化学物質の子どもの脳への影響

環境脳神経科学情報センター
黒田洋一郎

- Sorry, will speak in Japanese. PTs in English
- おことわり：

  国際シンポジュウムなのですが、聞いてくださる方の大部分は日本人なので話すのは日本語にします。そのかわりPTは英語にし、日本語を最小限加えます。

November 24 2013, International Symposium:

Increase of Autism, ADHD, LD triggered by Developmental Neurotoxic Chemicals on genetically determined vulnerability of specific synapses

自閉症,ADHD,LDの増加は遺伝子背景で決まるシナプスの脆弱性の上に発達神経毒性をもった化学物質が発症の引き金を引いたのが主な原因

Environmental Neuroscience Information Center
Yoichiro Kuroda
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Developmental Disorders and Diagnosis by DSM-5 ,"SYNAPTIC DISEASE"
発達障害とDSM-5による診断、「シナプス病」

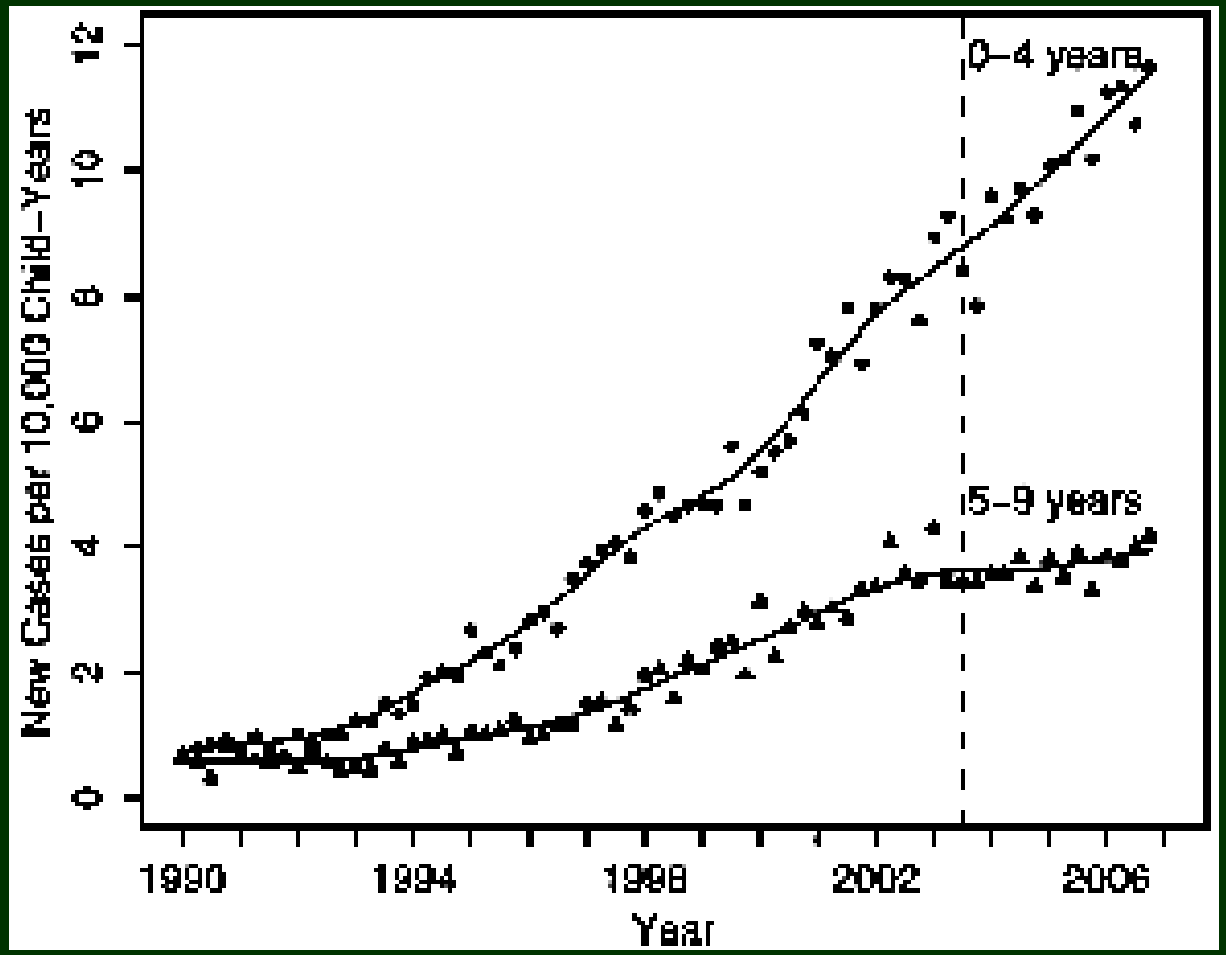
1. Autism Spectrum Disorders ： 自閉症スペクトラム
   = in DSM-IV, Pervasive Developmental Disorders, including Asperger syndrome
   Poor communications, Repetitive behaviors
2. Attention Deficit Hyperactive Disorders ：ADHD
   Lack of attention. Hyperactive
3. Learning Disorders (LD)：学習障害
   Poor reading, Poor mathematics

"SYNAPTIC DISEASE"=Abnormal synapses in specific neuronal circuits corresponding to the each symptom
（Formation, Plasticity, Maintenance)

Increase of autism children registered in California, USA (1990-2006）

米国カリフォルニア州における自閉症児の増加

~7 times increase, but ~40% come from wider diagnosis



Increase of Autistic Children in US and Japan showed the causes are not genetic but environmental factors
自閉症児増加の原因は遺伝ではなく環境要因

1. In Japan, a recent epidemiological study reported that autistic children increased several times, up to 1% in these 2 decades.

   日本ではここ２０年で数倍、全児童の１%になった

2. Real increased numbers of autistic children showed the causes are not genetic but environmental, because, if genetic, such a population-wide change needs hundreds, or thousands years

Genetic factors for autism appear to be hundreds of “related” genes (genetic background) which express vulnerability of the child to autism

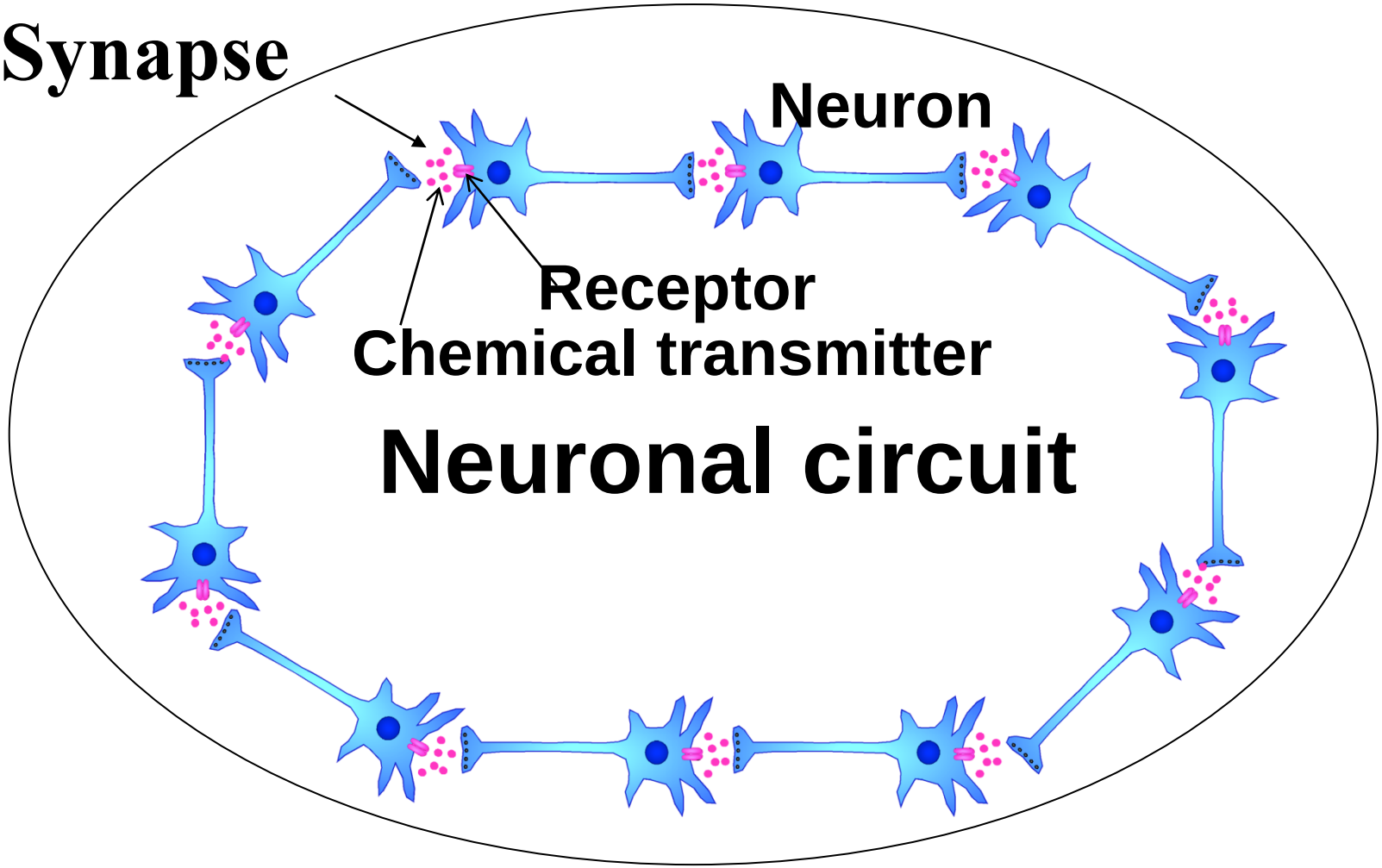
自閉症関連遺伝子は数百以上あり、発症しやすさの遺伝子背景を作り出す（シナプス関係がほとんど）

- Up to now, Autism- related genes listed in a data base are more than 3000 !!
- Strong candidates: more than 200.
- Functions of most strong candidate genes are formation, plasticity, maintenance of SYNAPSE,

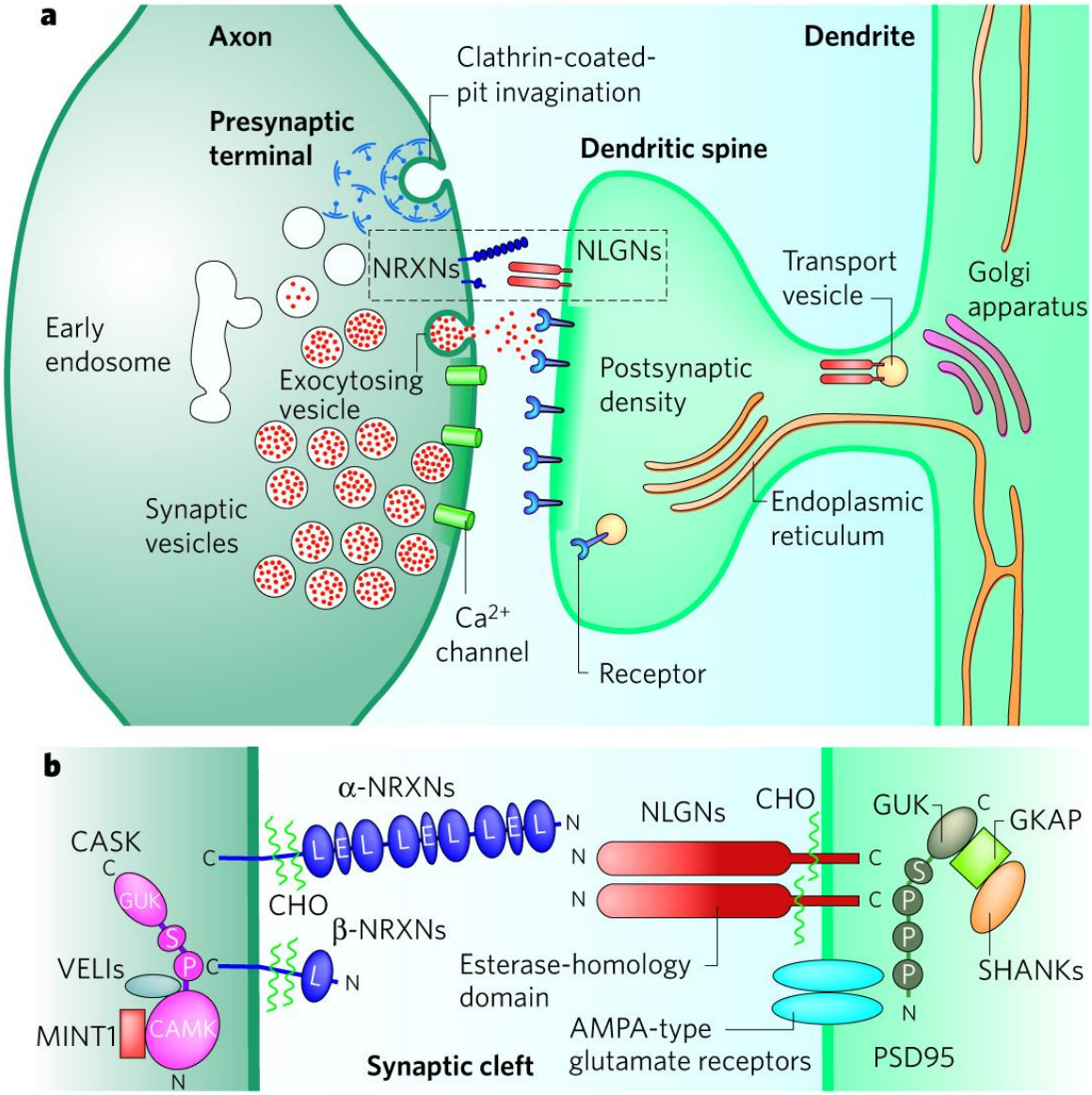
  directly or indirectly.

Neuronal circuit for behaviors, synapse, neurotransmitter

行動のもとになる神経回路とシナプス、神経伝達化学物質



Synaptic Structure and Connecting Proteins
シナプスの構造と結合タンパク質



(Sudhof 2008, Nature)

Autism-related genes (1) Synapse related
自閉症関連遺伝子（１）シナプス関係

Synapse related genes **from SFARI gene**

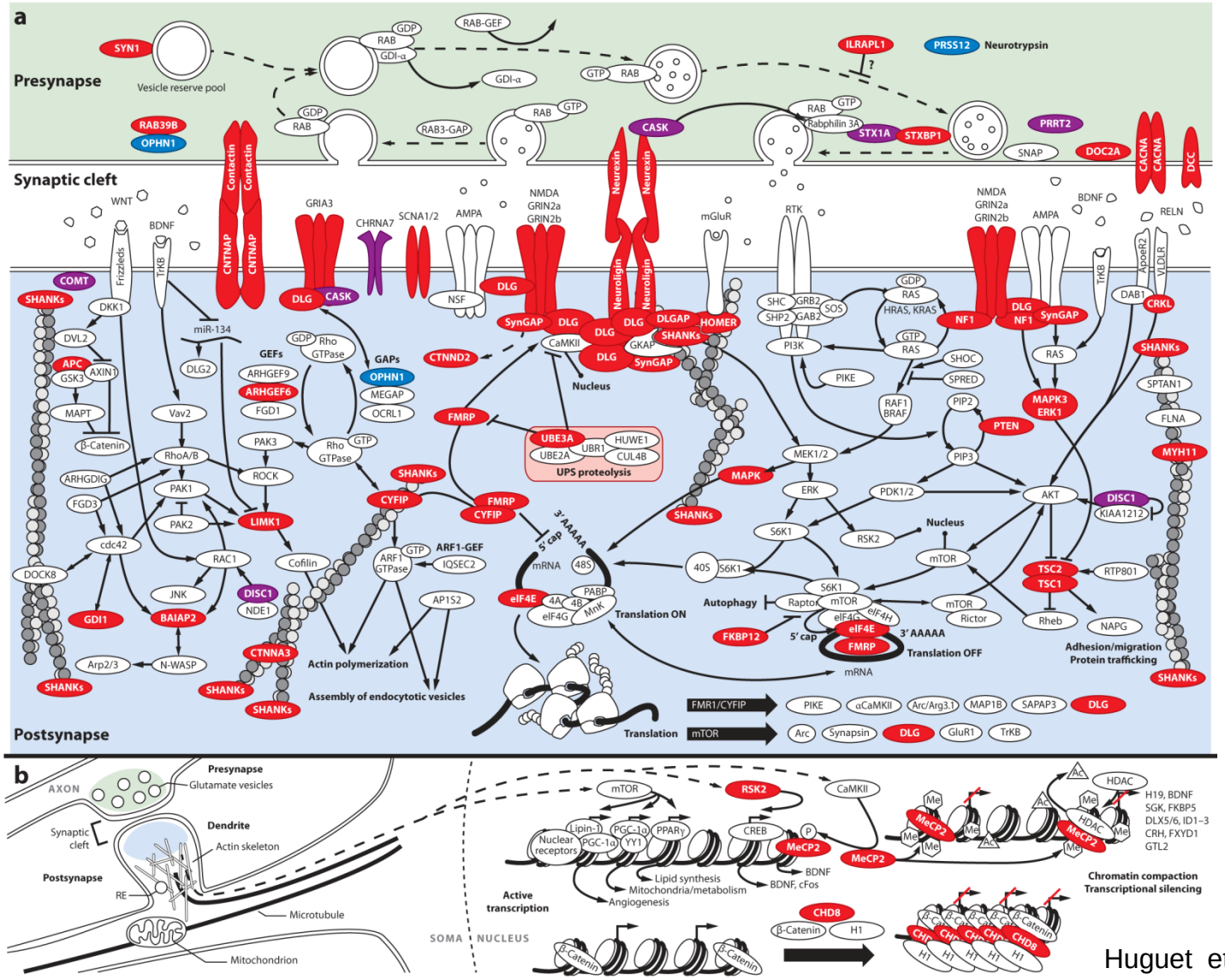
Gene symbol	Description
NRXN1	neurexin1
NRXN2	neurexin 2
NRXN3	neurexin 3
NLGN1	neuroligin 1
NLGN3	neuroligin3
NLGN4	neuroligin4
CNTN3	contactin 3 (plasmacytoma associated)
CNTN4	contactin 4
CNTNAP2	contactin associated protein-like 2
CNTNAP5	contactin associated protein-like 5
CTTNBP2	cortactin binding protein 2
CNTNAP3	contactin associated protein-like 3
SHANK3	SH3 and multiple ankyrin repeat domains 3
SHANK2	SH3 and multiple ankyrin repeat domains 2
SYNE1	spectrin (Synaptic nuclear envelope protein)
SYN1	synapsin 1
SYNGAP1	synaptic Ras GTPase activating protein 1
SYT17	synaptotagmin XVII

Autism related genes (2) Neurotransmitter related
自閉症関連遺伝子（２）神経伝達物質関係

Neurotranmitter related genes **from SFARI gene**

Gene symbol	Description
CHRNA7	nicotinic acetylcholine receptor, alpha 7
GRIN2B	glutamate receptor, ionotropic, N-methyl D-aspartate 2B
GRIN2A	glutamate receptor, ionotropic, N-methyl D-aspartate 2A
GRID2	glutamate receptor, ionotropic, delta 2
GRIK2	glutamate receptor, ionotropic, kainate 2
GRM8	glutamate receptor, metabotropic 8
GRIP1	glutamate receptor interacting protein 1
GABRA4	gamma-aminobutyric acid (GABA) A receptor, alpha 4
GABRB1	gamma-aminobutyric acid (GABA) A receptor, beta 1
GABRB3	gamma-aminobutyric acid (GABA) A receptor, beta 3
HTR1B	5-hydroxytryptamine (serotonin) receptor 1B
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A
HTR3A	5-hydroxytryptamine (serotonin) receptor 3A
CACNA1H	calcium channel, voltage-dependent, T type, alpha 1H
CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C

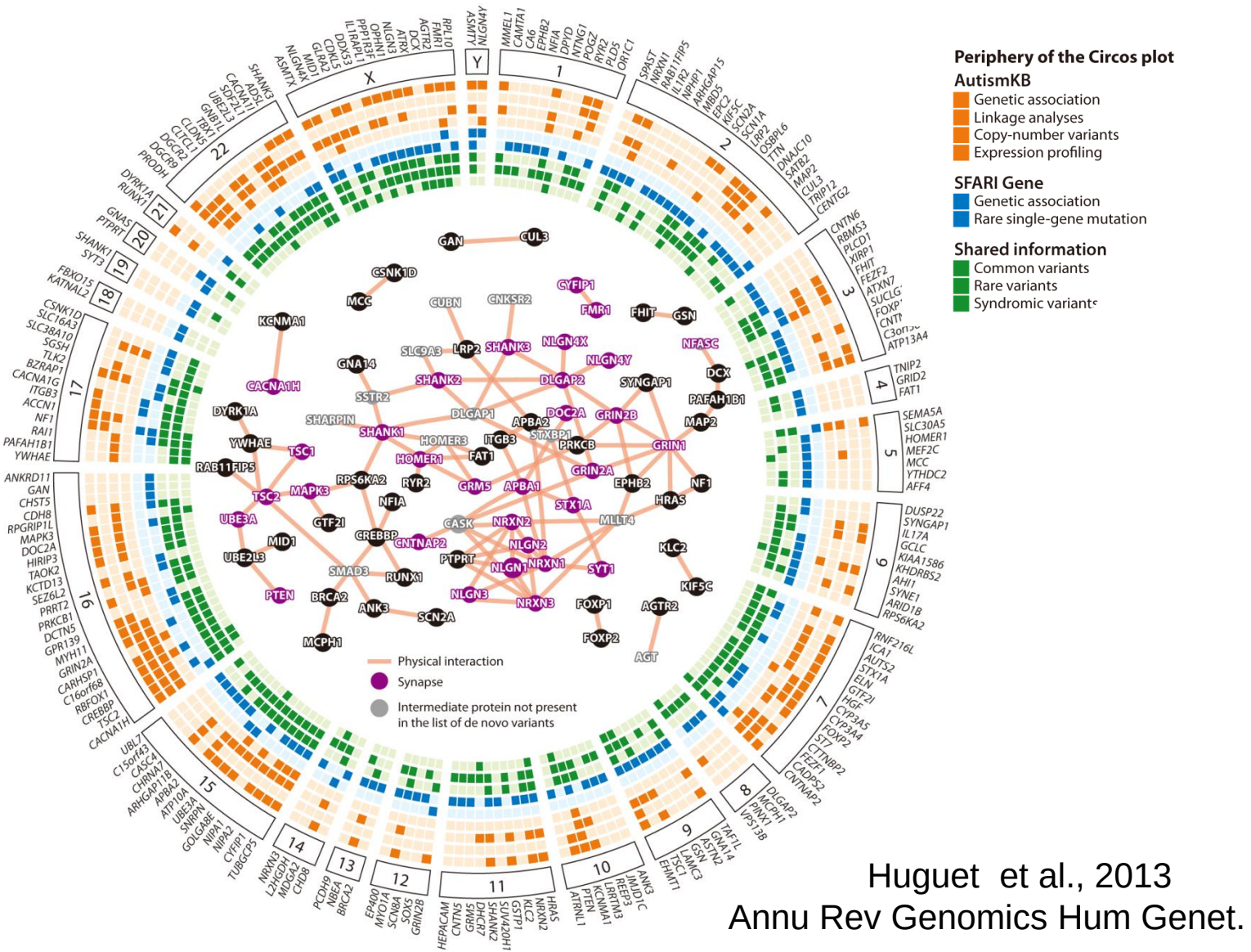
Complex Molecular (Gene) Structures in Synapse and Gene Expression System



Huguet et al., 2013
Annu Rev Genomics Hum Genet.

Hundreds of Autism-related Genes are expressed as complex networks work consisting genetic background

数百の自閉症関連遺伝子がネットワーク状に発現し遺伝子背景を構成している



Huguet et al., 2013
Annu Rev Genomics Hum Genet.

Children with Developmental Disorders have abnormal synapses in a specific neuronal circuit

自閉症など発達障害の子どもは症状に関連した神経回路のシナプスに異常がある

1． Each behavior acquired by establishing a specific neuronal circuit for it.

全ての行動は対応する神経回路の活動による

- When a child failed to develop the normal synapses of neuronal circuit corresponding to social communications, he will become autism. ある神経回路のシナプスに異常があれば、対応する行動が異常になる

Environmental Factors which can cause Developmental Disorders (Autism, ADHD, LD）
自閉症,ADHD,LDの原因となる環境要因

1. Developmental neurotoxic chemicals in the brain：
   脳内に侵入した発達神経毒性をもつ化学物質
   Valproic Acid, Thalidomide：バルプロ酸、サリドマイド
   Lead, Mercury, other Metals：鉛,水銀など重金属
   PCBs, Dioxins：PCB,ダイオキシン
   Nicotine：ニコチン（喫煙）
   Organophosphates, Neonichotinoids：
   有機リン系、ネオニコチノイド系農薬
2. Troubles in Perinatal Periods (low body weight birth etc)：
   低体重など出産前後のトラブル
3. Troubles in Developmental Periods (nurture)：養育期のトラブル

Valproic acid and Thalidomide
バルプロ酸とサリドマイド

- Anti-epileptic drug　抗てんかん薬
- When mothers take these drugs during early pregnant period, babies will have a risk of autism.
  妊娠初期の服用は自閉症のリスク
- Both drugs have some epigenetic developmental toxicities.
- エピジェネティックな発達毒性をもつ

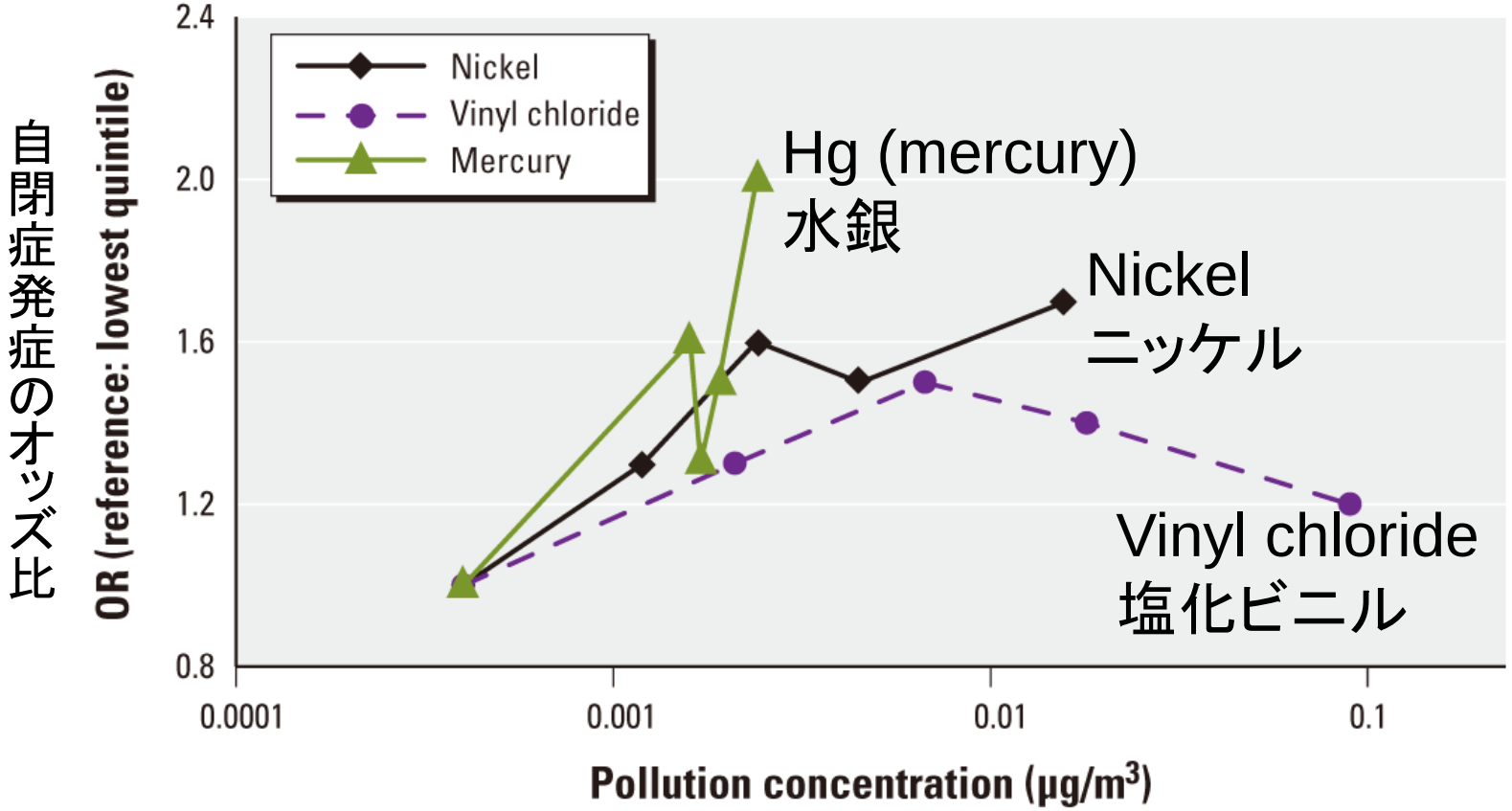
Mercury has general developmental neurotoxicities
水銀は広範な発達神経毒性を持つ

- Autism risk from Hg in air pollutants seems to be significant.

  大気汚染からの水銀はリスク有意に高い
- Autism risk from Hg in old vaccines seems to be very low, comparing to Hg from environment. New vaccines contain no Hg.

  古いタイプのワクチンでも水銀含量は微量で、環境からの水銀汚染に比べ約１０分の１量で、ワクチンだけのリスクは非常に低い。現在のワクチンには水銀は含まれていない。

Mercury in Air Pollutants Related to Autism
大気汚染物質中の水銀は、自閉症のリスクがある



Roberts, et al. 2013, EHP

Increase of autism and/or ADHD seems to be caused by pesticides

自閉症やADHDの増加は農薬が原因の可能性

1. Epidemiological studies show the relationship between exposures of organophosphate pesticides and ADHD.

疫学データは有機リン系農薬曝露とADHD、作業記憶などの行動異常との相関を示した。

2. Experimental studies demonstrate organophosphate and neonichotinoid pesticides have developmental neurotoxicities.

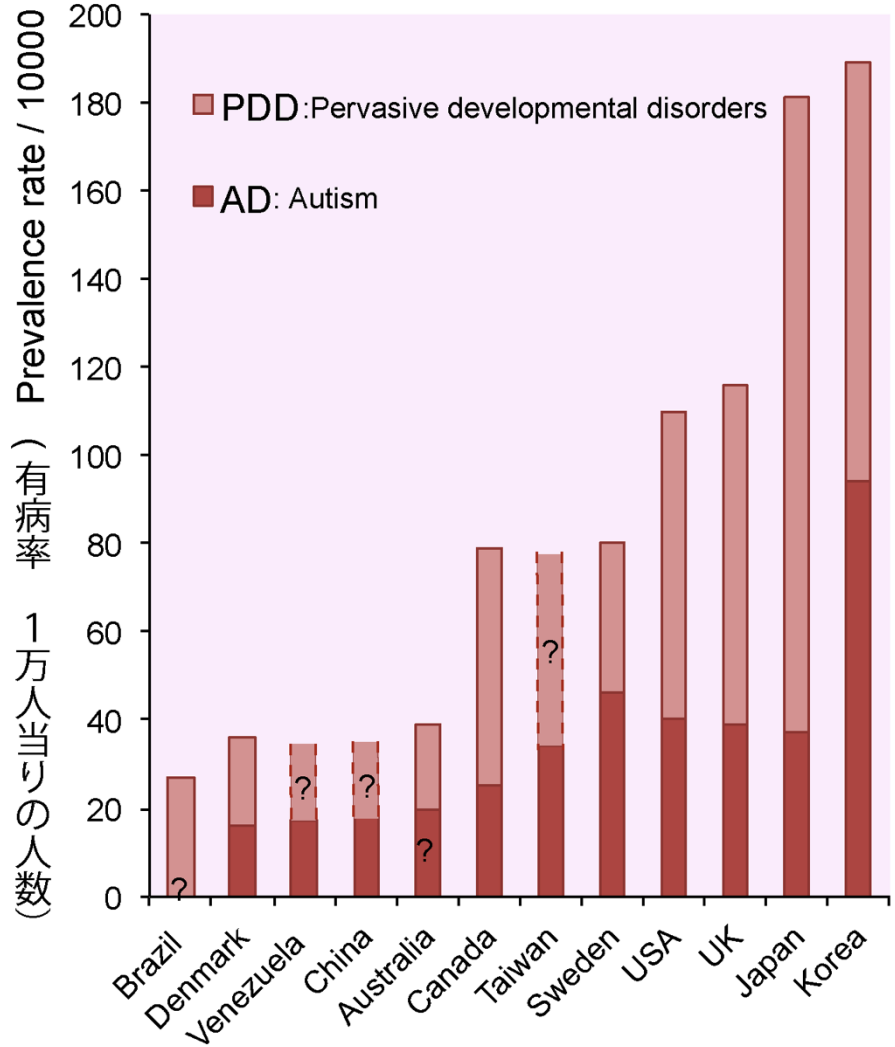
実験的に有機リン、ネオニコの発達神経毒性がしめされている

3. Toxicogenomic studies show the change of synapse-related gene expressions in neurons, after exposure of neonicotinoids

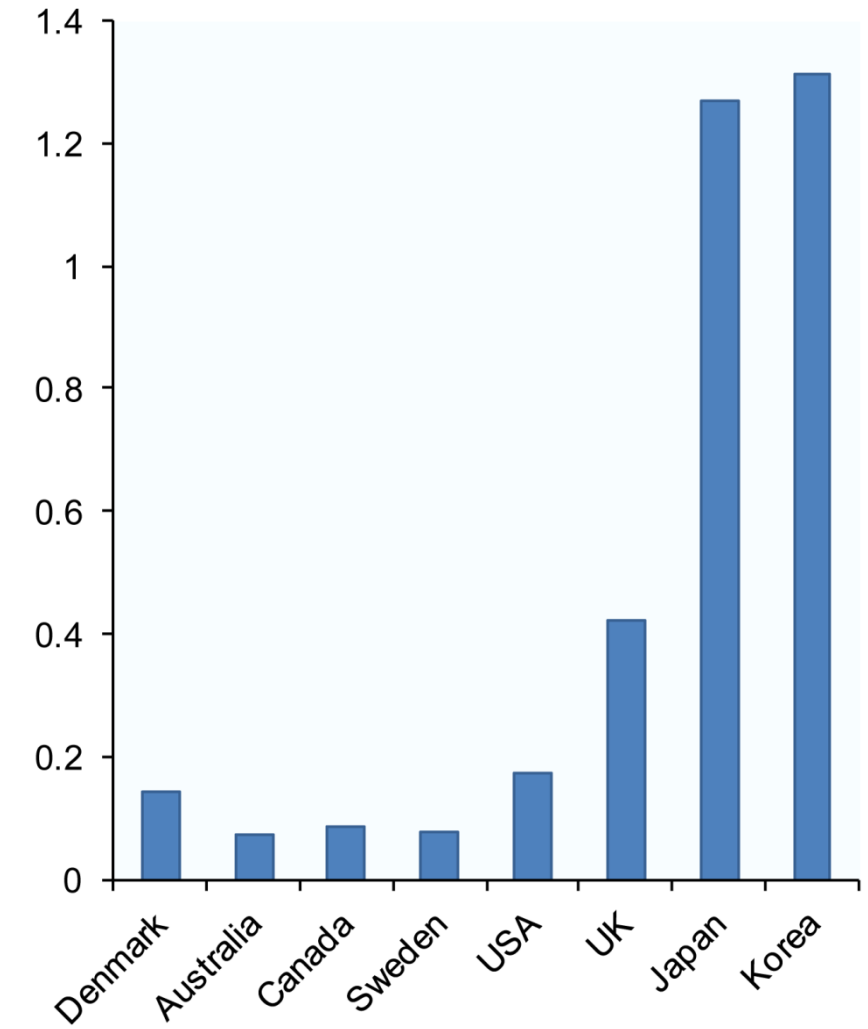
ネオニコ投与は神経細胞でのシナプス関連遺伝子の発現を変化させる

Korea and Japan were top countries of autism, suggesting pesticides use was main cause

Prevalence rates of Autism & PDD
自閉症、広汎性発達障害の有病率
(Elsabbagh, et al. 2012, Autism Res)



Total pesticides used /agricultural area
単位面積当たり農薬使用量
(OECD 2008)



Epidemiological Data showed: Children intoxicated with organophosphates tend to become ADHD and express lower IQ and working memory deficits

有機リン系農薬に曝露された子はADHDになりやすくIQや作業記憶能力が低下

- Pediatrics（２０１０）；children urine pesticide vs ADHD, risk =twice 子どもの尿中の有機リンが高いと、生まれた子がADHDに約２倍なりやすい
- Environ. Health Persp（２０１０）mother urine pesticides vs 7 points lower IQ 母親の尿中の有機リンが高いと、生まれた子の知能（IQ）が平均７．０下がる。
- Environ. Health Persp (２０１１) 2.8% deficit of working memory.母親の有機リンレベルが高いと，生まれた子の作業記憶が２．８％悪くなる。

To develop neuronal circuit, a huge number of gene expressions controlled by chemical information are necessary

脳の機能神経回路を発達させるには非常に多くの情報化学物質が正常に働くことが必須.

- Such physiologic chemicals are

  Hormones: thyroid hormone,　etc

  Neurotransmitters: acetylcholine, glutamate

- そのような情報化学物質には、甲状腺ホルモンなどのホルモン類やアセチルコリン、グルタミン酸などの神経伝達物質（神経ホルモン）がある

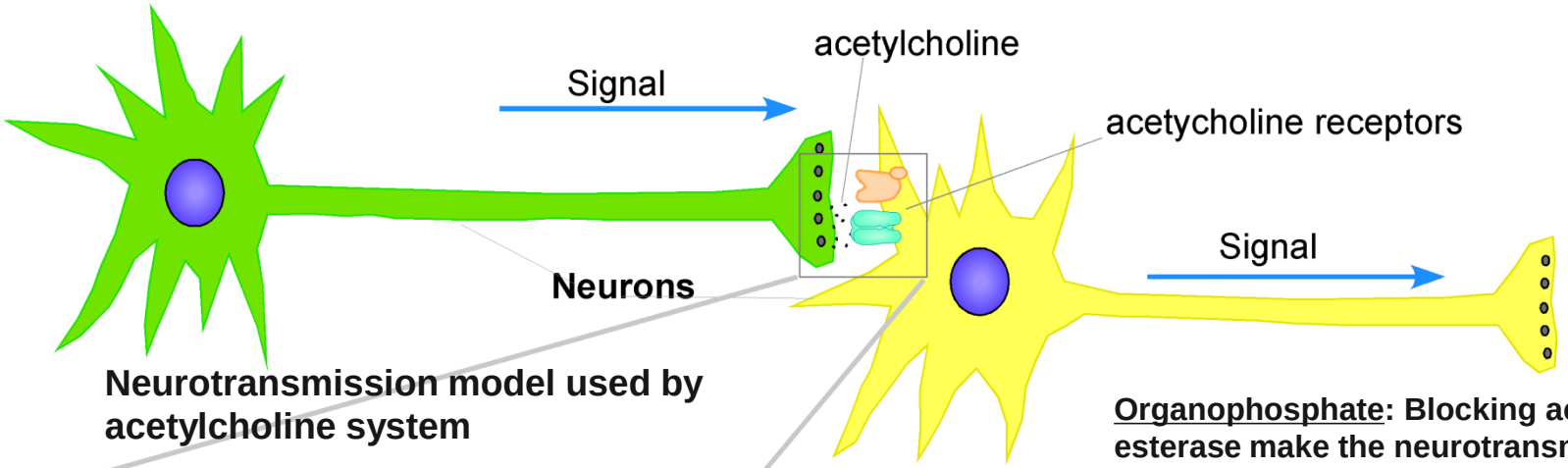
Acetylcholine is known as a critical information chemical to control gene expressions for neuronal circuits of higher functions such as attention and memory

アセチルコリンは注意や記憶などの神経回路を正常につくる遺伝子の発現に必須の情報化学物質

Acetylcholine binds to nicotinic acetylcholine receptors and control physiologic processes.

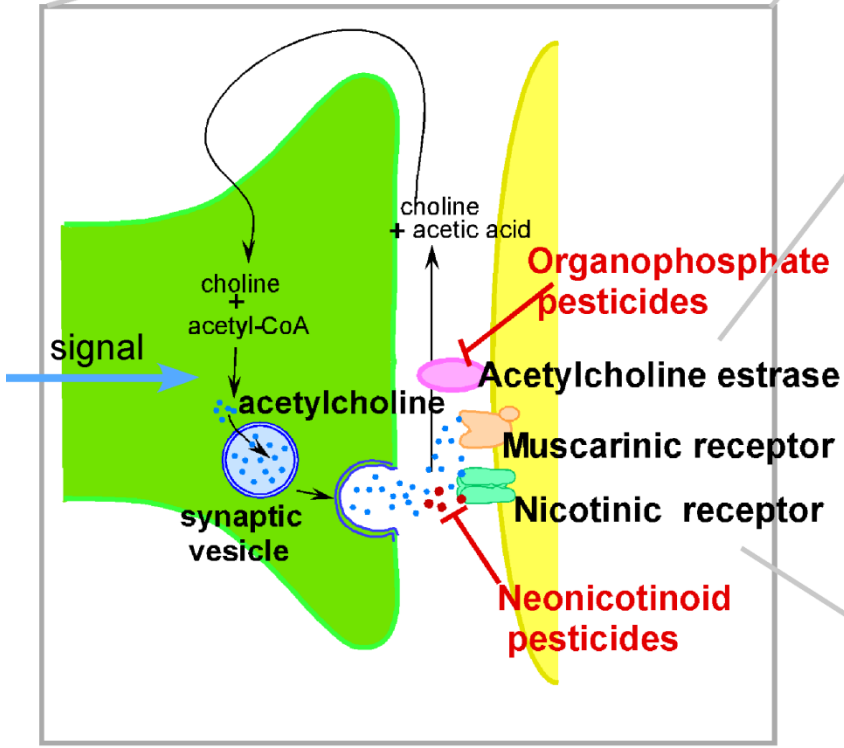
アセチルコリンはニコチン性（ニコチンも良く結合する）アセチルコリン受容体に結合して、様々な生理過程を進行させる

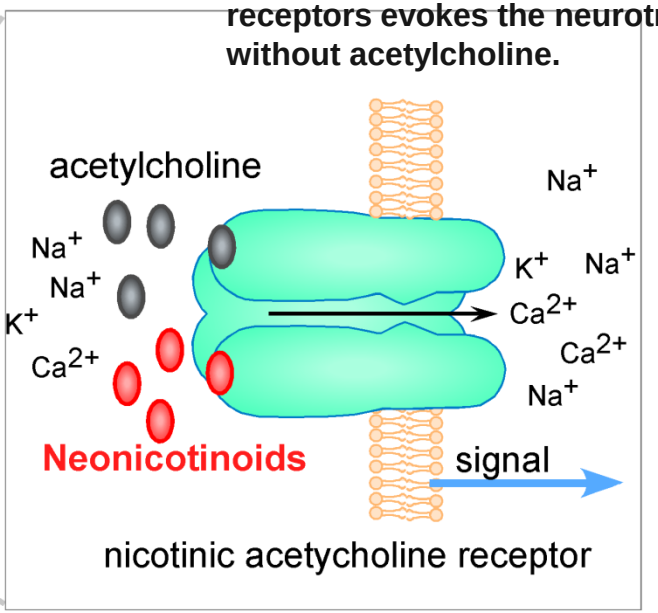
Organophosphate & neonicotinoid pesticides disrupt acetylcholine system
アセチルコリンによる神経伝達を撹乱するネオニコチノイドと有機リン系農薬



Neurotransmission model used by acetylcholine system

Organophosphate: Blocking acetylcholine esterase make the neurotransmission still on. The Sarin, nerve gas, has same effects.
Neonicotinoid: Agonist of nicotinic receptors evokes the neurotransmission, without acetylcholine.



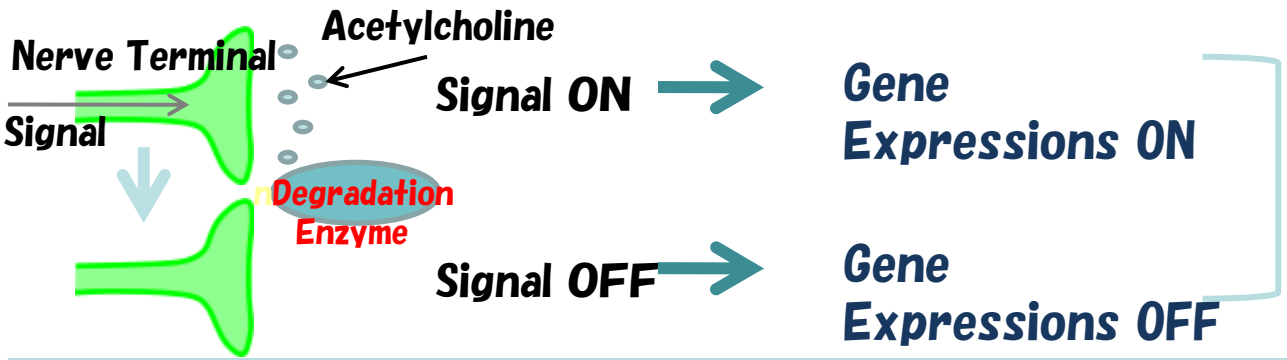


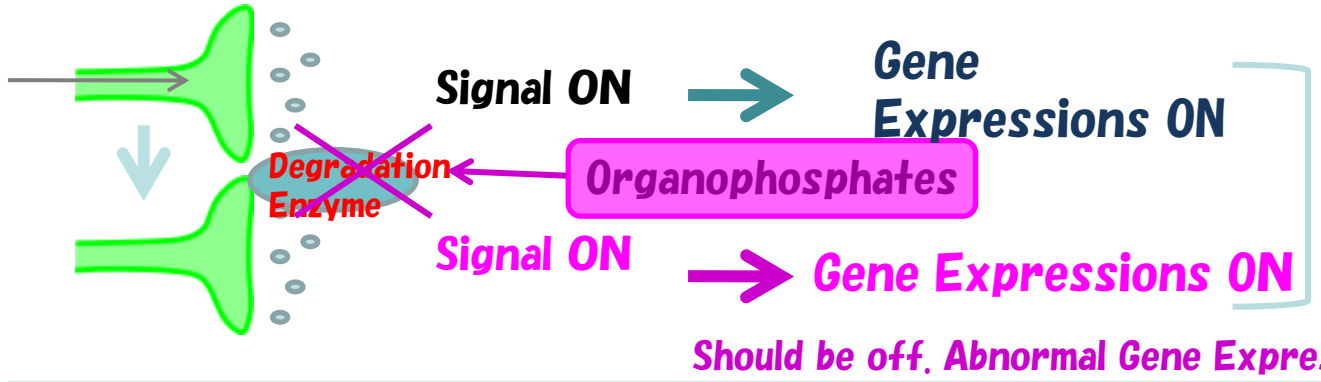
Organophosphates /Neonicotinoids disrupt acetylcholine information system indirectly / directly to control gene expressions for the neuronal circuits

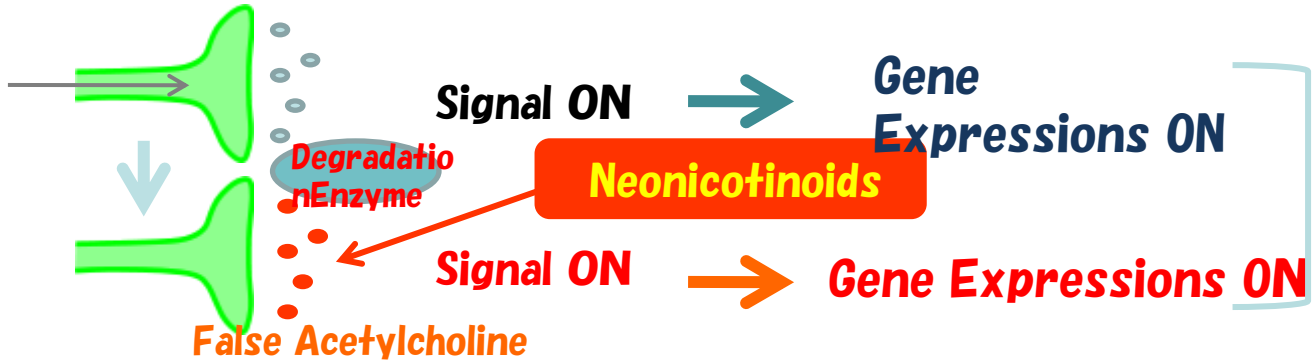
有機リン系/ネオニコ系農薬はアセチルコリンによる化学情報伝達に間接的/直接的に異常をおこし神経回路形成のための遺伝子発現をかく乱する

- Organophosphates: Block degradation enzymes cause continuous binding of acetylcholine
- Neonicotinoids: Bind to Nicotinic acetylcholine receptor work false-acetylcholine

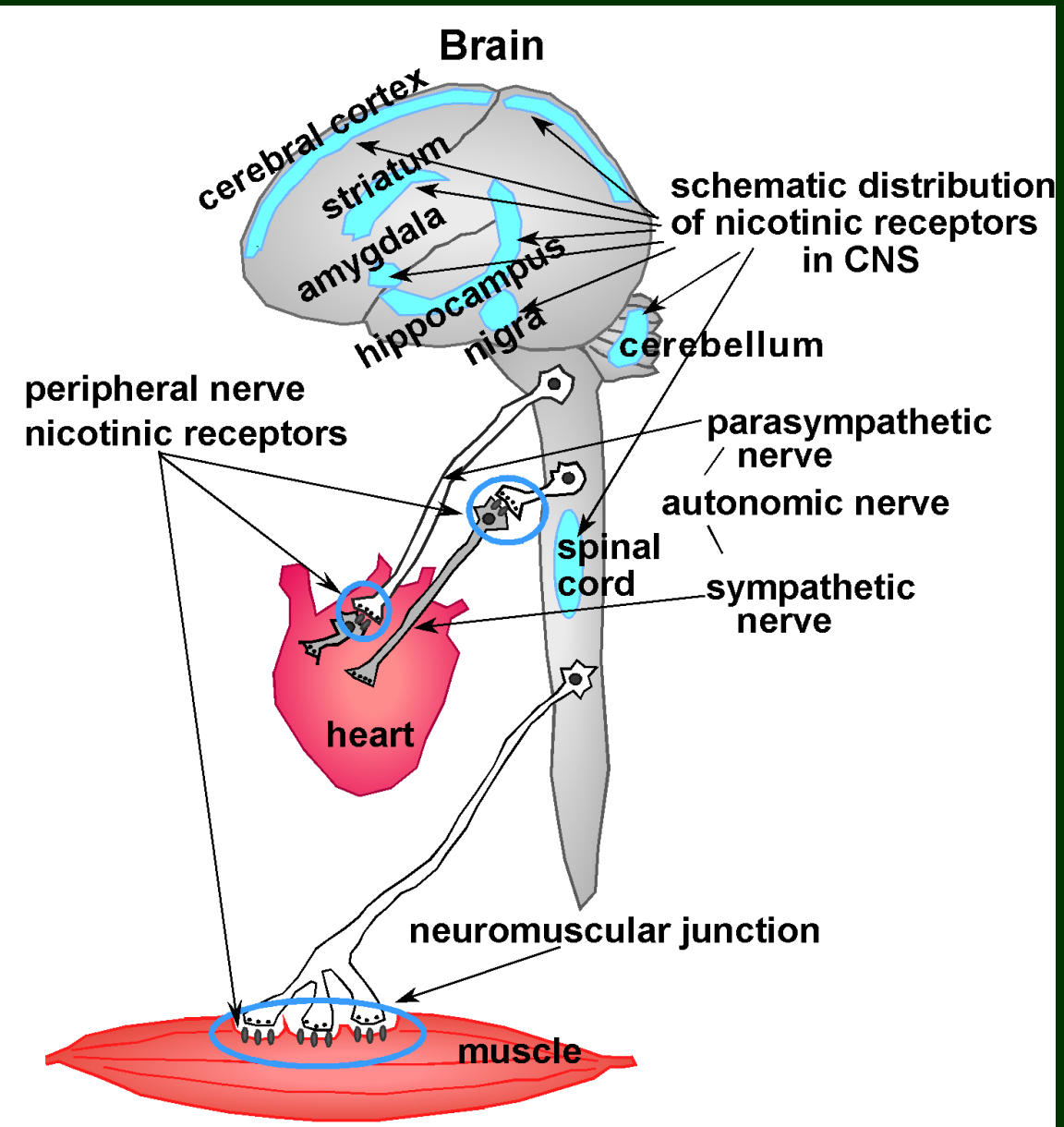
A Possible Mechanism of Abnormal Brain Development by Organophosphates and Neonicotinoids







Nicotinic acetylcholine receptors in nerve system.
ニコチン性受容体のヒト神経系での発現領域



Central nerve system 中枢神経

Functions:
Increase learning and memory
学習、記憶に関連

Related diseases:
depression, shizophrenia,
Alzheimer diseases, autism
鬱病、統合失調症、
アルツハイマー病、自閉症と関連

Peripheral nerve system 末梢神経

Functions:
autonomic nerve,
neuromuscular junction
自律神経系、神経筋接合部

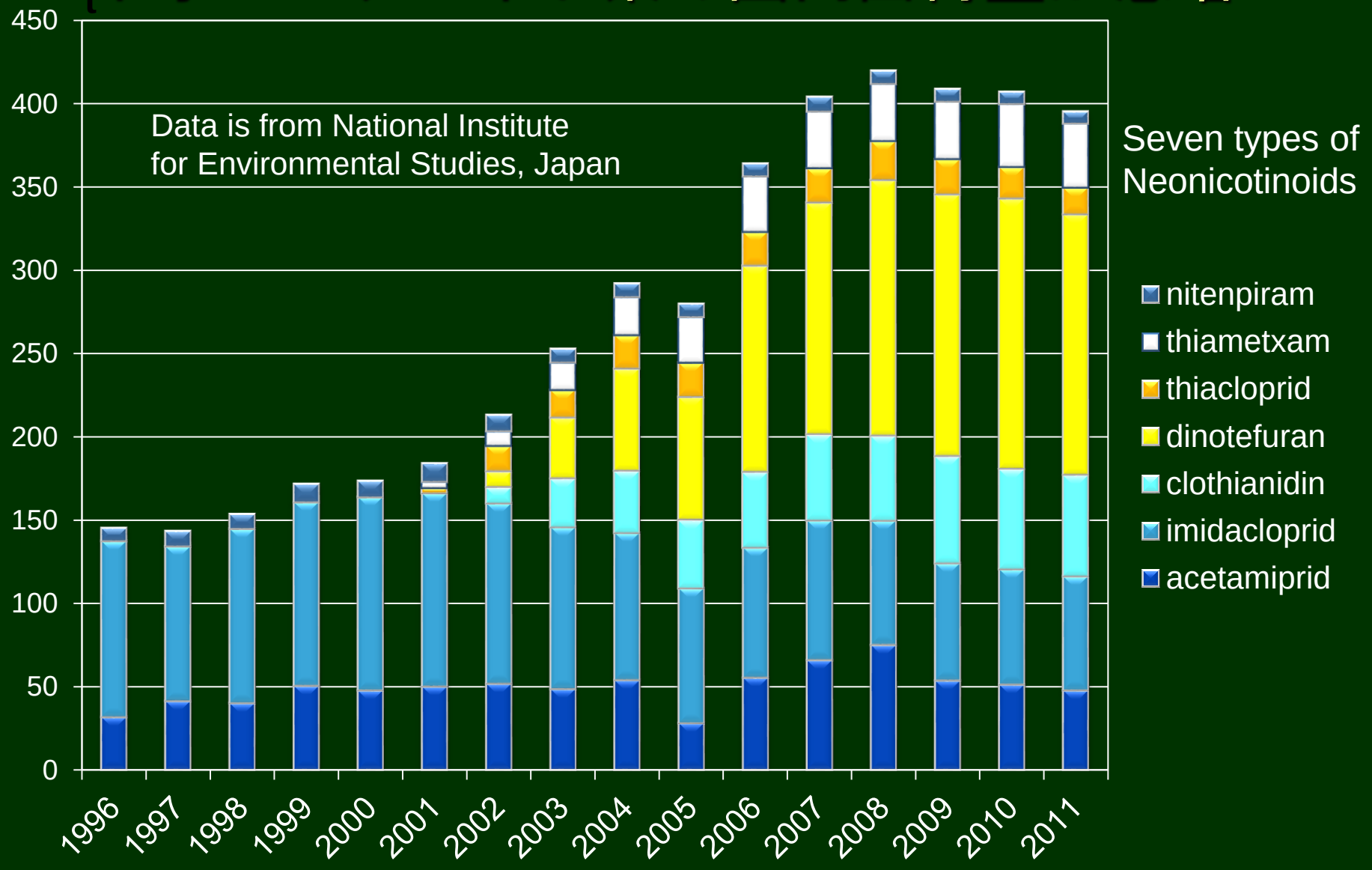
Nicotinic receptors are important for both of central and peripheral nervous system.

Honeybee Colony Collapse Disorder is caused by Neonicotinoid Pesticides

ミツバチ大量死はネオニコが原因と判明

- Recently, many honeybees died in US, EU, Japan .
- In 2012, 3 papers (2 in [Science] and 1 in [Nature]) reported that neonicotinoid /pyrethroid pesticides intoxicate highly developed memory system of worker bee brain and the bees cannot get back to their nest and take care of queen bee.
- Neonicotinoids can disrupt memory system of worker bees and/or its development in the brain during larval stages at lower concentrations.
- Developmental disorders of honey bees brain !!
- ネオニコによるミツバチの子の脳の発達障害！！

Neonicotinoids are rapidly increasing in Japan
ネオニコチノイド系の国内出荷量が急増



Maximum residue levels of neonicotinoids pesticides are very high in Japan

日本のネオニコチノイド系農薬の残留基準は極めて緩い

Maximal residue level of acetamiprid (ppm) アセタミプリドの農薬残留基準

Food	Japan	USA	EU	Food	Japan	USA	EU
Strawberry	3	0.6	0.5	tea dried	30	**	0.1*
apple	2	1.0	0.7	tomato	2	0.2	0.15
pear	2	1.0	0.7	cucumber	2	0.5	0.3
grape	5	0.35	0.2	cabbage	3	1.2	0.6
water melon	0.3	0.5	0.01*	broccoli	2	1.2	0.3
melon	0.5	0.5	0.01*	pepper	1	0.2	0.3

* minimum level of detection **In the US, 50ppm is set only for imported teas

Neonicotinoids are enough toxic to human brain and its functions (behavior)

ネオニコはヒトの脳や行動に有害である

- Pesticide companies defend that neonicotinoids are not so toxic to human,　compare to insects.

  農薬会社は昆虫は死ぬが、ヒトには安全と宣伝

- But no safety tests have been carried out on developmental neurotoxicities in mammals.

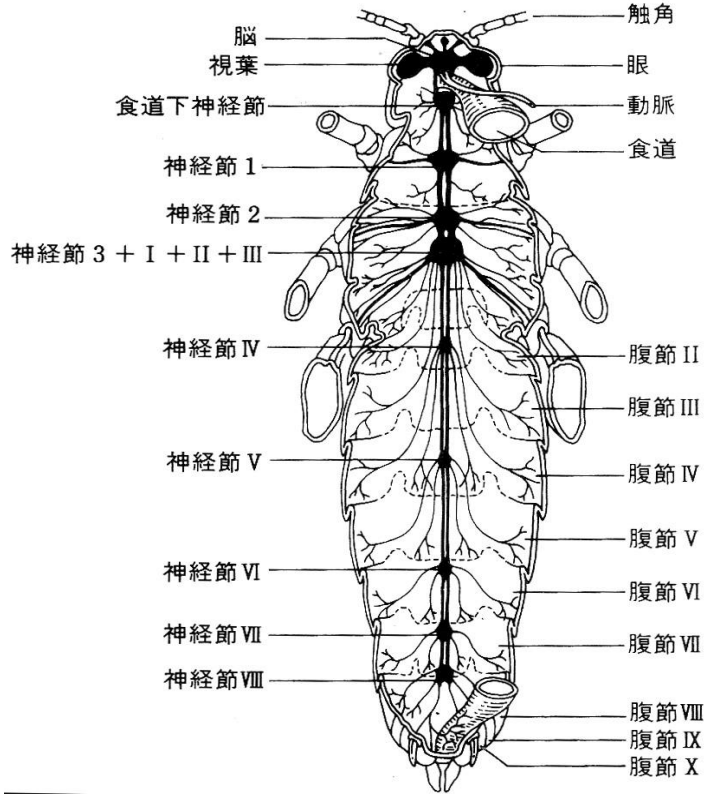
  しかし、発達神経毒性の安全試験はきちんとやっていない。

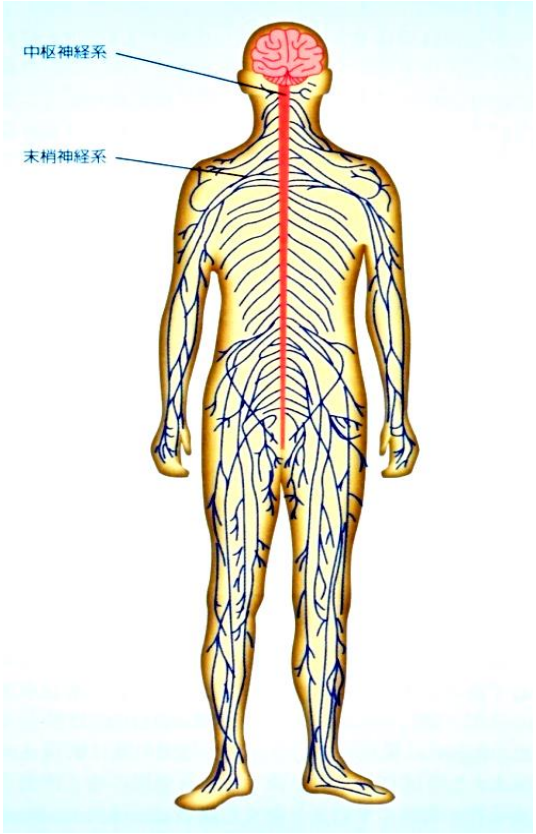
- Toxicities to human are so strong to cause acute nicotinic symptoms, even death cases.

  ヒトへの毒性はニコチン性急性症状や死亡例があるほど強い。

Basic structure and functions of human brain are similar to insect brain

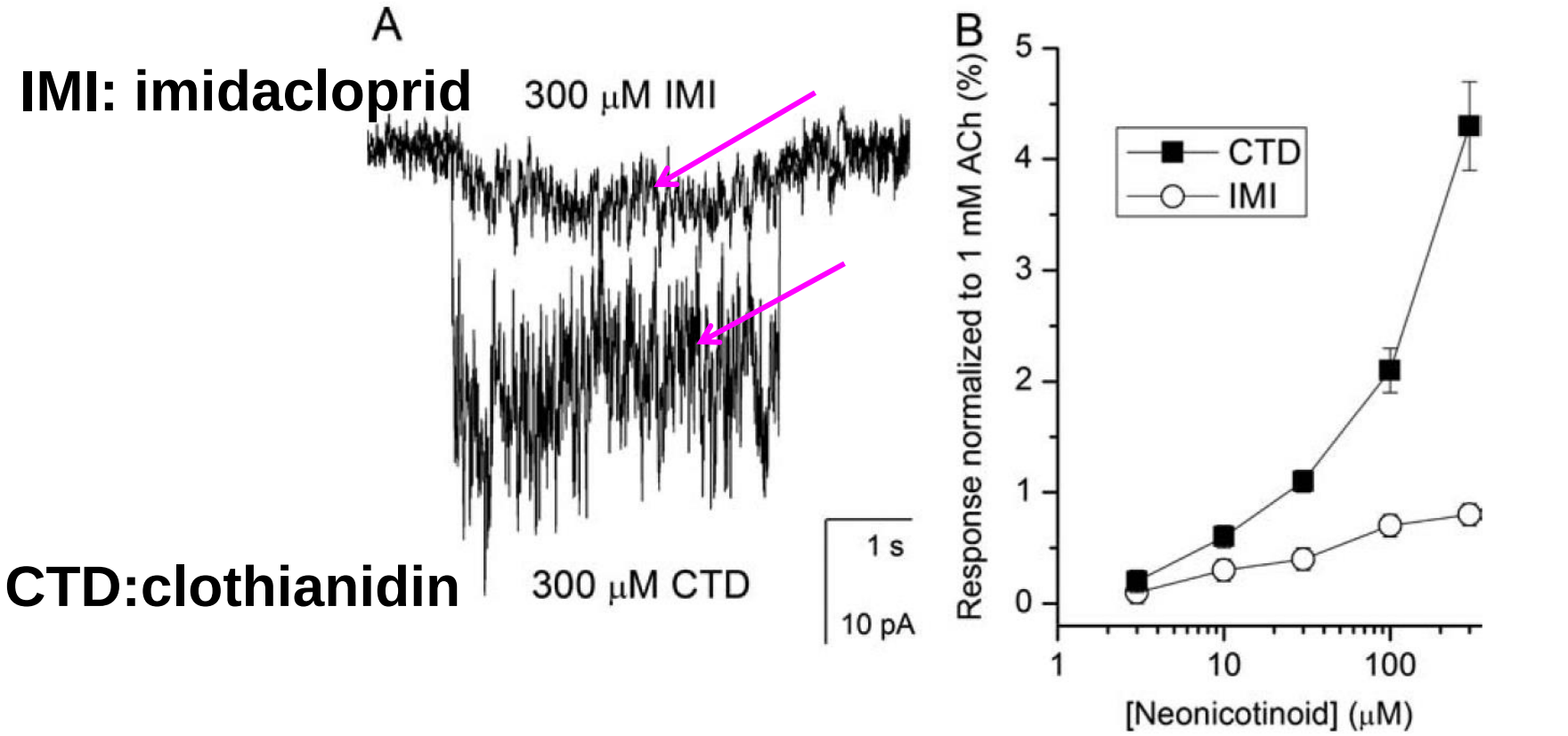
ヒトと昆虫の脳神経系の構造と機能は基本的に同じ





Major information chemicals (for example acetylcholine, glutamate)are same in human and in insect　主要.情報化学物質はアセチルコリン、グルタミン酸など全て同じ

Human : Neonicotinoids act as agonists binding to human nicotinic receptors
ネオニコはヒトのニコチン性受容体に作用する

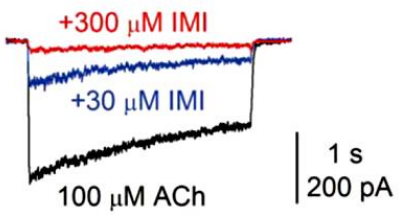


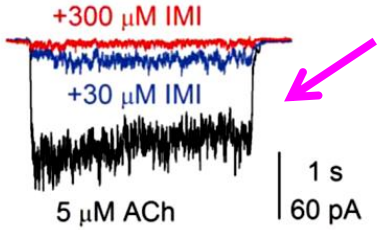
In HEK (human embryonic kidney) cells expressed human α4β2 nicotinic receptors, imidaclopriod and clothianidin cause excitations.

Li P., et al., *J. Neurosci Res.*, 89, 1295-1301 (2011)

Neonicotinoids disrupt normal functions of acetylcholine via human nicotinic receptors
ネオニコはヒトのニコチン受容体に作用し アセチルコリンの作用をかく乱する

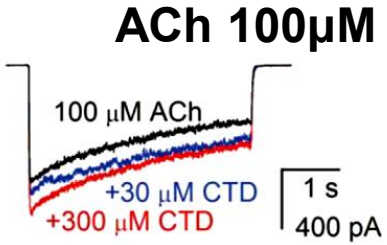
IMI: imidacloprid

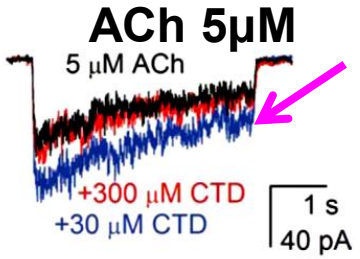




Inhibition
抑制

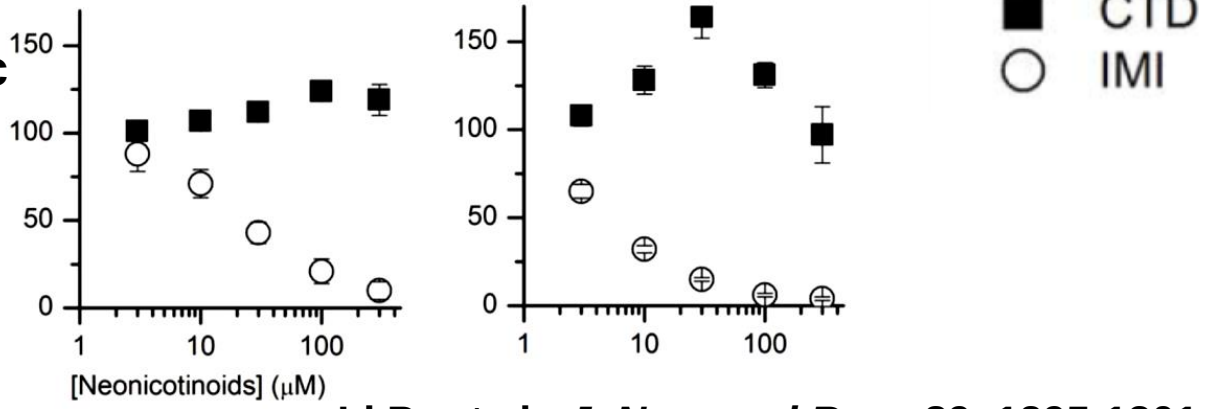
CTD: clothianidin





Enhancement
増強

HEK (human embryonic kidney) cells expressed human α4β2 nicotinic receptors



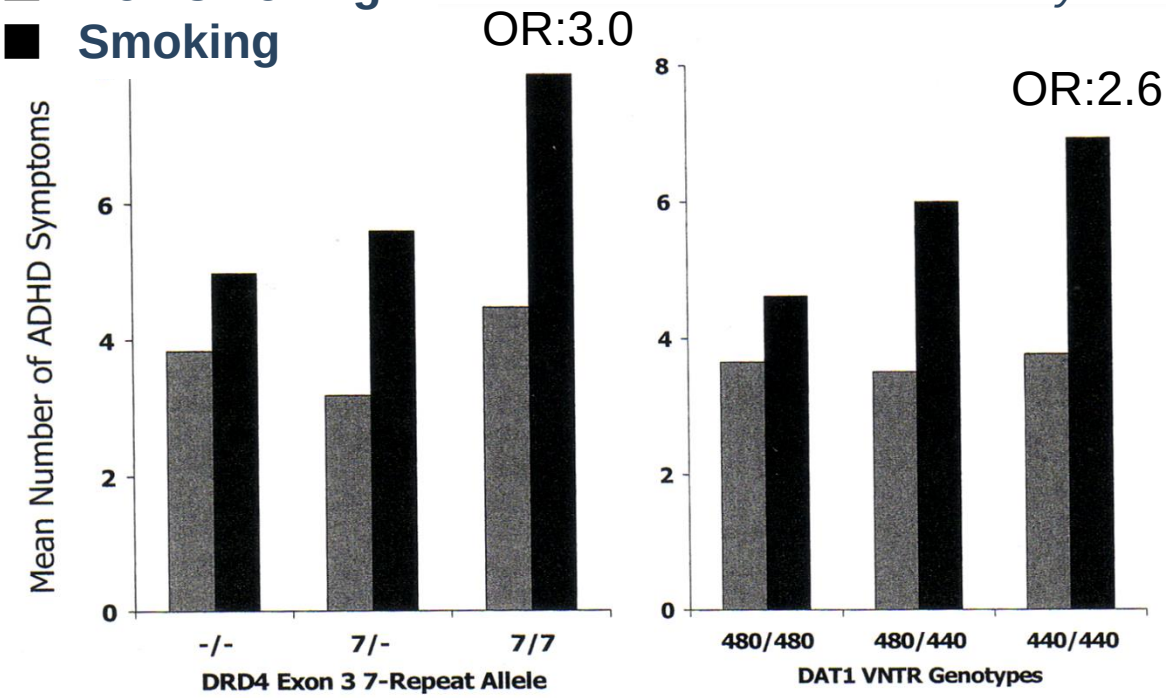
Li P., et al., *J. Neurosci Res.*, 89, 1295-1301 (2011)

Smoking during pregnancy is a high risk of ADHD
受動喫煙はADHDの高リスク・ファクター

ADHD発症

Non smoking
Smoking

Neuman et al. *Biol. Psychiatry* 2007, 61:1320-



Dopamine receptor polymorph
ドーパミン受容体遺伝子多型

Dopamine trasnsporter polymorph
ドーパミン輸送体遺伝子多型

- **Smoking during pregnancy is a high risk of ADHD, especially mothers with specific genetic polymorphisms are at a particularly high risk.**
- 喫煙がADHDのリスクとなる疫学報告は多いが、特にドーパミン系の特定な遺伝子多型をもつ母親が喫煙すると、ADHDにかかるリスクが顕著に上がる報告は注目されている。

Effects of neonicotinoids on cerebellar neurons from neonatal rats.

Methods:

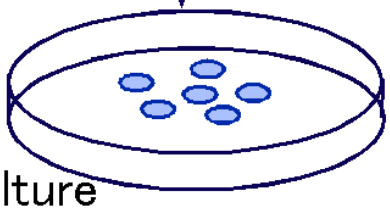
Homology of nAChRs between human and rat are more than 90%.

Rat cerebellum (post 1 day)

↓

α3,α4,α7nAChRs are expressed in cerebellum, especially developmental stage.

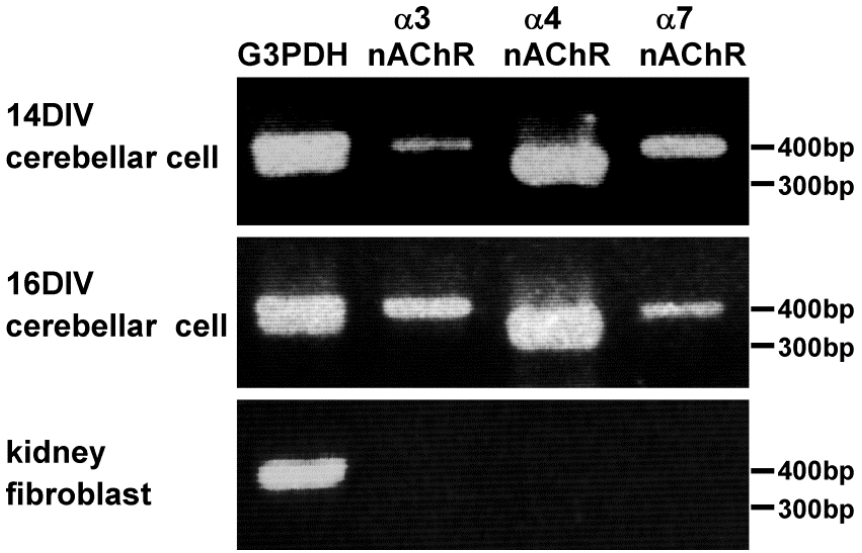
↓



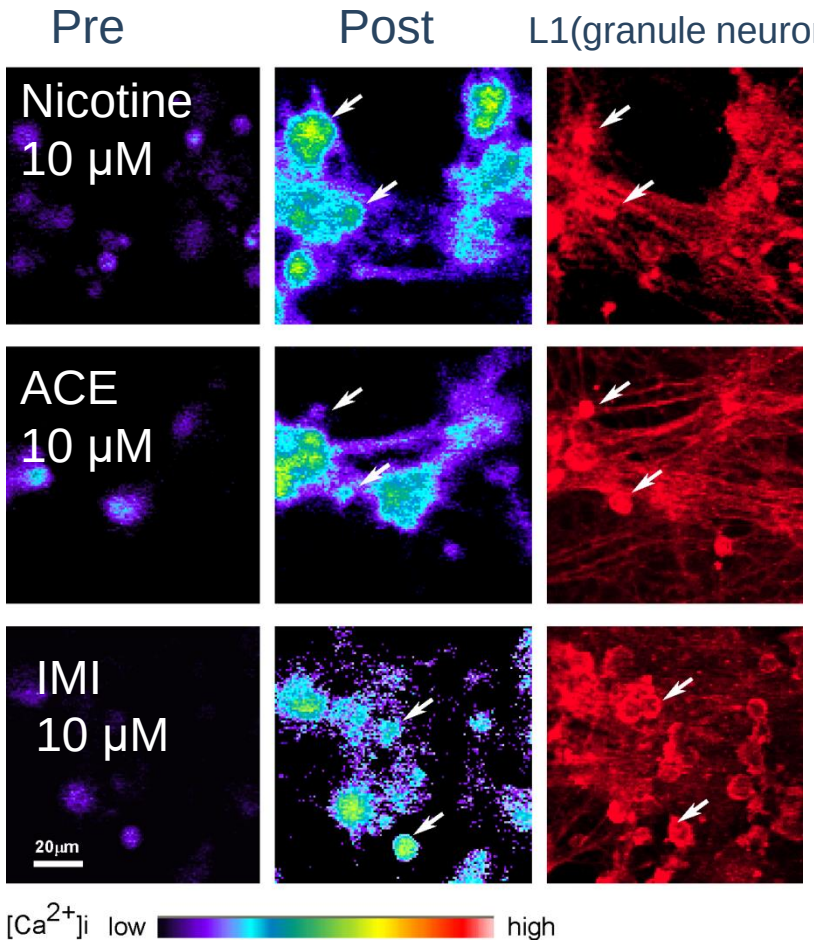
14−16day culture

↓

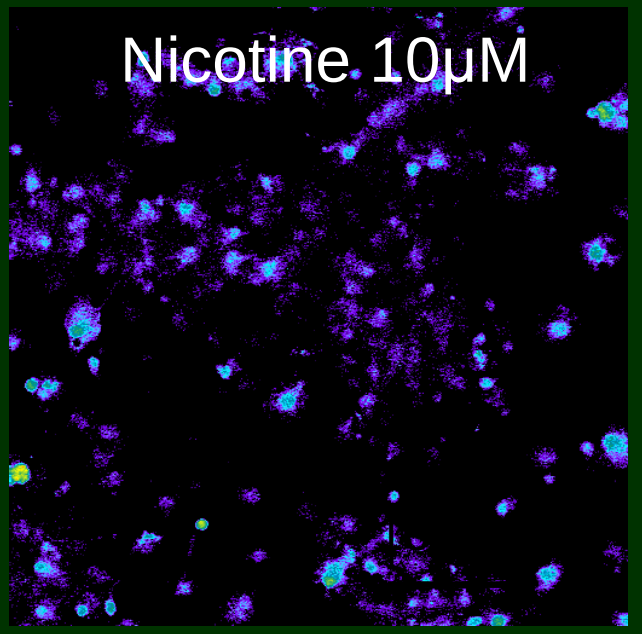
Cerebellar cultures were loaded with FLuo4, Ca^{2+} indicator.
Time-lapse images of Fluo4 fluorescence in neuronal cells were obtained using confocal laser scanning microscope.
To minimize desensitization of the nAChRs by drug leaking from the pipette tip, the pipette used for the pressure application was placed at a target position approximately 100 μm from the neuron.

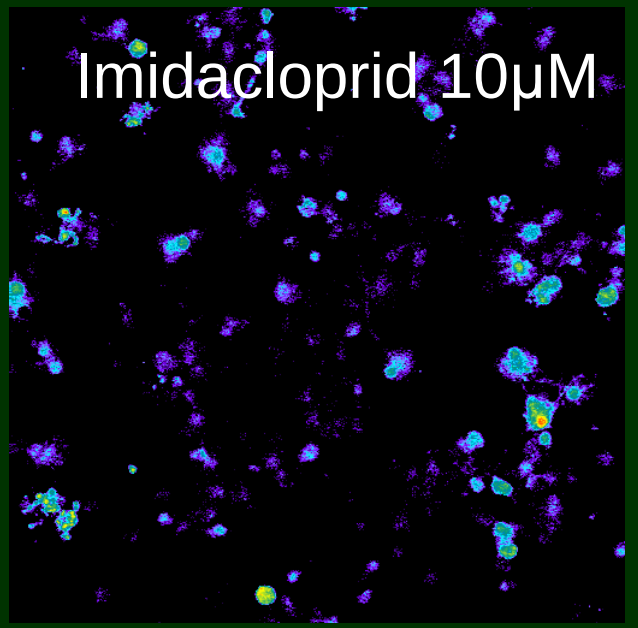


Neonicotinoids induce excitatory Ca^{2+} influx in cerebellar neurons　ネオニコチノイドは発達期の小脳神経細胞に興奮作用を起こす

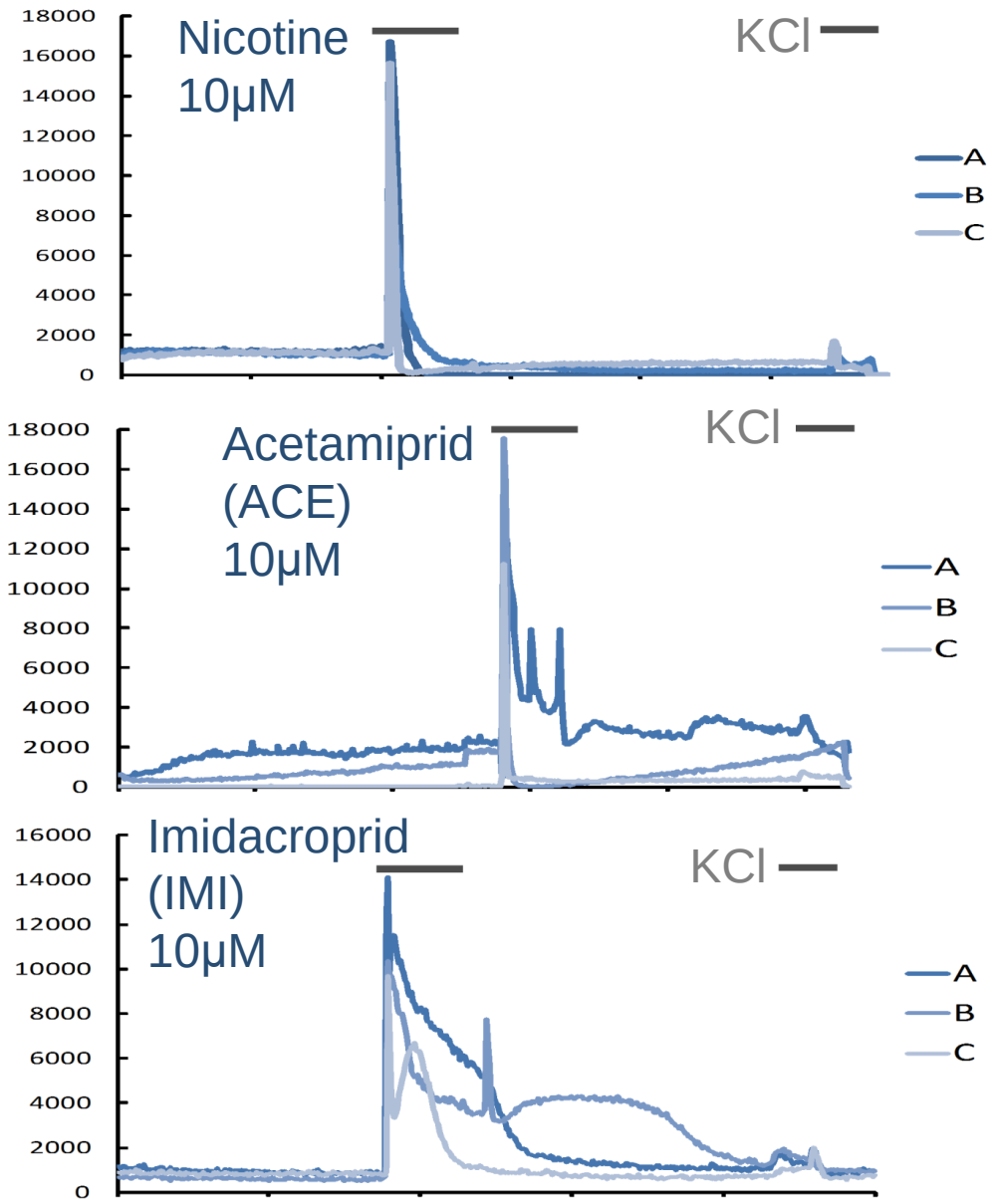


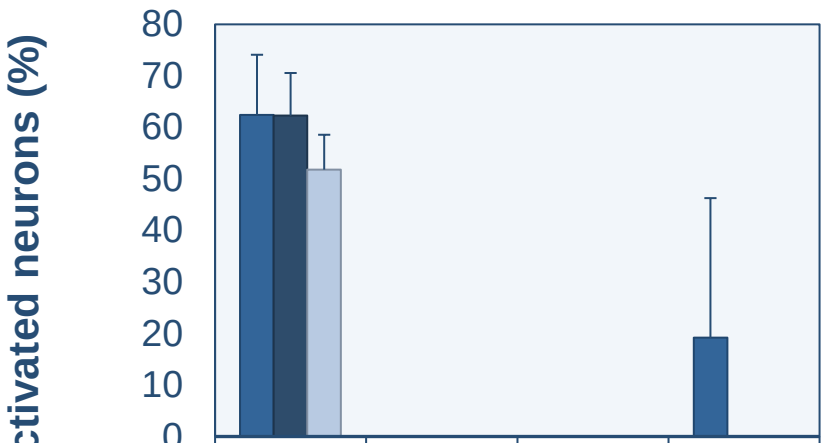
Cerebellar neurons from neonatal rat
ACE；acetamiprid
IMI;　imidacloprid





Neonicotinoids bind to rat acetylcholine receptors and excite neurons



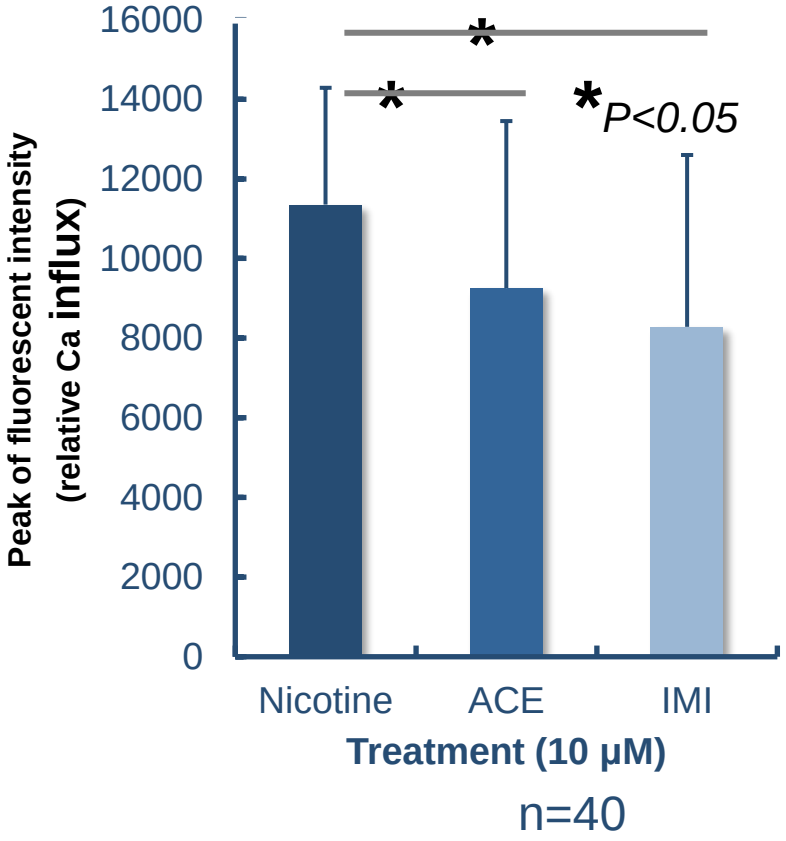


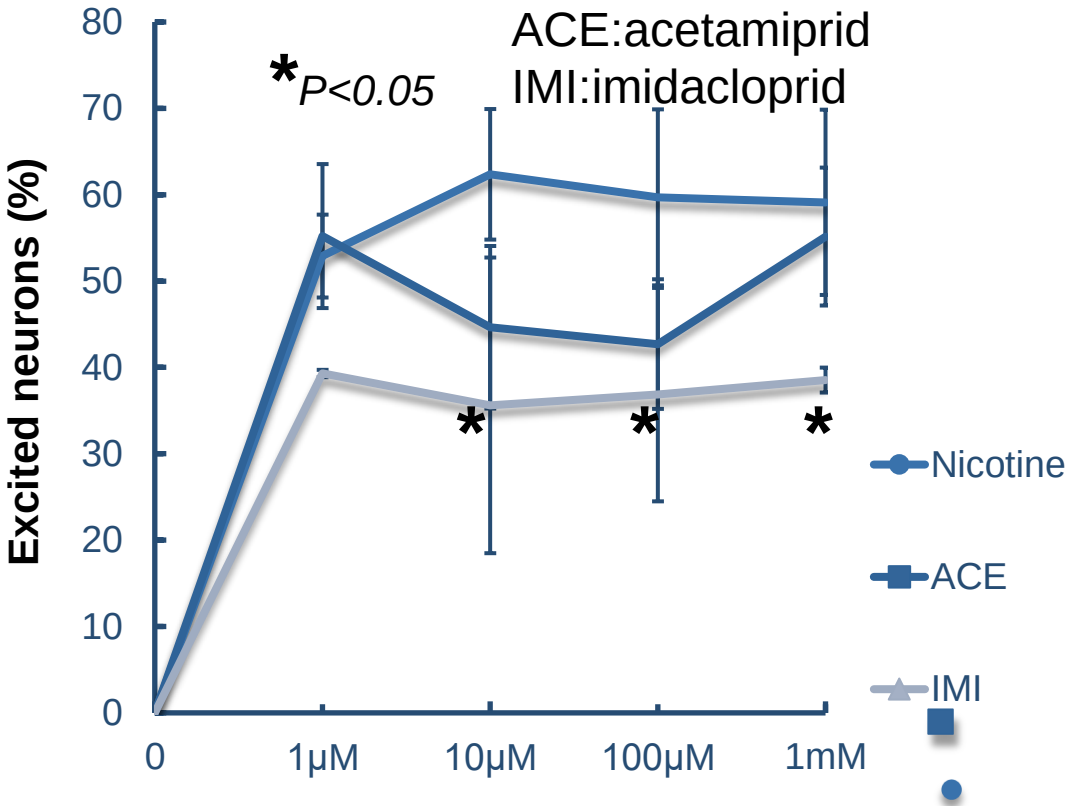
	None	MEC	α-BT	DHβE
Nicotine	62.4	0	0	19.2
ACE	62.3	0	0	0
IMI	51.8	0	0	0

（100μM）

The nAChR specific antagonists Inhibit Ca influx Induced by neonicotinoids and nicotine.
MEC: mecamylamine　100 μM
α-BT:　α-bungarotoxin（α7specific）1 μM
DHβE: dihydro- β-erythroidine 1 μM
（α4β2specific）

Neonicotinoids excite rat neurons like nicotine





The firing patterns, proportion of excited neurons, and peak excitatory Ca^{2+} influxes induced by ACE and IMI showed differences from those induced by nicotine. However, ACE and IMI had greater effects on mammalian neurons than those previously reported in binding assay studies. Kimura-kuroda et al, Plos One, 2012

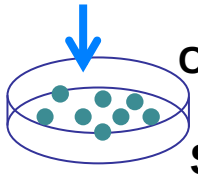
Gene expression analysis by whole genome DNA microarray

ネオニコチノイドの遺伝子発現への影響：DNAマイクロアレイ

Methods

Rat neonatal cerebellar cells

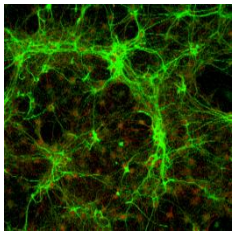
1 or 10 μM Nicotine, Acetamiprid, Imidacloprid



Culture for 10-14 days

Synapse formation

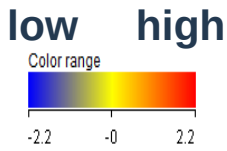
Neurons about 65 %
Glia about 30 %

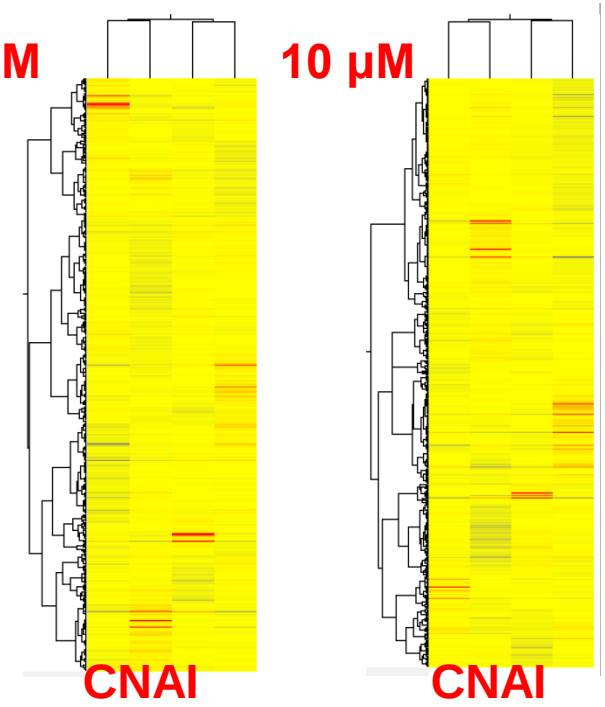


Total RNAs extraction & purification

Agilent DNA microarray
Whole rat genome V.3
30367genes
By SureScan Microarray Scanner

Analysis
Gene Spring GX（Agilent ）
reliable signals
About 20,000 genes

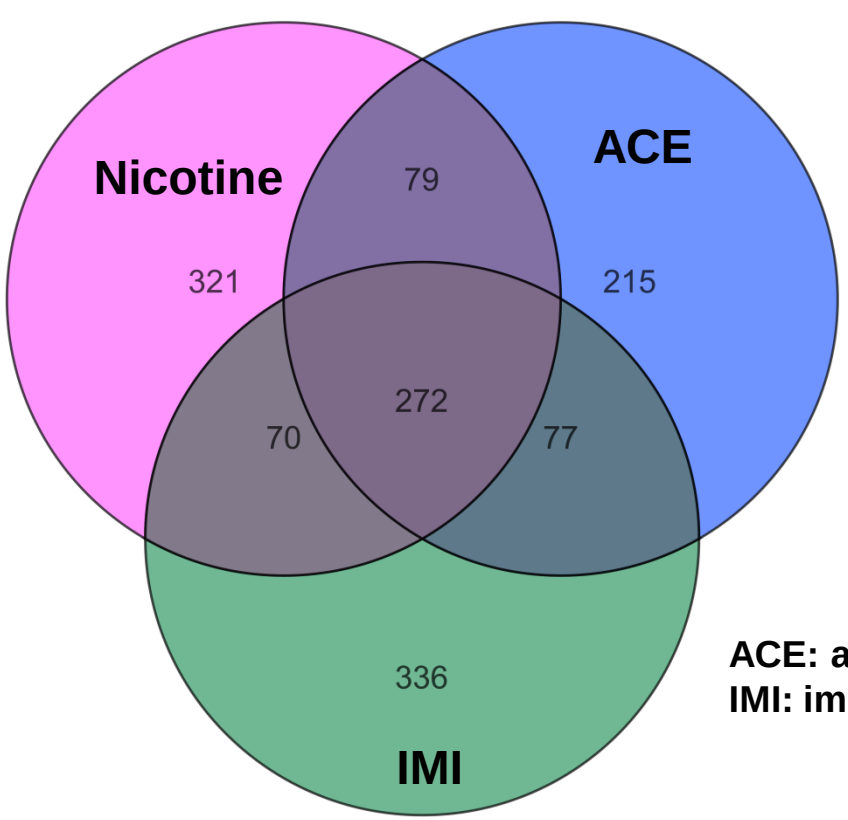




C: control
N: nicotine
A: acetamiprid
I: imidacloprid

Neonicotinoid or nicotine disrupts gene expressions of developmental brain ネオニコチノイド、ニコチン曝露は発達期脳の遺伝子発現を撹乱する

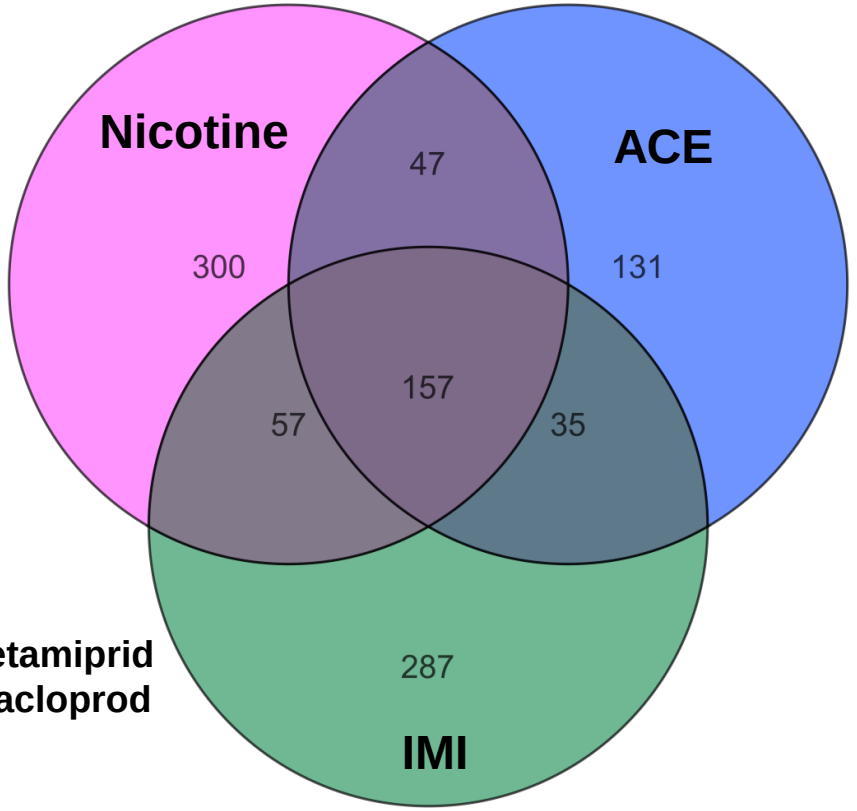
1μM exposure for 14days



Total 1370 genomes were up or down.

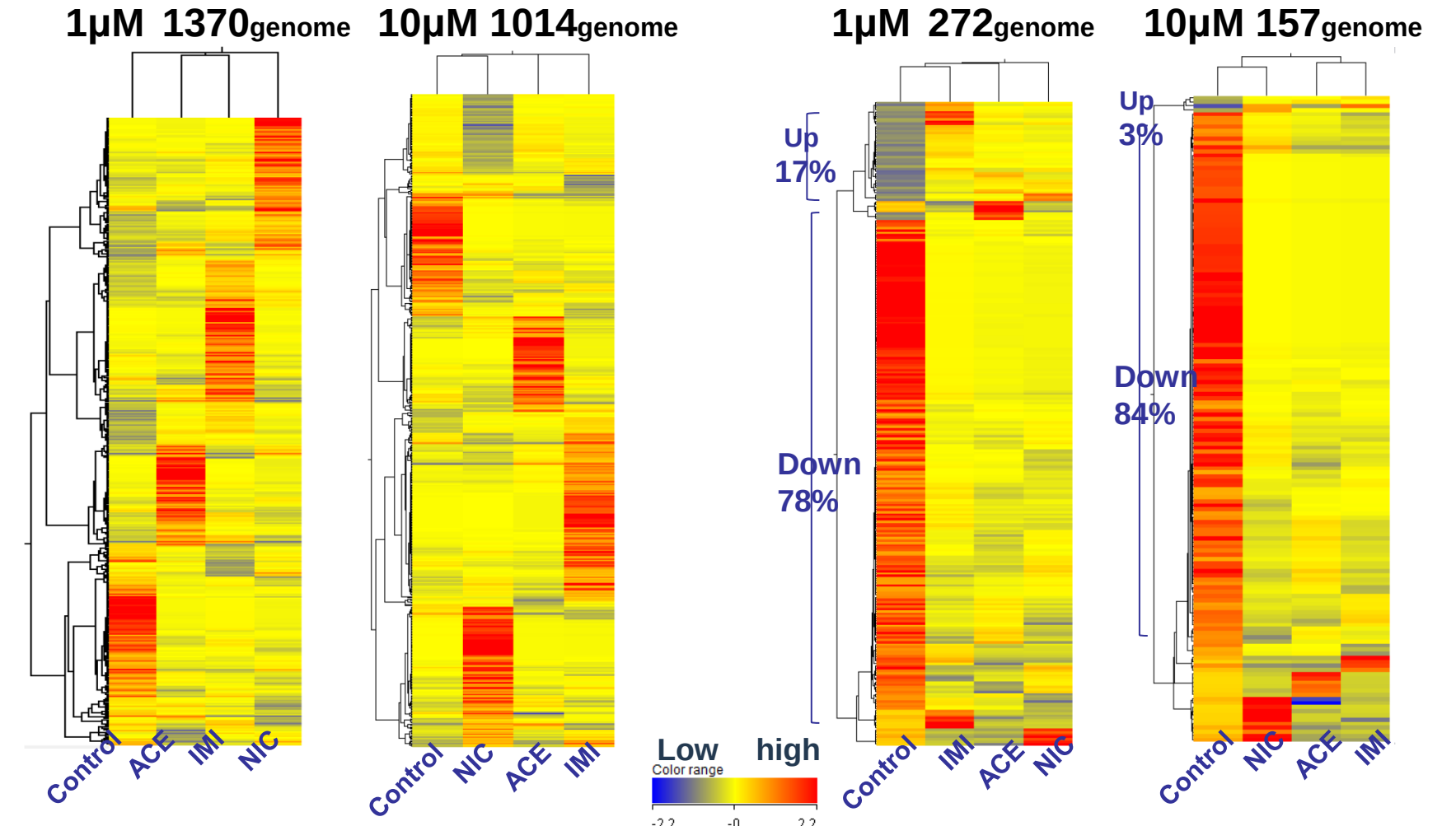
ACE: acetamiprid
IMI: imidacloprod

10μM exposure for 14 days



Total 1014 genomes were up or down.

Disruption of gene expressions of developmental cerebellum ネオニコチノイド曝露による遺伝子発現の変化



Disrupted gene expressions by NIC, ACE &/or IMI

Disrupted gene expressions common to three

Almost common genes were down regulated. ネオニコ、ニコチンで共通に変動する遺伝子はDown regulationされるものが大部分であった。

Genomes, their expressions were disrupted by nicotine, neonicotinoids
ネオニコチノイド、ニコチン曝露で発現が変化する遺伝子

Function 機能	Common to Nicotine, ACE & IMI 3群で共通に変化	Specific to ACE and/or IMI ネオニコチノイド2群 or 1群で変化
Synapse & Neurotransmitter シナプス＆ 神経伝達関連因子	Stx1b syntaxin1B	Gabra5 GABA A receptor α5 Npvf Neuropeptide VW Htr7 Serotonin receptor 7 Gpr63 G protein-coupled receptor 63
Hormone related ホルモン関連因子	Hsd3b5 hydroxy-δ-5-steroid dehydrogenase	Avpr1b Arginine vasopressin receptor 1B
Signal transduction シグナル伝達系	Aatk apoptosis-associated tyrosine kinase	Asip agouti signaling protein tGap1 GTPase activating protein
Transcriptional regulator 転写因子	Foxc2 Fork head box C2 Fbxw12 F-box and WD repeat domain protein 12	Ccdc68 coiled-coil domain containing 68 Rnf152 ring finger protein 152 Fbxl16 F-box and leucine-rich repeat protein 16 Ptch2 patched homolog 2

Nicotin, ACE, IMI,1μM

down regulation, up regulation

From these data, neonicotinoids may adversely affect developing brain, not only like the nicotine's effects, but also some their specific effects.

Summary of Neonicotinoid (ネオニコの要約)

1. Neonicotinoids bind to human acetylcholine receptors like nicotine, have neurotoxicities to developing human brain.
   ネオニコはヒトのアセチルコリン受容体にニコチンと同様結合し、特に発達期のヒト脳に神経毒性がある
2. Their toxicities are similar to nicotine, which cause ADHD , but also show their specific effects.
   ネオニコチノイドは既にADHDの発症が知られているニコチンに類似しているが、特異的な影響も起こす。
3. Combined toxic effects of organophosphate、neonicotinoid pesticides and radioactivities are concerned for children's developmental brain.
   有機リン系,ネオニコチノイド系農薬と放射線被爆の発達期の子どもの脳への複合影響が懸念される。