[15:40 – 16:10]

# Development of Inhibitors of β-Amyloid Plaque Formation

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Alzheimer's disease (AD) is the most common form of dementia in the aging population and is clinically characterized by a progressive loss of cognitive abilities. Pathologically, it is defined by the appearance of senile plaques - extracellular insoluble, congophilic protein aggregates composed of amyloid β (Aβ) and neurofibrillary tangles (NFTs) – inyracellular lesions consisting of paired helical filaments from hyperphosphorylated cytoskeletal tau protein as described by Alois Alzheimer a century ago. These hallmarks still serve as the major criteria for a definite diagnosis of the disease. Consequently, one of the key strategy for drug development in this disease area focuses on reducing the concentration of cerebral Aβ plaque by using substances that inhibit Aβ fibril formation.

We focused on developing inhibitors by synthesizing several kinds of aromatic molecules. The synthetic compounds were initially screened to evaluate the effective compound by tioflavin T fluorescence assay. The selected effective compounds were tested cytotoxicity and protective effect from Aβ-induced neuronal toxicity by cell based MTT assay with HT22 hippocampal neurons. The BBB permeability on effectors was also tested in in vitro co-culture model (HUVEC/C6 cell line). The behavior test was carried out in mutant APP/PS1 transgenic mouse model of Alzheimer's disease. And inhibition of Aβ fibril formation by the effective compound was monitored with transmitted electron microscopic images.

# Development of Inhibitors of $\beta$-Amyloid Plaque Formation

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2006. 4. 26

Dong Jin Kim

**1994년 11월 5일** 그는 "내 생애의 황혼으로 이끌어 갈 여행을 시작한다"며 **알츠하이머병**을 앓고 있음을 알린 후 **10년만인 2004년 6월5일** 그 생을 마감하며 미 캘리포니아주 시미밸리의 '로널드 레이건

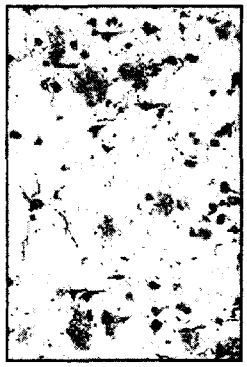
친애하는 미국인 여러분

나는 최근에 본인이 **알쯔하이머병**에 걸린 **수백만 미국인들** 중의 한명이 되었다는 이야기를 들었습니다. 이 사실을 알고 낸시와 나는 이 사실을 우리의 개인적인 비밀로 할 것인가 아니면 여러 사람들에게 알릴 것인가를 결정해야 했습니다. (중략) 우리는 이러한 것을 여러분들과 나누는 것이 중요하다고 느꼈습니다. 우리는 내가 알쯔하이머병에 걸렸다는 사실을 여러분들에게 알림으로써 이 병에 대한 보다 많은 관심이 유발되기를 진심으로 바랍니다. 이렇게 함으로써 이병으로 **고생하는 환자와 그 가족들에** 대한 이해를 높일 수 있을 것입니다. (중략) 불행하게도 내가 앓고 있는 알쯔하이머병이 점차 심해지면 **가족들이 힘든 고통을 겪을 것입니다.** 나는 내 아내 낸시를 이 고통스러운 경험에서 구할 수 있는 어떤 방법이 있기를 바랍니다. 그 때가 오면 여러분들의 도움으로 그녀는 믿음과 용기를 가지고 굳게 맞설 것이라고 믿습니다.

마지막으로 나에게 이 나라의 대통령으로써 일할 수 있었던 큰 영광을 준 여러분들께 감사드립니다. 언제일지 모르나 하나님께서 당신의 집으로 나를 부를 때, 나는 조국에 대한 깊은 사랑과 조국의 장래에 대한 영원한 희망을 가지고 떠날 것입니다. 이제 나는 **내 인생의 황혼기로의 여행을** 시작합니다. 미국의 앞날에는 항상 밝은 아침이 있을 것임을 믿습니다. 감사합니다. 친구들! 신의 축복이 있기를 기원합니다. 로널드 레이건

# Alzheimer's Disease (AD)

- 1907년 독일인 의사
  Alois Alzheimer 에 의해 발견
- 기억력 장애와 편집증적인
  망상을 보인 뇌신경질환을
  앓다가 사망한 51세의 환자
- 병리학적인 부검소견상
  - Senile Plaque (노인성 반)
    *β-Amyloid (Aβ)*
  - Neurofibrillary Tangle
    (NFT, 신경섬유 뭉치)
    *Tau protein*



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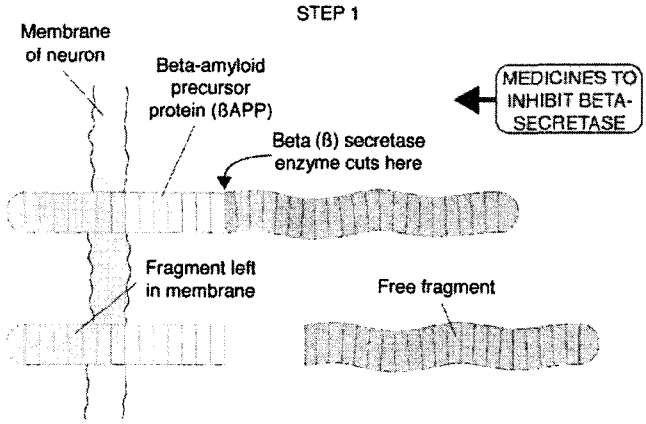
# β-Amyloid

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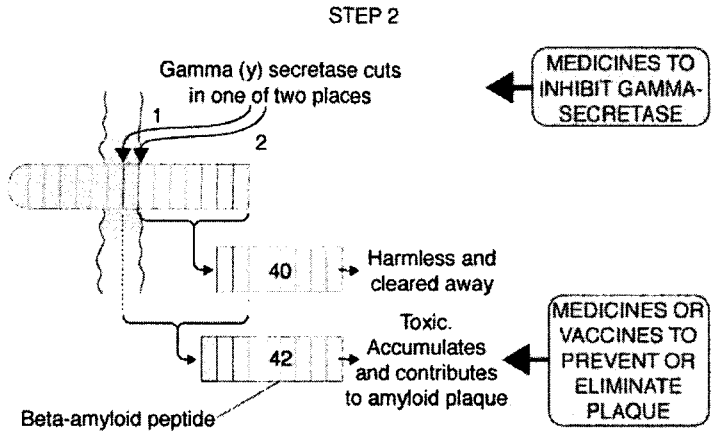
# β-Amyloid

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# Anti-amyloid strategies

| Drug Class | Aim | Pros | Cons |
|---|---|---|---|
| β-Secretase inhibitors | Decrease Aβ synthesis | BACE KO mouse are normal | In human ? |
| γ-Secretase inhibitors | Decrease Aβ synthesis | Decrease Aβ in mouse model | Side-effects associated with lack of other membrane proteins |
| Metal chelators | Prevention of aggregate formation by metal chelation (Zn, Cu) | In mouse model, decrease Aβ plaque | Harm the brain<br>Clioquinol in combination with Vitamin B12 is in clinical. |
| Aβ fibril inhibitor | Prevention of aggregate formation | In vitro, decrease Aβ plaque | ? |
| Aβ vaccination | Immune response against Aβ peptide | In TG mouse, decrease Aβ plaque improve cognitive function | Phase II suspended (brain inflammation) |
| Statins | Decrease Aβ by reducing cholesterol (mechanism unknown) | Reduced risk of developing AD in patients treated with statins | ? |
| NSAIDs | Inhibition of Aβ generation | Reduced risk of developing AD in patients treated with NSAIDs | Side-effects at the gastrointestinal tract following prolonged treatment |

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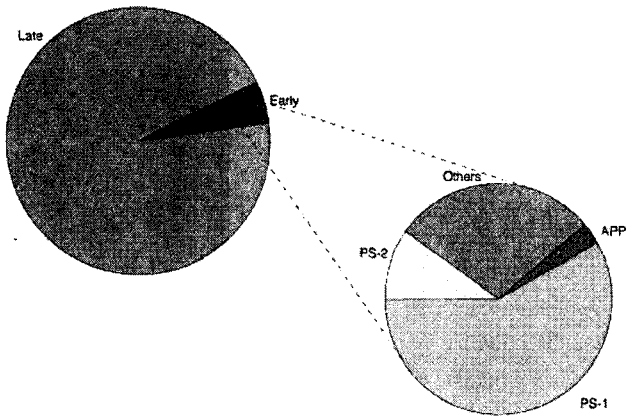
## Relative Frequency Related to Gene Mutation



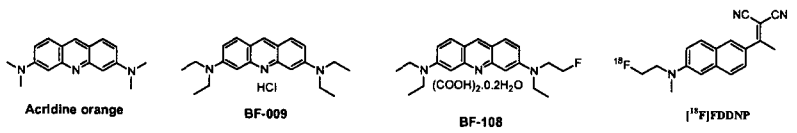

## Animal Model (TG Mouse)

| Model | Characteristics | Remark |
|---|---|---|
| Apoe E4 | Impaired spatial learning & memory in 14 months | Jackson Lab. |
| Psen I | Major gene early-onset familiar AD No Aβ deposits | Jackson Lab. |
| App2576 | Aβ deposits in 12 months | Jackson Lab. |
| App-Psen I | Aβ deposits in 6-7 months | Jackson Lab. |
| Psen II | No Aβ deposits | KFDA |
| App-Psen II | Aβ deposits in 8 months | KFDA |

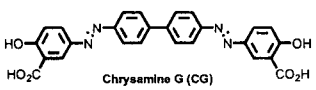
## Inhibitors of Aβ Fibril Formation

| Compound | IC50 | Aβ | Remarks |
|---|---|---|---|
| Acridine orange | 32nM[a] | Aβ40 (5μM) | *Neuroscience Research*, 2004, 65. |
| BF-009 | 167nM[a] | Aβ40 (5μM) | |
| BF-008 | 135nM[a] | Aβ40 (5μM) | |
| [18F]FDDNP | 457nM[a] | Aβ40 (5μM) | |
| X-34 | 452nM[a] | Aβ40 (5μM) | |
| HMPBr | 150μM[b] | Aβ40 (11.6μM) | *Biochem. J.* 1999, 283. |
| Doxorubicin | 30μM[b] | Aβ40 (11.6μM) | |
| Carvedilol | 30μM[b] | Aβ40 (11.6μM) | |
| SKF-74652 | 28μM[b] | Aβ40 (11.6μM) | |
| Daunomycin | 30μM[b] | Aβ40 (11.6μM) | |
| Rolitetracyclin | 59μM[b] | Aβ40 (11.6μM) | |
| Benzoquinone | 47μM[b] | Aβ40 (11.6μM) | |

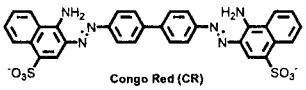
a : ThT assay
b : Immunoassay



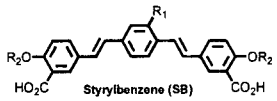
## Amyloid-fibril specific Agents




Chrysamine G (CG)

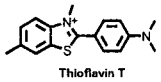



Congo Red (CR)

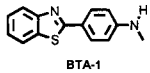



Styrylbenzene (SB)

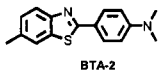
| | $R_1$ | $R_2$ |
|---|---|---|
| X-34 | H | H |
| X-04 | OMe | H |
| BSB | Br | H |
| ISB | I | H |
| IMSB | I | Me |



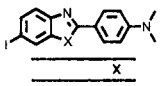
Thioflavin T



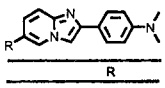
BTA-1



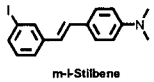
BTA-2



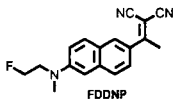
| | X |
|---|---|
| TZDM | S |
| IBOX | O |



| | R |
|---|---|
| IMPY/H | H |
| IMPY/Me | Me |
| IMPY | I or [$^{125}$I/$^{123}$I] |

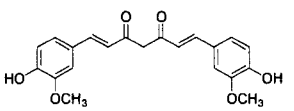


m-I-Stilbene

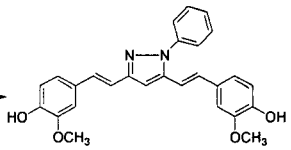



FDDNP

## Curcumin




curcumin



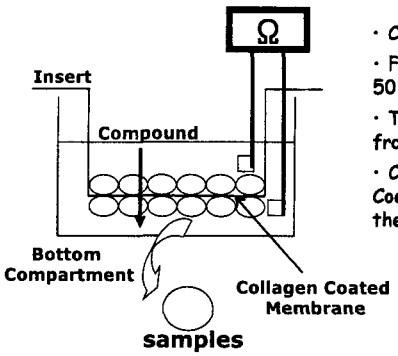

11-001

**WO 2005/006945**

- Neuroprotective Effect
  - HT22 cell, glutamate oxidative stress
  - Rat primary cortical neurons cultured
  - Rat primary cortical neurons cultured
- BBB penetration in mouse

# In Vitro BBB Model

→ In Vitro Co-Culture Models of the BBB




- Control compound → Rhodamine 123
- Final concentration of the compounds → 50 μM
- The samples → every 20min for 2 hours from the bottom compartment
- Caculation of the Papp (Permeability Coefficient) value for the permeability of the compounds

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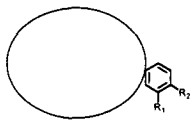
# *In silico* BBB




| X | $R_1$ | AlogP $R_2$ | | | BBB | | |
|---|---|---|---|---|---|---|---|
| | | $NH_2$ | $NHCH_3$ | $N(CH_3)_2$ | $NH_2$ | $NHCH_3$ | $N(CH_3)_2$ |
| N | $OCH_3$ | 4.0 | 5.1 | 5.8 | 0.06 | 4.3 | 0.32 |
| C | $OCH_3$ | 4.3 | 5.4 | 6.1 | 0.26 | 10.0 | 3.5 |

| X | $R_2$ | AlogP $R_1$ | | | BBB | | |
|---|---|---|---|---|---|---|---|
| | | $NH_2$ | $NHCH_3$ | $N(CH_3)_2$ | $NH_2$ | $NHCH_3$ | $N(CH_3)_2$ |
| N | $OCH_3$ | 4.0 | 5.1 | 5.8 | 0.06 | 4.3 | 0.32 |
| C | $OCH_3$ | 4.3 | 5.4 | 6.1 | 0.26 | 10.0 | 3.4 |

$NH_2 < NHCH_3 < NH(CH_3)_2$

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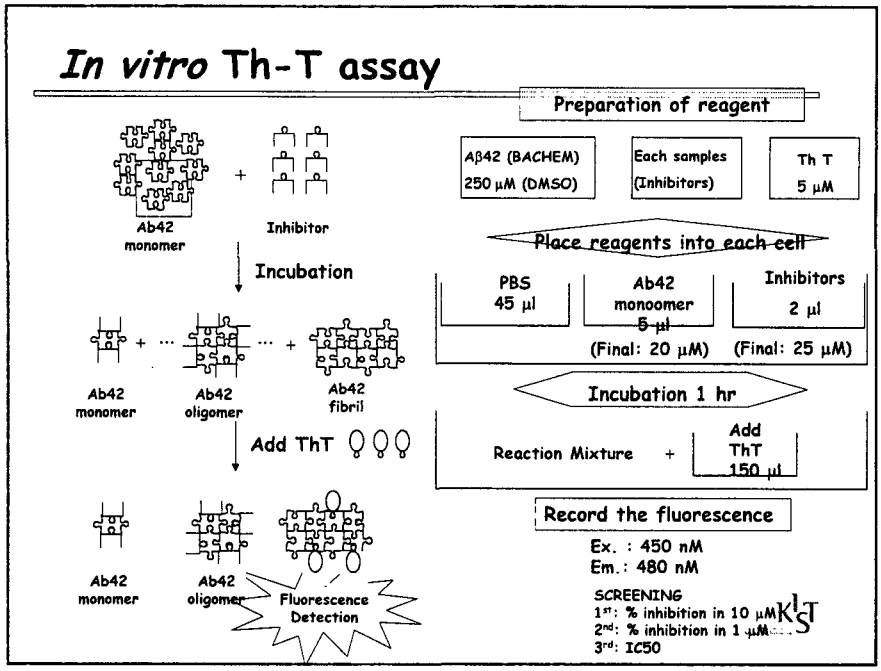
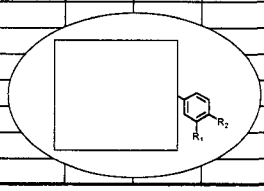



* Aβ42 (25 μM)

| Compound | X=N | | X=C | | $IC_{50}$ * 1 (mM) | *In silico* AlogP | *In vitro* BBB (papp) |
|---|---|---|---|---|---|---|---|
| | R1 | R2 | R1 | R2 | | | |
| KMS80007 | OMe | OH | | | 0.8 | | |
| KMS80018 | OMe | $NH_2$ | | | 0.7 | 4.0 | |
| KMS80029 | OMe | NHMe | | | 0.5 | 5.1 | |
| KMS80019 | OMe | $NMe_2$ | | | 0.8 | 5.8 | 6.6 |
| KMS80017 | $NH_2$ | OMe | | | 0.6 | 4.0 | |
| KMS80034 | NHMe | OMe | | | 1.1 | 5.1 | |
| KMS80035 | $NMe_2$ | OMe | | | 1.1 | 5.8 | 16.0 |
| KMS80012 | | | OMe | $NH_2$ | 0.6 | 4.3 | |
| KMS80030 | | | OMe | NHMe | 0.6 | 5.4 | |
| KMS80013 | | | OMe | $NMe_2$ | 1.0 | 6.1 | 67.0 |
| KMS80014 | | | $NH_2$ | OMe | 0.6 | 4.3 | |
| KMS80015 | | | NHMe | OMe | 0.6 | 5.4 | 7.2 |
| KMS80036 | | | $NMe_2$ | OMe | 1.1 | 6.1 | |
| HMPBr | | | | | 90.0 | | |
| IMSB | | | | | 8 | | |
| Acridine orange | | | | | 0.6 | | 1.03 |
| Curcumine | | | | | 0.5~0.8 | | 3.75 |
| FDDNP | | | | | 0.4 | | |
| PIB | | | | | 1.2 | | |
| 11-011 | | | | | 1.1 | | |

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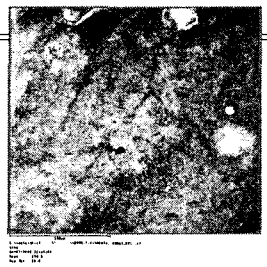
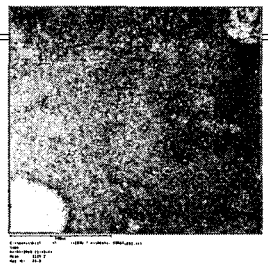
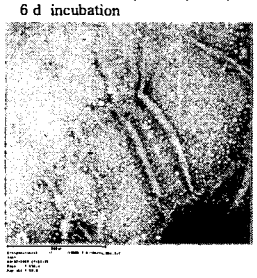
## TEM



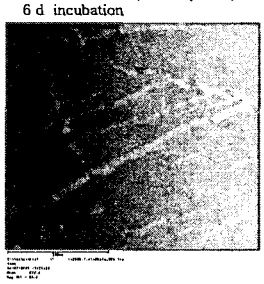
a) KMS 80013 120 μM + Aβ 200 μM
6 d incubation



b) KMS 80013 120 μM + Aβ 200 μM
6 d incubation



c) Aβ 200 μM only
6 d incubation



d) Aβ 200 μM only
6 d incubation

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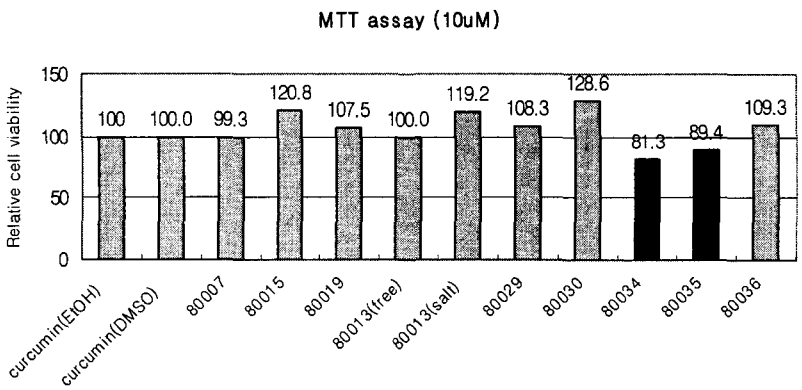
## Cytotoxicity (HT-22)

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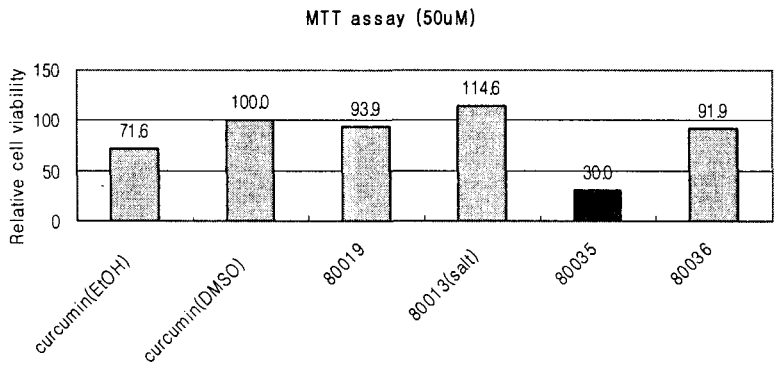
# Cytotoxicity (HT-22)

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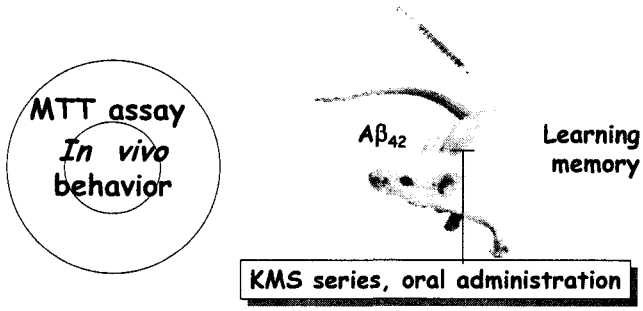
# *In vivo* behavior test

In *in vivo* learning assay, KMS series(KMS80013, KMS80019, KMS90036) were orally administered to APP/PSII transgenic mouse (Aβ deposits in 3 months).

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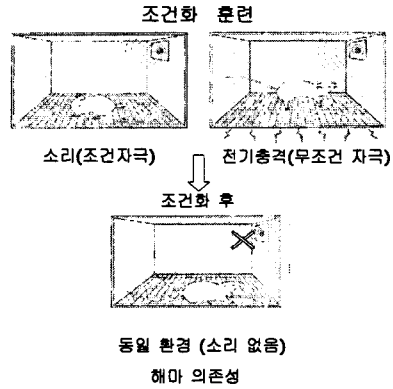
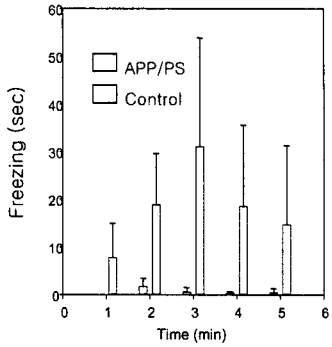
# Fear Conditioning

**Contextual conditioning**

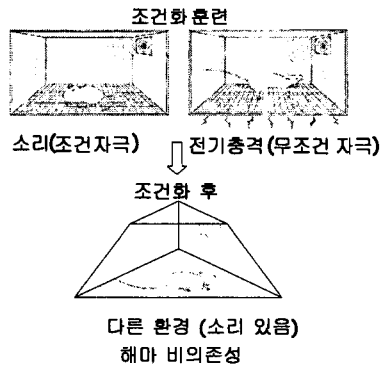


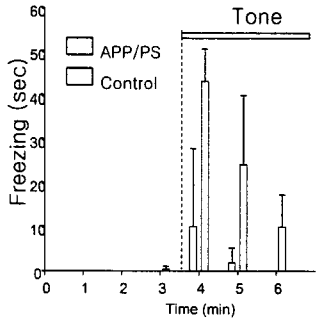




조건화 훈련 후 동일 환경(소리없음)에 처한 경우 **control mouse**는 **freezing**현상을 보이는 반면, **APP/PS mouse**는 거의 **freezing** 현상을 보이지 않음.

# Fear Conditioning

**Cue conditioning**




조건화 훈련 후 다른 환경(소리있음)에 처한 경우 **control mouse**는 **freezing**현상을 보이는 반면, **APP/PS mouse**는 거의 **freezing** 현상을 보이지 않음.

## Acknowledgement

Seong Rim Byeon
Ji Hoon Lee
Hye Yun Kim
Ye Lim Kim

Prof. Inhee Mook-Jung (SNU)
Prof. Dae Soo Kim (KAIST)
PhD. Hanjo Kim (BMD)