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2. 学会発表

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H. 知的財産所有権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

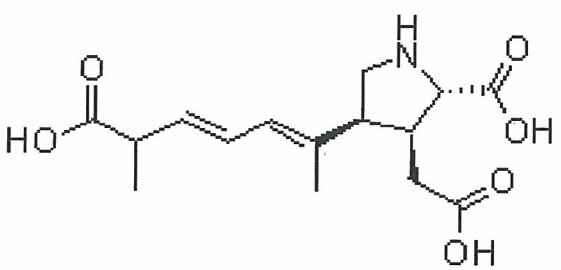
3. その他

なし

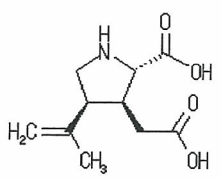
Domoic acid

- Domoic acid（DA）は、駆虫薬として用いられる紅藻中の、グルタミン酸のアゴニスト。グルタミン酸受容体と強く結合して結果的に麻痺による駆虫作用を示す （用量30mg程度）。
- 類似物質には、Kainic acid カイニン酸
- Domoic acidは異常繁殖した珪藻が活動を停止する際に作り出される。生物濃縮によって貝類やカニ、アンチョビなどに取り込まれる。現在では魚介類の輸出入において検査が行われるようになって来ている。
- カナダのプリンスエドワード島（1987年）での養殖ムラサキイガイによる食中毒の原因物質。（貝100g当たり31mg～128mgのdomoic acid）
- 被害者107人中4人が死亡、12人が重度の記憶障害に陥った。
- 推定中毒量は60mg～290mgと、致死量は300mg/60kg。
- 推定死因：海馬に大量のdomoic acidが取り込まれてグルタミン酸受容体と結合したために脳細胞が興奮・死滅し、中枢神経が侵された
- カナダのdomoic acid規制値は20ppmである。

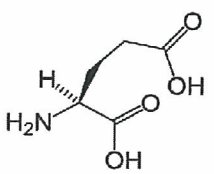
Domoic acid



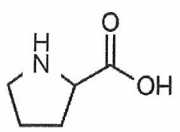
分子式 C15H21NO6
分子量 311.33
IUPAC名
[2S-[2a,3b,4b(1Z,3E,5R)]]-2-カルボキシ-4-（5-カルボキシ-1-メチル-1,3-ヘキサジエニル）-3-ピロロリジン酢酸
CAS登録番号 14277-97-5
プロリンの誘導体でもある。
単体は融点 213–217 ℃
無色の結晶性粉末
水によく溶け、有機溶媒に不溶



Kainic acid



Glutamic acid



Proline

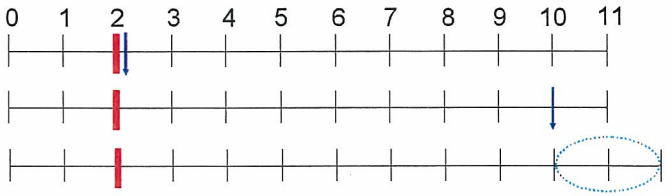
実験概要

DA 0.3mg/kg i.p. （C57BL/6 male mouse）

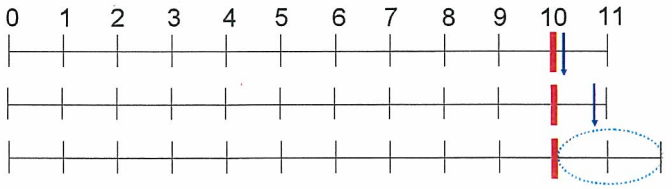
検体採取（大脳皮質(CX)と海馬(HC)）

行動解析

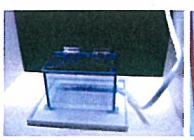
子ども期投与： 2W



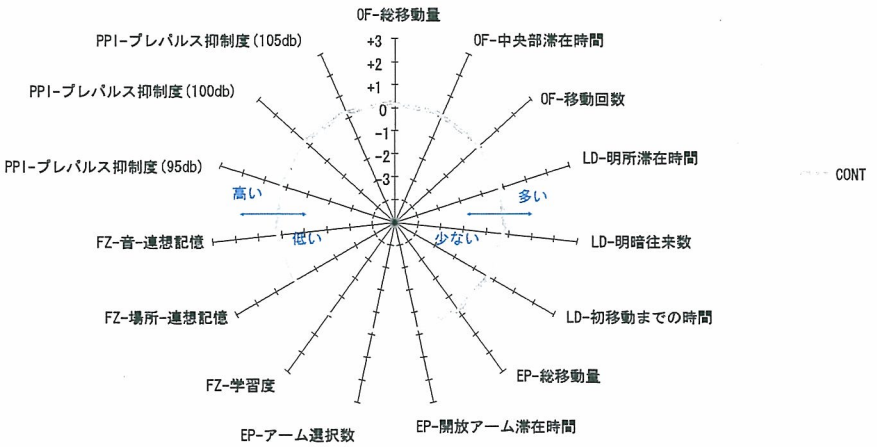
大人期投与： 10W



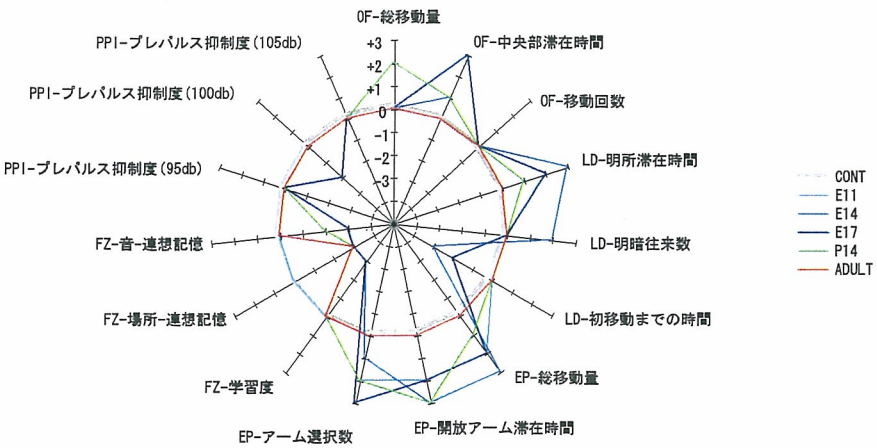
B6マウス（生後14日齢）
ドーモイ酸（0.3mg/kg）腹腔投与：対照群には生理食塩水を腹腔投与
→4週齢時に離乳＆雌雄判別
→神経行動毒性試験（♂マウス：10-11週齢）

OF	LD	EP	FZ	PPI
				
オープンフィールド試験 10分	明暗往来試験 5分	高架式十字迷路試験 10分	恐怖条件付け試験 6分・３日間	プレパルス驚愕抑制試験 30分
検定項目 総移動量 中央部-滞在時間 移動回数	検定項目 明所滞在時間 明暗往来数 初移動時	検定項目 総移動量 アーム選択数 開放アーム-滞在時間	検定項目 学習度（1日目） 場所-連想記憶（2日目） 音-連想記憶（3日目）	検定項目 プレパルス驚愕抑制度 (80-105db:120db)

OF→LD→EP→FZ→PPI



±1 : p<0.1
±2 : p<0.05
±3 : p<0.01



±1 : p<0.1
±2 : p<0.05
±3 : p<0.01

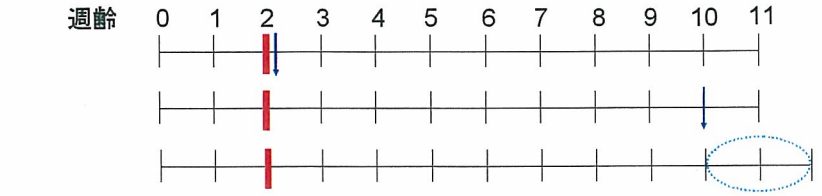
Precellome data

DA 0.3mg/kg i.p. (C57BL/6 male mouse)

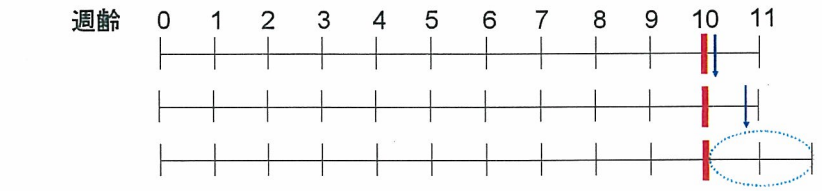
検体採取(大脳皮質(CX)と海馬(HC))

行動解析

子ども期投与: 2W

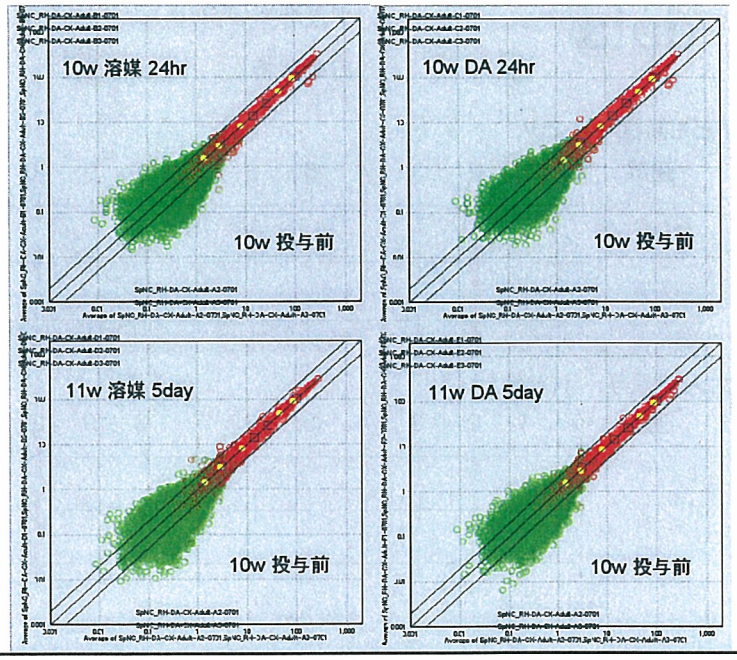


大人期投与: 10W

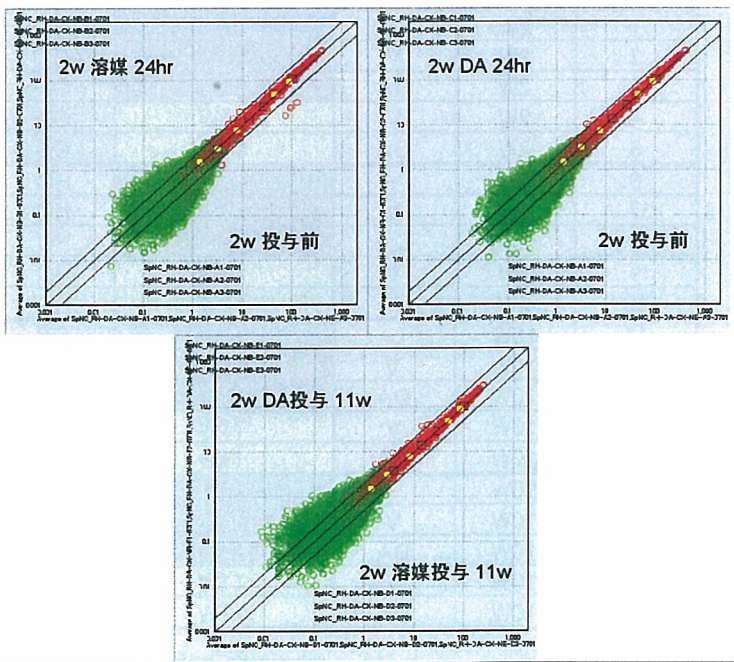


	Age	Treatment	Sampling Time	Tissue
1	2W	–	2W	CX
2	2W	V	2W+24hr	CX
3	2W	DA	2W+24hr	CX
4	2W	V	11W	CX
5	2W	DA	11W	CX
6	2W	–	2W	HC
7	2W	V	2W+24hr	HC
8	2W	DA	2W+24hr	HC
9	2W	V	11W	HC
10	2W	DA	11W	HC
11	10W	–	10W	CX
12	10W	V	10W+24hr	CX
13	10W	DA	10W+24hr	CX
14	10W	V	11W	CX
15	10W	DA	11W	CX
16	10W	–	10W	HC
17	10W	V	10W+24hr	HC
18	10W	DA	10W+24hr	HC
19	10W	V	11W	HC
20	10W	DA	11W	HC

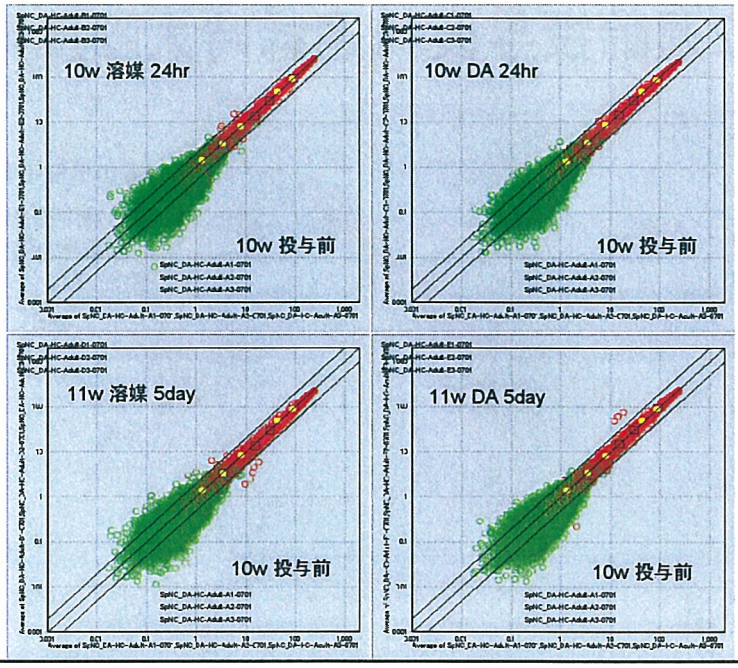
Adult投与 大脳皮質



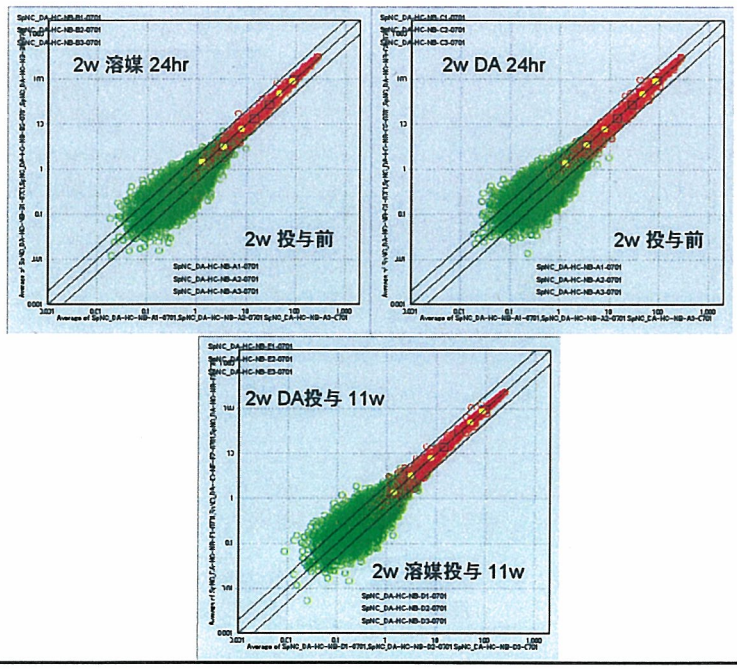
2週齢投与 大脳皮質



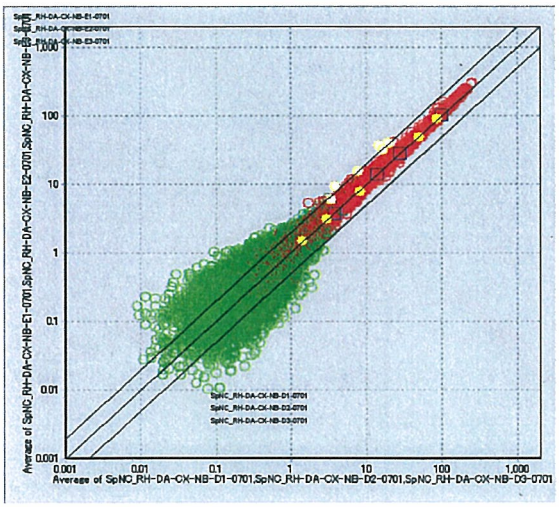
Adult投与 海馬



2週齢投与 海馬

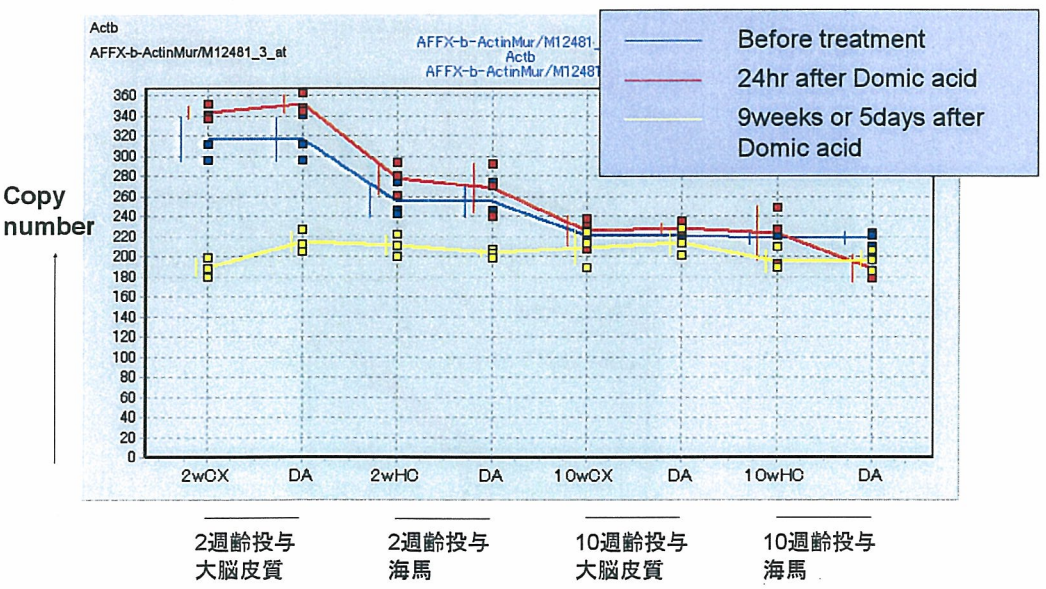


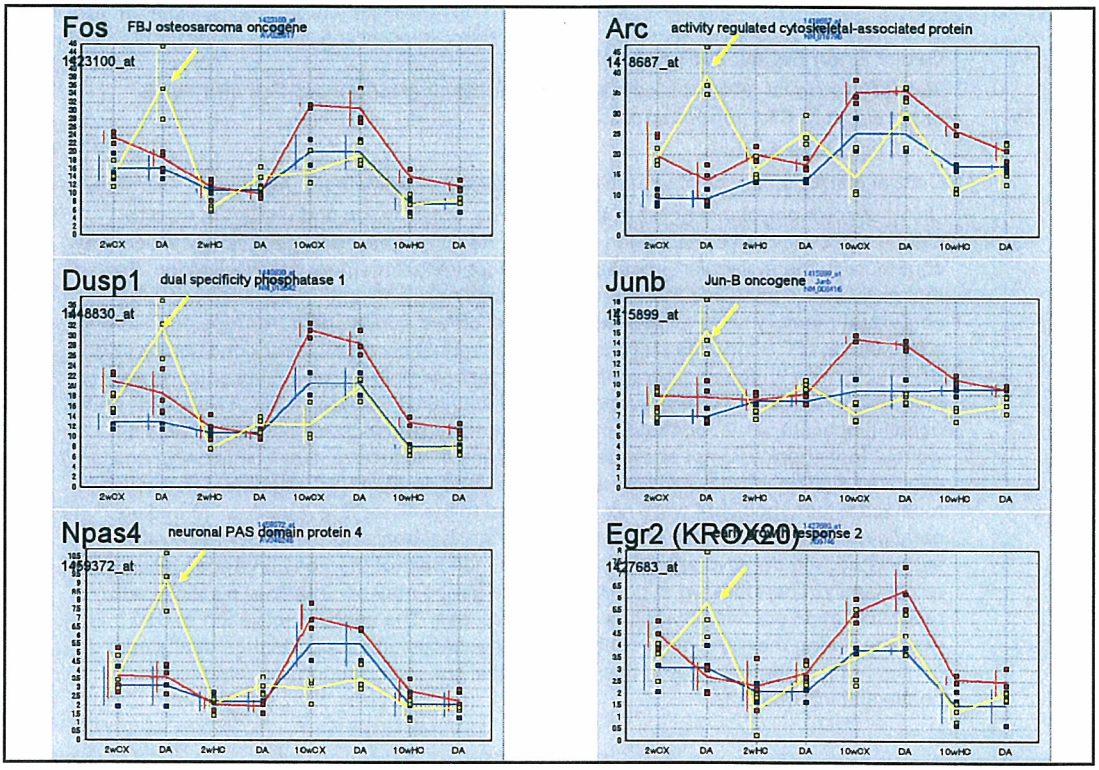
Scatter plotからの遺伝子選択：2週齢投与後11週齢時大脳皮質



- 6ヶの遺伝子を選択した。
- 他の群での比較では統計的な条件を満たす遺伝子は無かった。

グラフ設定

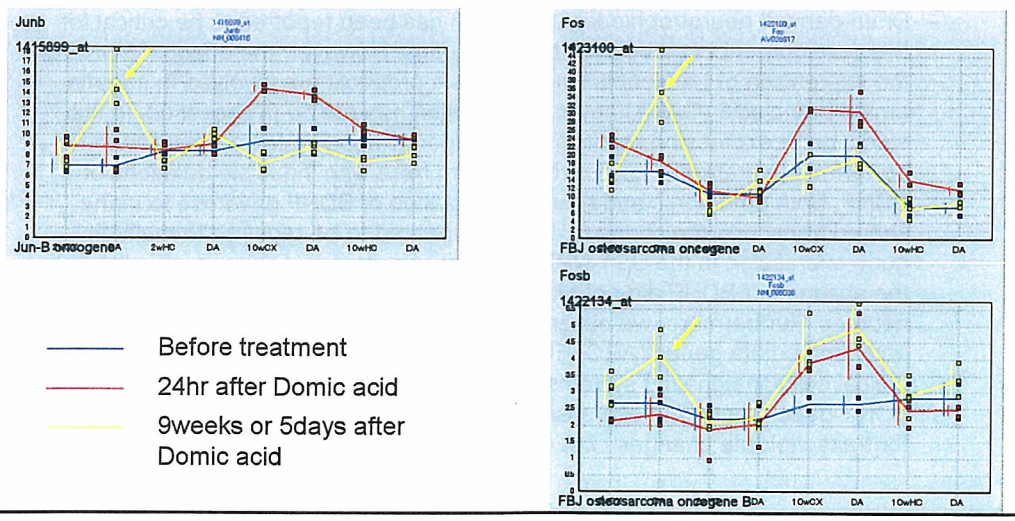




Jun, Fos family

Jun, FosがDA投与(ip)により4時間以内に誘導されることが報告されている(Neuroscience. 2005;136(4):1121-32.)。

→2週齢時投与後9週(11週齢)でJunb, Fosの発現上昇が認められた



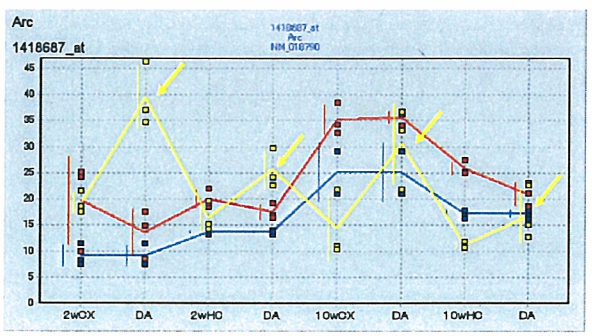
- **Genes Brain Behav. 2003 Feb;2(1):3-10.**
- **Memory retrieval after contextual fear conditioning induces c-Fos and JunB expression in CA1 hippocampus.**
  - Strekalova T, et al.
- Central Institut of Mental Health (CIMA), University of Heidelberg, Mannheim, Germany.
- Using specific polyclonal antisera against c-Fos, JunB, c-Jun and JunD, we tried to identify the candidate transcription factors of the immediate early gene family which may contribute to the molecular processes during contextual memory reconsolidation. For that purpose we analyzed the expression of these proteins in the hippocampus after contextual memory retrieval in a mouse model of fear conditioning. A single exposure to a foot shock of 0.8 mA was sufficient to induce robust contextual fear conditioning in C57Bl/6N mice. In these mice context dependent memory retrieval evoked a marked induction of c-Fos and JunB, but not of c-Jun and JunD, in pyramidal CA1 neurons of the dorsal hippocampus. In contrast, mice exposed and re-exposed only to the context, without foot shock, did not show behavioral signs of contextual fear conditioning and exhibited significantly less expression of c-Fos and JunB in CA1 neurons. Mice which received a foot shock but were not re-exposed to the context revealed no immediate early gene induction. These results demonstrate that contextual memory retrieval is associated with de novo synthesis of specific members of the Fos/Jun transcription factor family. Therefore we suggest that these genes may contribute to plasticity and reconsolidation accompanying the retrieval process. The specific activation of CA1 neurons during the retrieval of contextual fear associations supports the postulated concept of a mnemonic role of this hippocampal subsector during the retrieval of contextual informations.

- **Mol Psychiatry. 2006 Jul;11(7):633-48. Epub 2006 May 9.**
- **Specificity and timing of neocortical transcriptome changes in response to BDNF gene ablation during embryogenesis or adulthood.**
  - Glorioso C, et al.
- Department of Psychiatry, University of Pittsburgh School of Medicine, PA 15261, USA.
- Brain-derived neurotrophic factor (BDNF) has been reported to be critical for the development of cortical inhibitory neurons. However, the effect of BDNF on the expression of transcripts whose protein products are involved in gamma amino butric acid (GABA) neurotransmission has not been assessed. In this study, gene expression profiling using oligonucleotide microarrays was performed in prefrontal cortical tissue from mice with inducible deletions of BDNF. Both embryonic and adulthood ablation of BDNF gave rise to many shared transcriptome changes. BDNF appeared to be required to maintain gene expression in the SST-NPY-TAC1 subclass of GABA neurons, although the absence of BDNF did not alter their general phenotype as inhibitory neurons. Furthermore, we observed expression alterations in genes encoding early-immediate genes (ARC, EGR1, EGR2, FOS, DUSP1, DUSP6) and critical cellular signaling systems (CDKN1c, CCND2, CAMK1g, RGS4). These BDNF-dependent gene expression changes may illuminate the biological basis for transcriptome changes observed in certain human brain disorders.

- **Eur J Neurosci. 2006 Nov;24(10):2705-20.**
- **Npas4, a novel helix-loop-helix PAS domain protein, is regulated in response to cerebral ischemia.**
- Shamloo M, et al
- AGY Therapeutics, South San Francisco, California 94080, USA. Mehrdad_shamloo@affymax.com
- Basic helix-loop-helix PAS domain proteins form a growing family of transcription factors. These proteins are involved in the process of adaptation to cellular stresses and environmental factors such as a change in oxygen concentration. We describe the identification and characterization of a recently cloned PAS domain protein termed Npas4 in ischemic rat brain. Using gene expression profiling following middle cerebral artery occlusion, we showed that the Npas4 mRNA is differentially expressed in ischemic tissue. The full-length gene was cloned from rat brain and its spatial and temporal expression characterized with in situ hybridization and Northern blotting. The Npas4 mRNA is specifically expressed in the brain and is highly up-regulated in ischemic tissues following both focal and global cerebral ischemic insults. Immunohistochemistry revealed a strong expression in the limbic system and thalamus, as well as in layers 3 and 5 in the cortex of the unchallenged brain. When overexpressed in HEK 293 cells, Npas4 appears as a protein of approximately 100 kDa. In brain samples, however, in addition to the 100 kDa band a specific 200 kDa immunoreactive band was also detected. Ischemic challenge lead to a decrease in the 200 kDa form and a simultaneous increase in the 100 kDa immunoreactivity. This could indicate a novel regulatory mechanism for activation and/or deactivation of this protein in response to ischemic brain injury.

Arc (Activity regulated cytoskeletal-associated protein)

AMPA receptorによる制御を受けることが報告されているArc(*)の発現が投与後一定時間経過後に上昇していた



* Nat Neurosci. 2006 Jul;9(7):887-95.
AMPA receptors regulate transcription of the plasticity-related immediate-early gene Arc.
Rao VR, Pintchovski SA, Chin J, Peebles CL, Mitra S, Finkbeiner S.

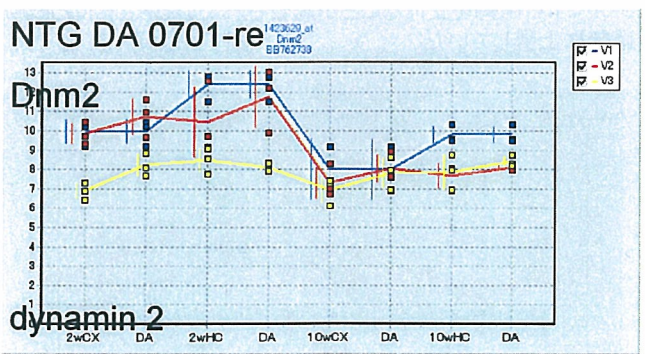
Arcと記憶の固定化

Neuron. 2006 Nov 9;52(3):403-7. Review.

Arc/Arg3.1: linking gene expression to synaptic plasticity and memory.

Tzingounis AV, Nicoll RA.

Arc/Arg3.1 is an effector immediate-early gene implicated in the consolidation of memories. Although cloned a decade ago, the physiological role of Arc/Arg3.1 in the brain has remained elusive. Four papers in this issue of Neuron address this function. These studies show that Arc/Arg3.1 regulates endophilin 3 and dynamin 2, two components of the endocytosis machinery. Genetic ablation of Arc/Arg3.1 in mice or overexpression in culture suggest that Arc/Arg3.1 regulates AMPA receptor trafficking and synaptic plasticity. Finally, Arc/Arg3.1 knockout mice show memory retention deficits. These recent developments provide new insights into the function of this popular activity-dependent neuronal marker.



- **Neuron. 2006 Nov 9;52(3):437-44.**
- **Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories.**
  - **Plath N**, et al.
- Molecular Neurobiology, Department of Biology-Chemistry-Pharmacy, Freie Universitat Berlin, 14195 Berlin, Germany.
- Arc/Arg3.1 is robustly induced by plasticity-producing stimulation and specifically targeted to stimulated synaptic areas. To investigate the role of Arc/Arg3.1 in synaptic plasticity and learning and memory, we generated Arc/Arg3.1 knockout mice. These animals fail to form long-lasting memories for implicit and explicit learning tasks, despite intact short-term memory. Moreover, they exhibit a biphasic alteration of hippocampal long-term potentiation in the dentate gyrus and area CA1 with an enhanced early and absent late phase. In addition, long-term depression is significantly impaired. Together, these results demonstrate a critical role for Arc/Arg3.1 in the consolidation of enduring synaptic plasticity and memory storage.

- **Brain Res Mol Brain Res. 1995 Jan;28(1):87-93.**
- **Krox20 may play a key role in the stabilization of long-term potentiation.**
- **Williams J**, et al
- Department of Pharmacology, School of Medicine, University of Auckland, New Zealand.
- Long-term potentiation-inducing stimulation of the perforant path was followed in dentate gyrus granule cells by a dramatic increase of mRNA and protein for Krox20, a zinc-finger-containing transcription factor. Induction of Krox20 required stimulation sufficient to induce LTP and was prevented by NMDA antagonists CPP and MK-801, which block LTP induction. Krox20 protein increased within 20 min of tetanization, was maximal between 1 and 8 h, and was still significantly elevated at 24 h after LTP induction. This prolonged appearance is in striking contrast with the more transient induction of the related molecule, Krox24. The elevation in the mRNA for Krox20 and Krox24 was of similar duration, suggesting that the Krox20 protein has a greater stability and may play a key role in the stabilization of long-term potentiation.

Scatter plotによる発現変化解析結果

- 大脳皮質、海馬ともに、DA投与に伴う発現変化の程度は小さかった
- 2週齢投与11週齢時において、6ヶの遺伝子の発現上昇が捉えられた

発現変化遺伝子のt検定による選択

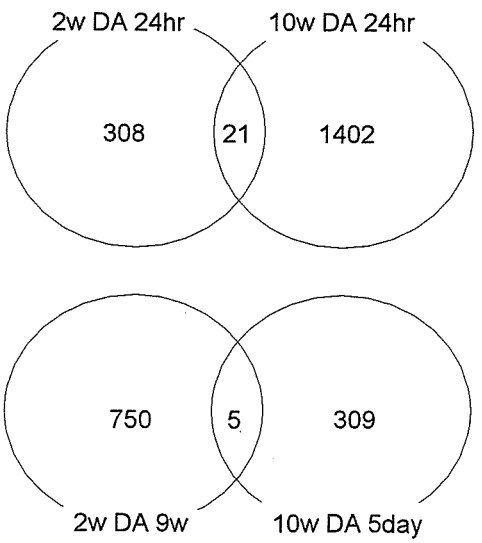
- t-test: $p<0.01$
  - 溶媒投与群を対照
- CV<20%
  - 各グループについて
    （例：2週齢投与後24時間皮質の場合
    1）2週齢投与前群
    2）溶媒投与群
    3）Domoic acid投与群
    の全てがCV<20%となる遺伝子に限る）

発現変化遺伝子数

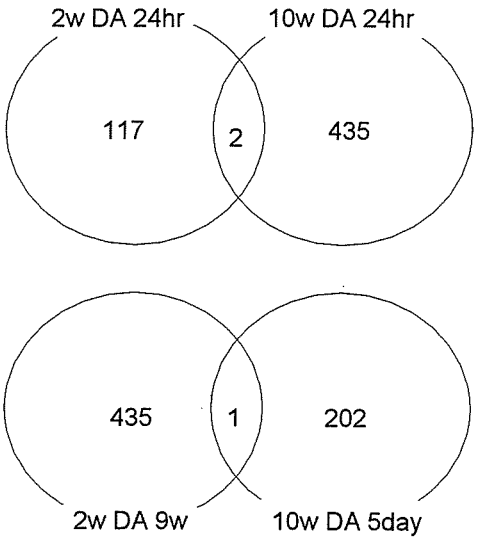
Group	Region	Number
2w DA 24hr	Cortex	308
	Hippocampus	117
2w DA 9w	Cortex	750
	Hippocampus	435
10w DA 24hr	Cortex	1402
	Hippocampus	435
11w DA 5day	Cortex	309
	Hippocampus	202

各群で多数の遺伝子が選択条件を満たした

発現変化遺伝子数: Cortex



発現変化遺伝子数: Hippocampus



発現変化遺伝子のt検定による選択

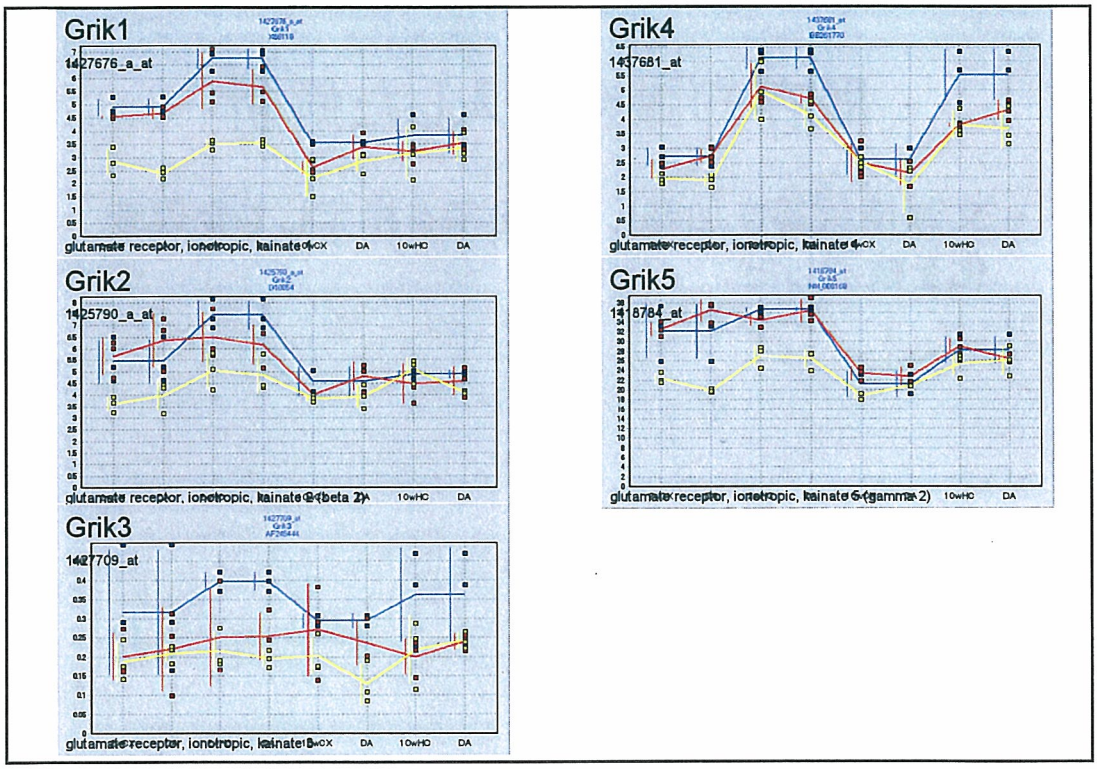
- 異なる投与時期で共通して発現変化した遺伝子はごく少なかった
- 各投与時期に特異的に発現変化した遺伝子をGene ontology解析にかけたが、特定の機能カテゴリーに集中する傾向は無かった

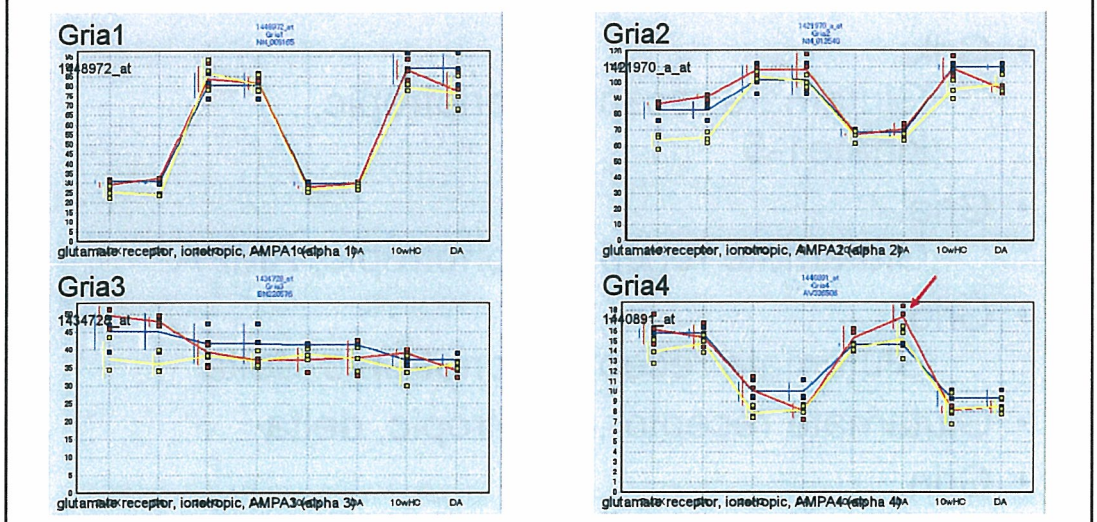
既存知識を導入した解析

- 文献情報等を加味し、遺伝子を指定して発現変化を検討した

グルタミン酸受容体の発現変化

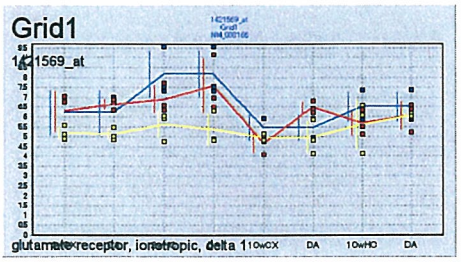
- Grik:
  Glutamate receptor, ionotropic, kinate1-5
- Gria:
  Glutamate receptor, ionotropic, AMPA
- Grid:
  Glutamate receptor, ionotropic, delta
- Glutamate receptor, ionotropic, delta
- Grin:
  Glutamate receptor, ionotropic, NMDA

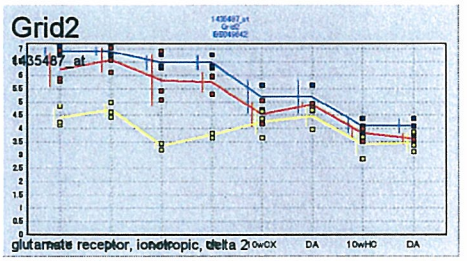


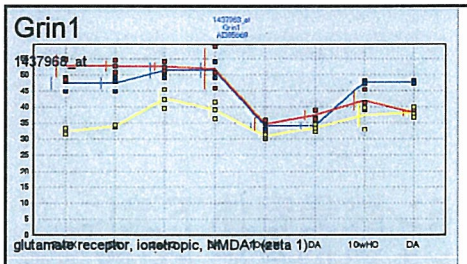


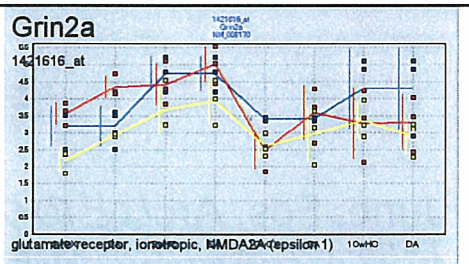
Gria4の発現が10週齢投与後24時間大脳皮質で上昇している

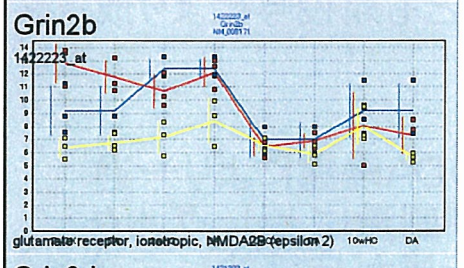
Glutamate receptor, ionotropic, delta

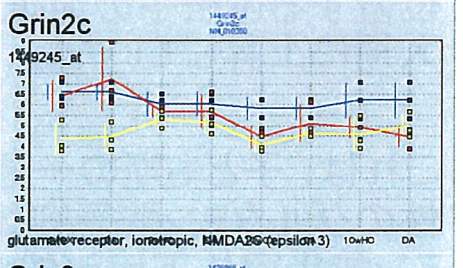


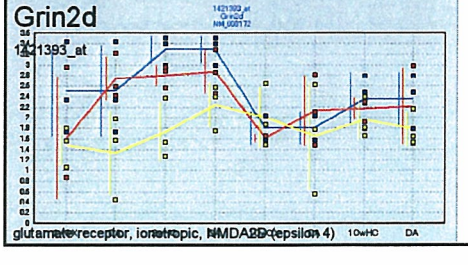


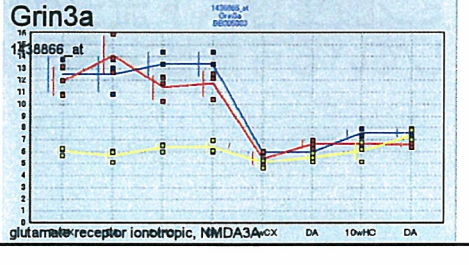




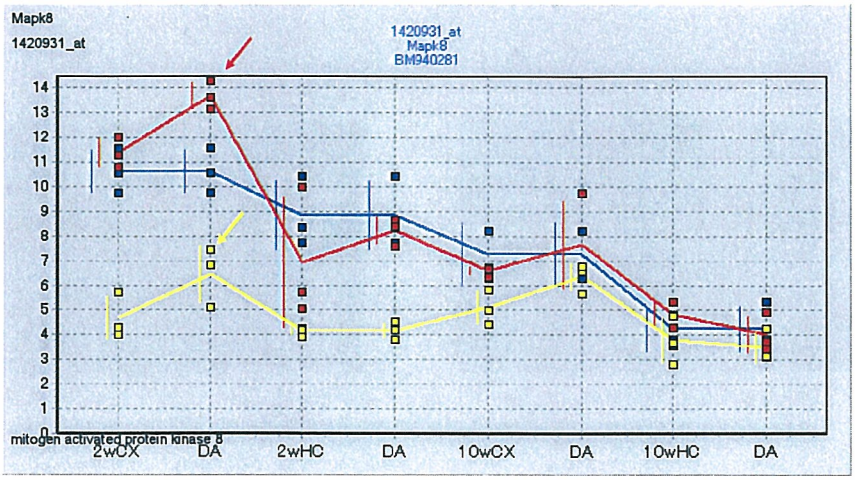








JNK1(MAPK8)



小胞体ストレスによるアポトーシス誘導に関わるJNK1の発現が2週齢投与24時間、11週齢時に発現上昇していた

考察

- Junb, Fos, Arcなどのグルタミン酸受容体下流に位置する遺伝子の発現が子ども期投与により成人期に上昇していた.
- これは、子ども期の一過性のDA曝露により一過性のグルタミン酸受容体過剰刺激により、グルタミン酸受容体下流シグナル伝達が持続的に亢進している可能性を示唆する