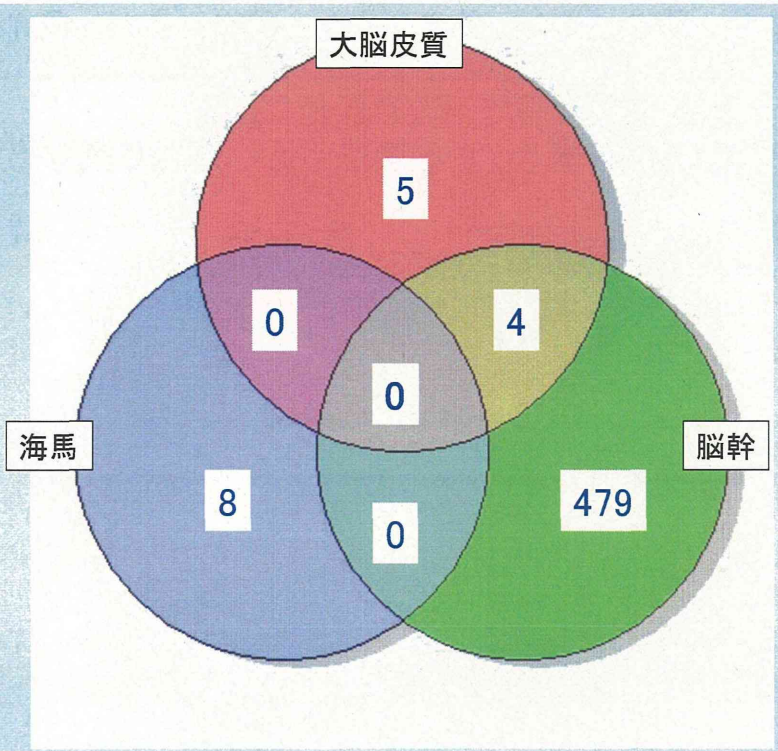
先行研究（平成24年度）

・脳3部位における、野生型及びERα欠失マウスの遺伝子発現の比較
細胞一個あたりの発現コピー数条件：
大脳皮質、海馬及び脳幹につき、それぞれ1.0、0.7及び0.8コピー以上

大脳皮質：	9 ps（増加）	767 ps（減少）
海馬：	8 ps（増加）	13 ps（減少）
脳幹：	483 ps（増加）	642 ps（減少）

先行研究（平成24年度）

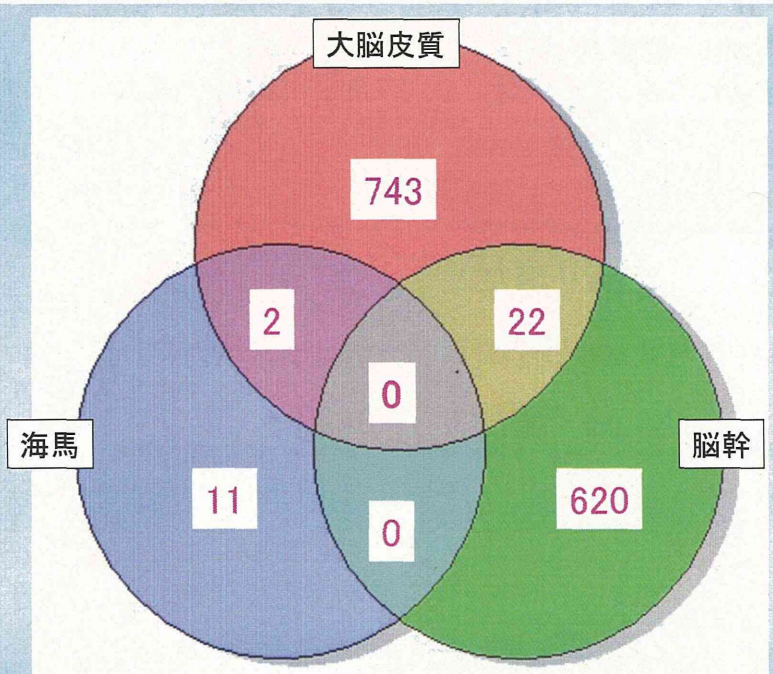
脳3部位の比較
野生型と比較し、ERα欠失マウスの場合に、発現が有意に増加する遺伝子数



先行研究（平成24年度）

脳3部位の比較

野生型と比較し、ERα欠失マウスの場合に、発現が有意に減少する遺伝子数



・3部位に共通して減少した遺伝子は認められなかった

・Esr1遺伝子が抽出されてくるべき
→海馬におけるEsr1遺伝子の発現が、野生型とERα欠失マウス間で、有意差がなかった為
→Esr1遺伝子は、大脳皮質と脳幹で、有意に発現減少

先行研究（平成24年度）

野生型とERα欠失マウス間で、脳の部位により、
有意に発現変動（増加・減少）する遺伝子が、かなり異なる

→ 部位ごとに分けて解析

・大脳皮質
・海馬
・脳幹

先行研究（平成24年度）

まとめ： 成熟期の雄性ERα欠失マウスの脳3部位（大脳皮質、海馬、脳幹）⇄野生型

- **大脳皮質**（＞細胞一個あたり1.0コピー）
  ERα欠失マウス：　776（増加：9 、減少：767 ）psの有意な発現変動
  RARシグナル伝達が低下し記憶障害が誘発する可能性、神経活動の活性化及び概日リズムが乱れる可能性を示唆
  （RAR関連遺伝子、カリウムチャネル、グルタミン酸受容体、セロトニン受容体、概日リズム関連遺伝子、アンドロゲン受容体、メチルCpG結合タンパク(MBD)関連遺伝子）

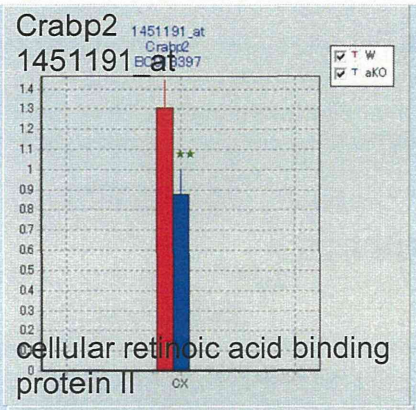
- **海馬**（＞細胞一個あたり0.7コピー）
  ERα欠失マウス: 21（増加：8 、減少：13）psの有意な発現変動
  野生型マウスとERα欠失マウスとの間に違いは、現時点では認められない

- **脳幹**（＞細胞一個あたり0.8コピー）
  ERα欠失マウス：　1,125（増加：483 、減少：642）psの有意な発現変動
  概日リズムが乱れる可能性及び、神経活動が活性化している可能性を示唆
  （概日リズム関連遺伝子、セロトニン受容体、カリウムチャネル、グルタミン酸受容体、GABA受容体、アンドロゲン受容体）

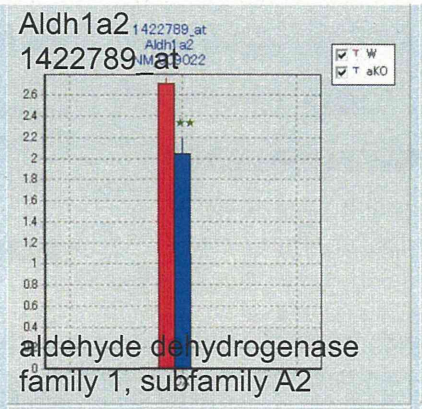
- アンドロゲン受容体の発現：　大脳皮質及び脳幹において低下

先行研究（平成24年度）

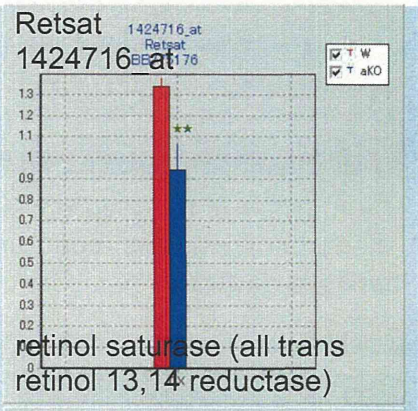
大脳皮質において、ERα欠失マウスで有意に発現減少を示すRARシグナル関連遺伝子



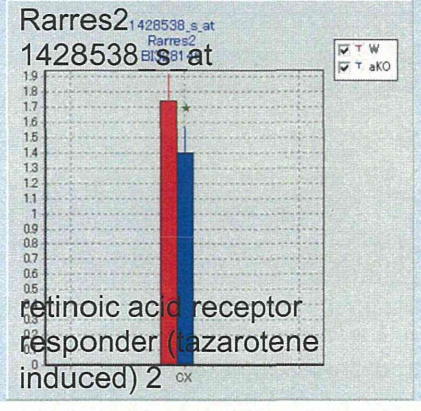
cellular retinoic acid binding protein II



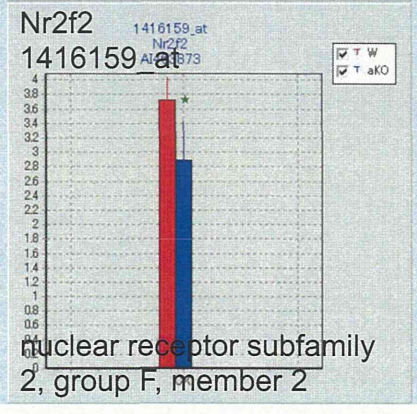
aldehyde dehydrogenase family 1, subfamily A2



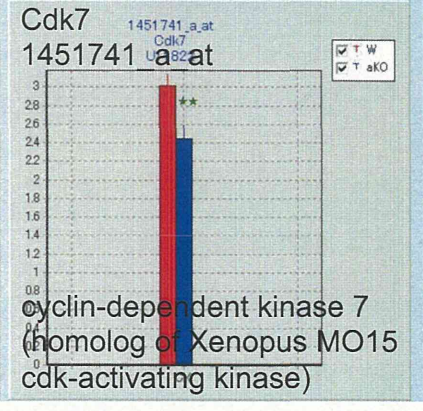
retinol saturase (all trans retinol 13,14 reductase)



retinoic acid receptor responder (tazarotene induced) 2



nuclear receptor subfamily 2, group F, member 2

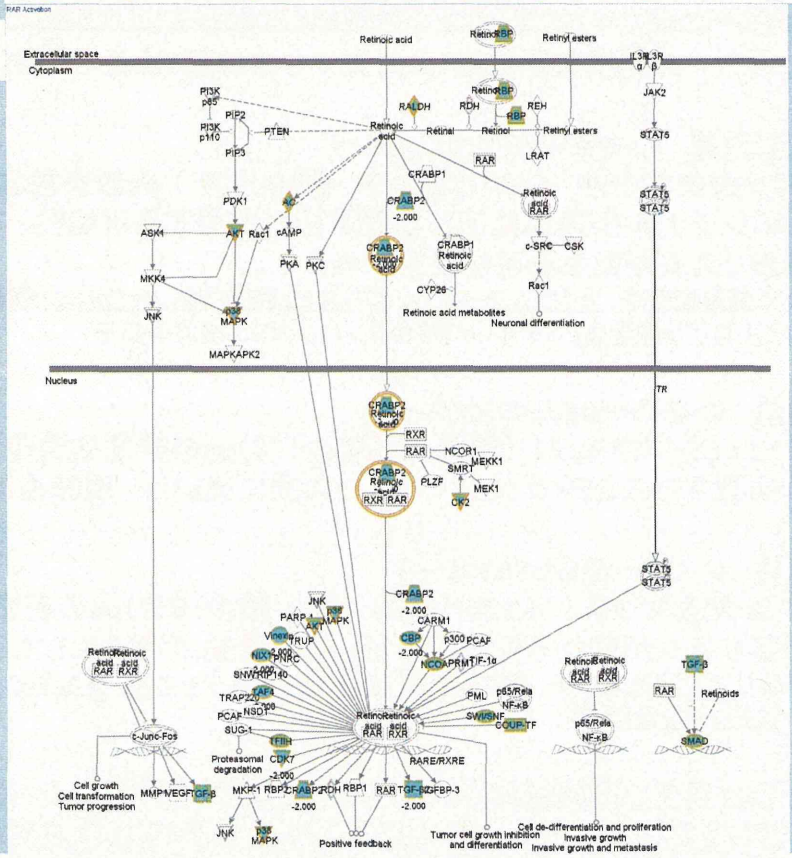


cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)

先行研究（平成24年度）

Ingenuity Pathways Analysis (IPA)　(Ingenuity Systems Inc.)

RAR Activation



先行研究（平成24年度）

dominant negativeなRARαを前脳特異的に発現させると、記憶障害（社会的記憶、空間記憶）が誘発される

Nomoto *et al. Molecular Brain* 2012, **5**:8
http://www.molecularbrain.com/content/5/1/8

Molecular Brain

RESEARCH　Open Access

Dysfunction of the RAR/RXR signaling pathway in the forebrain impairs hippocampal memory and synaptic plasticity

Masanori Nomoto[1,2], Yohei Takeda[1], Shusaku Uchida[1], Koji Mitsuda[1], Hatsune Enomoto[1], Kaori Saito[1], Tesu Choi[1], Ayako M Watabe[3,4], Shizuka Kobayashi[3], Shoichi Masushige[1], Toshiya Manabe[3] and Satoshi Kida[1,2*]

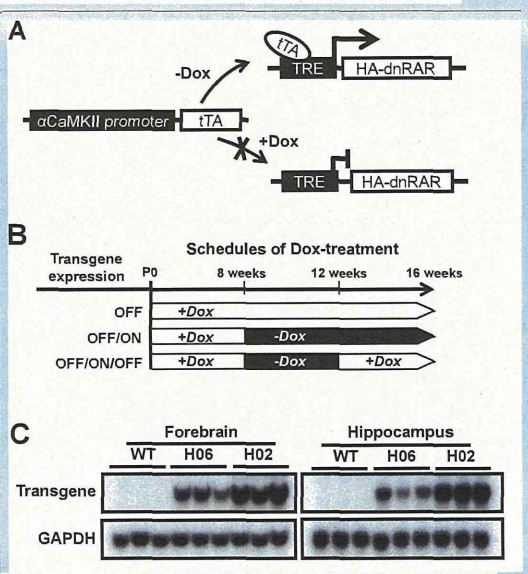
Abstract

Background: Retinoid signaling pathways mediated by retinoic acid receptor (RAR)/retinoid × receptor (RXR)-mediated transcription play critical roles in hippocampal synaptic plasticity. Furthermore, recent studies have shown that treatment with retinoic acid alleviates age-related deficits in hippocampal long-term potentiation (LTP) and memory performance and, furthermore, memory deficits in a transgenic mouse model of Alzheimer's disease. However, the roles of the RAR/RXR signaling pathway in learning and memory at the behavioral level have still not been well characterized in the adult brain. We here show essential roles for RAR/RXR in hippocampus-dependent learning and memory. In the current study, we generated transgenic mice in which the expression of dominant-negative RAR (dnRAR) could be induced in the mature brain using a tetracycline-dependent transcription factor and examined the effects of RAR/RXR loss.

Results: The expression of dnRAR in the forebrain down-regulated the expression of RARβ, a target gene of RAR/RXR, indicating that dnRAR mice exhibit dysfunction of the RAR/RXR signaling pathway. Similar with previous findings, dnRAR mice displayed impaired LTP and AMPA-mediated synaptic transmission in the hippocampus. More importantly, these mutant mice displayed impaired hippocampus-dependent social recognition and spatial memory. However, these deficits of LTP and memory performance were rescued by stronger conditioning stimulation and spaced training, respectively. Finally, we found that pharmacological blockade of RARα in the hippocampus impairs social recognition memory.

Conclusions: From these observations, we concluded that the RAR/RXR signaling pathway greatly contributes to learning and memory, and LTP in the hippocampus in the adult brain.

Nomoto M et al, Mol Brain 5:8-, 2012



・dnRAR: C末端を欠き、RXRとヘテロダイマー形成できるがDNAに結合できない
・αCaMKII プロモーター下、テトラサイクリンプロモーターとdnRARをつなぎTg作製

先行研究（平成24年度）

Morris water maze test

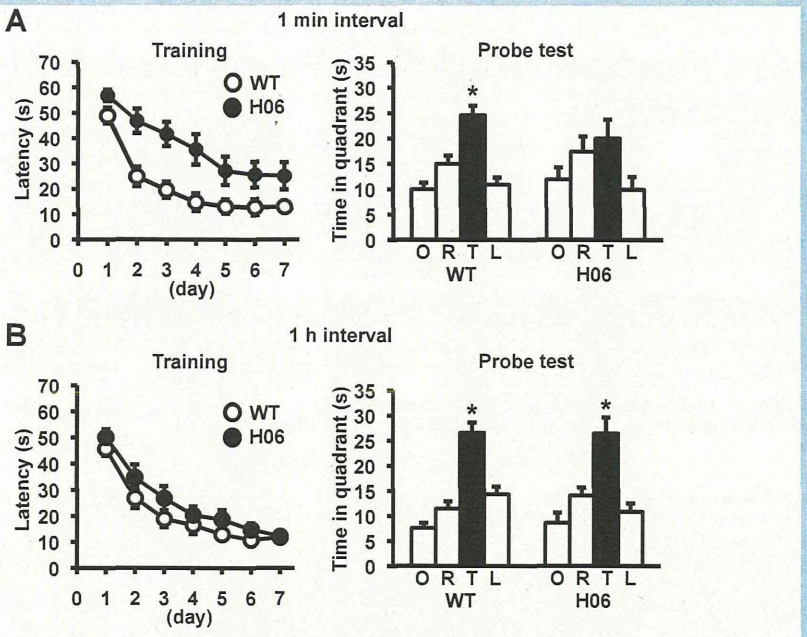


Figure 4 Impaired spatial memory in dnRAR mice and its rescue by spaced training. (A) Escape latencies during training with a 1 min interval (left panel; WT, n = 18; H06, n = 12). Data are indicated in blocks of 2 trials. Probe test at day 8 (right panel). *p < 0.05 compared with the other 3 quadrants. (B) Escape latencies during training with a 1 h interval (left panel; WT, n = 15; H06, n = 12). Data are indicated in blocks of 2 trials. Probe test at day 8 (right panel). *p < 0.05 compared with the other 3 quadrants. Error bars indicate SEM.

先行研究（平成24年度）

RARβ−RXRβ, RARβ−RXRγ, RXRβ−RXRγダブルミュータントマウス：自発運動能低下

Impaired Locomotion and Dopamine Signaling in Retinoid Receptor Mutant Mice

Wojciech Krężel, Norbert Ghyselinck,* Tarek A. Samad,* Valérie Dupé, Philippe Kastner, Emiliana Borrelli, Pierre Chambon†

Science. 279: 863-7, 1998

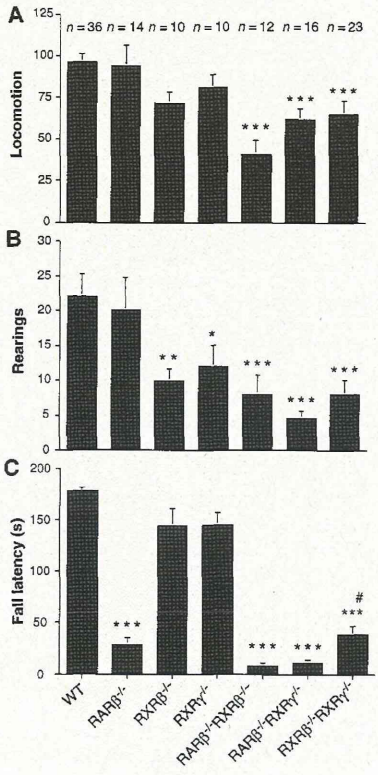


Fig. 1. Locomotor activity of RARβ$^{-/-}$, RXRβ$^{-/-}$, RXRγ$^{-/-}$, RARβ$^{-/-}$RXRβ$^{-/-}$, RARβ$^{-/-}$RXRγ$^{-/-}$, and RXRβ$^{-/-}$RXRγ$^{-/-}$ null mutant animals. In the open field test forward locomotion (**A**) (measured as the number of squares crossed) and the number of rearings (**B**) were scored during a 5-min test period. Rotarod performance (**C**) was determined as the time spent on the rotating rod. To avoid the possible effects of a mixed genetic background, we used large numbers (*n*) of animals in these tests. Data are expressed as means ± SEM, and groups were compared by one-way analysis of variance (ANOVA) with Welch correction [$F_{locomotion}(6,37) = 8.36$; $F_{rearings}(6,39) = 12.92$; $F_{latency}(6,59) = 12.62$]. Post hoc analysis was performed with the Bonferroni multiple *t* test with all possible 21 comparisons (BMDP) (*25*); $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ relative to wild-type (WT) littermates; $^{\#}P < 0.1$, relative to the RARβ$^{-/-}$RXRγ$^{-/-}$ group.

先行研究（平成24年度）

細胞性レチノール結合タンパク質タイプ II（CRBP2）の発現制御は？

Upstream Regulator (IPA検索)→ Retinoic acid–RAR–RXR

Upstream R	Other Molecule T	Predicted A	Activation z	Notes	p-value of c	Target molecules in dataset
HNF4A	transcriptio		0.055	bias	3.76E-08	AAR2,ABCB9,AGPAT1,AGXT2L1,AMACR,ANAPC15,ANKRA2,ARG2,ARHGEF19,ASB7,AVPI1,BOLA1,C2,C2orf29,C2orf43,CAP1,CD3EAP,CDH
ABCB6	transporter		1.633	bias	6.90E-05	CAT,HAGH,SLC25A37,SLC25A39,SLC25A51,TXNRD2
HTT	transcriptio				8.27E-04	AKT2,AMACR,ARNTL,ATP2B3,BCAP31,CASP3,CBX1,CIT,CLOCK,COX6B2,CREBBP,DNAJC3,DUSP6,FKBP4,GLO1,HSPA9,ITGB1,MGP,MMP
Retinoic ac	complex				2.80E-02	CRABP2,TGFB2

CRBP2の発現は、直接エストロゲンにより制御される

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Cellular Retinoic Acid-binding Protein II Gene Expression Is Directly Induced by Estrogen, but Not Retinoic Acid, in Rat Uterus*

Received for publication, March 12, 2003, and in revised form, June 2, 2003
Published, JBC Papers in Press, July 3, 2003, DOI 10.1074/jbc.M302551200

Xiao-Hong Li and David E. Ong‡
From the Department of Biochemistry, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232

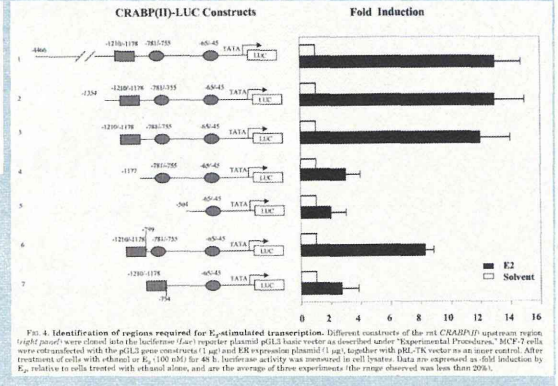
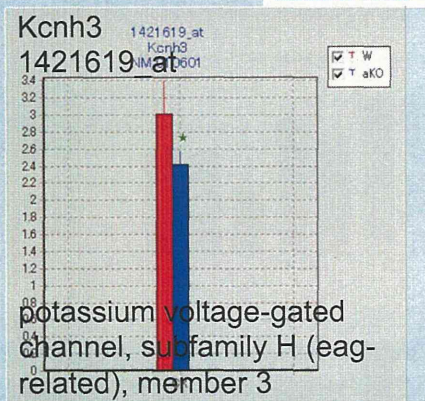


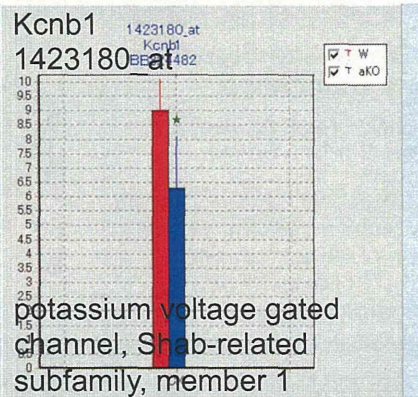
FIG. 4. **Identification of regions required for E_2-stimulated transcription.** Different constructs of the rat *CRABP(II)* upstream region (*right panel*) were cloned into the luciferase (*Luc*) reporter plasmid pGL3 basic vector as described under "Experimental Procedures." MCF-7 cells were cotransfected with the pGL3 gene constructs (1 μg) and ER expression plasmid (1 μg), together with pRL-TK vector as an inner control. After treatment of cells with ethanol or E_2 (100 nM) for 48 h, luciferase activity was measured in cell lysates. Data are expressed as -fold induction by E_2 relative to cells treated with ethanol alone, and are the average of three experiments (the range observed was less than 20%).

先行研究（平成24年度）

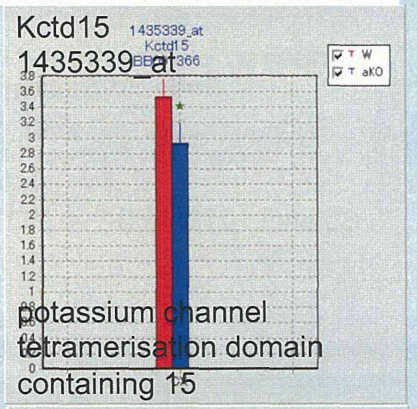
大脳皮質において、発現が有意に減少するカリウムチャネル遺伝子



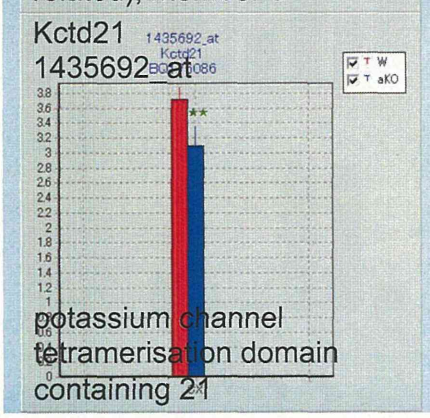
potassium voltage-gated channel, subfamily H (eag-related), member 3



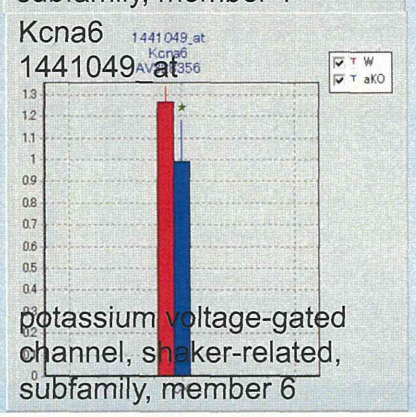
potassium voltage gated channel, Shab-related subfamily, member 1



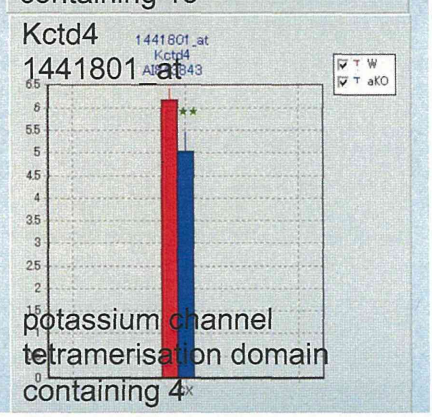
potassium channel tetramerisation domain containing 15



potassium channel tetramerisation domain containing 21



potassium voltage-gated channel, shaker-related, subfamily, member 6



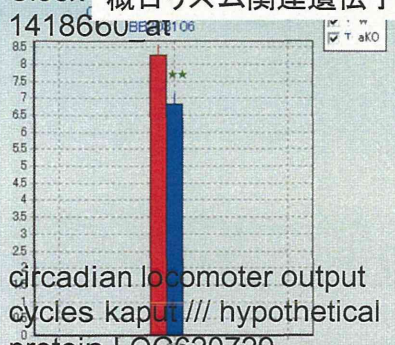
potassium channel tetramerisation domain containing 4

先行研究（平成24年度）

大脳皮質において、発現が有意に減少する概日リズム関連遺伝子

概日リズム関連遺伝子

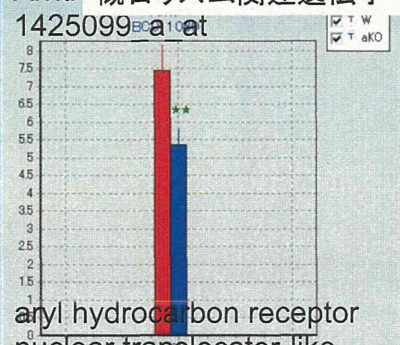
Clock
1418600_at



circadian locomoter output cycles kaput /// hypothetical protein LOC620729

概日リズム関連遺伝子

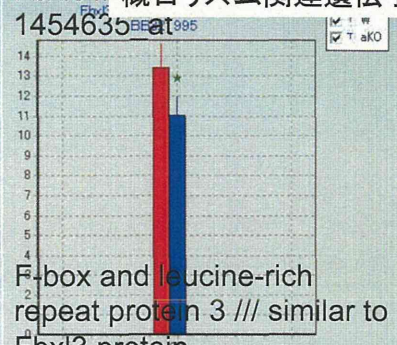
Arntl
1425099_a_at



aryl hydrocarbon receptor nuclear translocator-like

概日リズム関連遺伝子

Fbxl3 /
1454635_at

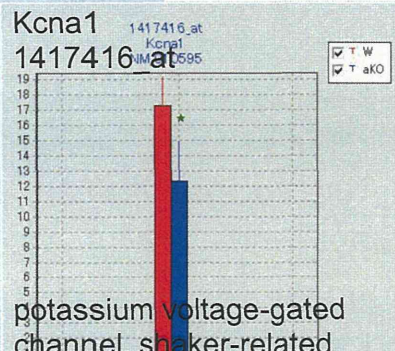


F-box and leucine-rich repeat protein 3 /// similar to Fbxl3 protein

先行研究（平成24年度）

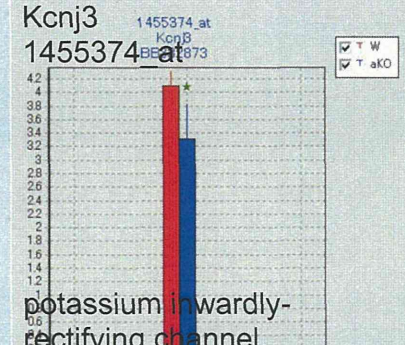
脳幹において、発現が有意に減少するカリウムチャネル遺伝子

Kcna1
1417416_at



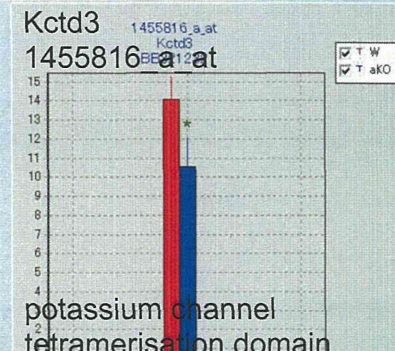
potassium voltage-gated channel, shaker-related subfamily, member 1

Kcnj3
1455374_at



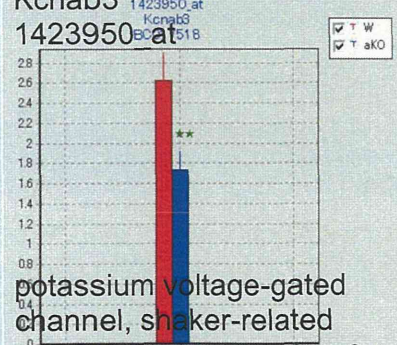
potassium inwardly-rectifying channel, subfamily J, member 3

Kctd3
1455816_a_at



potassium channel tetramerisation domain containing 3

Kcnab3
1423950_at

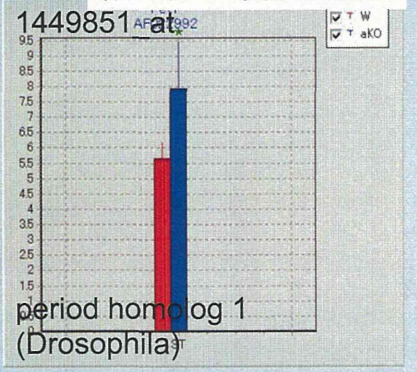


potassium voltage-gated channel, shaker-related subfamily, beta member 3

先行研究（平成24年度）

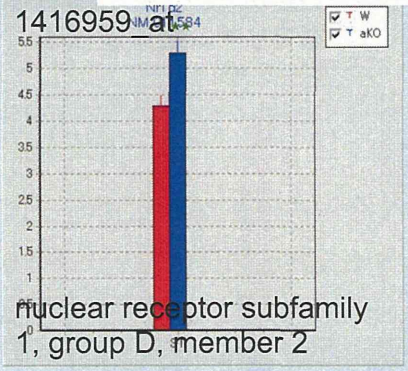
脳幹において、発現が有意に増加する概日リズム関連遺伝子

Per1 概日リズム関連遺伝子
1449851_at



period homolog 1 (Drosophila)

Nr1d2 概日リズム関連遺伝子
1416959_at



nuclear receptor subfamily 1, group D, member 2

先行研究（平成24年度）

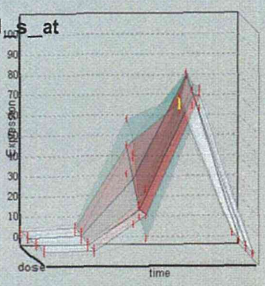
Circadian Rhythm 関連遺伝子の、野生型とERα欠失マウスとの発現変動比較 成熟期・肝

TTG183L BisA Wild	TTG184L BisA ERαKO
TTG185L EE Wild	TTG186L EE ERα KO

先行研究（平成24年度）

TTG183-L_SpNC_0 BisA Wild

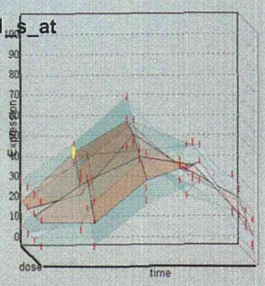
Dbp
1438211_s_at



D site albumin promoter binding protein

TTG184-L_SpN BisA ERαKO

Dbp
1438211_s_at



D site albumin promoter binding protein

TTG185-L(B)_SpNC_0 EE Wild

Dbp
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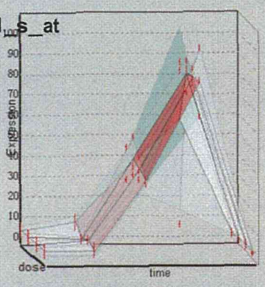

D site albumin promoter binding protein

TTG186-L_SpNC EE ERα KO

Dbp
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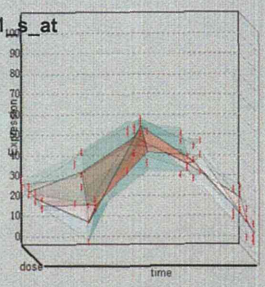
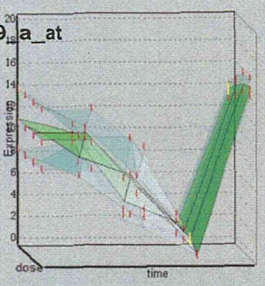


D site albumin promoter binding protein

先行研究（平成24年度）

TTG183-L_SpNC_0 BisA Wild

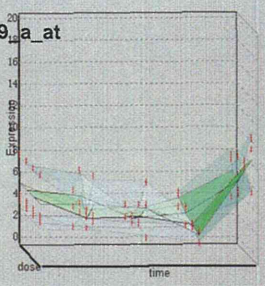
Arntl
1425099_a_at



aryl hydrocarbon receptor nuclear translocator-like

TTG184-L_SpN BisA ERαKO

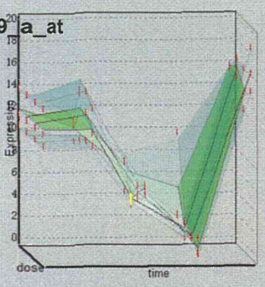
Arntl
1425099_a_at



aryl hydrocarbon receptor nuclear translocator-like

TTG185-L(B)_SpNC_0 EE Wild

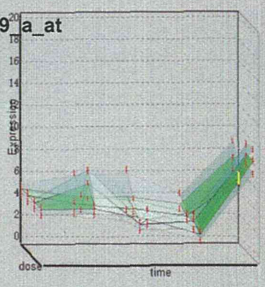
Arntl
1425099_a_at



aryl hydrocarbon receptor nuclear translocator-like

TTG186-L_SpNC EE ERα KO

Arntl
1425099_a_at

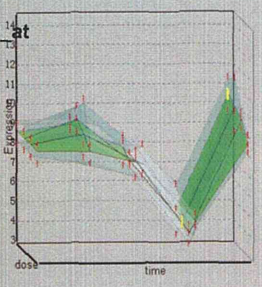


aryl hydrocarbon receptor nuclear translocator-like

先行研究（平成24年度）

TTG183-L_SpNC_0 BisA Wild

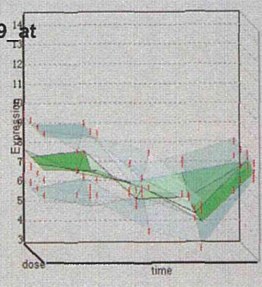
Clock
1418659_at



circadian locomotor output cycles kaput

TTG184-L_SpN BisA ERαKO

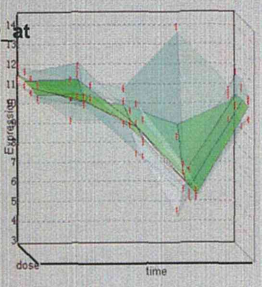
Clock
1418659_at



circadian locomotor output cycles kaput

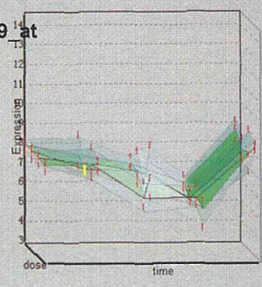
TTG185-L(B)_SpNC_0 EE Wild

Clock
1418659_at



TTG186-L_SpNC EE ERα KO

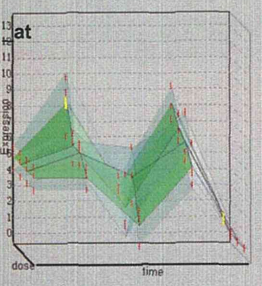
Clock
1418659_at



先行研究（平成24年度）

TTG183-L_SpNC_0 BisA Wild

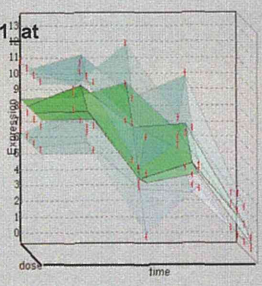
Per1
1449851_at



period homolog 1 (Drosophila)

TTG184-L_SpN BisA ERαKO

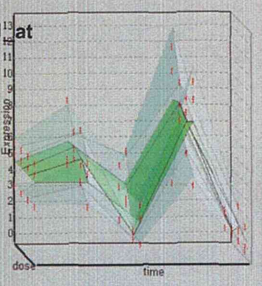
Per1
1449851_at



period homolog 1 (Drosophila)

TTG185-L(B)_SpNC_0 EE Wild

Per1
1449851_at



TTG186-L_SpNC EE ERα KO

Per1
1449851_at