April 11. 2003 (Friday) 10:10~10:40

마약중독시 변화하는 NMDA 수용체의 기능 및 발현

좌장 : 고광호(서울대학교)

오 세 관

(교수, 이화여자대학교 의과대학)

Modulation of NMDA Receptor Function and Expression in Drug Abuse

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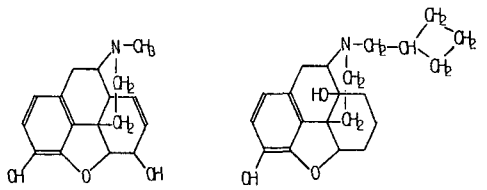
Butorphanol



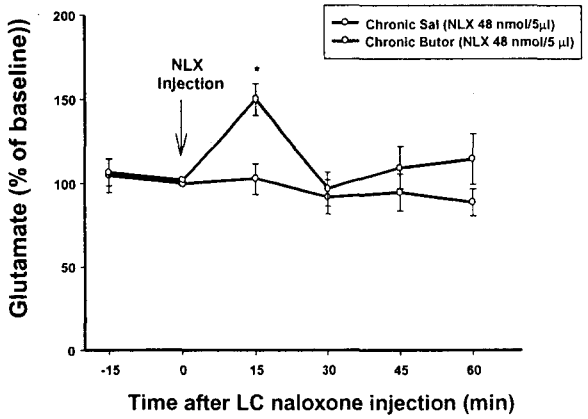
Morphine Butorphanol

1. Clinically used opioid analgesic agent
2. Mixed agonist/antagonist acts on opioid receptor
   (agonist on kappa receptor, antagonist on mu receptor)
3. Low abuse potential
4. Chronic use: development of tolerance and dependence

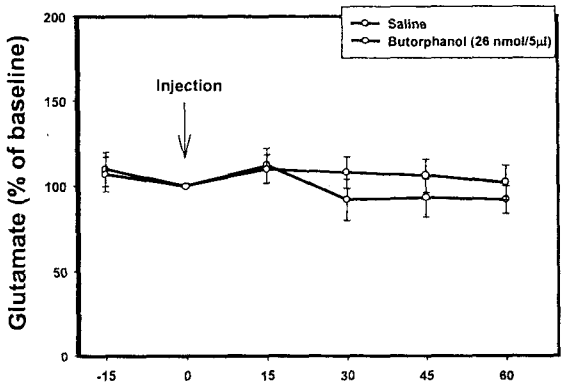
Withdrawal signs elicited in morphine- or butorphanol-dependent rats by naloxone injection

Withdrawal signs	Saline	Morphine	Butorphanol
Escape behavior	0/8	5/8*	4/8*
Wet dog shakes	2/8	8/8**	8/8**
Teeth chattering	0/8	7/8**	8/8**
Rearing	0/8	8/8**	7/8**
Locomotion	1/8	7/8*	6/8*
Stretching	0/8	6/8**	5/8*
Scratching	0/8	6/8**	7/8**
Salivation	0/8	6/8**	5/8*
Penis licking	0/8	7/8**	7/8**
Ptosis	0/8	6/8**	6/8**

Increases of extracellular glutamate within the locus coeruleus during butrophanol withdrawal



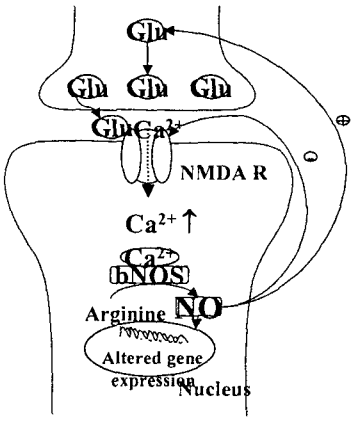
Acute i.c.v. injection of butorphanol does not affect extracellular glutamate within the locus coeruleus



1. Glutamate: Major excitatory neurotransmitter in CNS
2. Glutamate receptor:

   NMDA, AMPA, Kainate
3. NMDA receptor

   : Involved in the phenomena of opioid dependence and withdrawal



Experimental Protocol

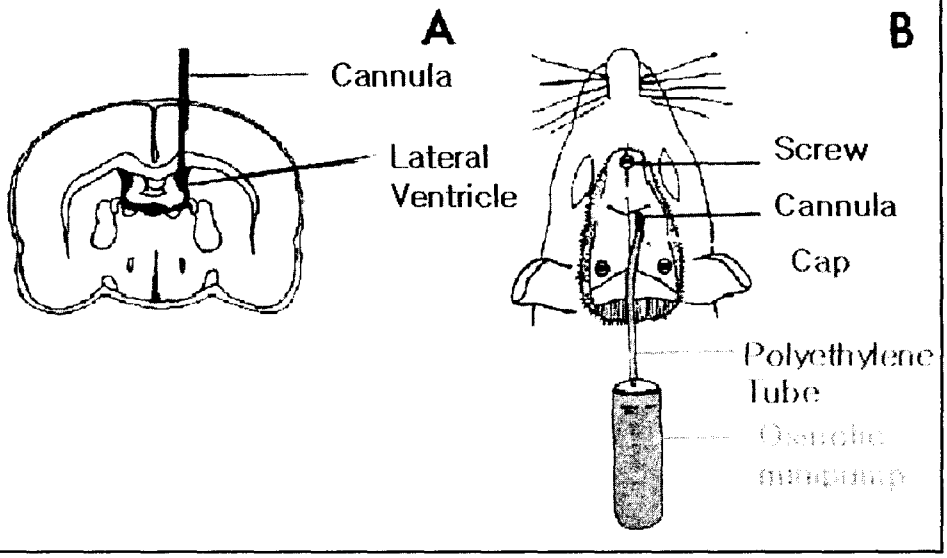
SD rats
220-240 g

Surgery for i.c.v.

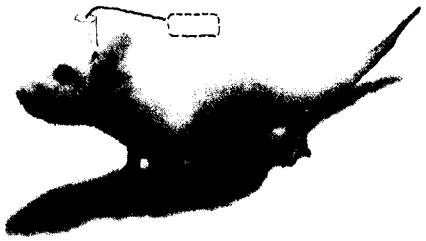
Implant Osmotic pump Butorphanol (26 nmol/ μl/hr)

Brain section (20 um) for Autoradiography *In situ* hybridization

Depiction of an i.c.v. infused rat



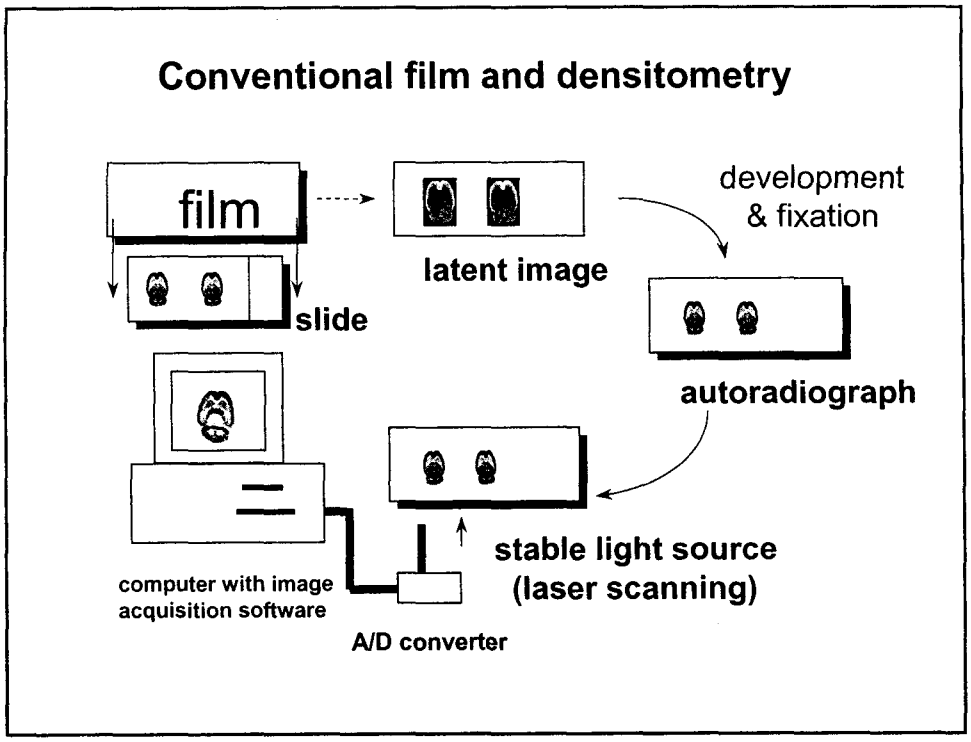
Chronic microinfusion into icv by using osmotic minipump

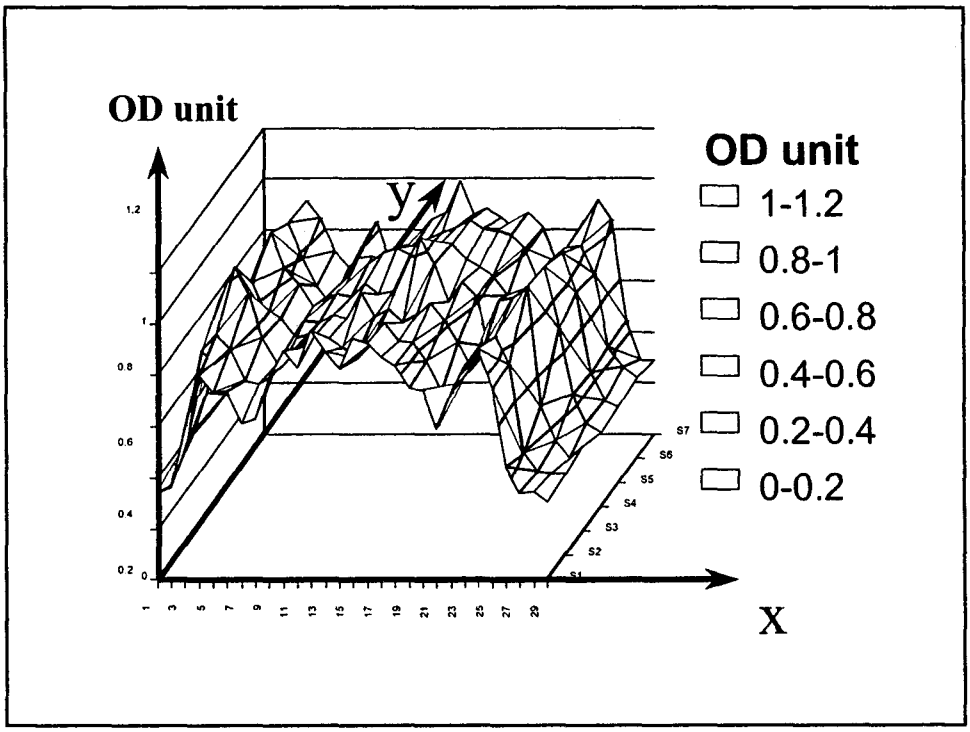


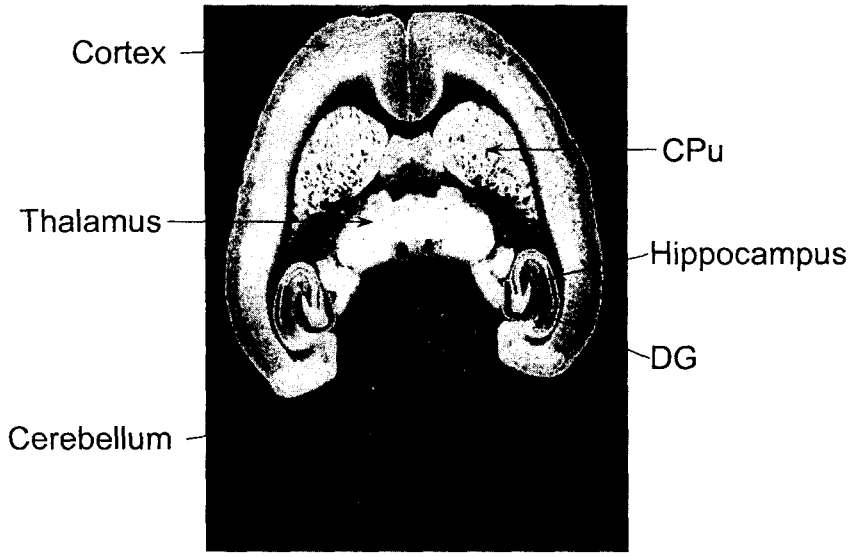
Butorphanol was infused (26 nmol/1μl/hr) for 3 days

Autoradiography of [^{3}H]MK-801

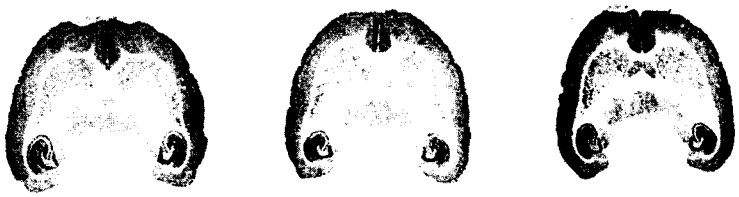
- 50 mM Tris-HCl
- 10 nM [^{3}H]MK-801
- Incubate : 120 min at 25 ℃
- Film expose : 4 weeks


Conventional film and densitometry
film
slide
latent image
development
& fixation
autoradiograph
stable light source
(laser scanning)
computer with image
acquisition software
A/D converter

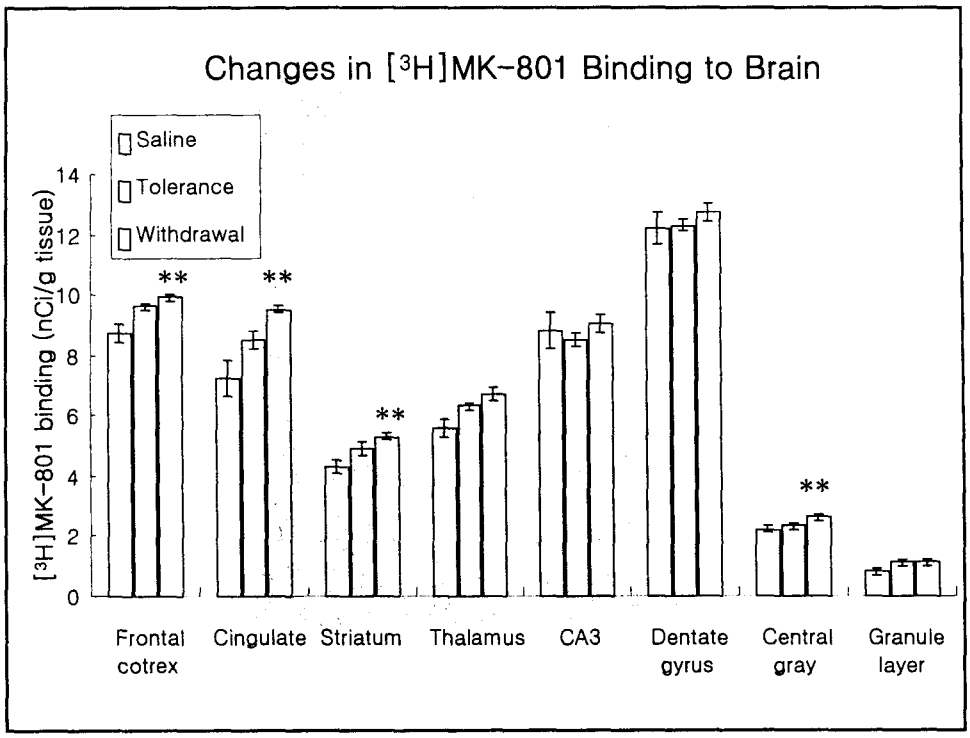

OD unit
y
X
OD unit
1-1.2
0.8-1
0.6-0.8
0.4-0.6
0.2-0.4
0-0.2

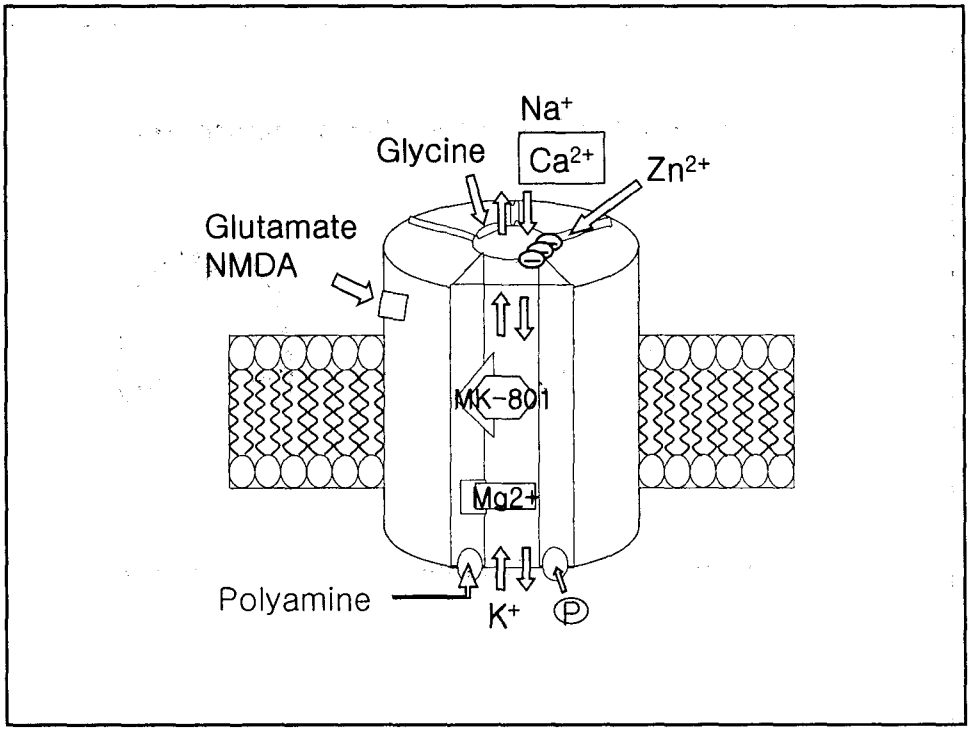


Changes of [^{3}H]MK-801 binding by butorphanol

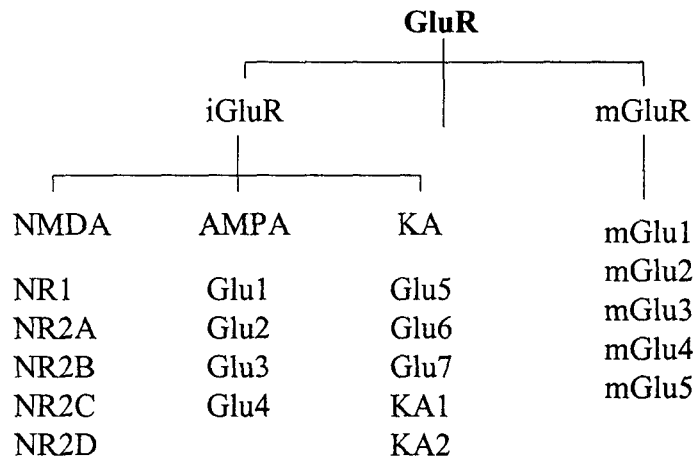


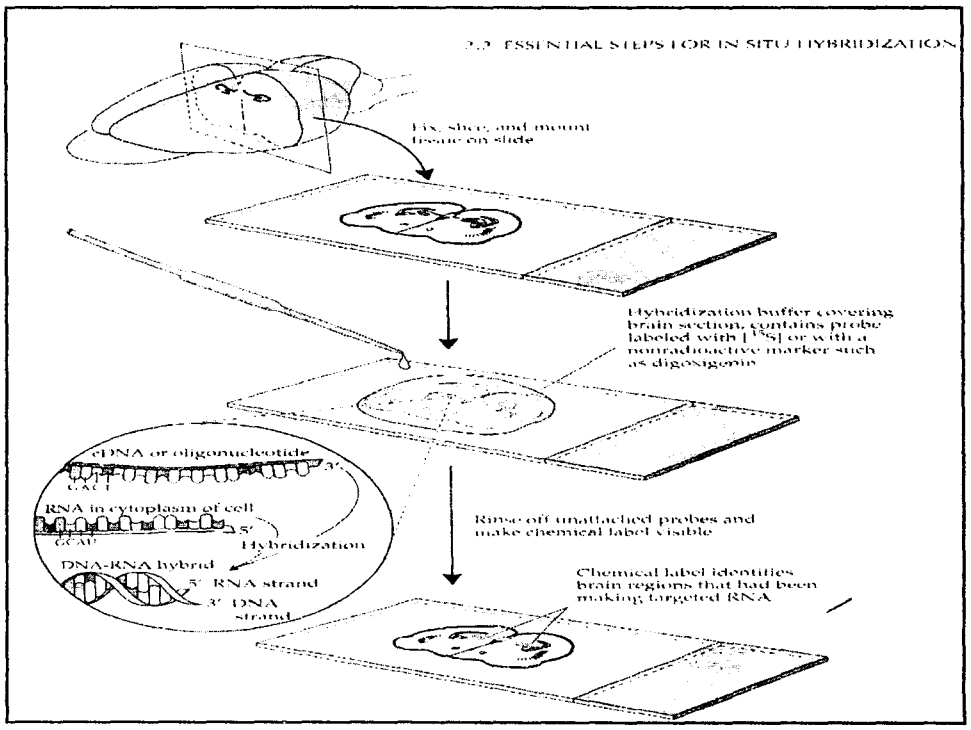
Control **Tolerance** **Withdrawal**


Changes in [3H]MK-801 Binding to Brain
Saline
Tolerance
Withdrawal
[3H]MK-801 binding (nCi/g tissue)
0
2
4
6
8
10
12
14
**
**
**
**
Frontal cotrex
Cingulate
Striatum
Thalamus
CA3
Dentate gyrus
Central gray
Granule layer

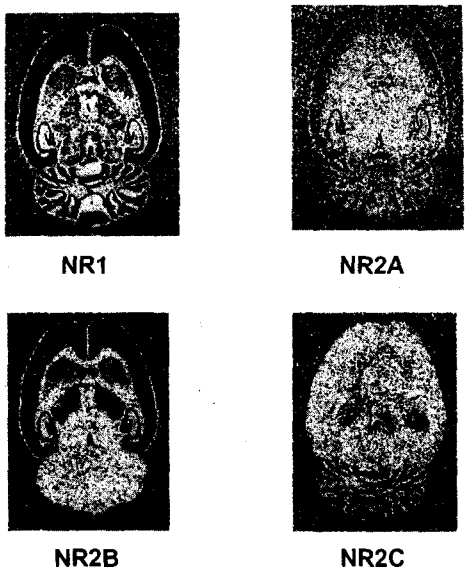

Na+
Glycine
Ca2+
Zn2+
Glutamate
NMDA
MK-801
Mg2+
Polyamine
K+
P

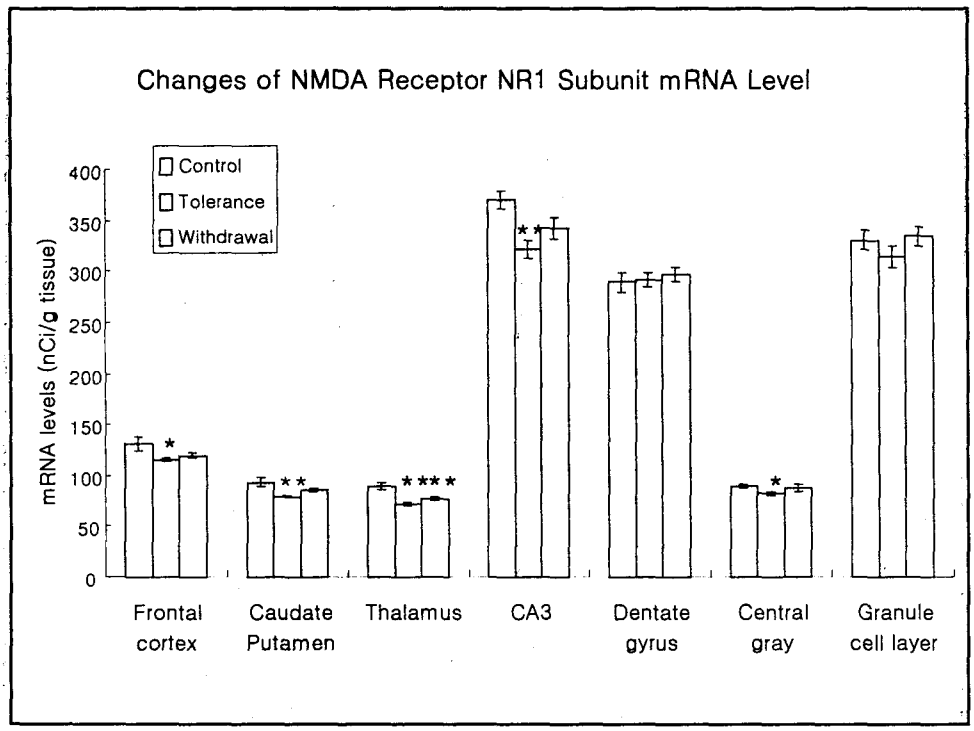
Subtypes of glutamate receptors

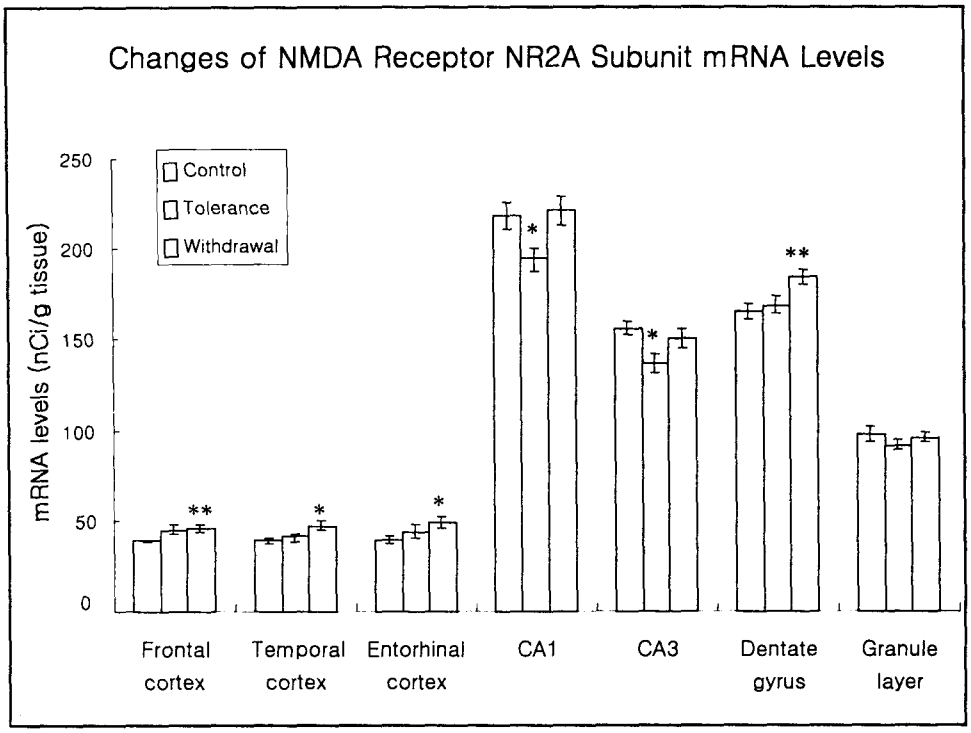


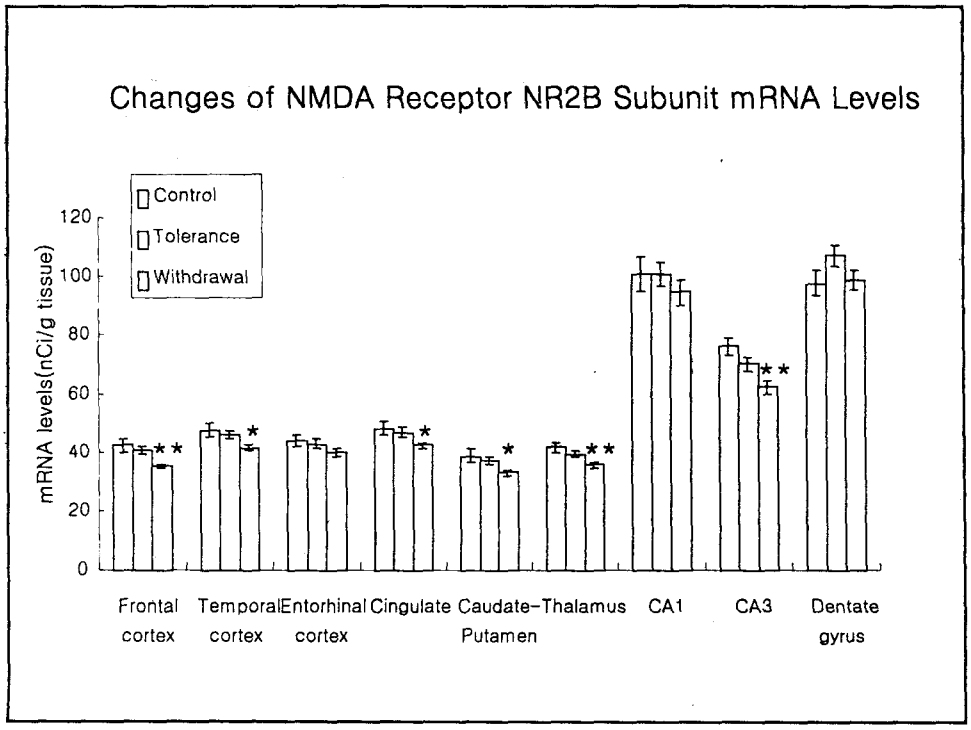


In situ hybridization images of NMDAR subunit mRNA

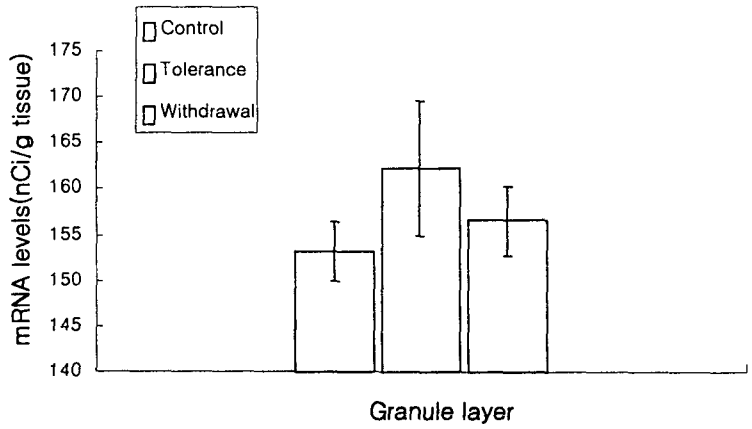


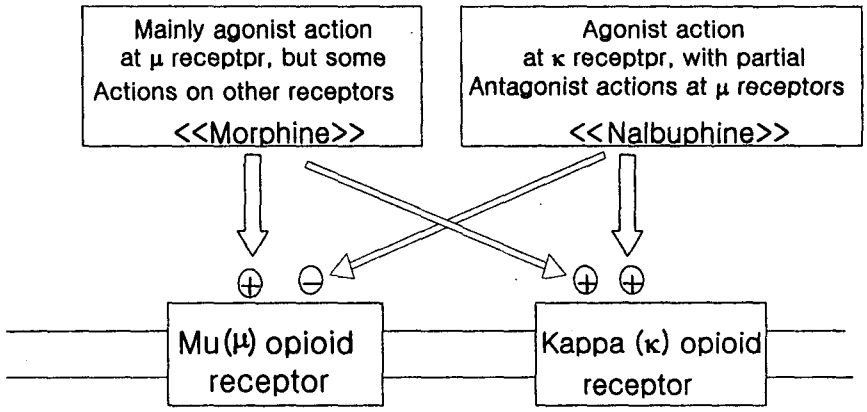



Changes of NMDA Receptor NR2A Subunit mRNA Levels
Control
Tolerance
Withdrawal
mRNA levels (nCi/g tissue)
250
200
150
100
50
0
Frontal cortex
Temporal cortex
Entorhinal cortex
CA1
CA3
Dentate gyrus
Granule layer


Changes of NMDA Receptor NR2B Subunit mRNA Levels
Control
Tolerance
Withdrawal
mRNA levels(nCi/g tissue)
120
100
80
60
40
20
0
Frontal cortex
Temporal cortex
Entorhinal cortex
Cingulate
Caudate-Putamen
Thalamus
CA1
CA3
Dentate gyrus

Changes of NMDA Receptor NR2C Subunit mRNA Levels

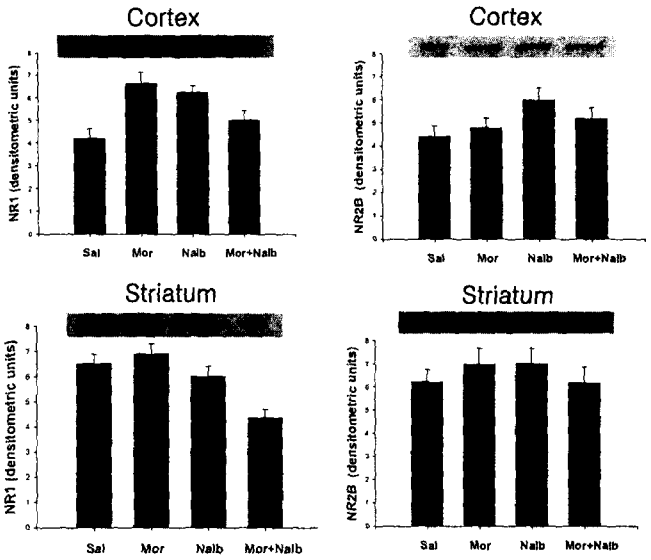




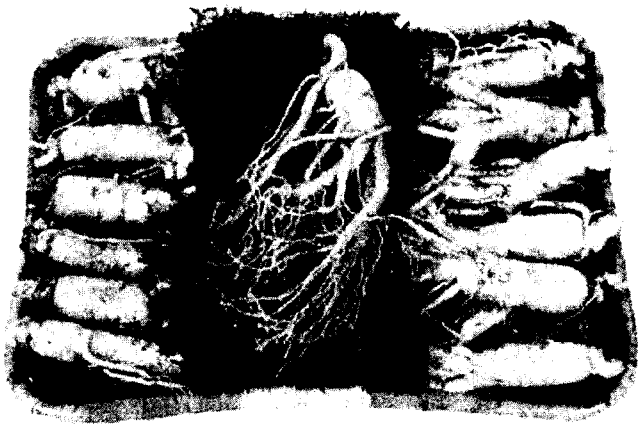
* Supraspinal analgesia
* Euphoria/sedation
* Physical dependence
* Pupil construction

* Spinal analgesia
* Sedation/dyaphoria
* Pupil construction

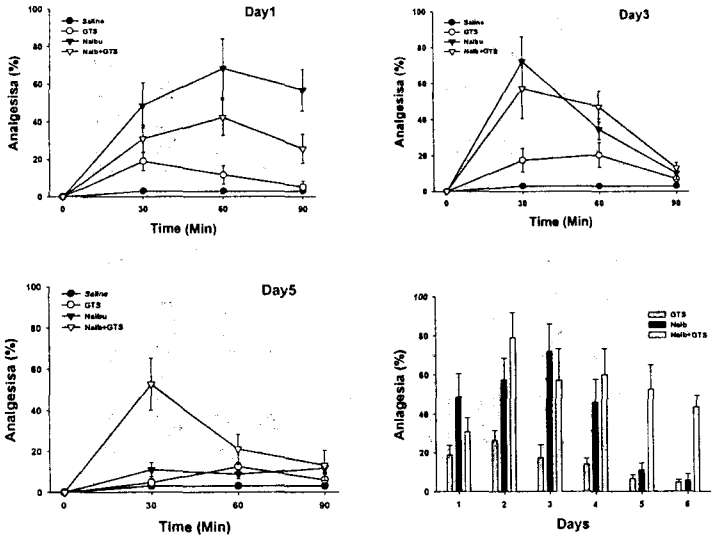
Downgerulation of NR1 expression by co-treatment



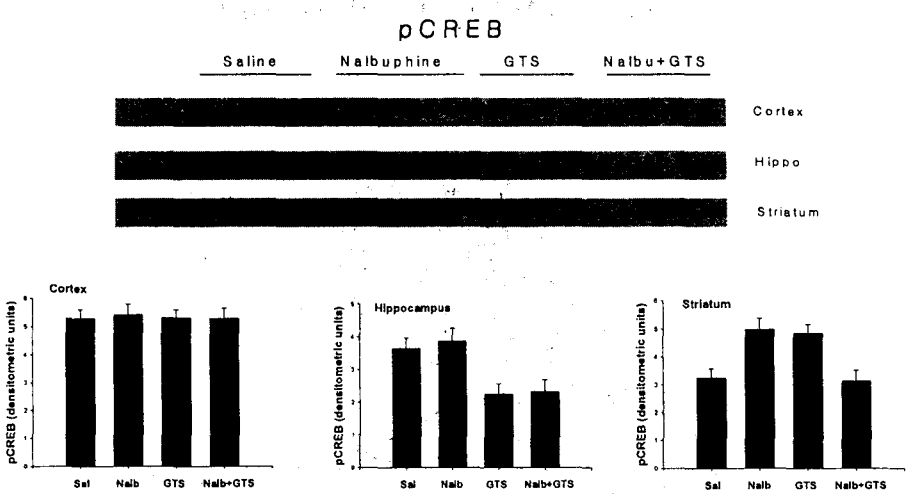
Red Ginseng Total Saponin



Ginseng saponin inhibits the nalbuphine tolerance



Ginseng saponin inhibits the nalbupine-induced pCREB expression



Greenlife World Inc.
(주) 그린라이프 월드

COEX (NA1700)

Prescription of COEX

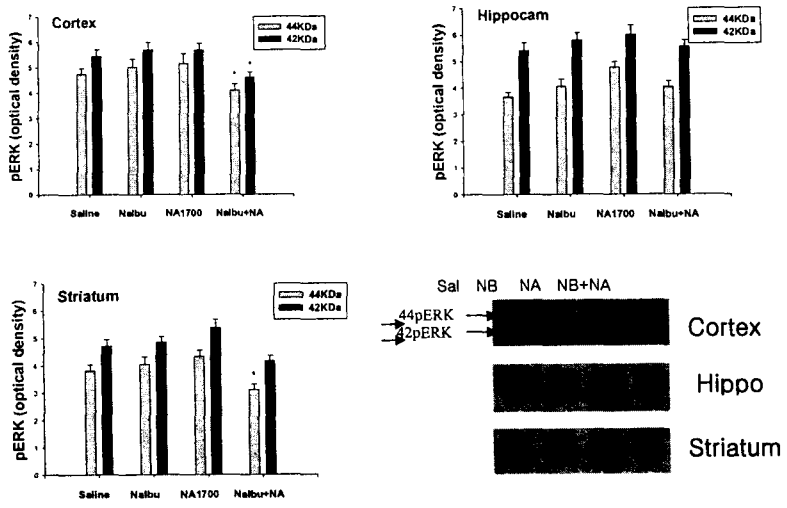
1.Orange Peel.....163mg
2.Penny Root.....160mg
3.Angelica Herb.....120mg
4.Sandalwood.....120mg
5.Poria.....80mg
6.Common Motherwort....80mg
7.Licorice.....80mg
8.Valeria Root..... 62mg
9.Geranium.....60mg
10.Piperis Nigris Fructus...35mg
11.Peppermint.....20mg
12.Cinnamon.....20mg
13.Cystein.....qs
14.Glutathione .....qs

Total 1000mg/capsule

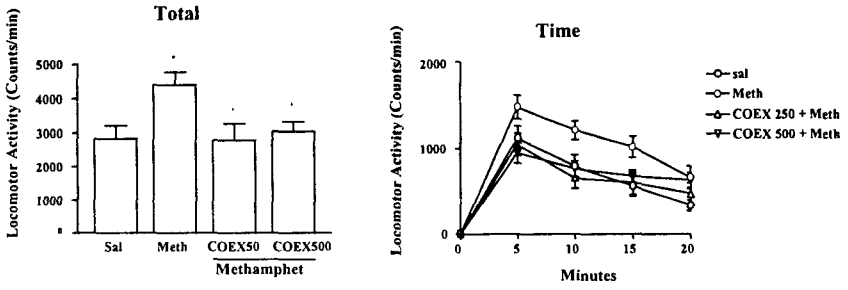
COEX suppress the nalbuphine-induced withdrawal signs

Withdrawal signs	Saline	Nalbuphine	Nalb+COEX
Escape behavior	0/10	1/10	1/10
Wet dog shakes	2/10	10/10#	3/10*
Teeth chattering	1/10	3/10	1/10
Rearing	1/10	9/10 #	3/10*
Locomotion	1/10	7/10 #	2/10*
Grooming	3/10	10/10#	7/10
Diarrhea	0/10	0/10	0/10
Penis licking	0/10	5/10#	0/10*
Ptosis	0/10	4/10#	1/10*

COEX inhibits the nalbuphine-induced pERK expression



COEX decreases the methamphetamine-induced locomotion



Withdrawal from butorphanol after continuous i.c.v. infusion

1) Elevates the binding of [^{3}H]MK-801 in the rat forebrain

2) Alters the level of NMDA receptor subunit (NR1, NR2) mRNA in a region-specific manner

Treatment approach for drug abuse

1) NA1700 shows good effects on the inhibition of nalbuphine- and methamphetamine-induced drug abuse symptoms

2) Ginseng total saponin suppress the nalbuphine-induced tolerance and withdrawal syndrome

These results indicate that

The selective modulation of NMDA receptor subunit gene expression may be involved in the development of tolerance to and withdrawal from butorphanol in different way.

The co-application of morphine and nalbuphine with a different ratio (eg. 10:1) would be a way of treatment to reduce drug abuse.

Natural product could be developed as good anti-narcotic medicine