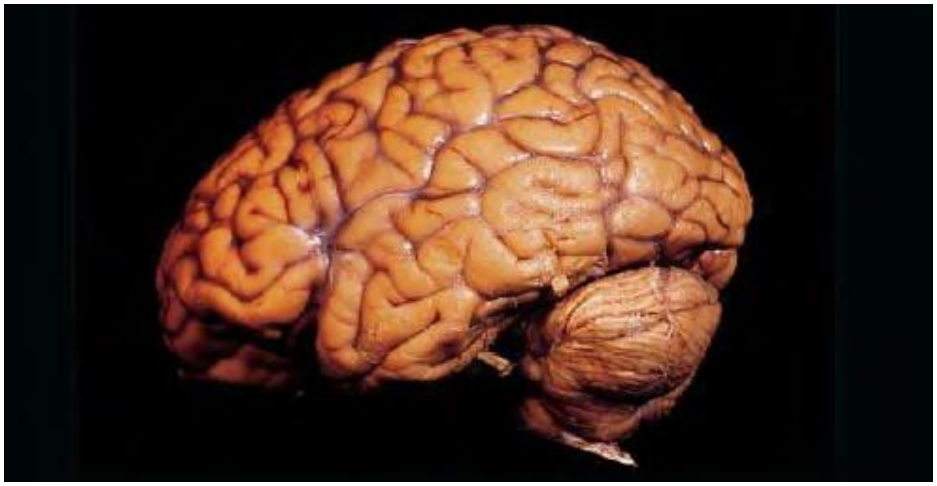
«Здоровый и больной мозг: от молекулярной физиологии к патологии, клинике и лечению»

Наиль Бурнашев



IV Патологические процессы в мозге и их коррекция

1. Основные патологические процессы в мозге.

2. Генетические заболевания.

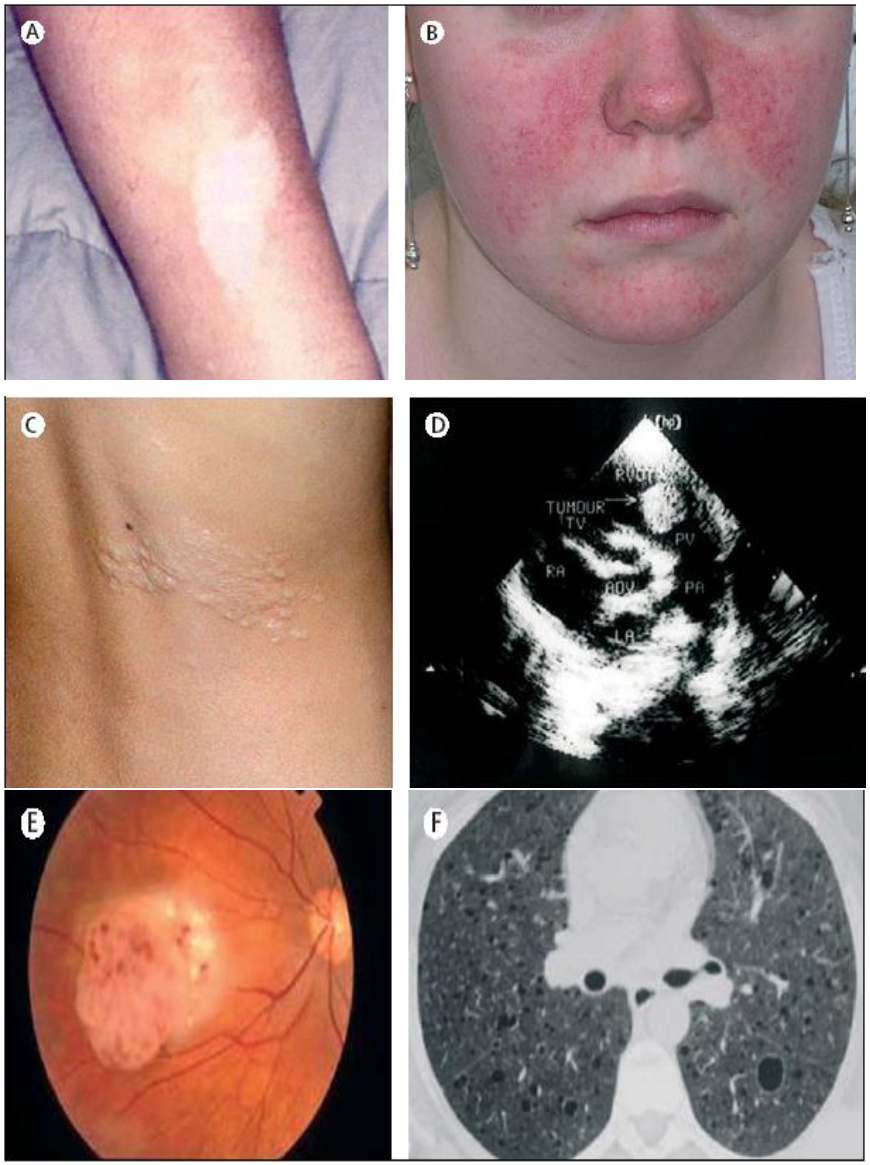
3. Клеточные и молекулярные механизмы развития эпилепсии

4. Синаптическая дисфункция при распространенных заболеваниях ЦНС. Болезнь Альцгеймера

5. Принципы разработки современных методов лечения заболеваний ЦНС

Туберозный склероз

Туберозный склероз (TSC)



Tuberous sclerosis **(TSC) The disorder--once known as *epiloia* or *Bourneville's disease*--was first identified by a French physician more than 100 years ago**

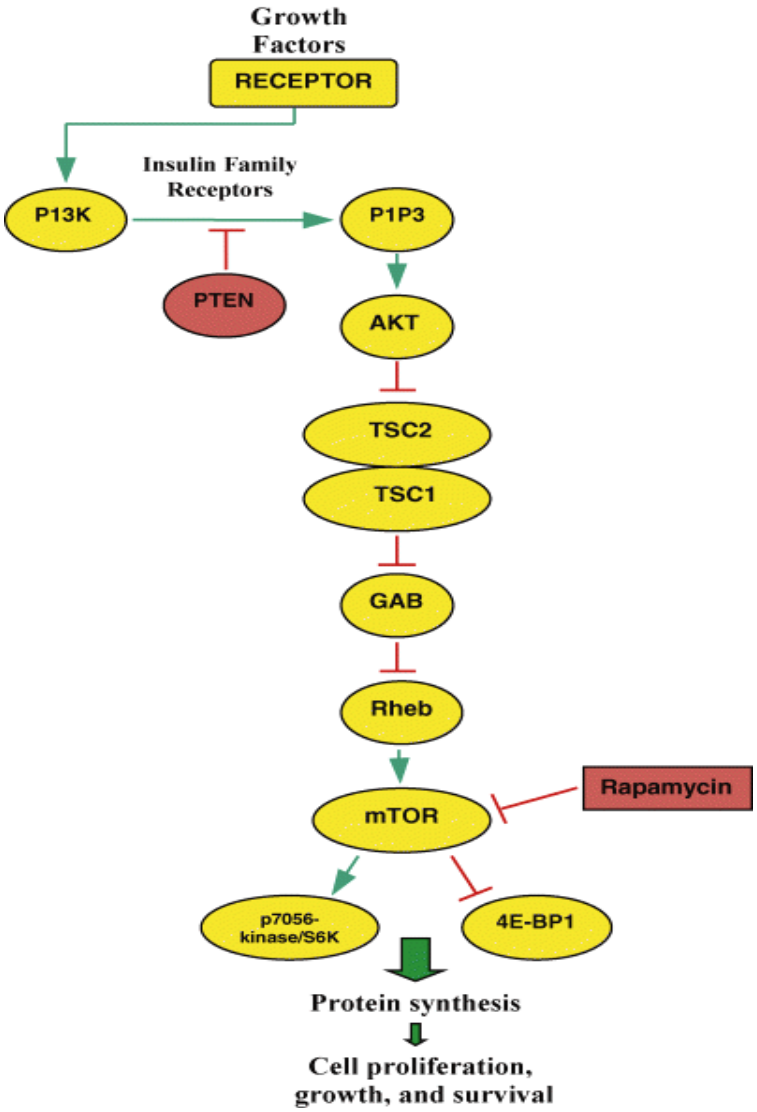
Tuberous sclerosis (TSC) is an multi-system autosomal dominant disease characterized by

- **mental retardation,**
- **epilepsy,**
- **developmental delay**
- **behavioural problems**
- **tumors of the skin, retina, heart, kidney, and brain.**

The disorder affects about 1 to 2 million individuals worldwide, with an estimated prevalence of one in 6,000 newborns.

TSC may be present at birth, but signs of the disorder can be subtle and full symptoms may take some time to develop. As a result, TSC can be unrecognized or misdiagnosed for years.

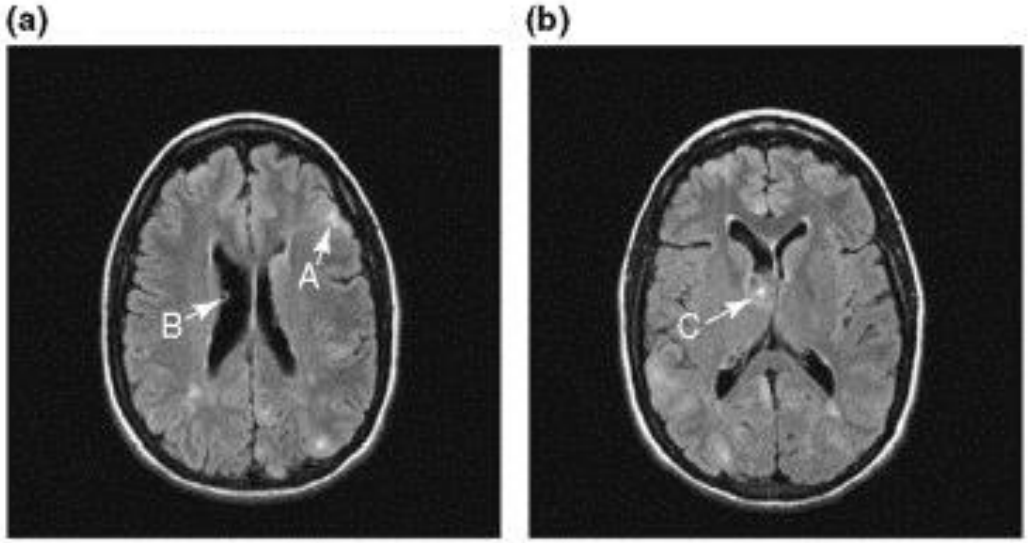
TSC вызывается инактивацией любого из двух генов-супрессоров опухолей: TSC1 или TSC2

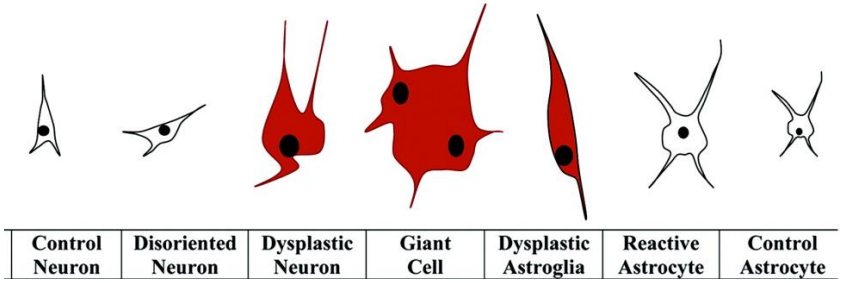


	TSC1	*TSC2*
Chromosomal location	9q34	16p13.3
Size	55 kb	40 kb
Number of exons	23	41
Transcript size	8·6 kb	5·5 kb
Mutation occurrence	10–15% of sporadic cases	75–80% of sporadic cases
Prevailing mutations	Small truncations (mostly nonsense mutations and small deletions), lack of hotspots	Large deletions and/or rearrangements involving *PKD1*, small truncations (mostly missense mutations or deletions), lack of hotspots
Phenotype	Less severe	More severe
LOH in affected organs	Rare	Frequent
Protein	Hamartin	Tuberin
Protein size	1164 aminoacids, 130 kDa	1807 aminoacids, 180 kDa
Protein function	Together with tuberin, hamartin regulates mTOR–S6K, and cell adhesion through interaction with ezrin and Rho	Together with hamartin, tuberin regulates mTOR–S6K and GTPase-activating proteins. Tuberin has a role in cell cycle

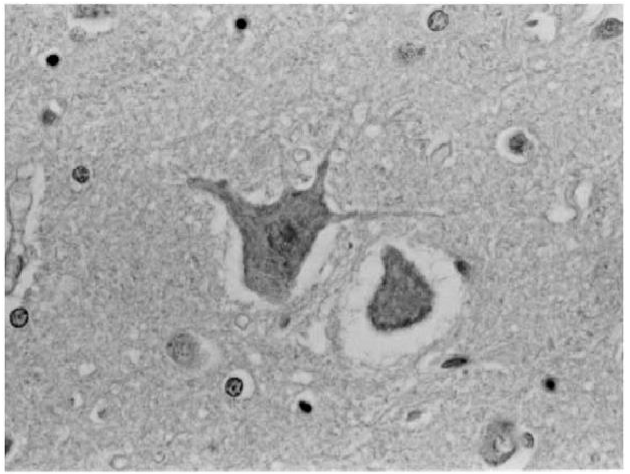
Три типа опухолей головного мозга связаны с TSC

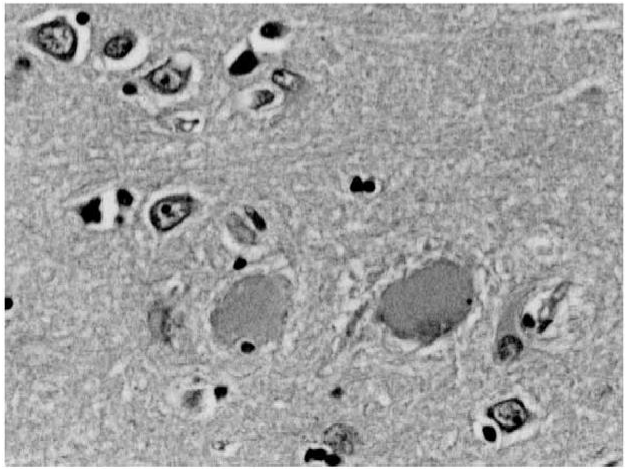
- cortical tubers,
- subependymal nodules, which form in the walls of the ventricles;
- giant-cell astrocytomas, a type of tumor that can block the flow of fluids within the brain.



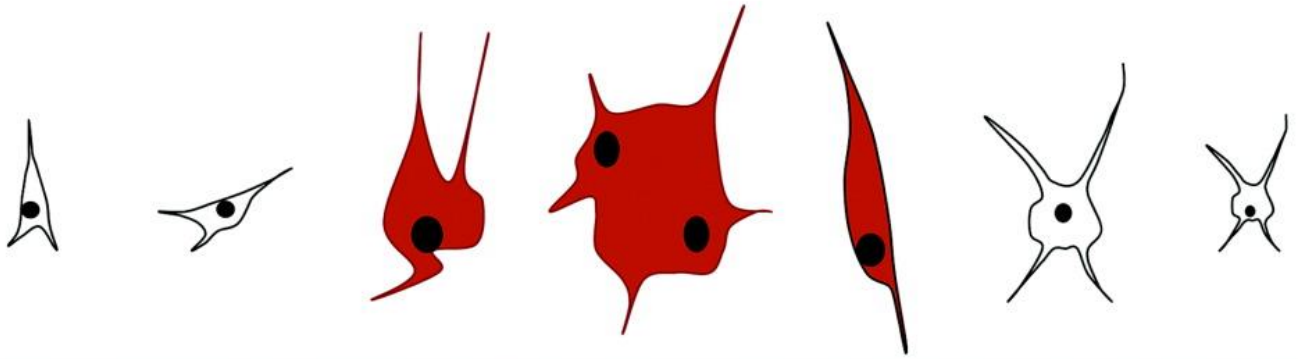


Abnormal giant cells in cortical tubers.





Диспластические клетки демонстрируют измененную экспрессию глутаматных и ГАМК-рецепторов, напоминающую экспрессию, в нормальных незрелых нейронах и глие



Protein Profile	Control Neuron	Disoriented Neuron	Dysplastic Neuron	Giant Cell	Dysplastic Astroglia	Reactive Astrocyte	Control Astrocyte
pS6	-	-	+	+	+	-	-
SMI 311	+	+	+	+	-	-	-
NeuN	+	+	+	-	-	-	-
Synapsin	+	+	+	-	-	-	-
c-Fos	-	+	+	-	-	-	-
GABA	-	-	-	-	-	-	-
Vimentin	-	-	-	+	+	+	-
GFAP	-	-	-	-	-	+	+
AMPARs	GluR2/3	↑ GluR1	↑GluR1 ↓GluR2	↑ GluR4 ↓ GluR2	↑GluR4 ↓GluR2	↑ GluR4	GluR2/3/4
NMDARs	NR1/2A	↑ NR2B	↑NR2B/3A ↓NR2A	↑NR2D/3A ↓NR2A	↑NR2A/2C	↑NR2A/2C	NR1/2C/3A

$GABA_AR$ α1, α2 are down regulated, α3- α6 are unaltered

Ап регуляция Ca2+ проницаемых АМПА рецепторов вTSC

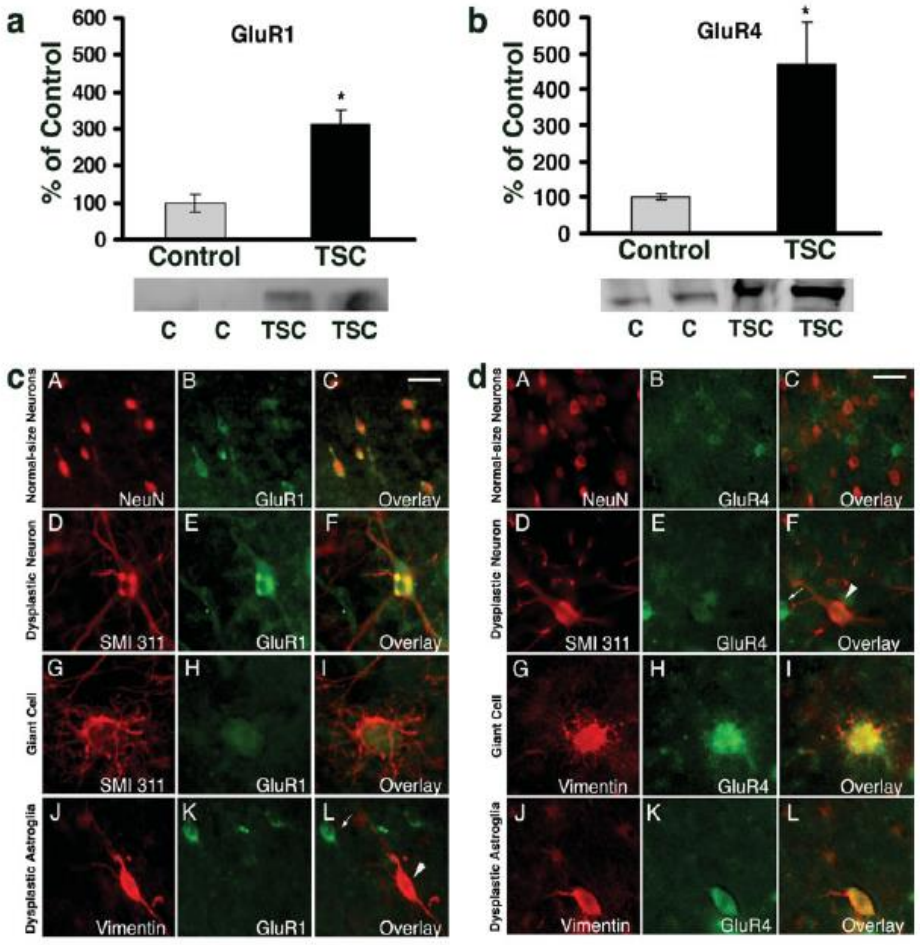


*Fig 2. Increased expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) subunits glutamate receptor 1 (GluR1) and GluR4 in human cortical tubers. (a, b) Western blot quantification of GluR1 (a) and GluR4 (b) expression demonstrates a significant increase in the tubers in comparison with control cortex (312% of control for GluR1; 468% for GluR4). * = p ≤ 0.05. Insets are representative Western blots for individual subunits. (c) Both normal-sized* (A–C) and *dysplastic neurons* (D–F, J–L, small arrow) *express high GluR1 levels, whereas* giant cells *(G–I) and* dysplastic astroglia *(J–L,* arrowhead*) demonstrate relatively low GluR1 expression. (d) In contrast with GluR1, GluR4 expression is low in* normal-sized *(A–C) and* dysplastic neurons *(D–F,* arrowhead*), but highly expressed in* giant cells *(G–I) and* dysplastic astroglia *(D–F,* small arrow; *J–L). Scale bars = 40μm. NeuN = neuron-specific nuclear protein; TSC = tuberous sclerosis complex.*

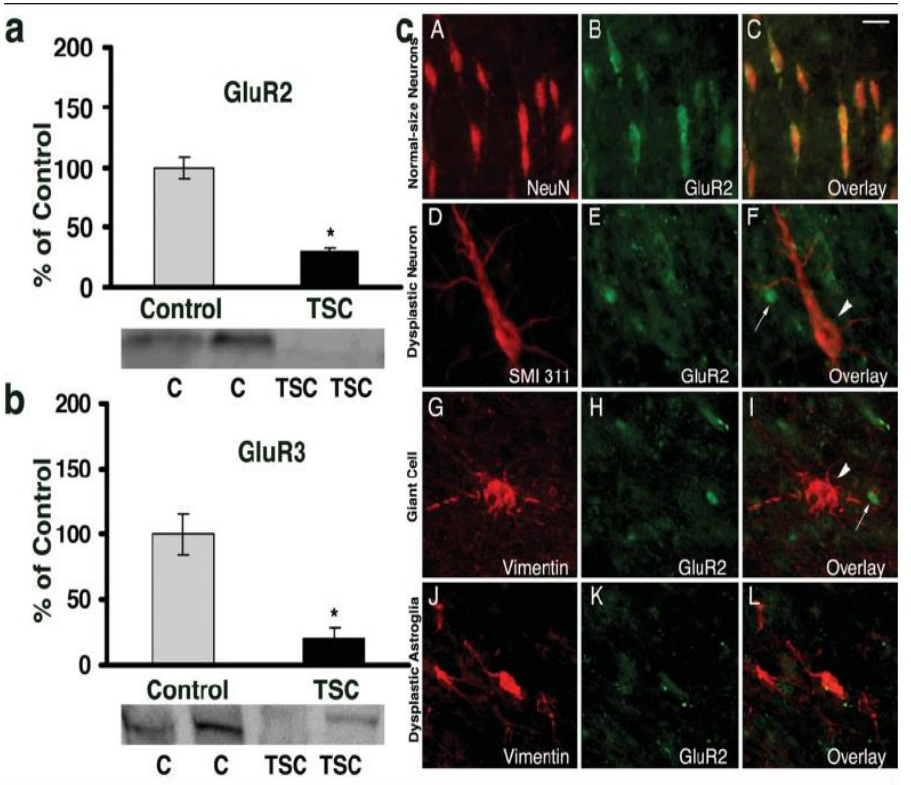
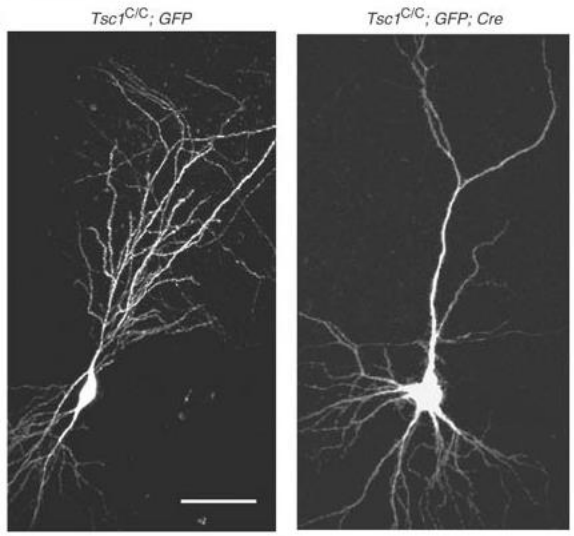
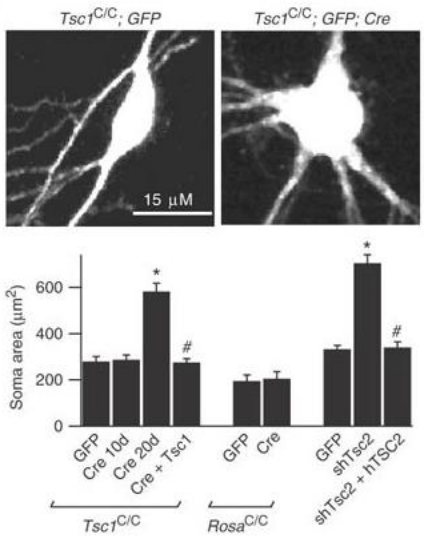


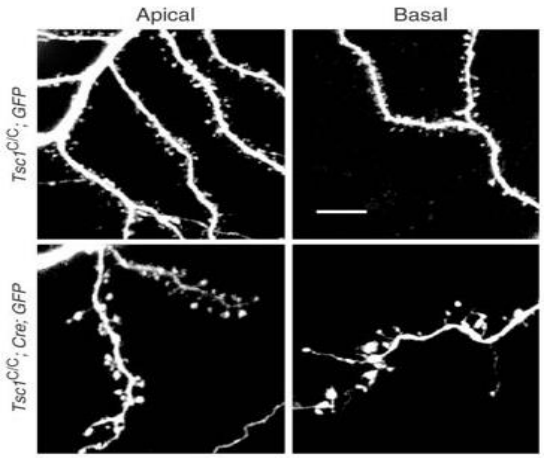
*Fig 3. Decreased expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) subunits glutamate receptor 2 (GluR2) and GluR3 in the tuberal tissue. (a, b) Western blot analysis of GluR2 (a) and GluR3 (b) subunits demonstrate that, in cortical tubers, there is significant decrease in expression of both subunits, relative to control (30% of control for GluR2; 20% of control for GluR3). * = p ≤ 0.05. Insets show representative Western blots for each subunit. (c)* Normal-sized neurons *(A–C) express high GluR2 levels, whereas* dysplastic neurons *(D–F,* arrowhead*),* giant cells *(G–I,* arrowhead*), and* dysplastic astroglia *(J–L) demonstrate lower GluR2 expression, compared with surrounding cells (*small arrows *in F, I). Scale bar = 20μm. NeuN = neuron-specific nuclear protein; TSC = tuberous sclerosis complex.*

Talos et al., Ann Neurol 2008;63:454–465,

Регуляция морфологии и функции нейронов в TSC

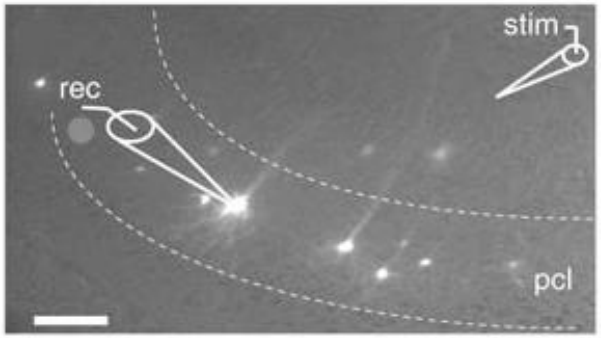


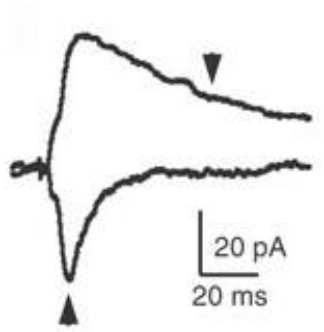


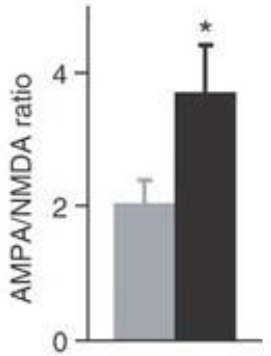


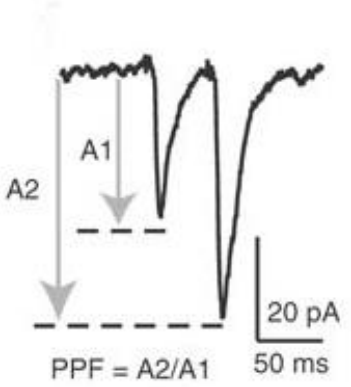
In post-mitotic, hippocampal pyramidal neurons of mice and rats, loss of Tsc1 or Tsc2 triggered enlargement of somas and dendritic spines and altered the properties of glutamatergic synapses

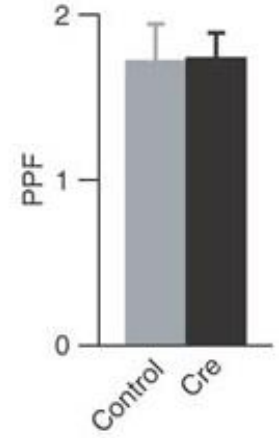
Application of rapamycin to neurons with reduced Tsc1 or Tsc2 levels restores neuronal soma and dendritic spine head sizes to control levels, consistent with mTOR being downstream of Tsc1/Tsc2.





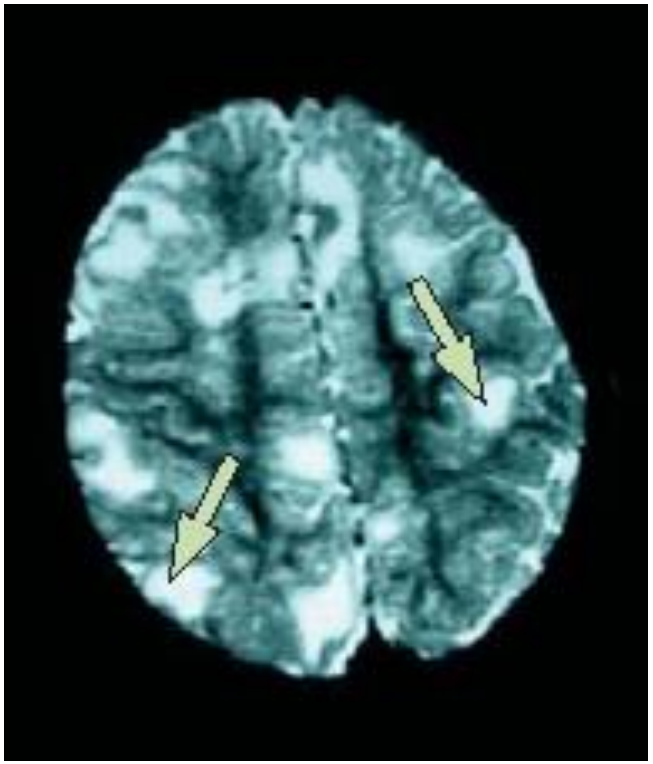


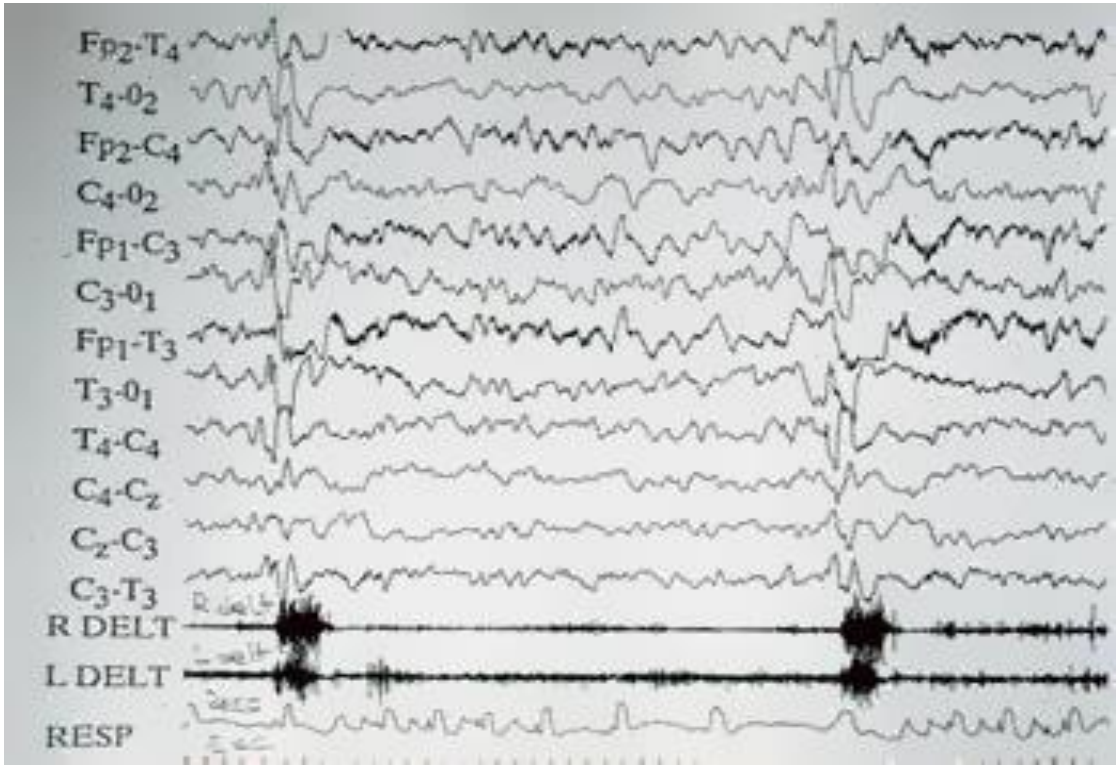




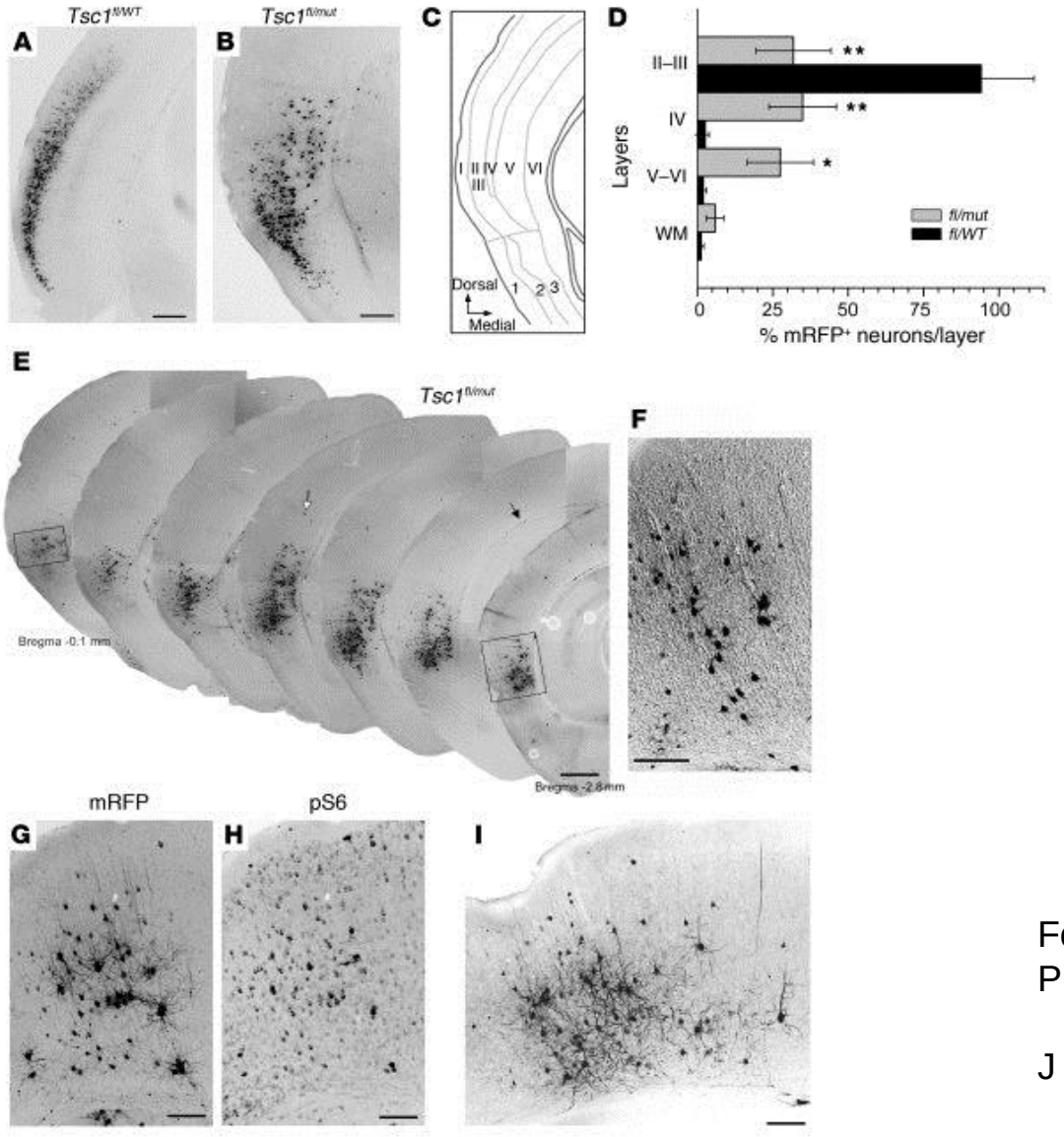
Nature Neuroscience **8**, 1727 - 1734 (2005), ***Tavazoie et al.,***

Клинически туберы часто упоминается как эпилептогенные очаги





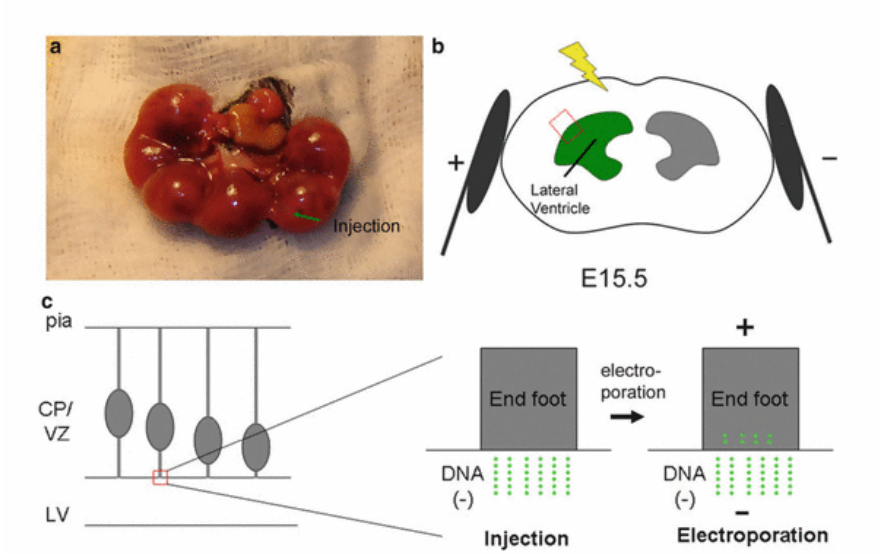
Нокаут *Tsc1* гена в отдельных клетках в кортикогенезе генерирует туберо-подобные повреждения

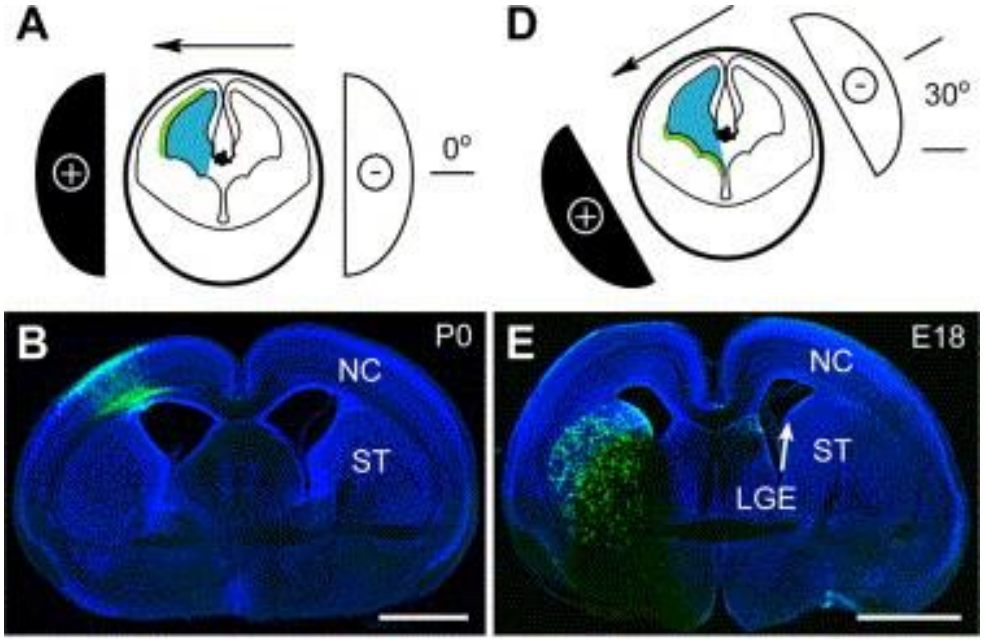


Feliciano DM, Su T, Lopez J, Platel JC, Bordey A.

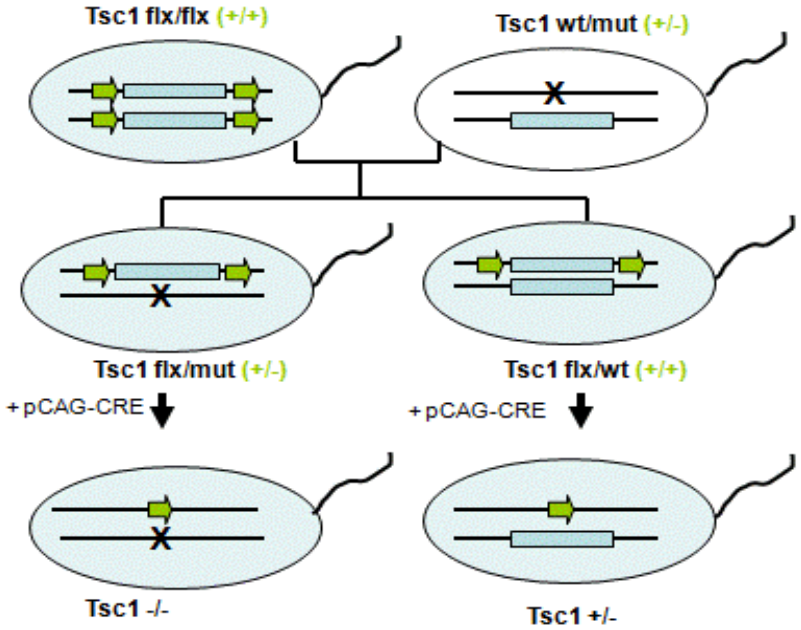
J Clin Invest. 2011

In utero электропорация



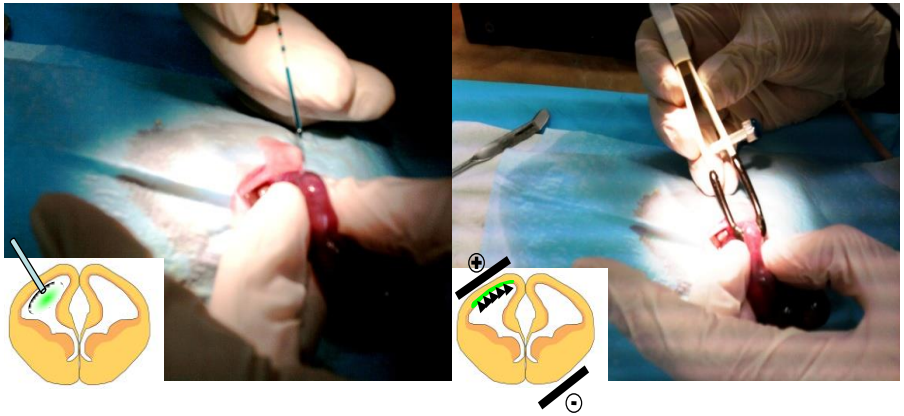


In utero электропорация

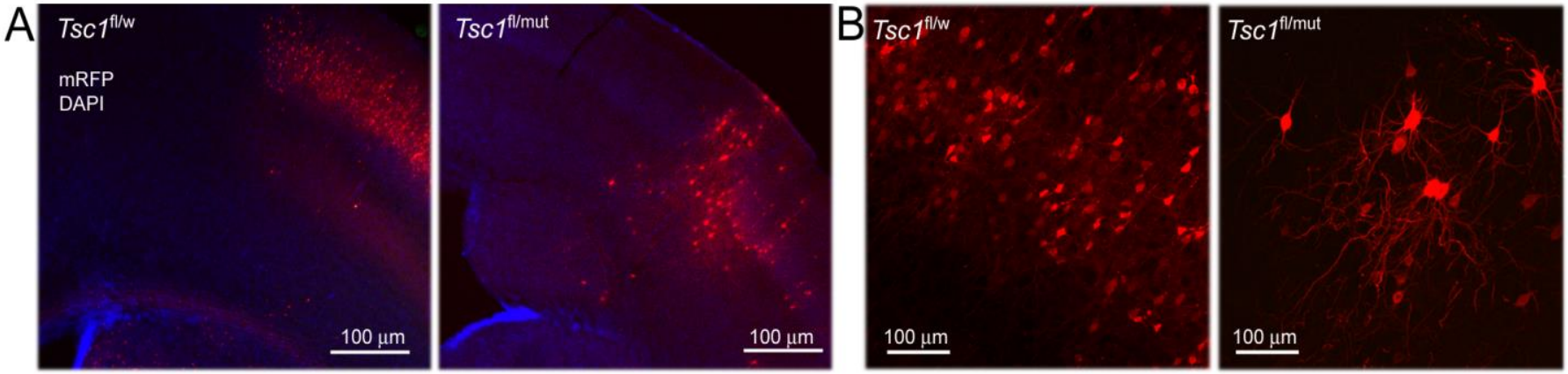


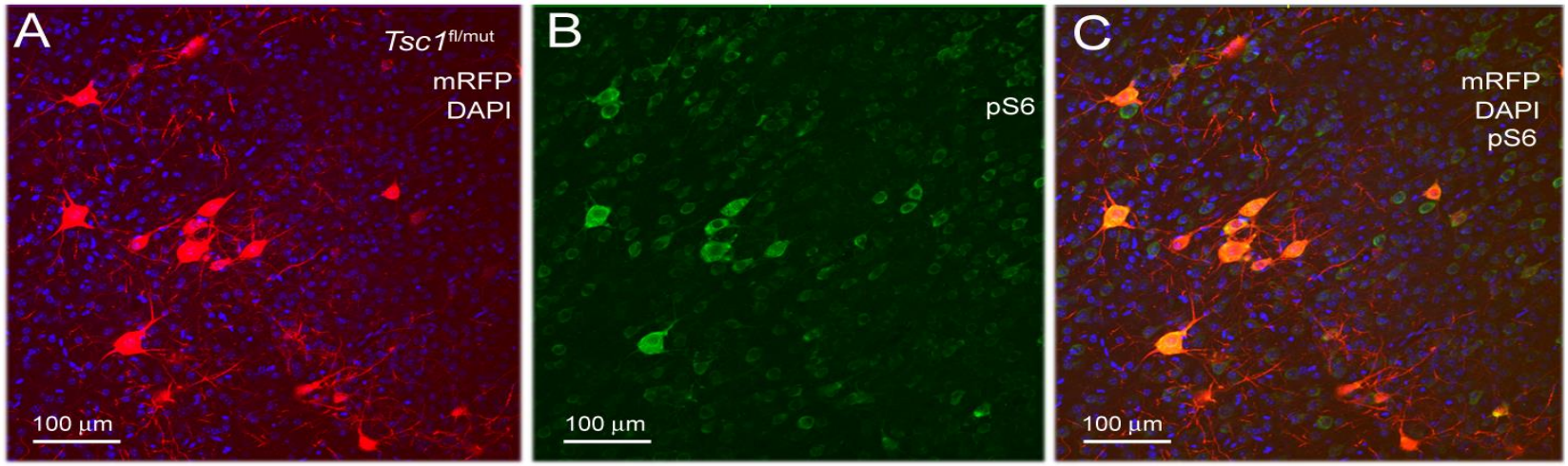
In utero Electroporation at E16

pCAG-Cre:GFP and pCAG-mRFP



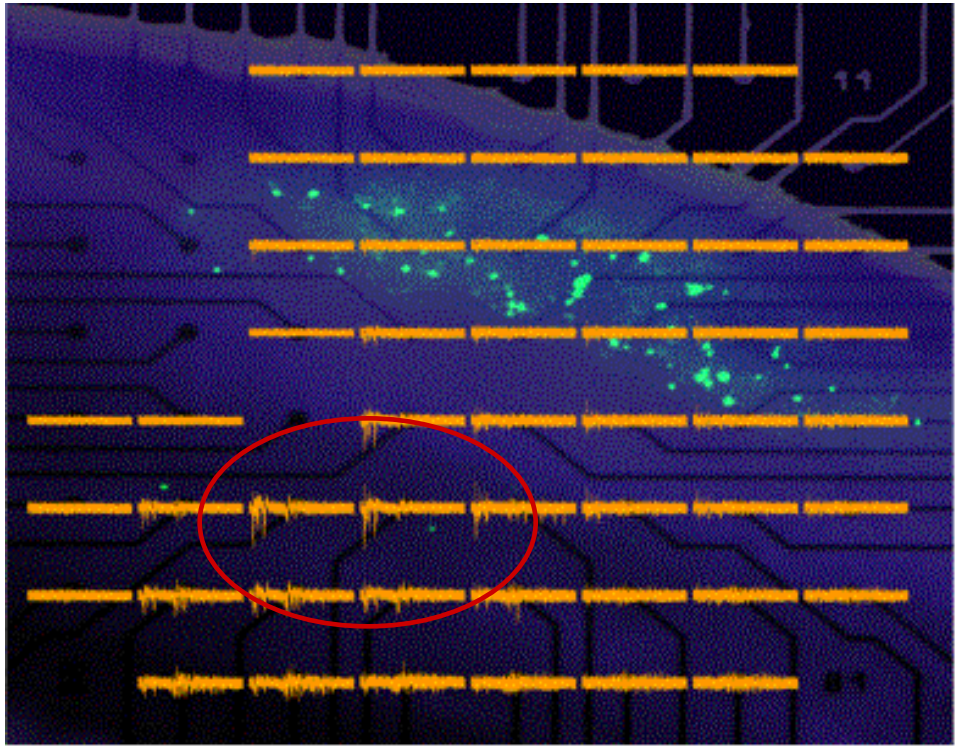
Валидация животной модели TSC1



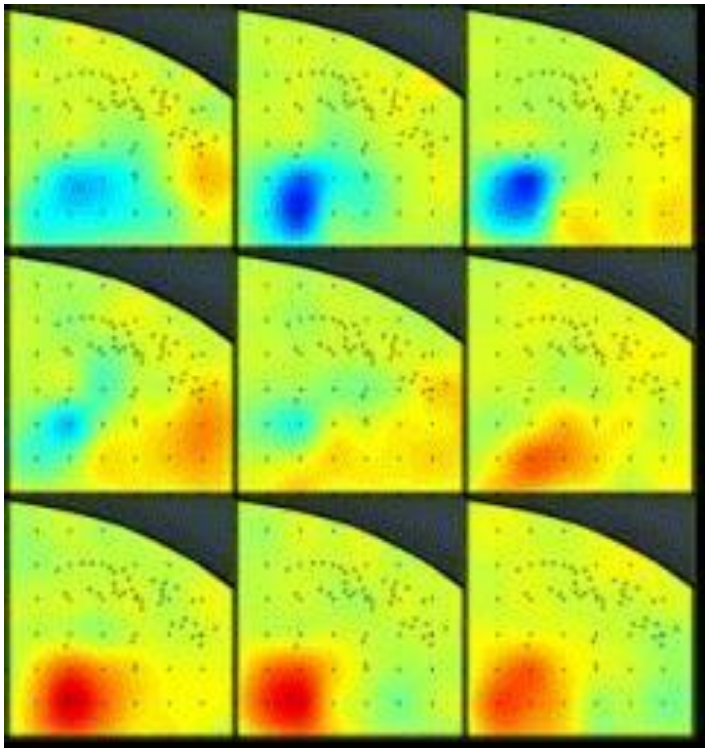


Эпилептиформная активность в неокортикальных срезах *flx/mut creTsc1-/-* электропорированных мышей

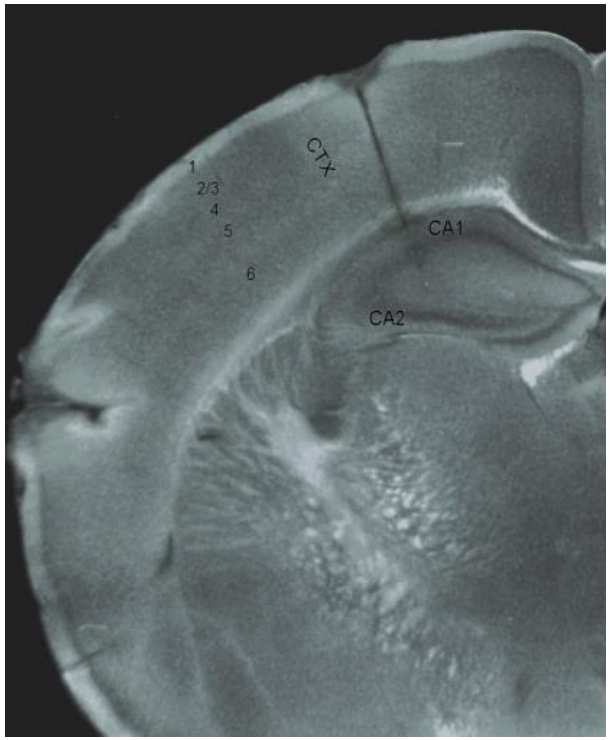
Multi-channel array extracellular recordings

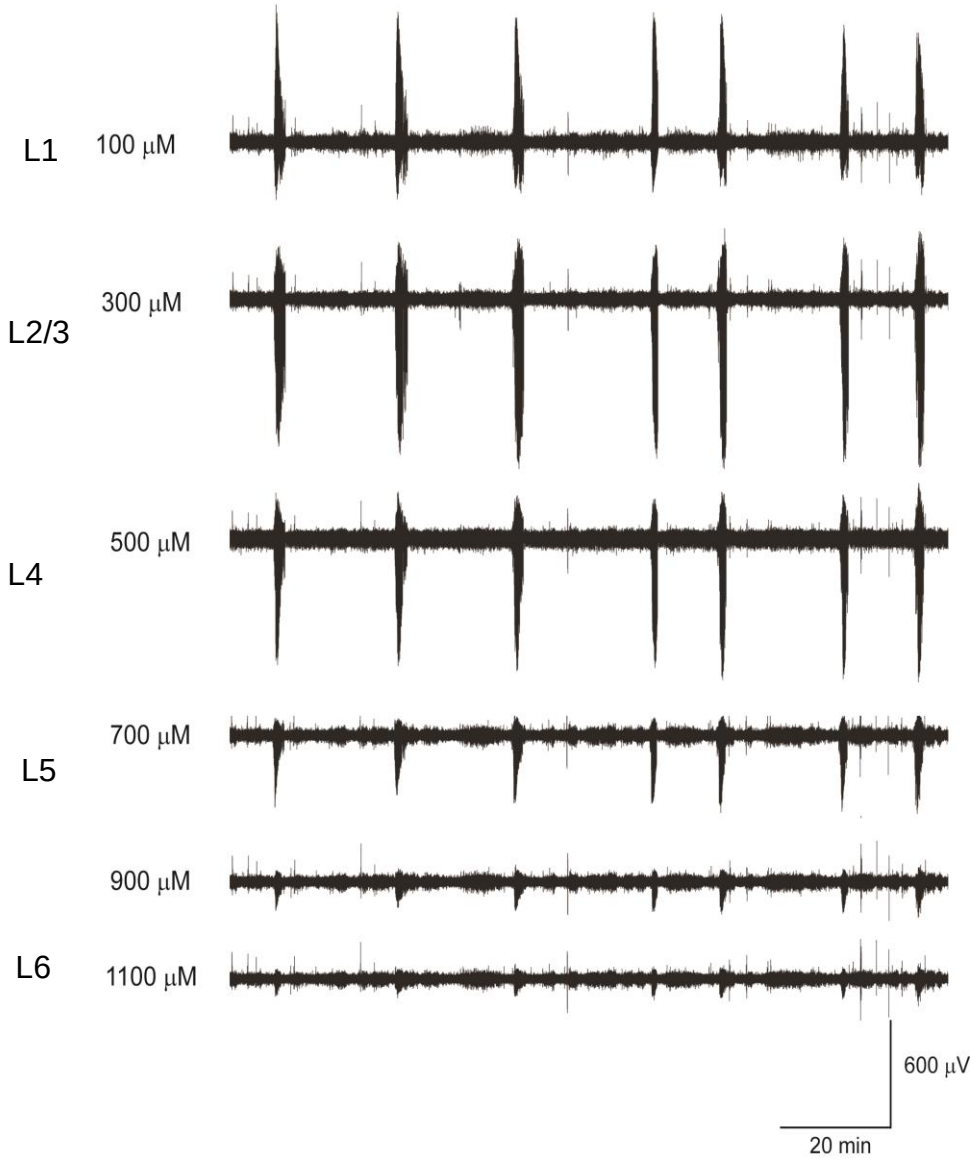


Current-source density analysis



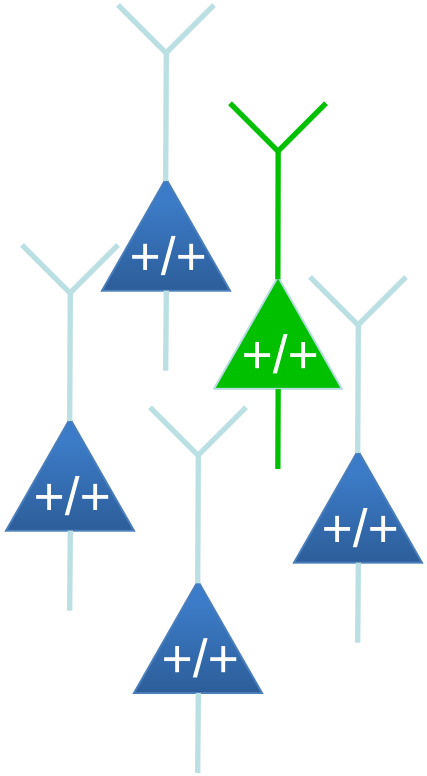
Спонтанная эпилептиформная активность в коре *Tsc1+ /* - мышей *in vivo*

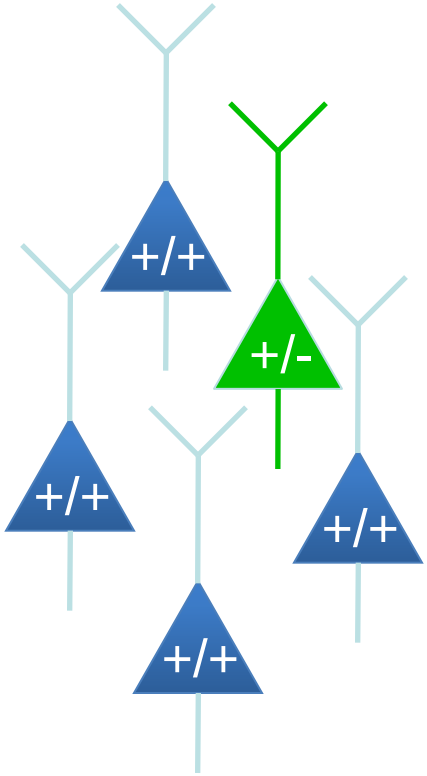


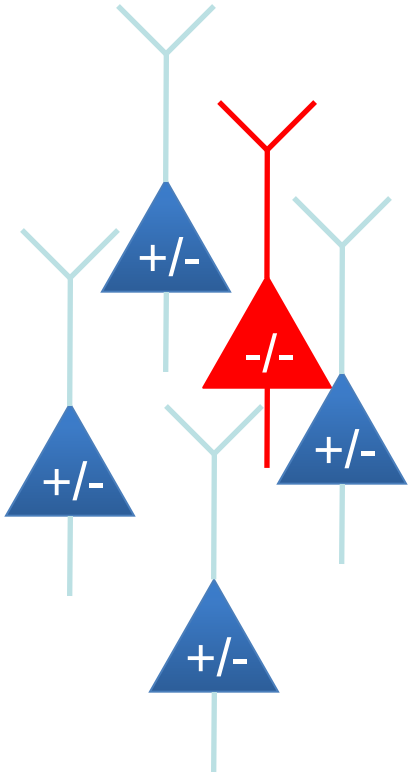


Животная модель TSC1

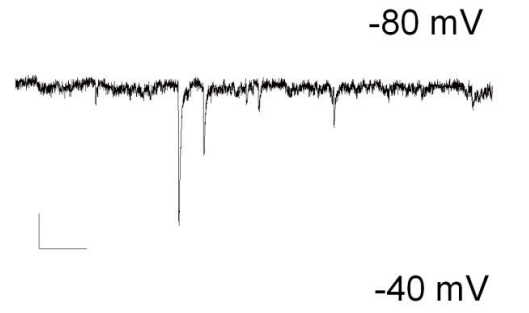
	flx/wt	*flx/wt creTSC1+/-*	*flx/mut creTSC1-/-*
electroporated	+/+	+/-	-/-
non electroporated	+/+	+/+	+/-

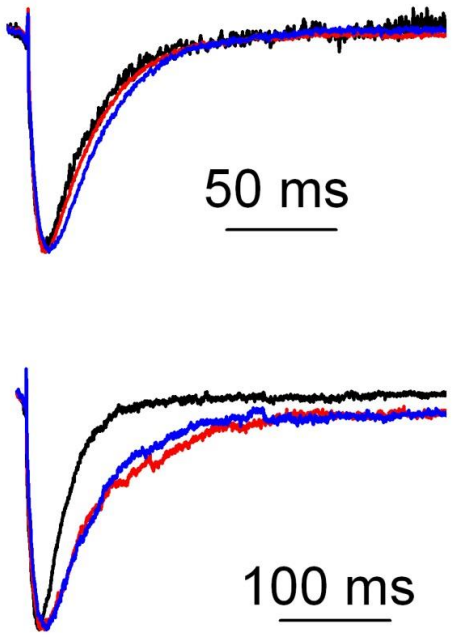


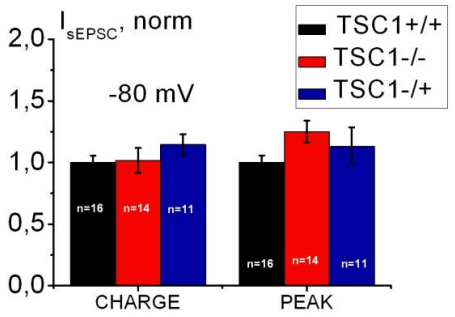


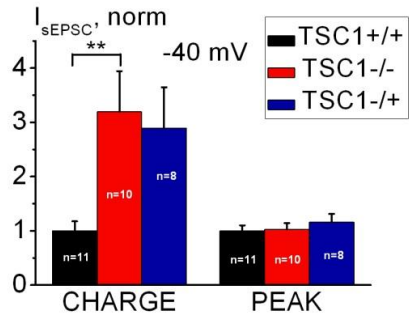


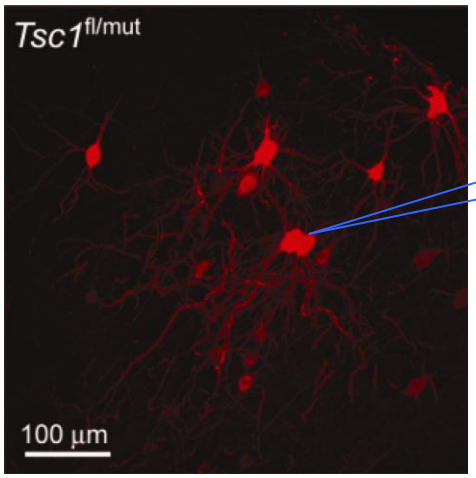
Замедление кинетики ВПСТ в TSC нейронах

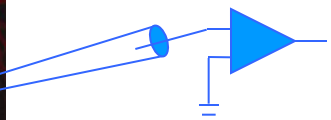






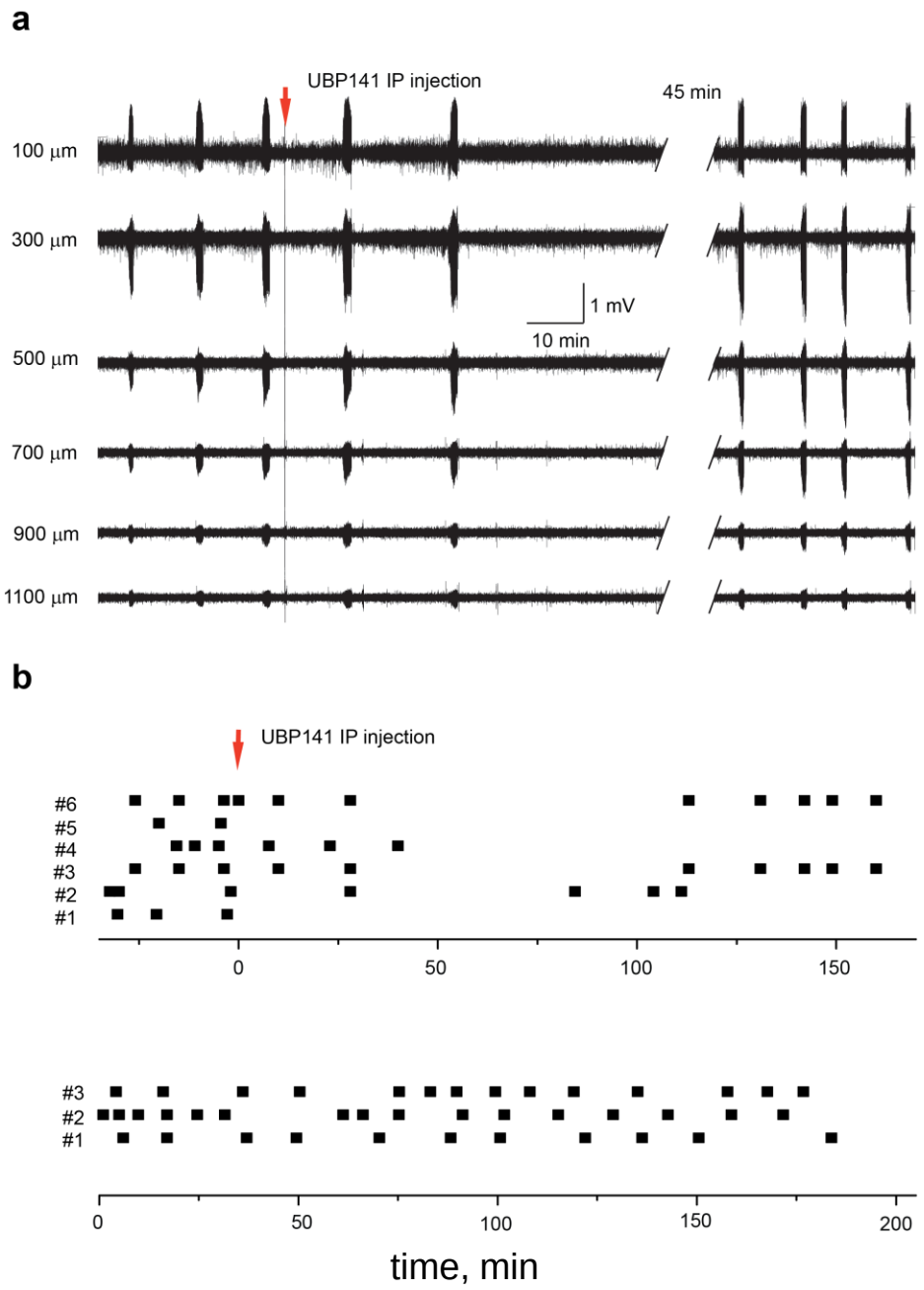




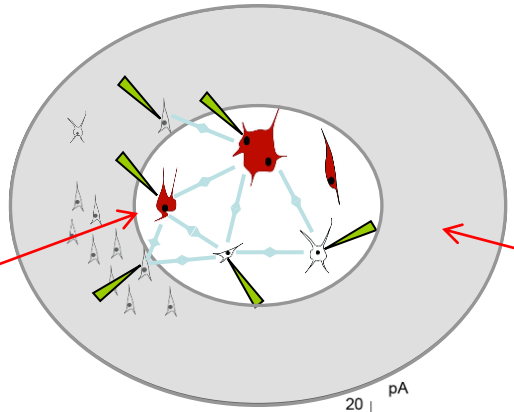


Кинетика затухания NMDAR опосредованного компонента ВПСТ в диспластических пирамидальных нейронах чувствительна к UBP141, селективному GluN2C / D антагонисту

Антиэпилептический эффект UBP141 *in vivo*



Patch clamp регистрация на срезах пост-операционной ткани TSC пациентов



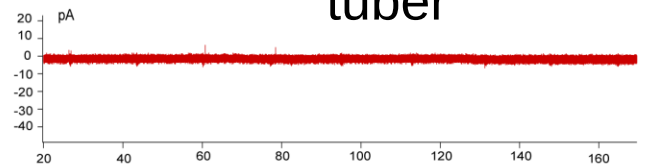
tuber

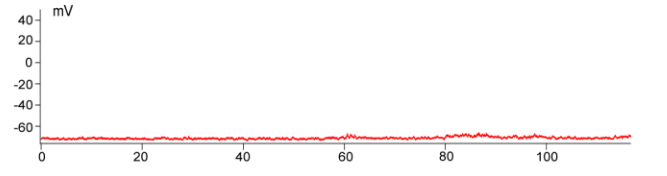
perituberal tissue

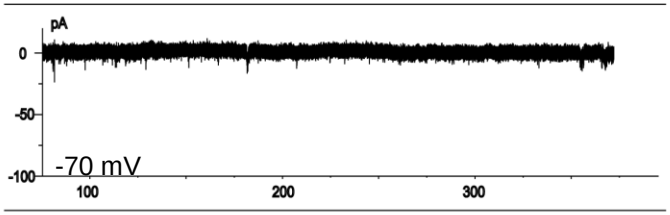
C-A

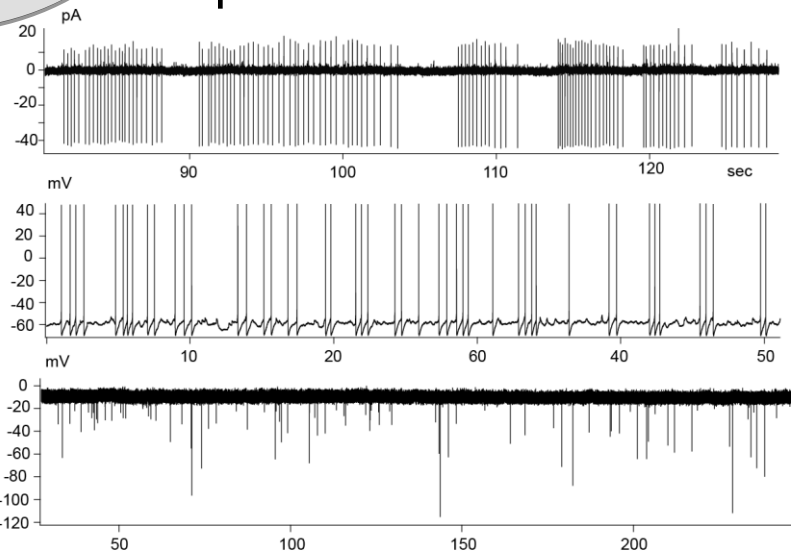
C-C

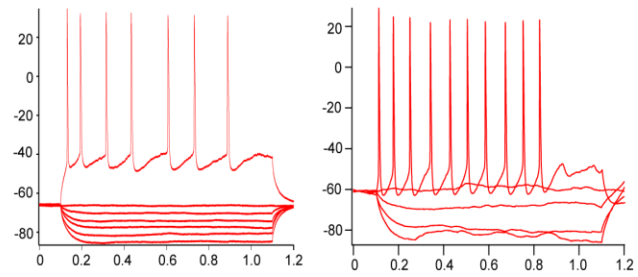
V-C

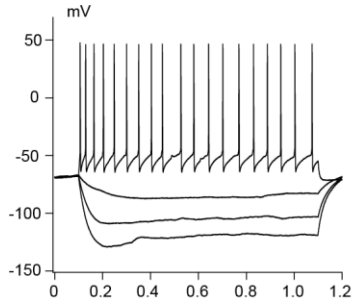




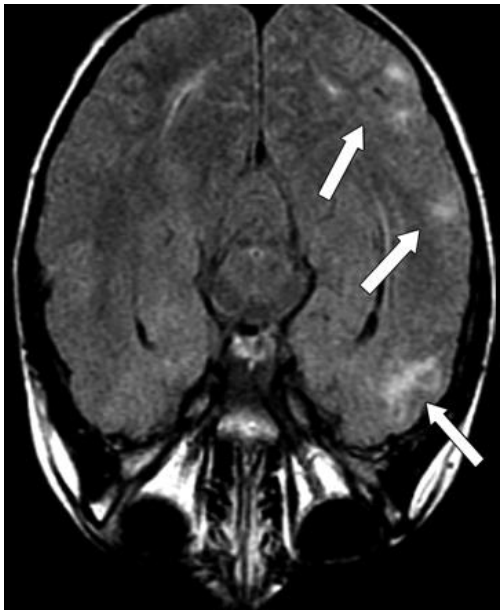


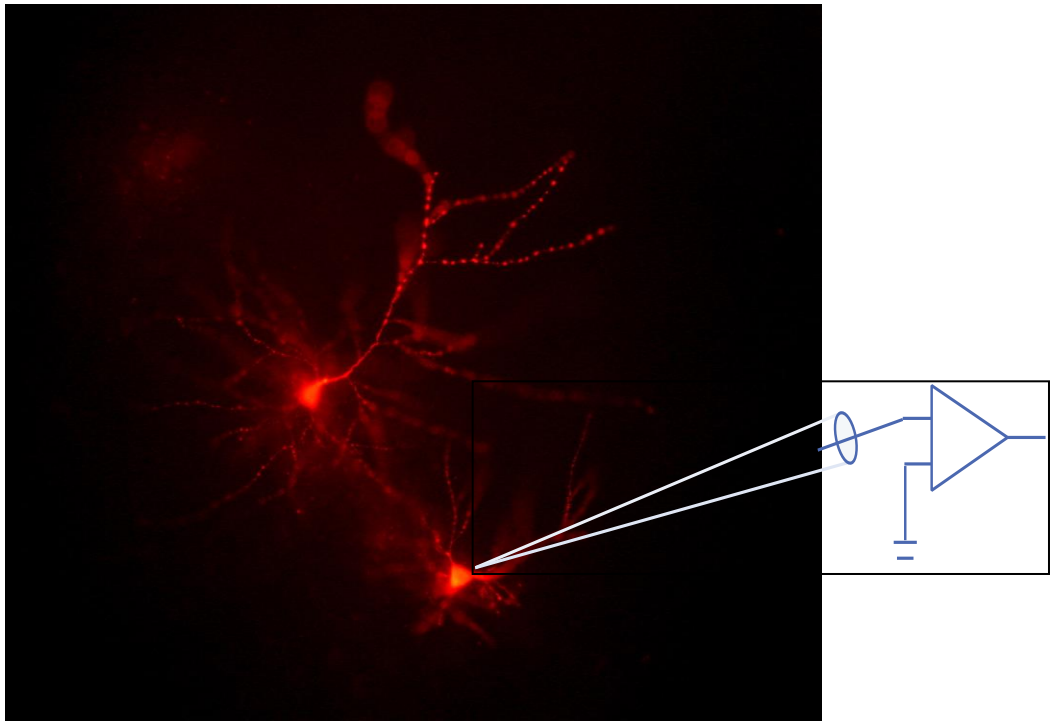


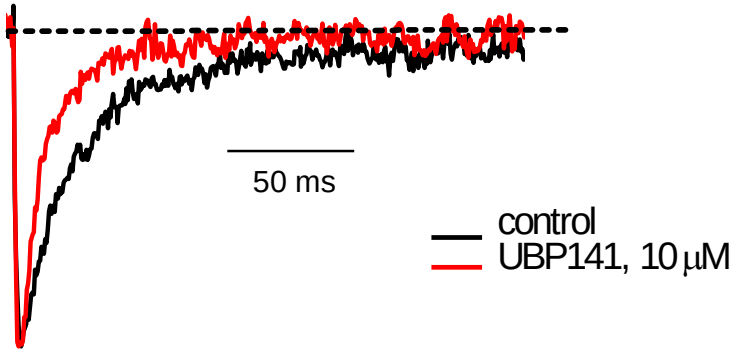




Кинетика затухания NMDAR опосредованного компонента ВПСТ в диспластических пирамидальных нейронах в послеоперационных срезах человеческого мозга чувствительна к UBP141







Синдром Fragile X

Клинические особенности Fragile X

Неврологические

- психические нарушения; начиная от трудностей при обучении до умственной отсталости

-синдром дефицита внимания и гиперактивность

-тревога и неустойчивое настроение

-аутистическое поведение

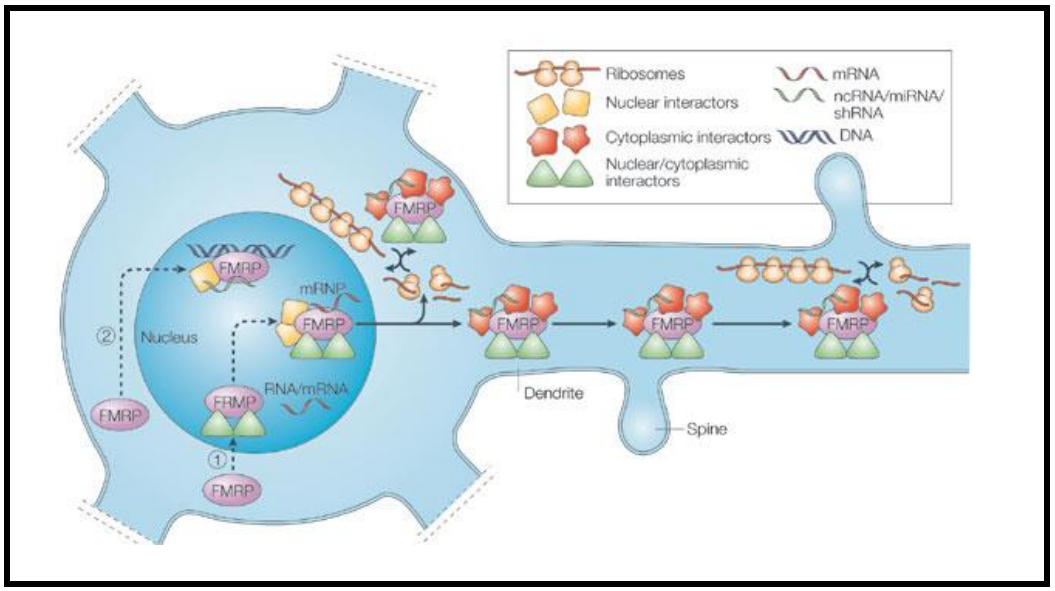
-эпилептические припадки у около 25% людей с **Fragile X**

Другие особенности

вытянутое лицо, большие уши, плоскостопие
увеличенные суставы, особенно на пальцах

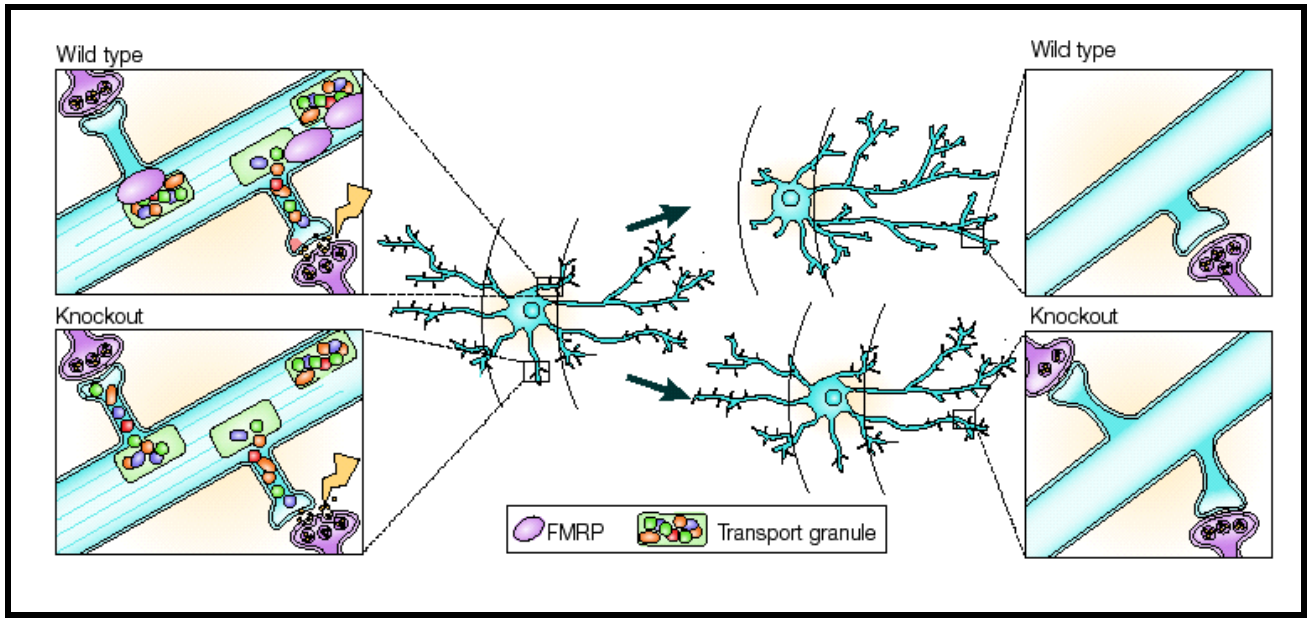
FRAX website; http://www.fraxa.org

The Fragile X Mental Retardation Protein, **FMRP** (***FMR1)***



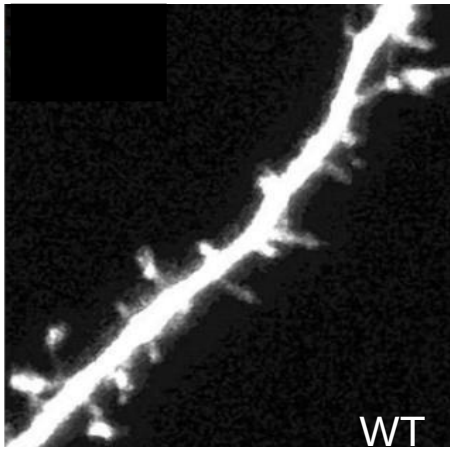
Регулятор транспорта и трансляции мРНК

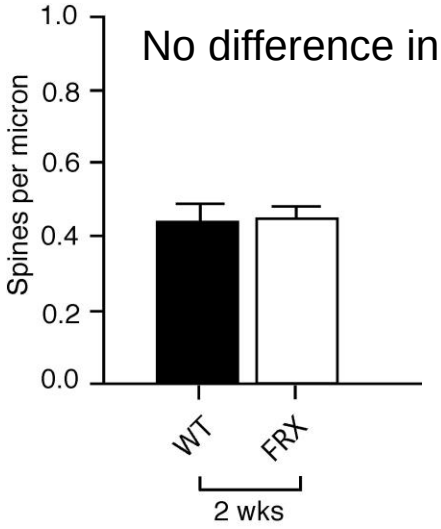
Отсутствие FMRP может привести к неизбирательной стабилизации незрелых шипиков

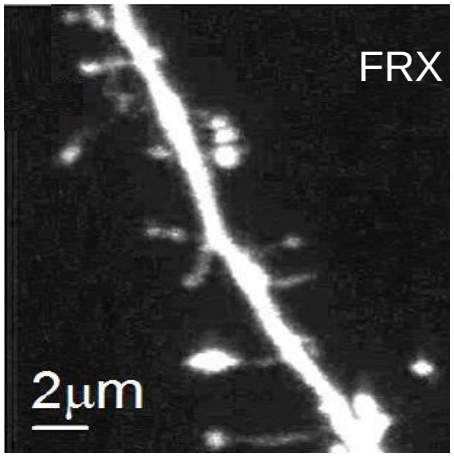


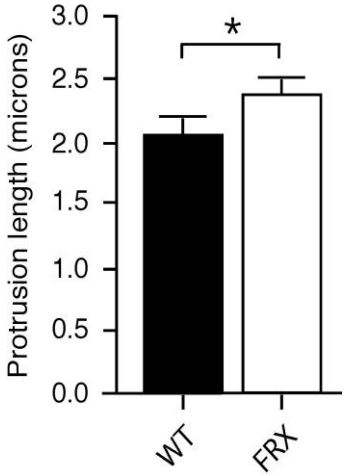
Bagni C & Greenough WT Nature Reviews Neuroscience 6, 376-387 (2005)

Шипики в префронтальной коре





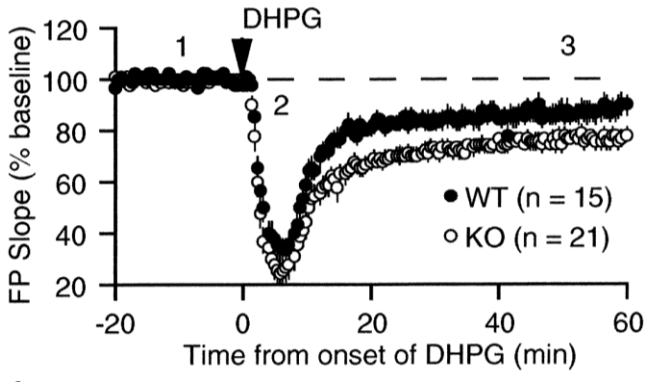




More elongated protrusions

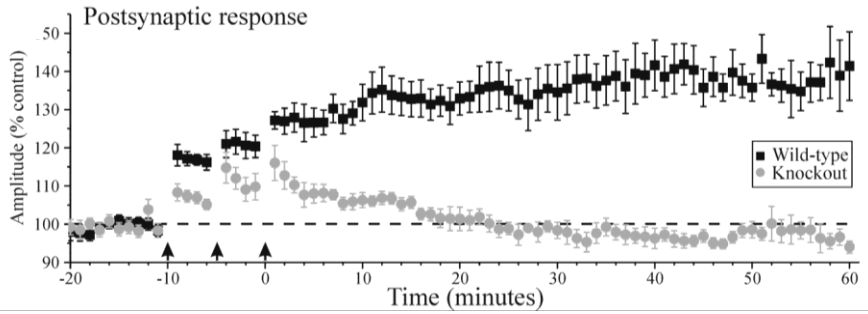
Дефицит синаптической пластичности у FMR1 KO мышей

LTD



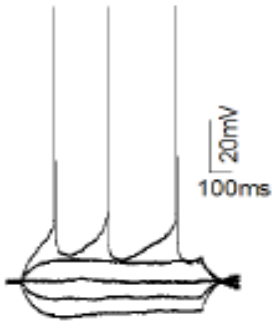
Hippocampus

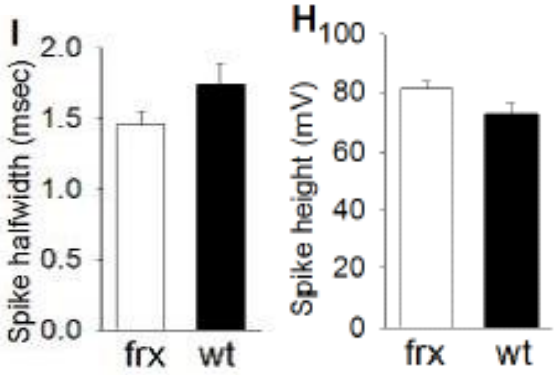
LTP



Neocortex

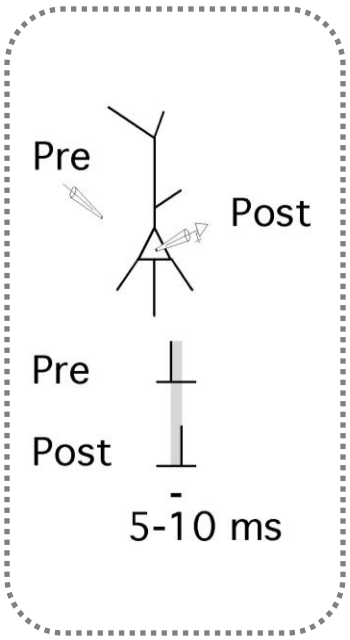
Нет разницы в базовых электрических параметрах:

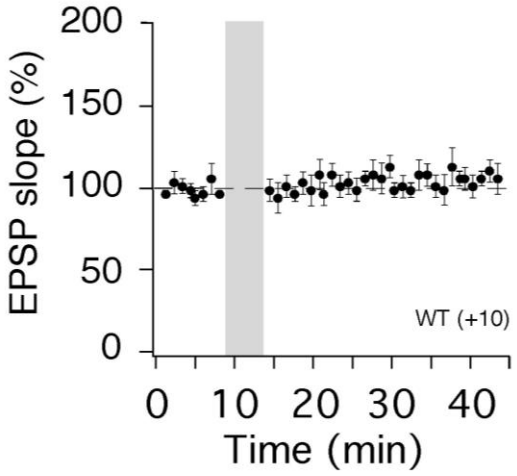


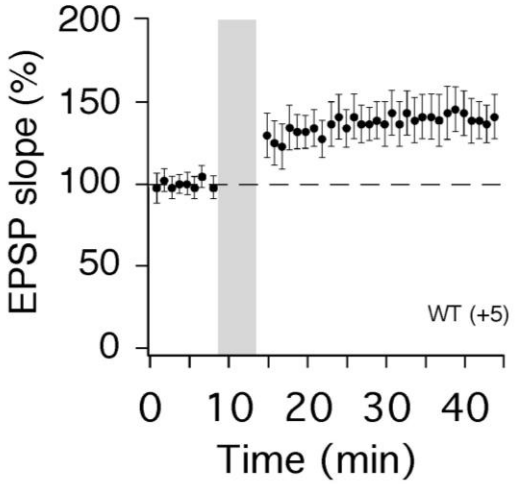


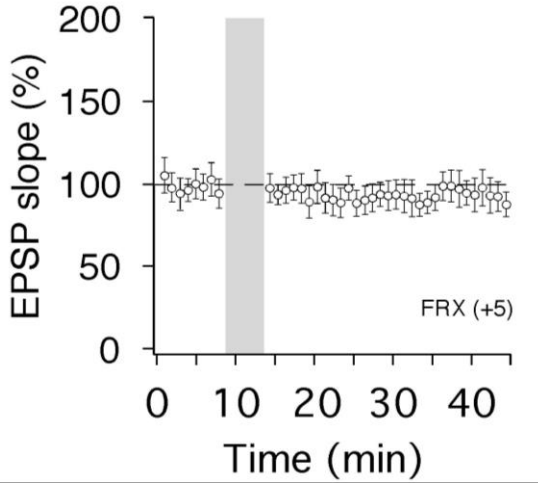
Входное сопротивление
Постоянная времени мембраны
Порог АР
Высота и продолжительность АР

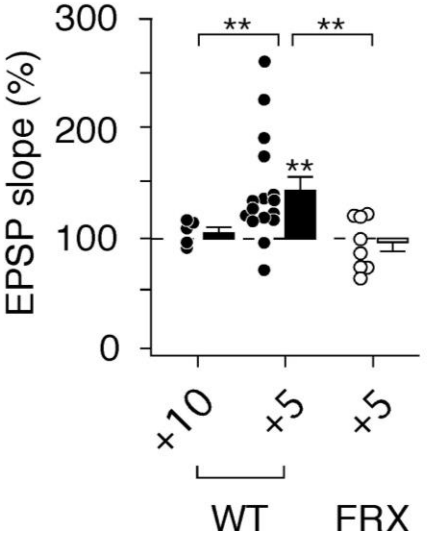
Отсутствие tLTP у мышей FRX



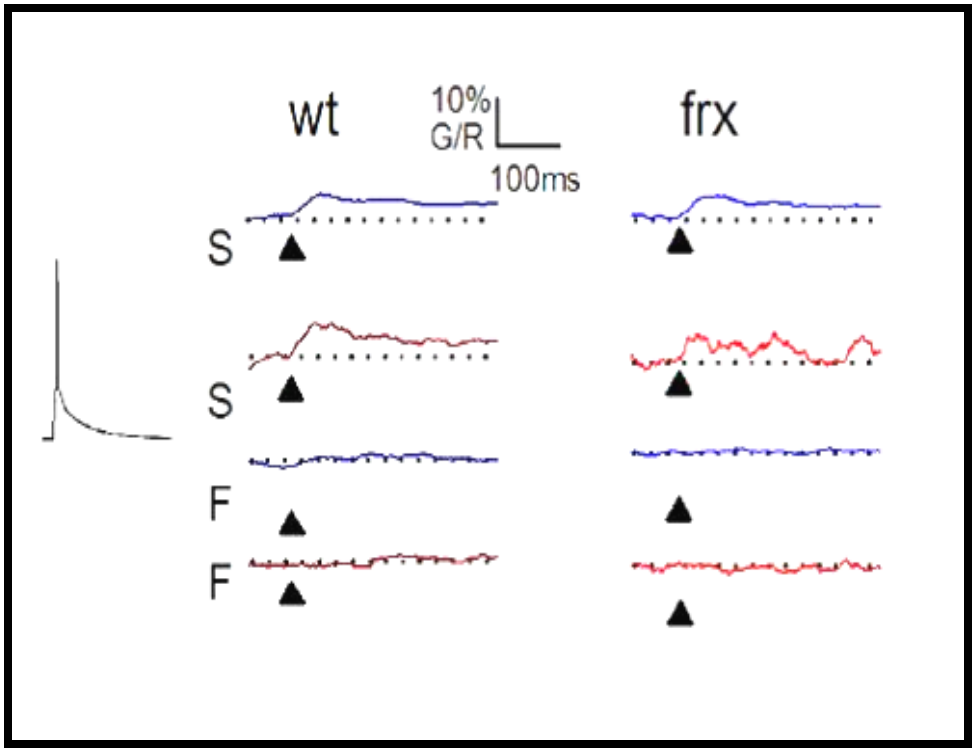


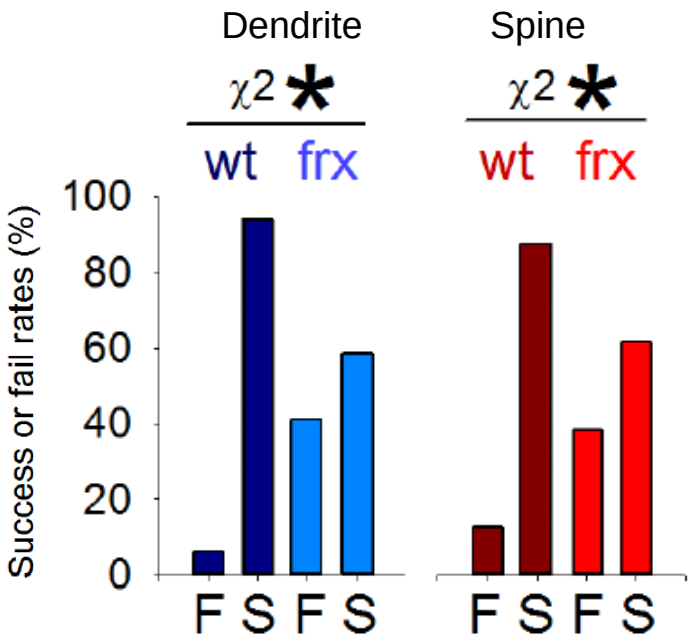




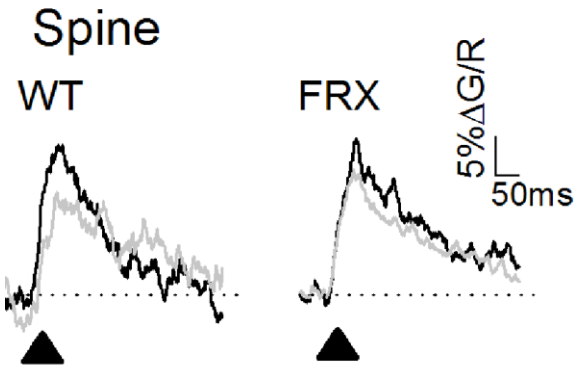


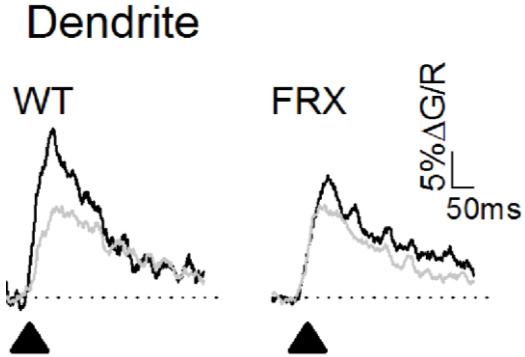
Сбои в кальциевой сигнализации в дендритах и спайнах FRX мышей

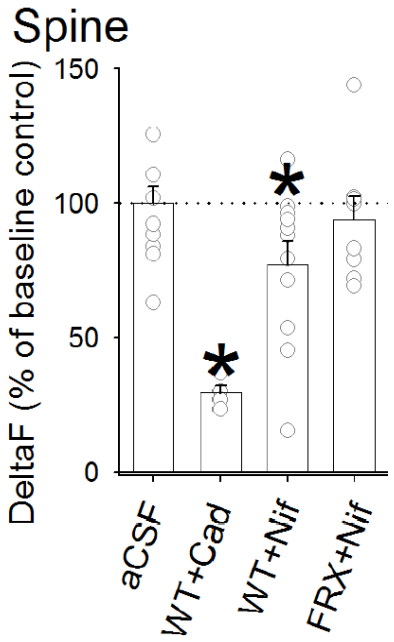


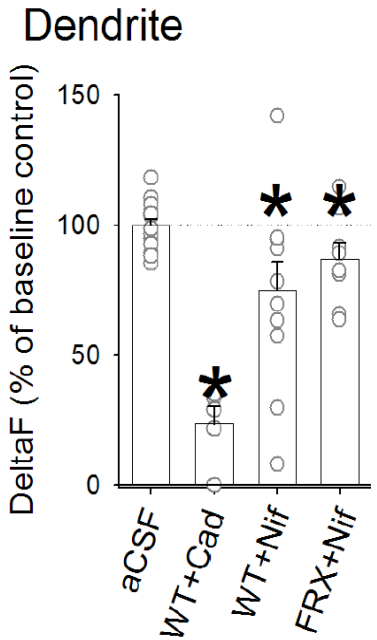


Снижение вклада Ca2+ каналов L-типа в FRX

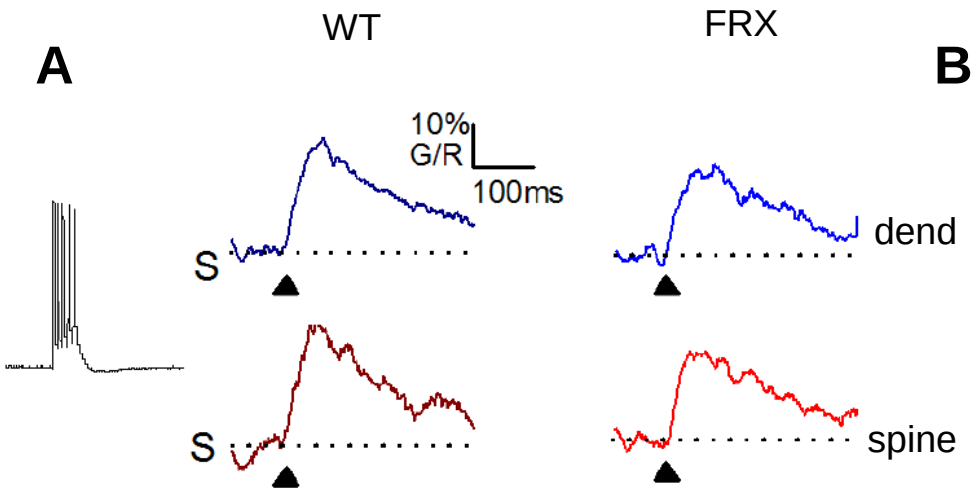


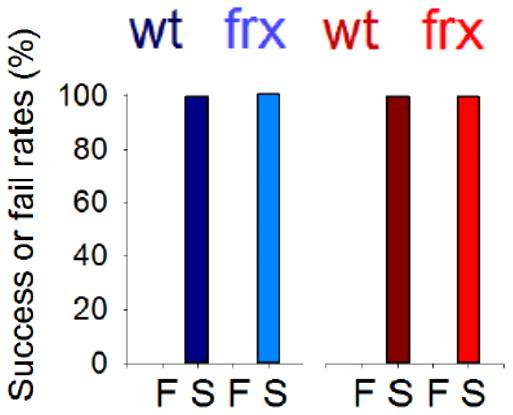


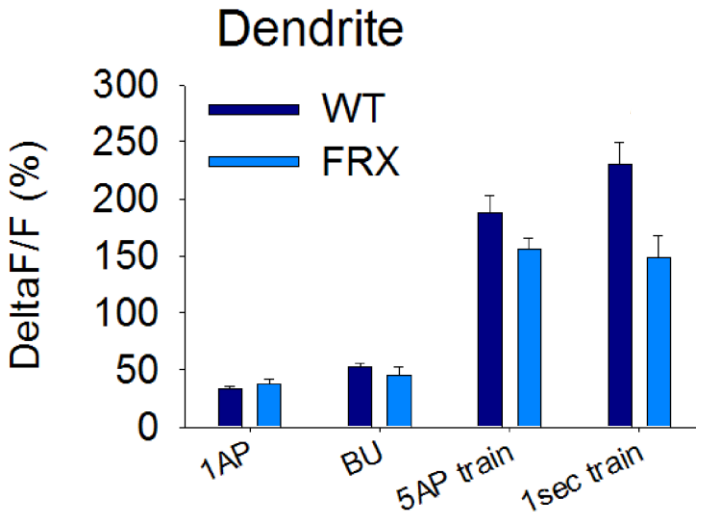


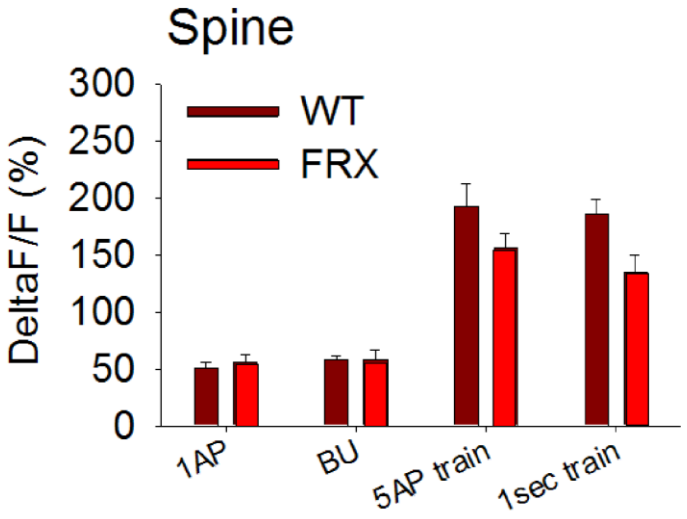


Передача сигналов кальция в шипиках и дендритах FRX успешна и увеличена при пачечной стимуляции

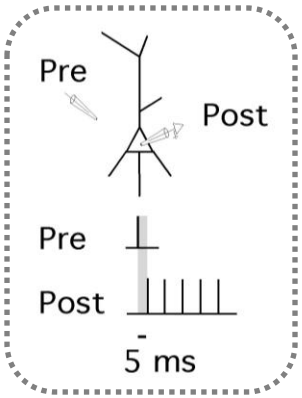


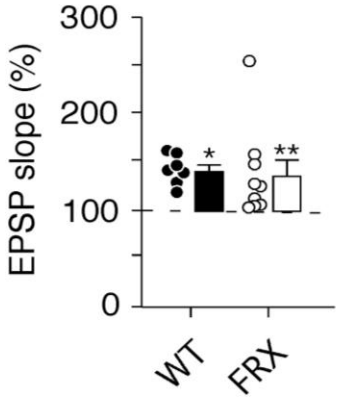


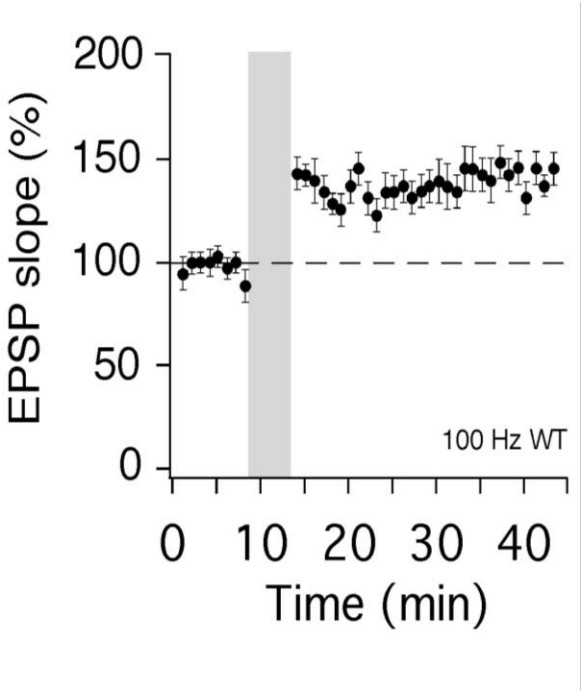


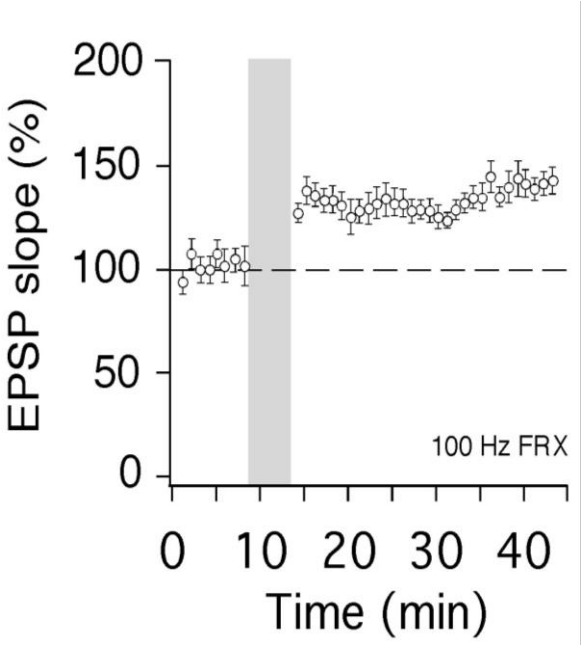


tLTP восстанавливается повышенной постсинаптической активностью







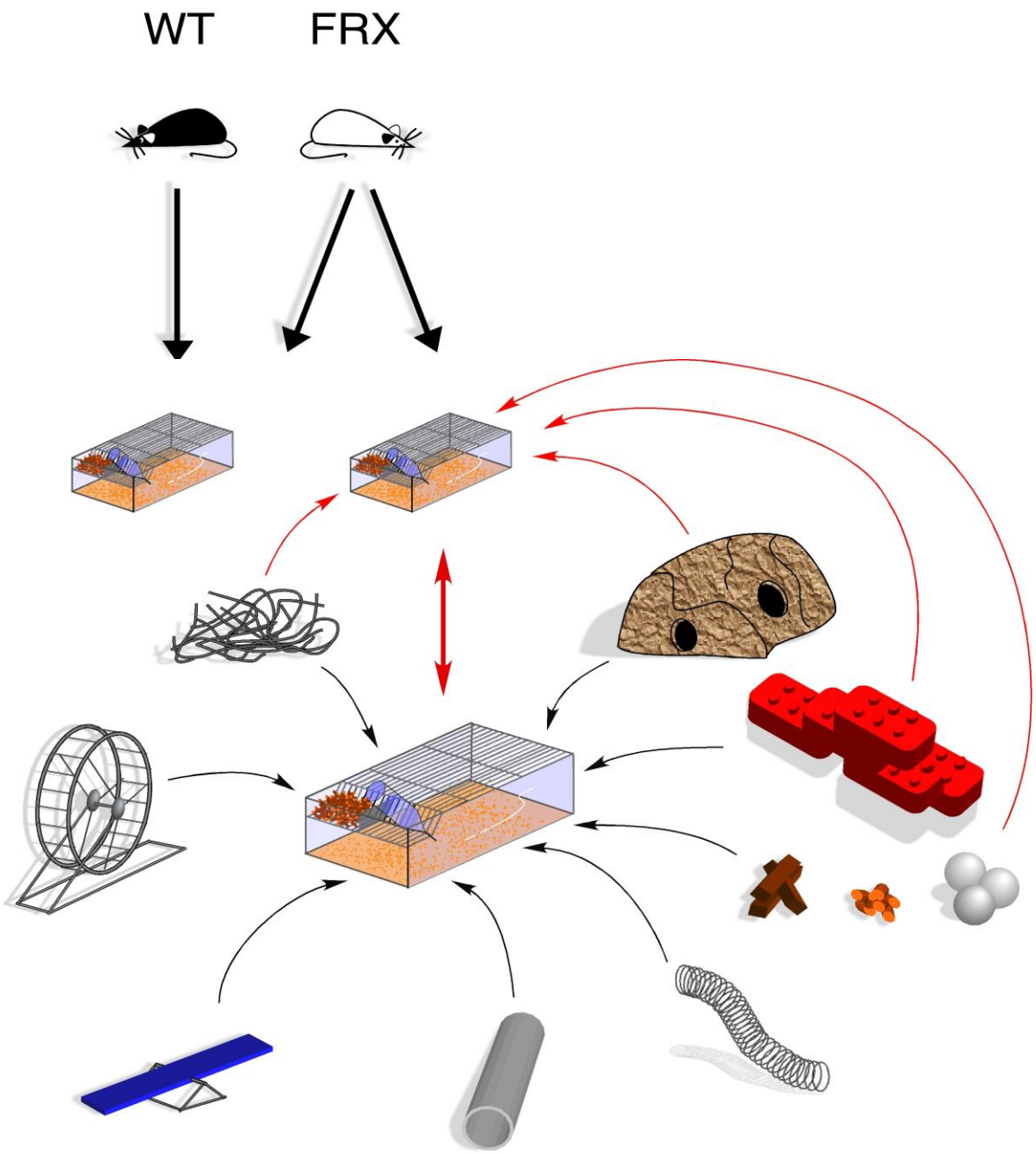


Environmental enrichment

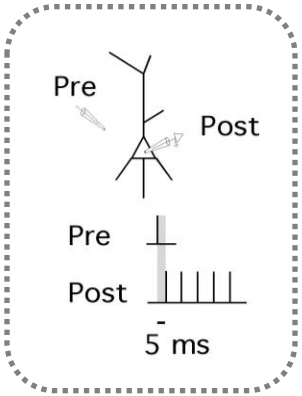
"Hebb (1949) noted that rats, which he had brought home for several weeks as pets for his children and later returned to the laboratory, showed better problem-solving ability than rats that had remained in the laboratory" [1].

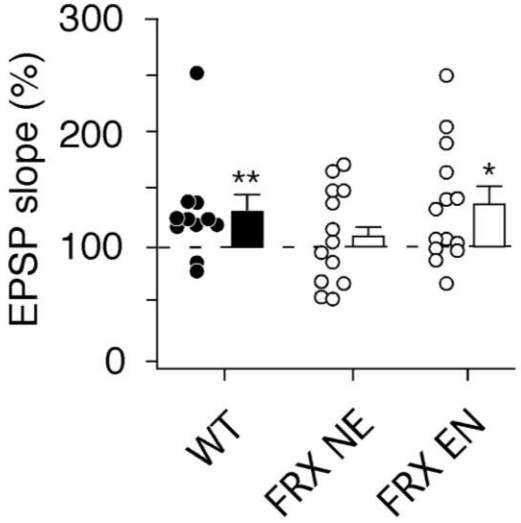
1. Quoted in *Neuropsychopharmacology* (2006) **31,** 36–46.

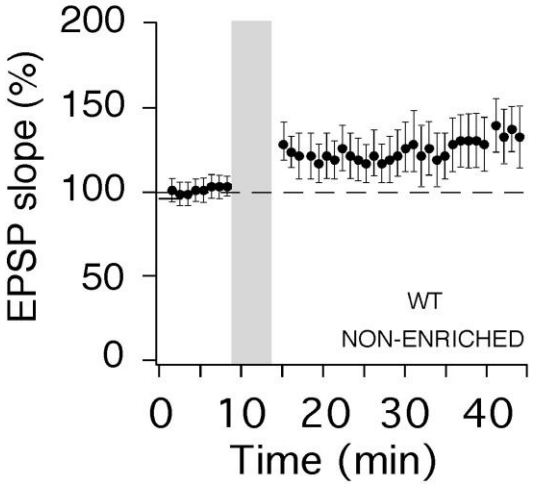
Парадигма обогащения окружающей среды

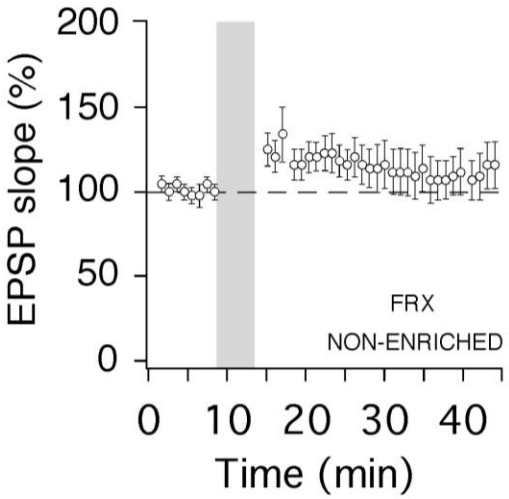


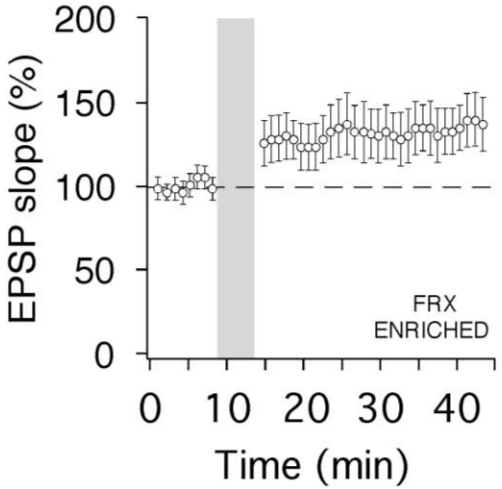
Обогащение окружающей среды восстанавливает tLTP у мышей FRX



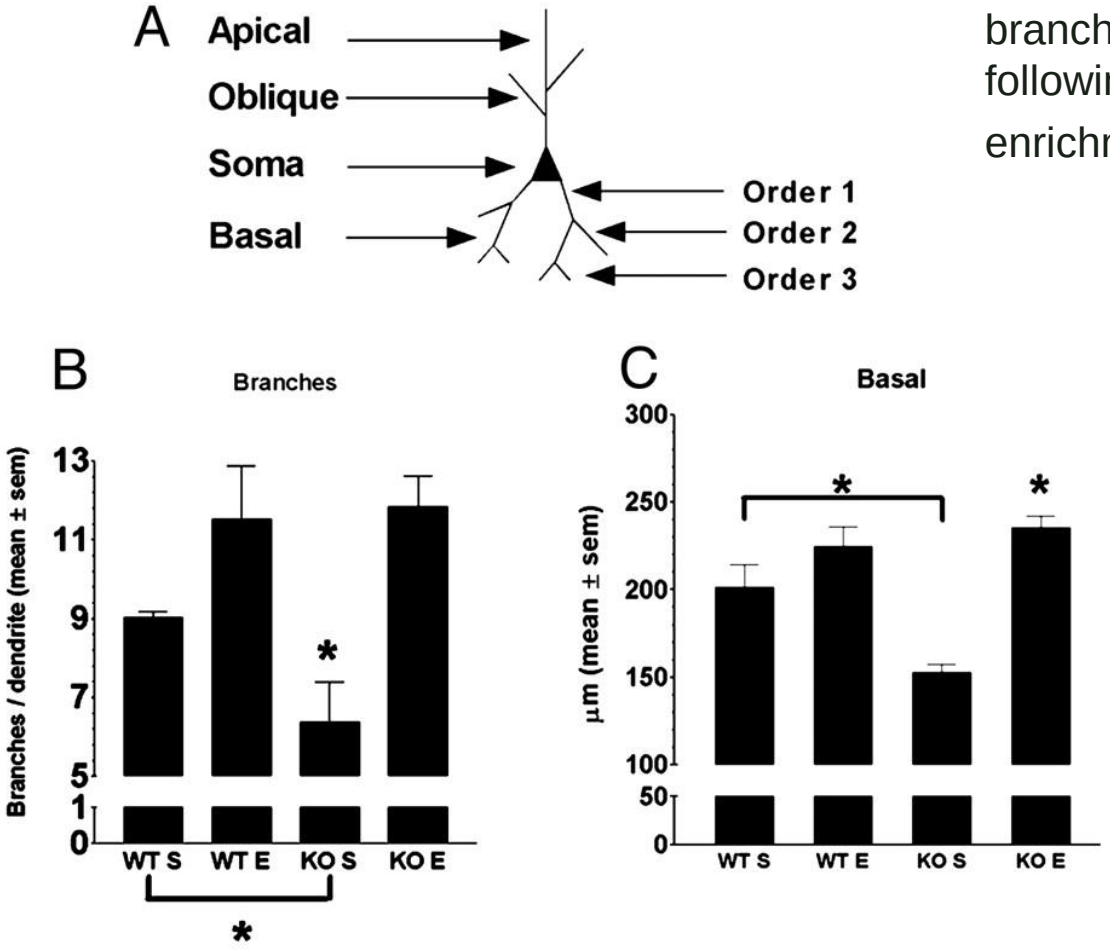








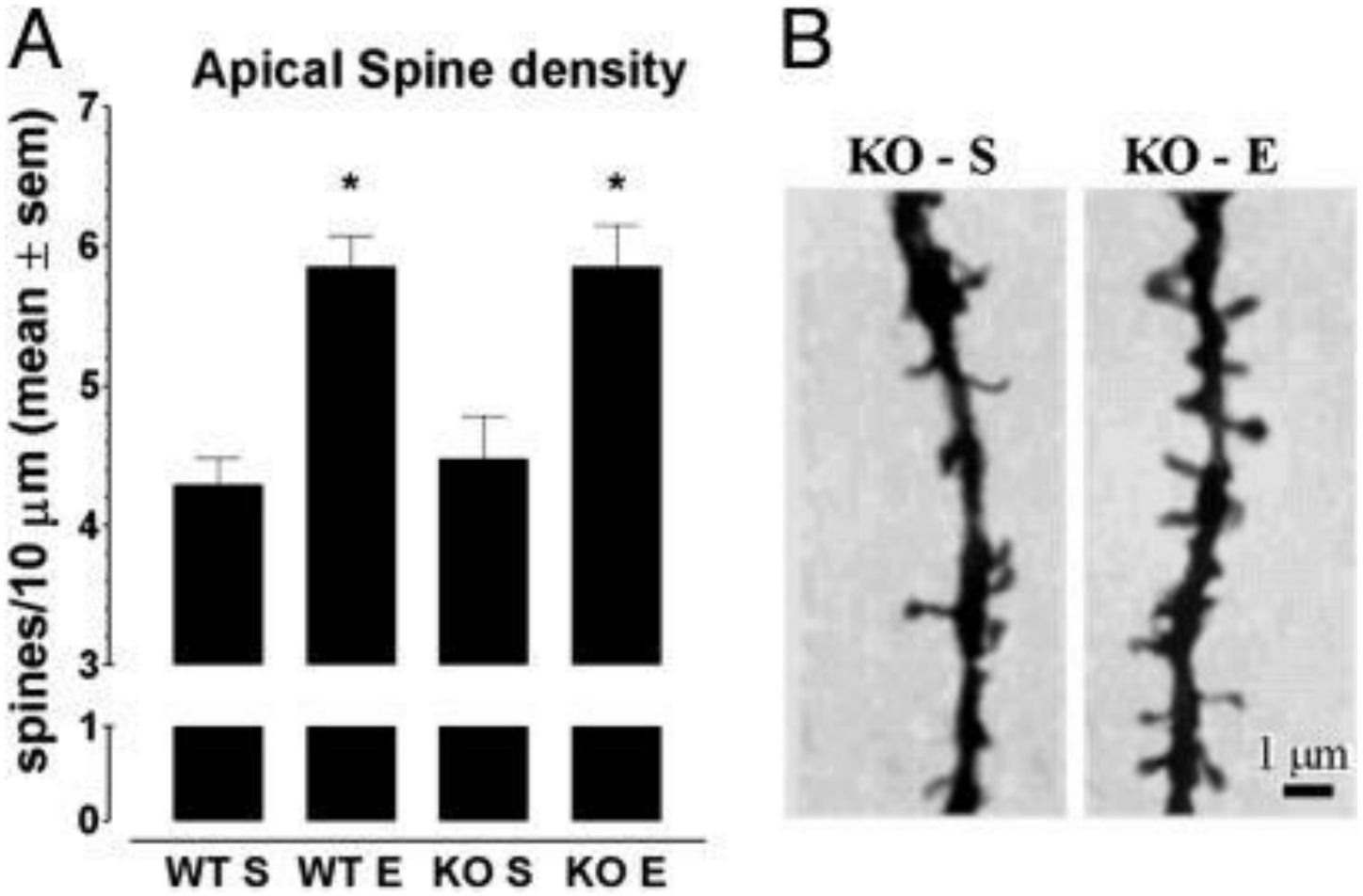
Влияние обогащения окружающей среды на нейронную морфологию в FRX



- Basal dendritic branching increased following environmental enrichment

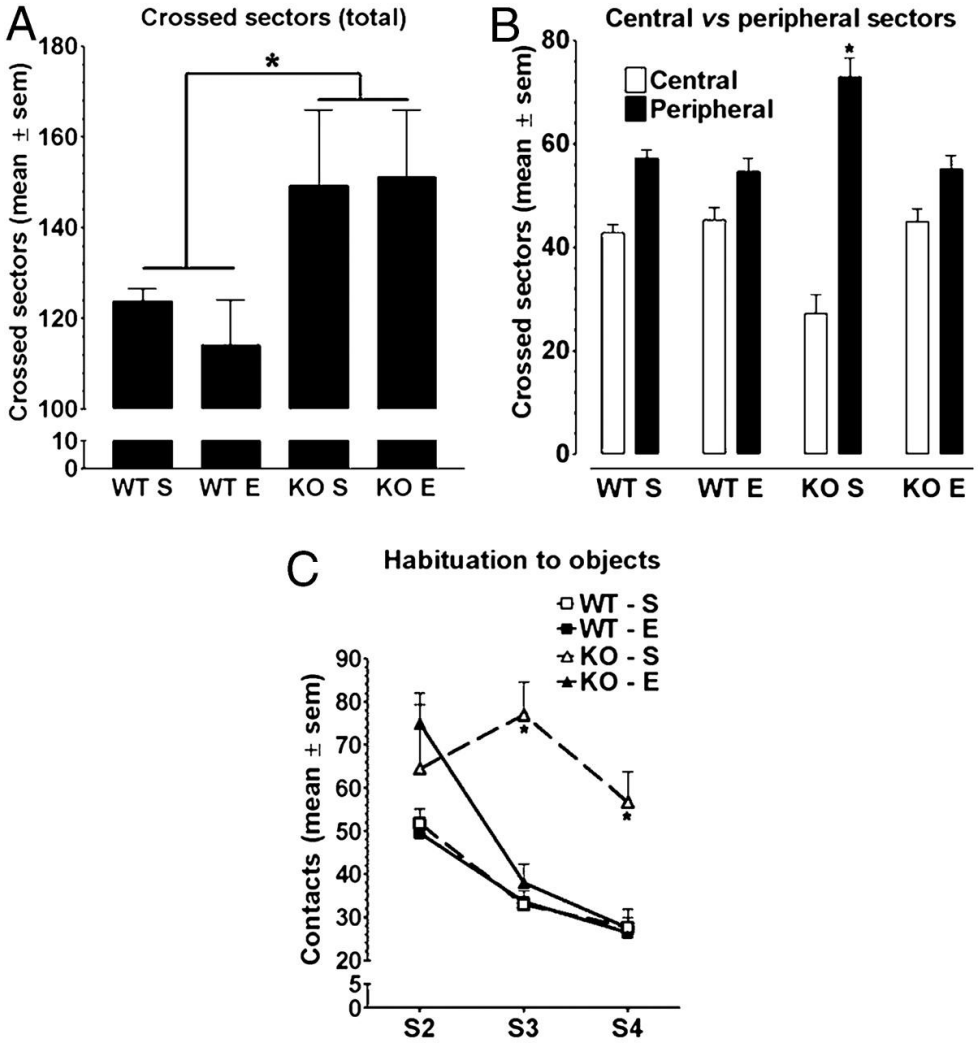
Restivo, Leonardo et al. (2005) Proc. Natl. Acad. Sci. USA 102, 11557-11562

Влияние обогащения окружающей среды на нейронную морфологию в FRX



Restivo, Leonardo et al. (2005) Proc. Natl. Acad. Sci. USA 102, 11557-11562

Влияние обогащения окружающей среды на поведение мыши FRX

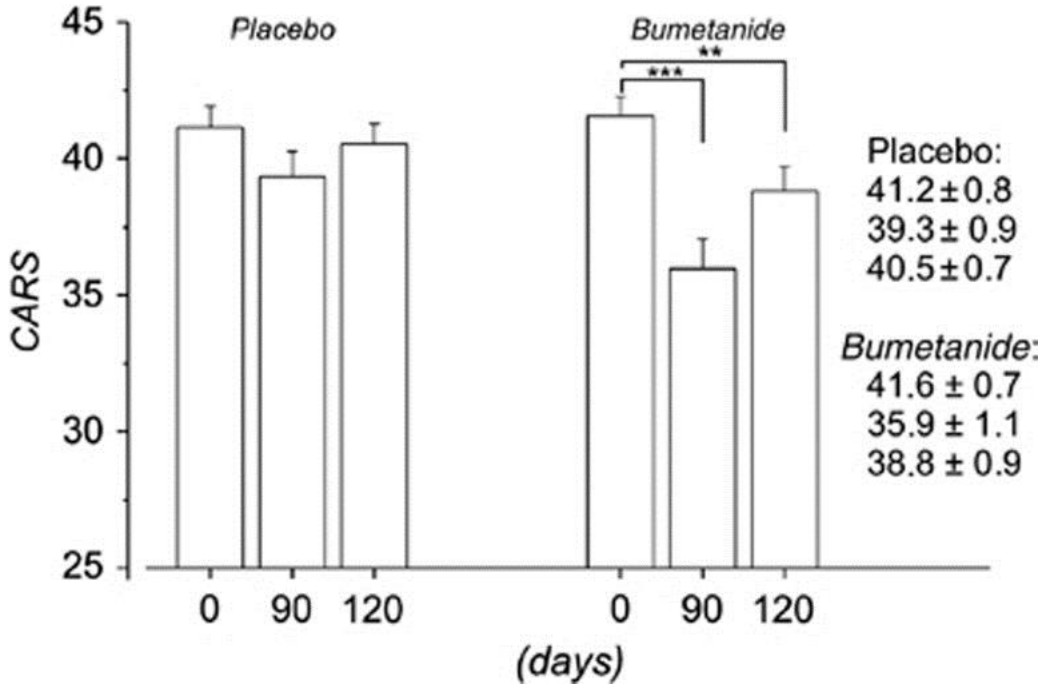


Restivo, Leonardo et al. (2005) Proc. Natl. Acad. Sci. USA 102, 11557-11562

Аутизм

Улучшение параметров тестов на аутичность при употреблении буметанида

1 мг в день



Lemonnier et al. 2012

Oxytocin-Mediated GABA Inhibition During Delivery Attenuates Autism Pathogenesis in Rodent Offspring

Roman Tyzio,[1,2]* Romain Nardou,[3]* Diana C. Ferrari,[3]* Timur Tsintsadze,[1,2,3] Amene Shahrokhi,[3]† Sanaz Eftekhari,[3]† Ilgam Khalilov,[1,2] Vera Tsintsadze,[1,2] Corinne Brouchoud,[1,2] Genevieve Chazal,[1,2] Eric Lemonnier,[4] Natalia Lozovaya,[1,2] Nail Burnashev,[1,2] Yehezkel Ben-Ari[1,2,3]‡

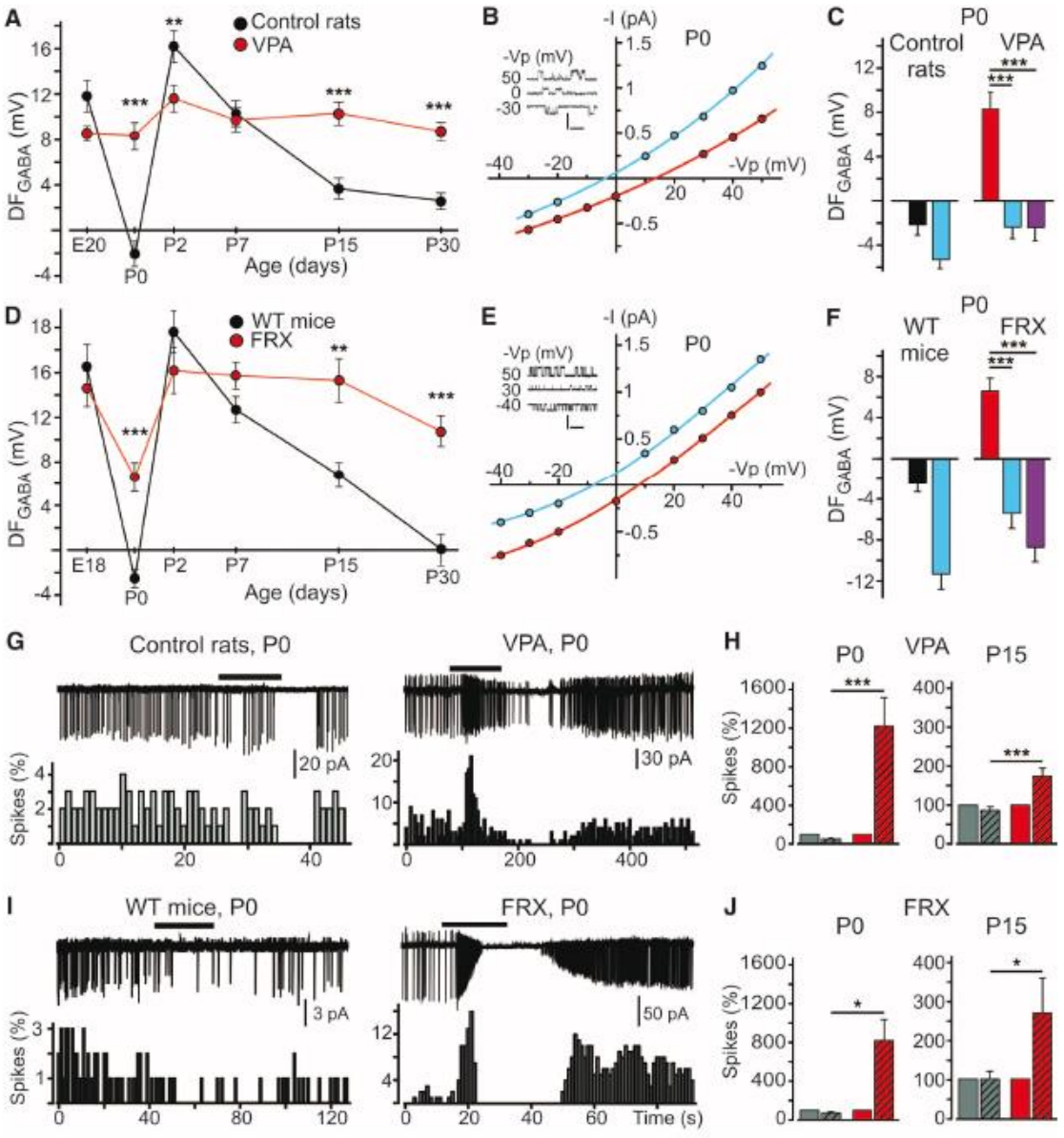


Fig. 1. Developmental excitatory-inhibitory GABA sequence is abolished in hippocampal CA3 pyramidal neurons in VPA rats and FRX mice.

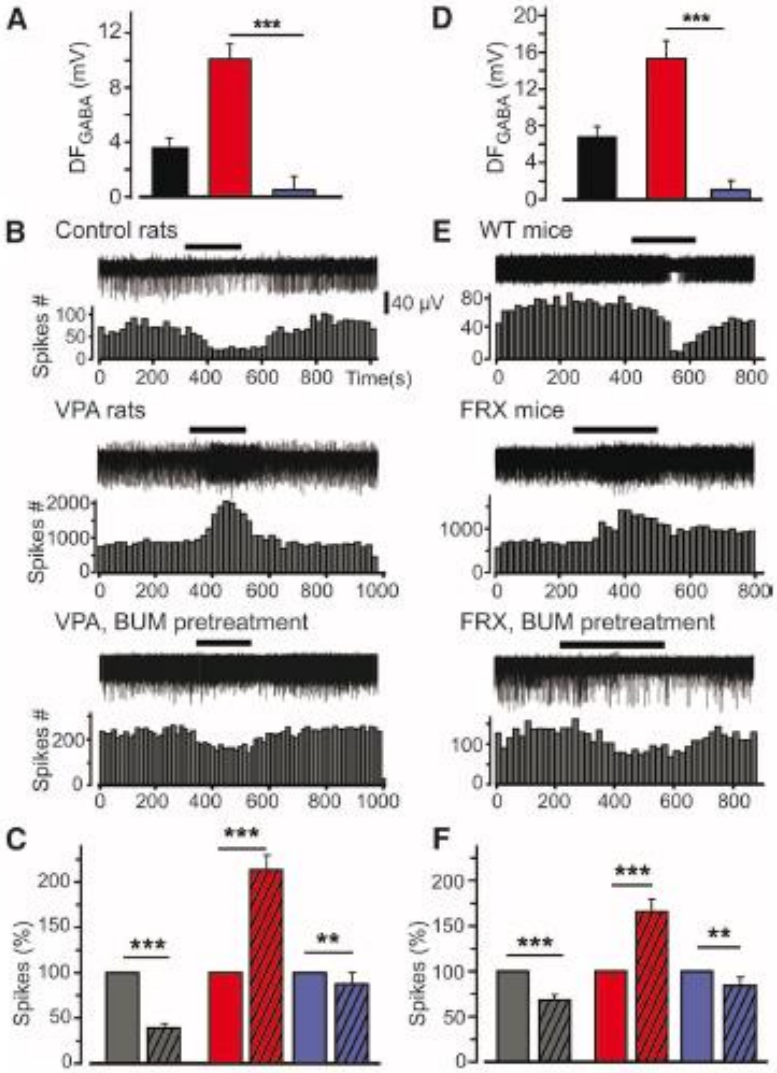


Fig. 2. Maternal pretreatment with bumetanide before delivery switches the action of GABA from excitatory to inhibitory in offspring in VPA and FRX rodents at P15.

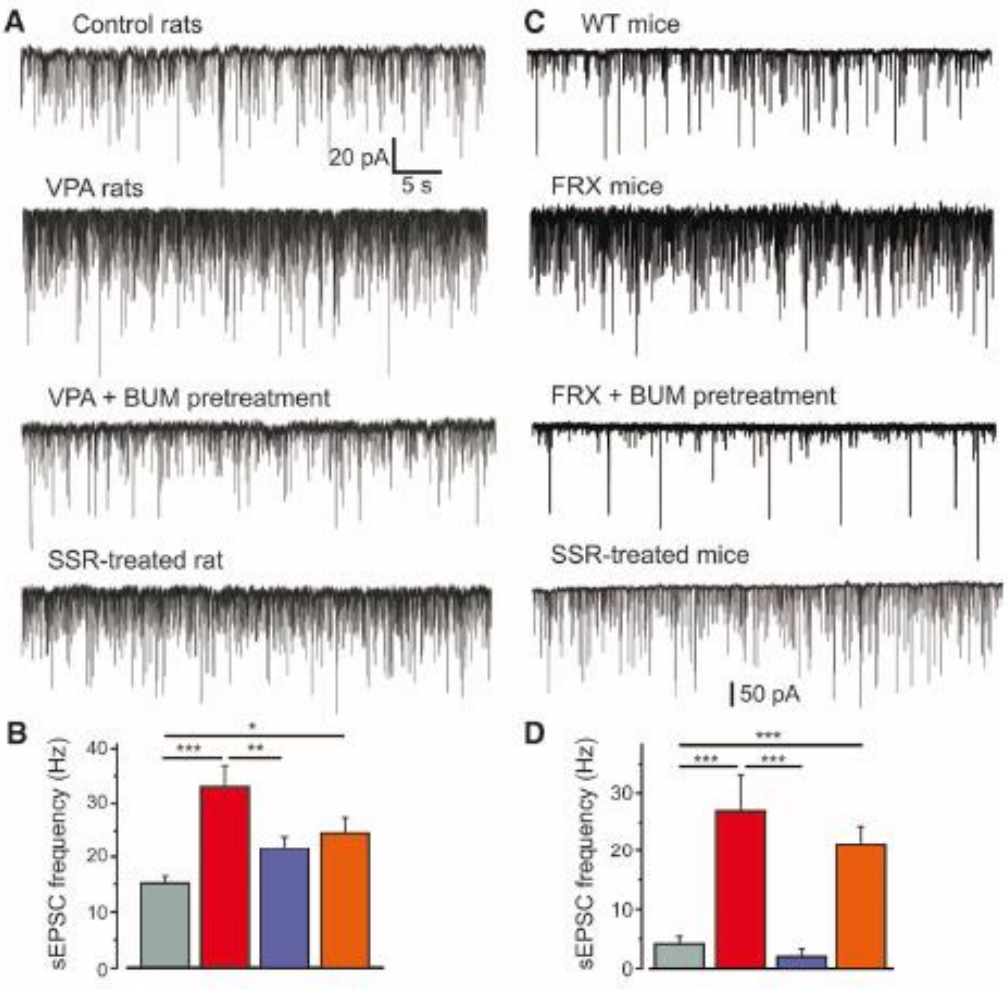


Fig. 3. Spontaneous activity is increased in VPA and FRX rodents at P15 and restored to control values by maternal pretreatment with bumetamide.

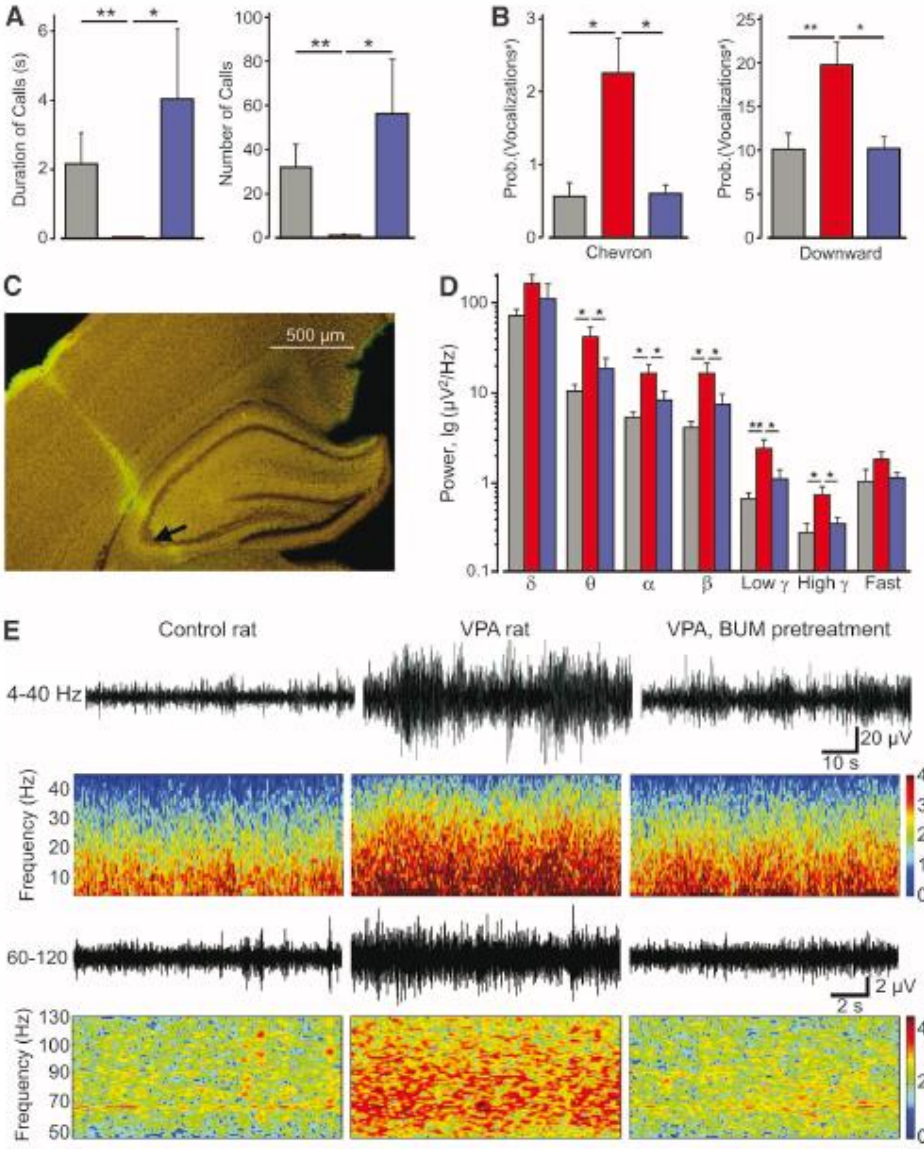


Fig. 4. Maternal pretreatment with bumetanide restores aberrant behavior and brain oscillations of animal models of autism.

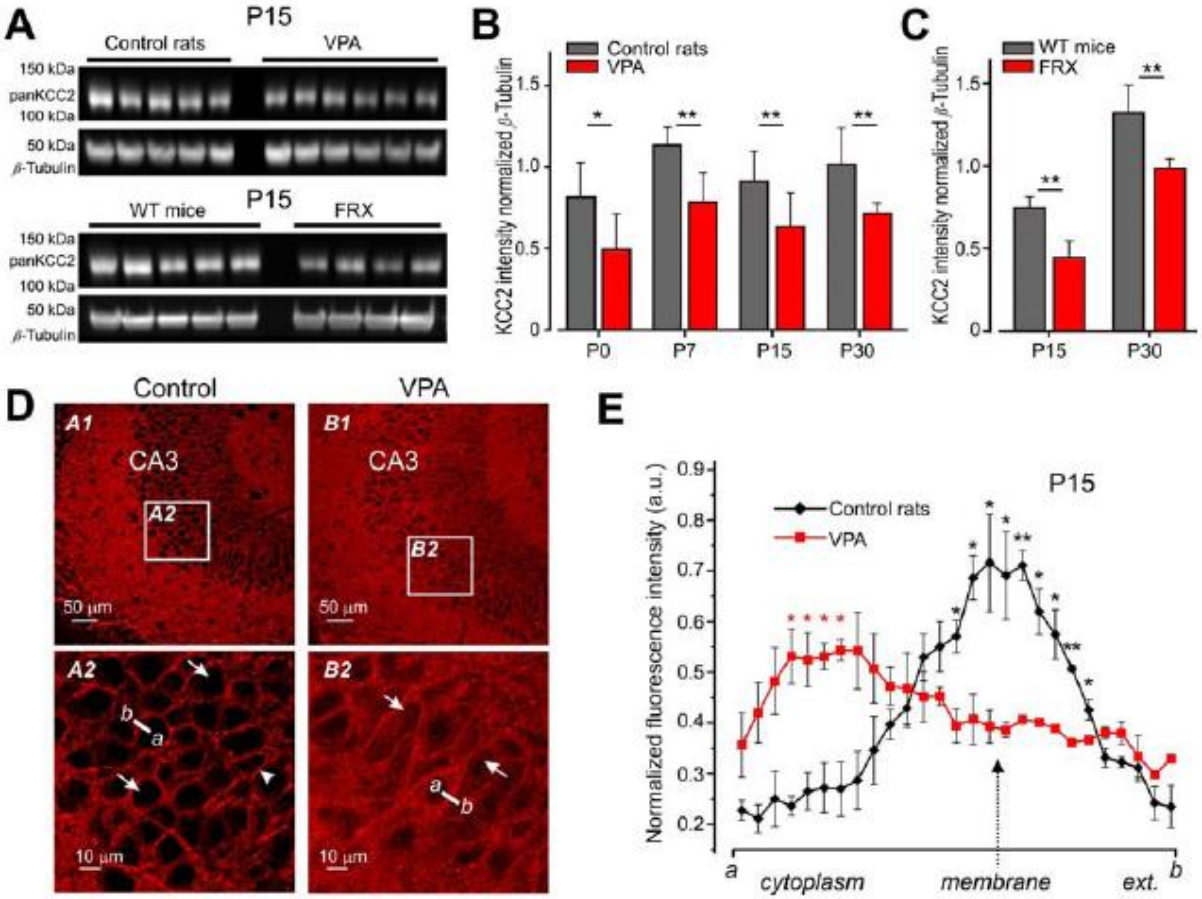
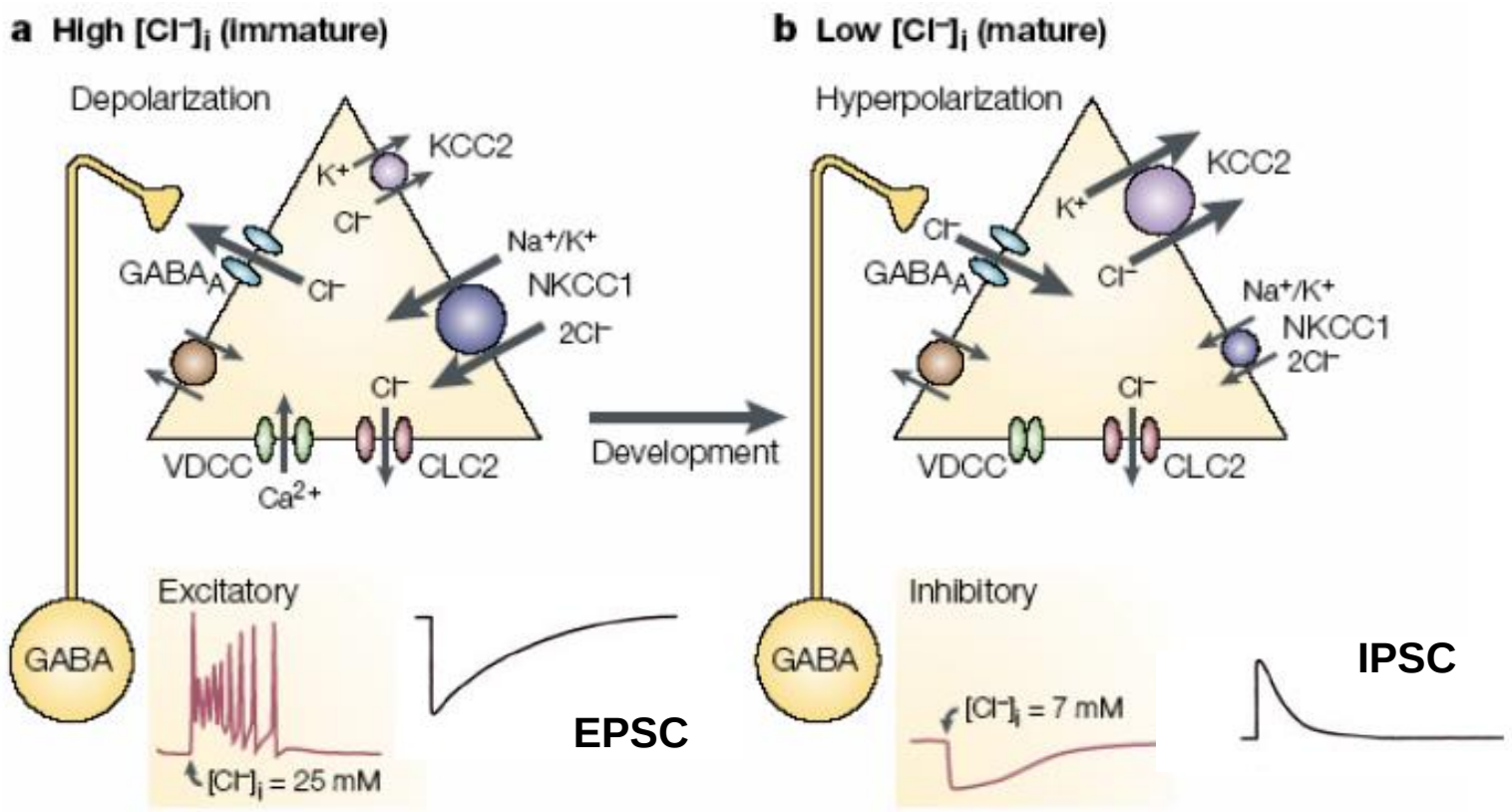


Fig. S1. KCC2 down regulation in VPA rats and FRX mice.

Изменение градиентов для Cl^- в процессе развития



Сдвиг в относительной экспрессии Cl^- транспортеров

Сначала экспрессируется Na^+-K^+-$2Cl^-$ котранспортер (NKCC 1), он увеличивает $[Cl^-]_i$ - ГАМК эффекты деполяризующие

Потом экспрессируется K^+-Cl^- котранспортер (KCC2) снижающий $[Cl^-]_i$ – ГАМК эффекты гиперполяризующие