D-AP5 : Stim PF high frequency-----
-slow EPSP(latency  80 ms)
Metabotropic Receptor, PF

Cerebellar motor learning: 1. VOR, OKR

2. Conditioned eye blink

Optokinetic response (HOKR, horizontal optokinetic)

Vestibuloocular reflex (VOR) ---- LTD (flocculus) (NO/ Hb) -----

Gain: eye movement/ screen oscillation

Neural circuit of OKR and VOR

Flocculus

Vestibular nuclei

Oculomotor nuclei and

a group of medial vestibular nucleus neurons
F.Shutoh, M.Ohki, H.Kitazawa, S.Itohara and S.Nagao
Ref. Neuroscience 139, 767-777,(2006)
Neuroscience 139, 767-777, (2006)
Trained mice/control mice: Stimulate vestibular nerves, recorded field potential in VN (medial)

-relationship between: increased VN neuronal activity in trained mice/long-term gain adaptation → VN is site where memory trace is stored (consolidation)

Short-term adaptation of HOKR----acquisition: site of learning at flocculus

Long-term adaptation of HOKR( link to LTD) site of memory consolidation in medial vestibular nucleus
Eye-blink conditioning

Types of blinking:

1. Spontaneous or involuntary blinking, protect eyes from dry (spread tears), Human 12/min-24/min, keep moisture

2. Voluntary blinking (self initiate, experimenter ask to response of stimuli)

3. Reflex blink (occur in response of stimuli—air puff, acoustic-click)
Eye blink: rabbit, cat, ferret---EMG(coil)---
cerebellum play major role

rat, mice…..Koekkoek et al develop
MDMT=Magnetic distance measurement technique
-use magnetic sensitive chips
-direct and precise detection of actual eyelid
movement (mice)…..mice high intensity

IN-Interposed nucleus
Experiment---------
Delay between tone(CS) condition stimuli—PF and Unconditioned stimuli-CF

Learning occurs between 150-500 ms (<100 ms, not learn)

V. Bracha, S. Zbarska, K. Parker, A. Carrel, G. Zenitsky, and J. R. Bloedel
PTX = Picrotoxin (chloride channel blocker)

Ref. J. Neurophysiol 91, 719-727, 2004

Aksenov D, Serdyukova N, Irvin K, Bracha V
Bold – site of plastic changes underlying eye blink

Star----- nucleus receive CS-US (site of learning)

/    ---- inactivate during training --> disrupt CR acquisition

BC = brachium conjunctivum

PM = premotor nucleus (including red nucleus)

Ref. Neuroscience 162, 787-796, 2009

V. Bracha et al.
Role of cerebellum in eye blink and VOR, OKR (cerebellum and motor learning)

Most people studied with site of learning and site of memory consolidation, however, we still do not know what form our memory is stored. May be

- morphology changes - increased spines (LTP)

-- biological molecules

--- proteins

--- genes

We have to find out.
Henk-Jan Boele, Sebastiaan K.E. Koekkoek and Cris I. De Zeeuw
Fig. 1. Optical setup for the imaging with oblique infrared LED illumination. (A) Infrared LED with its horizontal holder was mounted on X–Y–Z micromanipulator (black) fixed on to the microscope stage on the place of the removed condenser. The manipulator holding the preparation chamber is not shown. The microscope rested on a X–Y–table (not shown). (B) Adjusting the shape of the solution meniscus in the shallow and deep chambers. The shallow chamber was used for ganglia or tissue slices, whereas the deep one for the spinal cord, brainstem and cerebellum. The region of interest in the preparation was positioned approximately at the level of the upper surface of the chamber covered with Vaseline (grey arrowhead). The chamber was continuously perfused with oxygenated ACSF and the width of the meniscus (black arrowhead) was determined by positioning the suction needle.
Fig. 8. Cell imaging in the cerebellum of the rat at P13. (A) An isolated cerebellum (middle part above, viewed from above). (B) Schematic drawing of organization of the cerebellar cortex. ML, molecular layer; PL, Purkinje cell layer; GL, granule cell layer. Images were taken from the surface (depth, 0 μm), the molecular layer with parallel fibers of the granule cells (depth, 55 μm), the Purkinje cell layer with densely packed large cell bodies (depth, 110 μm), and the granule cell layer (depth, 135 μm). A single Purkinje cell with a different orientation is shown at the depth of 115 μm (bottom left).
References:

1. Cerebellar function: coordination, learning or timing
   M.D. Mauk, J.F. Medina, W.L. Nores and T. Ohyama
   Current Biology 10: R522-525

2. Cerebellar and extracerebellar involvement in mouse eyeblink conditioning: the ACDC model
   Henk-Jan Boele, Sebastiaan K.E. Koekkoek and Chris I. De Zeeuw

3. Memory trace of motor learning shifts transsynaptically from cerebellar cortex to Nuclei for consolidation
   F. Shutoh, M. Ohki, H. Kitazawa, S. Itohara and S. Nagao
   Neuroscience 139, 767-777, (2006)

4. The cerebellum and eye-blink conditioning: learning versus network performance hypotheses
   V. Bracha, S. Zbarska, K. Parker, A. Carrel, G. Zenitsky and J.R. Bloedel
   Neuroscience 162, 787-796, (2009)