교육과학기술부
21세기 프론티어
연구개발사업

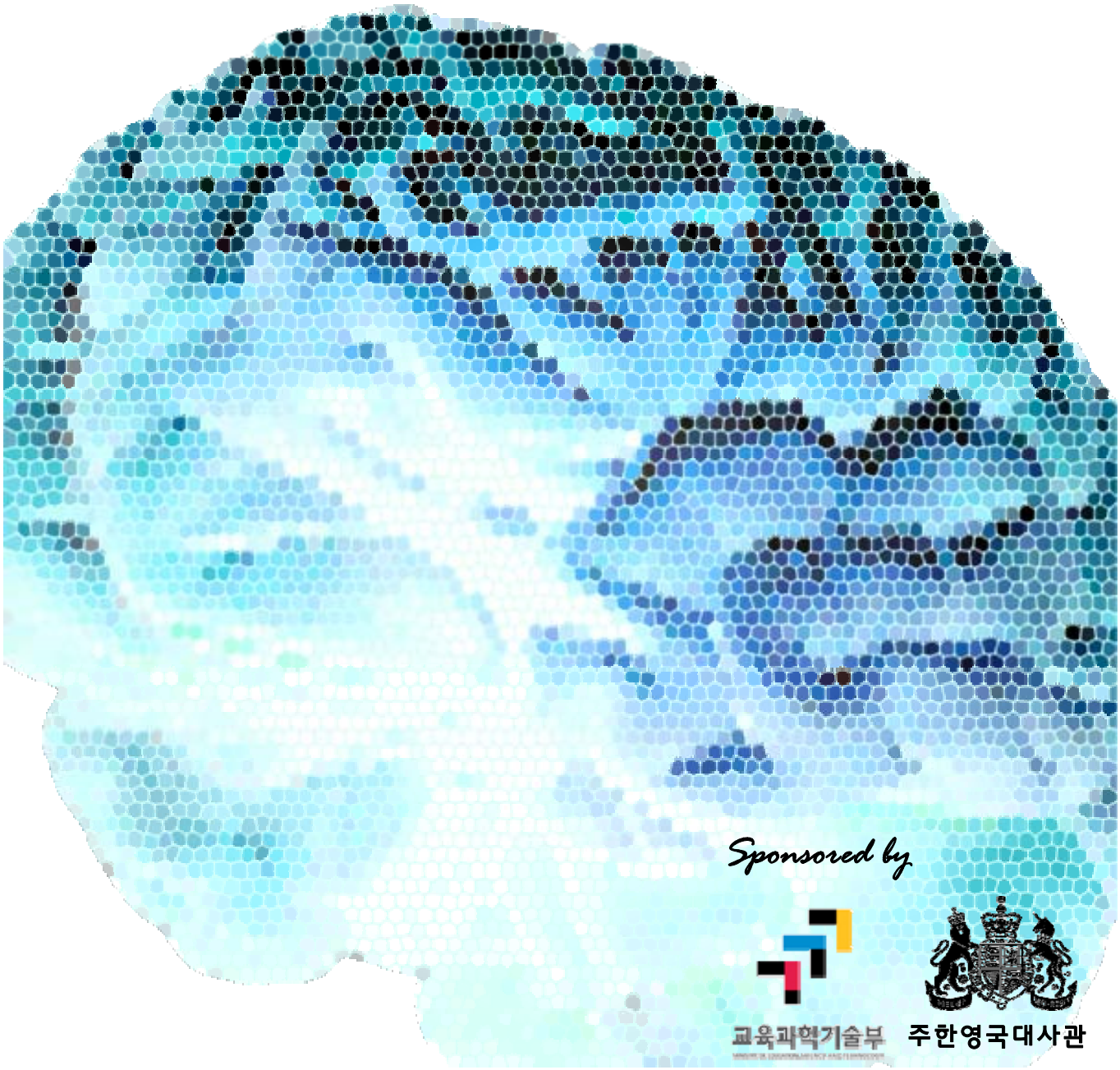
Brain Research Center
뇌기능활용및뇌질환
치료기술개발연구사업단

3rd Korea-UK Joint Symposium on Neuroscience

2008. 11. 27

Convention Center, Hoam Faculty House,
Seoul National University, KOREA

"Brain in sickness and in health"



3rd Korea-UK Joint Symposium on Neuroscience
제 3회 한 · 영 신경과학 국제심포지움

2008. 11. 27
Convention Center, Hoam Faculty House
Seoul National University, KOREA

- **Hosted by**
  - **Brain Research Center, MEST 21st Frontier Program on Neuroscience**
- **Sponsored by**
  - **Ministry of Education, Science and Technology**
  - **British Embassy in the Republic of Korea**

Welcoming Address

Dear colleagues,

Crisp morning air and leaves of red and gold remind us that autumn, the season for harvest, peace and reflection, is upon us.

In May 2004, the Brain Research Center entered into an agreement for collaboration for R&D in neuroscience with renowned institutions in the United Kingdom including the University of Bristol and the University of Manchester. We have since pursued with vigor our goal of international cooperation through bilateral exchange of ideas and resources.

It is thus with pleasure that I invite you to the 3rd Korea-UK Joint Symposium on Neuroscience to be held on November 27, 2008 at the Hoam Convention Center of Seoul National University. This is in continuation of the very memorable 2nd Symposium held in 2006 at the historic Royal Society in London.

I hope the 3rd Symposium will again be an arena where information and ideas can be shared and mutually beneficial collaborations can be established. Your input is always welcome, and I thank you in advance for your active support and participation.

Nov. 2008

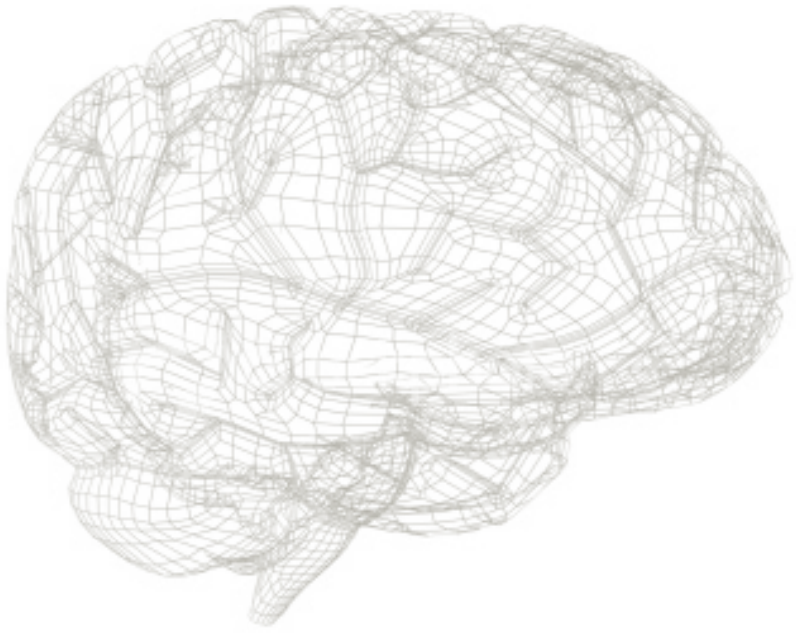
Director of Brain Research Center
Kyungjin Kim

Kyungjin Kim

Symposium Outline

Title	**3rd Korea-UK Joint Symposium on Neuroscience**
Date	**Nov 27th 2008**
Theme	**Brain in Sickness and in Health**
Organizing Committee	**Co-organizers : Yong-Keun Jung (Seoul Nat'l Univ.)** **Kei Cho (Bristol Univ.)** Ja-Hyun Baik (Korea Univ.) Jaesang Kim (Ewha Womans Univ.) Joohong Ahnn (Hanyang Univ.) Onyou Hwang (Univ. of Ulsan College of Medicine)
Office	**#308, 501 dong, College of Natural Sciences, Seoul National University** **Gwanak-ro 599, Gwanak-gu, Seoul 151-742** **Tel. +82-2-872-9100,9114** **Fax +82-2-872-9108** **E-mail : brc@brainfrontier.or.kr** **Website : www.brainfrontier.or.kr**

3rd Korea-UK Joint Symposium on Neuroscience



Time Table

Time			Programm	Location
am	pm	Min.		
8		30	Registration	2F Lobby
		50	Opening Ceremony	Mo Goong Wha
9		00	**Session 1** Synaptic Plasticity and Memory	Mo Goong Wha
10				
		40	Coffee Break	2F Lobby
11		10	**Session 2** Receptors and Synaptic Plasticity	Mo Goong Wha
12				
		45	Lunch Time & Poster Session	Soo Ryun Dong Baek Chang Pho
1				
2		00	**Session 3** Hormone and Brain Functions	Mo Goong Wha
3				
		40	Coffee Break	2F Lobby
4		00	**Session 4** Brain Imaging and Disorders	Mo Goong Wha
5		15	Poster Session	Soo Ryun Dong Baek Chang Pho
6		15	Closing Remarks & Dinner (buffet)	Mo Goong Wha & 2F Lobby

Programme

Opening Ceremony

08:50~09:00

Opening Address ..... Kyungjin Kim
(Director, Brain Research Center)

Congratulatory Remark ..... Graham Collingridge, FRS
(Director, MRC Centre for Synaptic Plasticity)

09:00~10:50

Session Ⅰ: Synaptic Plasticity and Memory

Chair : Kei Cho

09:00 **Synaptic plasticity and memory : future directions** ..... Tim Bliss, FRS
(National Institute for Medical Research, London)

09:25 **Synaptic protein degradation in memory reconsolidation and extinction** ..... Bong-Kiun Kaang
(Seoul National Univ.)

09:50 **Glutamate receptors and synaptic plasticity in the hippocampus** ..... Graham Collingridge, FRS
(Bristol Univ.)

10:15 **Role of presynaptic mitochondria in post-tetanic potentiation at central glutamatergic synapses** ..... Suk-Ho Lee
(Seoul National Univ.)

10:40 ~ 11:10 Coffee Break

11:10~12:45

Session Ⅱ: Receptors and Synaptic Plasticity

Chair : Jae-Young Koh

11:10 **Neuronal calcium sensor and synaptic plasticity** ..... Kei Cho
(Bristol Univ.)

11:35 **Amygdala depotentiation and fear extinction** ..... Sukwoo Choi
(Seoul National Univ.)

12:00 **Muscarinic receptor and synaptic plasticity in the hippocampus** ..... Jihoon Jo & Gi Hoon Son
(Bristol Univ.)

12:20 **Role of kainate receptors and mGluRs in hippocampal mossy fibre LTP : simultaneous 2-photon calcium imaging and electrophysiology** ..... Sheila Dargan
(Bristol Univ.)

12:45 ~ 14:00 Lunch Time & Poster Presentation

Programme

14:00~15:40

Session Ⅲ: Hormone and Brain Functions

Chair : Graham Collingridge, FRS

14:00	**Circadian clocks throughout the brain**	Hugh Piggins (Manchester Univ.)
14:25	**Adrenal peripheral clock in generating the circadian rhythm of glucocorticoid**	Kyungjin Kim (Seoul National Univ.)
14:50	**Rapid glucocorticoid signalling in the brain**	Stafford Lightman (Bristol Univ.)
15:15	**Increased sensitivity and anxiety-like behaviors upon chronic stress in mice lacking dopamine D2 receptors**	Ja-Hyun Baik (Korea Univ.)

15:40 ~ 16:00 Coffee Break

16:00~17:15

Session Ⅳ: Brain Imaging and Disorders

Chair : Kyungjin Kim

16:00	**Neural mechanisms of response selection**	Kyungmin Lee (Seoul National Univ.)
16:25	**New insights into the genetics of schizophrenia**	Michael Owen (Cardiff Univ.)
16:50	**Protection of methamphetamine dopaminergic toxicity by glutathione peroxidase mimics**	Hyoung-Chun Kim (Kangwon National Univ.)

17:15~18:15

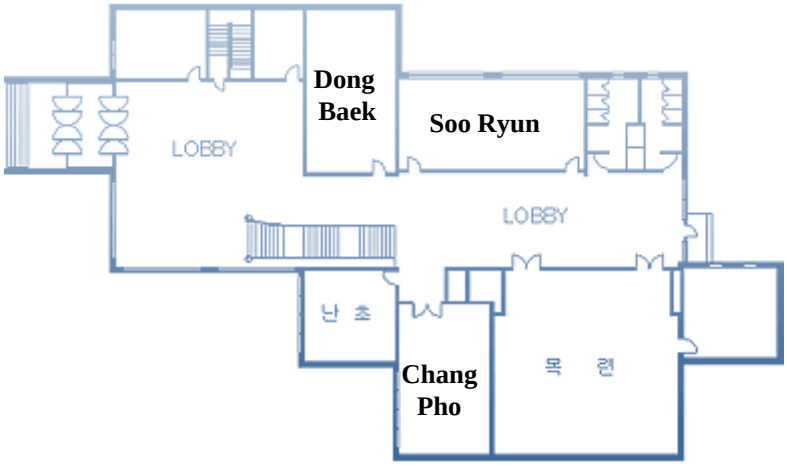
Poster Session Ⅴ

18:15 ~ Closing Remarks and Dinner (Buffet)

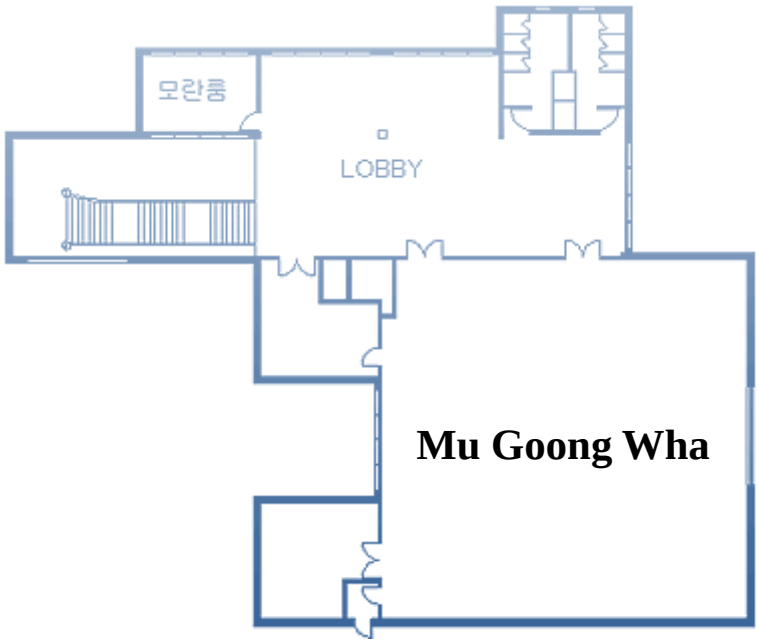
General Information

❖ Convention Center

1F : Poster



2F : Session



Office

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Session Ⅰ : Synaptic Plasticity and Memory

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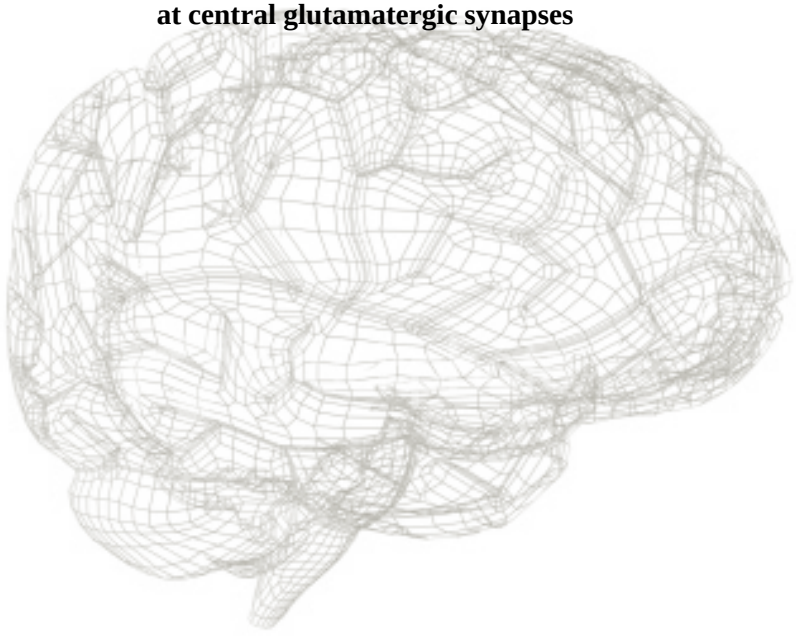

Tim V.P. Bliss, FRS

Prof. of Division of Neurophysiology
National Institute for Medical Research

Contact

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- Tel. : +44 181 959 3666 extension 2382

Education

- BSc in Physiology, McGill University, Montreal, Canada (63)
- PhD in Physiology, McGill University, Montreal, Canada (67)

Fellowship & Employment

- Member of Scientific Staff, Medical Research Council, National Institute for Medical Research (67 ~ 06)
- Head, Division of Neurophysiology, NIMR (88 ~ 06)
- Head, Neurosciences Group, NIMR (96 ~ 06)
- Visiting worker, Division of Neurophysiology, NIMR (06 ~ present)
- Scientific Advisory Committees Centre for Synaptic Plasticity, University of Bristol (Chair), Feldberg Foundation, Lister Foundation, Fondation Louis Jeantet

Honors

- Bristol Myers Squibb Award for Neuroscience (with ER Kandel) (91)
- Feldberg Prize (awarded by the Feldberg Foundation for the promotion of Anglo-German cooperation in biological and medical science) (94)
- Fellow of the Royal Society (94)
- Founding Fellow of the Academy of Medical Sciences (98)
- Annual Award for Contributions to British Neuroscience, British Neuroscience Society (03)

Synaptic plasticity and memory: future directions

Tim Bliss, FRS

National Institute for Medical Research, London

The hypothesis that memory is encoded by persistent changes in synaptic efficacy first emerged in the 19th century, but it was not until the discovery of long-term potentiation (LTP) in 1973 that an experimentally tractable model of synaptic plasticity became available. In the following decades much has been learnt about the cellular and molecular basis of LTP and its counterpart long-term depression (LTD), but the link between LTP, LTD and hippocampus-dependent memory has remained stubbornly hard to pin down. I will argue that the recent development of transgenic molecular devices will encourage a shift from mechanistic investigations of synaptic plasticity in single neurons towards an analysis of how networks of neurons encode and represent memory, and I will suggest ways in which this might be achieved. In the process, the hypothesis that synaptic plasticity is necessary and sufficient for information storage in the brain may finally be validated.

Bong-Kiun Kaang

- Prof. of Neurobiology Laboratory,
  Dept. of Biological Sciences, College of
  Natural Sciences Seoul Nat'l Univ.

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Education

- BS, Seoul Nat'l Univ. (84)
- MS, Seoul Nat'l Univ. (86)
- PhD, Columbia Univ., USA (92) * Sponsor: Nobel Laureate Eric R. Kandel, M.D.

Fellowship & Employment

- Post-Doc., Center for Neurobiology and Behavior Columbia Univ. (92 ~ 94)
- Prof. / Associate Prof./ Assistant Prof. / Dept. of Biological Sciences College of Natural Sciences, Seoul Nat'l Univ. (94 ~ Present)

Selected Publication

- Lee YS, Craig H Bailey, Eric R Kandel and Kaang BK, Transcriptional regulation of long-term memory in the marine snail Aplysia. Mol Brain. 2008;1:3.
- Park H, Pack C, Kinjo M, Kaang BK. In vivo quantitative analysis of PKA subunit interaction and cAMP level by dual color fluorescence cross correlation spectroscopy. Mol Cells. 2008; 26(1):87-92.
- Lee SH, Lim CS, Park H, Lee JA, Han JH, Kim H, Cheang YH, Lee SH, Lee YS, Ko HG, Jang DH, Kim H, Miniaci MC, Bartsch D, Kim E, Bailey CH, Kandel ER, Kaang BK. Nuclear translocation of CAM-associated protein activates transcription for long-term facilitation in Aplysia. Cell. 2007;129(4):801-12.
- Ko J, Kim S, Chung HS, Kim K, Han K, Kim H, Jun H, Kaang BK, Kim E.SALM synaptic cell adhesion-like molecules regulate the differentiation of excitatory synapses. Neuron. 2006;50(2):233-45.

Honors

- Prominent Research Award: College of Natural Sciences, Seoul Nat'l Univ. (07)
- Distinguished Scientist Award: Korean Ministry of Science (07)

Synaptic protein degradation in memory reconsoli dation and extinction

Bong-Kiun Kaang

Dept. of Biological Sciences, College of Natural Sciences, Seoul National Univ., Seoul, Korea

Background Memory is not only a reconstruction of the past influenced by numerous factors, but also is a dynamic process. For instance, the recollection process of long-term memory is dynamic and requires de novo protein synthesis. Long-term memories are not static, but are dynamic and become labile particularly during its retrieval. Thus, memory retrieval is thought to be a step required for incorporation of new information into preexisting memories. Thus, we have investigated whether or not protein degradation is involved in the reorganization of retrieved memory.

Results Specific postsynaptic proteins were degraded in the hippocampus by polyubiquitination after retrieval of contextual fear memory. We also found that the infusion of proteasome inhibitor into the hippocampus immediately after the memory retrieval prevented anisomycin (protein synthesis inhibitor)-induced memory impairment. This indicates that ubiquitin/proteasome-dependent protein degradation underlies destabilization processes after the memory retrieval. It also suggests preexisting memory is disrupted by proteasome-mediated protein degradation, and that updated memory is reconsolidated or strengthened by protein synthesis. Protein degradation is not required for memory consolidation, whereas it is critical for memory extinction. Synaptic protein degradation also plays a key role in fear memory extinction.

Conclusion Ubiquitin/proteasome-dependent protein degradation underlies destabilization processes after fear memory retrieval. Memory reorganization occurs via breakdown of original memories while new elements are incorporated by new protein synthesis. Taken together, synaptic protein degradation is critical in the reorganization of memory

Synaptic protein degradation in memory reconsolidation and extinction

Bong-Kiun Kaang

Department of Biological Sciences
Seoul National University

- Learning

  -the process by which we acquire knowledge about the world

- Memory

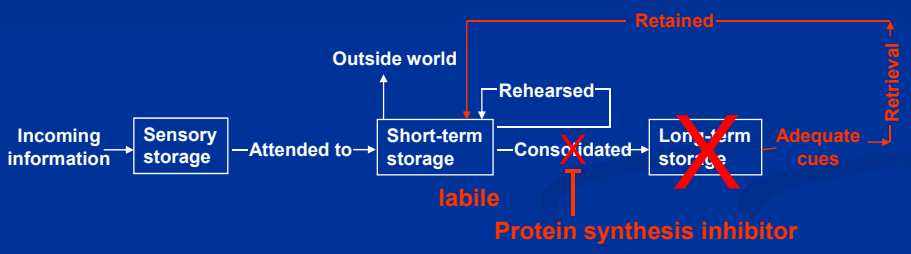
  -the process by which that knowledge is encoded, stored, and later retrieved

- We are who we are largely because of what we learn and what we remember.

-Kandel *et al*. 2000
In *Principles of Neural Science*

- Traditionally, memory is a stable record of the event, which can be recalled in the same form it was learned.
- Reasons to question this common sense idea
  : Memory changes with time
  : a dynamic and mutable process, a reconstruction of the past influenced by many factors.
- Not like computer files or libraries.

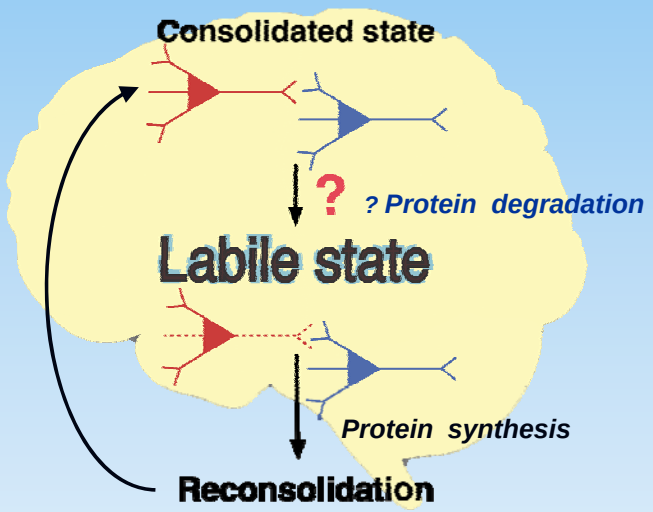
Reconsolidation after memory retrieval

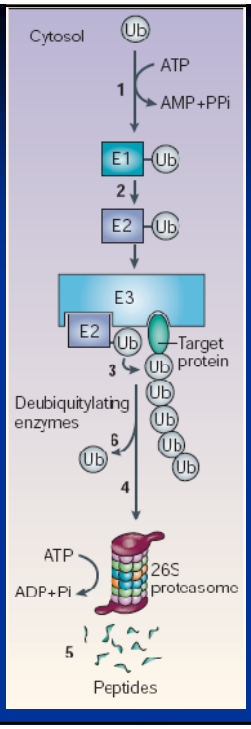


Reactivated memory undergoes a rebuilding process that depends on de novo protein synthesis.

(Nader et al., *Nature* 2000)

Hypothesis

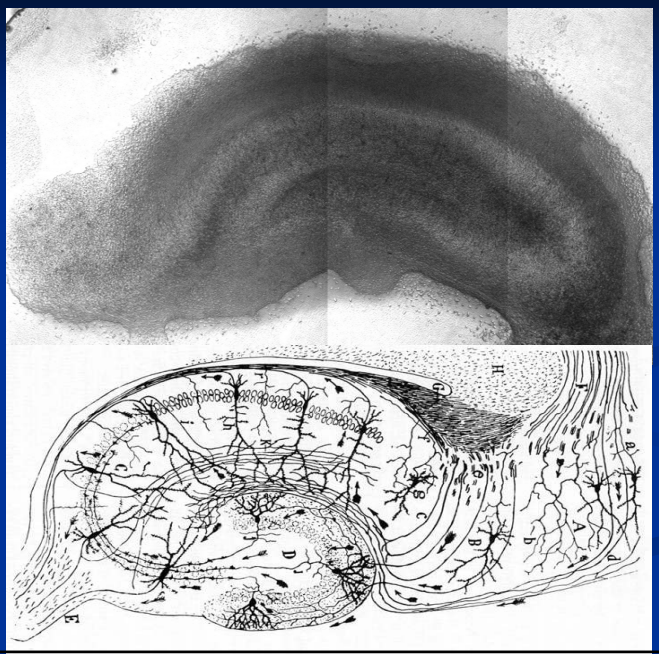




Ubiquitin-proteasome system

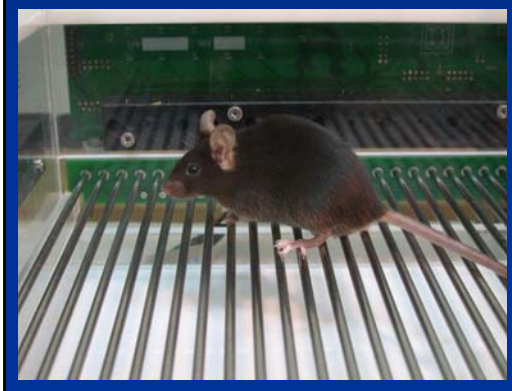
(Ciechanover, 2005)

Hippocampus



Cajal, 1901

Contextual fear conditioning



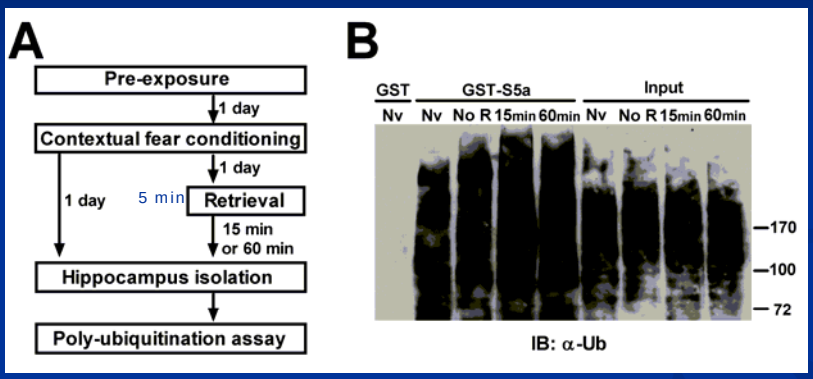
Context — Electric shock
Association

↓

Freezing behavior

: *Hippocampus*-dependent memory

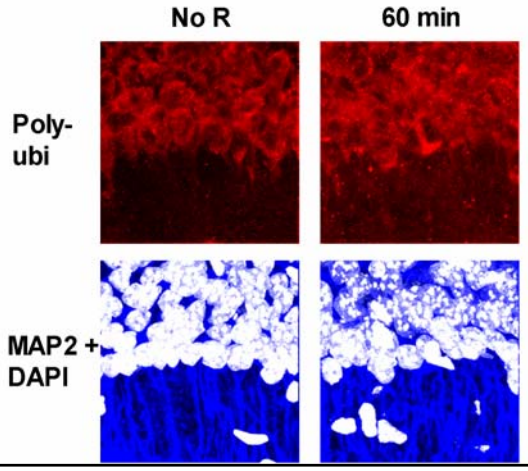
Protein polyubiquitination after memory retrieval

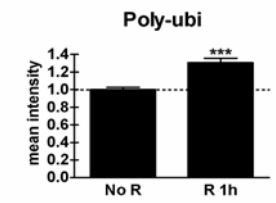


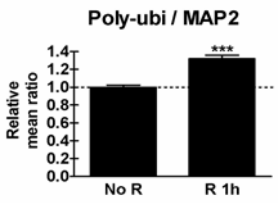
Fear memory retrieval increased polyubiquitination in the hippocampal synaptosomal membrane fraction.

Polyubiquitinated protein level after memory retrieval

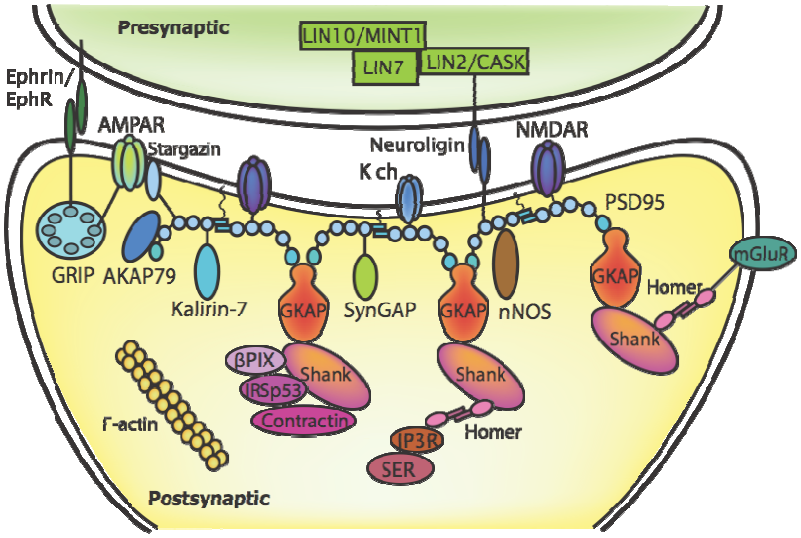
Contextual fear conditioning $\xrightarrow{24h}$ retrieval $\xrightarrow{1h}$ brain fixation







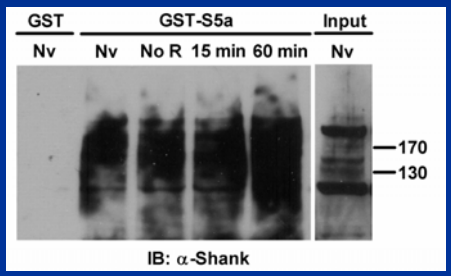
Which proteins?

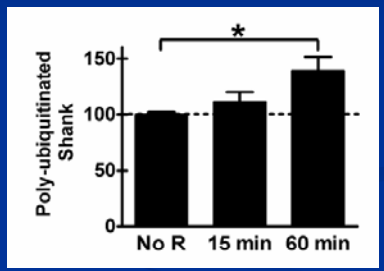


Excitatory Synapse

modified from Kim and Sheng (2004)

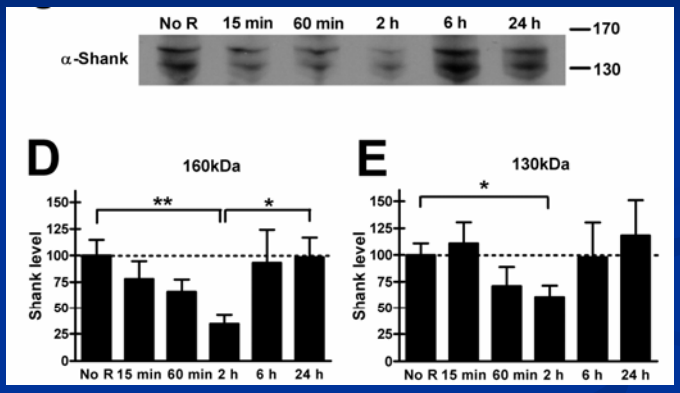
The polyubiquitination of Shank after memory retrieval





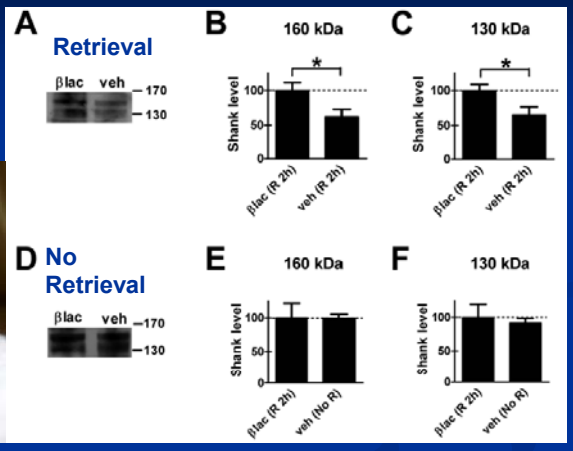
Polyubiquitinated Shank isoforms were increased 1 hour after retrieval.

Dynamics of endogenous Shank proteins after memory retrieval



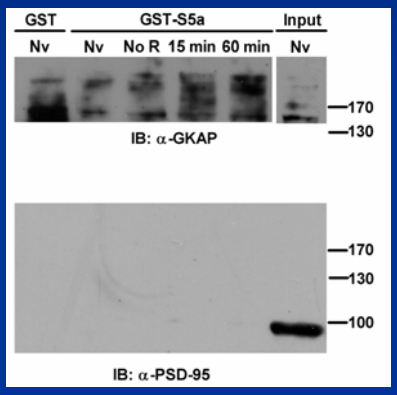
The time course of the increase of Shank polyubiquitination coincided with the time course of the reduction of the amount of endogenous Shank.

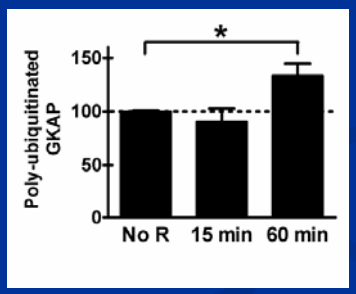
Degradation of Shank by proteasome



◆ The proteasome inhibitor clasto-lactacystin- β-lactone (βlac) blocked the decrease of the amount of Shank at 2 hours after retrieval.
◆ β lac infusion did not affect the basal level of Shank in control animal without retrieval.

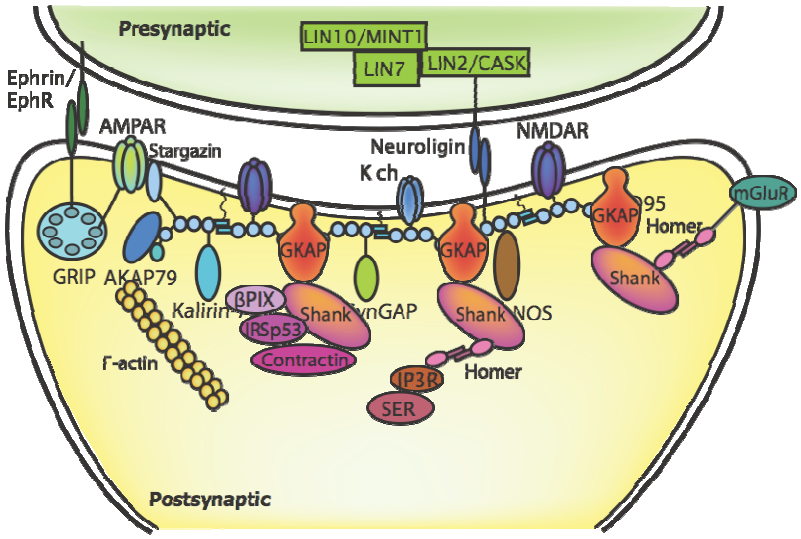
The polyubiquitination of other PSD proteins after memory retrieval





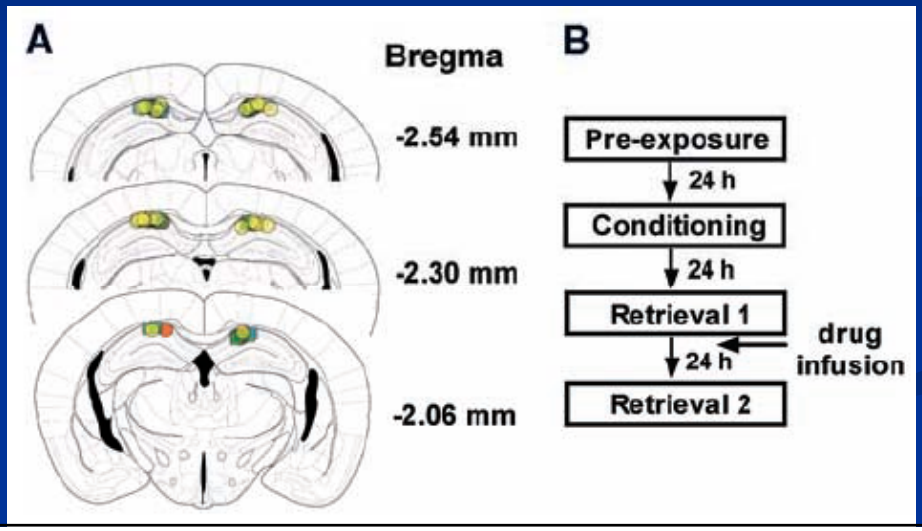
Polyubiquitinated GKAP, but not PSD-95 increased after memory retrieval.

Protein degradation and resynthesis in the synapse induced by memory retrieval

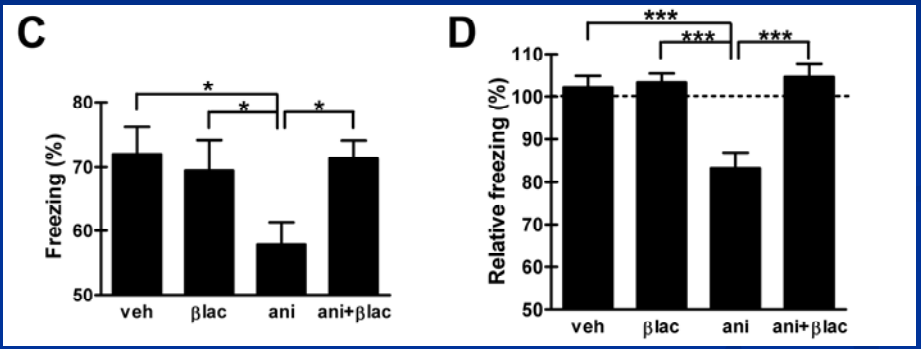


What is the physiological function of protein degradation after retrieval?

The role of protein degradation in memory reconsolidation

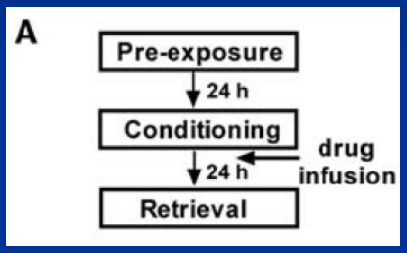


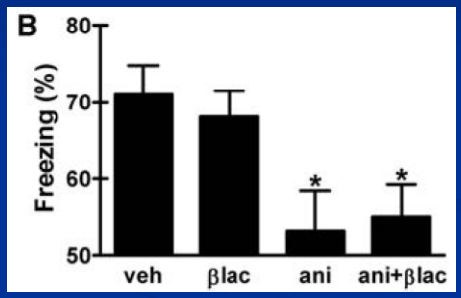
The labile state during memory retrieval was induced by protein degradation



Proteasome inhibitor blocked the anisomycin-induced memory impairment after retrieval.

The role of protein degradation during new learning





Protein degradation was not required for memory consolidation.

Memory Extinction: Reversal learning

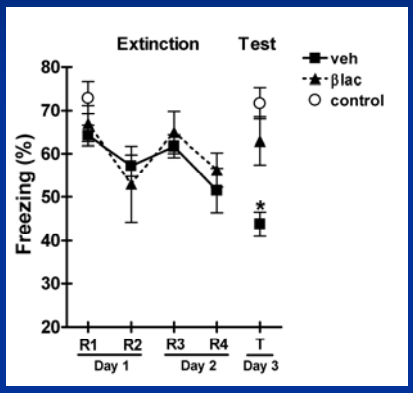
Repeated reexposure to the context without shock

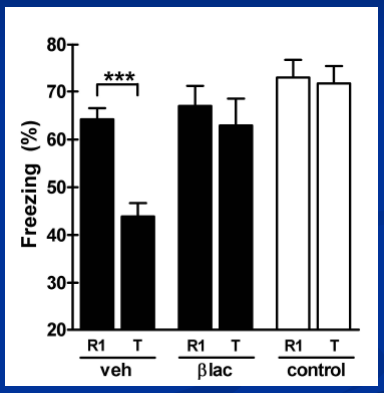
↓

Decreased freezing behavior: Memory Extinction

Simple model of memory reorganization

Protein degradation is critical for memory extinction





The infusion of proteasome inhibitor suppressed the extinction of contextual fear memory.

Memory Extinction Mechanisms

- Unlearning: *Erasing*
  - -Synaptic Degradation
  - -Synaptic Depotentiation

MEMORY

- Relearning: *Masking*
  - -Medial prefrontal inhibition
  - -Amygdala intercalated inhibitory neurons
  - -Local inhibitory neurons

Conclusion

◆ Specific postsynaptic proteins were degraded in the hippocampus by polyubiquitination after retrieval of contextual fear memory.

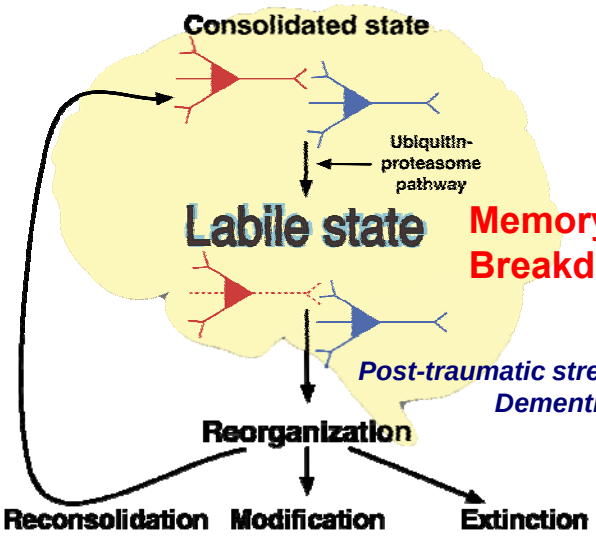
◆ The infusion of proteasome inhibitor into the CA1 region immediately after retrieval prevented anisomycin-induced memory impairment.

◆ Protein degradation is not required for memory consolidation, whereas it is critical for memory extinction.

◆ Ubiquitin/proteasome-dependent protein degradation underlies destabilization processes after fear memory retrieval.

◆ Memory reorganization occurs via breakdown of original memories while new elements are incorporated by new protein synthesis.

Working Model for Memory Reorganization



Powerful mechanism for memory update

Thanks to:

Sue-Hyun Lee
Jun-Hyeok Choi
Nuribalhae Lee
Hye-Ryeon Lee
Jae-Ick Kim
Nam-Kyung Yu
Sun-Lim Choi
Seung-Hee Lee
Hyoung Kim

Eunjoon Kim, KAIST

Graham L. Collingridge, FRS

- Director of the MRC Centre for Synaptic Plasticity

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- BSc in Pharmacology, Univ. of Bristol, UK (77)
- PhD in Pharmacology, Univ. of London, UK (80)

Fellowship & Employment

- Post-Doc. Research Fellow, Dept. of Physiology, Univ. of Brit. Columbia, Canada (80 ~ 82)
- Senior Research Officer, Dept. of Physiology & Pharmacology, Univ. of New South Wales, Australia (83)
- Lecturer, Reader, Dept. of Pharmacology, Univ. of Bristol, UK (83 ~ 90)
- Prof. and Head of Dept. of Pharmacology, Univ. of Birmingham, UK (90 ~ 94)
- Prof. of Neuroscience (94 ~ present), Head of Dept. (96 ~ 98), Director of MRC Centre for Synaptic Plasticity (98 ~ present), Dept. of Anatomy, Univ. of Bristol, UK
- Visiting Prof., Dept. of Medicine (03), Dept. of Psychiatry (07 ~ present), Brain Research Centre, Univ. of British Columbia, Canada

Selected Publication

- Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 2003;361:31-9.
- Collingridge GL, Kehl SJ, McLennan H. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J Physiol. 1983;334:33-46.

Honors

- Award, The Santiago Grisolia Prize (08)
- Elected President, British Neuroscience Association (07)
- Author of the most cited paper in neuroscience during the “Decade of the Brain” (00)
- Founder Fellow, Academy of Medical Sciences; Director, First MRC Centre in the UK (98)
- Founder Fellow, European DANA Alliance (97)
- Elected Fellow, The Royal Society (01)

Glutamate receptors and synaptic plasticity in the hippocampus

Graham L Collingridge, FRS

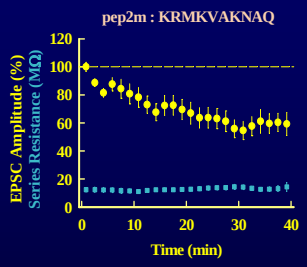
MRC Centre for Synaptic Plasticity, University of Bristol, UK

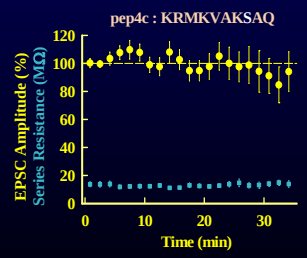
The primary mechanism for information storage in the vertebrate nervous system is believed to be alterations in the efficiency of synaptic transmission. The two most extensively studied forms of synaptic plasticity are long-term potentiation (LTP) and long-term depression (LTD) of excitatory synaptic transmission. The principal excitatory neurotransmitter, L-glutamate, act on four main subtypes of receptor – AMPA, NMDA, kainate and mGlu.

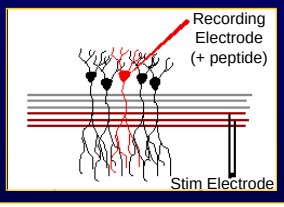
AMPA receptors mediate fast excitatory synaptic transmission. LTP and LTD are expressed as changes in the number and properties of AMPA receptors at synapses. NMDA receptors are the primary trigger for LTP, where they endow synapses with “hebbian” properties, and for one form of LTD. NMDA receptors mediate a slower component of synaptic transmission which itself if capable of undergoing LTP and LTD. mGlu receptors are the trigger for another major form of LTD. They can also mediate and / or modulate certain forms of LTP. Finally, kainate receptors can also trigger both LTP and LTD at certain synapses in the brain.

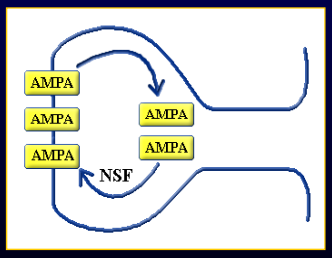
NMDA receptor-dependent LTP involves activation of protein kinases, such as CaMKII and PKC, whilst NMDA receptor-independent LTP involves PKA. In contrast, NMDA receptor-dependent LTD involves activation of ser/thr protein phosphatases, such as PP1 and calcineurin, whilst mGlu receptor-dependent LTD involves activation of tyrosine phosphatases. In addition, both forms of LTD also require activation of protein kinases (GSK-3 and p38 MAPK, respectively). Interestingly, GSK-3 is inhibited following LTP thereby providing a molecular mechanism of regulation of LTD by LTP. Potentially dysregulation of this process may contribute to psychiatric and neurodegenerative disorders.

Rapid cycling of AMPA receptors at synapses







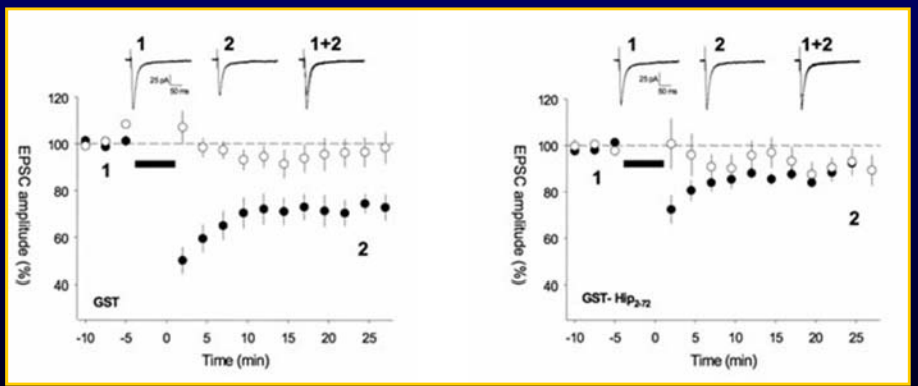


MRC *Centre for Synaptic Plasticity*

from: Nishimune *et al*, (1998) *Neuron* **21;** 87-97

University of BRISTOL

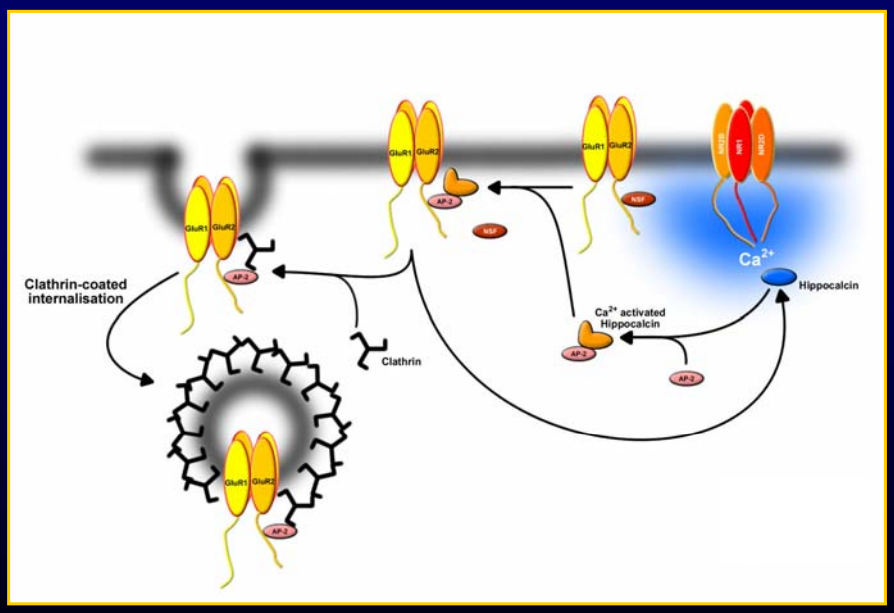
Hippocalcin is required for LTD



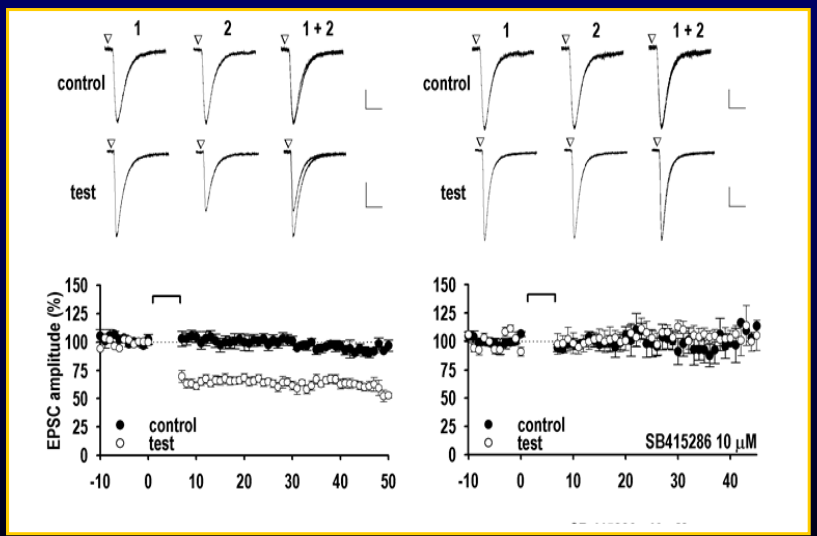
MRC *Centre for Synaptic Plasticity*

from: Palmer *et al* (2005) *Neuron* **47;** 487–494.

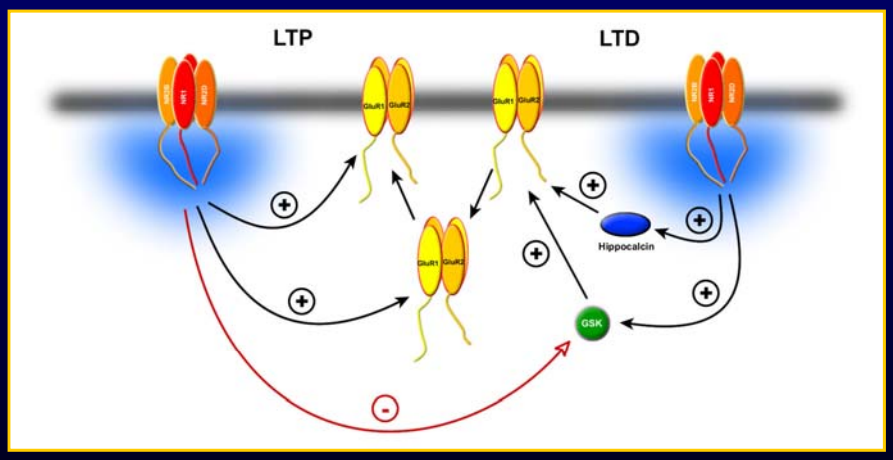
University of BRISTOL



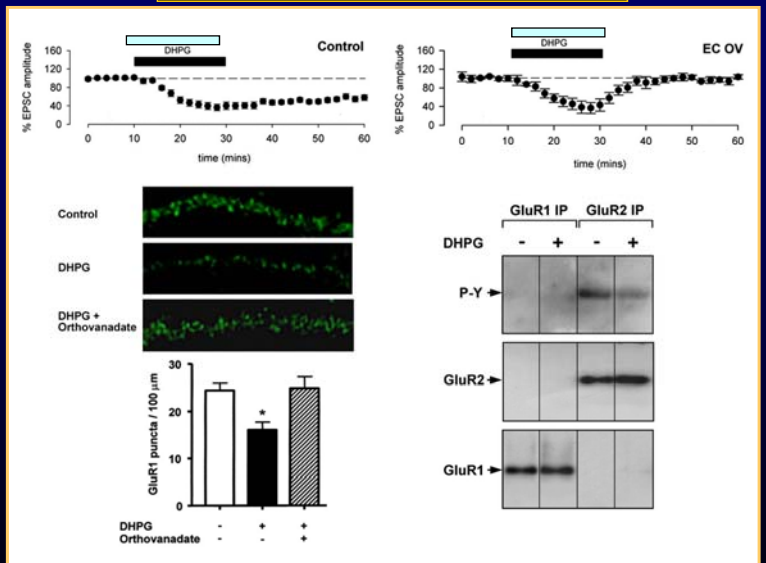
Postsynaptic application of a GSK3 inhibitor blocks LTD



from: Peineau *et al* (2007) *Neuron* 53; 703-717

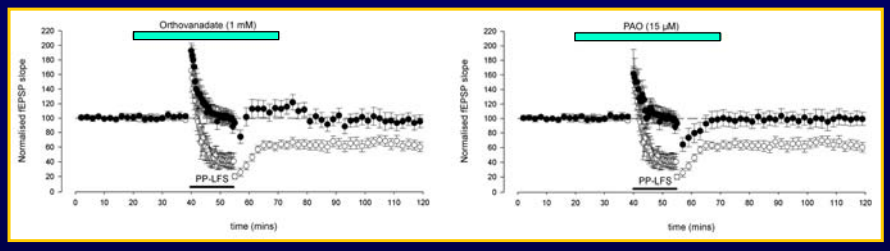


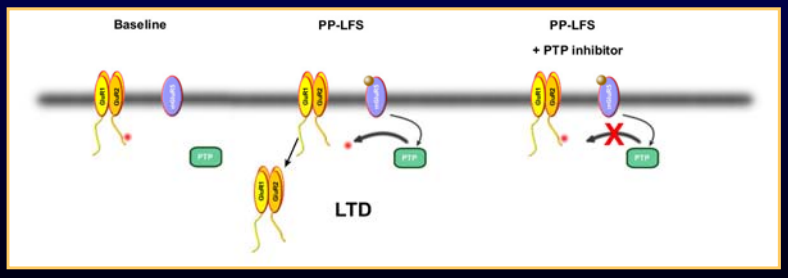
mGluR-LTD requires activation of PTPs



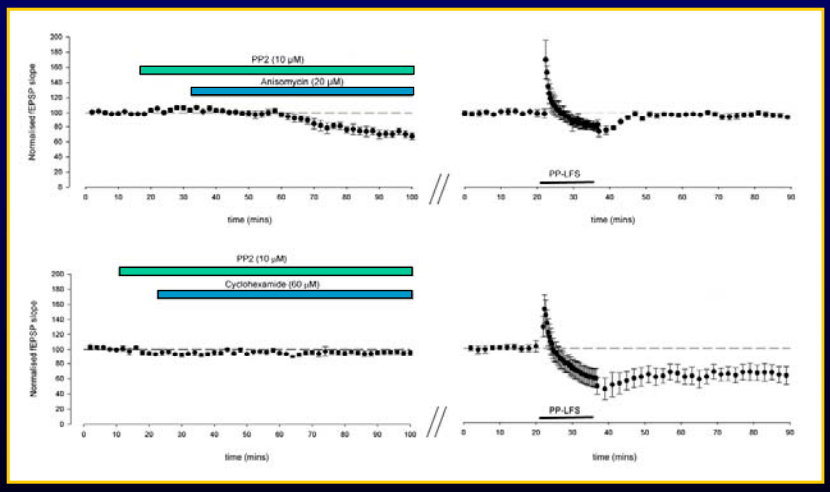
from: Moult *et al*, (2006) *J. Neurosci.* **26;** 2544-2554

Synaptic mGluR-LTD requires activation of PTPs

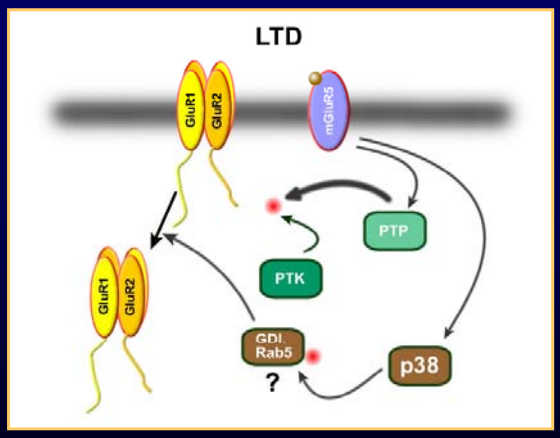




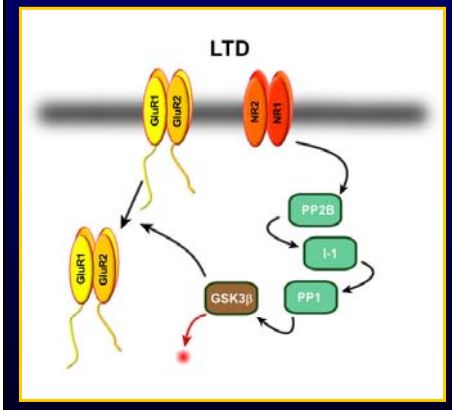
Inhibition of PTKs and activation of p38 MAPK mimics and occludes LTD

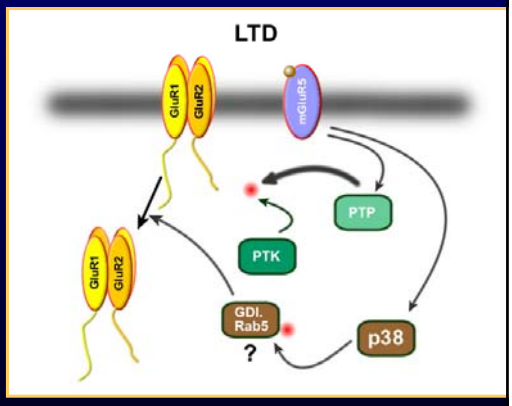


Role of PTKs and p38 MAPK in LTD



Summary; Different LTD mechanisms





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- Post-Doc. fellow in Max-Planck Institute (Prof. Neher's Lab, Goettingen, Germany) (97 ~ 99)
- Assistant Professor, Dept. of Physiology, Seoul Nat'l Univ. (99 ~ 03)
- Associate Professor, Dept. of Physiology, Seoul Nat'l Univ. (04 ~ present)

Selected Publication

- Lee JS, Kim MH, Ho WK, LeeSH . The release probability and the readily-releasable pool size are regulated by two independent mechanisms during post-tetanic potentiation at the calyx of Held synapse. J Neurosci. 2008;28(32):7945-53.
- Lee DY, Lee KH, Ho WK, LeeSH. Target cell-specific involvement of presynaptic mitochondria in post-tetanic potentiation at hippocampal mossy fiber synapses. J Neurosci. 2007;27(50):13603-13
- Lee SH, Kim MH, Lee JY, Lee SH, Lee D, Park KH, Ho WK. Na+/Ca2+ exchange and Ca2+ homeostasis in axon terminals of Mammalian central neurons. Ann N Y Acad Sci. 2007;1099:396-412.
- Cho H, Kim YA, Yoon JY, Lee D, Kim JH, Lee SH, Ho WK. Low mobility of phosphatidylinositol 4,5-bisphosphate underlies receptor specificity of Gq-mediated ion channel regulation in atrial myocytes. PNAS. 2005;102(42): 15241-6.

Role of presynaptic mitochondria in post-tetanic potentiation at central glutamatergic synapses

Jae-Sung Lee, Doyun Lee, Myoung-Hwan Kim, Won-Kyung Ho, **Suk-Ho Lee**

Dept. of Physiology, College of Medicine, Seoul National Univ., Seoul, Korea

Background Short-term plasticity is supposed to play a crucial role in synaptic computation. Global calcium in presynaptic terminal has profound influence on the short-term plasticiy. Previously, we studied the calcium clearance mechanisms at large presynaptic teriminals of brain stem glutamatergic synapse, calyces of Held and reported that Na/Ca exchanger is a major player in calcium clearance, but it is saturated at high [Ca2+]i (>2.5 M), at which mitochondria begin to take part in clearance. Post-tetanic mitochondrial Ca2+ release subsequent to Ca2+ uptake during tatanic stimulation was proposed as a mechanism for post-tetanic potentiation (PTP) at neuromuscular junction, but not studied at central synapses. Instead, recent studies suggest that activation of protein kinase C is responsible for PTP at central synapses. Nevertheless, it has been widely recognized that presynaptic terminals are heterogeneous, even among terminals originated from the same axon fiber. Electron microscopy studies revealed that presynaptic terminals harbour different nubmer of mitochondria, suggesting that mitochondria might have different influence on calcium dynamics and short-term plasticity depending on the type of presyantpic terminals. Here, we investigated the contribution of mitochondria on post-tetanic potentiation at synapses innervated by two types mossy fiber boutons (MFBs) and calxy of Held.

Results At large MFBs and the calyx of Held (referred to as large teriminals), mitochondria limited the calcium build-up during high frequency stimulation (HFS), whereas at small MFBs mitochondrial inhibition does not significantly affect the [Ca2+]i buildup, indicating that mitochondira take up Ca2+ during HFS at large terminals. Consistently, an elevation of resting [Ca2+]i during tens of seconds after the cessation of HFS (referred to as residual calcium) was significantly diminished by inhibitors of mitochondrial Na/Ca exchanger (mNCX) at large terminals but not at small MFBs. These results indicate that mitochondiral calcium uptake and subsequent post-tetanic calcium release via Na/Ca exchanger (mNCX) resulted in residual calcium at large terminals.

To test whether mitochondria-dependent residual calcium underlies the mechanism for PTP, we investigated effects of inhibitors of mitochondrial Ca2+ uniporter or mNCX on PTP. These drugs largely inhibited PTP at the mossy fiber (MF) synapses on mossy cell and at the calyx of Held synapses, which large terminals are involved. Moreover, the magnitude of PTP linearly correlated with residual calcium level, indicating that presynaptic residual calcium is responsible for PTP. In constrast, PTP at the MF synapse on hilar interneurons, on which small MFBs terminate, was not reduced by mNCX blockers. Instead, PTP at these syanpse was inhibited by PKC inhibitors, which had no effect on PTP at the synapse of large terminals.

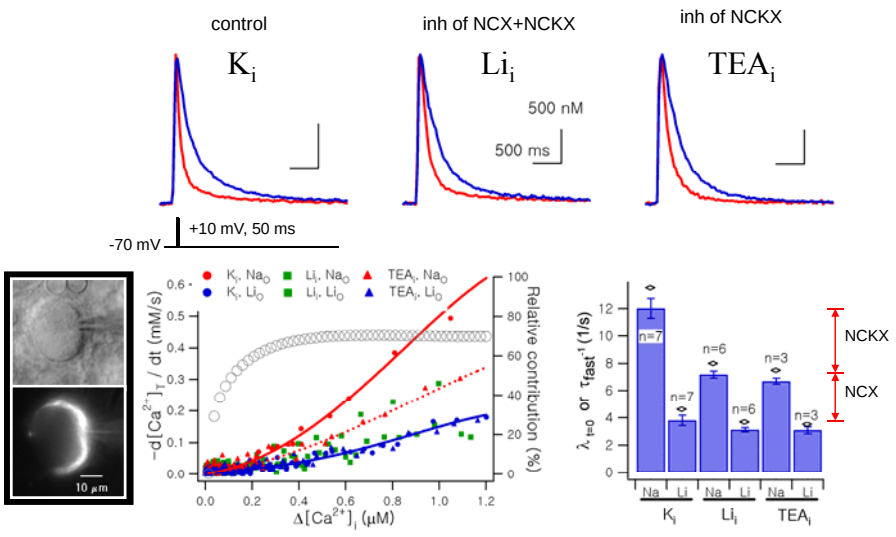
The magnitude of EPSC is determined by release probability (Pr) multiplied by the size of readily releasable pool (RRP). Analysis of Pr and RRP size before and after HFS revealed that PTP was accompanied with post-tetanic increases both in Pr and in the RRP size. Inhibition of mitochndria-derived residual calcium suppressed specifically the post-tetanic increase in Pr, which constiututes the fast phase of PTP. On the other hand, inhibitors of myosin light chain kinase (MLCK) or myosin ATPase suppressed specifically the increase in the RRP size.

Conclusion 1) Mitochondria- and PKC-dependent PTP are expressed at distinct presynaptic terminals; 2) PTP at large synaptic terminals depends on mitochondria-dependent residual calcium and activation of MLCK

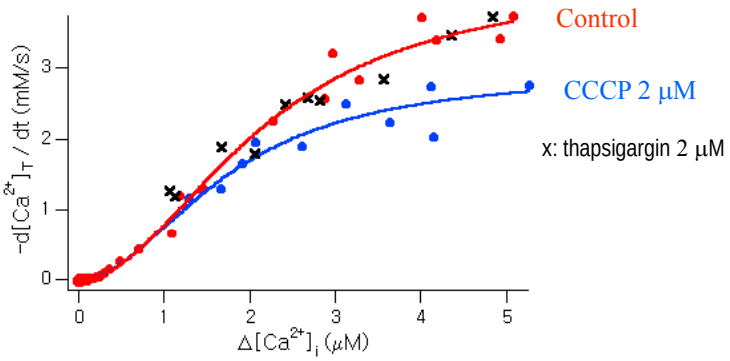
Role of presynaptic mitochondria in post-tetanic potentiation at central glutamatergic synapses

Lee, Suk-Ho
Department of Physiology
Seoul National Univ. College of Medicien

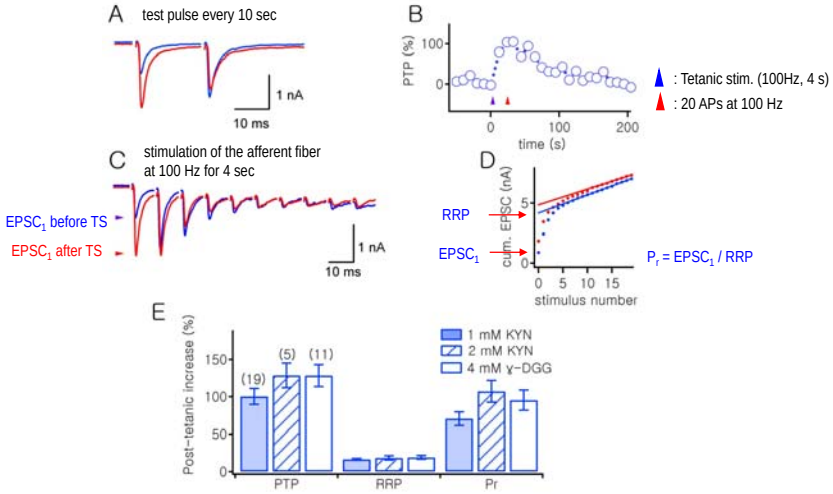
Na^+/Ca^{2+} exchanger is a major Ca^{2+} clearance mechanism at the calyx of Held



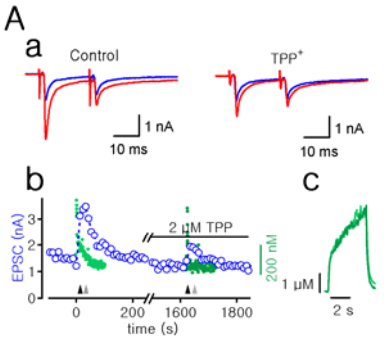
Mitochondria contribute to Ca^{2+} clearance when the Ca^{2+} load is high enough to saturate Na/CaX

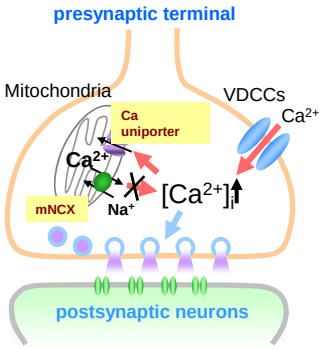


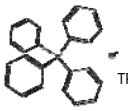
PTP is accompanied by increases in P_r and the RRP size



Block of post-tetanic mitochondrial calcium release abolishes residual calcium and inhibits PTP

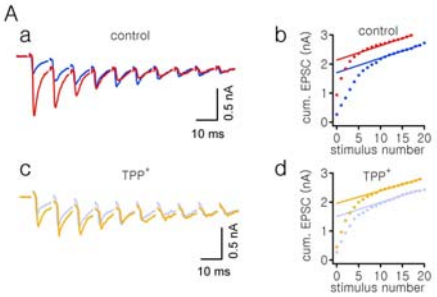




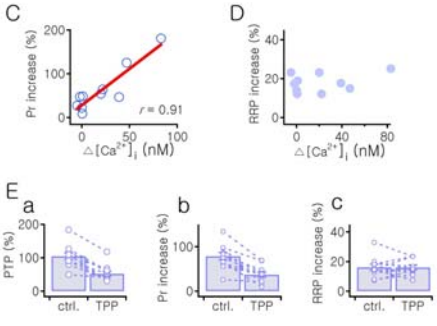


TPP+(tetraphenylphosphonium): mitoNCX inhibitor

Residual calcium contributes to the increase in P_r with no effect on the RRP size

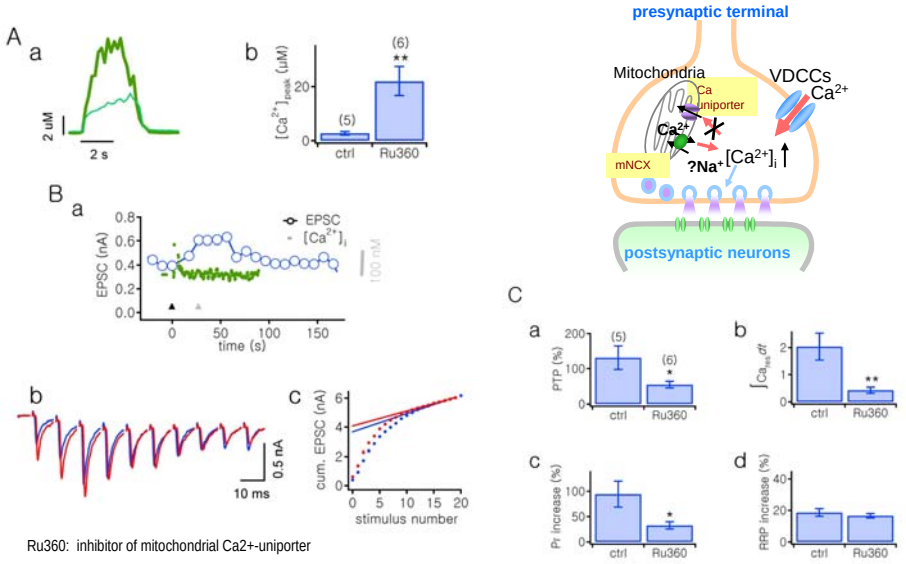


4 mM DGG + preloading of fura-4



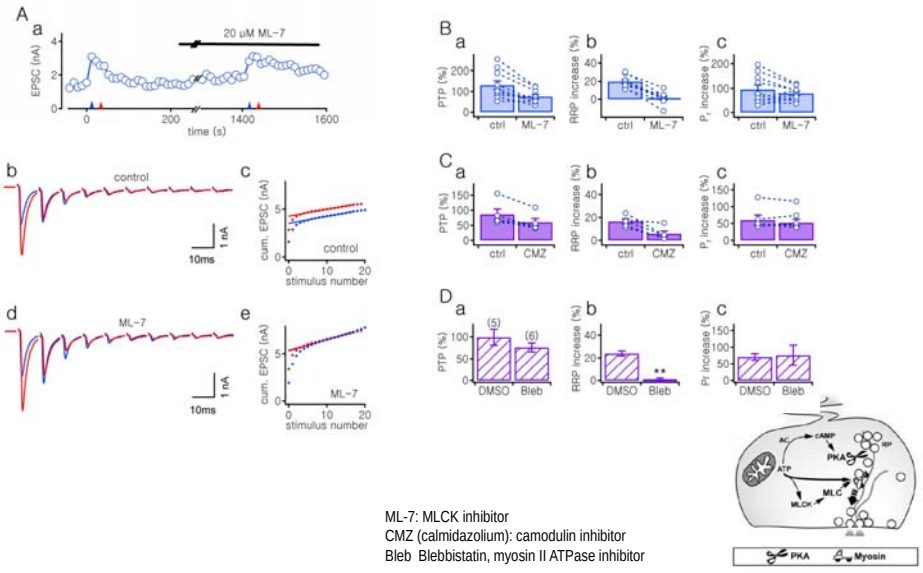
1 mM KYN

Block of mitochondrial calcium uptake abolishes the residual calcium and suppresses the increase in P_r but not in the RRP size



Ru360: inhibitor of mitochondrial Ca2+-uniporter

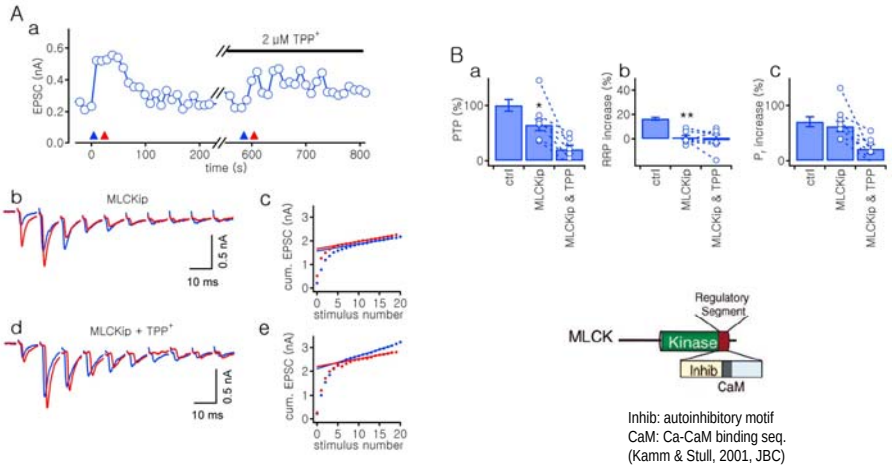
Post-tetanic increase in the RRP size is mediated by MLCK



ML-7: MLCK inhibitor
CMZ (calmidazolium): camodulin inhibitor
Bleb Blebbistatin, myosin II ATPase inhibitor

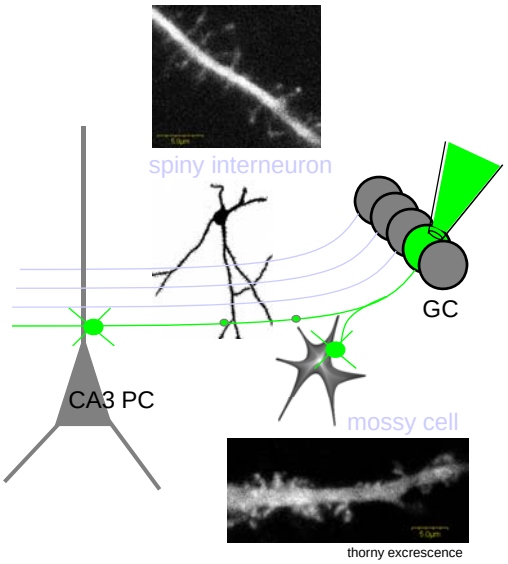
Verstrecken et al. (2005) Neuron

Preload of MLCK inhibitory peptide-18 (MLCKip) suppresses specifically the post-tetanic increase in the RRP size



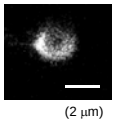
Inhib: autoinhibitory motif
CaM: Ca-CaM binding seq.
(Kamm & Stull, 2001, JBC)

Hilar neurons are innervated by MFBs with distinct morphology




(1 µm)

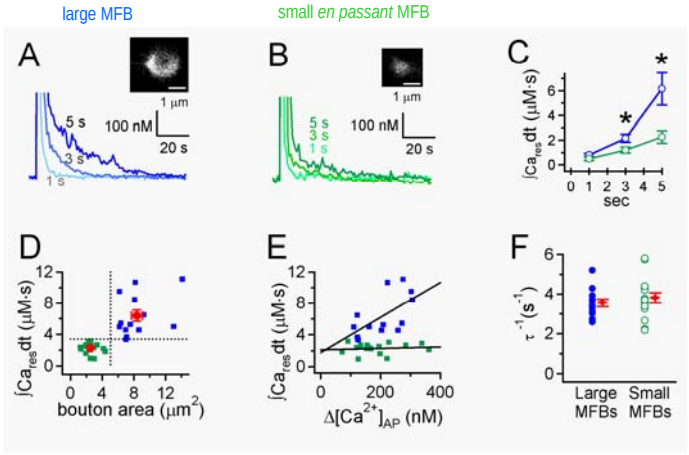
GC innervates hilar interneurons via filopodial extensions or small *en passant* MFBs


(2 µm)

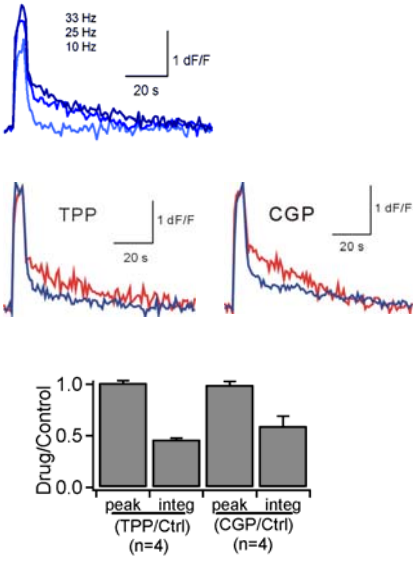
GC innervates hilar mossy cells and CA3 pyramidal neurons via large MFBs

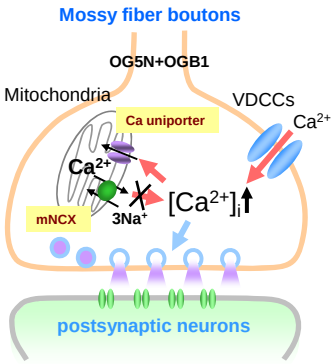
Acsady et al. (1998)

Dependence of post-tetanic residual calcium on
the number and the frequency of HFS at large and small MFBs.

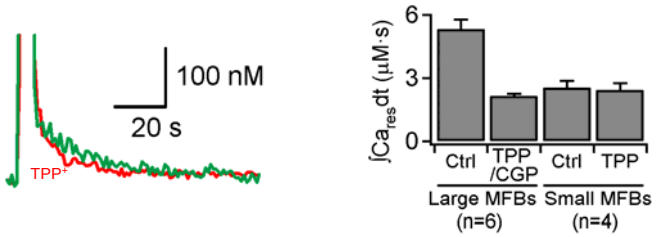


mNCX inhibitors have little effects on the Ca^{2+}-buildup druing tetanic stimulation

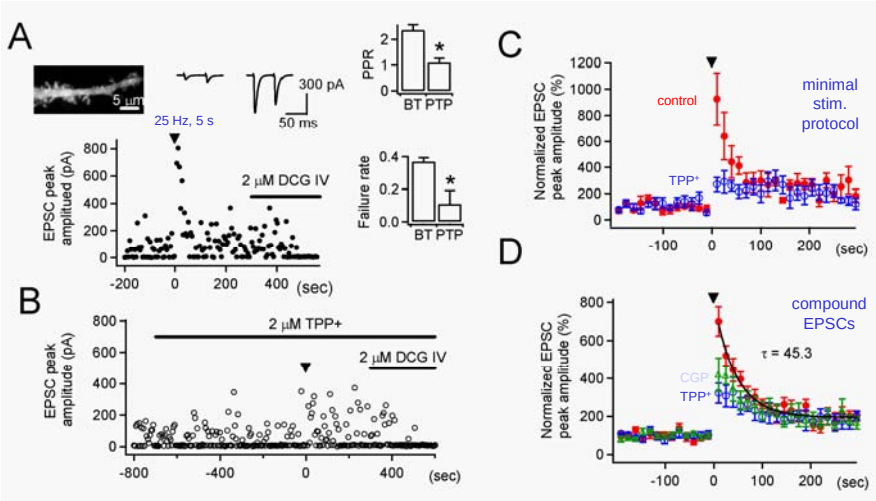




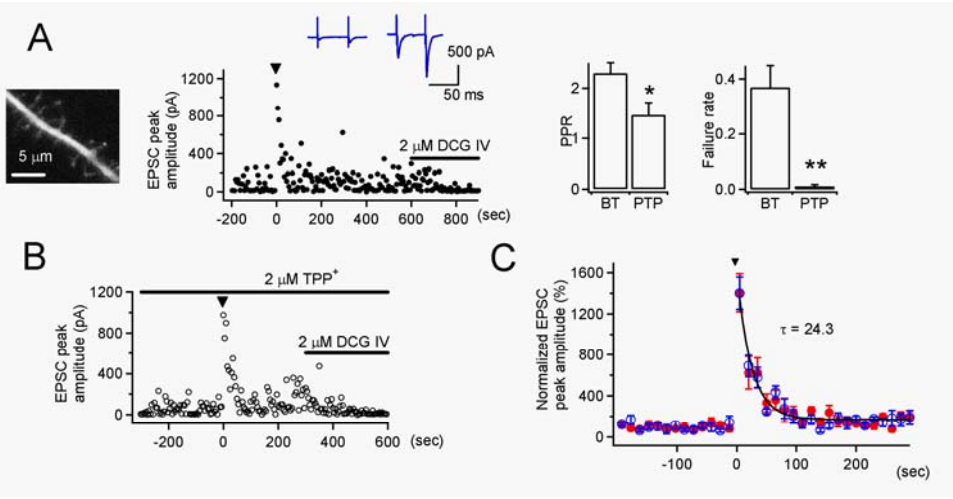
Inhibition of mitochondrial NCX has no effect on the residual Ca^{2+} at small MFBs



Post-tetanic Ca^{2+} release from mitochondria contributes to post-tetanic potentiation at the mossy fiber synapse onto hilar mossy cell



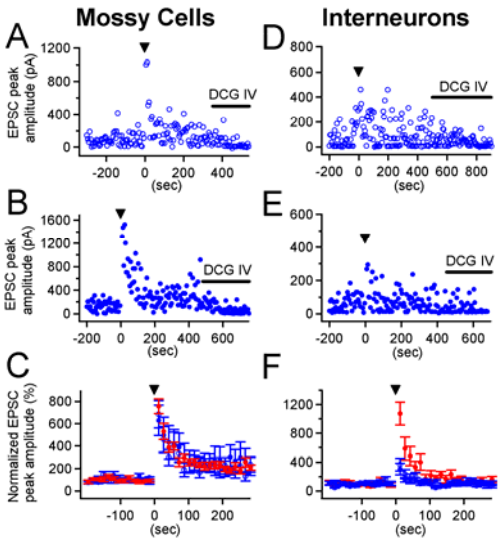
Post-tetanic mitochondrial Ca^{2+} release is not essential for the PTP at mossy fiber synapses onto hilar spiny interneurons



PKC inhibitors have differential effects on the PTP at MF synapses onto mossy cell and onto hilar interneurons

Chelerythrine

Ro-31-8220



Session Ⅱ : Receptors and Synaptic Plasticity

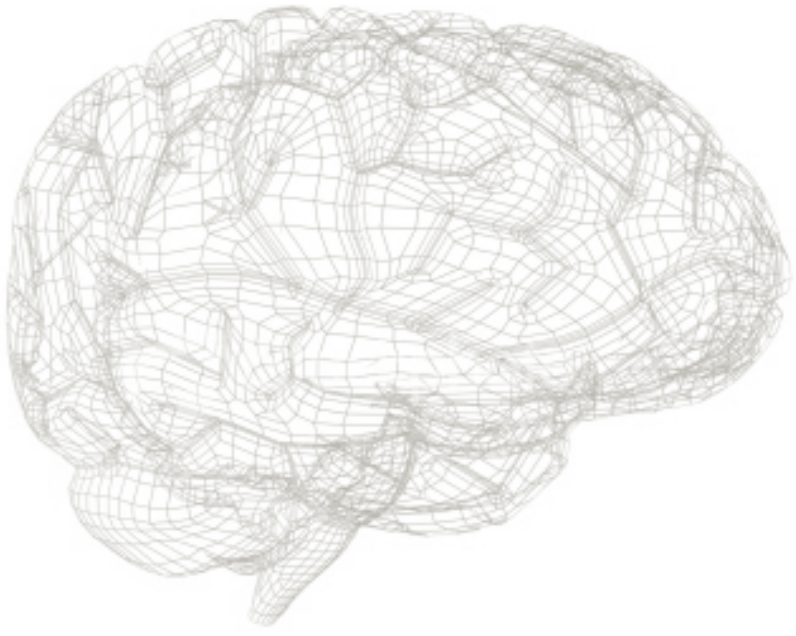
Chair : Jae-Young Koh

11:10 **Neuronal calcium sensor and synaptic plasticity** ..... Kei Cho (Bristol Univ.)

11:35 **Amygdala depotentiation and fear extinction** ..... Sukwoo Choi (Seoul National Univ.)

12:00 **Muscarinic receptor and synaptic plasticity in the hippocampus** ..... Jihoon Jo & Gi Hoon Son (Bristol Univ.)

12:20 **Role of kainate receptors and mGluRs in hippocampal mossy fibre LTP : simultaneous 2-photon calcium imaging and electrophysiology** ..... Sheila Dargan (Bristol Univ.)



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Selected Publication

- Jo J. Seok H. Kim MJ. Son GH. Park Y. Henley JM. Weiss JL. Sheng M. Collingridge GL. and Cho K. Metabotropic glutamate receptor-mediated LTD involves two interacting Ca2+ sensors, NCS-1 and PICK1. Neuron. 2008 (In press)
- Clewes O. Fahey MS. Tyler SJ. Watson JL. Seok H. Catania C. Cho K. Dawbarn D. and Allen SJ. Human proNGF: biological effects and binding profiles at TrkA p75NTR and sortilin. J Neurochem 2008 (In press).
- Gilpin H. Whitcomb D. and Cho K. Atypical evening cortisol profile induces visual recognition memory deficit in healthy human subjects. Mol Brain 2008;1:4
- Kim MJ, Futai K, Jo J, Hayashi Y, Cho K, Sheng M. Synaptic accumulation of PSD-95 and synaptic function regulated by phosphorylation of serin-295 of PSD-95. Neuron. 2007;56: 488-502
- Park H., Varadi A., Seok H., Jo J., Gilpin H., Liew C.G., Jung S., Andrews P.W., Molnar E. & Cho K. mGluR5 is involved in dendritic differentiation and excitatory synaptic transmission in NTERA2 human embryonic carcinoma cell-derived neurons. Neurophamacology 2007;52:1403-14.
- Jo J, Ball SM, Seok H, Oh SB, Massey PV, Molnar E, Bashir ZI, Cho K. Experience-dependent modification of mechanisms of long-term depression. Nature Neurosci. 2006;9: 170-2.
- Park Y, Jo J, Isaac JTR, Cho K. Long-term depression of kainate receptor-mediated synaptic transmission. Neuron . 2006;49:95-106.
- Sim JA, Chaumont S, Jo J, Ulmann L, Young M, Cho K, Buell G, North RA Rassendren F. Altered hippocampal synaptic potentiation in P2X4 knock-out mice. J Neurosci. 2006;26:9006-9.
- Harris SL, Cho K, Bashir ZI, Molnar. Metabotropic glutamate receptor signalling in perirhinal cortical neurons. Mol. Cell. Neurosci . 2004;25:275-87.
- Warburton EC, Koder K, Cho K, Massey PV, Duguid G, Baker GRI, Aggleton JP, Bashir ZI, Brown MW. Cholinergic neurotransmission is essential for perirhinal cortical plasticity and recognition memory. Neuron . 2003;38:987-96

Neuronal calcium sensors and synaptic plasticity

Kei Cho

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There are two major forms of long-term depression (LTD) of synaptic transmission in the central nervous system, which require activation of either N-methyl-D-aspartate receptors (NMDARs) or metabotropic glutamate receptors (mGluRs). In synapses in the perirhinal cortex we have directly compared the Ca2+ signalling mechanisms involved in NMDAR-LTD and mGluR-LTD. Whilst both forms of LTD involve Ca2+ release from intracellular stores the Ca2+ sensors involved are different; NMDAR-LTD involves calmodulin, whilst mGluR-LTD involves the neuronal Ca2+ sensor (NCS) protein NCS-1. In addition, there is a specific requirement for IP3 and PKC as well as protein interacting with C-kinase (PICK-1) in mGluR-LTD. NCS-1 binds directly to PICK1, via its BAR domain, in a Ca2+-dependent manner. Furthermore, the NCS-1-PICK1 association is stimulated by activation of mGluRs, but not NMDARs, and introduction of a PICK1 BAR domain fusion protein specifically blocks mGluR-LTD. Thus, NCS-1 is a component of a novel mechanism involved in mGluR-LTD

Metabotropic glutamate receptor-mediated LTD involves two interacting Ca^{2+} sensors, NCS-1 and PICK1.

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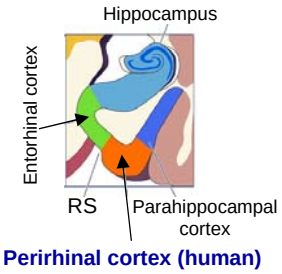
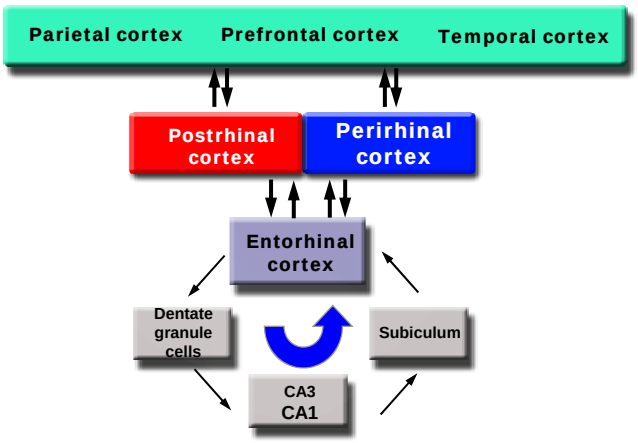
The perirhinal cortex and neuroanatomy



Perirhinal cortex (human)

Coronal view of Rat Brain



Importance of the perirhinal cortex

⏭ Atrophy of perirhinal and entorhinal cortices, the areas affected **earliest** and **most severely** by the abnormal protein fragments (amyloid) (*Baraak & Baraak, 1991; Jutton et al., 1998*)

⏭ Neurofibrillary tangles in the perirhinal cortex is correlated with the severity of Alzheimer's disease (*Blaizot et al., 2002*)

Ca^{2+}-binding protein and synaptic plasticity

Genin et al., (2001) Regulated expression of the **neuronal calcium sensor-1** gene during long-term potentiation in the dentate gyrus in vivo. *Neurosci 106, 571-577*.

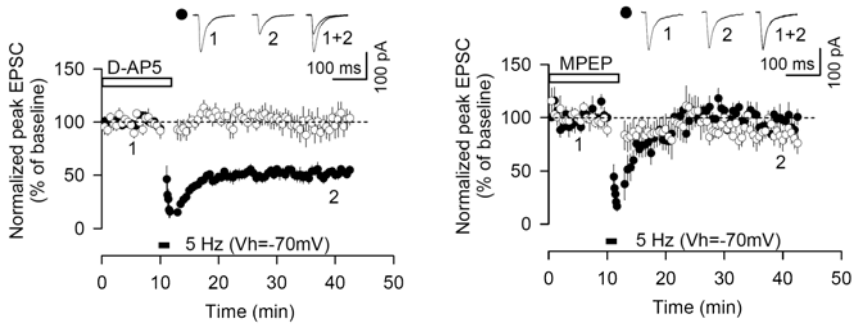
Gomez et al., (2001) Ca^{2+} signalling via the neuronal calcium sensor-1 regulates associative learning and memory in C elegans. *Neuron30, 241-248*.

Pamler et al., (2005) **Hippocalcin** functions as a calcium sensor in hippocampal LTD. *Neuron 47, 487-494*.

Hanley and Henley (2005) **PICK1** is a calcium-sensor for NMDA-induced AMPA receptor trafficking. *Embo J 24, 3266-3278*.

Terashima et al., (2008) An essential role for **PICK1** in NMDA receptor-dependent bidirectional synaptic plasticity. Neuron 57, 872-882.

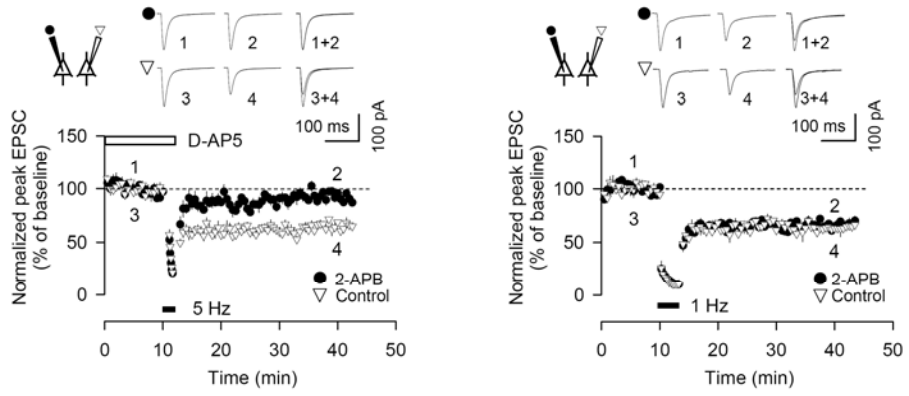
5Hz afferent stimulation induces mGluR-dependent LTD in the perirhinal cortex (P7~P12)



Jo et al., Neuron, In-press, 2008

Distinct role of Ca^{2+} signal in LTD

Dual-patch recordings

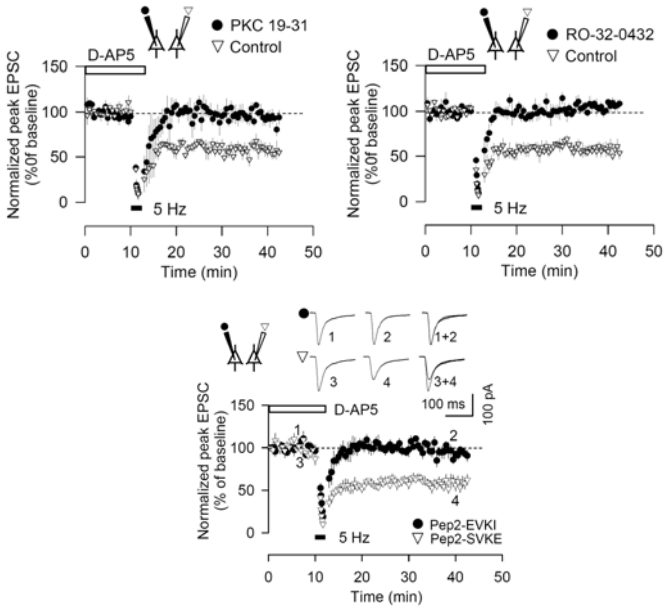


2-APB: IP3 receptor antagonist

Jo et al., Neuron, In-press, 2008

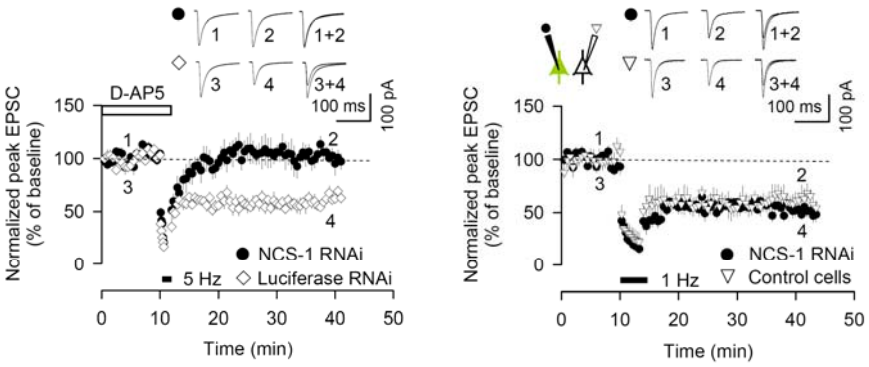
The role of PICK1 in 5 Hz-LTD (mGluR-LTD)

Dual-patch recordings



Jo et al., Neuron, In-press, 2008

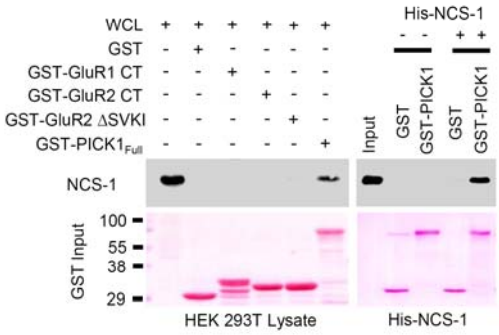
NCS-1 RNAi blocks 5Hz-LTD but has no effect on 1Hz-LTD



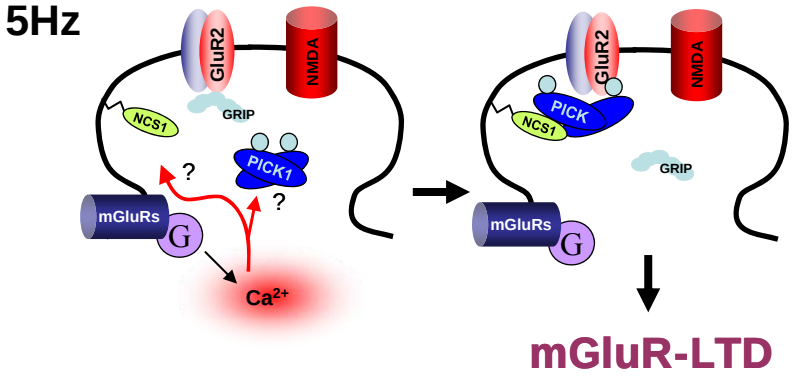
Dual-patch-recording

Jo et al., Neuron, In-press, 2008

NSC-1 associates with PICK1 and this may be vital for mGluR-LTD



Jo et al., Neuron, In-press, 2008



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- MA, Dept. of Zoology, College of Natural Sciences, Seoul Nat'l Univ. (91)
- PhD, Vanderbilt Univ., Nashvile, USA (97)

Fellowship & Employment

- Post-Doc. Fellow, Stanford Univ. (97 ~ 00)
- Assistant Prof., Ewha Womans Univ. (00 ~ 03)
- Associate Prof., Seoul National Univ. (03 ~ present)

Selected Publication

- Kim J et al.. Amygdala depotentiation and fear extinction. Proc Natl Acad Sci USA. 2007;104:20955
- Harata NC et al.. Frequency dependent kinetics and prevalence of kiss-and-run and reuse at at hippocampal synapses studied with novel quenching methods. Neuron. 2006;49:243.
- Choi S, Klingauf J, Tsien RW. Fusion pore modulation as a presynaptic mechanism contributing to expression of long-term potentiation. Philos Trans R Soc Lond B Biol Sci. 2003;358:695. (review)
- Lee SC, Choi S, Lee T, Kim HL, Chin H, Shin HS. Molecular basis of R-type calcium channels in central amygdala neurons of the mouse. Proc Natl Acad Sci USA. 2002;99:3276.
- Choi S, Klingauf J, Tsien RW. Postfusional regulation of cleft glutamate concentration during LTP at 'silent synapses'. Nature Neurosci. 2000;3:330.

Amygdala Depotentiation And Fear Extinction

Sukwoo Choi

School of Biological Sciences, College of Natural Sciences, Seoul National Univ., Seoul, Korea

Background The cortical and thalamic input synapses on the lateral amygdala (LA) carry auditiory information from the auditory cortex and the auditory thalamus onto the LA (1). Long-term potentiation (LTP) requiring the synaptic delivery of AMPA receptors (AMPARs) in these pathways appears to be necessary for establishing consolidated fear memory (2). In the present study we tested the hypothesis that depotentiation of conditioning-induced potentiation at excitatory synapses in the lateral amygdala underlies extinction of consolidated fear memory. Synaptic weights were monitored ex vivo using whole-cell (or field potential) recordings in amydala slices prepared from behavior-trained rats.

Results Fear conditioning resulted in a potentiation in the input-output relation of the excitatory postsynaptic current (EPSC) amplitude at thalamic input synapses onto the LA, consistent with a previous study (3). Extinction of consolidated fear memory produced apparent reversal of the conditioning-induced potentiation. In order to identify cellular mechanisms underlying the extinction-induced reversal of conditioning-induced potentiation, we searched for ex vivo depotentiation; that is where depotentiation stimuli produced reversal of in vivo synaptic potentiation preserved in amygdala slices Ex vivo depotentiation needs to satisfy the following two criteria for it to be a viable mechanism underlying the extinction-induced reversal: 1) depotentiating stimuli should produce synaptic depression in amygdala slices prepared only from fear conditioned rats, but not form naïve and unpaired controls, and 2) the stimulation-induced depression should be lower in extinction-group amygdala slices than in conditioned-group slices, so as to ensure that extinction occludes ex vivo depotentiation. Successful occlusion would be indicate that the above two processese involve the same (or similar mechanisms. In fact, synaptic depression induced either by paired-pulse stimulation or by DHPG application was found to meet the criteria for ex vivo depotentiation. In order to determine whether the DHPG-induced, ex vivo depotentiation is mediated by internalization of GluR2-containing AMPARs, a GluR2-derived peptide blocker (4) for AMPAR internalization, GluR23Y, were used. This peptide impaired the DHPG-induced, ex vivo depotentiation, but a control peptide did not produce any significant effects on the depotentiation. Accordingly, we used a cell permeable form of the peptide, Tat-GluR3Y, to determine whether AMPAR internalization plays a critical role in fear extinction. We performed intracranial microinjection of the peptides or saline into the LA. As predicted, microinjection of GluR23Y into the LA impaired fear extinction as compared with control peptide-injected groups. The attenuating effect of GluR13Y was evident on both short-term and long-term extinction.

Conclusion We have shown that fear extinction results in the reversal of conditioning-induced potentiation that has been consolidated at T-LA synapses. This reversal is mediated by a novel form of depotentiation that depends upon activation of NMDARs and mGluRs. A GluR2-derived peptide, a blocker for regulated AMPAR endocytosis, attenuated both depotentiation and extinction, supporting a link between the two events

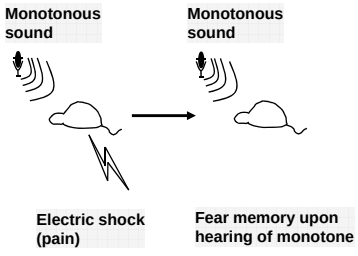
Ref)(1) LeDoux, J.E.(2000) Annual Review of Neuroscience 23, 155-84; (2) Maren, S. (2205) Neuron 47, 783-6; (3)Mckernan, M. G. & Shinnick-Gallagher, P. (1009) Nature 390, 607-11; (4)Ahmadian, G. et al., (2004) EMBO J 23, 1040-50

Amygdala Depotentiation and Fear Extinction

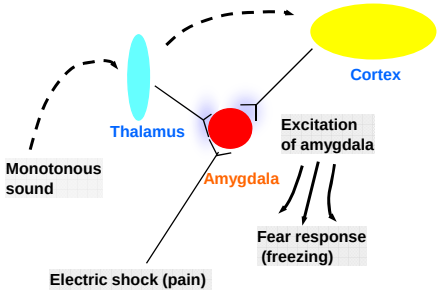
Sukwoo Choi
School of Biological Sciences
Seoul National University

Cellular Mechanism for Fear Conditioning:
Potentiation at Amygdala Synapses

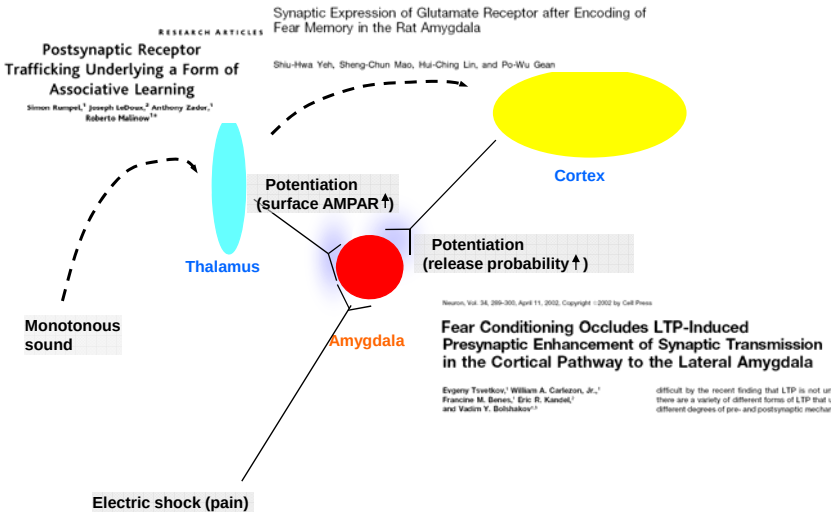
Fear Conditioning



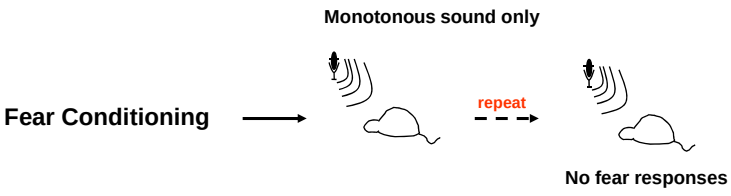
Neural Circuits for Fear Conditioning



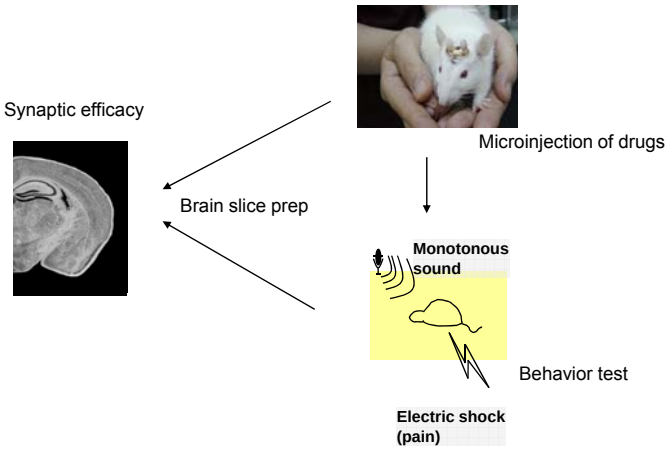
Fear Memory Traces:
Pre vs. Post



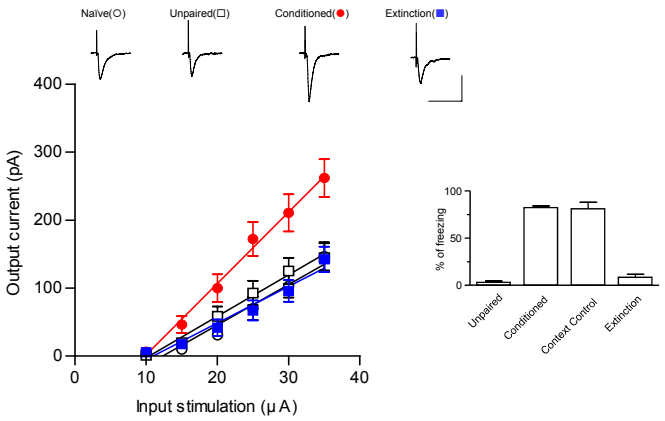
Fear Extinction: Cellular Mechanism?
Inhibition vs. Erasure



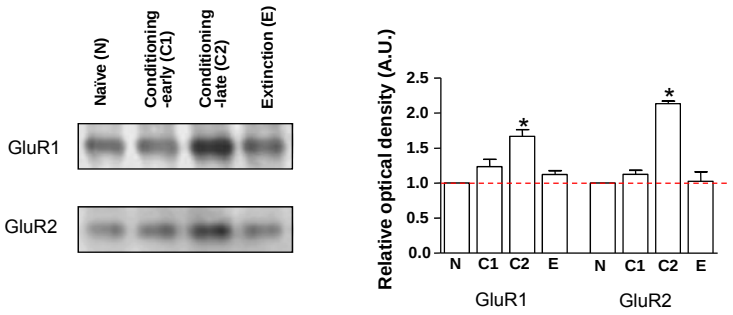
Experimental Schemes: Ex Vivo Approach



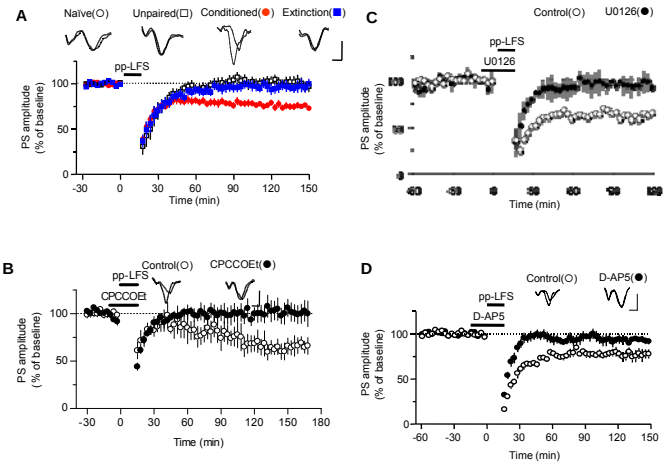
Fear Extinction Results in Apparent Reversal of Conditioning-Induced Potentiation



Fear Extinction Reverses Conditioning-Induced Enhancement of Surface Expression of AMPAR Subunits



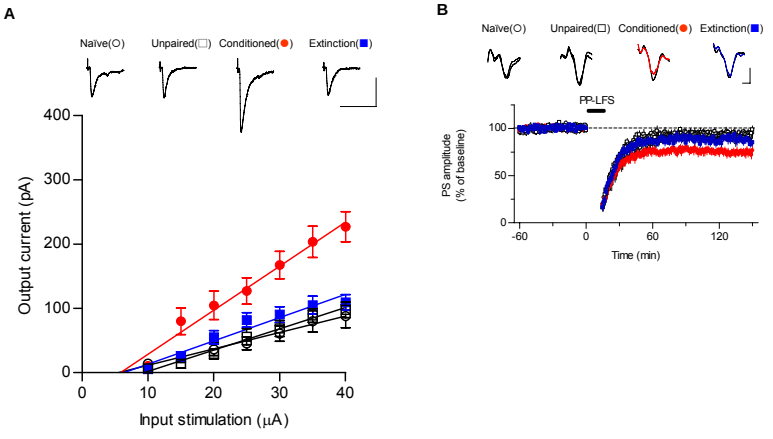
Pp-LFS produces *Ex vivo* Depotentiation at Thalamo-Amygala Synapses



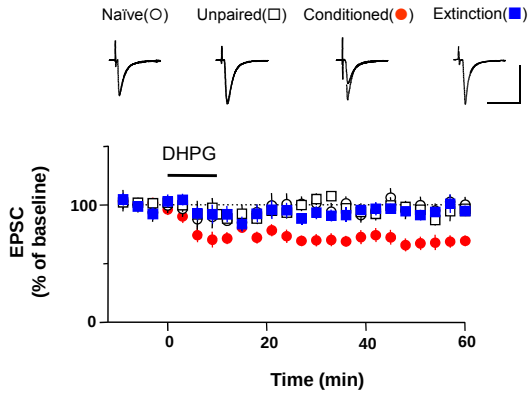
Effects of Drug Manipulations of the Lateral Amygdala on Extinction and Depotentiation

	Extinction	Depotentiation
NMDAR	YES	YES
mGluR1	YES	YES
MAPK	YES	YES (?)
Protein Synthesis	YES	Not determined

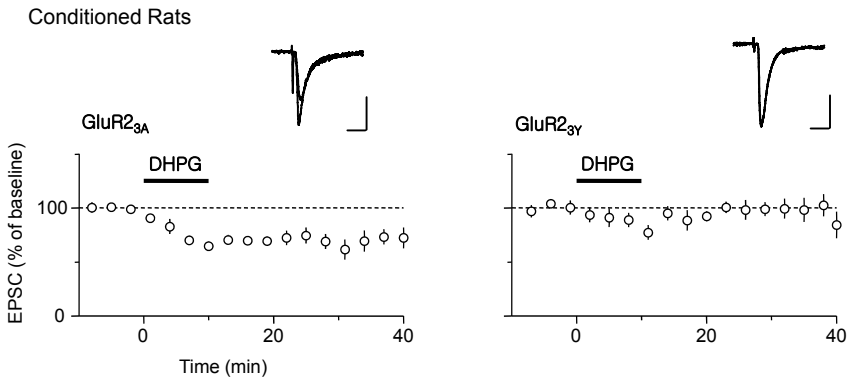
Pp-LFS produces *Ex vivo* Depotentiation at Cortico-Amygala Synapses



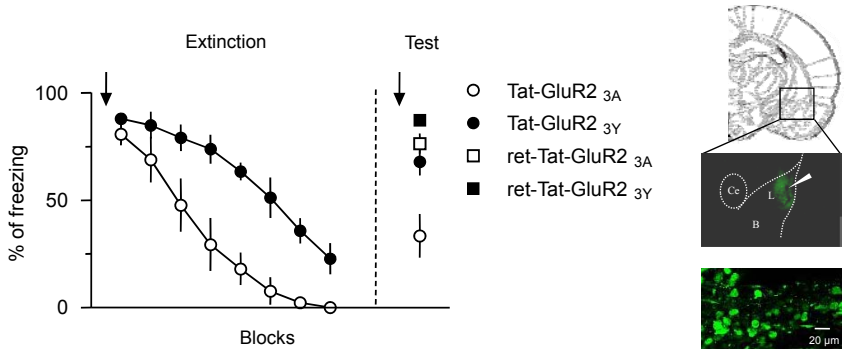
Depotentiation as a mechanism for the extinction-induced reversal of conditioning-induced potentiation



A GluR2-derived Peptide, a Blocker for Regulated AMPAR Endocytosis, Blocks Depotentiation



A GluR2-derived Peptide, a Blocker for Regulated AMPAR Endocytosis, Attenunates Fear Extinction



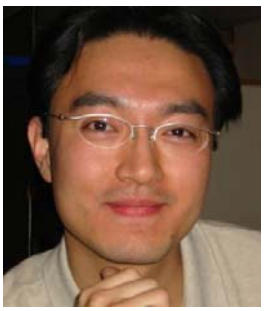
Conclusion

1. **Our findings support cause-and-effect relation between LA depotentiation and extinction.**

2. **AMPAR internalization at LA excitatory synapses appears to underlie fear extinction.**

3. **Strength of LA fear memory traces is correlated with that of conditioned fear**

Jihoon, Jo



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- PhD in Neuroscience, Univ. of Sheffield, UK (06)

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- Research Assistant, Inha Univ., Korea (01~02)
- Research Assistant, the Univ. of Sheffield, UK (05~06)
- Post-Doc. Research Associate, the Univ. of Sheffield, UK (06)
- Tenured Research Fellow, the Univ. of Bristol, UK (06~Present)

Selected Publication

- Jo J, Seok H, Kim MJ, Son GH, Park YK, Jamie W., Jeremy H., Morgan S., G. Collingridge and Cho KW. Differential postsynaptic Ca2+-signaling mechanisms determine NMDA receptor- versus metabotropic glutamate receptor-mediated LTD. Neuron. 2008. (in press)
- Jo J, Simon M Ball, Seok H, Oh SB, Peter V Massey, Elek Molnar, Zafar I Bashir and Cho KW. Experience-dependent modification of mechanisms of long-term depression. Nat Neurosci. 2006;9:170-2.
- Kim MJ, Kensuke F., Jo J, Yasunori H., Cho KW and Morgan Sheng. Synaptic accumulation of PSD-95 and synaptic function regulated by phosphorylation of serine-295 of PSD-95. Neuron. 2007;56:488-502.
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- Park Y, Jo J, John T.R. Isaac and Cho KW. Long-term depression of kainate receptor-mediated synaptic transmission. Neuron. 2006;49:95-106.

Muscarinic receptor and synaptic plasticity in the hippocampus

Jihoon Jo[1,2,*], Gi Hoon Son[1,2], Graham Collingridge[2] and Kwangwook Cho[1,2]

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[2]The MRC Centre for Synaptic Plasticity, Faculty of Medicine and Dentistry, Univ. of Bristol, UK.
[*]Presenting author

Background Synaptic NMDA receptors play a critical role during brain development, synaptic plasticity and neurodegeneration such as Alzheimer's disease. Growing evidences suggested that muscarinic acetylcholine (mACh) receptors are involved in learning and memory and can be targeted to pharmacological therapy for Alzheimer's disease. Activation of mACh receptors by carbachol (CCh) produces long-lasting depression (LLD) of synaptic transmission Analysis of changes in the coefficient of variation of AMPAR EPSCs and NMDAR EPSCs suggests that the mechanisms underlying LTD of AMPAR EPSCs and NMDAR EPSCs differ. However, little is known about the triggering and expression mechanisms that underlie LTD of NMDAR-mediated synaptic transmission. Therefore, this study identifies key aspects of the mechanism of CCh induced LTD in NMDAR-mediated current in the hippocampus.

Results Bath application of CCh (50μM for 10 minutes) induces LTD in NMDA receptor-mediated EPSCs in the CA1 region of the hippocampus. Application of 500nM pirenzepine, a M1 muscarinic receptor antagonist, blocked CCh-induced LTD signifying a role for the M1 mAChR. Postsynaptic inclusion of the Ca2+ chelator BAPTA (10 mM) prevented LTD. However, okadaic acid, which blocks activation of protein phosphatases (PP) 1 and 2a, and cyclosporine A, PP2b (calcineurin) and a PKC inhibitor Ro-32-0432inhibitors had no effect on LTD. Somehow the postsynaptic calcium event involves mAChR-mediated NMDAR-LTD. Therefore, the present study tested whether calcium-sensors are involved this LTD. Calmodulin inhibitory peptide MLCK had no effect on LTD. Next we tested whether hippocalcin, a high-affinity calcium sensor, had a role in LTD. Postsynaptic inclusion of GST-tagged N-terminal fragment of hippocalcin (GST-HIP2-27), which showed a Ca2+-independent binding affinity to their binding partners, prevented LTD, a phenomenon not seen when GST was used alone.

Conclusion These results suggest that activation of mACh receptors by carbachol (CCh) produces LTD in NMDAR-mediated current in the hippocampus and this LTD is M1 muscarinic receptor dependent. Also, this LTD requires postsynaptic Ca2+ and hippocalcin dependent mechanism.

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Education

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- MS, Dept. of Molecular Biology, College of Natural Sciences, Seoul Nat'l Univ. (00)
- PhD, Dept. of Molecular Biology, College of Natural Sciences, Seoul Nat'l Univ. (04)

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- Post-Doc. research associate, HW-LINE, Dept. of Clinical Sciences, Univ. of Bristol, UK (07 ~ present)

Selected Publication

- Jo J, Seok H, Kim MJ, Son GH, Park YK, Jamie W., Jeremy H., Morgan S., G. Collingridge and Cho KW. Differential postsynaptic Ca2+-signaling mechanisms determine NMDA receptor- versus metabotropic glutamate receptor-mediated LTD. Neuron. 2008. (in press)
- Son GH, Chung S, Geum D, Kang SS, Choi WS, Kim K, Choi S. Hyperactivity and alteration of the midbrain dopaminergic system in maternally stressed male mice offspring. Biochem Biophys Res Commun. 2007;352: 823-9.
- Son GH, Geum D, Chung S, Kim EJ, Jo JH, Kim CM, Lee KH, Kim H, Choi S, Kim HT, Lee CJ, Kim K. Maternal stress produces learning deficits associated with impairment of NMDA receptor-mediated synaptic plasticity. J Neurosci. 2006;26: 3309-18.
- Chung S, Son GH, Park SH, Park E, Lee KH, Geum D, Kim K. Differential adaptive responses to chronic stress of maternally stressed male mice offspring. Endocrinology 2005;146:3202-10.
- Son GH, Park E, Jung H, Han J, Lee KH, Seong JY, Kim K. GnRH pre-mRNA splicing: solving the mystery of a nature's knockout, hpg mouse. Biochem Biophys Res Commun. 2005;326: 261-7.

Honors

- Young Investigator's Award of the Korean Society of Endocrinology (06)

Muscarinic receptor and synaptic plasticity in the hippocampus: molecular mechanism underlying a novel form of long-term depression

Gi Hoon Son[1,2*], Jihoon Jo[1, 2], Graham Collingridge[2] and Kwangwook Cho[1, 2]

[1]Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, Faculty of Medicine & Dentistry, University of Bristol, Bristol BS1 3NY, UK;
[2]MRC Centre for Synaptic Plasticity, Department of Anatomy, University of Bristol, Bristol BS8 1TD, UK;
*Presenting author.

Background Muscarinic acetylcholine receptors (mAChR) are heavily involved in learning, memory and synaptic plasticity. Recently, we have shown a novel hippocampal long-term depression (LTD) in NMDA receptor-mediated excitatory postsynaptic currents (EPSCs), which is evoked by M1 mAchR receptor activation and subsequent intracellular Ca2+ rise. In the present study, we intended to elucidate the molecular mechanism underlying this novel form of LTD.

Results At first we examined whether carbachol (CCh), a mAChR agonist-induced LTD in NMDAR-mediated EPSCs is accompanied by receptor internalization. Surface biotinylation and immunoprecipitation experiments revealed that NMDAR subunits were internalized during CCh-evoked LTD; they were dissociated from PSD95 and appeared to be guided to endocytic pathway. It should be noted that M1 receptor-evoked LTD in NMDAR EPSCs was not mediated by well-established Ca2+-depedent pathways for LTD such as calmodulin, PKC and Ca2+-depenent protein phosphatases in spite of its requirement of Ca2+ signaling to suggest the presence of a novel mechanism. Therefore we focused on high sensitive neuronal calcium sensor family of proteins. Among members of this family, hippocalcin and NCS-1 is known to abundantly express in adult hippocampal neurons. Interestingly, hippocalcin, but not NCS-1 associated with both NR1 subunit of NMDAR and PSD95. Further molecular dissection showed that hippocalcin could directly bind to PSD95 via its N-terminal motif. CCh promoted a rapid dissociation of hippocalcin from both NR1 and PSD95, and this could be also inhibited by mAChR antagonist.

Conclusion Together with electrophysiological evidences, our findings strongly suggest that hippocalcin contributes to the stabilization of synaptic NMDAR complex at basal state, and then its dissociation can serve as a molecular switch to initiate mAChR-evoked NMDAR internalization.

Sheila Dargan

- Research Associate , MRC Centre for Synaptic Plasticity, The Dept. of Anatomy School of Medical Science

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Education

- PhD in Biophysics in labs of Prof. Alan Dawson / Edward Lea. Univ. of East Anglia, Norwich, UK. (01)

Fellowship & Membership

- Research Assistant in lab of Prof. Ian Parker. Dept. Neurobiology, Univ. of California, Irvine, CA, USA (02 ~ 04)
- Research Associate in labs of Prof. Graham Collingridge / David Jane. MRC Centre for Synaptic Plasticity, Univ. of Bristol, UK (04 ~ present)
- PENS school (2008) 26/108 applicants: in vivo 2-photon imaging (Carl Petersen) and Transmission Electron Microscopy (Graham Knott), Lausanne, Switzerland. (08)
- Education committee member, Biochemical Society (06 ~ 08)
- Travel Grant review panel, Biochemical Society (06 ~ 08)
- Chair, Univ. of Bristol's Research Staff Committee (07 ~ 08)
- Scientific meeting organization committees: USA (03) and UK (07, 08).

Selected Publication

- Dargan SL, Clarke VR.J, et al. ACET is a highly potent and specific kainate receptor antagonist: Characterisation and effects on hippocampal mossy fibre function. Neuropharmacology. 2008. (Epub)
- Bartlett TE, Bannister NJ, Collett VJ, Dargan SL, et al. Differential roles of NR2A and NR2B-containing NMDA receptors in LTP and LTD in the CA1 region of two-week old rat hippocampus. Neuropharmacology. 2007;52(1):60-70

Honors

- Young neuroscientists day, Bristol (07) *Invited theme speaker and *Poster Prize
- Life Sciences and YLS, Glasgow (07) *Selected oral communication *Poster Prize
- Calcium workshop. Chile (03) *Won NIH travel fellowship
- Gordon Conference, South Hadley, MA, USA (03) * selected abstract

Two-photon calcium imaging: Shedding light on the roles of pre-synaptic kainate and metabotropic glutamate receptors in hippocampal mossy fibre LTP

Sheila Dargan, David Jane and Graham Collingridge

MRC Centre for Synaptic Plasticity, University of Bristol, UK

Long-term potentiation (LTP) is a well-established experimental model used to investigate the synaptic basis of learning and memory. Although several candidate mechanisms have been proposed for LTP induction, frequency facilitation and pre-synaptic calcium signalling at mossy fibre - CA3 synapses in the hippocampus, these processes remain poorly understood. We are currently combining 2-photon microscopy, patch-clamp electrophysiology and pre-synaptic calcium imaging to investigate the role of kainate receptors, metabotropic glutamate receptors (mGluRs) and intracellular calcium stores at individual pre-synaptic terminals. In agreement with Scott et al. (Proc Physiol Soc, 2006) we find that short-term facilitation of pre-synaptic calcium transients following repetitive spikes is reduced in the presence of kainate receptor antagonists, consistent with previous electrophysiological data (Lauri et al, Neuron, 2003). Recent findings from field potential recordings performed in our lab suggest that group 1 mGluRs and GluK1-containing kainate receptors play a synergistic role in mediating LTP induction (Nistico et al., unpublished). Our 2-photon imaging experiments show that this novel form of LTP requires calcium release from intracellular stores and may manifest itself as a sustained rise in basal calcium within the pre-synaptic terminal.

Session Ⅲ : Hormone and Brain Functions

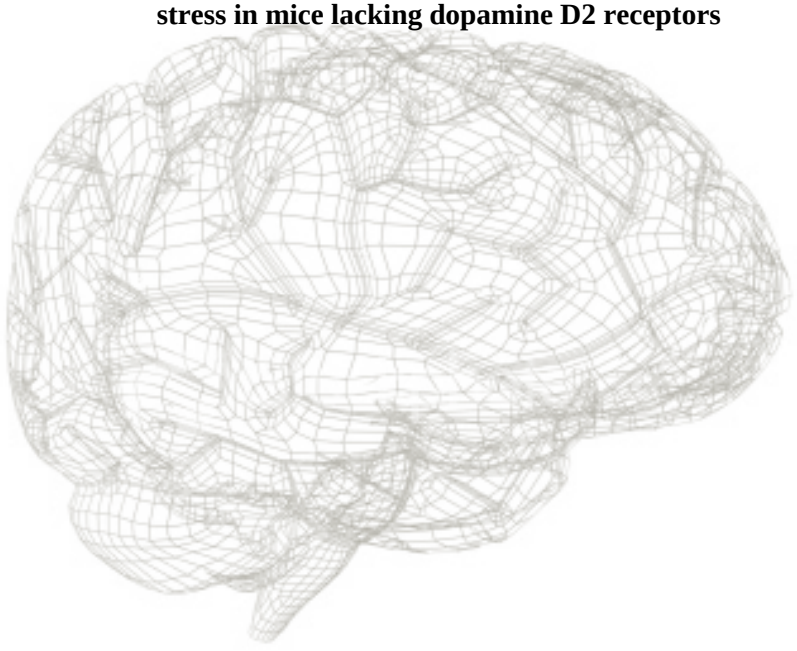
Chair : Graham Collingridge, FRS

14:00 **Circadian clocks throughout the brain** ..... Hugh Piggins (Manchester Univ.)

14:25 **Adrenal peripheral clock in generating the circadian rhythm of glucocorticoid** ..... Kyungjin Kim (Seoul National Univ.)

14:50 **Rapid glucocorticoid signalling in the brain** ..... Stafford Lightman (Bristol Univ.)

15:15 **Increased sensitivity and anxiety-like behaviors upon chronic stress in mice lacking dopamine D2 receptors** ..... Ja-Hyun Baik (Korea Univ.)



Hugh David Piggins

- Prof., Faculty of Life Sciences,
  Univ. of Manchester, UK

Contact

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Education

- BSc (Hons-Upper Second) in Psychology, Univ. of Edinburgh, Edinburgh, Scotland (85)
- PhD in Experimental Psychology, Univ. of Ottawa, Ottawa, Canada (91)

Fellowship & Employment

- Province of Ontario Postgraduate Scholarship (86 ~ 88)
- Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarship (88 ~ 90)
- Medical Research Council of Canada Doctoral Studentship (90 ~ 91)
- Natural Sciences and Engineering Research Council of Canada Post-doc. Fellowship (91 ~ 93)
- Medical Research Council of Canada Post-doc. Fellowship (93 ~ 96)

Selected Publication

- Brown T.M. and Piggins, H.D. Spatiotemporal heterogeneity in the electrical activity of suprachiasmatic nuclei neurons and their response to photoperiod. J Biol Rhyth. 2009. (In press)
- Brown T.M., Coogan, A.N., Cutler, D.J., Hughes, A.T., and Piggins, H.D. Electrophysiological actions of orexins on rat suprachiasmatic neurons in vitro. Neurosci Lett. 2009. (In press)
- Hughes, A.T., Guilding, C., Lennox, L., Samuels, R.E., McMahon, D.G., and Piggins, H.D. Live imaging of altered period 1 expression in the suprachiasmatic nuclei Vipr2-/- mice. J Neurochem. 2008;106:1646-57
- Hughes, A.T. and Piggins, H.D. Behavioral responses of Vipr2-/- mice to light. J Biol Rhyth. 2008;23: 211-9
- Brown, T.M., Mclachlan, E., and Piggins, H.D. Angiotensin II regulates the activity of mouse suprachiasmatic nuclei neurons. Neuroscience. 2008;154: 839-47.

Are circadian clocks present throughout the brain?

Hugh D. Piggins

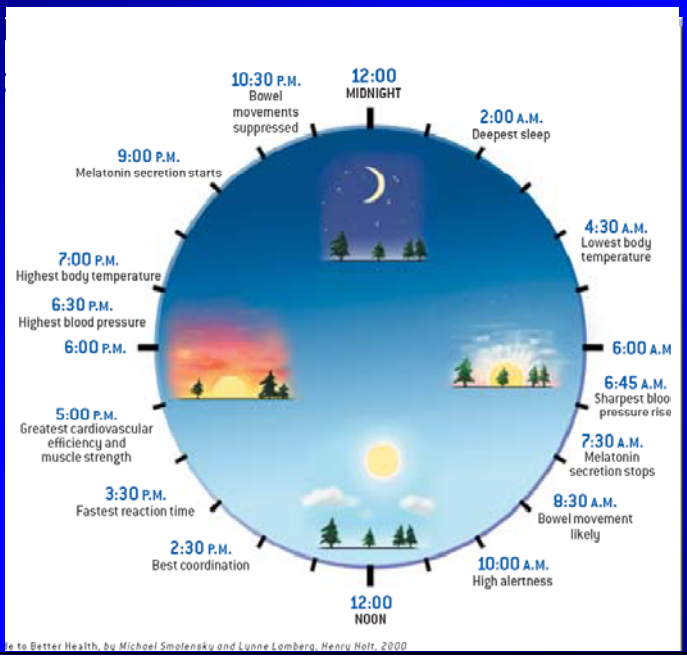
Faculty of Life Sciences, University of Manchester, Manchester, UK

Endogenous near 24h (circadian) rhythms pervade all aspects of our physiology and behaviour. Until recently, it was thought that these rhythms were generated exclusively by a master circadian clock in the suprachiasmatic nuclei (SCN) of the hypothalamus. However, with identification of the molecular basis of the SCN clock ('clock' genes such as Per1, Per2, Cry1, etc) it was determined that clock gene expression is widespread in the brain. At present it is unclear if extra-SCN neurons possess robust circadian clock properties. Through photovideomicroscopy and luminometry, we are exploring this in brain slices from the Per2Luc mouse in which PER2 protein expression is reported by luciferase (PER2::LUC bioluminescence). Circadian oscillations in PER2::LUC bioluminescence are detectable in a number of brain regions including the amygdala, habenula, and mediobasal hypothalamus (MBH). These oscillations are readily resolved to individual cells in the MBH, but unlike SCN neurons, MBH cell oscillators fail to sustain synchronized PER2::LUC expression and instead rapidly drift out of phase with one another, resulting in damped tissue-wide oscillations. We conclude that some extra-SCN brain sites do indeed possess single cell circadian oscillators; however, they lack key synchronisation properties of SCN neurons. Supported by the BBSRC.

Are Circadian Clocks Present Throughout the Brain?

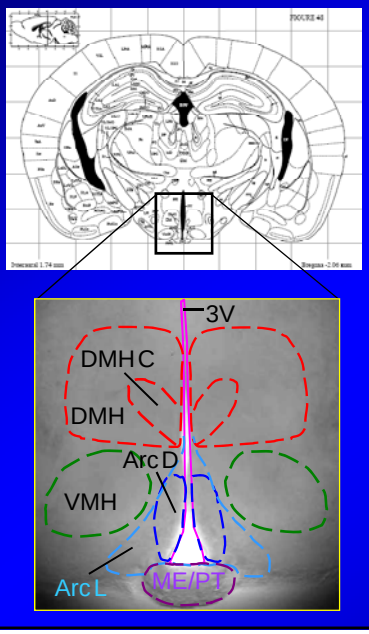
Hugh Piggins

Circadian rhythms pervade mammalian physiology



e to Better Health, by Michael Smolensky and Lynne Lamberg, Henry Holt, 2000

The mediobasal hypothalamus (MBH)



Includes:

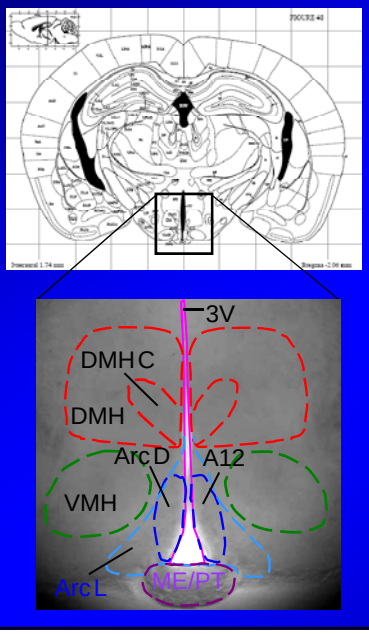
- Arcuate nuclei (Arc)
- Dorsomedial nuclei (DMH)
- Ventromedial nuclei (VMH)
- Median eminence/ Pars tuberalis (ME/PT)

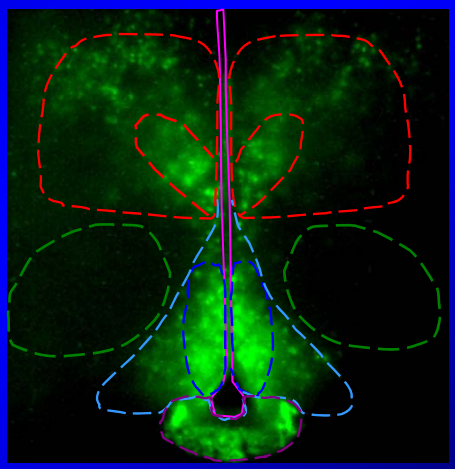
Functions:

Serves as the bodies main regulator of internal homeostasis.

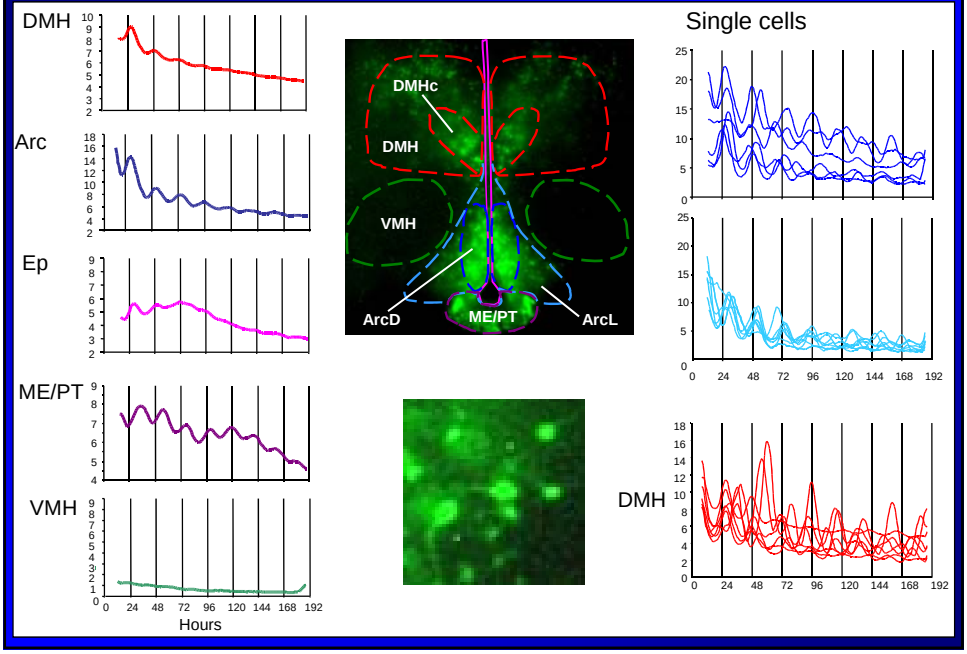
- appetite control
- reproduction

Clocks in the mediobasal hypothalamus?

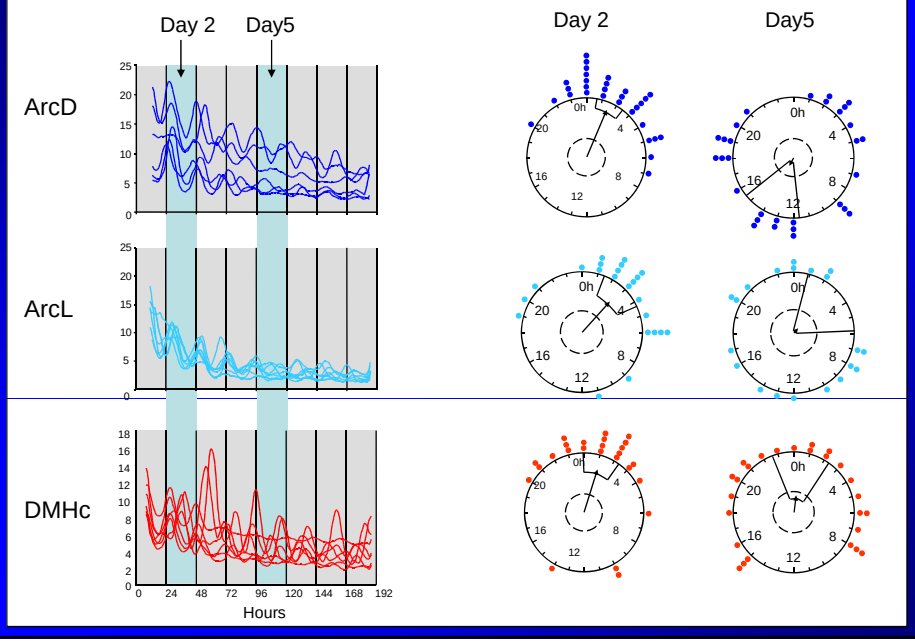




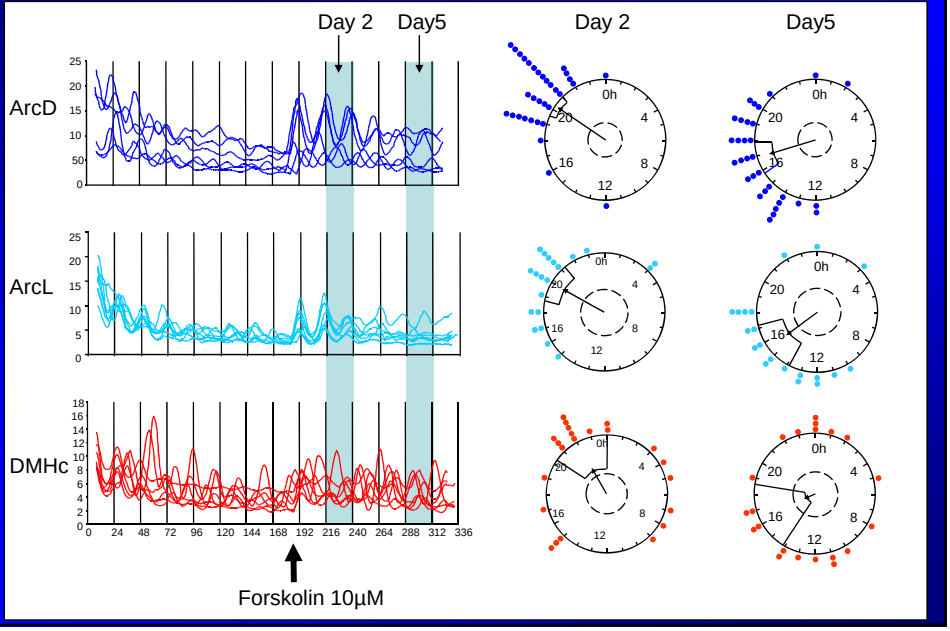
Circadian rhythms of PER2 in single cells of Arc and DMH



Cells become desynchronised by 5 days after culture



Forskolin resynchronises cells and strengthens rhythms



Conclusions

A hierarchy of circadian oscillators exist in the MBH

ME/PT expresses robust circadian rhythms in PER2

Arc expresses circadian rhythms in PER2 and cell firing

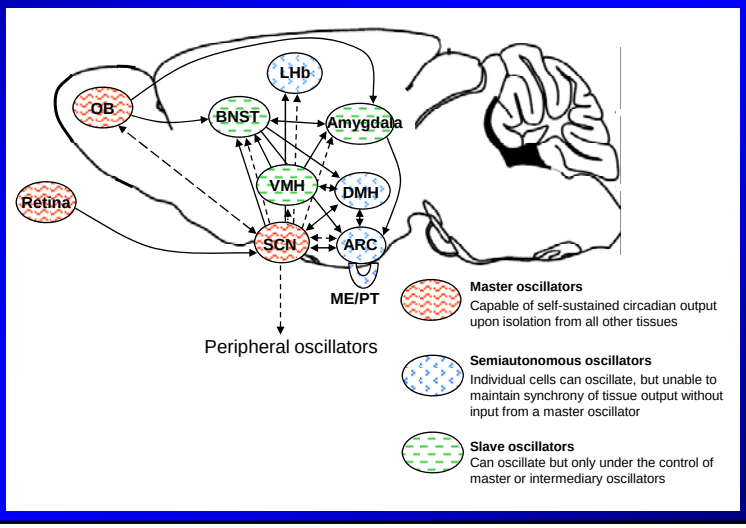
DMH expresses circadian rhythms in PER2 and cell firing

VMH does not expresses circadian rhythms in PER2 and cell firing

Ependymal cell layer expresses circadian rhythms in PER2

Conclusions

An array of circadian oscillators exist in the MBH



Acknowledgements

The Piggins Lab

Clare Guilding
Alun Hughes
Tim Brown
Mino Belle
Robert Bendall
Sara Namvar
Andrea Power
Rayna Samuels
Fiona Scott

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Education

- BS, Magma Cum Laude, Dept. of Zoology, Seoul Nat'l Univ. (75)
- MS, graduate School (Developmental Biology), Seoul Nat'l Univ. (79)
- PhD, Dept. of Physiology & Biophysics, Univ. of Illinois (84)

Fellowship & Employment

- Postdoctoral Fellow, Univ. of Illinois (84-85)
- Postdoctoral Fellow, Columbia Univ.(85)
- Alexander von Humboldt Fellow (Univ. of Goettingen) (92-93)
- Assistant, Associate and Full Professor at Seoul Nat'l Univ. (85-Present)

Selected Publication

- Park E, Han J, Son GH, Lee MS, Chung S. Park SH, Park K, Lee KH, Cho S, Seong JY Kim K. Cooperative actions of tra2alpha with 9G8 and SRp30c in the RNA splicing of the gonadotropin-releasing hormone gene transcript. J Biol Chem. 2006;281:401-9.
- Son GH, Geum D, Son GH, Chung S, Kim EJ, Jo J-H, Kim C-M, Lee KH, Kim H, Choi S, Kim HT, Lee CJ, Kim K Maternal. stress produces learning deficits associated with impairment of NMDA receptor-mediated synaptic plasticity. J Neurosci. 2006;27:3309-18.
- Kwon I, Lee J, Chang SH, Jung NC, Lee BJ, Son GH, Lee KH, Kim K. BMAL1 shuttling controls transactivation and degradation of the CLOCK/BMAL1 heterodimer. Mol Cell Biol. 2006;26:7318-30.
- Shim HS, Kim H, Lee J, Son GH, Cho S, Og TH, Kang SH, Seen DS, Lee KH, Kim K. Rapid activation of CLOCK by Ca+2-dependent protein kinaseC mediates resetting of the mammalian circadian clock. EMBO Rept. 2007;8:366-71.
- Lee J,Lee Y, Lee MJ, Park E, Kang SW, Chung CH, Lee KH, Kim K Dual modification by SUMO2/3 and ubiquitin promotes circadian activation of the CLOCK/BMAL1 complex. Mol Cell Biol 2008;28:6056-65.

Adrenal Peripheral Clock in Generating the Circadian Rhythm of Glucocorticoid

Sooyoung Chung, Gi Hoon Son and **Kyungjin Kim**

Department of Biological Sciences, Brain Research Center, 21st Frontier Program in Neuroscience, Seoul National University, Seoul 151-742, Korea

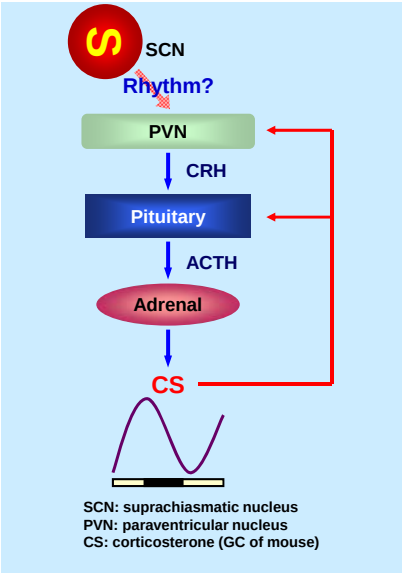
Glucocorticoid (GC) is an adrenal steroid with diverse physiological and behavioral effects including gluconeogenesis, lipid metabolism, immune function, and stress response. This blood-borne signal undergoes a robust daily oscillation, which has been thought to be driven by the master circadian clock in the suprachiasmatic nucleus (SCN) via the hypothalamus-pituitary-adrenal (HPA) axis. However, the source of the inherent rhythm of GC synthesis and secretion from adrenal gland is a long-standing issue which remains yet unresolved. We provide strong evidence showing that the peripheral oscillator intrinsic to the adrenocortical GC-producing cells is prerequisite for the generation of the robust circadian GC profiles by causing rhythmic steroid production. In this process, the steroidogenic acute-regulatory protein(StAR) links the molecular clock and steroidogenesis as a clock-controlled gene. Examination of mice with adrenal-specific knockdown of BMAL1 protein revealed that the adrenal clock machinery is required for rhythmic GC production. Furthermore, behavioral consequences are drastically affected in these animals, together with altered expression of Period1 in several peripheral organs. We conclude that the adrenal peripheral clock plays an essential role in harmonizing the circadian timing system by producing a robust GC rhythm.

Adrenal Peripheral Oscillator in Generating the Circadian Rhythm of Glucocorticoid

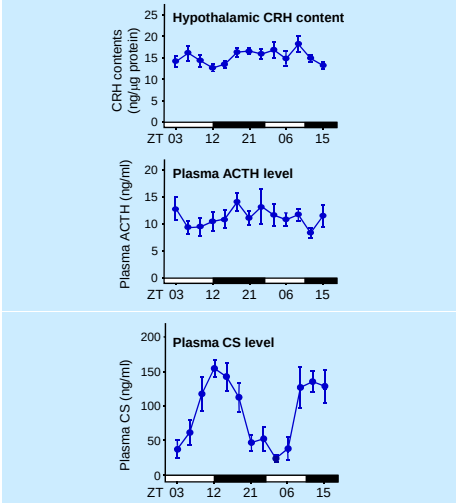
Kyungjin Kim

Department of Biological Sciences, Seoul National University;
21st Frontier Program in Neuroscience, Seoul, KOREA

Circadian rhythm of GC

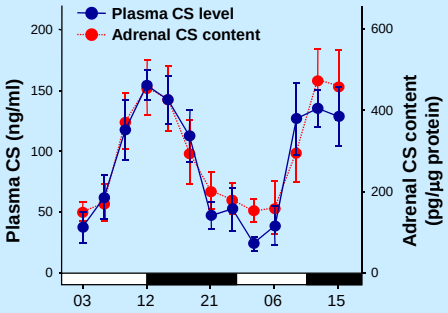


■ Daily rhythm of HPA-axis



Daily rhythm of adrenal CS biosynthesis

■ Circadian rhythms of CS content in adrenal gland

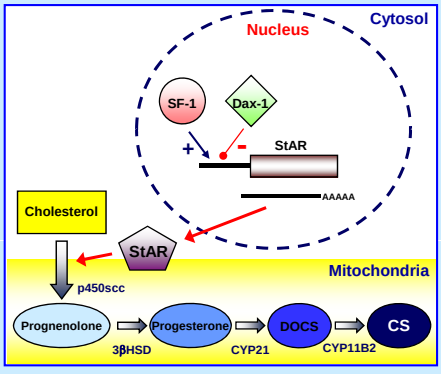


Questions

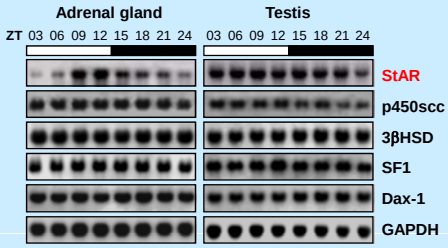
- What is the adrenal-specific factor responsible for oscillatory CS biosynthesis?
- Is the expression of this factor dependent on peripheral clock machinery?

Circadian rhythm of StAR gene expression

■ Steroidogenesis in the adrenal gland



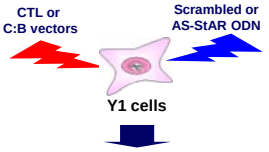
■ mRNA levels of steroidogenic genes



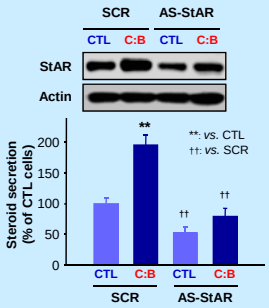
SF-1 : Steroidogenic factor-1
Dax-1 : Dosage-sensitive sex reversal adrenal hypoplasia congenita, critical region on the X chromosome gene-1
StAR : Steroidogenic acute regulatory protein
p450scc : Side-chain cleavage enzyme
3βHSD : 3β-hydroxysteroid dehydrogenase
CYP11B2: Cytochrome P450, subfamily 11B2
CYP21: Cytochrome P450, subfamily 21 (21-hydroxylase)

StAR: a link between steroidogenesis and clockworks

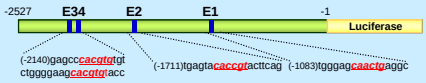
Experimental scheme



- StAR protein: immunoblotting
- Steroids production



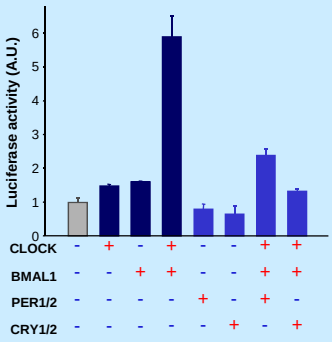
StAR promoter (E-box: CANNTG)



(-2140)gagcc**cacgtg**tgt
ctggggaag**cacgtg**tacc

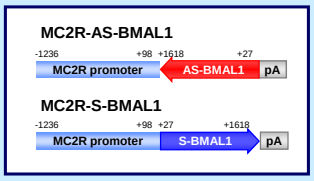
(-1711)tgagta**cacctg**acttcag (-1083)tgggag**caactg**aggc

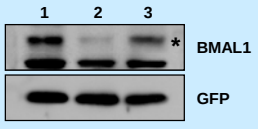
Luciferase assay



Disruption of clock machinery by MC2R-AS-BMAL1 *in vitro*

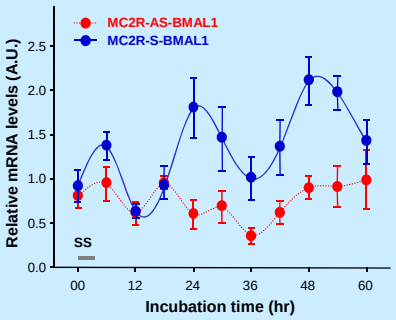
BMAL1 knock-down in Y1 cells





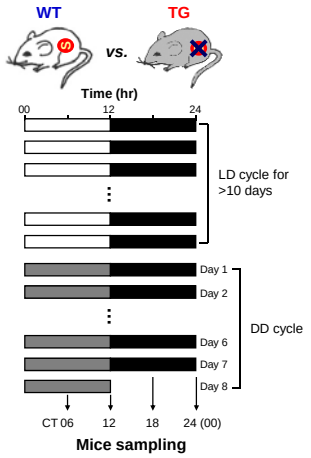
1. pcDNA
2. MC2R-AS-BMAL1
3. MC2R-S-BMAL1

StAR mRNA levels

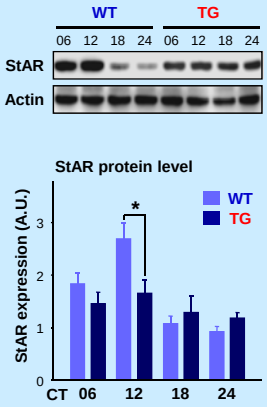


Arrhythmic CS production and dampened secretion

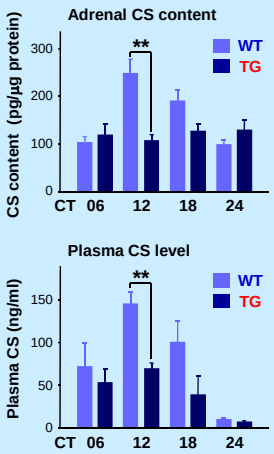
Experimental scheme



Adrenal StAR expression

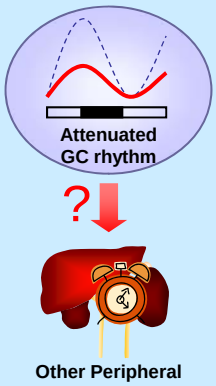


CS level



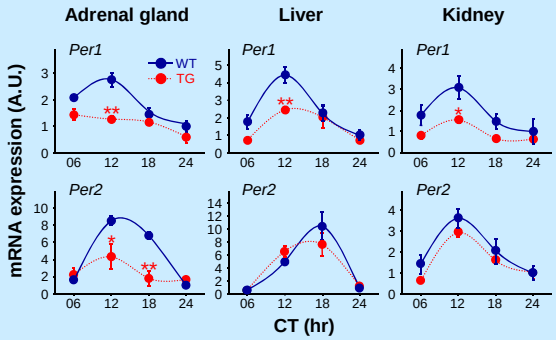
Dampened *Per1* expression in peripheral organs

Question



Per1 and Per2 mRNA expression

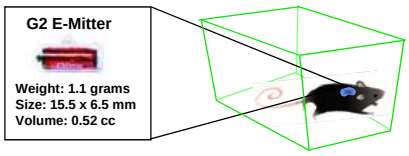
: Real-time RT-PCR



• Per1 but not Per2 gene sequences contains functional GRE element (Yamamoto *et al.*, 2005, J Biol Chem 280: 42036)

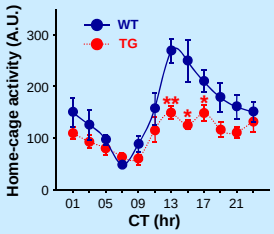
Attenuated behavioral rhythm in the TG mice

Measuring locomotor activity

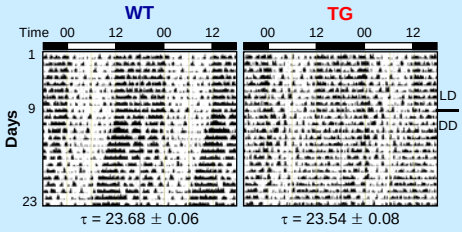


Monitoring behavioral rhythms

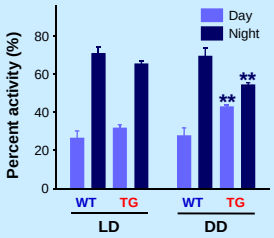
Home-cage activity profile



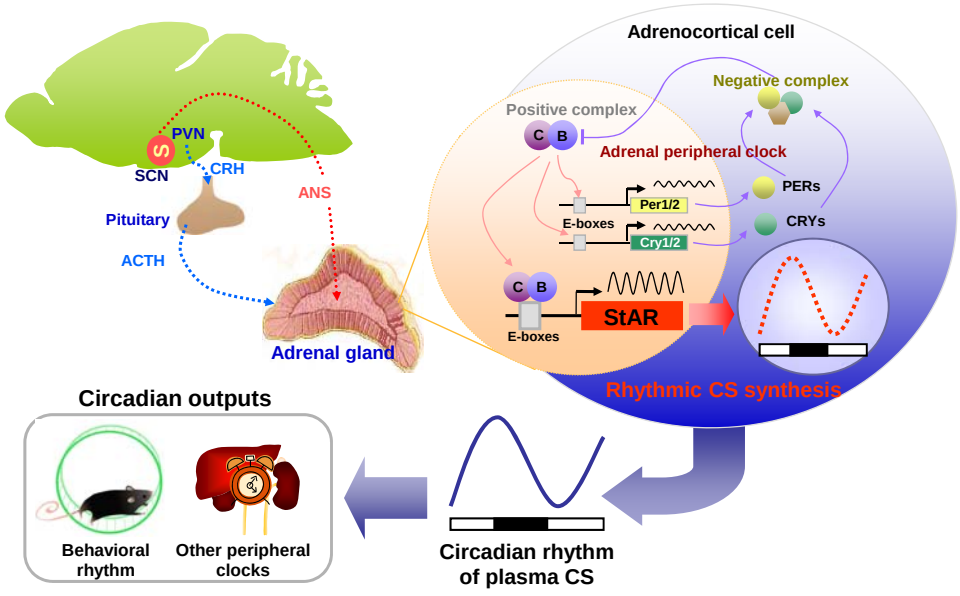
Actogram



Day/night difference



Hypothetical Model



Circadian outputs

Behavioral rhythm

Other peripheral clocks

Circadian rhythm of plasma CS

Stafford Lightman

- Prof. of Medicine, Henry Wellcome LINE
  Faculty of Medicine and Dentistry,
  the Univ. of Bristol

Contact

- E-mail : Stafford.Lightman@bristol.ac.uk

Education

- Qualified in Medicine in Cambridge (72)
- PhD, The University of Cambridge (84)

Fellowship & Employment & Membership

- Visiting Senior Scientist, Medical Research Council Neuro-Pharmacy Unit, Cambridge (80 ~ 81)
- Wellcome Trust Senior Lecturer, St. Mary's Hospital Medical School and Honorary Consultant Physician and Endocrinologist, St Mary's Hospital (81 ~ 82)
- Charing Cross and Westminster Medical School : Reader in Medicine (82 ~ 88)
  Prof. of Clinical Neuroendocrinology, Consultant Physician and Endocrinologist (88 ~ 92)
- Honorary Senior Research Fellow, Institute of Neurology and Consultant Endocrinologist to the National Hospital for Neurology and Neurosurgery (88 ~ Present)
- Editor-in-Chief, Journal of Neuroendocrinology (89 ~ 96)
- Chairman, Pituitary Foundation (95 ~ Present)
- Founder FMedSci (98 ~ present)

Selected Publication

- Spiga F, Harrison L, Wood S, Knight D, Macsweeney C, Thomson F, Craighead M, Lightman S. Blockade of the V1b receptor reduces ACTH, but not corticosterone, secretion induced by stress without effecting basal HPA axis activity. J Endocrinol. 2008. (e-Pub)
- Atkinson HC, Wood SA, Castrique ES, Kershaw YM, Wiles CC, Lightman SL. Corticosteroids mediate fast feedback of the rat hypothalamic-pituitary-adrenal axis via the mineralocorticoid receptor. Am J Physiol Endocrinol Metab. 2008 ;294(6):E1011-22.

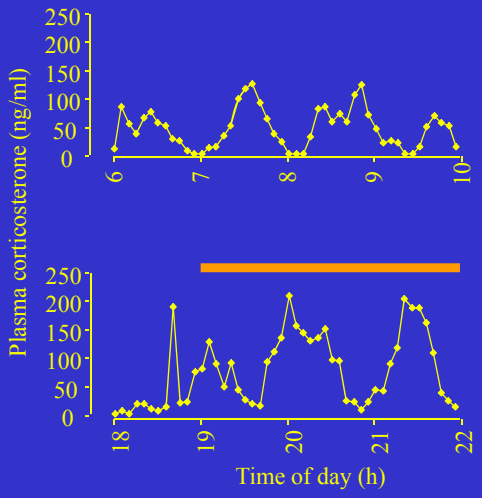
Rapid Glucocorticoid Signaling in the Brain

Stafford Lightman

Henry Wellcome LINE, University of Bristol, UK

The cell biology of both prokaryotic and eukaryotic organisms is characterised by stochastic processes which create phasic activation of biological processes. These processes become increasingly organised and more complex in multicellular animals and in mammals we find whole body rhythms of hormone activity, basal body temperature and of course wakening and sleeping. The stress responsive hypothalamo-pituitary-adrenal (HPA) axis is a perfect example of an environmentally entrained system upon which can be superimposed acute responses to external and internal stressors. We have demonstrated that the well characterised circadian rhythm of the HPA axis is actually made up of multiple circhoral pulses of glucocorticoid secretion. This ultradian rhythm of corticosterone secretion is not only found in the plasma but is also found in individual tissues such as the brain, where we measure it by intracranial microdialysis. At the cellular level, each pulse of glucocorticoid results in the rapid translocation of glucorticoid receptors from the cytoplasm to the nucleus, binding to glucocorticoid response elements on the DNA and initiation of HnRNA transcription. The frequency of ultradian pulses regulates differential activation of glucocorticoid (GR) and mineralocorticoid (MR) receptors – with responses to stress resulting in a prolonged activation of both MR and GR. We have evidence for a rapid intranuclear shuttling of GRs which provides scope for digital signalling in response to different patterns of basal and stress induced activity of the HPA axis.

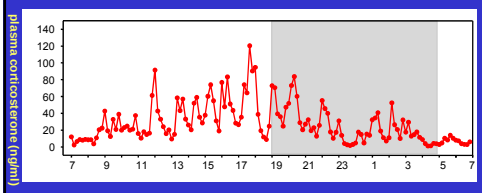
FEMALE SPRAGUE DAWLEY (5 MIN SAMPLING)



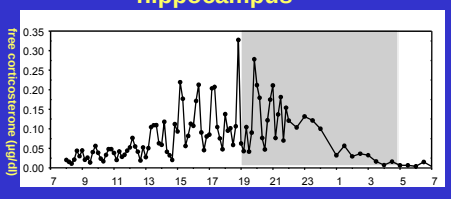
Is the pulsatile pattern synchronized between plasma and brain?

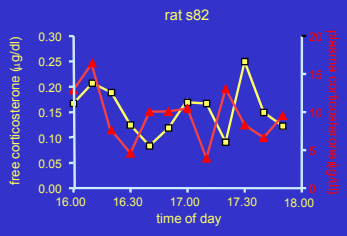
1. baseline

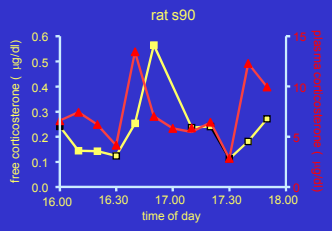
blood



hippocampus

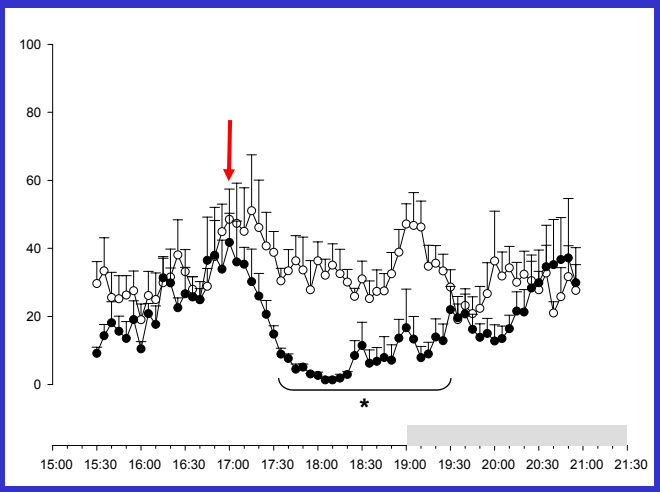






Droste, de Groote, Atkinson, Lightman, Reul, Linthorst, in prep 2007

Effect of methylprednisolone on basal corticosterone release

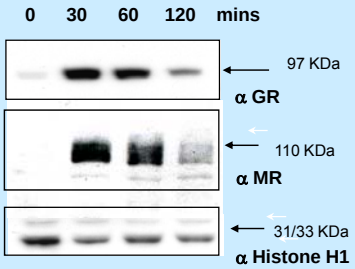


Saline (0.1ml, iv)
Methylprednisolone (50 µg/0.1ml, iv)

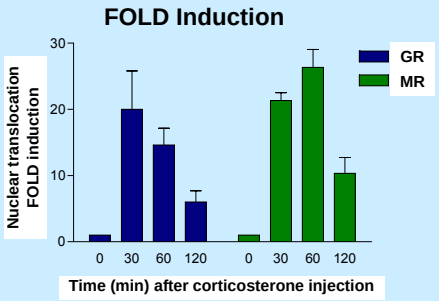
*Significant difference respect saline; $P \leq 0.05$.

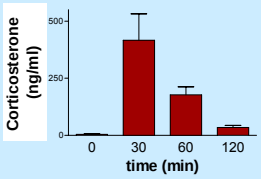
Spiga et al

GR and MR nuclear translocation in the HC after 1 bolus IP injection of corticosterone



20 µg NE/lane

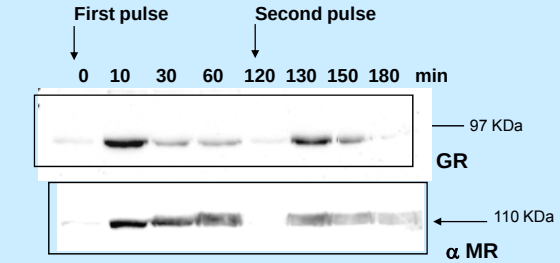


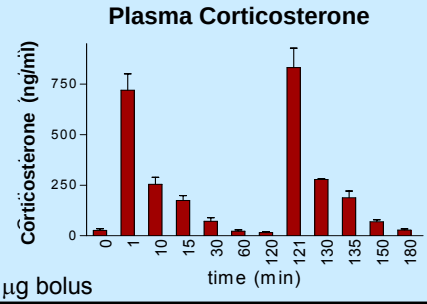


B = 1 mg bolus

B.L. Conway-Campbell

Two corticosterone "pulses" via IV injection

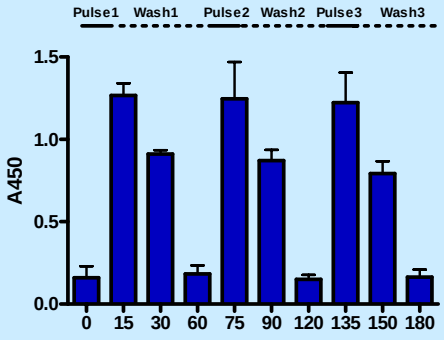




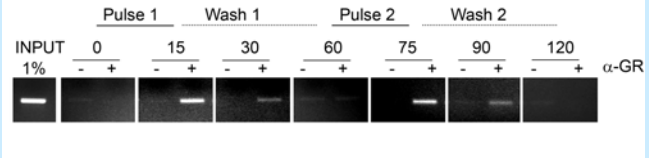
B = 2 X 100 μg bolus

B.L. Conway-Campbell

GR binding to GRE during pulsatile corticosterone treatment (100nM) in atT20 cells



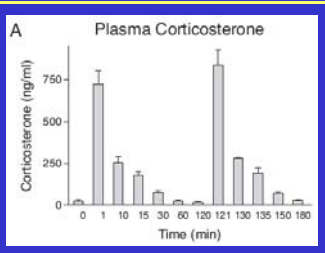
Timecourse:15min pulse every hour.

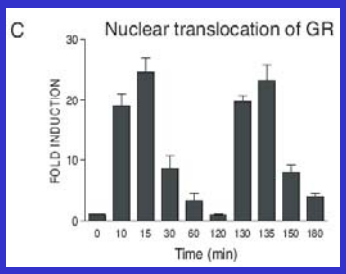


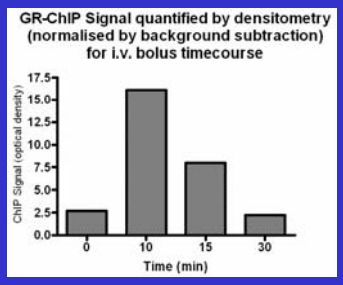
GR ChIP with GRE in mus MT1 promoter in atT20 cells

- John Pooley

Dynamics of GR Recruitment – IV Injection

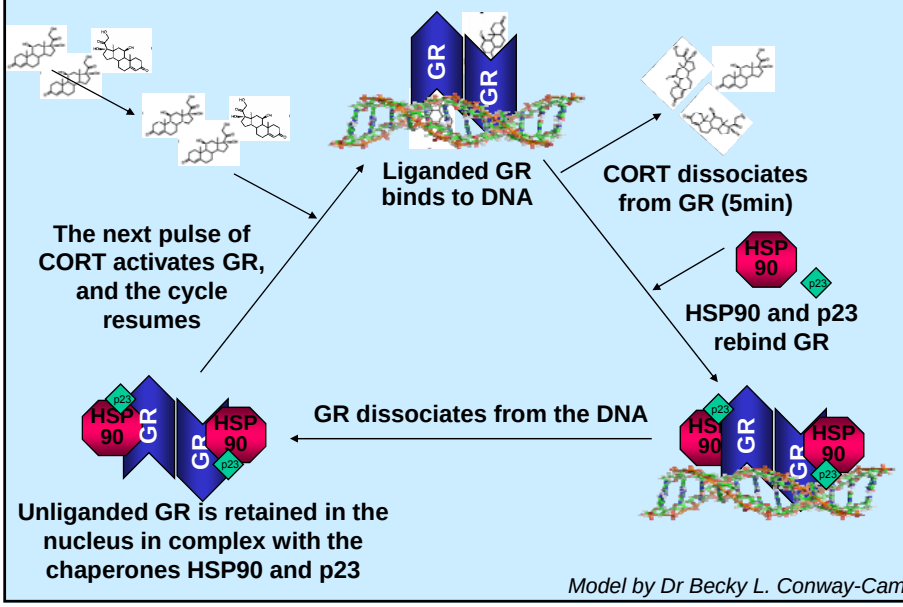






- John Pooley

Intra-nuclear cycle of GR activation



Model by Dr Becky L. Conway-Campbell

Ja-Hyun, Baik

- Prof. of School of Life Sciences and Biotechnology, Korea Univ.

Contact

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Education

- BS in Biochemistry, Dept. of Biochemistry, Yonsei Univ., Seoul, Korea (85)
- MS in Biochemistry, University Paris 6, Paris, France (87)
- PhD in Molecular and Cellular Pharmacology, Univ. de Paris VI (Pierre et Marie Curie), Paris, France (92)

Fellowship & Employment

- Post-Doc. fellow, IGBMC (Institut de Genetique et de Biologie Moleculaire et Cellulaire, Director: Dr. Pierre Chambon; Supervisor, Dr. Emiliana Borrelli), CNRS, Strasbourg, France (92 ~ 95)
- Assistant Prof., Medical Research Center, College of Medicine, Yonsei Univ. (96 ~ 02)
- Associate Prof., Medical Research Center, College of Medicine, Yonsei Univ. (02 ~ 03)
- Associate Prof., School of Life Sciences and Biotechnology, Korea Univ. (03 ~ 06)
- Full Prof., School of Life Sciences and Biotechnology, Korea Univ. (06 ~ present)

Selected Publication

- Kim SY, Lee HJ, Kim YN, Yoon S, Lee JE, Sun W, Choi EJ, Baik J-H. Striatal-enriched protein tyrosine phosphatase regulates dopaminergic neuronal development via extracellular signal-regulated kinase signaling. Exp. Neurology. 2008;214(1):69-77.
- Kim SY, Choi KC, Chang MS, Kim MH, Kim SY, Na YS, Lee JE, Jin BK, Lee BH, Baik JH.. The dopamine D2 receptor regulates the development of dopaminergic neurons via extracellular signal-regulated sinase and Nurr1 activation. J Neurosci. 2006;26(17):4567-76.
- S. Aoyama, H. Kase, J-H. Baik and E. Borrelli. Knockout mice in the study of dopaminergic diseases in "Adenosine receptors and parkinson's disease", Academic press, 2000;171-91.

Honors

- L’oreal Honor for Women in Bioscience, Young Investigator Honor 2002, from Women’s Bioscience Forum, Korea (02)
- AstraZeneca Virtual Research Institute Research Grant Award (06)
- AstraZeneca Virtual Research Institute Research Grant Award (07)

Increased sensitivity and anxiety-like behaviors upon chronic stress in mice lacking dopamine D2 receptors

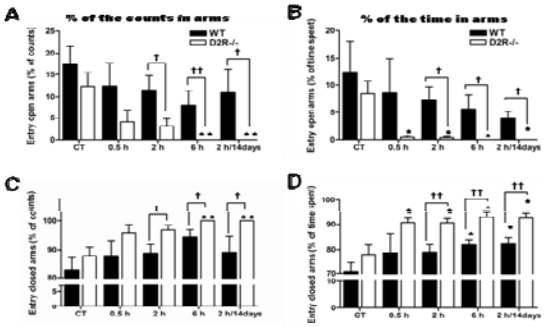
Eun Young Kang , Sa Yong Kim, Hyeri Sim,, Hyo Jin Lee, Mi hyun Choi , Sung Yul Kim, Tong Hee Koh, Se Hyun Yoon and **Ja-Hyun Baik**

School of Life Sciences and Biotechnology, Korea Univ., Seoul, South Korea

Background Dopamine regulates emotional and motivational behaviors through the mesolimbic dopaminergic pathways, which project from the ventral tegmental area (VTA) to the limbic region, including the nucleus accumbens (NAc). Disturbances of this pathway can result in reduced motivation, and anhedonia, which can be translated into depression. Therefore, dysfunction of dopaminergic mesolimbic system is closely associated with the pathophysiology of depression.

The dopamine D2 receptor (D2R) is highly expressed in the central nervous system and the pituitary gland, playing an important role in the regulation of diverse dopaminergic control of neural function. One of key interesting features of D2R is that D2R can act not only as postsynaptic receptors but also as the autoreceptors residing in presynaptic regions such as in the SN or in VTA in midbrain. Therefore, D2R may play an important role in the central regulation of homeostasis of dopaminergic neurotransmission in different aspects, regarding not only the control of locomotor activity but also of the motivational and emotional behaviors. To address the role of D2R in neural stress circuits, we have investigated the behavioral stress response of dopamine D2R knockout (D2R-/-) mice.

Results Mice assigned to 'stressed' groups were subjected to the restraint stress, being immobilized in a restrainer for 30min, 2h, and 6h for acute stress while chronically stressed mice were immobilized for 2h daily for 14 days. After stress session, the anxiety-like behavioral of mice were measured by the elevated plus maze. The D2R-/-mice displayed a significant stronger decrease in the percentage of entries to the open arms (Fig. 1A) and in the percentage of time spent to the open arms (Fig. 1B) as compared to the WT mice after stress.). In contrast the entry and the time spent in the closed arms were increased (Fig1C, D). These results show that the D2R-/- mice exhibited an increased anxiety in response to stress. The WT and D2R-/- mice after chronic stress were assigned to the forced swimming test (FST),



to measure the level of depression.

Figure1. Increased stress-induced anxiety in the absence of D2 receptor

The immobility time in WT mice after chronic stress were increased as compared to the control. The D2R-/- mice showed a significant decrease in the immobility time than WT mice after chronic stress (Fig.2).

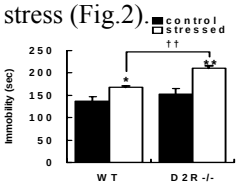


Figure 2. Immobility time in Forced swimming test following chronic restraint stress in WT and D2R-/- mice

Conclusion Here, we have investigated the behavioral stress response of Dopamine D2R-/- mice. We found that D2R-/- mice present increased despair and sensitivity to stress. By examining gene expression profiling changed differentially upon stress by microarray analysis in WT and D2R-/- mice, we found that many of genes involved in neuronal/synaptic plasticity are differentially regulated in WT and D2R-/- mice. These results suggest that D2R serves as an important mediator of regulation of plasticity in control of stress-responses. In addition, D2R can activate ERK signaling and it has recently been reported that STEP (striatal-enriched protein tyrosine phosphatase) may affect the D2R-mediated ERK signaling. We are currently exploring whether alterations in STEP activity via ERK signaling may have profound effects on networks of dopaminergic neurotransmission, including stress-related behaviors and drug addiction.

3rd Korea-UK
Joint Symposium on Neuroscience

Increased sensitivity and anxiety-like behaviors upon chronic stress in mice lacking dopamine D2 receptors

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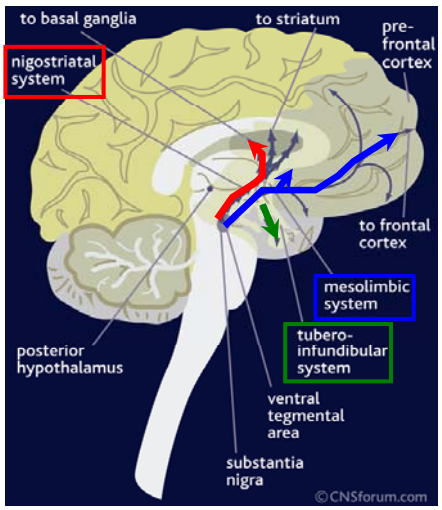
Molecular Neurobiology Laboratory,
School of Life Sciences and Biotechnology
Korea University
Seoul, South Korea

Dopaminergic neurotransmission system

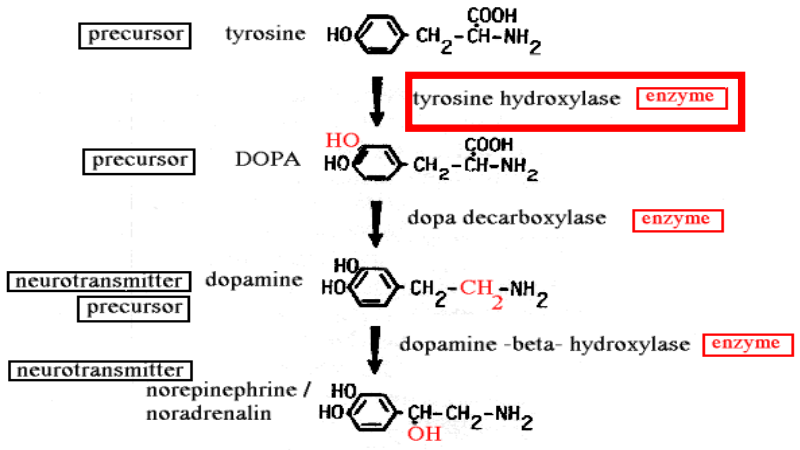


Nigrostriatal pathway connects the substantia nigra with the striatum; involved in the production of *movement*, as part of the basal ganglia motor loop.

Mesolimbic mesocortical pathway : the ventral tegmentum in the midbrain to the nucleus accumbens / the frontal cortex; involved in the initiation and maintenance of goal directed and *reward related behaviors* and may also play a role in regulation of affective expression

Tuberoinfundibular system: Major role in the regulation of some *hypothalamic and pituitary peptides*

Dopamine synthesis



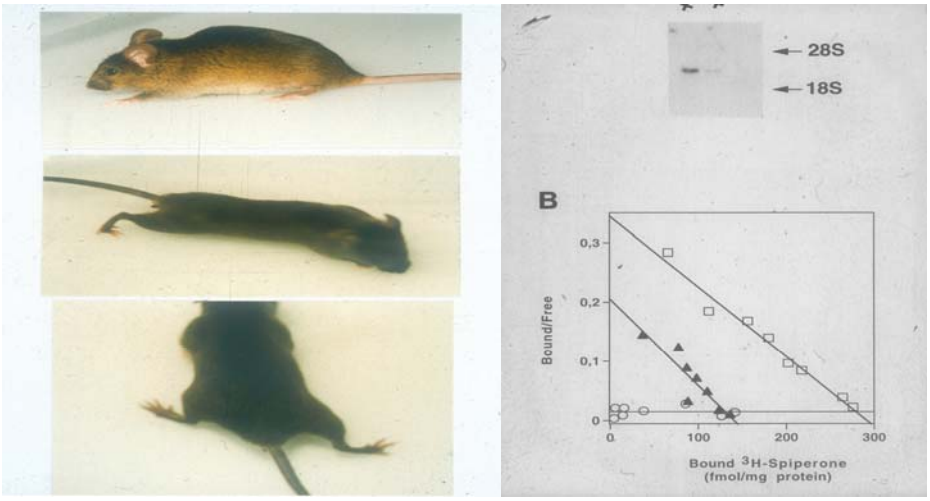
Dopamine receptors

- The dopamine receptors belong to the family of G-protein coupled receptor
- D1 like receptor : Gαs coupling (D1, D5)
- D2 like receptor : Gαi coupling (D2, D3, D4)

D2R :

- Highly expressed in the striatum and pituitary gland
- Crucial for the regulation of various physiological functions in dopaminergic neurotransmission
- also act as presynaptic receptor in midbrain

Dopamine D2 receptor knockout mice

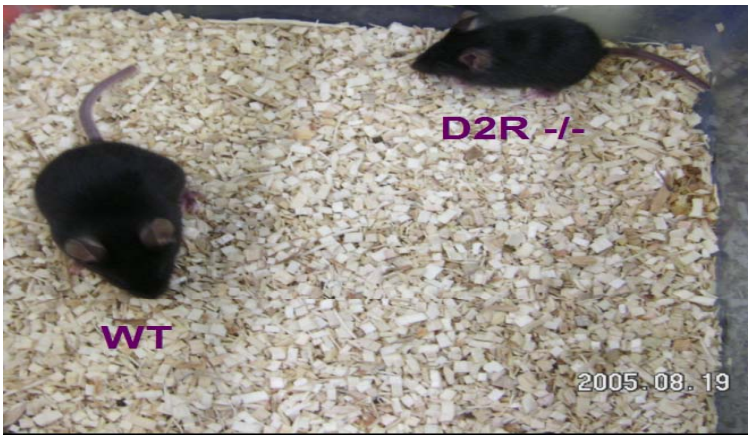


Baik et al., 1995, Nature

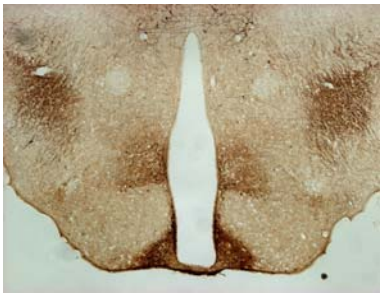
Dopamine D2 receptors

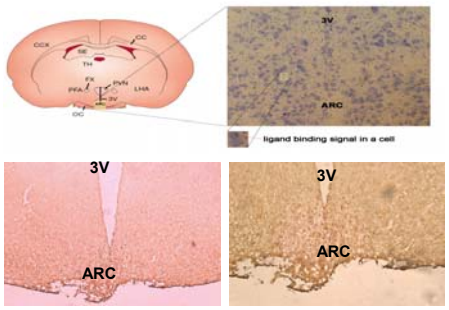
- **D2R might play a key role in the control of pituitary cell proliferation and maintenance.** *Neuron. 1997*
- **Both D2L and D2S mediated a rapid and transient activation of MAPK with dominant activation of p42-kDa MAPK.** *Biochem Biophys Res Commun. 1999*
- **The signaling through D2R was found to be crucial for anti-proliferative effects and cell death in pituitary tumor cells.** *Molecular and Cellular Endocrinology. 2003*
- **Distinct regulation of internalization and mitogen-activated protein kinase activation by two isoforms of the dopamine D2 receptor.** *Mol Endocrinol. 2004*
- **The absence of D2R alters the GABAergic neurotransmission.** *Mol Cell Neurosci. 2004*
- **The Dopamine D2 receptor Regulates the Development of Dopaminergic Neurons via ERK and Nurr1 Activation.** *Journal of Neuroscience. 2006*
- *Striatal-enriched protein tyrosine phosphatase regulates dopaminergic neuronal development via extracellular signal-regulated kinase signaling* *Experimental Neurology, 2008*

D2R-/- mice are lean



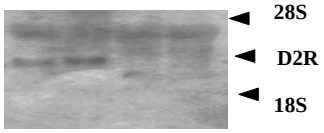
TH and D2R expression in hypothalamus



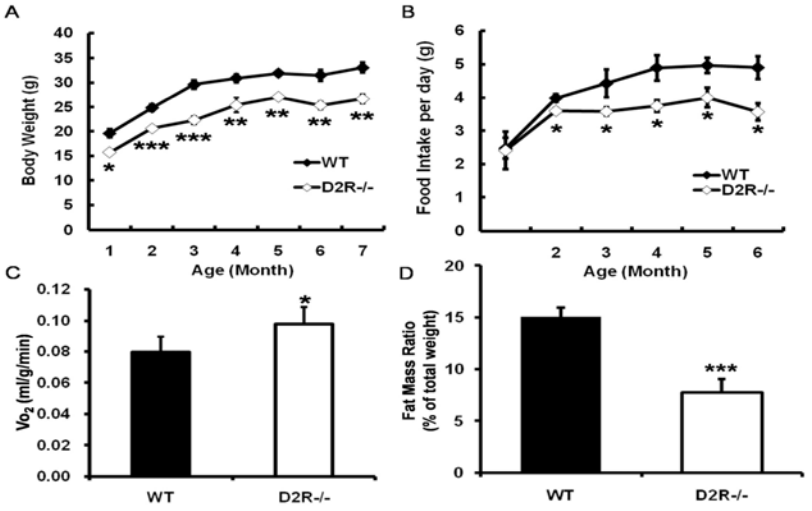


TH staining in ARC (Arcuate nucleus) of hypothalamus

WT D2R -/-

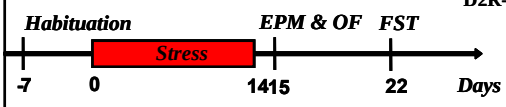


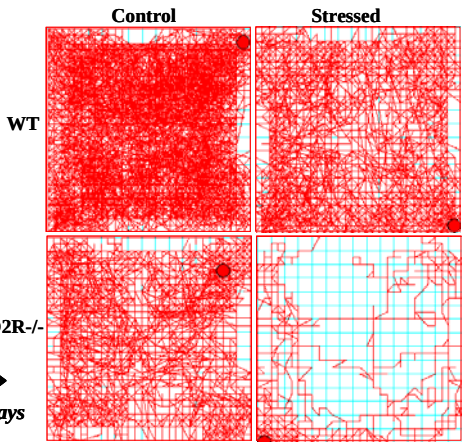
Reduced body weight and food intake in D2R-/- mice



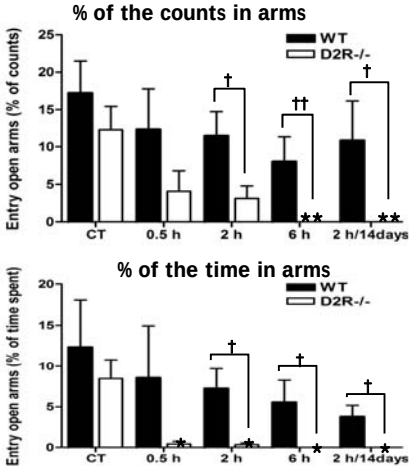
Altered stress-related behaviors in D2R-/- mice

Restraint stress

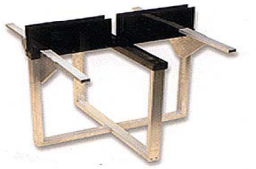




Effect of restraint stress on Elevated-Plus Maze Performance in WT and D2R-/- Mice



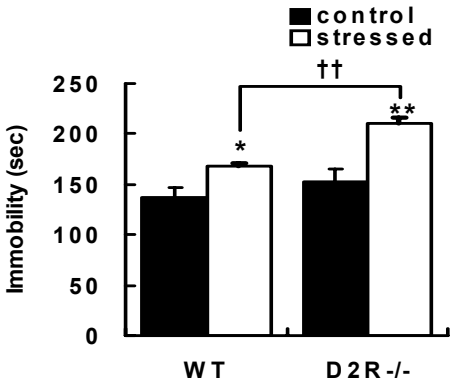
Elevated Plus Maze



Increased behavioral despair and anxiety to stress in D2R-/- mice

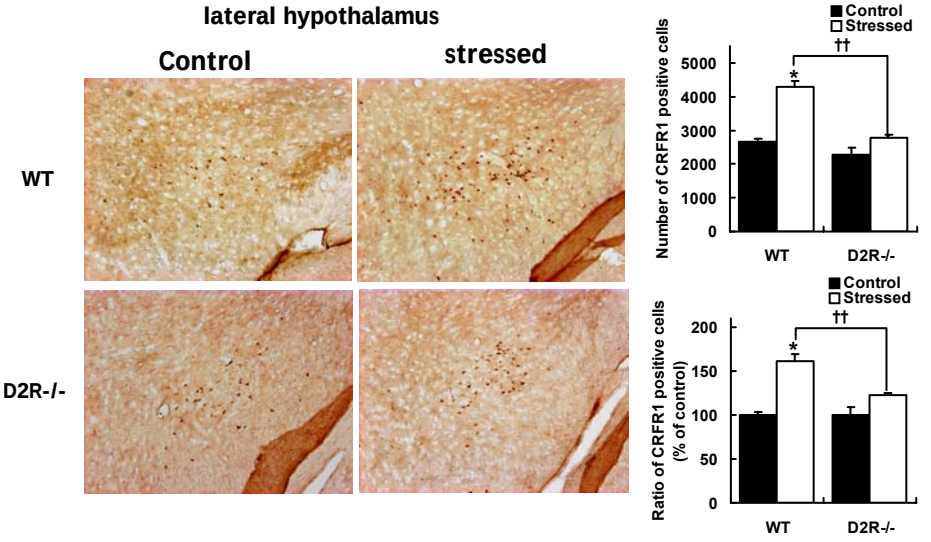
Effects of chronic stress on the FST

Forced Swim test

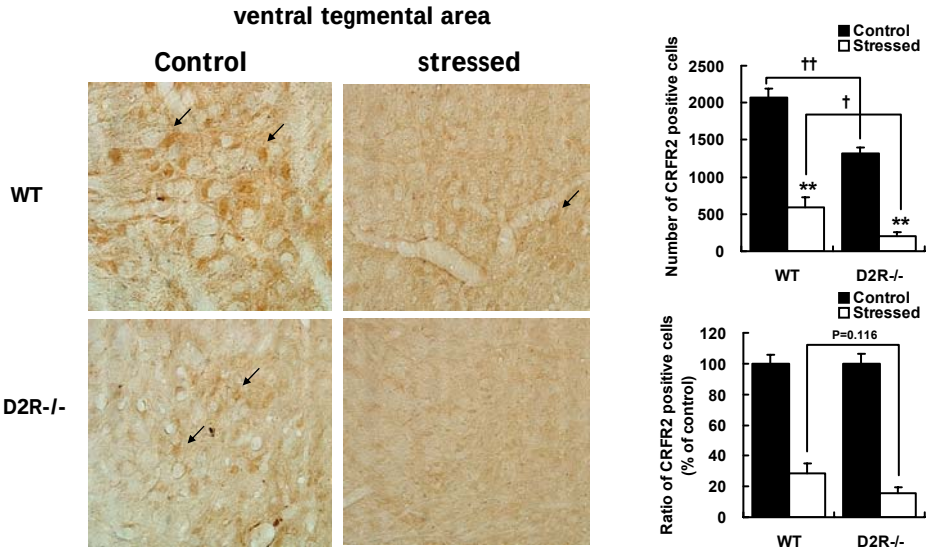


Increased behavioral despair and anxiety to stress in D2R-/- mice

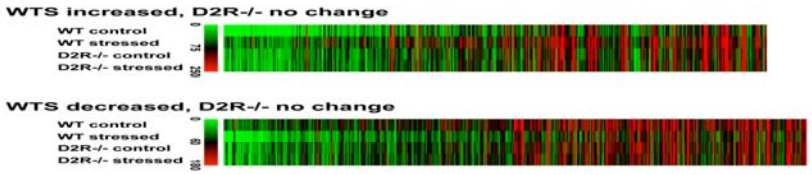
CRFR1 expression in WT and D2R-/- mice



CRFR2 expression in WT and D2R-/- mice

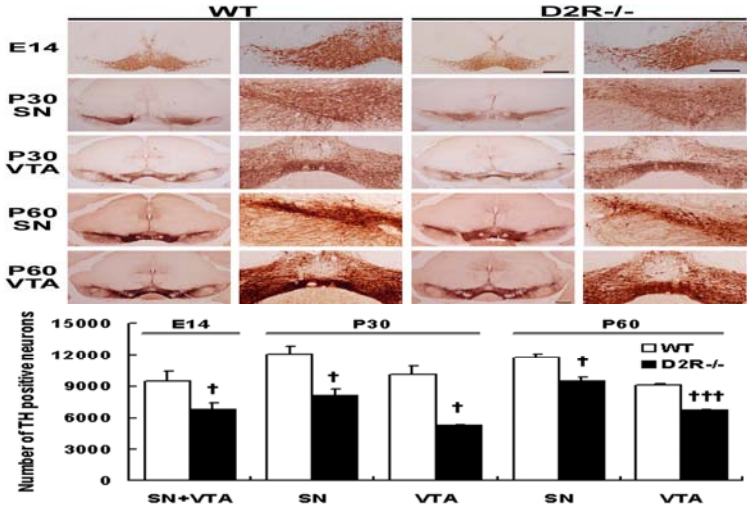


Microarray Analysis After stress in WT and D2R-/- mice



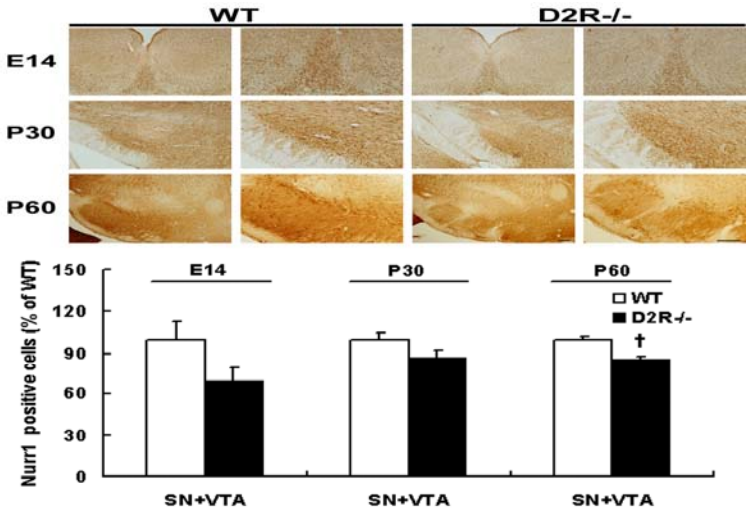
Gene Title	Gene Symbol	Stress Induced Changes		Basal Change
		WT	D2R-/-	
transducin-like enhancer of split 3, homolog of Drosophila E(spl)	Tle3	N/A	2.8	N/A
regulator of G-protein signaling 1	Rgs1	N/A	4.9	3.0
ubiquitin D	Ubd	N/A	9.2	N/A
amyloid beta (A4) precursor protein	App	N/A	0.3	0.3
neurotrophic tyrosine kinase, receptor, type 2	Ntrk2	N/A	N/A	N/A
protein phosphatase, EF hand calcium-binding domain 2	Ppef2	N/A	26.0	N/A
tissue inhibitor of metalloproteinase 2	Timp2	N/A	0.3	N/A
annexin A4	Anxa4	19.7	N/A	N/A
wingless-related MMTV integration site 3	Wnt3	16.0	N/A	N/A
thymoma viral proto-oncogene 3	Akt3	16.0	N/A	N/A
platelet-derived growth factor D	Pdgfd	14.9	N/A	N/A
fibroblast growth factor 18	Fgf18	13.9	N/A	N/A
heat shock protein 90kDa alpha (cytosolic), class A member 1	Hsp90aa1	9.2	0.2	0.0
wingless-related MMTV integration site 5A	Wnt5a	8.0	N/A	N/A
guanine nucleotide binding protein, alpha transducing 2	Gnat2	8.0	N/A	N/A
RAB2, member RAS oncogene family	Rab2	8.0	N/A	0.3
PDZ-RGS3 protein	Rgs3	7.0	N/A	N/A
amyloid beta (A4) precursor-like protein 2	Aplp2	5.7	N/A	N/A
adrenergic receptor, beta 2	Adrb2	5.7	N/A	N/A
amyloid beta (A4) precursor-like protein 2	Aplp2	5.7	N/A	0.2
spleen tyrosine kinase	Syk	4.9	N/A	N/A
wingless-related MMTV integration site 2	Wnt2	4.0	N/A	N/A
phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)	Pik3r1	4.0	N/A	N/A
Hedgehog-interacting protein	Hhip	3.7	N/A	N/A
megakaryocyte-associated tyrosine kinase	Matk	3.7	N/A	N/A
neural precursor cell expressed, developmentally down-regulated gene 4-like	Nedd4l	3.7	14.9	N/A
mitogen activated protein kinase kinase 4	Map2k4	3.5	0.4	N/A
mitogen activated protein kinase 8 interacting protein 1	Mapk8ip1	2.5	N/A	N/A

Decreased number of TH-positive neurons in D2R-/- mice



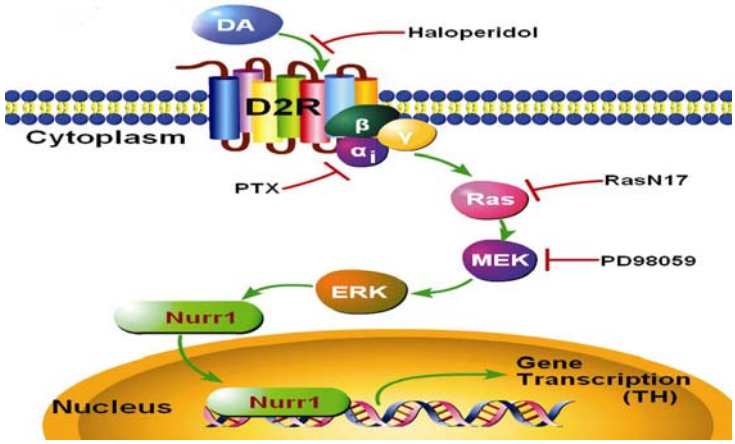
Kim et al., J.Neurosci., 2006

Stereological analysis of Nurr1 positive cells in WT and D2R-/- mice

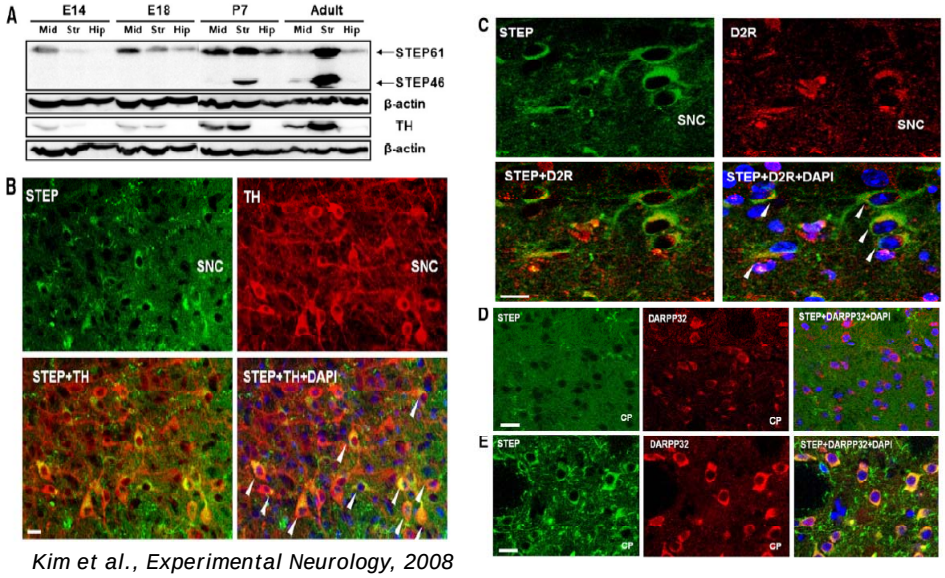


Kim et al., J.Neurosci., 2006

Proposed signaling pathways and the role of mesencephalic D2R in the development of mesencephalic dopaminergic neurons

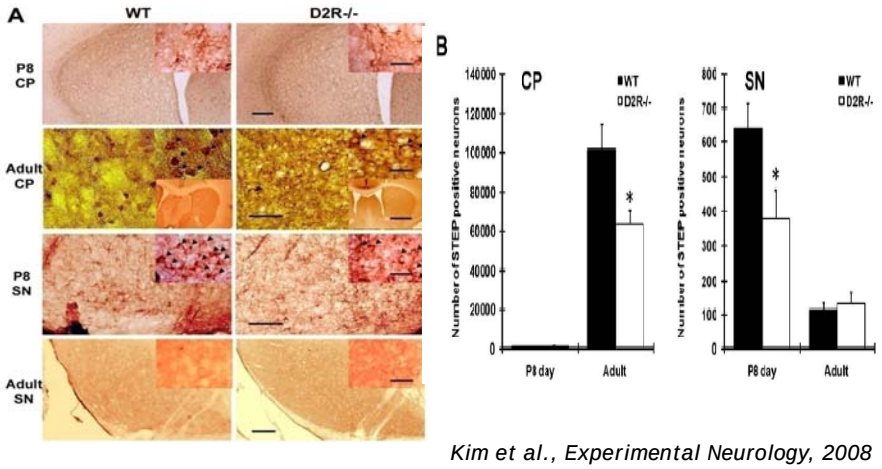


Expression of STEP, TH, D2R, and DARPP-32 in mouse brain



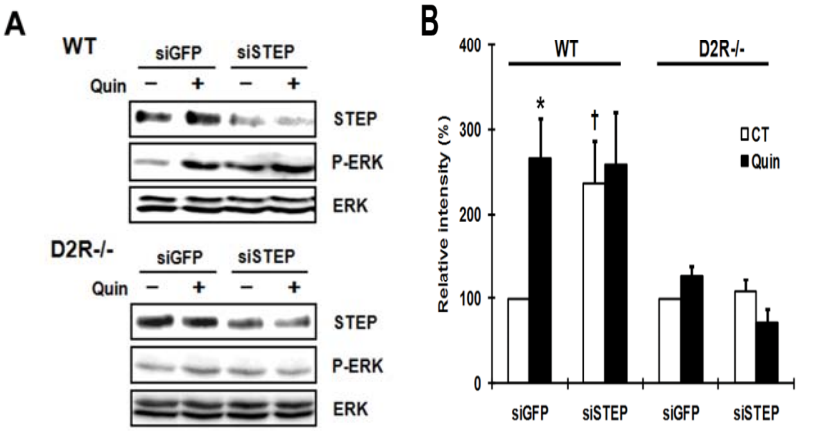
Kim et al., Experimental Neurology, 2008

Stereological analysis of number of Striatal-Enriched Protein Tyrosine Phosphatase (STEP)-positive cells in WT and D2R-/- mice.



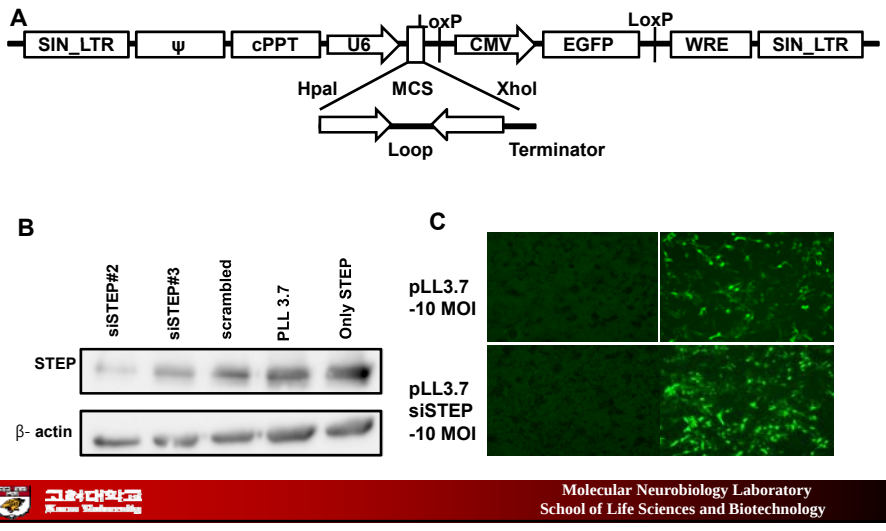
Kim et al., Experimental Neurology, 2008

STEP regulates ERK activation induced by D2R stimulation in mesencephalic dopaminergic neurons



Kim et al., Experimental Neurology, 2008

Role of STEP in Adult?



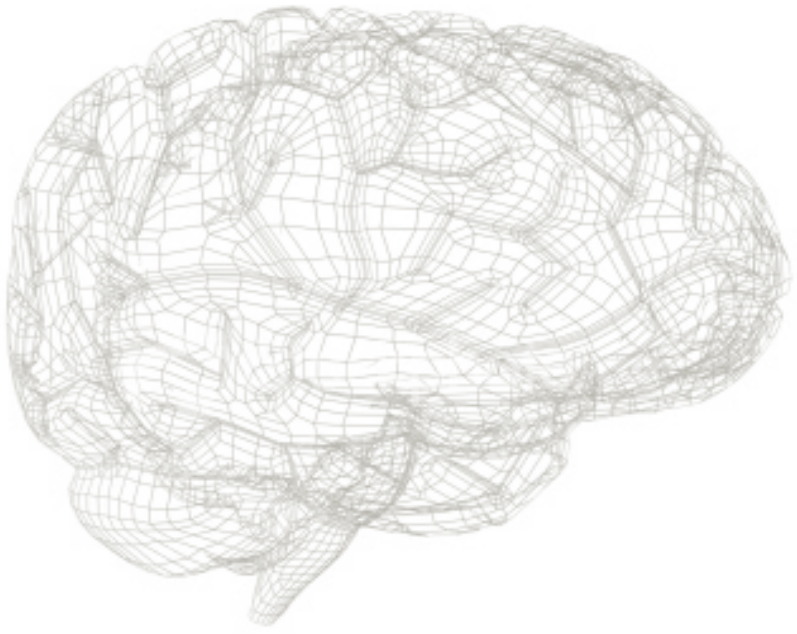
Session Ⅳ: Brain Imaging and Disorders

Chair : Kyungjin Kim

16:00 **Neural mechanisms of response selection** ..... Kyungmin Lee (Seoul National Univ.)

16:25 **New insights into the genetics of schizophrenia** ..... Michael Owen (Cardiff Univ.)

16:50 **Protection of methamphetamine dopaminergic toxicity by glutathione peroxidase mimics** ..... Hyoung-Chun Kim (Kangwon National Univ.)



Kyoung-Min Lee

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Education

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Fellowship & Employment

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- Fellowship (behavioral neurology / functional neuroimaging), Dept. of Neurology Cornell Univ. Medical College & Memorial Sloan-Kettering Cancer Center (96 ~ 97)
- Prof. of Dept. of Neurology, Seoul Nat'l Univ. (97 ~ present)
- Scientist, Smith-Kettlewell Eye Research Institute (05 ~ present)

Selected Publication

- Lee KM, Keler E.R. Neural activity in the frontal eye fields modulated by the number of alternatives in target choice. J Neurosci. 2008;28(9):2242-51.
- Woo SH, Lee KM, Effect of the number of response alternatives on brain activity in response selection. Hum Brain Mapp. 2007;28(10):950-8.
- Lee KM, Lai AP, Brodale J, Jampolsky A. Sideslip of the medial rectus muscle during vertical eye rotation. Invest Ophthalmol Vis Sci. 2007;48(10):4527-33.
- Lee KM, Alex R. Wade, Lee BT. Differential correlation of frontal and parietal activity with the number of alternatives for cued choice saccades. NeuroImage. 2006; 33(1):307-15.

Neural mechanism of saccadic response selection

Kyoung-Min Lee

Dept. of Neurology, College of Medicine, Seoul National Univ., Seoul, Korea

Background Since Hick's original description, many subsequent studies have confirmed the logarithmic relationship that exists between response time and the number of alternatives (NA) for a choice response. In other words, the time taken to choose a response appropriate for a cue increases with the number of potential response options. In the present study a novel paradigm was used to quantify saccade response time as a function of NA. In the task, human and non-human primates made a saccade to the remembered location of a visual target whose color was specified by a centrally located cue. The paradigm thus required a stimulus response transformation similar to that used by Hick. Using this task paradigm, we performed event-related fMRI experiments to identify brain areas carrying out the translation from a cue stimulus to response. We also conducted neuro-physiological investigations on one of the areas that showed NA-correlation in fMRI experiment, namely, the frontal eye fields (FEF), to elucidate neural mechanisms for choice behavior.

Results Behavioral experiments with normal humans and monkeys established that, when a color-to-location transformation was required for response selection, a logarithmic relationship was found for saccadic response time. Functional MRI results revealed activities associated with choice saccades at FEF and intraparietal sulcus (IPS). Within these regions, signals were correlated with NA at the medial part of the FEF and the posterior part of the IPS, but not at the lateral FEF and anterior IPS, indicating functional differences between these sub-regions and suggesting different sub-networks for response selection and saccade execution. Single-unit recordings from FEF revealed that visual responses to alternative targets decreased as NA increased, whereas perisaccade activities increased with NA. These modulations of FEF activities seem closely related to the choice process because the activity enhancements coincided with the timing of target selection, and the neural modulation was greater as NA increased, features expected of neural correlates for a choice process from the perspective of Hick's law. Following chemical inactivations of FEF by muscimol, the percentage of choice errors increased as a function of NA. In contrast, the percentage of dysmetric saccades (saccades that landed in the correct quadrant but were inaccurate) did not vary with NA. Saccade latency increased postlesion but did not increase with NA. We also made simultaneous inactivations in both FEFs. The results following bilateral lesions showed approximately twice as many choice errors. We also observed that saccades based on associative memory were impaired in a similar manner to those generated by visuospatial working memory, but visual target information survived to program saccades in directions outside of the lesioned field.

Conclusion This series of experiments with psychophysics, functional neuroimaging, single-unit recording, and reversible inactivation methods together firmly established that FEF is the cortical area responsible for saccadic choice based on associative memory. The experiments also demonstrated functional sub-regions in human FEF, which underlie each step of the stimulus–response translation and response execution, separately. Our neurophysiology data suggest two novel notions of FEF neuronal behavior that have not been reported previously: (1) cells called "phasic visual" that do not discharge in the perisaccade interval in a delayed-saccade paradigm show such activity in a choice response task at the time of the saccade; and (2) the activity in FEF visuomotor cells display an inverse relationship between perisaccadic activity and the time of saccade triggering with higher levels of activity leading to longer saccade reaction times. These findings support the area's involvement in sensory-motor translation for target selection through coactivation and competitive interaction of neural populations that code for alternative action sets. The dramatic effect of NA on choice errors, but the lack of an effect of NA on motor errors or response latency, suggests that two types of processing are interrupted by FEF lesions. The first involves the formation of a saccadic intention vector from associate memory inputs, and the second, the execution of the saccade from the intention vector. An alternative interpretation of the first result is that a role of the FEF may be to suppress incorrect responses. The doubling of choice errors following bilateral FEF lesions suggests that the effect of unilateral lesions is not caused by a general inhibition of the lesioned side by the intact side. Our findings also demonstrate that the area is necessary for the formation of saccadic intention to a memory-specified target, rather than the memory itself.

Neural mechanism of response selection

Kyoung-Min Lee
Seoul National University, Seoul, Korea
Smith-Kettlewell Eye Research Institute, San Francisco, CA

Response selection

- Two modes of response selection
  - Simple reaction
    - Reflexive, often hard-wired
    - All-or-none control
    - Driven by perceptual saliency
  - Choice response
    - Associative memory of stimulus and response
    - Voluntary control of response selection
    - Driven by “conceptual relevance” meaning-based: symbols, environmental cues

A mistake in response selection !

Hick's law

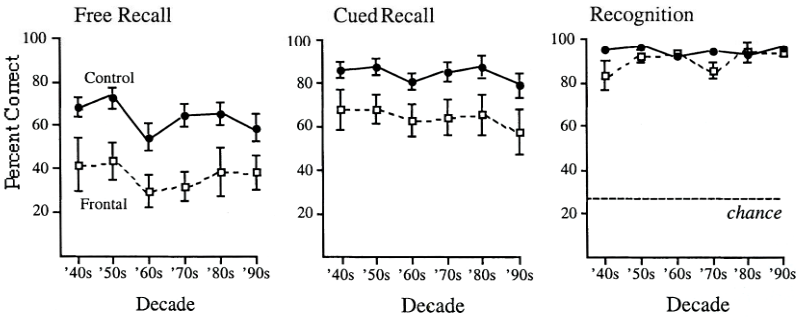
- Choice response time increases as a logarithmic function of choice alternatives:

  CRT = a * log (number of alternatives) + b

- A temporal characteristic of choice response
- Consistent across a wide variety of choice situations

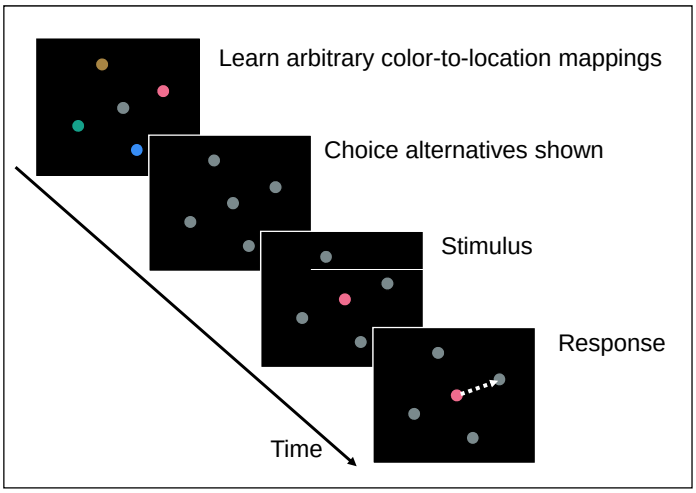
Memory and the frontal lobe

1) Retrieval of stored information



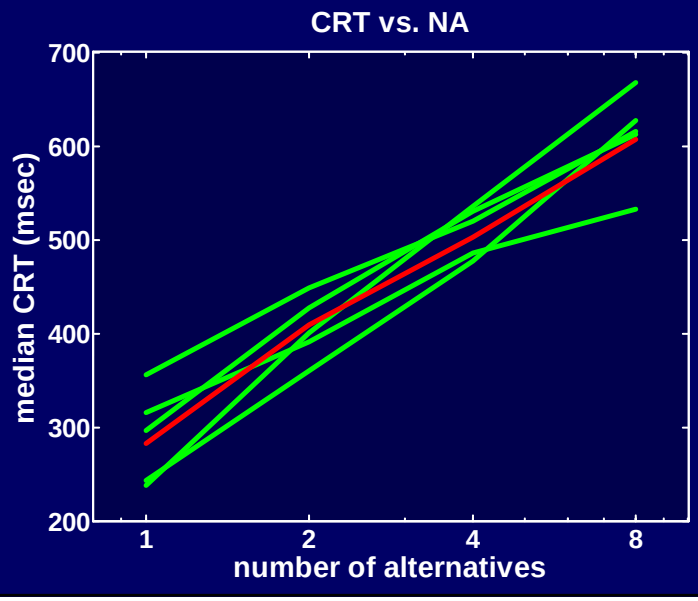
2) Working memory & Working with memory

- The choice saccade task:
  - Stimulus: color change at the center
  - Response: saccadic eye movement to a target that were the same color



memory color matching
8 alternatives

Hick's law obeyed in choice saccade task



NA correlation within areas activated in complete trials

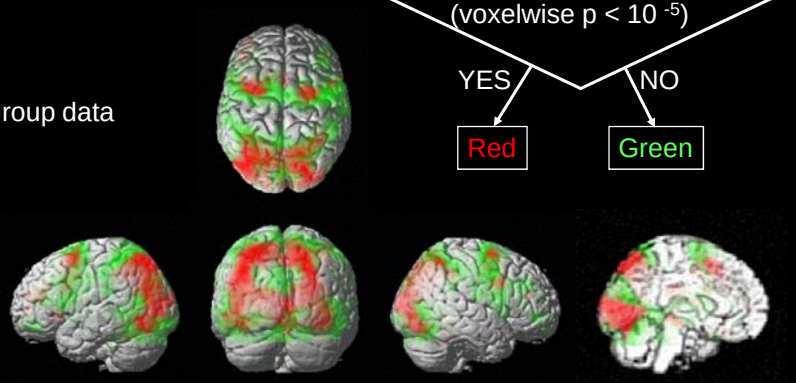
CT of NA 1 > baseline, corrected $p < 10^{-3}$

Correlated with NA?
(voxelwise $p < 10^{-5}$)

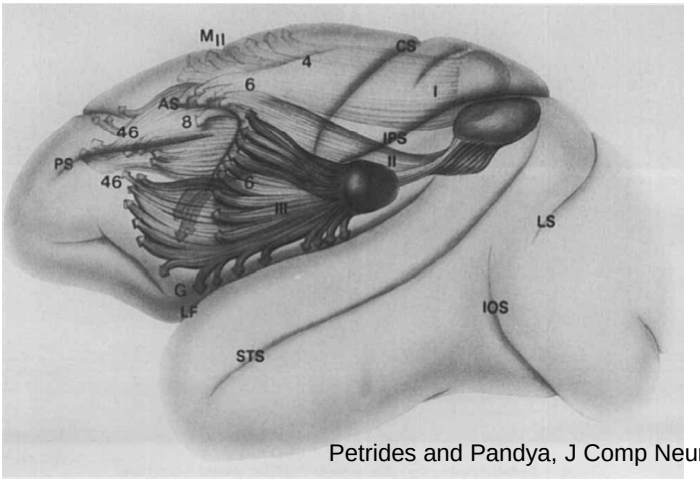
YES → Red

NO → Green

Group data



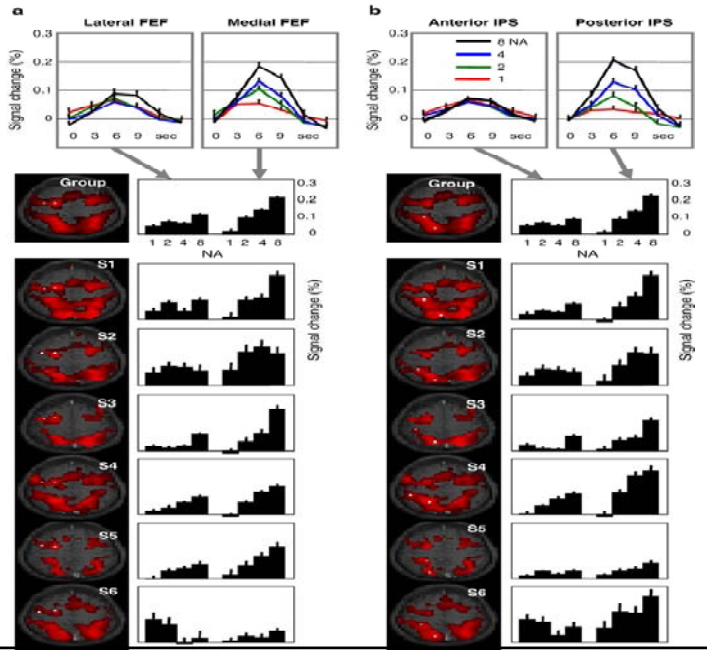
Distinct sub-networks between parietal and frontal areas



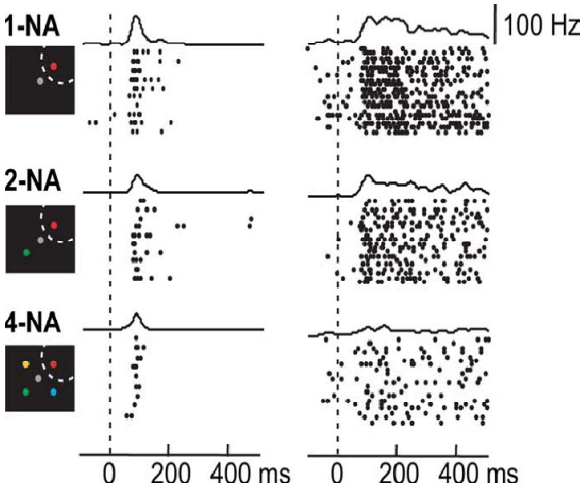
Petrides and Pandya, J Comp Neurol 1984

-human homologue of FEF
-functional divisions in FEF/SEF/IPS
-layout of cognitive stages in the cortices

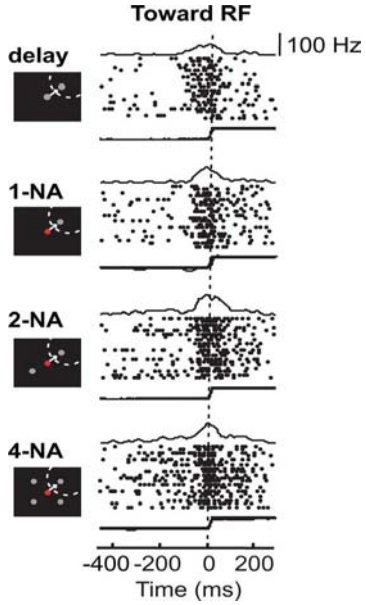
Sub-regions within FEF and IPS differentiated by NA correlation



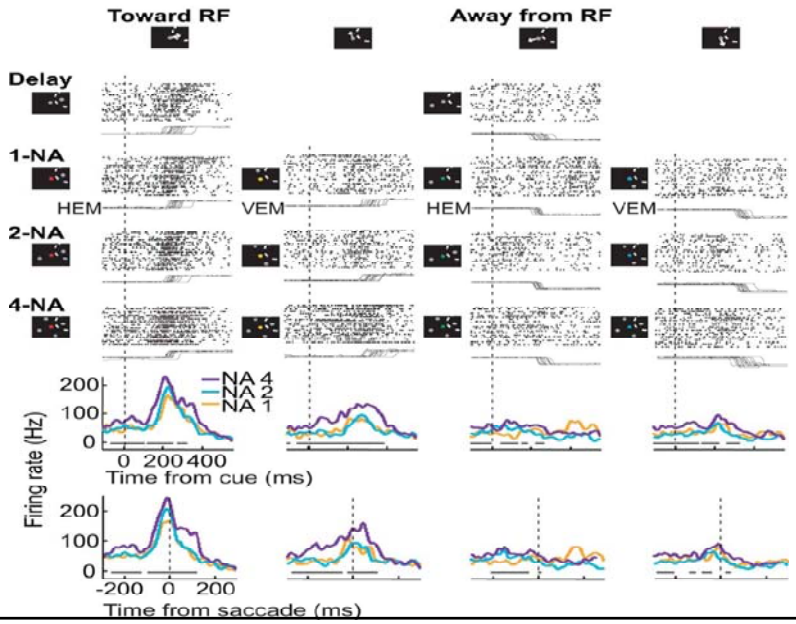
Visual FEF cells show modulation by NA in choice saccade.

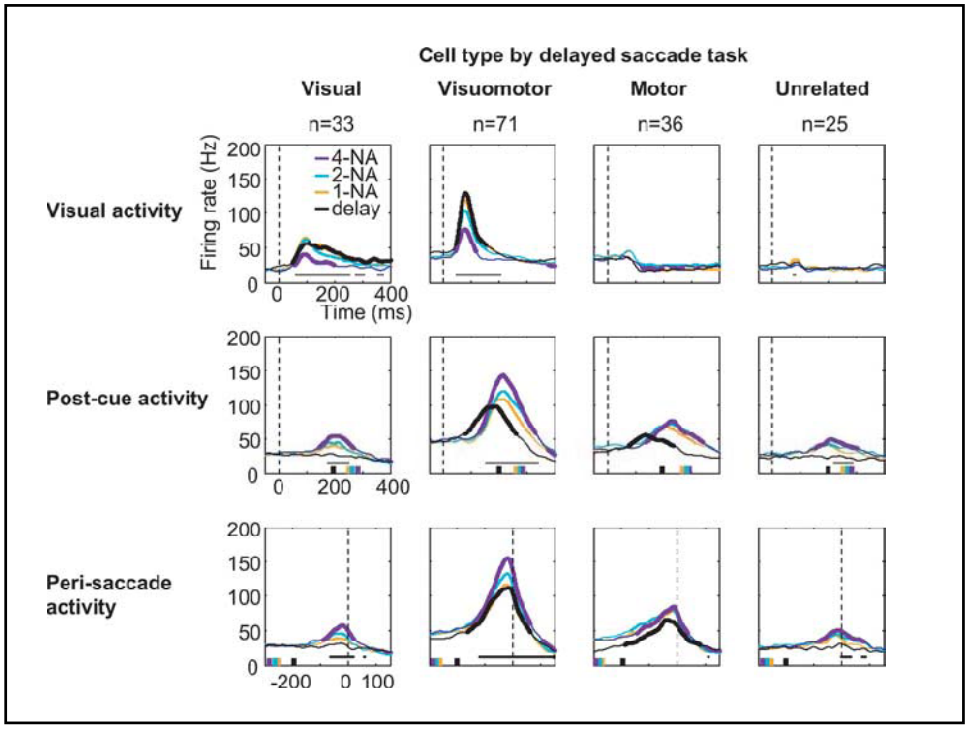


Motor FEF cells do not show modulation by NA.

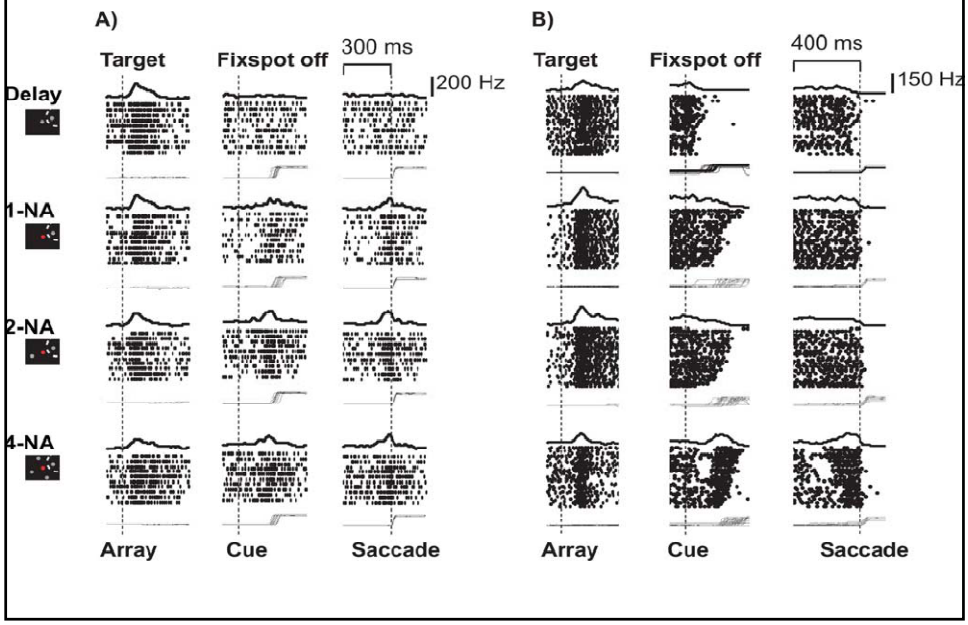


Visuo-motor FEF cells get modulated by NA in choice.

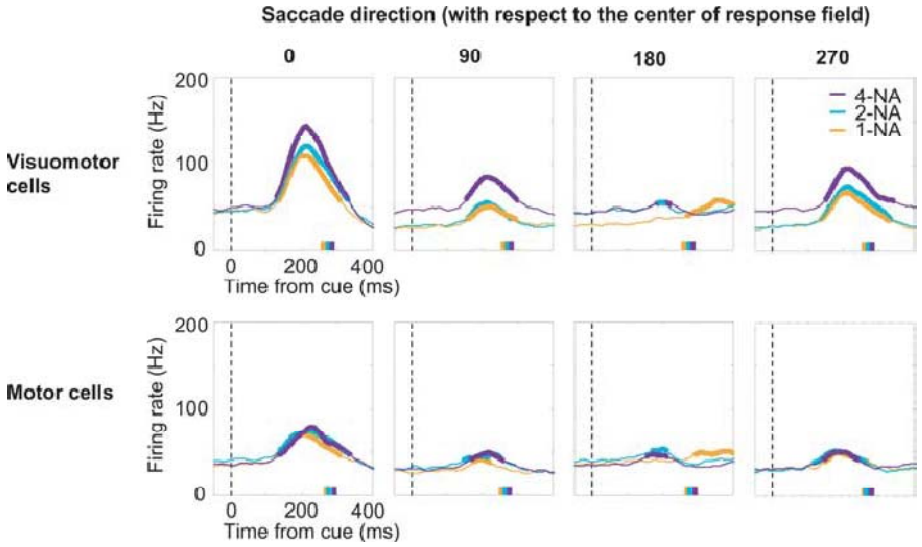




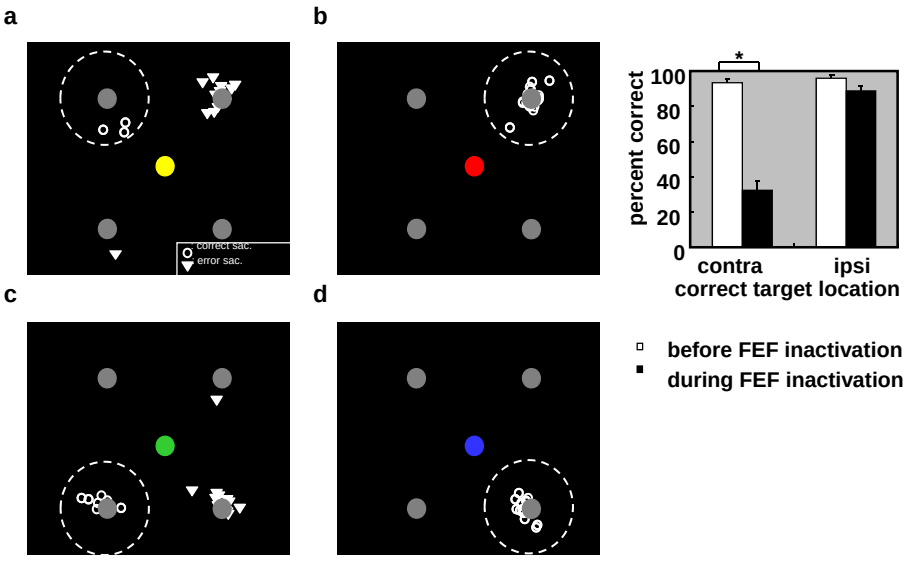
Saccade-related activities shown only in difficult choice tasks.



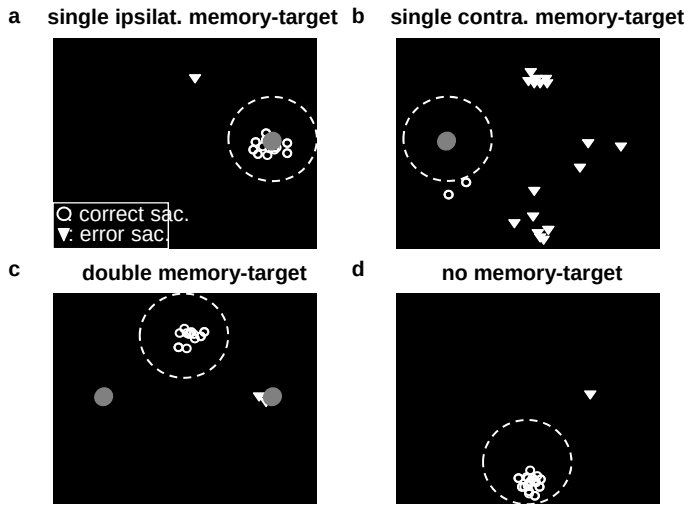
FEF activities reflect potential alternatives in upcoming choice.

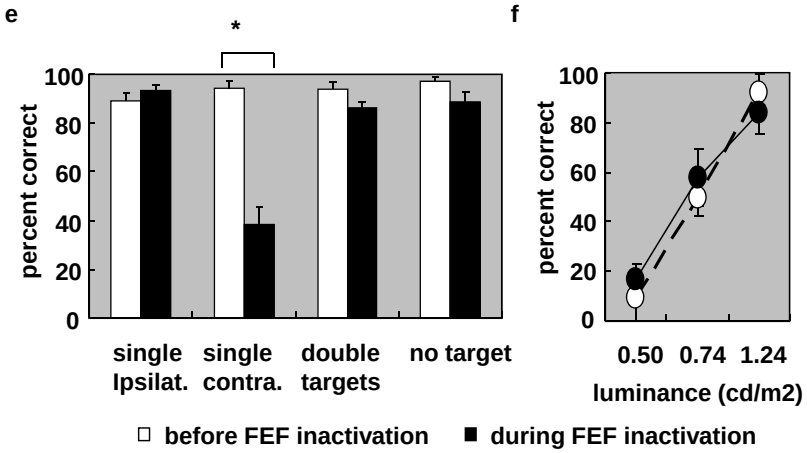


FEF inactivation by muscimol disrupts choice saccades.



FEF inactivation disrupts saccade intention,
without affecting attention or visual working memory.





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Education

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- PhD in Neuroscience (82)
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Employment & Membership

- Senior Lecturer in Neuropsychiatric Genetics at the Univ. of Wales College of Medicine (UWCM) (90)
- MRC Training Fellow at the Dept. of Biochemistry and Molecular Genetics at Imperial College, London (97)
- Personal Chair of UWCM (95)
- Prof. of Psychological Medicine and Head of Dept. of Psychological Medicine (98 ~ present)
- President of the International Society of Psychiatric Genetics (00 ~ 05)
- Pro Vice Chancellor for Research at UWCM (01 ~ 05)
- Member of the Council of the Academy of Medical Sciences (01 ~ 04)
- Consultant in General Adult Psychiatry (present)
- Head of Cardiff Neuropsychiatric Genetics Group (present)
- Dean and Head of the School of Medicine (present)

Selected Publication

- Williams NM, Preece A, Morris DW, Spurlock G, Bray NJ, Stephens M, Norton N, Williams H, Clement M, Dwyer S, Curran C, Wilkinson J, Moskvina V, Waddington JL, Gill M, Corvin AP, Zammit S, Kirov G, Owen MJ, O'Donovan MC. Identification in two independent samples of a novel schizophrenia risk haplotype of the dystobrevin binding protein gene (DTNBP1). Archives of General Psychiatry . 2004;61: 336-44
- Georgieva L, Moskvina V, Peirce T, Norton N, Bray NJ, Jones L, Holmans P, MacGregor S, Wilkinson J, Williams H, Nikolov I, Williams N, Ivanov D, Davis KL, Haroutunian V, BuxbaumJD, Craddock N, Kirov G, Owen MJ, O'Donovan MC. Convergent evidence that oligodendrocyte lineage transcription factor 2 and interacting genes influence susceptibility to schizophrenia. PNAS. 2006;103: 12469-74.
- Paylor P, Glaser B, Mupo A, Ataliotis P, Spencer C, Sobotka A, Sparks C, Choi C-H, Oghalai J, Curran S, Murphy K, Williams N, O'Donovan MC, Owen MJ, Scambler P, Lindsay E. Tbx1 haploinsufficiency is linked to behavioral disorders in mice and humans: Implications for 22q11 deletion syndrome. PNAS. 2006;103:7729-34

New Insights into the Genetics of Schizophrenia

Michael J Owen

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Schizophrenia (SZ) is a severe psychiatric disorder with a lifetime risk of approximately 1%. Early onset, poor response to medication, frequent relapse and chronic course impose considerable burdens on sufferers, their families and society. There remains therefore a pressing need for new and more effective therapies, and these will require advances in our understanding of disease mechanisms. Genetic epidemiology reveals that genes account for more than 80% of the population variance in risk. However, like other common diseases genetic influences are complex and heterogeneous, and are likely to involve a combination multiple common (< 1%) risk alleles of small effect and rare alleles of large effect. Advances in human genetics are at last allowing the specific risk genes for complex disorders such as schizophrenia to be identified. Early studies using these platforms have essentially confirmed that risk of schizophrenia reflects the operation of multiple common alleles of small effect (OR≤1.1) and sub-microscopic genomic abnormalities known as copy number variants which have larger effects on risk (OR>20) but which are individually rare (<0.001%). It seems likely that the continued application of emerging technologies to larger clinical samples will to result in further advances over the next few years, but many challenges remain.

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Education

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- PhD in Pharmacology, Graduate School, Chung-Ang Univ. (87)

Fellowship & Employment

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- Research Associate of Psychopharmacology, Department of Psychology, Texas A & M Univ., USA (92 ~ 93)
- Research Fellow, Section of Neuropharmacology, NIEHS/NIH, USA (93 ~ 94)
- Department Head, Department of Pharmacy, College of Pharmacy, Kangwon National Univ. (95 ~ 97)
- Director, Institute of Pharmaceutical Science, Kangwon Nat'l Univ. (97 ~ 99)
- Dean, College of Pharmacy, Kangwon Nat'l Univ. (01 ~ 03)
- Director, Institute of Pharmaceutical Science, Kangwon Nat'l Univ. (06 ~ 08)

Selected Publication

- Shin EJ, Jeong JH, Kim AY, Koh YH, Nah SY, Kim WK, Ko KH, Kim HJ, Wie MB, Kwon YS, Yoneda Y, Kim HC. Protection against kainate neurotoxicity by ginsenosides: Attenuation of convulsive behavior, mitochondrial dysfunction, and oxidative stress. J Neurosci Res. 2008. (in press)
- Shin EJ, Jeong JH, Bing G, Park ES, Chae JS, Yen TPH, Kim WK, Wie MB, Jung BD, Kim HJ, Lee SY, Kim HC. Kainate-induced mitochondrial oxidative stress contributes to hippocampal degeneration in senescence-accelerated mice. Cell Signal. 2008;20(4):645-58.
- Zhang W, Shin EJ, Wang T, Lee PH, Pang H, Wie MB, Kim WK, Kim SJ, Huang WH, Wang Y, Zhang W, Hong JS, Kim HC, 3-Hydroxymorphinan, a metabolite of dextromethorphan, protects nigrostriatal pathway against MPTP-elicited damage both in vivo and in vitro. FASEB J. 2006;20(14):2496-511.

Protection of methamphetamine dopamine toxicity by glutathione peroxidase mimics

Eun-Joo Shin, Duong Xuan Chu, Min Soo Kim, Kyo Hwan Koo, **Hyoung-Chun Kim**

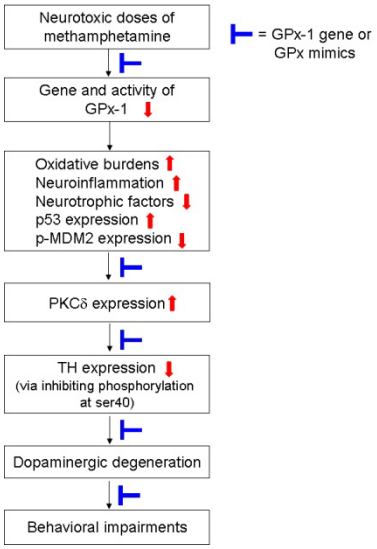
Neuropsychopharmacology and Toxicology Program, College of Pharmacy, Kangwon National University, Chunchon 200-701, Korea

Background Escalating evidence suggests that oxidative stress is involved in methamphetamine (MA)-induced neurotoxicity, and that peroxides including H2O2 play a crucial role in this neurotoxicity. A selenium-dependent glutathione peroxidase (GPx-1) out of GPx isozymes is considered as a major H2O2 scavenger in the brain. As the role of another cerebral H2O2 scavenger peroxiredoxin II was less pronounced than GPx-1 gene in response to MA, we explored in the present study whether GPx-1 gene or GPx-mimic compound affects MA-induced dopaminergic neurotoxicity. In addition, we asked whether protein kinase C (PKC) gene affects this toxicity, since it has been suggested that PKC contributes to cerebral oxidative stress and dopaminergic toxicity.

Results Treatment with MA (8.0 mg/kg, i.p. 4) resulted in the decrease in striatal glutathione peroxidase-1-like immunoreactivity (GPx-IR) in the GPx-1 (+/+) mice. MA treatment produced hyperthermia, dopaminergic toxicity, oxidative stress, neuroinflammation, loss of neurotrophic factors. Intrastriatal infusion with chelerythrine, a pan-inhibitor of PKC or rottlerin, an inhibitor of PKC delta, but not with any other PKC inhibitors, attenuated MA-induced toxicity. Treatment with MA resulted in significantly increased striatal expressions of PKC delta, whereas there was no significant change in the expression of any other PKC isozymes. These findings were more pronounced in GPx-1(-/-)-than GPx-1 (+/+) mice.

As shown in case of chelerythrine or rottlerin, GPx-mimic compound ebselen significantly attenuated MA-induced toxic effects, such as hyperthermia, deficits in behaviour, impairments in dopaminergic system, increases in the striatal expressions of the PKC delta, and decreases in the expression of neurotrophic factor. Treatment with rottlerin or ebselen significantly prevented MA-induced decreases in the phospho-mouse double minute (MDM)-2 expression as well as increases in the P53 expression in the striatum. The attenuating effects of rottlerin or ebselen were less pronounced in GPx-1 (-/-) mice than GPx-1 (+/+) mice.

GPx-IR was restored by intrastriatal GPx-1 gene transfection with GPx-1 gene-encoded adenovirus vector in GPx-1 (-/-) mice. Intrastriatal GPx-1 gene transfected GPx-1 (-/-) mice were less susceptible to MA-induced toxicity as compared with control vector-transfected GPx-1 (-/-) mice. In addition, MA-induced dopaminergic toxicity and reduction in GPx-1 expression were less pronounced in the PKC delta (-/-) mice than PKC delta (+/+) mice.



<Flow chart depicting our working hypothesis on the pharmacological action of GPx-1 gene or GPx-mimics in response to methamphetamine insult>

Conclusion The results suggest that GPx-1 gene is an essential factor for blocking MA-induced dopaminergic toxicity, and that PKC delta gene is contribute to this toxicity. The GPx-mimic compound attenuates MA-induced neurotoxicity via inhibiting PKC delta expression, oxidative stress and neuroinflammation.

Protection of methamphetamine dopaminergic toxicity by glutathione peroxidase mimics

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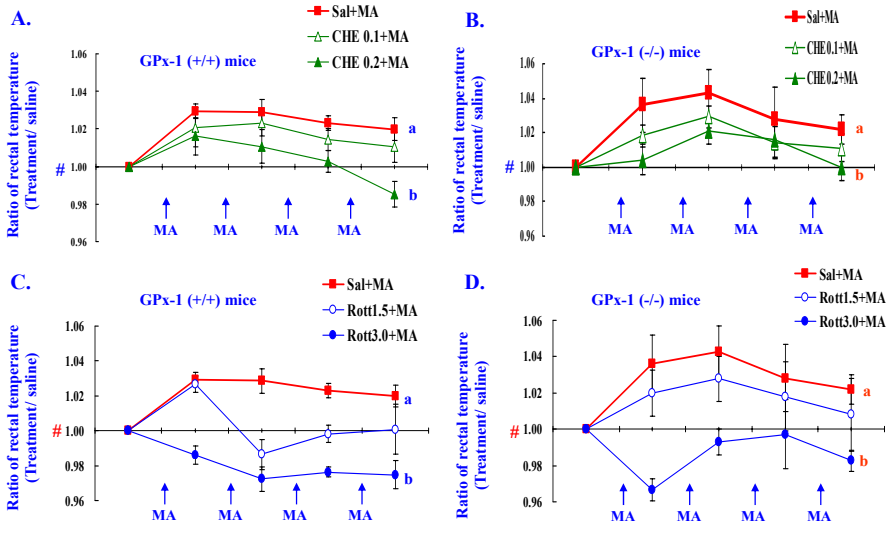
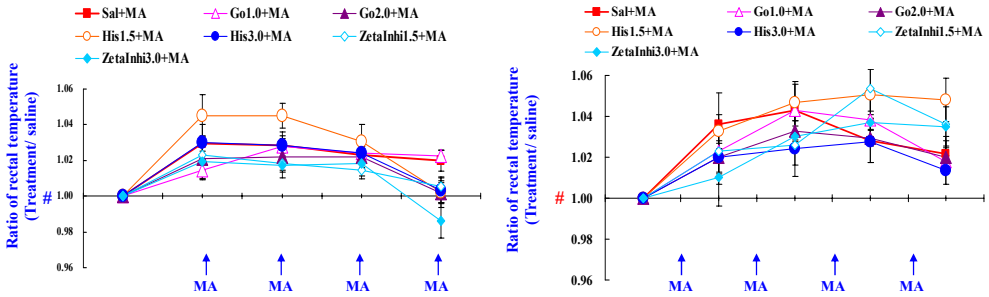
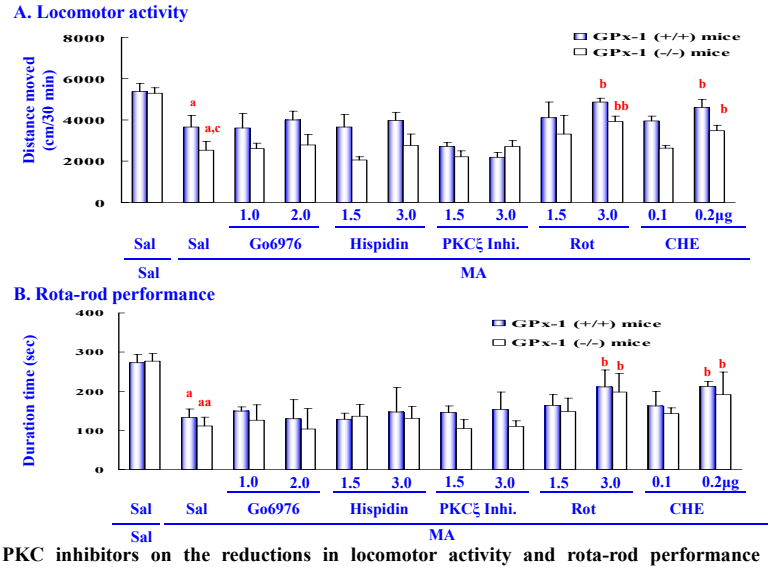


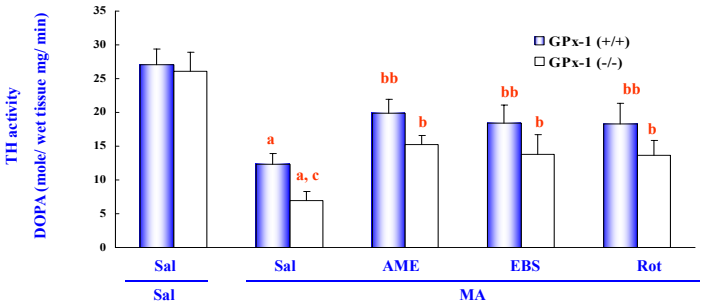
Effect of PKC inhibitors on the MA-induced hyperthermia in GPx-1 (+/+)- and GPx-1 (-/-)- mice. CHE = Chelerythrine chloride, a pan-PKC inhibitor (A and B). Rot = rottlerin, a PKCδ inhibitor (C and D). Each value is mean ± S.E.M. of 12 animals. Ambient temperature = 21±1 ^{0}C. $^{a}p<0.01$ vs. respective Sal + Sal, $^{b}p<0.01$ vs. respective Sal + MA (ANOVA for repeated measures followed by Bonferroni's test). # = Changes in rectal temperature (^{O}C) for 8 hours in saline-treated GPx-1 (+/+) mice are as follows; 36.6 ± 0.40, 36.07 ± 0.20, 36.72 ± 0.25, 36.6 ± 0.38 and 36.54 ± 0.16 and those of saline-treated GPx-1 (-/-) mice are as follows; 36.64 ± 0.20, 36.68 ± 0.20, 36.62 ± 0.23, 36.57 ± 0.18 and 36.41 ± 0.17 .



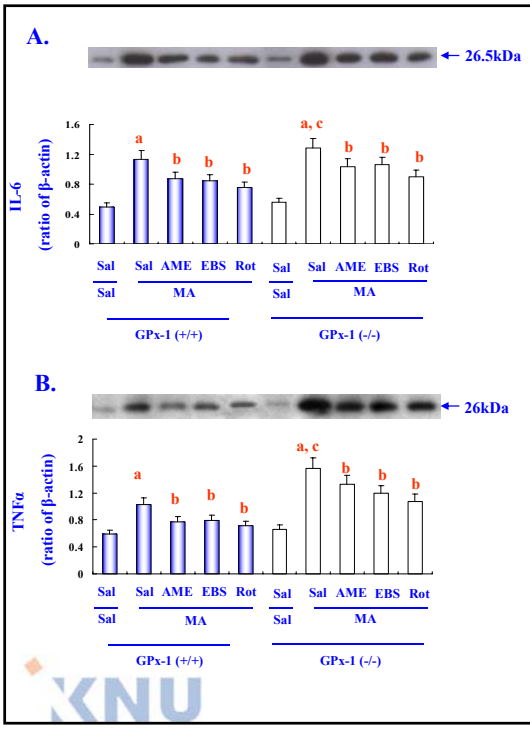
Effect of PKC inhibitors on the MA-induced rectal temperature in GPx-1 (+/+) (A) and GPx-1 (-/-) mice (B). Go = Go6976, PKCα and β inhibitor. His = Hispidin, PKCβ inhibitor. Zeta Inhi. = PKCξ pseudosubstrate inhibitor. Each value is mean ± S.E.M. of 12 animals. Ambient temperature = 21±1 ^{0}C. # = Changes in rectal temperature (^{O}C) for 8 hours in saline-treated GPx-1 (+/+) mice are as follows; 36.6 ± 0.40, 36.07 ± 0.20, 36.72 ± 0.25, 36.6 ± 0.38 and 36.54 ± 0.16; and those of saline-treated GPx-1 (-/-) mice are as follows; 36.64 ± 0.20, 36.68 ± 0.20, 36.62 ± 0.23, 36.57 ± 0.18 and 36.41 ± 0.17 .



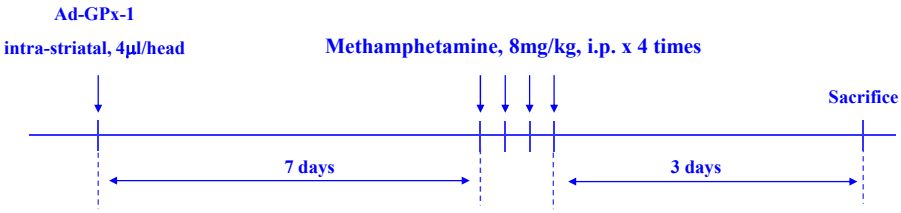
Effect of PKC inhibitors on the reductions in locomotor activity and rota-rod performance induced by methamphetamine (MA, 8mg/kg, i.p. x 4) in GPx-1 (+/+)- and GPx-1 (-/-)- mice. CHE = chelerythrine chloride, pan-PKC inhibitor, Go6976 = PKCα and β inhibitor, Hispidin = PKCβ inhibitor, PKCξ inhibitor = PKCζ pseudosubstrate inhibitor, Rot = Rottlerin, PKCδ inhibitor. Each value is mean ± S.E.M. of 8 animals. ^{a}p<0.05, ^{aa}p<0.01 vs. respective Sal + Sal, ^{b}p<0.05, ^{bb}p<0.01 vs. respective Sal + MA, ^{c}p<0.01 vs. Sal + MA of GPx-1 (+/+) mice (ANOVA followed by Fischer's PLSD test).



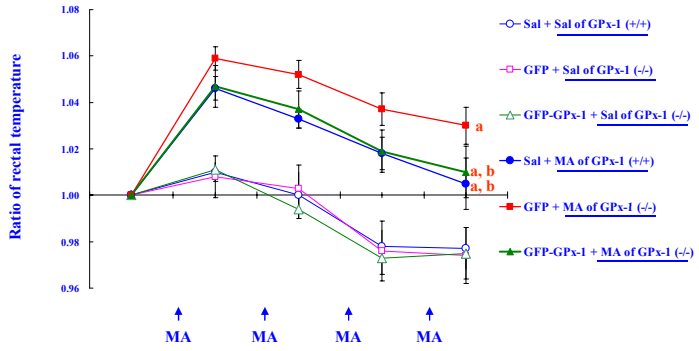
Effects of orally-administered acetylsalicylic maltol ester (AME, 50 mg/kg), ebselen (EBS, 20 mg/kg) or rottlerin (Rot, 20 mg/kg) on the reduction of tyrosine hydroxylase (TH) activity induced by MA in GPx-1 (+/+)- and GPx-1 (-/-)-mice. Sal = saline. Each value is the mean ± S.E.M of 6 animals. [a]p<0.01 vs. respective Sal + Sal, [b]p<0.05, [bb]p<0.01 vs. respective Sal + MA, [c]p<0.05 vs. Sal + MA of GPx-1 (+/+) mice (ANOVA followed by Fischer's PLSD test).



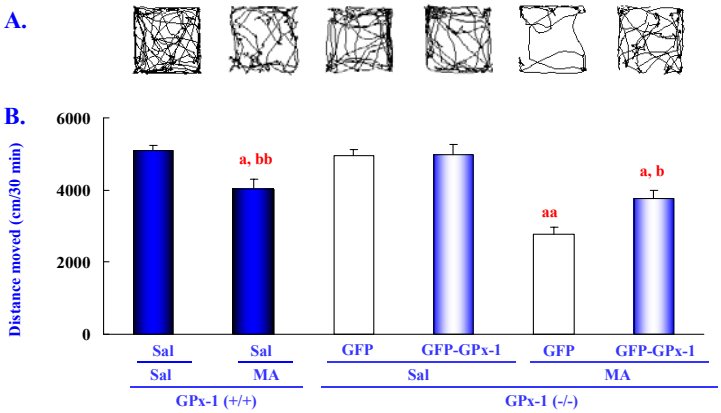
Effects of orally-administered acetylsalicylic maltol ester (AME, 50 mg/kg), ebselen (EBS, 20 mg/kg) or rottlerin (Rot, 20 mg/kg) on the IL-6, TNFα, and IFNγ expressions induced by MA in the GPx-1 (+/+)- and GPx-1 (-/-)-mice. Sal = saline. Each value is the mean ± S.E.M. of 4 animals. [a]p<0.01 vs. respective Sal + Sal, [b]p<0.05 vs. respective Sal + MA, [c]p<0.05 vs. Sal + MA of GPx-1 (+/+) mice (ANOVA followed by Fischer's PLSD test).



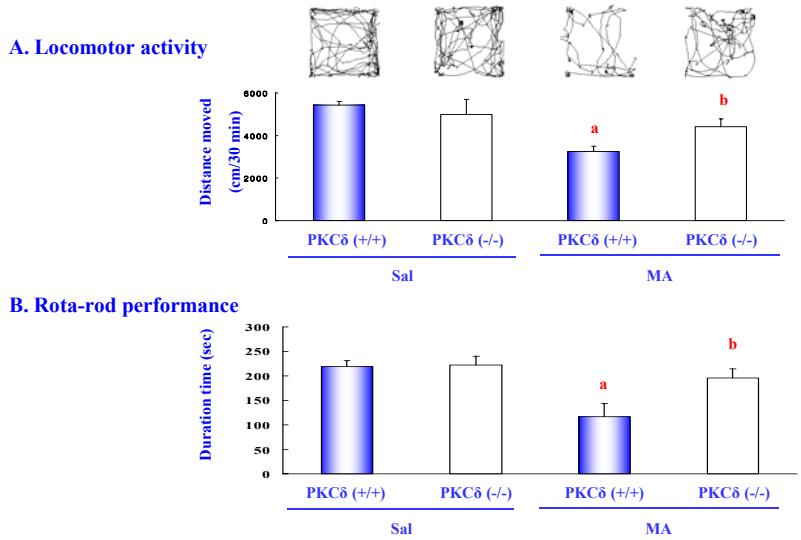
Experimental paradigm for intra-striatal administration of GPx-1 gene-encoded adenovirus vector on the MA-induced dopaminergic toxicity in GPx-1 (-/-) mice. Seven days after intra-striatal administration of GPx-1 gene-encoded adenovirus vector, MA (8mg/kg, i.p. x 4) was administered four times as 2 hours' interval.



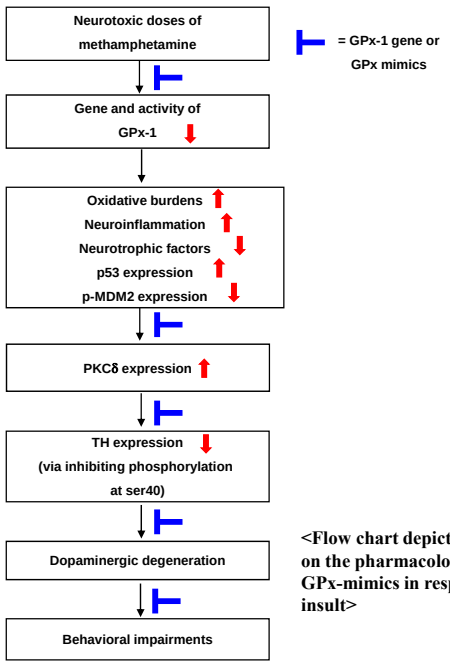
Effect of intra-striatal administration of GPx-1 gene-encoded adenovirus vector on the MA-induced hyperthermia in GPx-1 (-/-) mice. GFP = green fluorescent protein, GFP-GPx-1 = GPx-1 gene-encoded adenovirus vector. Starting rectal temperature (^{O}C) in each group is as follows; Sal + Sal of GPx-1 (+/+) = 37.5 ± 0.20, Sal + MA of GPx-1 (+/+) = 38.06 ± 0.12, GFP + Sal of GPx-1 (-/-) = 37.44 ± 0.42; GFP + MA of GPx-1 (-/-) = 37.17 ± 0.20, GFP-GPx-1 + Sal of GPx-1 (-/-) = 37.6 ± 0.10, and GFP-GPx-1 + MA of GPx-1 (-/-) = 38.01 ± 0.23. Each value is the ± S.E.M. of 8 animals. $^{a}p<0.05$ vs. respective Sal, $^{b}p<0.05$ vs. GFP + MA of GPx-1 (-/-) (ANOVA for repeated measures followed by Bonferroni's test).



Effect of intra-striatal administration of GPx-1 gene-encoded adenovirus vector on the MA-induced hypolocomotor activity (B) with tracing patterns (A) 3 days after the last MA injection in GPx-1 (+/+)- and GPx-1 (-/-)-mice. GFP = green fluorescent protein, GFP-GPx-1 = GPx-1 gene-encoded adenovirus vector. Each value is the ± S.E.M. of 8 animals. $^{a}p<0.05$, $^{aa}p<0.01$ vs. respective Sal, $^{b}p<0.05$, $^{bb}p<0.01$ vs. GFP + MA of GPx-1 (-/-) (ANOVA followed by Fischer's PLSD test).



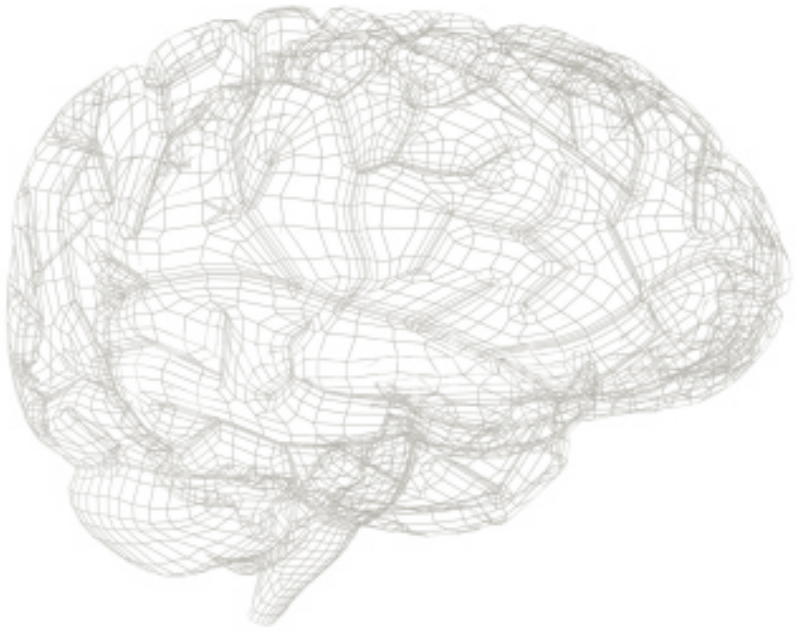
Locomotor activity with tracing patterns (A) and rota-rod performance (B) induced by methamphetamine (MA) in PKCδ (+/+)- and PKCδ (-/-)-mice. Each value is the ± S.E.M. of 10 animals. $^{a}p<0.05$ vs. respective Sal of PKCδ (+/+) , $^{b}p<0.05$ vs. MA of PKCδ (+/+) (ANOVA followed by Fischer's PLSD test).



<Flow chart depicting our working hypothesis on the pharmacological action of GPx-1 gene or GPx-mimics in response to methamphetamine insult>

Appendix

Session Ⅴ: Poster Session



P-1	dRich Independently Regulates Synaptic Growth and Postsynaptic Organization at the *Drosophila* Neuromuscular Junction	Seungbok Lee
P-2	DEN1 deneddylates non-cullin proteins in vivo	Jeongbin Yim
P-3	*twenty-four*, a Novel Clock Gene, is Required for Circadian Rhythms and PERIOD Expression in *Drosophila* Pacemaker Neurons	Joonho Choi
P-4	cAMP signalling in mushroom bodies modulates temperature preference behaviour in *Drosophila*	Jaeseob Kim
P-5	Essential role of AIMP in amyloid-β_{1-42} neurotoxicity and tau hyperphosphorylation	Yong-Keun Jung
P-6	Cereblon-evoked endoplasmic reticulum stress is critical in ischemic neuronal damage	Chul-Seung Park
P-7	Overexpression of a new anti-apoptotic protein protects motor neuron death and improves motor performance and mortality in a transgenic mouse model of ALS	Eui Ju Choi
P-8	Identification of ligands for orphan G protein-coupled receptor GPR92 and functional characterization of GPR92	Jae Young Seong
P-9	Molecular interaction between kisspeptin decapeptide and a lipid membrane	Jae Il Kim
P-10	Jab1 enhances cell survival through a direct interaction with 5-HT_6 receptors	Hyewhon Rhim
P-11	Identification of a Novel Enhancer for Tissue Specific Expression of the *Nestin* Gene in Neural Stem Cells	Haeyoung Suh-Kim
P-12	Identification and functional validation of direct regulatory targets of the transcription factor Sox2	Jaesang Kim
P-13	Fate choice of neural stem cells to cholinergic cells and regeneration in the memory-deficient rat model	Yunhee Kim Kwon
P-14	Regulation of the retinocollicular topography by endocytosis of the Eph-ephrin signaling complexes	Soo chul Park
P-15	*SRG3* (SWI3-related gene) in the mouse hippocampal neurogenesis	Hyeon Son
p-16	NMDARs is stabilized at the synapses by neuroligin-1 via its direct interaction with PSD-95	Joung-Hun Kim
P-17	Targeted disruption of mouse βPix gene results in early embryonic development failure and altered anxiety-related behavior	Dongeun Park
P-18	NFDP1, a highly enriched RGS for Gαs in brain, regulates G-protein coupled receptor signaling	Sunghoe Chang
P-19	Lysophosphatidic acid-induced survival of immortalized hippocampal progenitor cells via LPA receptor-mediated glycogen synthase kinase-3β inactivation	Sung-Oh Huh
P-20	Role of Immune Cells in Cerebral Ischemic Injury: Neuroprotective Effect of WCN-81	Won-Ki Kim
P-21	Enhanced expression of SOCS-2 in the rat hippocampus following transient forebrain ischemia	Mun-Yong Lee
P-22	The effect of VKORC1 and CYP2C9 genotyoe variation on warfarin dosing;Genetics on warfarin dosing in Korean stroke patients (GENWAKE) study	Jong Seong Kim
P-23	Transient receptor potential vanilloid subtype 1(TRPV1) rescues nigrostriatal dopamimergic neurons by inhibiting microglial activation in MPTP model of Parkinson's disease	Byung Kwan Jin

P-24	Elucidation of mechanisms for dopaminergic cell vulnerability and development of protective agents	Onyou Hwang
P-25	Molecular Interaction between Parkin and PINK1 in Mammalian Neuronal Cells	Kwang Chul Chung
P-26	Astrocytes in injury states rapidly produce anti-inflammatory factors to prevent excessive microglial inflammatory responses	Eun-hye Joe
P-27	Exploration of endophenotypes associated with pathophysiology of schizophrenia using multi-modal imaging techniques	Jun Soo Kwon
p-28	Surface-based analytical methods for multimodal MRI data and brain connectivity analysis, and application to the study of psychiatric disorders	Jong-Min Lee
P-29	Effect of neonatal treatment of MK-801 on ERK1/2-p70S6K-S6 signal pathway in the frontal cortex of developing rat brain with long-term behavioral changes	Yong Sik Kim
P-30	Potential role of TRPV1 receptors in drug addiction	Choon-Gon Jang
P-31	Mice lacking adenylyl cyclase-5 badly cope with restraint stress and show the anxiolytic-like behaviors	Pyung-Lim Han
P-32	Involvement of the hippocampal CAMK-IIα and ERK1/2 in the regulation of pain transmission in mice	Hong-Won Suh
P-33	Pro-inflammatory responses of spinal microglia induce the progressive reduction of morphine analgesic efficacy over time following nerve injury in rats	Heung Sik Na
P-34	Functional significance of TRPV1 in pain pathways: peripheral nociceptors and pre-/post-synaptic neurons	Seog Bae Oh
P-35	Increase of the spinal dehydroepiandrosterone sulfate (DHEAS) enhances nociceptive signaling: the involvement of sigma-1 receptor and $GABA_A$ receptor	Jang-Hern Lee
P-36	Prostaglandin E2 potentiated acetaldehyde-evoked nociception via TRPA1 and PKC dependent mechanism	Sun Wook Hwang
P-37	Anatomo-functional dysconnectivity of frontal-motor system in children with attention deficit/hyperactivity disorder	Dong Soo Lee
P-38	Where is a wake-promoting region in human brain? : rCBF changes by modafinil: a randomized double blind study	Seung Bong Hong
P-39	Language lateralization using MEG beta frequency desynchronization during auditory oddball stimulation with one-syllable words	Eun joo Kang
P-40	Monetary incentives can reduce the Amygdala activation from negative emotion faces	Min-Shik Kim
P-41	Hierarchy of cortical responses underlying binocular rivalry	Sang-Hun Lee
P-42	Harm Avoidance is associated with increased metabolism of a default network in the elderly	Jeanyung Chey
P-43	Sigma-1 receptor antagonist as a target for novel analgesics	Kee Won Kim
P-44	Optimization of sigma1 receptor antagonist using medicinal chemical approach	Lak Shin Jung
P-45	*In vitro and in vivo* Pharmacology of CGBI0472, a Dual Inhibitor against COX-2 and CAs for the Application to the Neuroinflammation Diseases	Ho-Jin Chang
P-46	*In vivo* performance evaluation of a totally implantable wireless neural signal transmission system (TiWiNets) for brain machine interface (BMI)	Hyung-Cheul Shin

P-47	Study on Cerebral Hemodynamics Using Near-Infrared Spectroscopy	Beop-Min Kim
P-48	Statistical standardization of EEG-based assessment tool for higher cognitive brain function	Jungmi Choi
P-49	Deep brain stimulation for neurpathic pain and dementia rat models	Jin Woo Chang
P-50	Optical measurement of neural activity in brain tissues	Sung June Kim
P-51	Arrayed microelectrodes for neurons to machine interface	Sang-Hoon Lee
P-52	Pyridoxine 5'-phosphate oxidase, not pyridoxal kinase, involves in long-term potentiation induction in the rat dentate gyrus	Soo-Young Choi
P-53	Oxidative-injury triggered autophagy in astrocytes	Jae-Young Koh
P-54	Genetic and Molecular Dissection of a Behaviour Circuit in *C. elegans*	Junho Lee
P-55	Mind Bomb 1-expressing intermediate progenitors generate Notch signaling to maintain radial glial cells	Young-yun Kong
P-56	A Novel mTOR Activator, Oxi-α Protects Dopamine Neurons By Repressing Autophagic Cell Death	Hyeung Jin Son
P-57	Intercellular propagation of α-synuclein pathology in Parkinson′s disease	Seung-Jae Lee
P-58	Inhibition of P25/CDK5 for Therapeutics of Alzheimer's Disease	Ilho Ha
P-59	A New Role of Calcineurin in Lifespan Regulation through Autophagy in *C. elegans*	Joohong Ahnn
P-60	Regional Cortical Thinning in Patients with Type 1 Diabetes: A Quantitative Morphometric Study of the Effects of Chronic Hyperglycemia on Brain	In Kyoon Lyoo
P-61	Glal-Derived Neurotrophic Factor is a direct target of transcriptional activation by Egr-1	Young Han Lee
P-62	Inositol 1,4,5-trisphosphate 3-kinase A functions as a scaffold for synaptic Rac signaling	Hyun Kim
P-63	Telomerase deficiency affects the functions of the olfactory bulbs and the hippocampus *in vivo*	Han-Woong Lee
P-64	Center For Brain Behavior Analysis Service (CBAS)	Hyun Taek Kim
P-65	Different expression of TASK-1 and -3 in electrically and pathologically proven hippocampal sclerosis	Chun Kee Chung
P-66	Anamorsin, an anti-apoptotic protein, is cleaved by caspase-3 during dopaminergic neuronal apoptosis	Young June Oh
P-67	Natural Products Bank	Young Choong Kim
P-68	New Brain Atlas: Ultra high resolution brain maps obtained by the 7.0 Tesla MRI and Cryomacrotome	Young-Bo Kim
p-69	Signal transformation in the primary visual cortex for perceptual decision	Choongkil Lee
P-70	Role of dentate gyrus in aligning cognitive map to external landmark	Min Whan Jung

dRich Independently Regulates Synaptic Growth and Postsynaptic Organization at the *Drosophila* Neuromuscular Junction

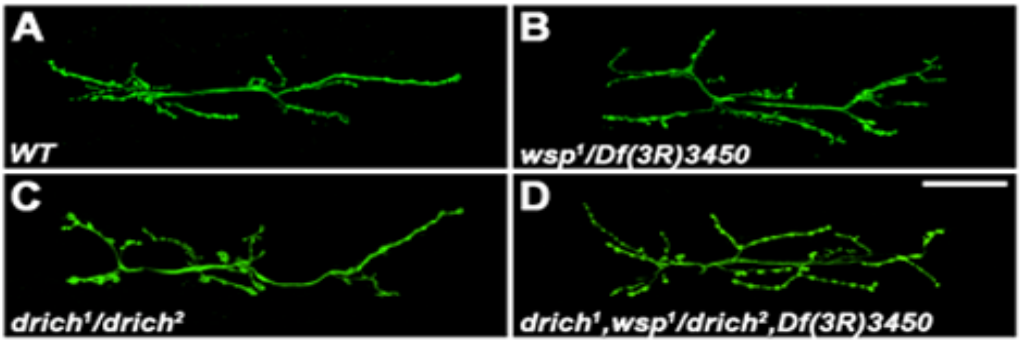
Seungbok Lee

Department of Cell and Developmental Biology, School of Dentistry, Seoul National University, Seoul, Korea

Background Synapses are specialized inter-cellular junctions required for communication between neurons and their targets. Once initially established, most synapses are remodeled in response to developmental programs and neuronal activity. Accumulating evidence suggests that actin, the major cytoskeleton in both pre- and postsynaptic compartments, plays an important role in the formation and morphological plasticity of synapses However, information on molecules and signaling pathways that regulate actin dynamics and organization at the synapse is still limited. *drich* encodes a protein that consist of an N-terminal BAR domain that is highly homologous to those of endophilin and amphiphysin, a central RhoGAP domain, and a C-terminal proline-rich domain (PRD). Here, we take the advantage of Drosophila genetics to study the role of dRich in synaptic growth at the neuromuscular junction (NMJ).

Results We show that the GTPase-activating protein (GAP) dRich promotes synaptic growth by down-regulating the postsynaptic Cdc42-Wsp pathway at the NMJ. dRich has selective GAP activity toward Cdc42 and is localized postsynaptically at the NMJ. Loss of dRich causes a significant reduction in the proliferation of synaptic boutons, while postsynaptic over-expression of dRich has the opposite effect. The synaptic undergrowth phenotype in *drich* is rescued by postsynaptic expression of wild-type dRich, but not by a GAP-deficient mutant. Moreover, *drich* bouton phenotypes are dominantly suppressed by mutations in *wsp*, which encodes a downstream effector of Cdc42. Consistent with this genetic interaction, dRich inhibits the recruitment of Wsp to Cdc42-interacting protein 4 (CIP4), a postsynaptic adaptor that binds the active form of Cdc42.
We also show that dRich regulates postsynaptic structure and organization independently of Cdc42. *drich* mutant NMJs display defects in the distribution of the PDZ protein Dlg and the scaffold spectrin, two postsynaptic proteins that play critical roles in the development of the postsynaptic specialization called the subsynaptic reticulum (SSR). Consistent with this result, the organization of the SSR in *drich* mutants is significantly changed at the ultrastructural level. In addition, synaptic GluRIIB is not only increased but also mislocalized in *drich* mutants.

Conclusion Our findings suggest that divergent dRich signaling can contribute to the coordination of presynaptic growth and postsynaptic organization.



DEN1 deneddylates non-cullin proteins in vivo

Yaru Chan[1], Jeongsook Yoon[2], June-Tai Wu[1,3], Hyung-Jun Kim[2], Kuan-Ting Pan[4], **Jeongbin Yim[2]** and Cheng-Ting Chien[1]

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[2]School of Biological Sciences, Seoul National University, 56-1 Seoul 151-741, Korea
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Background Conjugation by the ubiquitin (Ub)-like protein (Ubl) modulates protein activity in diverse cellular processes. Ubls are synthesized as precursors and are proteolytically processed to expose the C-terminal Gly that forms an isopeotide bond with Lys in target proteins. This conjugation process is catalyzed sequentially by E1 activating enzymes, E2 conjugating enzymes and, in many cases, E3 ligases that recognize target proteins. Conjugation of SUMO (small Ub-like modifier, also known as Sentrin) regulates the activities of a large array of cellular proteins. The conjugated SUMO moiety can be removed by Sentrin-specific proteases (SENPs) that also process SUMO precursors. The other Ubl, Nedd8, modifies and activates cullin-RING Ub ligase (CRLs) complexes. The process of neddylation has also been shown to regulate several cellular proteins and among these are the tumor suppressor protein p53, the breast cancer-associated protein BCA3 and ribosomal proteins. However, the repertoire of neddylated proteins and the regulation of their neddylation are not known.

Results In this study, we show that the *Drosophila* DEN1 protease specifically interacts with Nedd8 and can cleave off the Nedd8 C-terminal tail, thus promoting the maturation of Nedd8. The DEN1 protease is also able to remove the Nedd8 moiety from conjugated cullin proteins in an in vitro assay. However, this deneddylation activity is not prominent when assayed in *DEN1* mutant extracts. Instead, we found the accumulation of several Nedd8-positive proteins of unknown identity in the *DEN1* mutants, Accumulation of these proteins requires the Nedd8-E1 activation enzyme, indicating the involvement of the neddylation process. We further show that these neddylated proteins are distinct from neddylated cullin proteins that appear in *CSN* mutants, suggesting that DEN1 and CSN activities are largely non-overlapping in vivo. The purified DEN1 protein but not the enzymatic-dead DEN1 mutant can deplete the accumulation of these neddylated proteins, supporting the activity of DEN1 in clipping the Nedd8 moiety off non-cullin protein. Finally, genetic suppression of *Nedd8* mutant lethality by the *DEN1* mutation implicates neddylation of non-cullin proteins as being important to animal viability.

Conclusion This study confirms the existence of many neddylated non-cullin proteins in vivo. SUMO conjugation modulates cellular activities of numerous target proteins, It is proposed that transient SUMO modification could have a long-lasting effect on target proteins. In the absence of the DEN1 activity, the accumulation of neddylated proteins suggests that many cellular proteins are indeed neddylated; these proteins may exist transiently or in a small fraction in Wild-type cells. As inferred from our genetic analyses, balanced neddylation and deneddylation by DEN1 on proteins play an essential role in animal viability. The spectrum of neddylated proteins whose Nedd8 moieties are efficiently removed by the evolutionarily conserved DEN1 has probably been underestimated and the effects of neddylation on target proteins await further exploration.

twenty-four, a Novel Clock Gene, is Required for Circadian Rhythms and PERIOD Expression in *Drosophila* Pacemaker Neurons

Jongbin Lee[1], Chunghun Lim[2], Ravi Allada[2], **Joonho Choe[1]**

[1]Department of Biological Sciences, KAIST, Daejeon, Korea
[2]Department of Neurobiology and Physiology, Northwestern University, U.S.A

Background The Earth's rotation causes daily environmental changes such as light and temperature. In such an environment, most of living organisms have evolved cell-autonomous, endogenous circadian clock system which consists of three parts- input pathway, core clock and output pathway. The core clock resides in a small subset of neurons and synchronizes circadian clock from peripheral tissues. At the molecular level, it is composed of two interlocked transcriptional feedback loops in which core clock genes are rhythmically expressed. *Drosophila* has proved to be a valuable system for studying the circadian clock. Locomotor activities and eclosion rhythms are representative behaviors of *Drosophila* circadian system. To identify genes involved in the circadian rhythm of *Drosophila*, we performed ectopic-expression screening using the locomotor monitoring system and identified several candidates.

Results CG4857, which is one of candidates, resulted in a long locomotor period when overexpressed by timeless specific GAL4. We designate this gene as *twenty-four* (*twf*, 4+8+5+7=24). We have two strong hypomorphic *twf* alleles,. *twf[e]* allele has piggyBac insertion in the first intron, while *twfΔ* allele includes ~2.5 kb deletion generated by imprecise excision of P element in a *twf* locus. *twf* hypomorphic alleles showed weak but long periods. *twf* knockdown in clock cells mimics the circadian phenotype of *twf* hopomorphic alleles.

-*twf* transcript level in wild-type fly heads was relatively constant at different time-points under LD cycles. Moreover, it was not affected in clock mutant flies, suggesting that *twf* gene transcription is not circadian clock-controlled.

We constructed a GAL4 line driven by *twf* gene promoter region from -3.0 kb to +0.5 kb and visualized its spatial expression using UAS-GFP transgene. The co-staining with anti-PER antibody revealed that *twf*-GAL4 is strongly expressed in PDF+ LN_vs and weakly in dorsal LNs.

Poor rhythms are accompanied by dramatically reduced PERIOD protein levels in pacemaker neurons. *twf* expression in the pacemaker ventral lateral neurons is sufficient to rescue free-running locomotor rhythms as well as PER oscillation. These effects on PER are specific as other clock proteins, CLOCK, CLOCKWORK ORANGE, PDP1, and PDF were not comparably affected.

PER expression driven by a heterologous promoter in S2 cellswas increased or decreased by TWF overexpression or *twf* knockdown, respectively.

Together these data demonstrate that the TWF-PER interaction may facilitate PER accumulation and sustain circadian behavioral rhythms.

Conclusion

- *twf* is a novel component in *Drosophila* circadian clock system required for robust behavioral rhythm under free-running condition.
- *twf* is functionally expressed in PDF+ pacemaker neurons.
- PER oscillation in pacemaker cells requires *twf* expression.
- *twf* may post-transcriptionally regulate PER expression.

cAMP signalling in mushroom bodies modulates temperature preference behaviour in *Drosophila*

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Background Homoiotherms, for example mammals, regulate their body temperature with physiological responses such as a change of metabolic rate and sweating. In contrast, the body temperature of poikilotherms, for example *Drosophila*, is the result of heat exchange with the surrounding environment as a result of the large ratio of surface area to volume of their bodies. Accordingly, these animals must instinctively move to places with an environmental temperature as close as possible to their genetically determined desired temperature. The temperature that *Drosophila* instinctively prefers has a function equivalent to the 'set point' temperature in mammals. Although various temperature-gated TRP channels have been discovered molecular and cellular components in *Drosophila* brain responsible for determining the desired temperature remain unknown.

Results We identified these components by performing a large-scale genetic screen of temperature preference behaviour (TPB) in Drosophila. In parallel, we mapped areas of the Drosophila brain controlling TPB by targeted inactivation of neurons with tetanus toxin and a potassium channel (Kir2.1) driven with various brain-specific GAL4s. Here we show that mushroom bodies (MBs) and the cyclic AMP–cAMP-dependent protein kinase A (cAMP–PKA) pathway are essential for controlling TPB. Furthermore, targeted expression of cAMP–PKA pathway components in only the MB was sufficient to rescue abnormal TPB of the corresponding mutants. Preferred temperatures were affected by the level of cAMP and PKA activity in the MBs in various PKA pathway mutants.

Conclusion cAMP/PKA signalling in the MB is necessary and sufficient for normal TPB, whereas normal levels of cAMP/PKA signalling in other parts of the body are dispensable. Our results raise the question of whether temperature sensation and interpretation require learning and memory (LM) processes. Our data do not exclude this possibility, but there are some differences between LM and TPB. This implies that temperature sensation and interpretation might share molecular and cellular mechanisms with learning and memory, but they do not use identical processes.

Essential role of AIMP in amyloid-β_{1-42} neurotoxicity and tau hyperphosphorylation

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Background Accumulation of amyloid-β (Aβ) in the brain is believed as the primary influence driving Alzheimer's disease (AD) pathogenesis, though its pathogenic mechanisms are poorly understood. To understand molecular mechanisms of Aβ-induced neuropathology, we performed the DNA microarray analysis and isolated a novel Aβ interacting membrane protein (AIMP) as a neuronal target protein of $A\beta_{1-42}$.

Results AIMP expression is strongly increased in Aβ-positive neurons in the brain of Tg2576 mice and AD patients. AIMP-deficient neurons are completely resistant to $A\beta_{1-42}$ neurotoxicity. Also, $A\beta_{1-42}$ potently binds to AIMP *in vitro* and Aβ neurotoxicity is blocked by synthetic peptides of AIMP interfering the interaction of AIMP and $A\beta_{1-42}$. Further, we isolated three AIMP-associated kinases (AAK1, 2, 3) which mediated cytotoxicity caused by $A\beta_{1-42}$ and AIMP agonistic antibody (K1). In addition, forced expression of AIMP and K1 antibody induced the accumulation of hyper-phosphorylated tau, a major component of neurofibrillary tangles, via an AAK1-dependant manner.

Conclusion Our results represent that AIMP is required for major pathogenic aspects of AD, neurodegeneration and tau hyper-phosphorylation caused by Aβ as a neuronal receptor. Moreover, the AAKs acting as critical mediators of AIMP/Aβ signaling are essential for tau hyperphosphorylation. Thus, AIMP and AAKs should be understood for AD therapeutic applications at the view of neurodegeneration and tau hyper-phosphorylation caused by Aβ.

Cereblon-evoked endoplasmic reticulum stress is critical in ischemic neuronal damage

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Background Autosomal recessive, non-syndromic mental retardation (ARNSMR) is defined as a type of mental retardation affecting 1-3% of the general population and individuals typically exhibit an intelligence quotient (IQ) of 50-70. Currently, five genes linked to ARNSMR have been identified and one mutation, in cereblon (CRBN), results in premature termination in the carboxyl-terminal region (C1274T CRBN mutation) in ARNSMR individuals. CRBN has been reported to modulate surface expression of the rat BK_{Ca} channel alpha-subunit, rSlo, and functional channel formation. However, how CRBN function is affected by the ARNSMR-associated mutation is completely unknown.

Results When neuronal cells were treated with various neurotoxic agents, hydrogen peroxide was found to potently induce CRBN expression. The induction of CRBN was further examined using an *in vivo* animal model of global cerebral ischemia by transient occlusion of the common carotid arteries. Neuronal loss induced by ischemia was detected by Nissl staining and robust induction of CRBN was concomitantly observed in the CA1 region of the rat hippocampus.
CRBN knockdown cells were highly resistant to H/R-induced cell death, suggesting that CRBN is required for H/R-induced neurotoxicity. In addition, expression of several proteins correlated with ER stress was examined in the CRBN KD cells. Induction of both the neuroprotective GRP78 and the neurotoxic GADD153/CHOP, a pro-apoptotic C/EBP homologue potently mediating neurotoxicity of ER stress in brain ischemia, were significantly suppressed in the CRBN knock-down cells. For further validation of the role of CRBN in ER stress and neuronal loss by H/R, we examined primary cultured neurons using lentivirus harboring CRBN shRNA (lenti-shCRBN). The lenti-shCRBN-infected neurons were highly resistant to H/R-induced toxicity.
Mouse hippocampal HT22 cells expressing CRBN showed pronounce cell shrinkage and nuclear condensation, and CRBN fused to red fluorescent protein (CRBN-RFP) co-localized with GRP78 in the perinuclear region. The activation of ER stress by CRBN was further assessed in terms of neuronal death. Cell death by CRBN expression was potently prevented by treatment with 50 μM salubrinal, an inhibitor of eIFα dephosphorylation known to protect cells from ER stress, while it was partially suppressed by treatment with z-VAD-fmk, a pan-caspase inhibitor, and calpeptin, a calpain inhibitor. Transient expression of CRBN in HT22 cells resulted not only in nuclear condensation but also in a strong accumulation of polyubiquitinated proteins. Hypoxia-ischemia also induced the accumulation of poly-ubiquitin deposits, which was effectively inhibited in CRBN KD cells.
To understand whether CRBN affects proteasome activity, we measured intracellular proteasome activity using green fluorescent protein fused to the CL1 degron, which has been used to analyze proteasome activity in mammalian cells. The number of degron-positive B103 cells increased more than 4-fold when cells were co-transfected with CRBN, compared to mock-transfected controls, further suggesting that CRBN can suppress proteasome activity in combination with inducing ER stress.

Conclusion CRBN has a crucial role in regulating ER stress and the ER-associated function of CRBN should be considered for potential therapeutic intervention against H/R damage and mental retardation.

Overexpression of a new anti-apoptotic protein protects motor neuron death and improves motor performance and mortality in a transgenic mouse model of ALS

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Background Mutated Cu/Zn-superoxide dismutase 1 (SOD1) contributes to degeneration of motor neurons in an animal model and possibly patients with motor neuron diseases such as amyotrophic lateral sclerosis (ALS). Previously, we described a newly developed anti-apoptotic protein, which effectively reduced oxidative stress-induced apoptosis in many types of cultured cells by blocking the stress-activated protein kinase signaling pathways.

Results We set out experiments to examine if the protein would alleviate the loss of motor neuron in transgenic mice overexpressing SOD1 mutation. Before symptom onset, we detected a significant down-regulation of the protein in motor neurons of the SOD1 mutant mice. Genetic manipulation of the anti-apoptotic protein improved motor neuron survival and delayed disease onset and motor defect. In addition, it had significant effect on extension of life expectancy.

Conclusion The present findings suggest that the novel anti-apoptotic protein plays a central role in the early stages of chronic motor neuron death.

Identification of ligands for orphan G protein-coupled receptor GPR92 and functional characterization of GPR92

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Background The superfamily of G protein-coupled receptors (GPCRs) is the largest and most diverse group of membrane-spanning proteins. It plays a variety of roles in pathophysiological processes by transmitting extracellular signals to cells via heterotrimeric G proteins. Completion of the human genome project revealed the presence of ~ 168 genes encoding established transmitter GPCRs, as well as 207 genes predicted to encode novel GPCRs for which the natural ligands remained to be identified, the so-called 'orphan' GPCRs. Eighty-seven of these orphans have now been paired to novel or previously known molecules, and 120 remain to be deorphaned. Recently, we screened a series of small compounds acting at the orphan G protein-coupled receptor GPR92 using a signaling pathway-specific reporter assay system.

Results Lipid-derived molecules including farnesyl pyrophosphate (FPP), and N-arachidonylglycine (NAG) and lysophosphatic acid (LPA) were found to activate GPR92. Computer-simulated modeling, combined with site-directed mutagenesis of GPR92, indicates that Thr^{97}, Gly^{98}, Phe^{101} and Arg^{267} of GPR92 are responsible for the interaction of GPR92 with FPP and NAG. RT-PCR analysis revealed that GPR92 mRNA is highly expressed in the dorsal root ganglia (DRG) but faintly in other brain regions. Peripheral tissues including, spleen, stomach, small intestine, and kidney also express GPR92 mRNA. Immunohistochemical analysis revealed that GPR92 is largely co-localized with TRPV1, a nonspecific cation channel that responds to noxious heat, in mouse and human DRGs. FPP and NAG increase intracellular Ca^{2+} levels in cultured DRG neurons.

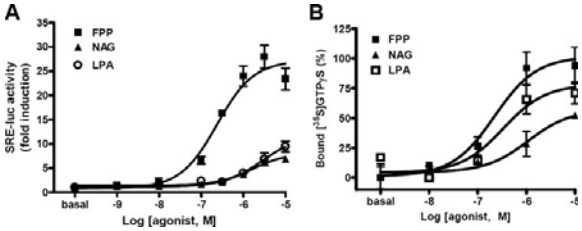

FIGURE 1. Response of GPR92 to FPP and NAG

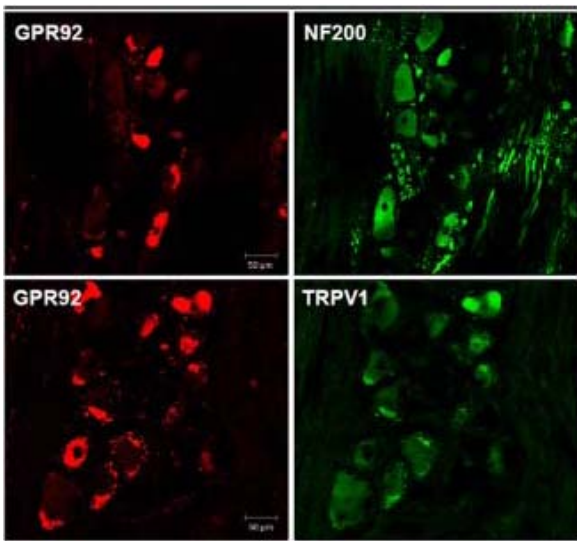

FIGURE 2. Immunohistochemistry of GRP92 in the human DRG

Conclusion We have demonstrated that multiple lipid-derived molecules can activate GPR92 with different potencies. The presence of multiple ligands for GPR92 portends a wide range of biological and medicinal roles for this receptor. The roles of FPP and NAG through activation of GPR92 in peripheral tissues and the DRG require further investigation.

Molecular interaction between kisspeptin decapeptide and a lipid membrane

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Background G protein-coupled receptors (GPCRs) are the largest family of cell surface membrane receptors and regulate a variety of physiological responses by mediating transmission of extracellular signals into cells. GPR54 is a GPCR family member that is widely expressed in the central nervous system and shares about 45% identity with the galanine receptor. Galanine, however, does not activate or even bind to GPR54. Instead, kisspeptin (also known as metastin), a 54-amino acid peptide fragment of the protein encoded by the *KiSS-1* metastasis suppressor gene, appears to be the cognate ligand for GPR54. It also was previously reported that the 10 C-terminal residues of kisspeptin (kisspeptin-10; Tyr45-Phe54) are critical for specific binding to GPR54 and exhibit agonist activity comparable to that of wild-type kisspeptin. Although it is known that GPR54 is a membrane-embedded protein, details of the molecular interaction between kisspeptin and membrane lipids remain unknown. Our aim in the present study was to assess whether the interaction between kisspeptin-10 and GPR54 is consistent with the "membrane compartment theory," proposed by Sargent and Schwyzer, who suggested that the membrane induces a ligand to assume a more ordered conformation preferable for interaction with the binding site on its receptor.

Results We performed functional and structural analyses using a set of alanine-scanning analogs to characterize the contribution made by each side-chain of kisspeptin-10 to its activity. We found that there is a strong correlation between lipid membrane binding and agonist activity. For instance, the F10A and non-amidated (NH_2→OH) analogs showed little or no GPR54-agonist activity and elicited no blue shift in tryptophan fluorescence. NMR analysis of kisspeptin-10's solution structure in the presence of DPC micelles revealed it to contain several tight turn structures, encompassing residues Trp3 to Phe10, but no helical conformation like that seen previously with SDS micelles (Fig.1).

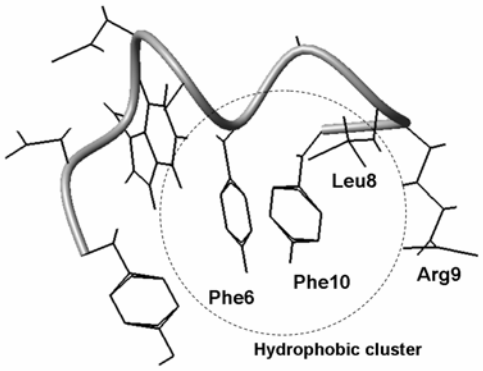

Fig.1. Model of the structure having the lowest energy value among the 22 converged structures showing the orientation of side chains. The important C-terminal residues are marked as the hydrophobic cluster.

Conclusion Our finding that there is a strong correlation between the membrane binding of kisspeptin-10 analogs and their agonist activity implies that endogenous kisspeptin likely activates GPR54 via a two-step ligand transportation mechanism.

Jab1 enhances cell survival through a direct interaction with 5-HT_6 receptors

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Background The human 5-HT_6 receptor (5-HT_6R), a Gs protein-coupled receptor for serotonin, is one of the latest cloned receptors among the known 5-HT receptors. To data, most findings suggest that 5-HT_6 receptors play a crucial role in the control of mood and emotion, and memory formation, but also contribute to the pathogenesis of neurological disorders, including learning and memory disorders, depression and Alzheimer disease. Nevertheless, the cellular mechanisms responsible for 5-HT_6R-mediated physiological responses are poorly elucidated. Therefore, in order to identify the function of 5-HT_6R and its cellular mechanisms in the CNS, we employed a yeast two-hybrid screen system on a human brain cDNA library and found Jab1 (Jun activation domain-binding protein 1) is bound to 5-HT_6R. Jab1 has been known to play roles in multiple intracellular signaling pathways, including regulation of the cell cycle by degrading p27 and p53, gene transcription by stabilizing c-Jun/DNA complex, and increase in HIF-1α stability under hypoxia conditions.

Results In the present study we found, using a yeast two-hybrid assay, that the intracellular loop 3 (iL3) and carboxyl-terminal region (CT) of 5-HT_6R interact with Jab1. Using a GST-pull down assay, we determined that the CT and the iL3 of 5-HT_6R interact with the N-terminal region (NT) and the CT of Jab1, respectively. We also confirmed the interaction by GST-pull down, FRET, and co-immunoprecipitation assays in transfected cell lines as well as in adult rat brains. Immunocyto(histo)chemistry also showed prominent co-localization between 5-HT_6R and Jab1 in transfected cell lines, primary neurons, and a similar distribution between 5-HT_6R and Jab1 in the rat brain. Based on these results, we next examined the functional and reciprocal modulation between these two proteins. In detail, we demonstrated 5-HT_6R- and Jab1-mediated-signal pathways and 5-HT_6R- and Jab1-mediated cell protection *in vitro* and *in vivo* studies.

Conclusion We demonstrated the specific role of Jab1 in the modulation of 5-HT_6R and its cellular signaling pathways via a direct interaction, and the correlation between 5-HT_6R and Jab1 under the hypoxia/ischemia conditions. These results provide new insights into the cellular level and the physiological roles of 5-HT_6R and Jab1 in the central nervous system.

Identification of a Novel Enhancer for Tissue Specific Expression of the *Nestin* Gene in Neural Stem Cells

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Background Nestin, an intermediate filament protein is considered as a hallmark of the neural stem cells during embryogenesis. Several studies have suggested that several *cis*- and *trans*-elements are responsible for the tissue specific expression of the nestin gene in the developing central nervous system.

Results In this study, we identified a novel E-box element that regulates tissue specific expression of the nestin gene in neural precursor cells. Mutation of E-box eliminated tissue specific expression of the LacZ gene that was driven by the nestin second intron in neural precursor cells. Proneural basic helix-loop-helix (bHLH) transcription factors such as Ngn1, Ngn2 and Mash1 could bind to this E-box during embryonic development.

Conclusion The data indicate critical roles of proneural bHLH transcription factors in regulating timely expression of the nestin gene in neuronal progenitor cells, and further suggest heterogeneous population of nestin-expressing precursor cells in the nervous system.

Identification and functional validation of direct regulatory targets of the transcription factor Sox2

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Background Transcription factors have emerged as a key tool in regulated induction of differentiation and fate determination of cells. Recently reported landmark studies including generation of induced pluripotent stem cells and transdifferentiaion of pancreatic β cells from pancreatic exocrine cells have been possible via combinatorial expression of transcription factors. In most cases, however, the function of transcription factors is understood only at the cellular or organism level.
Sox2 is a member of the Sox transcription factor family whose defining characteristics is the HMG-box DNA binding domain. Although it is well-known for its expression and function in embryonic stem cells, Sox2 is also expressed in neural stem cells and the ventricular zone of developing nervous system. The role of Sox2 in neural tissues is to prevent premature neuronal differentiation and maintain the growth of cells.

Results Our goal was to identify direct regulatory targets of Sox2, a key determinant and regulator of neural stem cells and neural development. We performed a ChIP-on-chip assay using human neural stem cell line REN cells which expresses Sox2 at a high level. Genomic DNA bound to Sox2 were immunoprecipitated and applied to a microarray chip on which oligonucleotides representing genomic DNA sequences near the promoter of human genes are present. We defined top 100 candidate sites and thus the target genes that likely are bound by Sox2.
We chose Sox6, ranked #2, for further analysis. Comparative genomic analysis showed that five highly conserved potential Sox2 binding sites are present within 200 base pair region. We confirmed a strong and specific binding between Sox2 and these sites by chromatin immunoprecipitation assay. Furthermore, we designed various luciferase based reporter constructs with Sox6 promoter and demonstrated that 4 of them strongly affect Sox2-driven Sox6 expression.
A series of experiments to confirm our finding in vivo has been initiated using in ovo chicken eletroporation system. Expression vectors encoding Sox2 and constitutively active and dominant negative derivatives of Sox2 will be introduced to developing neural tube of chicken embryos. After electroporation, the effect of the overexpression will be assessed by examining the neural tube with various markers for neurons and glial cells as well as Sox6 either by immunohistochemistry or by RNA in situ hybridization. We also plan to examine the epistatic relationship between Sox2 and Sox6 by co-expression .

Conclusion Our results led to identification of numerous potential direct regulatory targets of Sox2. The strategy has been validated by functional assays showing that Sox6 is indeed regulated by Sox2 through conserved cis-elements present in the Sox6 promoter region. It is envisaged that defining regulatory relationship between Sox2 and target genes and determining the role of target genes will reveal the functional network of genes that in turn govern neural stem cells and neural development.

Fate choice of neural stem cells to cholinergic cells and regeneration in the memory-deficient rat model

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Background Fate choice of neuronal cells is believed to be regulated by transcription factors and chromatin modification factors. Delicate control of the expressions of such factors is probably orchestrated by signaling of growth factors, kinase, and transcription cofactors in time and dosage dependent manners in developmental programs.
We screened transcription factors affecting development of cholinergic, glutamatergic, and GABAergic neurons. We found overexpression of homeodomain transcription factor, pax6 and bHLH factors NeuroD and Ngn1 is effective to induce ChAT+, VGluT1+ and GAD+ cells, respectively. In the nervous system, Pax6 is expressed in the future motor neurons in the developing spinal cord and are reported to be phosphorylated by homeodomain interacting kinases2 (HIPK2). We are interested in the mechanism controlling induction of cholinergic fate by HIPK2 and Pax6. We investigated the expression of downstream genes by microarray analysis and by using HIPK2 KO mice and pax6-transgenic mice. We also examined the role of chromatin remodeling factors in the fate choice of neural cells and pax6 on regeneration of neural stem cells transplanted into the hippocampus of the memory deficient rat model.

Results To evaluate the effect of Pax6 and HIPK2 on the transcriptional activation of Hes1, we used a luciferase reporter assay. When we infected adenoviruses of Pax6 and HIPK2 with Hes1 promoter, the expression of hes1 was increased. Interaction of Pax6 and HIPK2 was shown by co-immunoprecipitation and binding of Pax6 to hes1 promoter was detected by chromatin immunoprecipitation. Protein and mRNA levels of Hes1 were upregulated by Pax6 or HIPK2 wildtype-adenovirus in a neuronal stem cell line, HiB5, while mutant type of those proteins upregulated the mRNA and protein expression of Mash1, which is well known to be repressed by Hes1. We also found overexpression of HIPK2 and Pax6 facilitated neural stem cells to differentiate into cholinergic cell fate.
To determine whether Pax6, HIPK2 and Hes1 participates fate choice in embryonic neurogenesis, we investigated the expression patterns of neuronal transcription factors in the mouse embryo. Hes1 protein is expressed in the SVZ in the embryo and HIPK2 and Pax6 were merged in the ChAT+ cells in wild type mouse while the expression of Hes1 and ChAT was reduced and expression of Mash1 and GAD+ cells increased in the HIPK2 KO mouse. Pax6-GFP signal was also merged with ChAT expression in pax8-GFP transgenic mouse.
Next we asked whether Pax6 and HIPK2 influence the fate of neuronal cells in hippocampus. We performed immunostaining with Pax6 transgenic mice and HIPK2 transgenic mice and the expression levels of ChAT, GAD and VGluT1 were investigated.
Finally, we transplanted Pax6 overexpressing NSCs in to the memory deficient rat model and found improved learning and memory through the behavior tests, such as Y-maze task and passive avoidance test. Six weeks after transplantation, Pax6 overexpressing cells were more differentiated into ChAT positive cells in the rat hippocampus.

Conclusion The interaction between Pax6 and HIPK2 plays an important role to regulate the Hes1 expressions and influence the fate choice of neuronal cells during development. Together with chromatin modification factors, these transcription factors and neurotrophic factors regulate the fate choice of neural stem cells during neurogenesis and improve neuronal regeneration.
This research was supported by a grant (M103KV010008-07K2201-00810) from Brain Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology.

Regulation of the retinocollicular topography by endocytosis of the Eph-ephrin signaling complexes

Sooyeon Ryu, Yujin Kim and **Soochul Park**

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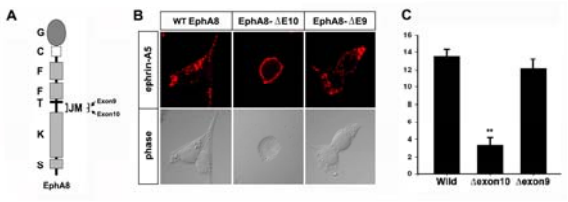
Background In the nervous system, a topographic map is common principle to organize various sensory inputs to the brain. Especially the retinocollicular projection has been the predominant model system for studying the developmental mechanism and the gradients of guidance molecules involved in the formation of topographic maps. Eph receptors and ephrins are both membrane-bound proteins and interact with one another at sites of cell-cell contract. The initial contact-mediated interaction between axon growth cone and target cell results in an adhesion between the ephrin and Eph; however, in many cases the contact subsequently promotes repulsion of the axon. With regards of the retinocollicular projection, axons with a high EphA receptor concentration (e.g., from the temporal retina) project to areas with a low ephrin-A concentration (e.g., the anterior SC), whereas axons with a high ephrin-A concentration (e.g., from the nasal reina) project to areas having low EphA concentrations (e.g., the posterior SC). Recent findings suggested that one mechanism responsible for this repulsive response is endocytosis, allowing detachment and retraction of contacted cells by eliminating ligand-receptor complexes from the cell surface. Although it is well known that repulsive effects of Eph/ephrin signaling is central to neuronal circuit formation, there has been no experimental evidences to account for the functional relevance between the endocytosis of Eph-ephrin complexes and the contact-mediated repulsion of them in vivo.

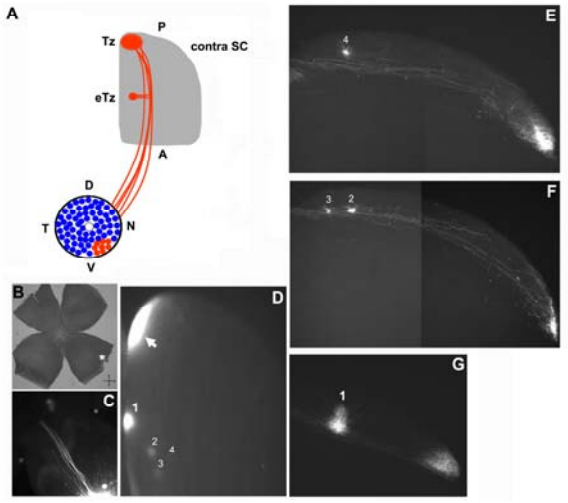
Results Here we show that EphA8 receptor is internalized by ephrinA5 ligand stimulation via clathrin-mediated mechanism. Interestingly, a specific region in the juxtamembrane domain of EphA8 encoded by exon10 of ephA8 is absolutely required for the endocytosis of EphA8-ephrinA5 complexes (*Fig.1*). Importantly, it is found that t he level of GTP-bound Rac is significantly reduced in cells

Fig.1



expressing the EphA8 mutant region. To further illustrate the role of the endocytosis-defective EphA8 mutant for the retinocollicular topography, we generated BAC transgenic mice expressing the endocytosis-defective EphA8 mutant. Anterograde tracing experiments revealed that retinocollicular topographic mapping is disturbed in mice expressing the endocytois-defective EphA8 mutant (*Fig.2*), suggesting that this EphA8 mutant act as a dominant negative form for other EphA receptors expressed in the anterior region of the superior colliculus.

Fig.2



Conclusion Therefore, our results are consistent with a hypothesis that endocytosis of Eph-ephrin complexes is one of the mechanisms underlying the inhibitory responses between the growing retinal ganglion axonal branches and the superior collicular cells.

SRG3 (SWI3-related gene) in the mouse hippocampal neurogenesis

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Background Mammalian SWI/SNF chromatin remodeling complex has been demonstrated to act as an important intrinsic factor in the proliferation and differentiation of mammalian neural stem cells. *SRG3* (SWI3-related gene) is a newly found core subunit of SWI/SNF complex and known to play a critical role in stabilizing the SWI/SNF complex by attenuating its proteasomal degradation. Accordingly, we speculated the disturbance of *SRG3* expression might disorganize both proliferation and differentiation of neural stem cells, thus resulting in reduced neurogenesis. To study the relationship of *SRG3* with neurogenesis, we performed loss-of-function and gain-of-function experiments using lentivirus expressing shRNA for *SRG3* (Lenti-srg3i-GFP) and retrovirus overexpressing *SRG3* (Retro-srg3-GFP), respectively.

Results 1.Knockdown of *SRG3* decreases proliferation of mouse embryonic hippocampal neural progenitor cells

To overcome the less transfection efficiency of siRNA oligomers in neural progenitor cells, we employed lentivirus expressing shRNA for *SRG3* (Lenti-srg3i-GFP). The efficiency of Lenti-srg3i-GFP virus in downregulating SRG3 expression in mouse embryonic hippocampal neural progenitor cells was confirmed by RT-PCR and western blotting for *SRG3*. When neural progenitor cells from E16(embryonic day 16) mouse hippocampus were transfected with Lenti-srg3i-GFP viruses and then pulsed with BrdU for 1 h after expansion in the presence of bFGF plus EGF, the percentage of cells colabelled for BrdU and GFP among total GFP^+ cells was significantly reduced in Lenti-srg3i-GFP virus treated cells as compared to control lentivirus treated cells. (Control-lentivirus, 45.1%; Lenti-srg3i-GFP virus, 30.8%, P=0.0081, n = 10)

2. Overexpression of *SRG3* in mouse hippocampal neural progenitor cells has no significant effects on proliferation

We investigated whether overexpression of *SRG3* in mouse neural progenitor cells could affect neurogenesis using retrovirus overexpressing srg3. The efficiency of Retro-srg3-GFP virus was confirmed by RT-PCR and western blotting for *SRG3* as in Lenti-srg3i-GFP virus treatments. Proliferation assay was performed in the same way as the one using Lenti-srg3i-GFP virus.

In contrast to Lenti-srg3i-GFP virus treatment, overexpression of *SRG3* using retrovirus in mouse neural stem cells showed no significant change in the percentage of cells colabelled for BrdU and GFP among total GFP^+ cells as compared to control retrovirus treated cells (Control-retrovirus, 43.1%; Retro-srg3-GFP virus, 54.4% , P=0.0759; n = 12)

Conclusion *SRG3* appears to be involved in mouse hippocampal neurogenesis, at least the proliferation of mouse neural progenitor cells. We are now proceeding to further investigate the mechanism by which *SRG3* plays a role in proliferation of neural progenitor cells in the adult hippocampus *in vivo.* We are also examining whether neuronal differentiation of mouse hippocampal neural progenitor cells involves *SRG3*.

NMDARs is stabilized at the synapses by neuroligin-1 via its direct interaction with PSD-95

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Background A cell adhesion molecule, neuroligin-1 is critical for the specification of excitatory synaptic connections, and it has recently been suggested that neuroligin-1 is required for sustaining NMDAR-mediated current in mature excitatory synapses. Despite evidence for a decrease in NMDAR-mediated currents derived by deletion or depletion of neuroligin-1, the underlying molecular mechanisms remain to be known.

Results We found that neuroligin-1 is physically bound to either NR2A or NR2B in heterologus expression system and also in synaptosomes prepared from adult animals. Surprisingly, the co-transfection of PSD95 significantly enhanced the interaction of neuroligin-1 and NR2A or NR2B as compared with in the absence of PSD95. Unlike PSD95, synaptic scaffolding molecule (S-SCAM), however, hardly enhanced the binding efficacy of neuroligin-1 and NR2A or NR2B. These results indicate that specific cooperation of PSD95 and neuroligin-1 facilitates the synaptic stabilization of NR2A and NR2B. Subsequent physiological studies exhibit that the lateral diffusion of NMDAR is significantly affected in neuroligin-1-depleted hippocampal neurons.

Conclusion These results support the notion that persistent expression of neuroligin-1 and its interaction with PSD95 stabilize NMDARs at the synapses, which contributes to NMDAR-dependent synaptic transmission in the mature excitatory synapses.

Targeted disruption of mouse βPix gene results in early embryonic development failure and altered anxiety-related behavior

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Background Cytoskeleton rearrangement is an essential step for a cell to act diverse biological functions, such as migration, proliferation and differentiation. The major signaling molecules that regulate cytoskeletal remodeling are Rho family small GTPases. These GTPases act as molecular switches, being inactive when bound to GDP and active when bound to GTP. Guanine nucleotide exchange factors (GEFs) act as positive regulators of GTPases by dissociation of bound GDP and subsequent replacement with GTP. βPak-interacting exchange factor (βPix) is a GEF for Rac/Cdc42 GTPase. In cell culture systems, βPix forms a multi-protein complex with Pak, GIT1, Paxillin, participating in the regulation of focal adhesion turnover and actin cytoskeleton. But the in vivo role of βPix is not well unknown. So, we generated βPix-deficient mice through gene targeting and investigated its physiological function in embryo development and behavior in learning and memory.

Results A targeting vector with neomycin-resistance cassette was introduced into ES cells. We obtained two independent ES clones in which the βPix genomic locus have been correctly targeted. Heterozygous mutant mice ($\beta Pix^{+/-}$) were generated from chimeras which carries this recombinant. To generate βPix-deficient mice ($\beta Pix^{-/-}$), heterozygous mice were intercrossed. $\beta Pix^{-/-}$ mice was inviable and deletion of βPix results in embryonic lethality around day 9-10 of gestation. At embryonic day 8.5, βPix-/ embryos were smaller in size and significant developmental delays.(Fig.1) Most embryos exhibit incompelet closure of neural tube from the forebrain to the hindbrain.(Fig.1C), and axial rotation was impared.(Fig.1B,D) Another feature is the failure of allantois-chorion fusion.(Fig.1D,F) Allantois of the null embryo was unattached to the chorion and formed a balloon shape structure.(Fig.1B,D,F)

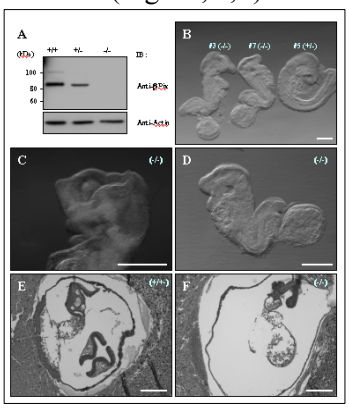

Fig.1 Analysis of Pix βPix-null embryos phenotype (A) Western blot analysis of Pix-null embryos in E8.5. (B-D) Whole mount analysis of Pix-null embryos. Incomplete closure of neural folds is seen in (C) and (D), while the allantois did not fuse and had a balloon shape (B, D). (E-F) Histological analysis of E8.5 embryos in deciduo; wild type (E) and Pix-null (F). Scale bars from (A) to (F) represent 1 mm.

Mouse embryonic fibroblasts(MEFs) at E8.5 were primary cultured. While wild type and heterozygous MEFs attached and spread well with prominent focal adhesions to the fibronectin-coated culture substrate, βPix null fibroblasts were poorly attached and spread.

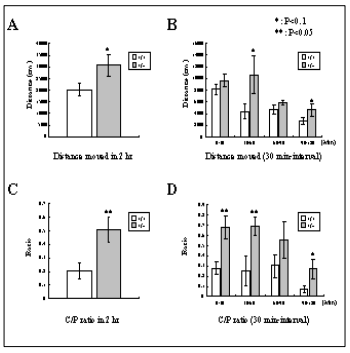

Fig.2 Analysis of anxiety-like behaviors of βPix heterozygous mice in the open field test. Distance traveled between 2 hours (A) or in 30-minute intervals (C) are shown. The ratio of time spent in the inner squares or periphery of the open field apparatus is shown during 2 hours (B) or in 30-minute intervals (D) are shown. Data are expressed as the mean ± SEM. * P<0 1 ** P<0 05

In open field test, hyperactivity and less anxiety were observed in $\beta Pix^{+/-}$ mice comparing to wt mice, indicated by traveled distance and retention time at the center.(Fig.2) Analysis in thirty minutes intervals showed that this hyperactivity and decreased anxious feature was just in early time.(Fig.2B,D)

Conclusion βPix is critical for early embryonic development and the lethality may due to impaired cell adhesion and failure of the placenta formation. βPix plays a potential role in anxiety-related behavior.

NFDP1, a highly enriched RGS for Gαs in brain, regulates G-protein coupled receptor signaling

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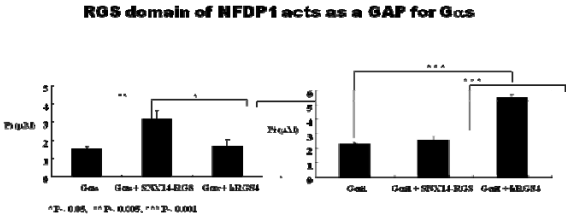
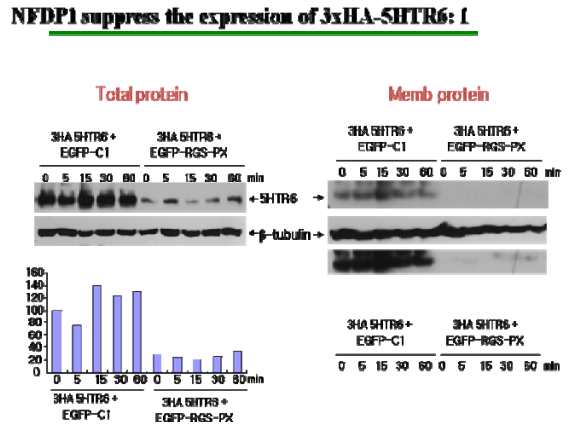
Background Heterotrimeric G proteins transduce signals from extracellular stimulations such as nutrients, hormones, neurotransmitters, chemokines and ligand stimulated-G protein coupled receptors (GPCRs) at cell surface to the intracellular environment. The RGS proteins for $G\alpha_i$, $G\alpha_q$, $G\alpha_{12/13}$ are well-characterized , but RGS GAP for Gαs are poorly understood. In nervous system, the signaling pathways mediated by Gαs-linked GPCRs such as 5-HT6 receptors and dopamine D1 receptors have known to be involved in various higher brain functions such as memory, cognition as well as in pathophysiological conditions such as depression, Alzheimer diseases, and drug addiction. So far, a few regulators have been found to regulate GPCR signaling in brains, but none of them has effect on Gαs-mediated signaling. We searched sequence databases to identify RGS proteins that act as GAPs for Gαs in brains.

Results NFDP1 is highly expressed in lung, testis, and brain regions such as hippocampus, cerebellum and cerebral cortex. NFDP1 weakly bound to wild type Gαs but strongly interacts with constitutively active form of Gαs (Q227L). Its interaction with wild type Gαs is increased in the presence of AMF. NFDP1 accelerated the catalytic rate of GTP hydrolysis of Gαs. The β2AR agonist isoproterenol- induced increase in the cellular cAMP level was almost completely abolished in cells expressing NFDP1.
LC-MS/MS results showed that RGS domain of NFDP1 was directly phosphorylated by PKA. Double mutation at both serines to alanines almost completely abolished phosphorylation of RGS domain by forskolin treatment. Furthermore, the interaction of RGS-PX with Gαs decreased dramatically by phosphorylation.
NFDP1 acts as a negative regulator for 5-HT6 receptor and dopamine D1 receptor signaling through its GAP activity, subsequently blocks the 5-HT6 receptor and dopamine D1 receptor signaling pathway and reduces the surface expression of both receptors.





Conclusion NFDP1 is a RGS GAP for Gαs being highly expressed in brain regions. The binding affinity of NFDP1 to Gαs drastically decreased by its phosphorylation in RGS domain though the feed-back regulation by PKA which abrogates NFDP1′s role as a GAP. NFDP1 also accelerated ligand-mediated postendocytic degradation of Gαs-linked 5-HT6 receptors and dopamine D1 receptors, suggesting that NFDP1 functions as a node between GPCRs signaling and sorting. Our results raise the possibility that NFDP1 could be a novel target for the treatment of psychiatric disorders and drug addiction.

Lysophosphatidic acid-induced survival of immortalized hippocampal progenitor cells via LPA receptor-mediated glycogen synthase kinase-3β inactivation

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Background Lysophosphatidic acid (LPA; 1-acyl-sn-glycerol-3-phosphate), one of the simplest natural phospholipids, exerts diverse biological effects, including cell proliferation and differentiation, platelet aggregation, protection from apoptosis, promotion of cell survival, stress fiber formation, tumor cell invasion, and cell morphological change. Recent studies have shown that various extracellular factors including LPA plays roles in controlling cell survival and apoptosis in early developing neurons. We have conducted studies aimed at identifying specific LPA receptor subtypes and signaling events that may mediate their actions in apoptosis.

Results We have conducted studies aimed at identifying specific LPA receptor subtypes and signaling events that may mediate their actions in apoptosis. To explore how LPA regulates cell apoptosis in developing neural cells, signaling cascades triggered by LPA in neuronal survival were investigated in H19-7 neuroprogenistor cells. When cultured in the presence of 5mM LPA, total numbers of cells were increased significantly compared to those cells cultured in the absence of LPA. BrdU incorporation assay revealed that LPA-induced increase of cell number was attributed to increase in cell survival instead of cell proliferation. Inhibition of p38 MAPK with SB203580, Gi protein with PTX, and GSK-3β with Bio efficiently blocked LPA-induced cell survival. But the inhibitor of PKA, Rp-cAMP, and PI-3K inhibitor, wortmannime, has no effect on LPA-promoted cell survival in H19-7 cells. Moreover, GSK-3β phosphorylation along with LPA-induced survival was suppressed by pertussis toxin (PTX) and by siRNA for LPA1 or LPA2.

Conclusion These results demonstrated that LPA induced-cell survival occurs through Gi/o coupling of the LPA receptors following inactivation of GSK-3β in H19-7 cells.

Role of Immune Cells in Cerebral Ischemic Injury: Neuroprotective Effect of WCN-81

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Background In cerebral ischemic insults, activated inflammatory cells produce various bioactive molecules that may modulate the extent of brain ischemic injury. After ischemic insult, microglial cells are first activated and are themselves injured in the ischemic core lesion. Soon later, monocytes migrate into the lesion. Gamma irradiation largely decreased the number of leucocytes, particularly macrophages, in blood and significantly reduced the infarct volume. These data indicate that infilterating activated macrophageal cells aggravate the ischemic brian injury via induction of inflammatory reactions. In the present study, therefore, we further investigated the role of activated immune cells in cerebral ischemic injury. We also recently discovered a new chemical to reduce the cerebral ischemic injury via regulation of inflammatory cell aactivities

Results To investigate the role of activated immune cells, we microinjected lipopolysaccharides (LPS) into rat corpus callosum. Isolectin B4-positive round-shaped cells were abundantly observed in ipsilateral side 1 day after LPS injection. However, the number of those immune cells was found much less in rats pre-exposed to gamma-irradiation. RPA, RT-PCR and ELISA showed that LPS microinjection rapidly increased mRNA and proteins expression for pro-inflammatory IL-1beta, TNF-alpha and iNOS, which were reduced by gamma irradiation. Microinjection of LPS markedly accelerated ischemic injury, which was reduced by pre-exposure to gamma-irradiation. The results indicate that activated macrophages/microglia modulate the extent of ischemic brain injury through expression of pro-inflammatory molecules. Our new drug KJ0529 (1 mg/kg, qid, i.v. injection) attenuated the cerebral ischemic injury and reduced the recruitment of monocytes in rat brain ischemic lesion. KJ529 (10 nM) inhibited the migration activity of monocytes through the down-regulation of Rho GTPases (including Rac, Cdc42 and Rho), chemotactic sensing and directed motility. Understanding the exact neuroprotective mechanism of KJ0529 may provide a therapeutic strategy for anti-inflammatory response in neurodegenerative diseases. We recently synthesized another new chemical WCN-81. WCN-81 (3 mg/kg, qid, i.v. injection) attenuated the cerebral ischemic injury. WCN-81 significantly reduced the size of infarction caused by MCAO/reperfusion. We found that WCN-81 decreased the migration/infiltration of microglia or monocytes at middle nanomolar concentrations. WCN-81 was found to inhibit the activity of caspase-3 and protect mitochondria from oxidative stress. However, WCN-81 did not inhibit the NMDA receptor-mediated excitotoxicity. WCN-81 siginificantly reduced the production of nitric oxide and reactive oxygen species in lipopolysaccharides-stimulated microglial cells. However, WCN-81 does not have direct scavenging effect against reactive oxygen species.

Conclusion In cerebral ischemic insults, activated inflammatory cells are involved in the progress and extent of brain ischemic injury. Thus, modulation of inflammatory reaction during and after cerebral ischemic insult would be beneficiary for better treatment of cerebral ischemic injury.

Enhanced expression of SOCS-2 in the rat hippocampus following transient forebrain ischemia

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Background Suppressor of cytokine signaling-2 (SOCS-2) has recently identified as an important regulator that is involved in neuronal differentiation and maturation. However, the role of SOCS-2 in ischemia-induced hippocampal neurogenesis still remains to be clarified. Here, we investigated spatiotemporal expression of SOCS-2 in the rat hippocampus following transient forebrain ischemia, and particular attention was paid to its changes in the dentate gyrus.

Results SOCS-2 mRNA was constitutively expressed in hippocampal neurons and astrocytes in control animals, however, its upregulation occurred specifically in reactive astrocytes in the hippocampus proper, in particular CA1 and the dentate hilar regions by day 3 after reperfusion, and was sustained for more than two weeks. This was compatible with the postischemic upregulation of SOCS-2 in the CA1 region as detected by the semi-quantitative reverse transcriptase–polymerase chain reaction analysis. In addition to the CA1 and hilar regions, SOCS-2 was transiently increased in the subgranular zone (SGZ) of the dentate gyrus on days 3-7 days after reperfusion. Most of the SOCS-2 expressing cells in the SGZ were colabeled with glial fibrillary acidic protein (GFAP), and a subpopulation of GFAP/SOCS-2 double labeled cells in the SGZ coexpressed neural progenitor marker nestin or proliferation marker proliferating cellular nuclear antigen. In addition, a subset of SOCS-2 labeled cells in the SGZ expressed immature neuronal marker polysialylic acid-neural cell adhesion molecule.

Conclusion These data suggest that SOCS-2 may be involved in glial reaction, and possibly in adult hippocampal neurogenesis during ischemic insults.

The effect of VKORC1 and CYP2C9 genotyoe variation on warfarin dosing;Genetics on warfarin dosing in Korean stroke patients (GENWAKE) study

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Background Variant alleles for CYP2C9 and VKORC1 may account for variability in anticoagulation response. However, results from homogeneous Asian patients are not available. We tested SNPs in VKORC1 and CYP2C9 and analyzed their effects on warfarin dosing for target INRs in stroke patients.

Method Between Nov, 2007 and Aug, 2008, we prospectively enrolled ischemic stroke patients who showed stable target INR (1.7 to 3.5) > 3 times during at least 3 months before enrollment in Asan Medical Center neurology outpatient clinics. We sampled patients' blood 12 hours after regular warfarin intake. We measured direct serum warfarin (R- and S-warfarin) and vitamin K levels. We divided the patients into high, intermediate, and low INR response groups using two cutoff values (33.3% and 66.6% quartile) according to the "INR response by warfarin dose" variable. We extracted DNA and tested SNPs in CYP2C9 (three sites; 1075, 1076, 430), VKORC1(ten sites; 2653 , 6484, 6853. 9041, 5808, 3673, 861, 381, 7566, 6009), GGCX (eight sites; 268, 13875, 8240, 6258, 8015, 8016, 8445, 412)using standard sequencing method.

Results All 171 patients (mean age; 66.2year±10.9, male; 60%) had excellent drug compliance, and had ischemic strokes caused by arrhythmia (52%), valvular replacement (25%) and others (23%). The mean INR was 2.22±0.56, the mean daily maintenance dose of warfarin 3.9±1.5g, and the mean R- and S- warfarin levels were 0.71±0.43 and 0.31±0.23 ug/ml, respectively. Among VKORC1 SNPs, six SNPs (6484T>C, 6853C>G. 9041G>A, 3673G>A, 381T>C, 7566C>T) were tightly linked among each sites. Therefore, the six SNPs showed the same degree of correlation with phenotype variables.
The high response group showed a high prevalence of six heterogeneous mutant SNP in VRORC1 which are tightly liked (mutant prevalence in the group of high response; 63.1%, intermediate; 26.3%, low; 10.5%, p=0.009) and the low response group showed high prevalence of mutant 1075A>C CYP2C9 polymorphism with marginal significance (mutant prevalence in the group of high response; 17.6%, intermediate; 23.5%, low; 58.8%, p=0.06). Low serum circulating R-warfarin level was significantly correlated with mutant 1075A>C CYP2C9 (wild; 0.73ug/ml±0.43) vs. heterogeneous mutant; 0.51ug/ml±0.31, p=0.01) without differences in INR level (wild; 2.22 ±0.56 vs. heterogeneous mutant; 2.16± 0.54).

Conclusion In homogeneous Korean patients, six SNPs in VKORC1 influences warfarin dosing, and any mutation would result in altered INR response by maintenance warfarin dose. On the other hand, mutant 1075A>C CYP2C9 appears to affect serum R-warfarin level without influencing on INR response.

Transient receptor potential vanilloid subtype 1(TRPV1) rescues nigrostriatal dopamimergic neurons by inhibiting microglial activation in MPTP model of Parkinson's disease

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Background Transient receptor potential vanilloid subtype 1 (TRPV1) is a nonselective cation channel activated by the vanilloids such as capsaicin (CAP), by its endogenous ligands such as anandamide (AEA) or *N*-arachidonoyl-dopamine. The widespread distribution of TRPV1 in the brain has suggested that this receptor plays a significant role in the CNS. This is supported by recent evidence of TRPV1-mediated activities in several regions of the rat brain, including the hypothalamus, locus coeruleus, hippocampus and substantia nigra (SN). Moreover, CAP has been reported to induce increased glutamate release from nigral slices, to enhance motor behavior, and to produce hypokinesia in parallel to decrease in the activity of nigrostriatal dopaminergic (DA) neurons, suggesting that TRPV1 has a functional role in the SN. This has suggested that the endovanilloid systems may be implicated in neurodegenerative disorders, including Parkinson's disease (PD) and Huntington's disease. We therefore examined whether transient receptor potential vanilloid subtype 1 (TRPV1) could contribute to prevention of dopaminergic neuronal death in the substantia nigra (SN) of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease (PD).

Results Immunocytochemical staining showed a significant loss of dopaminergic neurons and their fibers, and microglial activation in the nigrostriatal pathway. RT-PCR analysis and double-label immunohistochemistry demon-trated that the upregulation of inducible nitric oxide synthase (iNOS), IL-1β and TNF-α in microglia in the SN in vivo, indicating the activation of inflammatory system. Reactive oxygen species (ROS) production, assessed by hydroethidine histochemistry, were observed in the SN area in which degeneration of dopainergic neurons occurred. However, treatment with capsaicin, TRPV1 agonist, increased survival of nigrostriatal dopaminergic neurons and dopamine levels in the striatum without inhibiting of MPTP metabolism. This neuroprotection is accompanied by inhibiting production of inflammatory cytokines and ROS. All of these neuroprotective effects were reversed by treatment with TRPV1 antagonist CZP and iodo-resinoferatoxin (I-RTX), indi-ative of TRPV1 mediation.

Conclusion CAP, TRPV1 agonist, protect snigrostriatal DA neurons from the MPTP neurotoxicity via TRPV1 receptor activation *in vivo* without disturbing MPTP metabolism. Also motor deficits and dopamine depletion-induced MPTP attenuated by TRPV1 receptor activation *in vivo*. Even though activation of TRPV1 did not prevent direct toxicity of MPP^+ *in vitro*, it suppressed the expression of proinflammatory cytokines and oxidative damage in SN. 4These results suggest that vanilloid system may be beneficial for the treatment of neuro-egenerative diseases, such as PD, that are associated with microglial activation.

Elucidation of mechanisms for dopaminergic cell vulnerability and development of protective agents

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Background Dopaminergic neurons are particularly vulnerable to cell stress, leading to their degeneration as in Parkinson's disease (PD). Molecules and enzymes inevitably required for dopaminergic neurotransmission in these cells may also contribute to the vulnerability. The present study sought to elucidate the cellular mechanisms involved in this vulnerability and to search, based on the knowledge thus gained, for agents that provide protection to the nigral dopaminergic neurons.

Results 1) We have found a novel role of the enzyme metalloproteinase-3 (MMP-3) in dopaminergic neurodegeneration. MMP-3 is induced in these cells in response to stress and contributes to cell death. Pharmacological inhibition, suppression of expression by siRNA, and the absence of MMP-3 gene all lead to protection against cell stress-induced dopaminergic cell death both in vitro and in vivo. The enzyme activity participates in the apoptotic signaling, acting upstream of caspase-3 and downstream of JNK. In addition, MMP-3 leads to induction of NADPH oxidase, thereby facilitating generation of superoxide and oxidative stress. Furthermore, MMP-3 also contributes to interfering with DJ-1, whose dysfunction has been recognized to be involved in PD pathophysiology. MMP-3 can cleave DJ-1 and deprive it of its anti-oxidant activity. 2) We have found that quinone reductase (QR), an enzyme known to function during phase II detoxification in the liver, can be induced to be expressed in dopaminergic cells and in turn alleviate the oxidative stress caused by the dopamine quinone buildup. The compounds sulforaphane and bromocriptine were found to induce QR in dopaminergic cells and to provide protection against dopaminergic toxins. Therefore, ways to induce QR may be utilized to develop protective therapy for PD. 3) While COX-2 inhibition has been shown to be neuroprotective, the mechanism by which COX-2 contributes to cell death has not been fully understood. We found that in dopaminergic neurons where dopamine is readily oxidized to the harmful dopamine quinone, the activity of COX-2 has an exacerbating effect due to its ability to facilitate superoxide generation, independent of its prostaglandin-synthesizing activity. COX-2 expression can be induced under the exposure of BH4, the endogenous molecule in dopaminergic cells required for dopamine synthesis, via a mechanism involving the CRE/AP-1 site in its gene. 4) We have designed and synthesized a series of compounds that may have protective effects on dopaminergic cells via mechanism(s) elucidated above. Novel candidate compounds that provide neuroprotection have been found. They have antioxidant activity, downregulate the induction of MMP-3 and COX-2 expression in dopaminergic cells, suppress neuroinflammatory responses, and alleviate the behavioral deficits in MPTP-induced animal model of PD. These compounds can penetrate the blood-brain barrier, have no apparent drug-drug interaction problems, hERG inhibiting problems, or drug toxicity problems, making them potentially good candidate drugs. We have also identified pre-existing compounds that have QR-inducing effects and therefore may potentially be used toward PD therapy.

Conclusion Understanding the cellular mechanism by which the nigral dopaminergic neurons are particularly sensitive to various cellular stress conditions allows development of ways to elevate the threshold level at which cell death is triggered and new targets against which protective compounds can be designed and tested. We have found MMP-3 and QR as such cellular targets and obtained several candidate compounds that provide neuroprotection to dopaminergic cells in PD models.

Molecular Interaction between Parkin and PINK1 in Mammalian Neuronal Cells

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Background Parkinson's disease (PD) is characterized by the deterioration of dopaminergic neurons in the pars compacta of substantia nigra and the formation of intraneuronal protein inclusions known as Lewy bodies (LBs). The etiology of PD is not known, but the recent identification of several mutation genes in familial PD has provided a rich understanding of the molecular mechanisms of PD pathology. Among these, mutations in *PTEN-induced putative kinase 1* (*PINK1*) and *parkin* are linked to early-onset autosomal recessive forms of familial PD. Parkin functions as an E3 ubiquitin ligase, which ubiquitinates several target proteins and enhances degrading them via ubiquitin-proteasome system (UPS), while *PINK1* gene encodes a serine/threonine kinase. Recent studies from *Drosophila* demonstrated that *PINK1* and *parkin* act in a linear genetic pathway required for the maintenance of mitochondrial function. However, it has not been determined whether PINK1 directly binds to parkin, or/and parkin could be a potential substrate for PINK1 kinase. The current study provides the first evidence of direct interaction between these two proteins.

Results To investigate the relationship between parkin and PINK1, we determined whether parkin binds to PINK1 in mammalian cells using co-immunoprecipitation assay. It is observed that PINK1 selectively binds to parkin in transformed neuronal cells and rat brain tissues, including substantia nigral and striatal regions (Fig. 1). In addition, we found that two functional consequences caused by the interaction between the two proteins. The first thing was that, when parkin was overexpressed in cells, the level of PINK1 was dramatically increased. When we checked the mRNA level of PINK1, there was no change of PINK1 mRNA in stable cell lines overexpressing wild type parkin compared to control cells, indicating that parkin does not affect the transcription of PINK1. To investigate the mechanism by which parkin upregulates PINK1, we performed *in vivo* ubiquitination assay in mammalian cells. As a result, PINK1 ubiquitination was reduced by parkin overexpression, which led to the upregulation and intracellular accumulation of PINK1. On the other hand, PINK1 induced the formation of parkin-containing MT-dependent cytoplasmic aggresomes.

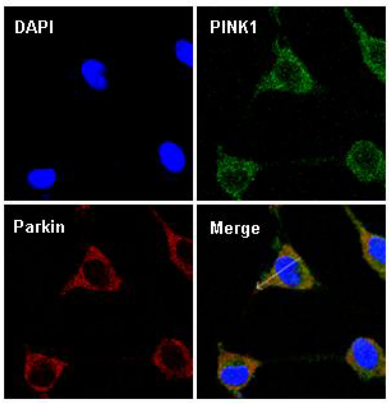

Fig. 1 Endogenous parkin binds to endogenous PINK1. SH-SY5Y cells were fixed, permeabilized and labeled with anti-parkin and anti-PINK1 antibodies. Cells were then stained with TRITC-conjugated and FITC-conjugated secondary antibodies and DAPI. Immunostained preparations were examined with a confocal microscope.

Conclusion The study demonstrates the biochemical interaction and functional relevance of PINK1 and parkin. The two proteins affect each other's stability, solubility and tendency to form cytoprotective microtubule-dependent aggresomes. Our report suggests that both PINK1 and parkin appear to play an important role to regulate the formation of LBs, and advances the current understanding of PD pathogenesis.

Astrocytes in injury states rapidly produce anti-inflammatory factors to prevent excessive microglial inflammatory responses

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Background Brain inflammation as well as systemic inflammation is a defense mechanism that protects injured brain from further infection. However, surrounding tissues are also damaged by inflammatory mediators. Therefore, there should be endogenous mechanisms that prevent excessive brain inflammation in injured brain.
Microglia, major inflammatory cells in the central nervous system, are activated in injured brain and express inflammatory mediators such as inducible nitric-oxide synthase (iNOS), tumor necrosis factor-alpha (TNF-α) and prostaglandins. Astrocytes, highly abundant cells in the brain, appear to modulate microglial inflammatory response. In the presence of astrocytes, microglial expression of iNOS, IL-12, and TNF-α etc., was significantly reduced compared with that in the absence of astrocytes. Recent studies from our laboratory reported that astrocytes in intact states exert anti-inflammatory effect through the expression of antioxidant enzymes such as heme oxygenase-1 in microglia. However, astrocytes are also influenced by the injury that causes neuronal death and microglial activation. Therefore, it is important to know whether and how astrocytes in injury states modulate microglial inflammatory responses.

Results Damaged astrocytes rapidly exert ed anti-inflammatory effect on IFN-γ-treated microglia. Astrocyte culture conditioned media prepared from astrocytes treated with oxygen-glucose deprivation (OGD-ACM) or H_2O_2 strongly inhibited expression of iNOS and other inflammatory mediators in microglia (Figure 1). The anti-inflammatory effect of OGD-ACM was detected within 5 min after OGD or H_2O_2 treatment while ACM prepared from healthy astrocytes showed anti-inflammatory effect at least 1-3 d after incubation (Figure 2). OGD-ACM directly reduced microglial inflammatory responses without protein synthesis. OGD-ACM appeared to inhibit IFN-γ signaling in the nuclei because OGD-ACM inhibited neither phosphorylation nor translocation of STAT-1/3, but inhibited binding of nuclear extract to GAS element and GAS-luciferase activity. The active component(s) in OGD-ACM penetrated 3kDa pore, and heat-insensitive.

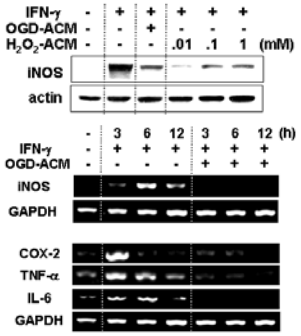

Figure 1. Effect of conditioned media prepared from damaged astrocytes on microglial activation.

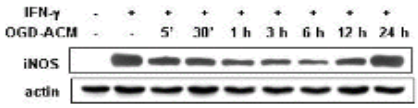

Figure 2. Effect of the extent of astrocyte damage on microglial activation.

Conclusion These results in this study suggest that damaged astrocytes rapidly release soluble factor(s) that reduce microglial inflammatory responses. Thus, astrocytes appear to be an important regulator to protect neuron from excessive inflammation in the brain.

Exploration of endophenotypes associated with pathophysiology of schizophrenia using multi-modal imaging techniques

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Background The pathophysiology of schizophrenia may be influenced by interactions of genetic, neurodevelopmental, environmental and/or psychological factors, making it difficult to elucidate the etiology of the disorder through clinical manifestations. Convergent line of evidence suggests that neurobiological deficits of schizophrenia already begin before onset of psychosis, indicating neurodevelopmental aspect, and these deficits progress according to the course of schizophrenia, representing neurodegenerative aspect. In order to explore endophenotypic markers associated with schizophrenia, we used multi-modal imaging techniques including functional magnetic resonance imaging (fMRI), magnetoencephalography (MEG) and magnetic resonance spectroscopy (MRS) in unaffected first-degree relatives of patients with schizophrenia, subjects at ultra-high risk (UHR) for schizophrenia experiencing prodromal symptoms, and established schizophrenia patients.

Results In fMRI study investigating neural correlates associated with spatial working memory, there were different activation patterns in brain regions among normal control (N=16), relatives of schizophrenia (N=17), UHR subjects (N=21) and schizophrenia patients (N=15). Decreased activations of the parietal and dorsolateral prefrontal cortex were already present before onset of schizophrenia and these deficits extended during disease progression into chronic schizophrenia. First-degree relatives of schizophrenia patients showed increased activations in the thalamus, caudate and hippocampus during spatial working memory tasks. Relatives of schizophrenia (N=22) also showed decreased levels of NAA, Cr and Cho in the thalamus compared with normal control with MRS. In addition, UHR subjects (N=16) showed a smaller right MMNm dipole moment in the superior temporal gyrus than those of normal control in MEG study, suggestive of functional deficits in the early stage of auditory processing before onset of schizophrenia. MEG study also revealed that UHR subjects (N=17) showed decreased alpha event-related desynchronization (ERD) compared with normal control, and schizophrenia patients (N=10) showed more diminished ERD than UHR subjects.

Conclusion These findings provide that functional deficits in the fronto-temporo-parietal network begin before the onset of schizophrenia and these deficits extend through the course of schizophrenia. Thalamic dysfunction in first-degree relatives of schizophrenia patients may represent a genetic liability to schizophrenia. Our studies suggest that spatial working memory related neural correlates, MMNm dipole moment, alpha ERD and neurochemicals associated with fronto-thalamo-temporo-parietal dysfunction may be endophenotypes for schizophrenia.

Surface-based analytical methods for multimodal MRI data and brain connectivity analysis, and application to the study of psychiatric disorders

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Background T1-weighted structural MRI (sMRI), diffusion tensor imaging (DTI), and functional MRI (fMRI) has been suggested as a useful tool for the analyses of gray matter (GM) and white matter structure, and brain function respectively in various psychiatric disorders such as schizophrenia and obsessive compulsive disorder (OCD). Recent studies have tried to analyze brain structure and function using a 3D surface-based method. However, its methodological framework was not established well and showed limitations in clinical application. In addition, it has been reported that understanding the changes of brain connectivity in disease group are important for pathology detection.
We suggested well-established surface-based methods for multimodal MRI data (sMRI, fMRI, DTI) and brain connectivity analysis. These techniques were applied to the study of psychiatric disorders to find abnormalities of brain structure and function.

Results A quantitative validation of surface-based sMRI analysis Our surface reconstruction algorithm showed the best geometric/topologic accuracy compared to other algorithms. The evaluation showed high accuracy of cortical thickness measure (mean RMS errors: 0.42±0.096mm). The reliability of our surface-based sMRI analysis was confirmed.
Surface-based fMRI analysis We aligned and mapped fMRI data to cortical surface model using multimodal image registration and two surface mapping techniques. Our surface-based method for fMRI analysis showed higher spatial accuracy (12% higher) of functional localization compared with a traditional volume-based method.
Surface-based DTI analysis Using cortical surface model, we showed that GM mean diffusivity was significantly biased by the cerebrospinal fluid contamination effect. We proposed a localized analysis framework for measuring GM mean diffusivity.
Surface-based cortical thickness analysis and pattern classification in schizophrenia and OCD We proposed classification method based on principal components of cortical thickness between schizophrenic patients and normal controls, showing high classification accuracy (93.6%). In OCD patients, we found significant cortical thinning in the left ventral cortex system.
Structural/functional connectivity analysis and abnormal pattern in OCD We suggested the surface-based analysis of structural relationship between cortical and subcortical areas. Significantly different structural relationship was found in OCD patients compared to normal controls, showing abnormal brain structural network. In fMRI analysis, ROI-based correlation matrix analysis showed the different pattern of functional connectivity between OCD patients and normal controls.

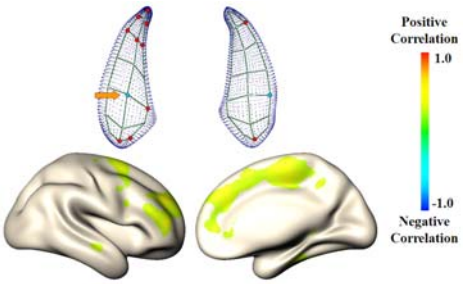

Fig. Structural relationship between cortical and subcortical areas in OCD brain

Conclusion These results suggest that our novel surface-based methods for sMRI, fMRI and DTI analysis are reliable. Application studies show abnormal structure in the cortical/subcortical areas and structural/functional connectivity pattern in schizophrenic and OCD patients, which is meaningful for pathology detection.

Effect of neonatal treatment of MK-801 on ERK1/2-p70S6K-S6 signal pathway in the frontal cortex of developing rat brain with long-term behavioral changes

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Background Systemic injection of MK-801, a selective NMDA receptor antagonist, on postnatal neonatal rat induces long-term neurochemical and behavioral changes, which has been suggested as the neurodevelopmental rat model of schizophrenia. Proper regulation of protein synthesis, which is operated by the signal transduction pathways of protein translation initiation, is one of the major requisites for the normal development of brain. In the signal pathways related to protein translation, protein translation initiation is tightly regulated via MAPK and Akt pathways, which in turn converges to the regulation of p70S6K. Previously, we reported the effect of MK-801 on ERK1/2 and Akt related mTOR/p70S6K pathways in the frontal cortex of adult rat brain. In this study, p70S6K related signal pathway were investigated after single or double treatments of MK-801 on PN7 in the frontal cortex of developing rat brain with long-term behavioral changes.

Results Single injection of MK-801, 0.5 and 1.0 mg/kg, induced significant decrease in the phosphorylation of p70S6K and S6 from 4 to 8hr and increase at 24hr, while 0.1 mg/kg induced similar changes with less degree. Two injections of MK-801, 8hr apart, induced similar pattern of changes more prominently. Phosphorylation of ERK1/2-p90RSK showed similar pattern of changes to p70S6K phosphorylation, but AKT-mTOR pathway was not affected significantly. In addition, phosphorylation of eIF4B, another substrate of p70S6K, was altered similarly, but phosphorylation of 4E-BP, down-stream of mTOR, was not affected. Phosphorylation of p70S6K at Thr389 or Thr421/Ser424 can be affected by either Akt-mTOR or ERK1/2 signal pathway depending on the cell type. After MK-801 treatment, phosphorylation of p70S6K at Thr389, but not at Thr421/Ser424, was changed with similar pattern to ERK1/2 phosphorylation. Moreover, phosphorylation of S6 at Ser235/236, which residues are exclusively phosphorylated by p90RSK, showed same pattern of changes. These findings implicate the effect of MK-801 on p70S6K-S6 pathways in the frontal cortex of PN7 rat is regulated by ERK1/2 pathway.
After single or two injections of MK-801 on PN7, measurement of locomotor activity and prepulse inhibition were performed at PN42. Single treatment of MK-801 showed tendency of decrease in locomotor activity and did not show significant changes in prepulse inhibition. However, rats treated with two injections of MK-801 showed increased locomotor activity and deficits in prepulse inhibition.

Conclusion Treatment of MK-801 on PN7 of rats induced dose- and time-dependent changes in ERK1/2-p70S6K-S6 signal pathway in the frontal cortex of developing rat brain, along with long-term behavioral changes reflected by increased locomotor activity and prepulse inhibition deficits. MK-801 treatment induced the decrease in the activity of the protein synthesis signal pathway on PN7 followed by increase on PN8, which may suggest the down-regulation of protein translation machinery on PN7 with compensatory up-regulation on PN8. In early developing brain, synaptogenesis, differentiation, and migration of neural cells occur, which needs tight regulation of protein synthesis. Therefore, dysfunctions in brain protein synthesis signal pathways during the critical postnatal period of neurodevelopment in response to the treatment of NMDA receptor antagonist could contribute to the long-term behavioral alterations, which resemble to the symptoms of schizophrenia.

Potential role of TRPV1 receptors in drug addiction

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Background Methamphetamine, cocaine, nicotine or morphine is known to induce psychomotor activity and psychological toxicity when it is used repeatedly. As a result, it produces potent physical dependence and psychological dependence. At present, however, there is no useful treatment for this problem. Furthermore, the putative mechanisms are not fully understood yet. Transient receptor potential vanilloid 1 (TRPV1) is the target of capsaicin, the pungent ingredient in chili peppers. TRPV1 is expressed in CNS areas such as the striatum, cortex, and hippocampus, and excites dopaminergic neurons and enhances chemical- and noxious-induced dopamine release in the nucleus accumbens. These data suggest that TRPV1 may modulate drug addiction. In this study, we investigated the involvement of TRPV1 receptors in neurobiological mechanisms of drug abuse. We also suggest the potential role of TRPV1 receptors as a novel therapeutic target for the treatment of drug abuse.

Results We evaluated whether changes in TRPV1 activation were involved in cocaine addiction expressed as the development of cocaine-induced conditioned place preference (CPP). Pretreatment with the TRPV1 antagonist, capsazepine (2.5 and 5 mg/kg, i.p.), significantly reduced the time on the cocaine side. Combined administration of lower dose of cocaine (7.5 mg/kg, i.p.) and capsaicin (0.3 mg/kg, i.p.) were significantly different than vehicle group or cocaine alone.

TRPV1 knock-out mice did not produce the cocaine-induced CPP and behavioral sensitization during the development session in mice. After development of CPP, cocaine (15 mg/kg) treatment significantly increased specific [^{3}H]resiniferatoxin binding in the cingulate cortex, striatum, and nucleus accumbens. Capsazepine (5 mg/kg) pretreatment blocked these cocaine-induced increases in the cingulate cortex, striatum, and nucleus accumbens. We also measured TRPV1 mRNA expression using quantitative real-time RT-PCR. Cocaine significantly increased levels of TRPV1 mRNA in the cortex, CPu, and hippocampus. These results suggest that the development of cocaine seeking is dependent on the increase of TRPV1 in previous brain regions. In dorsal root ganglion (DRG) neurons, acute treatment with 100 μM cocaine before a 1-day application of capsaicin (5 μM) enhanced Ca^{2+} influx. These data suggest that changes in Ca^{2+} influx after TRPV1 activation may play a role in the expression of cocaine-induced addictive behaviors. Thus, Ca^{2+} influx by TRPV1 activation may contribute to enhanced DA release in the NAc of animals sensitized to cocaine, consistent with a previous Ca^{2+} channel study. In addition, novel TRPV1 antagonists significantly blocked cocaine-induced CPP.

Acute treatment with capsazepine augmented morphine analgesia in mice. Pretreatment with capsazepine inhibited analgesic tolerance to and dependence on morphine. Novel TRPV1 antagonists significantly inhibited morphine-induced analgesic tolerance and withdrawal syndrome.

Conclusion Our results show that blockade of TRPV1 activation can reduce the cocaine-induced development of craving and relapse, and TRPV1 activation potentiates cocaine induced development and reinstatement. Furthermore, changes in TRPV1-mediated Ca^{2+} influx may modulate the DA release associated with drug reward. Global TRPV1 deficiency yields deficits in cocaine addiction. TRPV1 antagonist blocks morphine-induced analgesic tolerance and dependence. Novel TRPV1 antagonists inhibit the cocaine and morphine dependence. Therefore, TRPV1 may be a new target for treatment and prevention of drug-induced dependence and relapse.

Mice lacking adenylyl cyclase-5 badly cope with restraint stress and show the anxiolytic-like behaviors

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Background Physiological responses to acute stress proceed with the activation of the many neurotransmitter systems. Many brain regions are known to modulate the HPA axis activation in stress responses, but the detailed neural circuits and signaling system in the upstream of the HPA axis need to be explored further. Moreover, despite previous studies of anxiety, information about the regulations and neural substrates of the anxiety still need to be studied more. Type 5 adenylyl cyclase (AC5) is highly concentrated in the dorsal striatum and nucleus accumbens, which are implicated in reward, emotion, and stress-related behavior. We studied whether the AC5 is involved in the regulation of stress and anxiety responses.

Results AC5-/- mice exposed to daily 2-hr restraint stress for only 3-5 days showed severely inferior stress-coping responses, including severe body weight loss, poor coat condition, respiratory difficulties, and phobia-like behavior. Plasma corticosterone levels during 2-hr stress sessions increased in AC5-/- mice compared to those of AC5+/+ mice. However, neither the glucocorticoid receptor antagonist RU486 nor the CRH receptor antagonist NBI27914 blocked their inferior stress-coping. Whereas the administration of the GABAA receptor allosteric modulator, diazepam, or the D1 dopamine receptor antagonist, SCH23390, prior to restraint stress sessions changed their stress-coping response to the stressed AC5+/+ mouse level. Stress-triggered c-Fos expression was blunted in the dorsal striatum of AC5-/-.

We also demonstrate that mice lacking AC5 display strong reductions in anxiety-like behavior in several paradigms. This anxiolytic behavior in AC5-/- mice was reduced by the D1 receptor antagonist SCH23390, and enhanced by the D1 dopamine receptor agonist DHX. DHX-stimulated c-Fos induction in AC5-/- mice was blunted in the dorso-lateral striatum, but it was over-activated in the dorso-medial striatum and NAc. The siRNA-mediated inhibition of AC5 levels within the NAc was sufficient to produce an anxiolytic-like response. Microarray and RT-PCR analyses revealed an up-regulation of prodynorphin and down-regulation of cholecystokinin (CCK) in the NAc of AC5-/- mice. Administration of a kappa opioid receptor antagonist or a CCK receptor agonist reversed the anxiolytic-like behavior exhibited by AC5-/- mutants. Moreover, for the long-term studies, we established the lentiviral vector delivery system (Fig. 1) to inhibit the specific gene expression in the target brain areas.

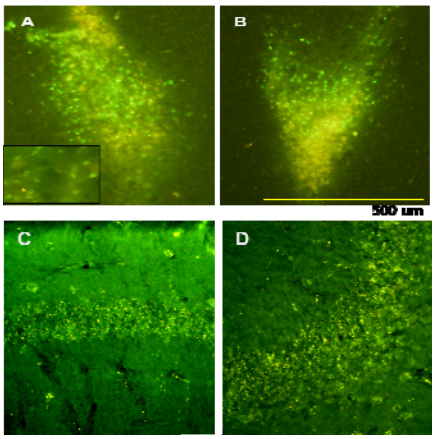

Fig.1. Photomicrographs showing expressions of siRNA-AC5+siRNA-GLO green (A and B) in neurons of the NAc and Lentiviral-GFP (C and D) in the pyramidal neurons of the CA1 (C) and CA3 (D) regions of the hippocampus.

Conclusion These results suggest that AC5-associated signal system and neural network are involved in the regulation of anxiety and stress-coping response. Particularly, our results suggest an essential role of AC5 in the NAc for maintaining normal levels of anxiety.

Involvement of the hippocampal CAMK-IIα and ERK1/2 in the regulation of pain transmission in mice

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Department of Pharmacology and Institute of Natural Medicine, College of Medicine, Hallym University, Chuncheon, Korea

Background Several lines of evidence have demonstrated that phosphotylation of Ca^{2+}/calmodulin dependent protein kinase II (CaMK-II) and extracellular signal-regulated protein kinase (ERK1 and ERK2) are involved in the regulation of nociception. It has been suggested that ERK1/2 activation is involved in spinal nociceptive processing and secondary hyperalgesia after carrageenan-induced inflammation. Furthermore, we previously reported that pERK expression significantly increased in the CA3 region of hippocampus induced by intrathecal (i.t.) substance P (SP) injection, but not by subcutaneous (s.c.) formalin injection. CaMK-II is well known as an important regulator for calcium signaling in synaptic transmission by phosphorylating various proteins, including neuronal membrane receptors and intracellular transcription factors. In addition, we also demonstrated that CaMK-II may play important role in the regulation of nociceptive processing elicited by i.t. SP as well as s.c. formalin injection. In this study, we examined the role of CaMK-IIα and ERK1/2 in nociceptive processing at the supraspinal levels (in hippocampus) in various pain models.

Results In the immunoblot assay, i.t. injection with SP, glutamate, TNF-α or IL-1β increased the pERK and pCaMK-IIα level in the hippocampus, and an immunohistochemical study also showed that the increase of pERK and pCaMK-IIα immunoreactivity mainly occurred in the stratum lucidum/radiatum layer of the CA3 region or dentate gyrus of hippocampus. Meanwhile, s.c. injection with formalin increased the pCaMK-IIα expression in the hippocampus, but it has no effect on the pERK level. Furthermore, intraperitoneal (i.p.) injection acetic acid (5%) did not affect on the pERK as well as pCaMK-IIα levels in the hippocampus at all. In the behavioral study, we also examined the effect of PD98059 (ERK inhibitor) and KN-93 (a CaMK-II inhibitor) on pain behaviors induced by several different stimuli using stereotaxic microinjection. Pretreatment of PD98059 in the hippocampal CA3 region (bregma: -2.25mm, medial-lateral: 2.50mm, depth: 2.25mm) showed antinociceptive effect in the pain behaviors induced by glutamate, TNF-α and IL-1β, but not in the formalin and writhing responses. Stereotaxic injection of KN-93 also attenuated nociceptive behaviors induced by s.c. formalin, i.t. glutamate, TNF-α, IL-1β injection, except i.p. acetic acid injection. We also confirmed the involvement of pERK and pCaMK-IIα in the pain processing of the hippocampus using the lentiviral ERK and CaMK-II overexpression vectors.

Conclusion These results suggest that pERK and pCaMK-IIα located at the hippocampus are an important regulator during the nociceptive processes induced by several pain models differently.

Pro-inflammatory responses of spinal microglia induce the progressive reduction of morphine analgesic efficacy over time following nerve injury in rats

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Neuroscience Research Institute and Department of Physiology, College of Medicine, Korea University, Seoul, Korea

Background Morphine has been considered as a potent analgesic, but its analgesic efficacy in neuropathic pain is highly controversial. Moreover, because of the severe adverse consequence, its use in clinical practices is strictly limited. Pain due to peripheral nerve injury is a dynamic and progressive process and its underlying mechanisms alter over time following nerve injury. Thus, the time course following nerve injury may be an important factor determining the efficacy of analgesics. In the present study, we examined whether analgesic efficacy of morphine against neuropathic pain changes over time following peripheral nerve injury. If so, we investigated whether this alteration is related to pro-inflammatory responses of spinal glia, which has been recently considered as a contributor to morphine tolerance, using the rat tail model of peripheral neuropathy.

Results Two doses of morphine (1 and 2 mg/kg, i.p.) were excellent in relief of mechanical allodynia at the early time (2 weeks) following nerve injury, whereas their anti-allodynic effect was significantly reduced at the late time (16 weeks). Decrease in morphine efficacy was observed to be progressive over time after nerve injury. RT-PCR results indicated that mRNA for Iba-1 (a marker for microglia) and pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6 were significantly increased in the spinal cord 2 weeks after nerve injury. Of pro-inflammatory cytokine transcripts, IL-1β and IL-6 were kept highly expressed up to 16 weeks following nerve injury. At both the early and late times, IL-6 immunoreactivity was observed to be co-localized with OX-42, a marker for microglia, in the spinal cord. To address the involvement of pro-inflammatory responses of spinal microglia in the progressive reduction of morphine efficacy, we examined whether inhibition of glia or neutralizing pro-inflammatory cytokines could recover the morphine efficacy at the late time. While single treatment of morphine (1 mg/kg, i.p.) did not attenuate mechanical allodynia, the co-administration of morphine with intrathecal, AV411 (2.5 ug/rat, i.t.) as a glia-specific phosphodiesterase inhibitor or IL-6 neutralizing antibody (0.01 ug/rat, i.t.) significantly suppressed mechanical allodynia at 16 weeks following nerve injury. Next, we examined why just the early pain is more susceptible to morphine treatment than the late pain despite under the glial pro-inflammatory influences throughout neuropathy. Systemic administration of naloxone (2 mg/kg, i.p.), an opioid receptor antagonist, enhanced mechanical allodynia at 2 weeks, but not 16 weeks following nerve injury, suggesting that opioid receptor-mediated analgesia overcomes anti-analgesic effect of pro-inflammatory cytokines at the early time contrary to the late time following nerve injury.

Conclusion Our results suggest that morphine efficacy in relief of neuropathic pain reduces over time following nerve injury and this alteration is involved, in part, in pro-inflammatory responses of spinal microglia to nerve injury.

Functional significance of TRPV1 in pain pathways: peripheral nociceptors and pre-/post-synaptic neurons

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Background Transient receptor potential vanilloid subtype 1 (TRPV1) is a key sensory transducer for various pain stimuli such as capsaicin, noxious heat, low pH, and endogenous lipids in the periphery. However, the identity of endogenous activators for TRPV1 under patho-physiological condition is still debated. Interestingly, TRPV1 is also expressed in several regions of the central nervous system (CNS) including superficial layers of spinal dorsal horn. However, very little is known about significance of functional expression of TRPV1 on the central presynpatic terminals of nociceptive neurons, and expression patterns of postsynaptic substantia gelatinosa (SG) neurons in the spinal dorsal horn.

Results We found that *1*-oleoyl-2-acetyl-*sn*-glycerol (OAG), a membrane-permeable diacylglycerol (DAG) analog, elicited intracellular Ca^{2+} transients, cationic currents and cobalt uptake that were blocked by TRPV1-selective antagonists, but not by inhibitors of PKC and DAG lipase in rat dorsal root ganglion (DRG) neurons or HEK 293 cells heterologously expressing TRPV1. OAG-induced responses were about one fifth of capsaicin induced signals, suggesting that OAG displays partial agonism. We also found that endogenously produced DAG can activate rat TRPV1 channels. Mutagenesis of rat TRPV1 revealed that DAG-binding site is at Y511, the same site for capsaicin binding, and PtdIns(4,5)P2 binding site may not be critical for the activation of rat TRPV1 by DAG in heterologous system. Next, we examined the modulation mechanism of TRPV1 by mGluR5 on the central presynaptic terminals of nociceptive neurons. (*R,S*)-3,5-dihydroxy-phenylglycine (DHPG)-induced pain behaviors such as spontaneous pain behaviors and mechanical allodynia were decreased in TRPV1-/- mice. The enhancement of mEPSC frequency, but not of the amplitude, induced by DHPG in SG neurons was significantly reduced by TRPV1 antagonism. *In vitro* results by using Ca^{2+} imaging and whole cell recordings (WCRs) showed that mGluR5, rather than mGluR1, is functionally coupled to TRPV1, and DHPG-induced Ca^{2+} responses result from direct activation of TRPV1 by DAG on the central presynaptic terminals.
Single-cell RT-PCR (scRT-PCR) analysis revealed the expression of TRPV1 mRNA in subsets of SG neurons (n = 23/30). EM analysis also detected TRPV1-immunoreactivity in the postsynaptic dendrites of SG neurons. Capsaicin (2 μM) evoked slow inward currents with reversal potential of 0 mV and outward rectification that were readily reversible. Capsaicin-evoked currents were blocked by a TRPV1 antagonist, capsazepine (20 μM). By performing scRT-PCR following WCRs, we found that capsaicin-responsive neurons express TRPV1 in SG neurons (n = 25).

Conclusion In the present study, we demonstrates that DAG directly activates TRPV1 channel in rat nociceptive neurons, and DAG mediates functional coupling of mGluR5 and TRPV1 in a membrane-delimited manner in central presynaptic terminals, thereby contributing to the modulation of nociceptive synaptic transmission to spinal SG neurons. We propose that DAG serves as an endogenous ligand for rat TRPV1, acting as an integrator of $G_q/_{11}$-coupled receptors and receptor tyrosine kinases that are linked to phospholipase C, and mGluR5-TRPV1 coupling on the central presynaptic terminals of nociceptive neurons could be an important mechanism underlying central sensitization under pathological pain conditions.
In addition, our results demonstrate TRPV1 expression in postsynaptic SG neurons of spinal dorsal horn. Studies on the functional role of TRPV1 in SG neurons are now in progress.

Increase of the spinal dehydroepiandrosterone sulfate (DHEAS) enhances nociceptive signaling: the involvement of sigma-1 receptor and $GABA_A$ receptor

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Background Several evidences have suggested that one of neurosteroids, DHEAS modulate central nervous system related functions by activating sigma-1 receptors (Sig-1Rs) and/or allosterically inhibiting $GABA_A$Rs. Both receptors play an important role in the spinal pain signaling. Moreover, with respect to the Sig-1Rs, it has been demonstrated that the activation of spinal Sig-1Rs induces facilitation of N-methyl-D-aspartate receptor (NMDA-R) activity via increase of protein kinase C (PKC) or protein kinase A (PKA)-dependent phosphorylation of NMDA-R subunit 1 (pNR1) which plays a key role in the enhancement of pain perception. In this regard, the present study hypothesized that intrathecally injected DHEAS enhances nociceptive signaling at the spinal cord level via activation of Sig-1R and inhibition of $GABA_A$Rs, thus increasing spinal pNR1. Firstly, we determined whether intrathecal (i.t.) DHEAS injection was able to affect nociceptive thresholds to peripheral mechanical stimulation. Secondly, we verified the effect of spinal DHEAS injection on spinal NMDA-R sensitivity using a model of NMDA-induced pain.

Results In the first part of experiments using a von Frey filament test, i.t. DHEAS injection dose-dependently decreased the nociceptive threshold to mechanical stimulation, thus producing mechanical allodynia. Moreover, this DHEAS-induced mechanical allodynia was significantly reduced by administration of the Sig-1R antagonist, BD-1047 or the $GABA_A$R agonist, muscimol. Additionally, this DHEAS-induced mechanical allodynia was suppressed by another neurosteroid, progesterone, which acts as an endogenous blocker of both Sig-1Rs and $GABA_A$Rs. These results suggested that increase of spinal DHEAS produced mechanical allodynia via activation of Sig-1Rs and inhibition of $GABA_A$Rs. In the second part of experiments using a model of NMDA-induced pain, i.t. treatment of DHEAS dose-dependently increased the i.t. NMDA-induced spontaneous behavior. Moreover, this facilitatory effect of DHEAS on NMDA-induced pain was significantly reduced by pretreatment of the Sig-1R antagonist but not $GABA_A$R agonist. Additionally, image analysis of PKC- or PKA-dependent pNR1 immunohistochemical staining in the spinal cord indicated that i.t. injection of the DHEAS enhanced pNR1 expression in the spinal dorsal horn. This increased pNR1 expression was significantly reduced by pretreatment with the Sig-1 R antagonist. These findings indicated that i.t. DHEAS facilitated spinal NMDA-induced pain via increase of PKA- and PKC dependent pNR1 which was mediated by Sig-1Rs.

Conclusion Present results indicated that increase of spinal neurosteroid, DHEAS could enhance the nociceptive signaling in normal mechanical stimulation and NMDA-induced pain. I.t. DHEAS produced mechanical allodynia which was mediated by Sig-1Rs and $GABA_A$Rs. Subsequently i.t. DHEAS facilitated spinal NMDA-induced spontaneous pain behaviors via activation of Sig-1Rs leading to increase of PKA- and PKC dependent pNR1. This study opens interesting possibilities for further investigations into the mechanisms underlying neurosteroid modulation of spinal pain transmission.

Prostaglandin E2 potentiated acetaldehyde-evoked nociception via TRPA1 and PKC dependent mechanism

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Background Prostaglandins and acetaldehyde have been recognized as important mediators for hangover pain after ethanol intake. The sensory mechanisms of the acetaldehyde-evoked hangover pain and of the contribution of prostaglandins are largely unknown. thermosensitive transient receptor potential (thermoTRP) ion channels in the primary afferents play a crucial role as body thermosensors and also as peripheral pain detectors. We hypothesized that acetaldehyde activates a sensory TRP channel thereby evoking nociceptive animal behaviors. We also examined whether this pain mechanism involves prostaglandin E2 and its related signaling.

Results We showed that acetaldehyde, an intermediate substance of ethanol metabolism, is able to activate mouse and human TRPA1 in HEK293T cell heterologous expression system and cultured mouse trigeminal neurons. Acetaldehyde did not activate other five temperature-sensitive TRP channels expressed in sensory neurons. A TRPA1 antagonist camphor and a general TRP blocker ruthenium red suppressed TRPA1 activation by acetaldehyde. These two blockers also suppressed acute nociceptive behaviors induced by intraplantarly administered acetaldehyde into the mouse hindpaws. Coapplication of prostaglandin E2 with acetaldehyde robustly potentiated the acetaldehyde-induced nociceptive responses both in vitro and in vivo. A EP1 receptor antagonist effectively suppressed the prostaglandin-induced potentiation. PKC but not PKA blockers prevented the prostaglandin E2 effects.

Conclusion Our data suggest that acetaldehyde evoked pain via activation of TRPA1. This effect of acetaldehyde was potentiated by prostaglandin E2 via PKC signaling. Our result may help elucidation of the hangover pain mechanism.

Anatomo-functional dysconnectivity of frontal-motor system in children with attention deficit/hyperactivity disorder

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Background Inadequate motor response such as, motor inhibition is one of the key symptoms of attention deficit/hyperactivity disorder (ADHD). Motor dysfunction in ADHD is associated with response inhibition rather than simple visuo-spatial sensory motor response. Response inhibition is involved in sensory motor control. We aimed to examine anatomo-functional dysconnectivity of frontal-motor system in children with ADHD. Anatomical change was examined by voxel based morphometry on gray matter and fractional anisotropy images and functional connectivity by interregional correlation on FDG-PET image.

Results In ADHD children (N=30) relative to age matched pediatric disease controls (N=10), decrease of gray matter volume on structural MR image was found in middle/inferior frontal, anterior cingulate, paracentral lobule, and inferior parietal regions by voxel-based morphometry analysis ($p<0.001$, uncorrected). Decrease of fractional anisotropy value was found in superior longitudinal fasciculus, corpus callosum, and cortico-pontine/cortico-spinal tracts by diffusion tensor image ($p<0.001$, uncorrected). In ADHD children (N=39) relative to age matched deaf children (N=39), absence of metabolic correlation with left frontal lobe (VOI) was found in right primary/sensory cortex and left precuneus ($P<0.001$, uncorrected).

Conclusion Anatomo-functional dysconnectivity of frontal-motor system was found in children with ADHD. These dysconnectivity might be associated with maturation delay of prefrontal regions in ADHD (Shaw et al., 2007). Functional metabolic dysconnectivity of frontal-motor system might be ascribed to anatomical disconnection in children with ADHD.

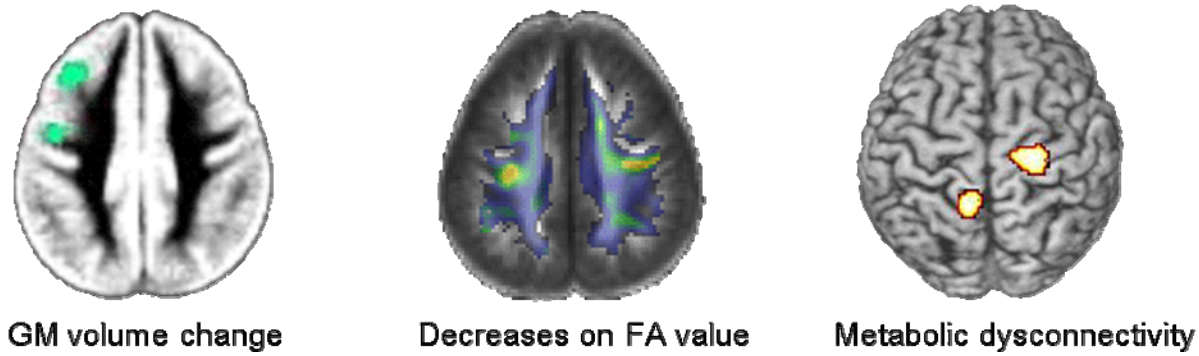

Figure 1. Anatomo-functional connectivity in ADHD children.

Where is a wake-promoting region in human brain? : rCBF changes by modafinil: a randomized double blind study

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Background Modafinil, 2-[(diphenylmethyl)-sulfinyl acetamide], is a novel wake-promoting agent that is used to treat excessive daytime sleepiness associated with sleep disorders. Previous placebo controlled trials have concluded that modafinil reduces objective excessive daytime sleepiness in narcoleptic patients, and in sleep-deprived normal subjects. To investigate the effects of a wake-promoting drug, modafinil on regional cerebral blood flow (rCBF) in healthy volunteers, we performed ^{99m}Tc-ethylcysteinate dimmer single photon emission computed tomography (SPECT) before and after modafinil or placebo administration.

Results Twenty-one healthy subjects received single doses of 400 mg modafinil or placebo in a double blind randomized crossover study design. Administrations of modafinil or placebo in a subject were separated by a 2-week washout. Brain SPECT was performed twice before and 3 h after modafinil or placebo administration. For statistical parametric mapping analysis, all SPECT images were spatially normalized to the standard SPECT template and then smoothed using a 12-mm full width at half-maximum Gaussian kernel. The paired t-test was used to compare pre- versus post-modafinil and pre- versus post-placebo SPECT images. Differences in rCBF between post-modafinil and post-placebo conditions were also tested. Modafinil decreased Epworth and Stanford sleepiness scales whereas placebo did not. The post-modafinil condition was associated with increased rCBF in bilateral thalami and dorsal pons, whereas the post-placebo condition showed increased rCBF in a smaller area of the dorsal pons when compared with the drug naïve baseline condition. Compared with the post-placebo condition, the post-modafinil condition showed higher rCBF in bilateral frontopolar, orbitofrontal, superior frontal, middle frontal gyri, short insular gyri, left cingulate gyrus, left middle inferior temporal gyri, left parahippocampal gyrus, and left pons.

Conclusion The present study demonstrates that a single dose of modafinil increased rCBF in multiple brain regions, which are related to arousal, attention, executive function, and emotion in healthy volunteers without sleep deprivation. This study is the first to investigate the effects of wake-promoting drug, modafinil on rCBF in healthy volunteers.

Language lateralization using MEG beta frequency desynchronization during auditory oddball stimulation with one-syllable words

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Background Some patients with epilepsy have difficulty performing complex language tasks due to the long duration of the disease and cognitive side effects of antiepileptic drugs. Therefore, a simple passive paradigm would be useful for determining the language dominance lateralization in epilepsy patients. The goal of this study was to develop an efficient and non-invasive analysis method for determining language dominance in epilepsy patients.

Results To this end, magnetoencephalography (MEG) was performed while an auditory stimulus sequence comprised of two one-syllable spoken words was presented to 17 subjects in an oddball paradigm without subject response. The time-frequency difference between deviant and standard sounds was then analyzed in the source space using a spatial filtering method that was based on minimum-norm estimation. The laterality index was estimated in language-related regions of interest (ROI). The results were compared to the traditional lateralization method using the Wada test. Beta band oscillation activity decreased during deviant stimulation, and the lateralization of the decrease was in good agreement with the Wada test, in the posterior part of the inferior frontal gyrus in 94% of the subjects and in the posterior part of the superior temporal gyrus in 71% of the subjects.

Conclusion In conclusion, the ROI-based time-frequency difference between deviant and standard sounds can be used to assess language lateralization in accordance with the Wada test.

Monetary incentives can reduce the Amygdala activation from negative emotion faces

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Background Considering social importance of recognizing emotions expressed by other peoples' faces, it is of no surprise that faces with negative emotions capture attention (Ohman et al., 2001) and are processed automatically even when attention is diverted from those stimuli and directed to elsewhere (Vuilleumier et al., 2001). There exist, however, other factors that can also strongly influence attentional processes and motivational incentive is one of those important factors. In particular, recent studies demonstrated that monetary incentives could modulate attentional selection (Libera and Chelazzi, 2006) and enhance processing in attention-related brain regions (Small et al., 2005). Do monetary incentives affect attentional capture caused by viewing faces with negative emotions and, therefore, brain regions mediating emotional expressions? We performed two experiments to answer these questions.

Results There were two experimental variables: the monetary incentive the participant would win if they gave a correct answer (0 or 250 KRW) and emotional valence of the distractor face (negative or neutral). In each trial, there appeared a scene, a face, and a scrambled picture on the screen. Participants indicated whether the scene was an indoor or an outdoor location with a button press, while ignoring the distractor face. The face always appeared in the middle and the others on its sides. The behavioral response and functional Magnetic Resonance Imaging data were collected in two separate experiments. Participant were slower at the "scene" task when a face expressed a negative emotion than when a face was neutral only for the incentive absent condition. For the incentive present condition, there was no difference in RTs between the trials with negative faces and trials with neutral faces (Figure 1). ROI analysis of the functional MRI data showed that the amygdala in the right

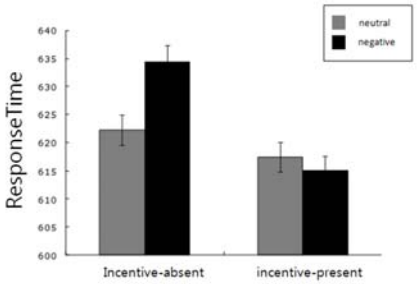

Figure 1. Behavioral data

hemisphere showed a greater activation when a face expressed a negative emotion than when a face was neutral only for the incentive absent condition. For the incentive present condition, activation in the right amygdala didn't show differential activations in response to negative and neutral faces (Figure 2).

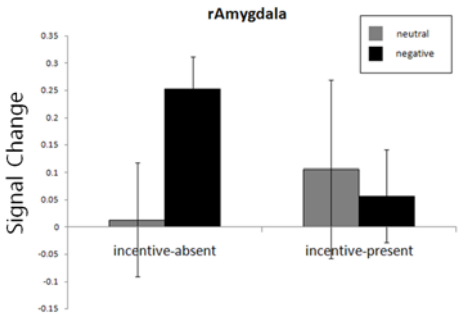

Figure 2. fMRI data (ROI analysis)

Conclusion Results from these two experiments showed that monetary incentives could indeed modulate the processing of negative emotional faces and influence the activation in the brain region mediating emotional facial expressions.

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Hierarchy of cortical responses underlying binocular rivalry

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Background When your eyes view dissimilar patterns, you experience a perceptual illusion called binocular rivalry. Rather than melding into a stable, single image, the two patterns compete for visibility, with one temporarily dominating perception for several seconds, only to be replaced in awareness by the other. During transitions in perceptual state, one typically sees a traveling wave in which the perceptual dominance of one pattern emerges locally and expands progressively as it renders the other pattern invisible. Here, we carried out fMRI experiments that used attention as a tool to dissociate the conscious perception of traveling waves during rivalry from the automatic neural processing underlying the initiation and propagation of these waves.

Results Human observers viewed a dichoptic display that was designed to induce perceptual waves. The rival images comprised a low-contrast carrier grating (viewed by one eye) and a high-contrast mask grating (viewed by the other eye). During each fMRI scanning session, observers carried out either a perceptual latency task or a diverted attention task. In the perceptual latency task, observers pressed a key when a perceptual wave reached a target area, thereby providing a measure of the arrival time of the perceptual wave. In the diverted attention task, the dichoptic rival gratings and the sequence of events were identical to those in the perceptual latency task, except that a rapid series of small, colored letters and numbers appeared at fixation. During each trial, one of the colored characters was repeated, and after each trial the observers reported whether the repeated item was a letter or a number. The observers were completely unaware of the dynamics of the traveling waves. We found that activities in V1, V2, and V3 all reflected the spatiotemporal dynamics of rivalry while observers attended the rival gratings and carried out the perceptual latency task. However, when attention was diverted, waves were preserved in V1, but eliminated in V2 and reversed in V3.
By conducting a series of control experiments, we ruled out the possibility that cortical waves were due to (i) inherent differences in response latencies between upper and lower visual-field quadrants, (ii) artificial responses induced by the contrast pulses, or (iii) the presence of a rapid stream of letters and numbers at the fixation.

Conclusion Our data imply that competition between two rival stimuli involves neural circuits in V1, and attention is crucial for the consequences of this neural competition to advance to higher visual areas and promote perceptual waves.
[Acknowledgements: This research was supported by a grant (M103KV010017-06K2201-01710) from Brain Research Center of the 21st Century Frontier Research Program funded by the Ministry of Education, Science and Technology, the Republic of Korea]

Harm Avoidance is associated with increased metabolism of a default network in the elderly

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Background The automatic and stable character of the Cloninger's temperament can be related to the resting state brain metabolism and may help one to facilitate an immediate response to stimuli.

Especially, the harm avoidance (HA) involves cautious, apprehensive, fearful, shy and fatigable traits and high HA group can exhibit high resting state brain metabolism because they tend to worry even when they rest.

In addition, a default network including the regions of the frontal region, precuneus, and posterior cingulate is known to collect both internal and external information during the resting state and influence one's readiness. Therefore we can expect that this network of high HA group would show high level of resting state metabolism.

Using FDG-PET and TCI (The Temperament and Character Inventory; Min et al., 2007), we investigated this relationship between HA and the metabolism in the frontal areas, precuneus and posterior cingulate in healthy elderly female subjects from a community center.

Results We found that high HA level correlates positively with the metabolism of frontal areas and precuneus, and negatively with the thalamus and hippocampus.

Conclusion These results suggest that people with high HA who tend to worry constantly show high level of resting state metabolism of the default network to be thoroughly prepared for future events. However, this excessive worry seems to affect negatively on the hippocampus function, reflected by low resting state metabolism of this region.

Sigma-1 receptor antagonist as a target for novel analgesics

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Background Sigma sites, originally proposed as opioid receptor subtypes, are currently thought to represent unique receptors with a specific pattern of drug selectivity, a well-established anatomical distribution and broad range of functional roles. Because activation of sigma-1 receptor modulates calcium mobilization from intracellular pools and several neurotransmitter releases including glutamate, these receptors are involved in learning and memory, response to stress and depression, psychostimulant induced sensitization, vulnerability to addiction and pain perception. Although previous reports have suggested that the sigma-1 receptor may be involved in pain sensation in formalin induced pain, potential role of sigma-1 receptor on other type of pain symptoms has not been fully elucidated. In the present study, we addressed the antinociceptive effect of sigma-1 receptor antagonist (BD1047) in various types of pain symptoms. Furthermore, we have synthesized new ligands to sigma-1 receptor with antagonistic activity and evaluate analgesic potency.

Results *Acute pain model***:** Although intrathecal BD-1047 has antinociceptive effect on the second phase of formalin induced pain, the intraperitoneal injection of BD-1047 has dose-dependent analgesic effect on both phase of formalin induced pain in mice. When it is determine the antinociceptive effect of systeBD1047 in the model of visceral acute tonic pain, the acetic-acid writhing test in mice, it has significant antinociceptive effect. In other type of inflammatory pain model, carrageenan induced thermal hyperalgesia also reduced by BD-1047.While intrathecal injection of sigma-1 receptor agonist (PRE-084) did not evoke any nociceptive pain behavior, it significantly augmented intrathecal NMDA induced pain behaviors. BD1047 selectively reversed PRE-084 induced pan behaviours in NMDA pain test. *Neuropathic pain model***:** Experimental neuropathic pain was induced by three different type of nerve injury including chronic compression of the L5-6 dorsal root ganglion (CCD), chronic constrictive injury (CCI) and diabetic neuropathy (DNP). In these neuropathic models, BD1047 successfully decreased mechanical allodynia as comparable with gabapentin. *Headache model***:** Noxious trigeminal nucleus caudalis stimulation with capsaicin (one of headache models) significantly increased Fos-like immunoreactivity which was dose-dependently reduced by intracisternal BD1047 pretreatment. When PRE084 was repeatedly administered by intracisternal infusion method over 1 week, Fos-like immunoreactivity in TNC was significantly increseasd as comparable with NMDA infusion. These overall results suggested that sigma-1 receptor was involved in the meningeal nociceptors activation. *Novel compound test***:** Finally, we addressed that our novel compound (LJ-1950, IC_{50}=18.8 nM) has more potent analgesic effect in the neuropathic pain models as compared with BD1047.

Conclusion Among several analgesic indications of sigma-1 receptor antagonist, neuropathic pain and headache remains a prevalent clinical problem because it is often poorly responsive to the currently used analgesics. Thus sigma-1 receptor antagonist represents a new target for novel analgesics to specifically various intractable pain symptoms.

Optimization of sigma1 receptor antagonist using medicinal chemical approach

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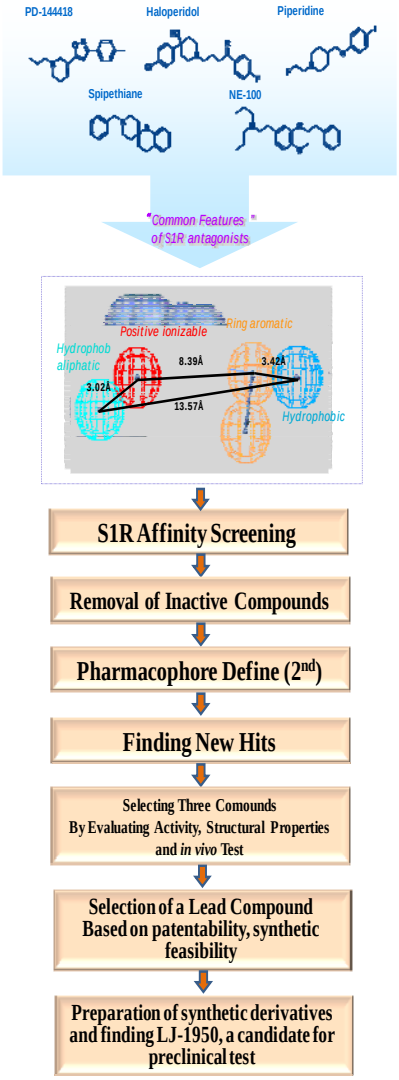
Background Novel therapeutic agents replacing traditional narcotic analgesic and non-steroidal anti-inflammatory drugs are necessary because there are several limits for controlling pains derived by a nerve disease. Sigma1 receptor is a novel and promising target which plays a crucial role in inducing pains. Virtual screening of chemical libraries based on a pharmacophore established by analyzing the structural features of known antagonists for sigma1 receptors afforded several hits. On the basis of this result, we carried out structure-activity relationships to develop preclinical candidates.

Results Selection of a lead compound among three compounds was accomplished through its novelty, patentability and a synthetic feasibility. Finally BMD-250196 was chosen as a lead compound. Based on BMD-250196, a key hit found by a virtual screening and in vitro test, many derivatives were designed and synthesized. By analyzing the structure-activity relationship of first thirty analogues some common structural features were derived. The structure of BMD-250196 could be thought to contain three parts. The first is the alkyl group on benzene ring and the second is the linker. The last one is an alkyl amine. The affinity of the synthetic analogues antagonizing sigma1 receptor seemed to be significantly affected by modification of these three parts. Various substituents on benzene ring, the size of the linker and a variety of amines were tested. Small size of substituents like halo, methyl or ethyl groups gave a low affinity. The molecular size was also an important factor in determining the affinity. Up to date the best result on *in vitro* and *in vivo* test was obtained when the derivative, LJ-1950. Several derivatives (LJ-1946, LJ-1950, LJ-1951, LJ-1952) also showed good affinities.



Conclusion Several sigma1 receptor antagonists were found to have a nanomolar affinity. Among them, LJ-1950 gave the best result in *in vivo* test. A cautious investigation would be necessary in determining the overall size of the molecules and preventing a metabolic problem. A research work for developing better antagonists for sigma1 receptor is underway.

In vitro and in vivo Pharmacology of CGBI0472, a Dual Inhibitor against COX-2 and CAs for the Application to the Neuroinflammation Diseases

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Background Alzheimer's disease (AD) is the main cause of dementia and characterized by the accumulation of extracellular amyloid plaques and intracellular tau induced neurofibrillary tangles. It has been reported NSAIDs are associated with around 80% reduction in the incidence of AD, showing brain inflammation is involved in the progression of AD. Recently the involvement of COX-2 has been report; cyclooxygenase-2 (COX-2) is induced by Aβ and PGE2 levels are found to be increased in AD. CrystalGenomics has developed COX-2 inhibitors as an anti-inflammatory agent and tried to expand the application of COX-2 inhibitors to the inflammatory brain diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). CGBI0472's inhibitory activity against Carbonic Anhydrases (CAs) may overcome side effects current COX-2 inhibitors have shown in the cardiovascular system. Various *in vitro & in vivo* PK/PD studies for CGBI0472 have been carried out for its development with AD indication.

Results CGBI 0472 shows an inhibitory activity against COX-2 with 0.003 ug/ml of an IC50 value and has high COX-2 selectivity over COX-1. It also inhibits hCAI and II activity with IC 50 values 0.31 and 0.028 uM, respectively. It shows its efficacy in Carrageenan induced rat foot edema as an acute inflammation animal model. *In vitro* microsomal study, CYP inhibitory assay, dose-dependent mouse PK study, 1 week toxicity and MTD studies, and BBB penetration have been performed. CGBI0472 shows good pharmacological properties. Furthermore it had a tendency to i) improve the behavior in familiar and altered context in CFC test and ii) reduce soluble A-beta 1-42 levels when compared to vehicle treated transgenic mice.

Conclusion CGBI0472 as a COX-2 inhibitor shows the efficacy in Carragenan induced arthritis animal model. As a CA inhibitor it may avoid severe cardiovascular adverse events such as myocardial infarction and thrombosis because CG510649, another dual inhibitor with the same scaffold did not show any side effects in phase I and II clinical studies. Along with its efficacy in Tg mouse AD model CGBI0472 is being developed for the treatment of AD.

In vivo performance evaluation of a totally implantable wireless neural signal transmission system (TiWiNets) for brain machine interface (BMI)

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Background Brain-machine interface (BMI) studies by decoding movement information from many single neurons in motor brain areas have raised hope for paralyzed people. However, one of obstacles to overcome for the development of neural prosthesis applicable to human patients is a realization of brain-implantable, small, wireless brain-machine interface chip.

Results We have developed a TiWiNets combined with advanced digital signal processing capable of realizing a totally implantable system for BMI. It consists of a preamplifier with only 2 opamps for each channel, bluetooth module (BM), a Labview-based monitor program, and 16 bit-RISC microcontroller. Digital finite impulse response (FIR) windowed sinc filter was implemented with hanning window (cosine), having a 3dB passband from 400 to 1500 Hz and a 48-dB stopband for other frequencies using the microcontroller. The real-time bandpass filter algorithm was completely executed by moving the digital samples (10k Hz) and 200-tap filter coefficients. Less than ±2% error was obtained between simulated and measured FIR results. Due to the design of the powerful FIR filter the TiWiNets size could be reduced dramatically to module dimension of 22*26*8mm including BM except for battery. An *in vivo* performance was evaluated by transmitting neural spikes from software-selectable two channels of the TiWiNets implanted in an intra-cranial brain areas sequentially in terms of long-term stability and activation of target devices, to PC via BM. In the monitor program on PC, a neural spike detection algorithm with auto-reference, time-frequency analysis, autocorrelation, fast fourier transform (FFT), waveform classification by slope calculation, and time-domain event histogram are developed to establish a directly single cell BMI algorithm for machine control strategy. It was shown that rate histogram or possible combinations as that strategy could become very useful control variables by allowing volitional control of robot or any target devices in real-time.

Conclusion We concluded that our system could fulfill *in vivo* requirements in diverse BMI-related fields.

Study on Cerebral Hemodynamics Using Near-Infrared Spectroscopy

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Background NIRS (near-infrared spectroscopy) is a relatively new, non-invasive method to measure optical properties (scattering and absorption coefficients) and physiological properties (concentration of oxy-, deoxy-hemoglobin, cytochrome c oxidase, etc) of biological tissues. In this study, we performed two different projects including NIRS-based BMI (Brain machine-interface) and in vivo seizure monitoring: (1) BMI is a method to control an external device by measuring physiological signals and translating the signals into the code which can be recognized by the device. Most of current BMI systems are based on the measurement of electrical activities using invasive electrodes. Invasive electrode method has problems such as infection and cannot be readily applied to humans. To overcome the drawbacks of current BMI systems, NIRS-based BMI is proposed as an alternative method. (2) Seizure is well-known to be the most catastrophic states of the brain and large portions of the brain are involved in an abnormally synchronized manner. We used animal mouse models to induce abnormal neuronal synchronized activities followed by maximum hemodynamic perturbation. The neuronal and hemodynamic responses using EEG and NIRS were measured simultaneously.

Results (1) We measured hemodynamic responses of the rat barrel cortex to whisker stimulation by using a frequency-domain NIRS system. We designed multiple optical probes comprising multi-mode optical fibers and manipulating arms, both of which can be easily applied to small animals. Various electrical stimulations were applied to rat whiskers at different voltage levels and stimulation frequencies. Our results show that the hemodynamic responses are highly dependent on the stimulation voltage level, and not so much on stimulation frequency.
(2) GBL (gamma-butyrolactone) and 4-AP (4-aminopyridine) were applied to induce absence and tonic-clonic seizures, respectively. The epileptic events were traced by skull EEG and the spatiotemporal hemodynamic changes were obtained by applying frequency-domain, multi-channel, two-wavelength NIRS. Our results show that the GBL systemically induces a hyperoxia state throughout the whole brain (4 out of 6 mice) while 4-AP induce sporadic blood inflows with no systemic drift in the oxygenated hemoglobin level (4 out of 6 mice). A linear correlation study shows that the inter-channel dependence increased as seizure developed but the synchronization level dose not coincide with the ratio of the epileptic responses which appeared on EEG. The hemodynamic responses to absence seizure were morphologically different in the intra-subject and inter-subject data. More than half of the absence seizure did not cause any significant changes in the hemodynamic variables whereas vasoconstriction or wash-out responses were observed in relatively earlier period and deoxy-hemoglobin increase was detected in relatively later period. The phase relationship between oxy and deoxy-hemoglobin shows regional dependence as well as brain-state dependence.

Conclusion Our successful monitoring of cerebral hemodynamics in the mouse and rat brain using frequency-domain multi-channel two-wavelength NIRS open a possibility to study the neurovascular coupling in genetically manipulated animals.

Statistical standardization of EEG-based assessment tool for higher cognitive brain function

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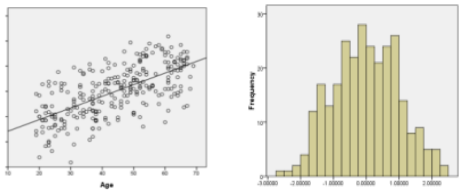
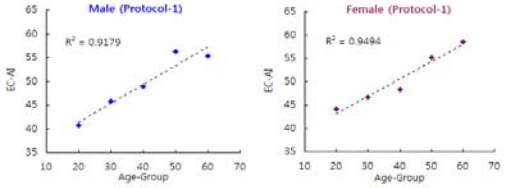
Background The higher cognitive function means the cognitive process including perception, attention, memory and reasoning, related to the cerebral cortex of brain. Here, we suggest the quantitative EEG (electroencephalogram)-based method for physiological assessment of the higher cognitive function. It comprises four protocols and each protocol is as follows. In the first protocol, the biological degeneration of brain function, generally caused by aging, damage or brain disease, is estimated by peak-frequency, peak-amplitude and brain-map pattern of intrinsic rhythm extracted from background EEG activity with eye-closed in a quiet place. In the second protocol, the low-level brain function such as arousal level is assessed by spectral distribution and asymmetric ratio in eye-open state. In the third protocol, the mid-level higher cognitive function related to the top-down attention is assessed by P300-latency and P300-amplitude of event-related potential in an easy active oddball task. In the fourth protocol, the neural efficiency of the complex higher cognitive function, combined together with memory and reasoning as well as perception and attention, is evaluated by latency and amplitude of the induced gamma response in a cognitive discrimination task. The assessment tool may be put to practical use for popularization because it can be realized in the noninvasive, portable and inexpensive form as well as in the automatically interpreted form using the statistical standardized T-score.

Results We conducted the clinical study for statistical standardization of quantitative EEG parameters based on healthy male/female 300 volunteers aged 19-69 yr.The detail procedure for clinical trial was approved by Asan Medical Center Institutional Review Board(with WHO FERCAP Certification).

The EEG activity of eight monopolar channels(Fp1,Fp2,F3,F4,T3,T4,O1,O2 referenced by A2) was recorded with 256Hz sampling frequency during about 5-min for each of above-mentioned 4-protocols using QEEG-8(LXE3208, LAXTHA Inc., Daejon, Korea) device.
Several hundred quantitative EEG-parameters were extracted from EEG data obtained for each protocol(p1=323, p2=283, p3:510, p4:351 parameters). To obtain the standard range of each parameter, statistical normality was examined by histogram, goodness-of-fit(Kolmogorov-Smirnov test), normal Q-Q plot and then the standard range of mean(-,+)standard deviation was calculated in each male/female age-groups.



Finally, each parameter may be transformed into clinically preferred assessment-scale such as standard T-score(50-M,10-SD) or Z-score(0-M,1-SD) by using the obtained standard range.



Also, we could find many parameters with highly correlated linear trend to chronicle age. These EEG-parameters may be of great worth as the biological aging-index of brain function.

Conclusion These results suggest that standardized EEG-based quantitative parameters can be useful for objective assessment of higher cognitive brain function and brain aging.

Deep brain stimulation for neurpathic pain and dementia rat models

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Background Deep brain stimulation (DBS) is a widely used clinical tool for neurodegenerative disease such as parkinson's disease, neuropathic pain and alzheimer's disease. DBS has potential as an effective treatment for neurological disorders for all that a successful clinical application has not yet been fully implemented.
Our study previously showed that electrical stimulation improved treadmill locomotion test in rats with 6-OHDA induced Parkinsonian rodent model. Based on the result, we have planed to test the effect of DBS from carrying on stimulation on animal models which have neurodegenerative disease such as neuropathic pain and dementia.

Results To generate an efficient rat model of neuropathic pain and dementia, we used to the TST method that tibial and sural nerves were completely transected and tightly ligated, while the peroneal nerve was left intact in left hind paw and injected 192 IgG-saporin in lateral ventrical (LV) for selectively destroyed of forebrain cholinergic neurons. Two weeks after the surgery, electrodes were inserted to neuropathic pain rat model each into PAG, ACC, and thalamus (VPL). And one week later, we stimulated and test the behavior.
In our results, in neuropathic pain rat model, electrode implant into PAG and DBS stimulation could be causing the worsened pain intensity in neuropathic pain rats. However, in ACC and thalamus stimulation, it shows slightly analgesic effect for the neuropathic pain rats. PAG has participated to nociception as it well known but it does not have great influence on the neuropathic pain rat model. Nevertheless, when in ACC and thalamus stimulation, as far as it has effect to decreasing the pain, it can be considered that limbic system still related to neuropathic pain to some extent.

Conclusion The result demonstrates that there can be beneficial effects of ACC or thalamus-DBS on neuropathic pain rat model and may suggest an appropriate rodent model for DBS study.

Optical measurement of neural activity in brain tissues

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Background Neural recording techniques have played an essential role in neuroscience and neural engineering. Recently, methods using light have been attracting attention because they have many advantages over the conventional methods. Intrinsic optical methods do not require any label, and are also free from the stimulus artifact. Particularly, optical fiber-based method will not be disturbed by sheath encapsulation arisen from the cellular response. The free space-based methods will make it possible to detect brain activity noninvasively with simple apparatus.
As the fundamental studies toward development of optical techniques offering advantages described above, we have conducted two experimental and one theoretical studies. First, we investigated how the optical transmittance of the brain tissue varied with neural activation. Based upon this study, the optical reflection was also monitored by using optical fibers located on the tissue. Finally, we developed and analyzed a novel neuron model to elucidate which neurophysiological event contributes to such optical changes.

Results In the first experiment monitoring the near-infrared transmission spectrum (NIRTS) of the brain tissue during neural activation, we found significant changes in the NIRTS associated with neural activity. When the field potential showed its maximum change, the NIRTS started increasing. This increase relaxed much slowly (~100 ms relaxation constant) than the field potential. The optical response exhibited a monophasic change, while the field potential showed biphasic shifts. Optical changes were repetitively observed in other preparations although their amplitude and wavelength dependency were slightly different from each other. In the control experiments conducted in the absence of the brain tissue or electrical stimulation, no optical change was found. It enables us to claim that the optical responses originated from neural activity.
We also examined how the optical reflectance of the brain tissue would change with neural activation. Through the optical fiber located on the tissue, the reflectance was monitored while the neural activity was electrically evoked and recorded. The reflectance increased at the onset of population spikes. This reflectance change was monophasic, similar to the NIRTS change. As the control, we conducted similar recording at other positions that had no neural pathway. We could not find any optical change in the control experiment.
The optical responses commonly showed monophasic and slow changes despite different optical properties under measurement. Moreover, such features were quite different from the electrical signals. To elucidate this difference, we hypothesized that the optical responses might originate from the cellular volume change (CVC) during neural activation. We constructed this hypothesis on the basis of the idea that the CVC would be also monophasic and slow relative to the membrane potential. Since the conventional Hodgkin-Huxley neuron model could not provide the CVC dynamics, we developed a new neuron model containing the variable cellular volume and intracellular concentrations. Numerical computation results of this model showed that the time course of the CVC was very similar to that of the optical response.

Conclusion In this study, we found that the transmittance and reflectance of the brain tissue change during neural activation. The optical changes were not mediated by the neurovascular coupling, thus much faster than conventional methods such as the functional near-infrared spectroscopy (fNIRS). In addition, we constructed a new neuron model suggesting that the optical signal is closely related to the cellular volume change. These findings, in the future, could be applied to development of new optical neuroimaging techniques.

Arrayed microelectrodes for neurons to machine interface

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Background Understanding how neural activity in sensory cortices relates to perception, how neural signals in motor cortices control the motion of the human body and how nerve conduction is related with a certain disease has been a goal of neuroscience. Recent developments in neural engineering have made tremendous strides towards the realization of this goal. Among these new technologies, the arrayed microelectrodes have played key roles and are broadly applied in clinical fields such as pacemaker, the cochlear implant, retinal prostheses, deep brain stimulators (DBS). In this paper, we introduce 3 types of electrode for measuring the neural signals. The first one is implantable electrode in the cortices and the second one is mountable electrode on the surface of cortices. The third one is the electrode for recording compound muscle action potential (CMAP) that reflects a summation of motor-unit potentials in a muscle will be introduced.

Results First, the implantable needle type microelectrodes (SU-8 (50, MicroChem, USA)) was fabricated with 5 electrode array using conventional photolithography and metal-patterning processes. For the easier separation of the completed SU-8 electrode from the underlying layer, the polyimide film was employed as temporary substrate. The electrodes were sufficiently strong to be penetrated into the cortices. The impedance of each electrode in saline solution was nominally 150 kΩ at 1 kHz and 1.5 kΩ at 1 kHz before and after Pt-black electroplating. Eight-week-old male SPF/VAF rat (215 g) was used for recording of cortical local field potential (LFP) signals. Simultaneous recordings from each of the five electrodes of the SU-8 microelectrode were obtained using a Grass 8-16 amplifier and Digidata 1440 Hz. From the histological test, the sizes of ipsilateral and contralateral hemispheres in stained sections were not significantly different; suggesting that implantation of the SU-8 microelectrode did not cause severe illness or inflammation. The local field potentials (LFP) were successfully recorded from each electrode. The signals from ach electrode were stably measured cortices without interference from neighboring electrodes.
Second, the 32 channel electrode to measure the signals from the surface of cortices was fabricated using polyimide as substrate. As feasibility test, the signals from skull of SPF/VAF rat (215 g) were successfully measured.
Third, a polydimethylsiloxane (PDMS) and silver ball based multichannel electrode was developed and test for the clinical applicability was carried out through the nerve conduction study in patients with diabetic polyneuropathy. This electrode used a flexible, biocompatible, non-toxic and non-flammable polymer as substrate material and can be applied for the long term monitoring of neuromuscular signals. We performed nerve conduction studies on normal healthy subjects and patients with diabetes mellitus, stimulating the median nerve and recording CMAPs from the abductor pollicis brevis (APB). The difference in latency between patients and controls was observed.

Conclusion Three types of multichannel microelectrodes were successfully fabricated using diverse microfabrication technology and the feasibility and clinical suitability were evaluated through the animal experiment and human studies.

Pyridoxine 5'-phosphate oxidase, not pyridoxal kinase, involves in long-term potentiation induction in the rat dentate gyrus

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Background In the present study, we investigated whether the expression of pyridoxal kinase (PLK) and pyridoxine-5'-phosphate oxidase (PNPO) are altered following long-term potentiation (LTP) induction, and whether Tat-PLK and Tat-PNPO transductions affect LTP induction and paired-pulse responses in the rat dentate gyrus.

Results PNPO immunoreactivity was markedly increased in dentate granule cells after the induction of LTP, but that of PLK was not. Tat-PNPO (20 and 200 μg/kg), but not Tat-PLK or vitamin B6 precursors, treatments, increased the efficiency of high frequency stimulus-induced potentiation of populations spike amplitude as compared to saline-, or Tat-protein-treated animals. These changes correlated with the alterations in PNPO activity and its immunoreactivity. In addition, Tat-PNPO transduction increased paired-pulse facilitation, but had no effect on the fast and late paired-pulse inhibitions.

Conclusion These findings suggest that PNPO may play a role in activity-dependent regulation of PLP level in the brain, which is involved in LTP induction and paired-pulse facilitation, but not in enhancement of GABAergic inhibition.

Oxidative-injury triggered autophagy in astrocytes

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Background Recently, we have demonstrated that H_2O_2 induces lysosomal membrane permeabilization (LMP) in cultured hippocampal neurons. We also showed lysosomal zinc accumulation may be a key event leading to LMP. In the present study, we investigated whether oxidative injury caused similar events in astrocytes, and whether these events involve autophagy.

Results Exposure of cultured astrocytes to H_2O_2 increased zinc levels in certain vesicles and the cytosol, as observed in neurons. Double staining revealed that most zinc-loaded vesicles were lysosomes as they were co-stained with lysoTracker. Interestingly, a marker for autophagy, LC-3, was induced following H_2O_2 exposure. Consistent with increased autophagy, astrocytes transfected with GFP-LC3 exhibited increased autophagic vacuole (AV) formation after exposure to H_2O_2 or zinc. LC3-positive AVs also contained labile zinc. Chelation of zinc with TPEN [tetrakis(2-pyridylmethyl)ethylene-diamine] greatly reduced AV formation, subsequent LMP, and cell death in astrocytes. Moreover, 3-methyladenine, an inhibitor of early AV formation, also completely blocked H_2O_2–induced AV formation, LMP, and cell death. On the other hand, bafilomycin A1, an inhibitor of vacuolar H^+ ATPase, which blocks fusion between AV and lysosomes, did not inhibit AV formation, but reduced LMP and H_2O_2– or zinc-induced neuronal death.

Conclusion Present results support the possibility that endogenous zinc may play a key role in early AV formation in response to oxidative stress in astrocytes. In addition, our results suggest that AV formation is a necessary preceding event for LMP and cell death in oxidative injury.

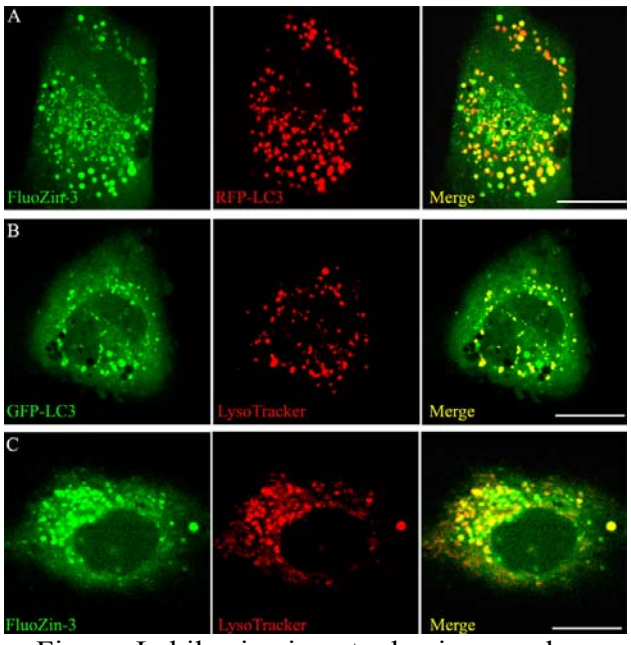

Figure. Labile zinc in autophagic vacuoles

Genetic and Molecular Dissection of a Behaviour Circuit in *C. elegans*

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Background When the food is limited or the population is too crowded, The soil nematode *C. elegans* enters an alternative stage called the dauer. The dauer larvae usually do not move and pharyngeal pumping is almost arrested. The dauer larvae show an unexpected behaviour, nictation. The dauer larvae climb up onto any projections and while standing by their posterior tip, wave in three-dimensional spirals and loops. (Croll, N.A., and Matthews, B. E., 977, Biology of nematodes) This behaviour is not shown by animals at any other developmental stage. It is known that there are specific neural circuits for specific behaviours such as forward and backward movements, thermotaxis and chemotaxis. However, the neural circuit involved in the nictation behaviour has not been studied.

Results We established the behavior assay system for nictation. We used the gauze and micro-post dirt chip. In the gauze assays, the frequency of the nictating dauers at a specific time point was about 20%, and if we removed the nictating dauers and waited, we found that over 95% of the dauers could eventually nictate.
Using the micro-post dirt chip, it was possible to observe single dauers initiate and keep nictation. We quantified the frequency and duration of nictation at the individual level.
We found that nictating dauers could move to a new environment by attaching to the fruit flies. Only nictating dauers could move to new plates suggesting that nictation is a behaviour reserved for transmission to new environments.
We examined which neurotransmitter is involved in nictation. We found that serotonin, glutamate, and dopamine are not involved. We found that dauers defective in these neurotransmission still nictated.
We found that acetylcholine is involved in nictation. Animals defective in acetylcholine synthesis, which can move pretty well, can not nictate. Cell-specific rescue experiments showed that production of acetylcholine in subsets of neurons in the head was able to restore the nictation behavior, suggesting that these neurons are involved in nctation.
From EMS mutagenesis, we identified a mutation that made adults able to perform nictation behaviour. We are mapping and cloning this gene. We finished SNP mapping, and now trying to perform sequencing to find the mutation.
From screening of pre-existing neuronal mutations, we found that a transcription factor required for many aspects of neuron development is required for nictation. We are trying to determine when and where this TF is required for nictation by cell-specific rescue and heat shock rescue.

Conclusion We established the behavior assay for nictation, by using the gauze and micro-post dirt chip. We showed that nictation is a survival instinct behaviour. By using the mutants that have defect in nictaton, we are identifying which neuron is important for nictation and we will elucidate the neural circuit involved in nictation. We identified a mutation that made adults able to perform nictation. If we find the gene, this gene will be a master regulator of nictation and we can say the complex behavior can be controlled by a single gene. Nictation is regulated by neurotransmitters and transcription factor that is important for neuronal development.

Mind Bomb 1-expressing intermediate progenitors generate Notch signaling to maintain radial glial cells

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School of Biological Sciences, Seoul National University, Seoul, Korea

Background Notch signaling is critical for the stemness of radial glial cells (RGCs) during embryonic neurogenesis. Although Notch signal-receiving events in RGCs have been well characterized, the signal-sending mechanism by the adjacent cells is poorly understood. Especially, what type of cells are relevant celluar source of Notch ligands and how the Notch-Notch ligand interactions are regulated in the repetitive divisions of RGCs are not characterized well.

Results Here we report that conditional inactivation of *mind bomb-1 (mib1)*, an essential component for Notch ligand endocytosis, in mice using the *nestin* and *hGFAP* promoters. These mutant mice display complete abrogation of Notch activation, defective RGC maintenance, and premature differentiation to intermediate progenitors (IPs) and finally neurons. These phenotypes were completely rescued by the introduction of active Notch1, demonstrating that the neurogenic phenotypes in the Mib1 mutant mice are caused by defective Notch activation. The monitoring of RGC divisions using DiI-labeling method revealed that Mib1 mutant RGCs exhibited the symmetric divisions that produce either two IPs or two neurons. Using several independent methods, we identified not only young neurons, but also IPs as Mib1-expressing cells. A FACS-based functional analysis further revealed that $Mib1^{+}$ cortical cells display only limited neurosphere-forming activity and efficiently trigger Notch signaling in the neighboring cells. Furthermore, retroviral-mediated gene manipulation at the single cell level showed that Notch signaling in the RGCs is activated by Mib1-expressing IPs produced by neighboring RGCs.

Conclusion These results demonstrate the role of Mib1-expressing IPs as an important cellular source of Notch signal to maintain the self-renewal of RGCs together with newborn neurons, and provide a novel mechanism to the RGC self-renewal and to expand the RGC pool in mammalian neurogenesis.

A Novel mTOR Activator, Oxi-α Protects Dopamine Neurons By Repressing Autophagic Cell Death

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Background Previously, we identified a novel protein, Oxi- , involved in oxidative stress (OS)-induced signaling in dopamine neurons by analyzing comparative gene expression profiles during OS-induced cell death in the dopamine cell line, SN4747 *(Yoo, M. et al. Neurochem. Res. 29: 1223 (2004))*. The SN4741 model was developed to overcome the paucity of dopamine neurons and dilution effect of non-dopamine cells in the substantia nigra midbrain region *(Son, J, et al. J. Neurosci. 19: 10 (1999))*.
Our strategy was to identify a target, whose expression was altered by OS with a previously unrecognized effect on dopamine cell death. Among the 36 significantly up- or downregulated genes, Oxi- , a member of the newly discovered Oxi gene family, was chosen as a prime candidate that contributes to OS-induced signaling in dopamine neurons. Oxi- is expressed specifically in brain dopamine neurons, and is significantly down-regulated by OS. Moreover, gene-specific knockdown of Oxi- significantly increased the neuronal susceptibility to OS, while Oxi- overexpression protected against OS-induced cell death *(Yoo, M. et al. Neurochem. Res. 29: 1223 (2004))*.
Herein we explore the molecular basis of Oxi- -mediated neuroprotection against OS. We show that Oxi- protects dopamine cells by the novel mechanism of mammalian target of rapamycin (mTOR) activation and subsequent inhibition of autophagic cell death. We also identify the novel signaling pathway activated by excessive OS, which involves downregulation of Oxi- , repression of mTOR activation, accumulation of AVs and increased autophagic cell death.

Results We find that the phylogenetically conserved Oxi- protects against OS by a novel mechanism: activation of the mTOR kinase and subsequent repression of autophagic vacuole (AV) accumulation and autophagic cell death. In contrast, the downregulation of Oxi- by OS suppresses the activation of mTOR kinase. The pathogenic effect of downregulated Oxi- was confirmed by gene-specific knockdown, which resulted in the repression of mTOR kinase as well as enhanced susceptibility to OS. In accordance with these observations, treatment with rapamycin, an mTOR inhibitor and autophagy inducer, potentiated OS-induced cell death, while similar treatment with an autophagy inhibitor, 3-methyladenine (3-MA) protected the dopamine cells. Finally, expression of two members of the newly identified Oxi gene family was regulated in temporal manner during OS-induced cell death, suggesting that both proteins could be potential targets for therapeutic intervention in dopamine neurons.

Conclusion The basis of the Oxi- -mediated neuroprotection is due primarily to its novel ability to activate mTOR kinase and subsequently repress autophagic cell death. In contrast, the OS-induced signaling includes down-regulation of Oxi- , suppression of mTOR activation, accumulation of AVs and increased autophagic cell death in the dopamine cells. Taken together, these and our previous findings suggest that Oxi- has an important regulatory role under OS, and is a potential target for future therapeutic intervention for dopamine neurons under excessive OS. Our data also implicate multiple cell death signaling pathways activated by OS in dopamine neurons. Further functional characterization of other members of the Oxi gene family will help elucidate the complex mechanism of OS-induced cell death in dopamine neurons.

Intercellular propagation of α-synuclein pathology in Parkinson′s disease

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Background Parkinson′s disease (PD) is primarily defined as a movement disorder associated with degeneration of dopaminergic neurons in the nigrostriatal system. However, its pathology, involving the progressive neuronal accumulation of aggregated α-synuclein and the formation of Lewy bodies (LB), affects various functional structures throughout human nervous system that leads to serious cognitive and behavioral alterations. In a large proportion of PD cases, accumulation of aggregated α-synuclein undergoes an ascending and highly predictable pattern of progression, spreading from lower brain stem and olfactory bulb into the limbic system and eventually to the neocortex, suggesting a mechanism involving pathological propagation, similar to the one observed in prion diseases. The idea of pathological propagation has recently gained much attention after two studies shown the host-to-graft propagation of α-synuclein positive Lewy-like pathology in long-term mesencephalic transplants in PD, results that have a profound impact in cell-based therapies. However, the underlying mechanism of the initiation and propagation of α-synuclein pathology are not fully understood yet, neither is it clear if similar transmission might occur *in vivo* into neuronal stem cells.

Results Here, we show that in neuronal cells a small amount of α-synuclein protein is translocated into vesicles and released from cells. Under protein folding stresses, such as oxidative stresses and quality control failure, aggregated forms of α-synuclein are also released from cells. Mass spectrometiry analysis showed increased carbonylation in secreted α-synuclein compared to the cellular protein. In co-culture systems, we demonstrated that these secreted α-synuclein aggregates are transmitted to the neighboring neurons and astroglia forming Lewy-like inclusions. This intercellular transmission and deposition of α-synuclein aggregates is dependent on the endocytosis. Lysosomal failure caused the accumulation of transmitted α-synuclein aggregates in the recipient cells. The transmission of α-synuclein aggregates to neighboring neurons and astroglia was also demonstrated in transgenic mouse models overexpressing human wildtype α-synuclein. Microarray analysis of astroglia that were exposed to secreted forms of α-synuclein showed changes in the gene expression profile reflecting an inflammatory response, such as the induction of cytokines and chemokines. There was a significant correlation between the cytokine induction and the accumulation of transmitted α-synuclein in glia.

Conclusion The data presented here suggest a novel mechanism leading to the formation and spread of neuronal and glial α-synuclein aggregates, providing strong evidence for direct cell-to-cell propagation of α-synuclein. This study also explains how failure of quality control systems contributes to the transmission of abnormal α-synuclein and thus the propagation of synuclein pathology. This may explain the recently reported α-synuclein positive Lewy-like pathology in long-term mesencephalic transplants in PD and the topographical progression of Lewy pathology in PD suggested by Braak and colleagues.

Inhibition of P25/CDK5 for Therapeutics of Alzheimer's Disease

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Background Alzheimer's disease (AD) is an irreversible, progressive brain disorder that is characterized by dementia. Two pathological hallmarks for AD are amyloid plaques and neurofibrillary tangles. Cdk5 and GSK3b are major kinases which hyper-phosphorylate tau protein and disrupt cytoskeleton which lead to neuronal cell death eventually. Cdk5 does not have kinase activity by itself and requires regulator, P35 or P39. In neurotoxic conditions P35 cleaves into P25 by calpain and resulting P25 overactivates cdk5. Many groups have reported that the amounts of p25 and cdk5 kinase activity are specifically upregulated in the brain of AD patients. Considerable evidence points to importance of p25/cdk5 in hyper-phosphorylation of tau leading to AD and the inhibitors of P25/cdk5 were considered as candidates for AD therapeutics. However the development of the compounds which inhibit cdk5 specifically have not been successful because they also inhibit other Cdks or other kinases. As a strategy to inhibit cdk5 specifically we developed the compounds which inhibit P25, an aberrant regulator of cdk5.

Results A three dimensional structure of P25/CDK were designed and 3.4 million small compounds were docked in the P25 of the P25/CDK5 complex by computer. The *in silico* screening selected 108 chemicals as P25 inhibitors and half of the compounds were test in *in vitro* cell-free kinase assay and cell-based assays. A few chemotypes are selected for further optimization. Many derivatives were synthesized for one chemotype and three of them show strong inhibition of P25/cdk5 in cell-based assay. The compounds have very low cytotoxicity and should be candidates for AD therapeutics.

Conclusion The compounds which specifically inhibit P25 have been developed and they should be good candidates for AD therapeutics.

A New Role of Calcineurin in Lifespan Regulation through Autophagy in *C. elegans*

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Background Previously, our studies on calcineurin mutants suggest that calcineurin is involved in egg-laying, fertility, growth, G-protein mediated signaling pathway in C. elegans. Recently, it has also been reported that the calcineurin loss-of-function mutants [*tax-6(p675)* and *cnb-1(ok276)*] and null mutant [*cnb-1(jh103)*] have extended lifespan in *C. elegans*. However, in the last few years there have been several reports on different mechanisms and pathways that operate to extend lifespan in *C. elegans* mutants. Besides signals from reproductive system and sensory neurons in *C. elegans* have been shown to influence the lifespan. Nevertheless, all the signals and resulting pathways have been shown to converge broadly into three pathways. One among them is dietary restriction/target of rapamycin (TOR) pathway, sensed by nutrients, which is dependent on autophagy. The other is the insulin/IGF-1 pathway, sensed by different environmental cues, also depend on autophagy in both dauer and adults. On the other hand, translational inhibition pathway in S6kinase/rsks-1 mutants does not involve autophagy. However, the genetic pathway or mechanism leading to extended lifespan in calcineurin mutants is still not well understood.

Results To assess the role of autophagy in the extended lifespan of calcineurin mutants, we used feeding RNAi to target two different autophagic genes: *bec-1* and *atg-7*. These genes are orthologs of yeast *ATG6* and *ATG7* respectively, functioning in autophagic vesicle nucleation (*bec-1*), and the autophagosome expansion and completion (*atg-7*). We performed feeding RNAi experiments with CnB null mutant [*cnb-1(jh103)*] since these mutants displayed maximum number of autophagositic bodies, as indicated by the punctated GFP::LGG-1 expression pattern. By means of semi-quantitative RT-PCR analysis, we confirmed that feeding RNAi was efficient and it selectively knocked down the mRNA levels of *bec-1* and *atg-7* genes (data not shown). When fed with bacteria expressing dsRNA for *bec-1* and *atg-7* genes, *cnb-1* null mutant showed significantly reduced autophagosome bodies. The significant reduction of GFP::LGG-1 expression in *cnb-1* null mutant fed with *bec-1* and *atg-7* RNAi suggested that autophagy process was indeed inhibited. Subsequently, we studied the effect of these genes on the extended lifespan of *cnb-1* null mutant in F1 generation. We found that wild type worms fed with *bec-1* had the comparable lifespan to the wild type worms fed with control vector only. But in the *cnb-1* null mutants, we observed that extended lifespan was completely reversed to the level of wild type worms when fed with bacteria expressing *bec-1* dsRNA. The effect of *atg-7* RNAi on *cnb-1* null mutant was considerably more as compared to *cnb-1* null mutants fed with *bec-1* RNAi. Interestingly, we observed that lifespan of wild type worms fed with *atg-7* RNAi was also shortened when compared to wild type worms fed with vector only.

Conclusion we report for the first time a role of calcineurin in regulating lifespan through autophagic process. In wild type worms under normal conditions, calcineurin maintains the normal level of autophagy. In the absence of calcineurin autophagic process is activated which in turn contributes to the lifespan extension. Thus, taken together, we suggest that pathway operating for lifespan extension in calcineurin mutants converge at the autophagic process. This activated autophagic process in calcineurin mutants may be due to the disruption in calcium signaling pathway, which remains to be elucidated by genetic analysis and mutant studies.

Regional Cortical Thinning in Patients with Type 1 Diabetes: A Quantitative Morphometric Study of the Effects of Chronic Hyperglycemia on Brain

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Background Research suggests that type 1 diabetes mellitus (T1DM) is associated with central nervous system (CNS)-related changes including cognitive and behavioral dysfunction. Chronic hyperglycemia and recurrent hypoglycemic episodes, frequently observed in T1DM, may play an important role in these CNS complications.

With remarkable progress on brain imaging methodology, an increasing number of studies have reported focal and global structural changes of brains. Even though there is an increasing number of studies that report focal and global structural changes of the brain in diabetic patients, none have examined cortical thickness, which is a very sensitive indicator of brain structure.

In this study, three dimensional cortical mapping was conducted to measure global and regional cortical thickness in T1DM patients and healthy control subjects.

We hypothesize that global and regional cortex would be thinner in T1DM patients compared to healthy controls and that cortical thinning would be associated with neurocognitive dysfunction in T1DM patients.

Results Magnetic resonance images were obtained from 44 patients with T1DM and 29 matched healthy volunteers. Brain surface reconstruction and measurement of cortical thickness was conducted by cortical surface-based analysis using automated procedures for segmentation. between T1DM and controls. White matter (WM) and intracranial volume did not differ between groups. Average cortical thickness in left and right hemisphere was thinner in T1DM patients than in controls. The voxel-wise statistical map of cortical thickness difference across the entire cerebrum demonstrated cortical thinning in dorsolateral and inferior prefrontal, and precentral cortices of left hemisphere and primary visual cortex of right hemisphere in T1DM patients relative to healthy controls.

Conclussion These findings indicate that there are global structural abnormalities in cerebral cortex in T1DM patients. Furthermore, the T1DM induced cortical thinning was prominent in prefrontal, motor, and visual cortices.

Glal-Derived Neurotrophic Factor is a direct target of transcriptional activation by Egr-1

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Background Early growth response-1 (Egr-1) is an immediate early response gene, which functions as a transcription factor. Egr-1 can be rapidly induced by diverse pharmacological and non-pharmacological stimuli such as the activation of glutamate and dopamine D1 receptors, antagonism of NMDA and dopamine D2 receptors, and brain injury and ischemia in individual brain areas. Activated Egr-1 can induce the expression of several target genes that play key roles in neuronal responses (e.g., differentiation, neuronal plasticity, and astrocyte cell proliferation), such as phenylethanolamine *N*-methyl transferase (PNMT), fibroblast growth factor-2 (FGF2), platelet-derived growth factor (PDGF), synapsin I and synapsin II, suggesting that Egr-1 may play a potential role in the nervous system. Astrocytes play important roles in regulating CNS neurogenesis and synaptogenesis, as well as controlling ionic and neurotransmitter environments of the central nervous system (CNS). Astrocytes also provide neuroprotection against various neuronal damage by release of various growth factors, such as bFGF and glia derived neurotrophic factor (GDNF). This study aims to investigate whether GDNF is regulated by Egr-1 in astrocytes.

Results Egr-1 expression was clearly induced by FGF2 as a function of time both in primary rat astrocytes and in C6 rat glioma cells. The GDNF gene promoter contains putative Egr-1-bindisng sequences. To characterize whether these Egr-1-binding sequences are responsible for conferring stimulation to the *GDNF* gene promoter after FGF2 treatment, we generated the reporter constructs represent either the wild type configuration or internal deletion of three Egr-1 motifs. This set of constructs was transfected into primary cultured rat astrocytes and treated with FGF2 for the luciferase gene induction. FGF2-induced promoter activity was increased by ~2-fold in wild-type reporters, but had no effect in mutant constructs lacking apparent Egr-1-binding sequences. EMSA and ChIP assay demonstrated that Egr-1 bound directly to the proximal promoter of the GDNF gene in response to FGF2. Forced expression of Egr-1 activated GDNF transcription. Transfection of mutant promoter constructs which lack Egr-1-binding sites lost the FGF2 inducibility. Furthermore, knockdown of endogenous Egr-1 expression by introduction of siRNA specific to Egr-1 mRNA reduced the ability of FGF2 induction of GDNF expression. The role of Egr-1 in GDNF expression was further verified in primary cultured astrocytes from Egr-1 -/- mice; FGF2 induction of GDNF expression was significantly reduced in Egr-1 -/- astrocytes compared with Egr-1 +/- astrocytes.

Conclusion These study showed for the first time that Egr-1 regulates GDNF transcription through Egr-1-binding sequences that act as a positive regulation of the GDNF promoter by FGF2 signaling in astrocytes.

Inositol 1,4,5-trisphosphate 3-kinase A functions as a scaffold for synaptic Rac signaling

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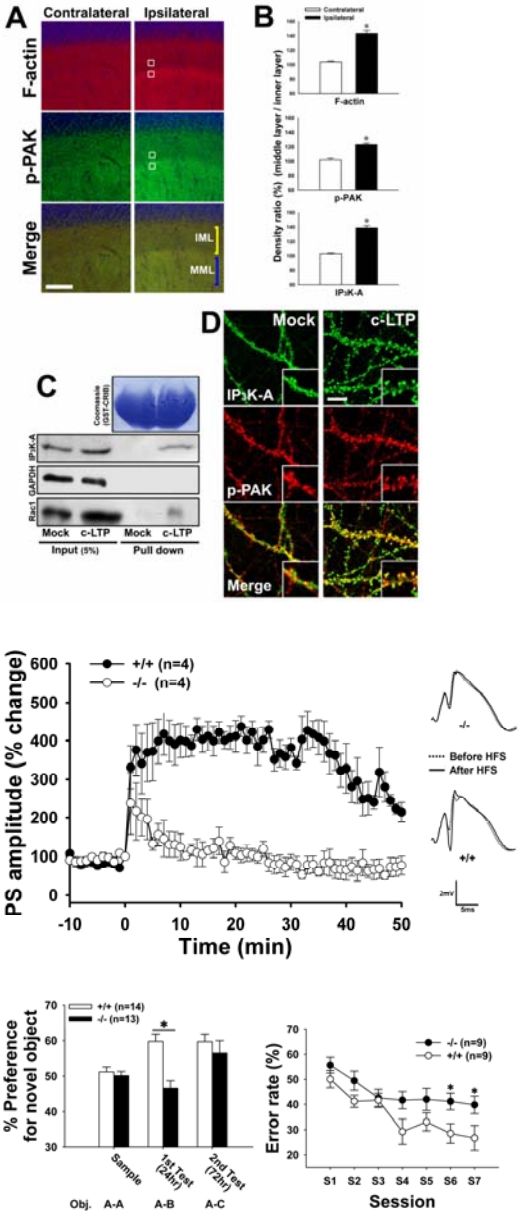
Background Activity-dependent alterations of synaptic contacts are crucial for synaptic plasticity. The formation of new dendritic spines and synapses is known to require actin cytoskeletal reorganization specifically during neural activation phases. Yet the site-specific and time-dependent mechanisms modulating actin dynamics in mature neurons are not well understood. The key regulators of actin reconstruction in dendritic spines are the small GTPase proteins such as Rho A, cdc42, and Rac1. In particular, Rac1 has been shown to regulate the morphogenesis of dendritic spines by affecting actin dynamics. The mechanism responsible for neuronal activity-dependent dynamic positioning of activated Rac1 to the dendritic actin cytoskeleton, where the Rac1 complex functions, remains unknown. In this study, we have delineated a role for inositol 1,4,5-trisphosphate 3-kinase A (IP_3K-A) in activity-dependent targeting of activated Rac1 to the F-actin fibers of neurons.

Results In this study, we show that actin dynamics in spines is regulated by a Rac anchoring and targeting function of IP_3K-A, independent of it's kinase activity. Upon neural activation, IP_3K-A bound directly to activated-Rac1 and recruited it to the actin cytoskeleton in the post-synaptic area. This focal targeting of activated Rac1 induced spine formation through actin dynamics downstream of Rac1 signaling. Consistent with the scaffolding role of IP_3K-A, IP_3K-A knockout mice exhibited defect in activity-dependent accumulation of PAK1, a downstream effector of Rac1. This deficiency resulted in a reduction in the reorganization of actin cytoskeletal structures in synaptic area of dentate gyrus. Moreover, IP_3K-A knockout mice showed deficits of synaptic plasticity in perforant path and in hippocampal-dependent memory performance.



Conclusion These data support a novel model in which IP_3K-A is critical for the spatial and temporal regulation of spine actin remodeling, synaptic plasticity, and learning and memory via an activity-dependent Rac scaffolding mechanism.

Telomerase deficiency affects the functions of the olfactory bulbs and the hippocampus *in vivo*

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Background Telomerase reverse transcriptase (TERT) has been implicated in cell proliferation and stem cell homeostasis in various tissues but not in the brain *in vivo*. Furthermore, TERT should have other roles independently of its catalytic activity. Recent studies have reported that TERT is induced to protect neuronal cells from apoptosis and cell death both *in vitro* and *in vivo*. Generally, both cell growth and death are not frequent in the brain, but the certain regions including the olfactory bulbs showed rapid cell turnover. Furthermore, the olfactory bulbs contain a population of stem and progenitor cells in which telomerase activity is detected. Based on them, we asked whether TERT exerts its roles in maintaining normal functions associated with the olfactory bulbs *in vivo*.

Results We observed the strong expression of TERT in the specific region of the adult brain such as the cerebellum, the hippocampus, and the olfactory bulbs. Interestingly, TERT expression was clearly distinguished from that of TERC in the specific brain regions including the hippocampus and the olfactory bulb. In the hippocampus, the expression of TERT, but not that of TERC, was detected, which is consistent with the absence of telomerase activity in this region. In fact, the acoustic startle, spontaneous locomotor activity and motor coordination that are linked to cerebellum function, were not altered in G1 TERT-/- mice, but olfactory bulb-related function including the odor-related performance and hippocampus-related functions such as anxiety-like behavior and recognition memory were changed. These lines of evidence indicate that telomerase may be involved in modulating normal functions of those brain regions.

Conclusion These results suggest that telomerase is constitutively expressed in the specific brain regions and may plays important roles in maintaining the normal behaviors and brain functions of the olfactory bulbs and the hippocampus, which may be independent of telomerase activity since TERC expression did not overlap with TERT in the specific regions of the olfactory bulbs and the hippocampus. More importantly, G1 TERT-/- or TERC-/- mice do not manifest any abnormality associated with telomere functions even in highly proliferative tissues. This is the first evidence showing the functional consequence of TERT deficiency in mouse brain. Although we cannot provide the molecular mechanisms governing these phenomena at this point, this study will open the new area of telomerase biology associated with brain functions.

Center For Brain Behavior Analysis Service (CBAS)

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Background In recent years, emphasis on behavioral research has been intensified for an obvious reason: Behavior is the ultimate product of the central nervous system. Advances in molecular and genetic study further increased the need for systematic assessment of animal behavior and its causal relation to the brain circuit. In addition, new approaches to drug discovery started to recognize benefits of *in vivo* screening strategies. The Center for Brain-Behavior Analysis Service (CBAS) is established to meet the increasing needs of quantitative phenotypic assessments. Our goal is (1) to screen for a broad range of behavioral phenotypes of normal and mutant mice and establish phenotyping protocols for functional analyses, (2) to characterize phenotypic abnormalities of diverse animal models of brain disease and develop behavior test protocols to evaluate therapeutic effect of experimental treatments, (3) to establish novel behavior test procedures and to develop test apparatus and customized programs for the collection and analysis of behavioral data, and (4) to offer training programs for researchers outside of behavioral neuroscience field to meet growing needs for assessing cognitive and emotional abilities in behaving animals.

Results CBAS has provided investigators with four different services.

First, we have conducted more than a dozen behavior test services for functional analyses of the brains of various model animals. We characterized a broad range of mutant mice and disease models using rats and mice. A total 19 behavior analysis services with over 100 behavior tests were performed within the last year.

Second, we have established behavior test protocols for carrying out standardized behavioral analyses. We have documented in detail all phenotyping procedures and protocols and formalized them into test manuals to provide a reference database for investigators in other laboratories. The result was two method books (published in Korean) and one illustrated manual (in preparation in English with a tentative title '*Protocols for Functional Analysis*').

Third, we have developed the apparatuses and software for the analysis of animal behavior. In addition to the two patented test apparatus (both in the process of registration), we developed a pain testing apparatus based on naturalistic rearing posture. The new patent was developed in collaboration with a company. We also developed a software tool to analyze eyeblink response for rodents and humans (Vision-Based Eyeblink Analysis Tool: V-BEAT), which is frequently to assess simple motor learning. The program is being applied for a patent.

Finally, we have been developing training programs for various researchers in different research fields for behavioral analyses. In an effort to disseminate our know-hows to the neuroscience community, we are preparing the the 3rd workshop for behavior analyses of brain function in December. Lectures on behavioral analysis and hands-on training workshops will be held.

Conclusion CBAS has helped neuroscientists working in various fields including molecular and genetic neuroscience, disease mechanism, drug discovery and pre-clinical industry by providing accessible options for behavioral phenotyping and screening in multiple occasions. We will continue our effort to bridge the gap between in vivo testing and in vitro approaches through test services, database sharing and training programs.

Different expression of TASK-1 and -3 in electrically and pathologically proven hippocampal sclerosis

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Background Conventional Electrocorticography (ECoG) analyses have been performed on a 2-D space comprised of intracranial electrodes. Thus, despite the fact that ECoG data have a much higher signal-to-noise ratio than electroencephalographic data, ECoG inherently requires a priori information to locate the electrodes, and it is difficult to estimate the depth of the epileptogenic focus using ECoG. A 3-D approach is needed to determine the presence of an epileptogenic focus in the complex structure of the cortex. We applied a spatiotemporal source localization technique using the first principal vectors. A directed transfer function was then employed for the time series of the potential ictal sources to compute the causal relationship between them, from which we could identify the primary sources responsible for the ictal onset. We selected sample from operated patients from Brain Bank, who had hippocampla sclerosis and seizure activity in a spatiotemporal source localization technique. We have recently reported that TASK-1 may play a role in epileptogenesis, and be targets of anti-epileptic drugs in experimental epilepsy model (Kim et al., 2008; Kim et al., 2007). We performed an analysis of **T**andem of P domains in a **W**eak **I**nwardly rectifying **K**$^+$ channel (TWIK)-related acid-sensitive K^+ (TASK)-1 and -3 channel immunoreactivity from samples from hippocampus of electrically/histologically proven medial temporal lobe epilepsy (hippocampal sclerosis; HS), that of non-HS, that of autopsy human, that of pilocarpine treated rat and normal rat, Here, we show the differential expression of TASK-1 and -3 in the hippocampi of epileptic patients and experimental epilepsy models, which extend our understanding of the roles of TASK1-3 regarding involvement in epileptogenesis and characterizing properties of the epileptic hippocampus.

Results The validated ictal source localization approach was applied to a number of ictal ECoG data sets recorded from six epilepsy patients, and the resultant 3-D ictal source locations coincided with the surgical resection area as well as the traditional 2-D electrode-based source estimates.
In the normal human hippocampus, TASK-1 and -3 immunoreactivity was mainly observed in hippocampal pyramidal neurons and dentate granule cells. In the hippocampi of patients with hippocampal sclerosis, TASK-1 and -3 immunoreactivity was rarely observed in neurons. However, TASK-1 immunoreactivity was observed in astrocytes, and TASK-3 immunoreactivity was detected in astrocytes as well as microglia. In the rat hippocampus, TASK-1 immunoreactivity was observed in astrocytes within normal and epileptic hippocampus. TASK-3 immunoreactivity was strongly detected in the CA1-3 pyramidal cell layer and the granule cell layer of the dentate gyrus within the normal hippocampus. Following status epilepticus, TASK-3 immunoreactivity was increased in astrocytes and microglia, while TASK-3 immunoreactivity was rarely observed in neurons.

Conclusion We developed new method to analyze surface ECoG and enlarged the method of interpreting conventional method. With this result, better localization of seizure onset zone is expected. We also may differentiate epileptogenic hippocampus from non epileptogenic one. From electrically proven hippocampus, we found different expression of TASK-1 and-3. These results may enlarge our understanding for epileptogenesis in hippocampus.

Anamorsin, an anti-apoptotic protein, is cleaved by caspase-3 during dopaminergic neuronal apoptosis

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Background Apoptosis is an active cell death process in which a family of cysteine proteases called caspases participates in so-called cascade process of enzyme activation. Caspase-3 is one of the well-established effector caspases in the process and once activated, it cleaves several important cellular proteins leading to cell death. To further screen potential endogenous caspase-3 substrates in apoptotic cell death in general and in the mouse dopaminergic neuronal cell line (MN9D) in particular, we developed a novel tool in conjunction with 2-dimensional electrophoresis and mass spectrometry.

Results

We identified approximately 45 putative caspase-3 substrates by using this method. Subsequent study revealed that most of identified putative substrates were confirmed to be cleaved following treatment of purified caspase-3 into in vitro transcribed and translated putative substrate in the presence or the absence of z-VAD, a pan-caspase inhibitor. Among the putative caspase-3 substrates, our further study was focused on Anamorsin, a newly identified protein with anti-apoptotic function which is indispensible in the definitive haematopoiesis process. We confirmed that Anamorsin is indeed one of caspase-3 substrates as determined by in vitro caspase-3 cleavage assay as well as 6-hydroxydopamine (6-OHDA)-treated cell-based caspase cleavage assay. By applying a point mutation onto the several consensus sequences for caspase-3 cleavage within the primary sequences, we mapped potential caspase-3-mediated cleavage site of Anamorsin. This was also confirmed by series of transient expression of each mutated cDNA sequences in MN9D cells treated with 6-OHDA. In the subsequent studies, cleavage of Anamorsin are detected in several postmortem brains or experimental models of the CNS disorders including Parkinson's disease, Alzheimer's disease, ischemia and spinal cord injury. Overexpression of Anamorsin into MN9D cells accelerated STS-induced apoptosis, whereas shRNA mediated knockdown of Anamorsin sensitized MN9D cells to STS-induced apoptosis.

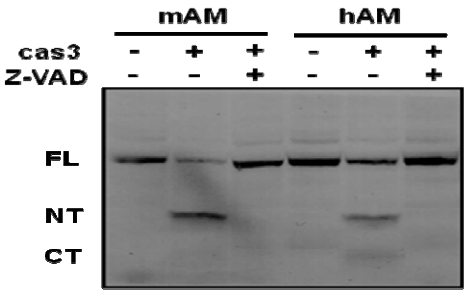

Figure 1. Both mouse and human Anamorsin are cleaved by caspase-3 *in vitro.*

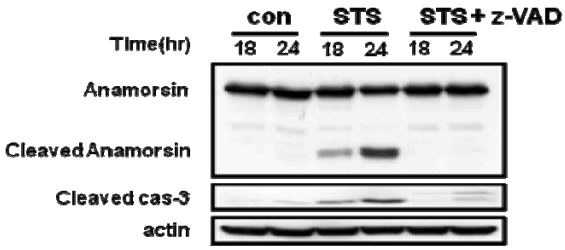

Figure 2. Anamorsin is cleaved during dopminergic neuronal apoptosis.

Conclusion

We identified Anamorsin as a novel substrate of caspase-3 and determined the cleavage sequence of Anamorsin by caspase-3. We have now conducted studies delineating functional roles of the caspase-3 substrates in the progression of dopaminergic neuronal death and subsequently its associated apoptotic mechanisms. Outcome of the study will help us to better understand the pathophysiology of the CNS diseases including Parkinson's disease.

Natural Products Bank

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Background Natural products have been the most successful source of drug discovery ever due to their chemical and pharmacological diversity. Recent development of molecular biology has provided efficient screening tools for various biological activities. Moreover, it has enabled us to elucidate the action mechanisms of active natural products. Accordingly, a need for a natural products bank which can provide various natural products for researchers has been growing. Thus, natural products bank should be equipped with a convenient operating system and expertise as a core facility in the brain research center.

Results The core facility established the natural products bank, consisting of 1000 plant extracts and 311 pure natural compounds. The extraction conditions for plant extracts were optimized, which led to ensure the reproducibility for extracting process. Also, the purification methods were further improved for the pilot scale production of bioactive compounds from natural products.
In addition, an efficient providing system was set up to offer important information of natural products including methods for extracts preparation and standardization, physicochemical properties, and bioactivities of compounds in the bank. Moreover, to raise the efficiency in finding new active compounds, " neuroprotective plant extracts kit", consisting of 50 different plant extracts which already known to show antioxidative, antiinflamatory, and/or neuroprotective activities was developed. It can offer a convenient providing services for efficient utilization.
Besides the establishment of natural products bank and its providing system, neuroprotective compounds were isolated and characterized from several plant extracts of the bank, *Stemona tuberose, Biota orientalis, Idesia polycarpa, Euscaphis japonica, Dictamnus dasycarpus* and Amomum tsao-ko by bioactivity-guided separation. Also, the action mechanisms of the compounds deposited in the bank, such as 15-metoxypinusolidic acid, decursinol, decursin and torilin were elucidated. The results suggest these extracts and compounds as therapeutic candidates for various neurodegenerative diseases.
In the meantime, the Natural Products Workshop was held for the scientists who were not familiar to natural products. The workshop introduced recent trends for the drug development from natural products, which suggests how bioactive compounds from natural products can be obtained and utilized for drug development.

Conclusion Based on this study, natural products bank which can provide various extracts and isolated compounds with reliable information for drug discovery was successfully established. The investigations on the bioactivities and action mechanisms of natural products were performed using the natural products bank. In addition, the database and infrastructure for the maximal use of the natural products bank were built up. The natural products bank, as one of the core facilities in the brain research center will play a key role by providing the new paradigm to develop new drug candidates from natural products.

New Brain Atlas: Ultra high resolution brain maps obtained by the 7.0 Tesla MRI and Cryomacrotome

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Background The advances in neuroanatomy are no longer of interest only to the anatomist. In the nervous system, structural organization is most essential in understanding the functional concepts. Therefore, a solid understanding of the subtle details of the brain structure became important for a lucid communication amongst the clinicians, scientists and other specialists working in the field of neuroscience and clinical medicine.Recent advances in MRI, especially those in the Ultra High Field (UHF) MRI of 7.0 Tesla (T), have attracted significant attention in the field of brain imaging for neuroscience research as well as in clinical applications. On another front, we have witnessed great advances in imaging of human cadavers using cryomacrotome with increased sophistication and techniques.With all these super resolution images that can be obtained with UHF MRI, such as 7.0T and the advanced cryomacrotome technology developed recently, we thought that development of a New Brain Atlas would be an important and timely contribution to the medical and neuroscience community.

Results We made a set of ultra-high resolution brain images obtained from a cadaver by cryomacrotome and by reconstructed partner images (sagittal and coronal), together with corresponding in vivo ultra-high resolution human brain images obtained by 7.0T MRI. Thus, we have been able to obtain a complete set for the entire brain atlas with axial, sagittal, and coronal images of the cadaver and a 7.0T MRI of the in vivo brain. We applied a reference system that can be applicable to both cadaver gross anatomy and MRI anatomy. A system of Cartesian axes is defined on the midpoint of central intercommissural distance, with coordinates expressed in mm and +/- in axial, coronal and sagittal This system will enable us to standardize the sectional planes and the levels of the slices in any brain that may be encountered in practice.For the reader's convenience, we have displayed a few selected view images with expanded views to visualize details of the images. We believe that the expanded or magnified images provide information not previously available.

Conclusion We hope this work will serve as a Road Map both in the clinical arena and for the neuroscience researchers. The ultra high resolution brain atlas will accelerate research in neuroscience, and aid in clinical applications such as drug discovery and investigation of dysmorphia and malformation of the human brain, among others.

Signal transformation in the primary visual cortex for perceptual decision

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Our everyday activity is full of visually-guided behavioral responses. One of the simplest is to detect a visual target and make a saccadic eye movement to it. A series of processing is thought to mediate this response: target information is collected by the sensory system, and based on this, a perceptual decision is made as to whether and where the target is present, and then a behavioral response is initiated by the motor system. The response time (RT) for saccades, i.e., the elapsed time from the onset of the stimulus to the onset of the saccadic eye movement, depends on surprisingly diverse factors. More surprising is the fact that RT also varies a great deal on a trial-to-trial basis even under identical conditions. Various models have been proposed to explain RT variability (1, *2*), and neural mechanisms of RT variability have usually been sought beyond the primary visual cortex (V1), such as in the frontal eye field (3).
Neuronal activity in V1 in saccade tasks shows considerable variability in response to the presentation of the identical saccade target across repeated trials. Regarding the functional significance of this variability, there are two scenarios. In the traditional view, the role of V1 for visually-guided responses is purely sensory, and signals related to the behavioral response are generated entirely in later stages, and thus, V1 variability is a noisy fluctuation around the mean visual response and uncorrelated with RT. In the second scenario, the variability in neuronal activity of V1 persists throughout subsequent processes, and the subsequent response initiation accordingly fluctuates, resulting in RT variability. The initial variability at sensory stages may grow as the variable signal crosses subsequent synapses, and the origin of response variability can be traced in sensory processing stages.
In the current study, we examined whether the origin of RT variability can be traced back to V1 by examining covariation of trial-to-trial variability in V1 response to visual target and RT.
We recorded the activity of single units in the monkey V1 while they detected a peripheral target of varying contrast and made saccadic eye movements to it. Measures of first spike timing and spike count were obtained, and their roles for behavioural response were analysed.

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Role of dentate gyrus in aligning cognitive map to external landmark

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Humans and animals form internal representations of external space based on their own body movement (dead reckoning) as well as external landmarks. It is poorly understood, however, how different types of information are integrated to form a unified map of external space (i.e., cognitive map). Because the medial and lateral entorhinal cortex, which appear to separately process spatial and nonspatial information, respectively, send converging projections to the dentate gyrus (DG), we hypothesized that one role of the DG is binding spatial and nonspatial information. To test this hypothesis, we conducted physiological and behavioral experiments in Bax knock-out (Bax-KO) mice, in which post-mitotic neuronal death is completely blocked and as a result newly generated granule cells continue to accumulate disrupting neural circuitry specifically in the DG.

Results Place-specific discharges of hippocampal neurons were dissociated from a distinct visual landmark in Bax-KO mice.

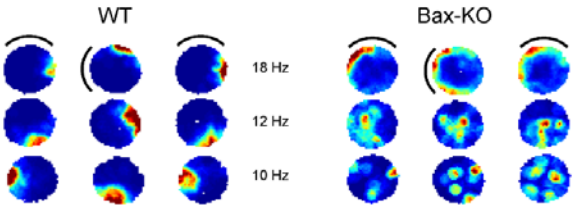

Figure 1. Spatial firing of hippocampal neurons across three recording sessions.
The black arcs indicate the location of a visual cue. Behaviorally, when target locations predicted by dead reckoning and external landmarks were made incongruent, these animals were impaired in finding a target location based on visual landmarks.

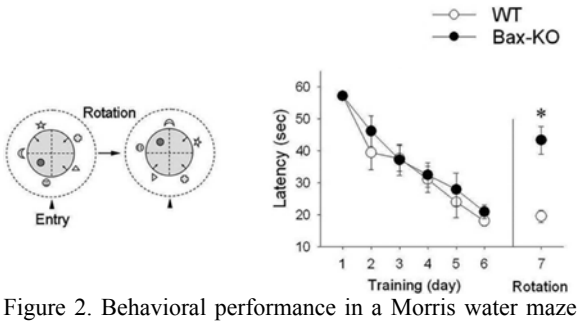

Figure 2. Behavioral performance in a Morris water maze task.
The graph shows the latency to find a hidden platform during six days of training. On probe trial (day 7), visual cues and the platform location were rotated 90° clockwise.

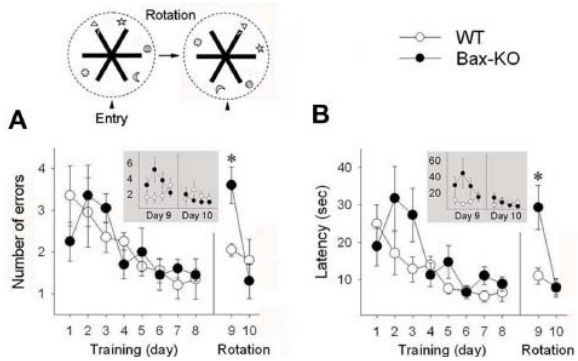

Figure 3. Performance in a spatial reference memory task on a six-arm radial maze.
Animals were trained to find water reward at the end of one arm. Following eight days of training, the maze, the target location and visual cues were rotated together randomly in steps of 60◦. Insets: trial-by-trial performance of the animals during the last two days on which the maze was randomly rotated.

Conclusion Our results show that intact DG is required for aligning the internal representation of space (cognitive map) to external landmarks. They are also consistent with the proposed role of DG in integrating spatial and nonspatial information in constructing context-specific spatial representations.

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