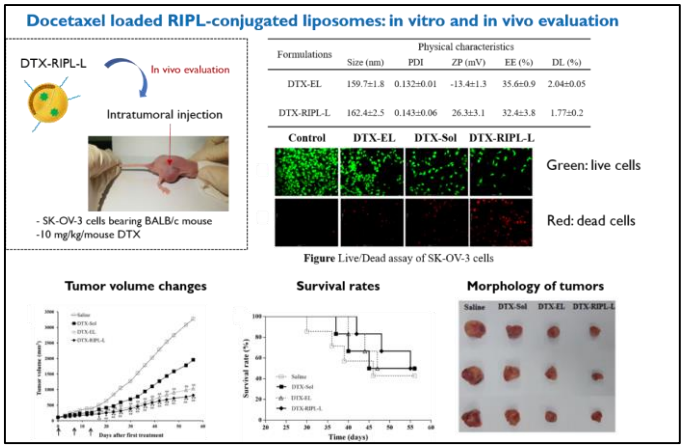
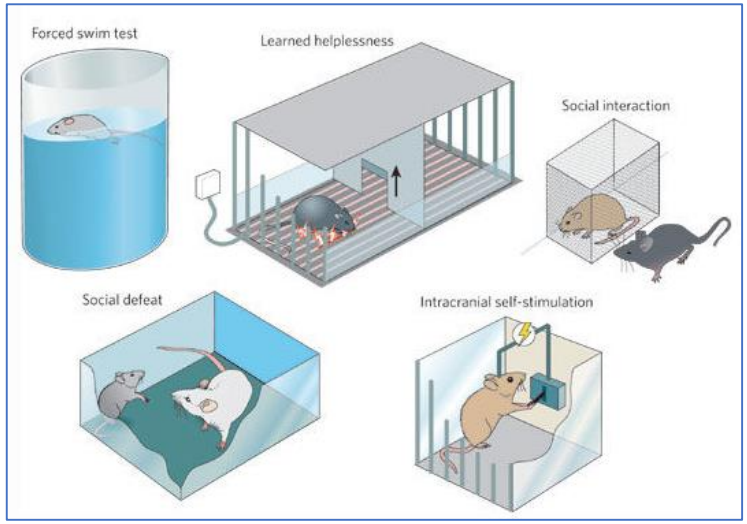
Neurotoxic assay with behavioral tests in animal model

- Learning and memory
- Recognition
- Social behavior

Introduction





**Animal model >>> Cognitive, Social, Sensorimotor…

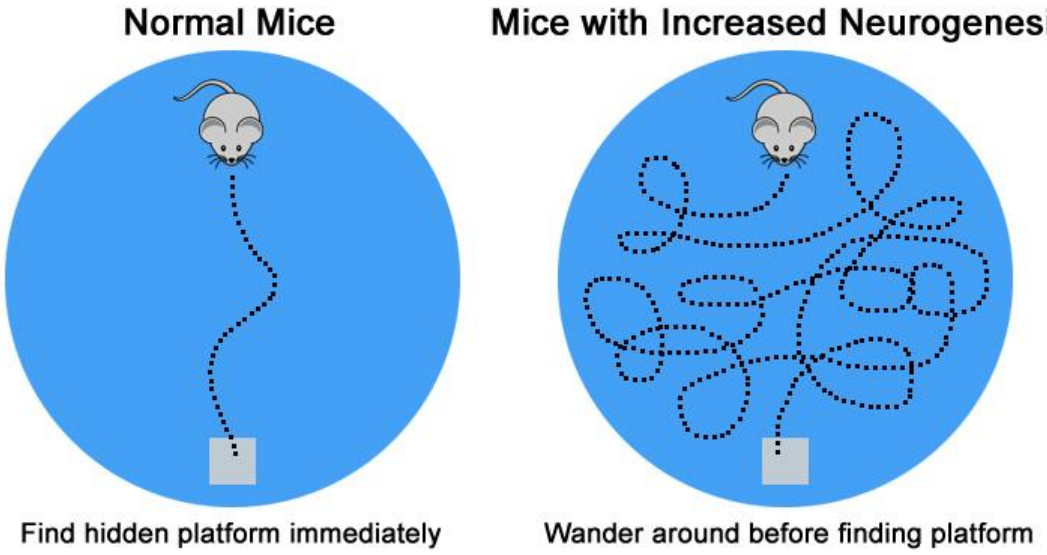
Cognitive:
Learning and memory

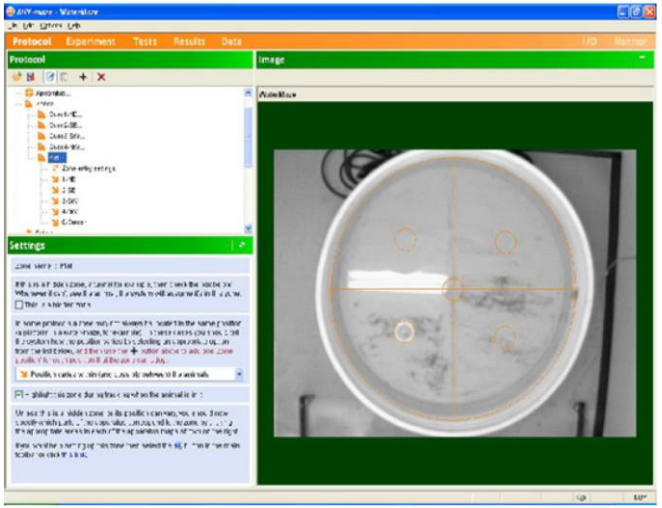
- Morris water maze
- Passive avoidance task
- Y-maze test
- Novel object recognition test
- Water finding test
- Conditioned fear learning test

- Three chamber test → Social :
General sociability

Morris water maze

- Established in 1981 by Richard G Morris (neuroscientist)
- Hippocampal-dependent learning - acquisition of spatial memory and long-term spatial memory
- Neurobiology and neuropharmacology of spatial learning and memory
- Neurocognitive disorders such as Alzheimer's disease
- Observing the subject's ability to find a hidden platform in an opaque water tank





J. Vis. Exp. (53), e2920, doi:10.3791/2920 (2011).

http://www.jove.com/video/2920

Methodological considerations

Animals

- Sex differences
  - Male vs Female
  - Sex hormones (Testosterone, Estradiol..)
- Species and strain differences
  - Mouse vs Rat
  - Between strains
  - Differences in MWM learning ability
  - Age → Age-related decline in spatial learning abilities
- Nutrition
- Stress and infection

Collected parameters for analysis

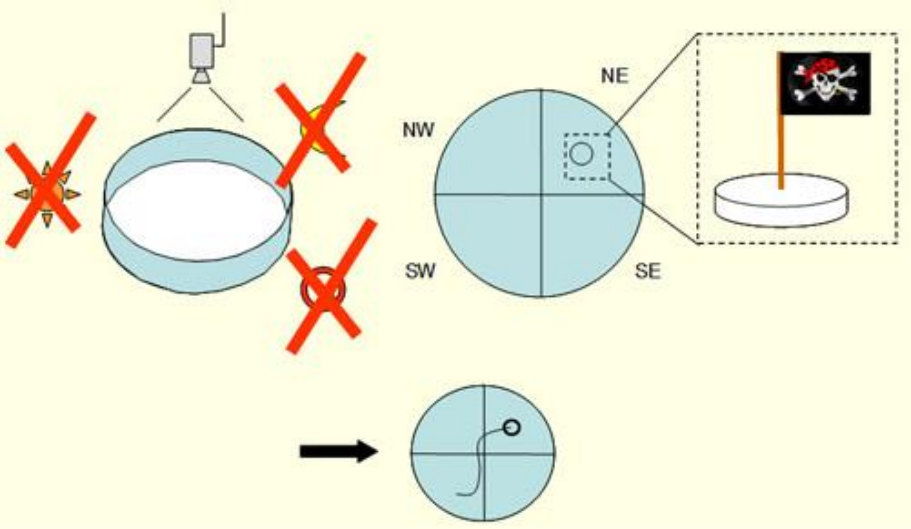
- Distance moved
- Mean velocity
- Time in each quadrant
- Percent time in each quadrant
- Escape latency
- Thigmotaxis

Applications

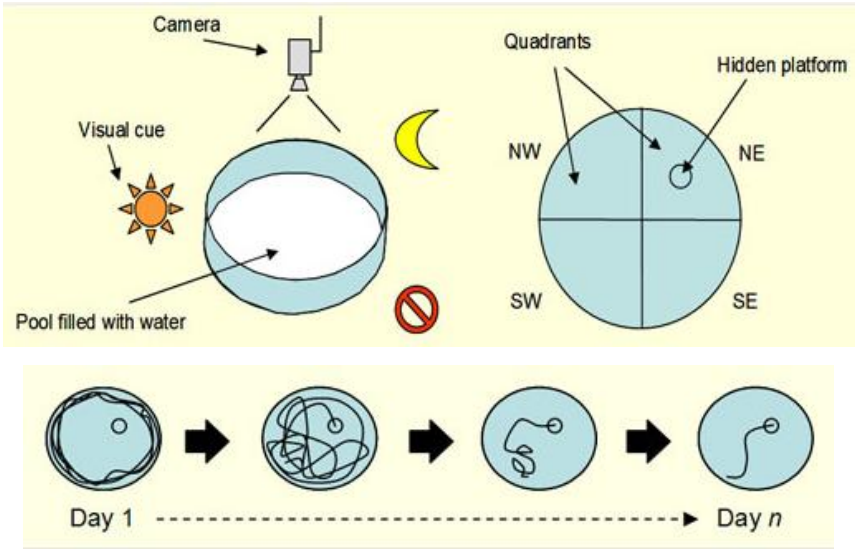
- Assessment of models for neurocognitive disorders
  - Cerebral vascular disease
  - Neurotrauma
  - Developmental disorders
  - Metabolic disorders with cognitive complications
  - Alzheimer's disease
  - AIDS dementia complex
  - Miscellaneous disorders with cognitive complications
- Behavioral, pharmacological, neurosurgical interventions

Procedure

Visible platform



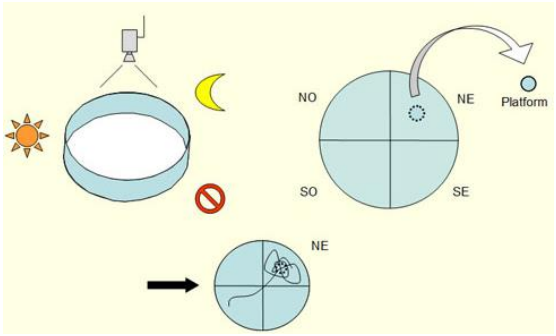
Hidden platform



- To assess nonspatial learning (independent of hippocampal function)
- To rule out the possibility that the spatial learning deficits detected might actually be a product of deficient escape motivation or impairment of vision and/or motor skills
- The platform location is indicated by a marker rising above the water (Visual cues)

- Place navigation → Learn to swim from any starting position
- Acquiring a long-term memory of the platform's spatial location
- Platform is rendered invisible by rendering the water opaque
- Time elapsed and/or the distance traversed to reach the hidden platform
- Various objects or geometric images are often placed in the testing room or hung on the wall
- Animals can use these visual cues as a means of navigating in the Maze
- Each subsequent entry → More efficient at locating the platform

Probe test



- Animal's tendency to persist around the platform's previous location
- "Measure of retention"
- Hidden Platform test

  → Removal of platform from the pool to measure spatial bias
- By measuring the time and distance traveled in each of the four quadrants
- Percentage of the total time elapsed

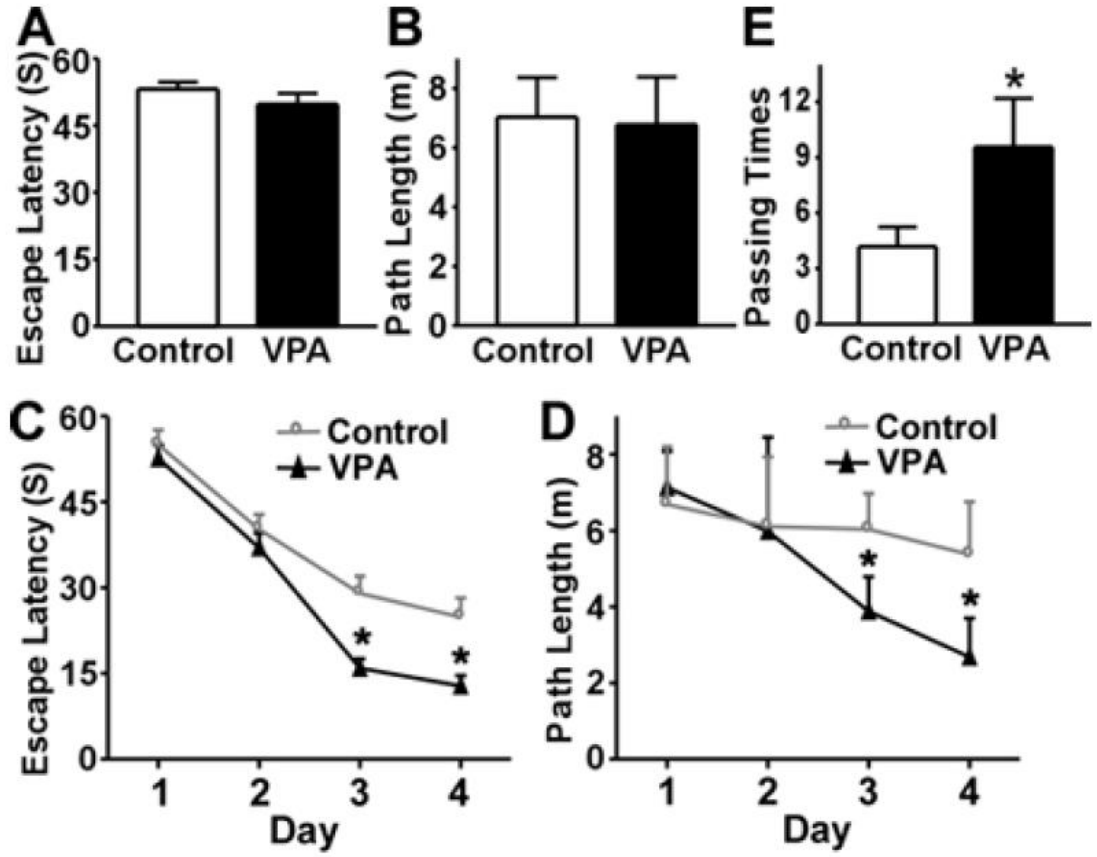
Reference memory test

- Last learning trial → Probe trial
- Cannot differentiate between short- and long-term memory
- Long interval between the last training trial and probe trail

  >>> Independent of memory of the last training

Working memory test

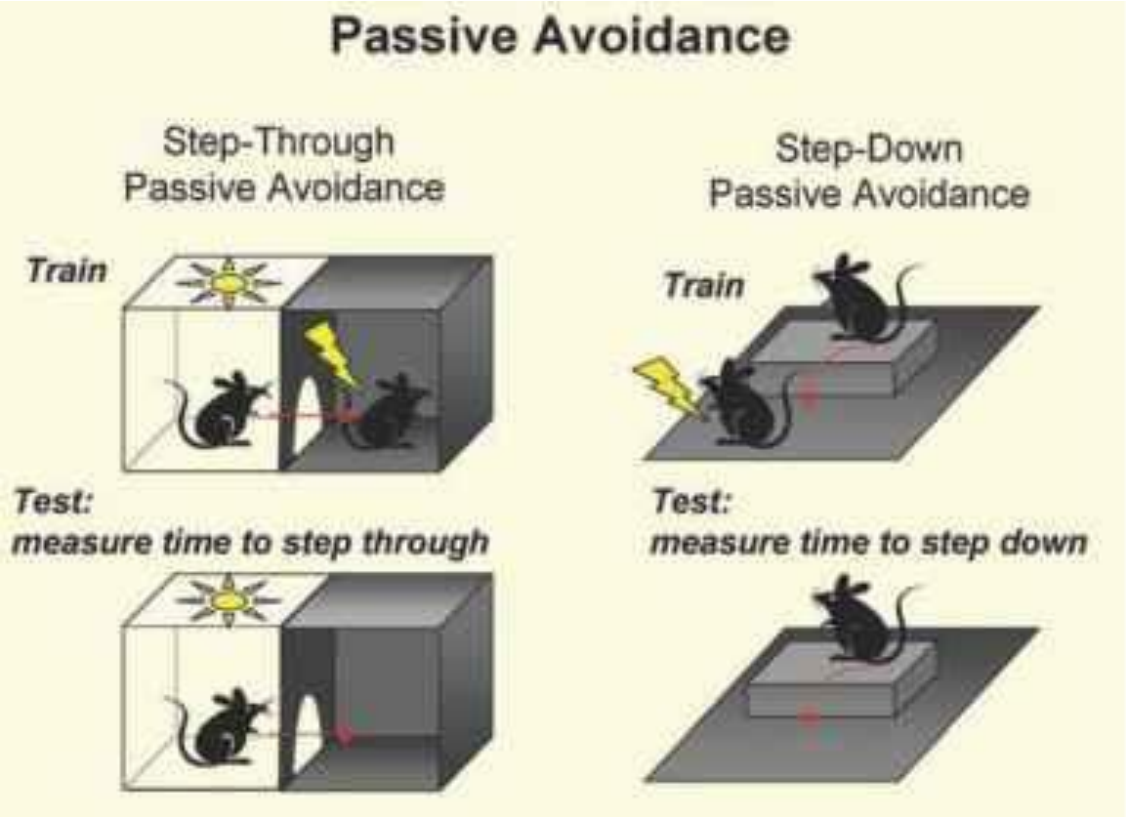
- Platform is relocated every day and animal is given two trials
- Trial 1 (sample) ---interval--- Trial 2 (test)



J. Vis. Exp. (53), e2920, doi:10.3791/2920 (2011).

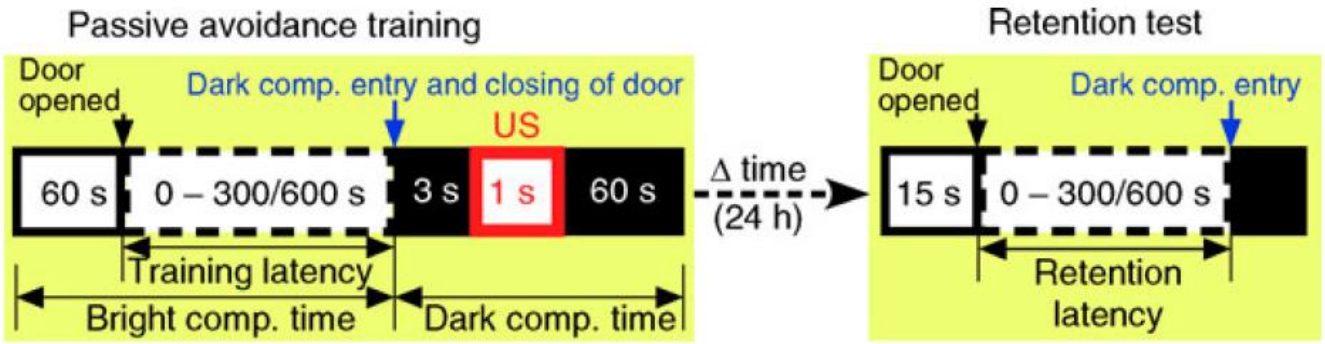
Figure 3. Representative results for the Morris Water Maze. The 7-month APP23 transgenic mice carrying human Swedish mutant APP gene were tested after one month of daily VPA (n=30 mice) or vehicle solution (n=30 mice) injections. **(A)** During the first day of visible platform tests, the VPA treated and control APP23 mice exhibited a similar latency to escape onto the visible platform. P>0.05 by student's t-test. **(B)** The VPA-treated and control APP23 mice had similar swimming distances before escaping onto the visible platform in the visible platform test. P>0.05 by student's t-test. **(C)** In hidden platform tests, VPA treated APP23 mice showed a shorter latency to escape onto the hidden platform on the 3rd and 4th day, P<0.001 by ANOVA. **(D)** The VPA-treated APP23 mice had a shorter swimming length before escaping onto the hidden platform on the 3rd and 4th day, P< 0.01 by ANOVA. **(E)** In the probe trial on the 6th day, the VPA-treated APP23 mice traveled into the third quadrant, where the hidden platform was previously placed, significantly more times than controls. * P<0.005 by student's t-test. (Adapted and reprinted from *The Journal of Experimental Medicine 205*, 2781-2789, 2008, Rockefeller University Press, Originally published in J. Exp. Med. doi:10.1084/jem.20081588.) (6).

Passive avoidance task



- An aversive (emotional) conditioning paradigm
- Subject learns to associate a particular context with the occurrence of an aversive event
  (e.g., an electrical shock, the unconditioned stimulus)
- Defined as the suppression of the innate preference for the dark compartment of the test apparatus
  (or stepping down from an elevated platform) following exposure to an inescapable shock

Procedure and experimental parameters



Procedure Steps:

Training Trial:

1. The testing apparatus is a trough-shaped alley divided into two distinct compartments that are separated by a sliding door. The white, brightly lit compartment is free of aversive stimulation whereas the black, dark compartment is equipped with shock capability. The apparatus is cleaned with 70% ethanol before use.
2. The training trial begins by placing the animal in the white compartment facing the door.
3. The door is opened to allow access to the dark compartment.
4. The latency to enter the dark compartment is recorded.
5. When the animal steps into the dark compartment with all four paws, the door is closed and a 1-2 second footshock is delivered (0.2-0.5 mA shock, minimum required to elicit flinching and/or vocalization).
6. The animal remains in the dark compartment for an additional 10 seconds after the termination of the aversive stimulus, before being removed and placed back into its home cage.
7. The apparatus is cleaned with 70% ethanol in between animals.

Testing Trial:

1. At the time of the testing trial (usually 1-7 days after training), the animal is again placed inside the white compartment and the door is raised to allow access to the dark compartment.
2. The latency to re-enter the dark compartment is recorded; however, there is no aversive stimulus applied to animal upon re-entry into the dark compartment during testing.

Pre-experimental procedures	Housing Transfer to the experimental room Handling Timing of drug treatment
Experimental procedures	Timing of retention testing (short-term & long term) Current Waiting/delaying time before access to the dark compartment Delay between dark compartment entry and US exposure to avoid escaping the bright compartment
Experimental parameters	Transfer latencies detection Total time spent per compartment US response assessment Activity measurements Time of testing

Y maze test

- Working memory is a system for temporarily storing and managing the information required to carry out complex cognitive tasks such as learning, reasoning , and comprehension

- Spatial working memory was assessed by spontaneous alternation behavior in y maze

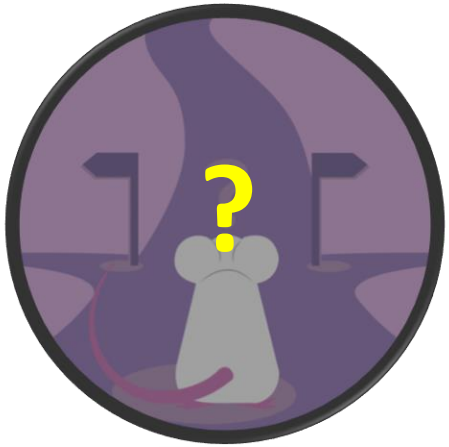
- Behavioral test for measuring the willingness of rodents to explore new environments

- Brain- hippocampus, septum, basal forebrain, and prefrontal cortex--are involved in this task.

- Y-shaped maze with three white, opaque plastic arms at a 120° angle from each other.
  35 cm x 7 cm x 10 cm (L x H x W)

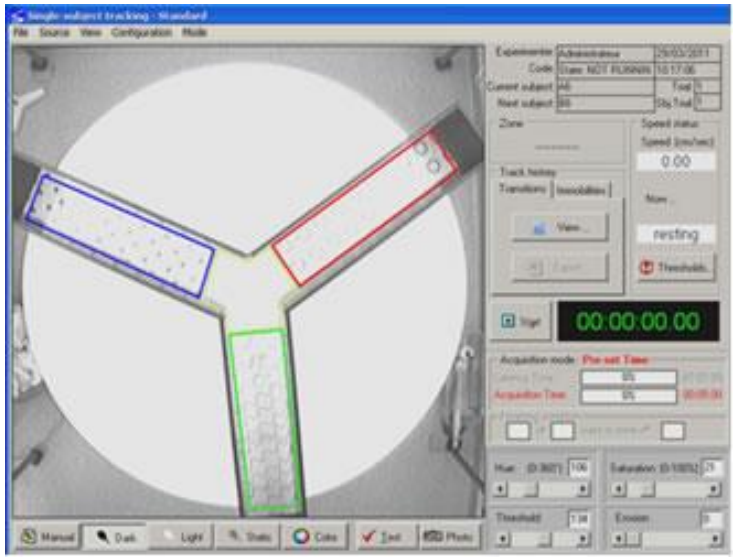
- After introduction to the center of the maze, the animal is allowed to freely explore the three arms.

- An entry occurs when all four limbs are within the arm.

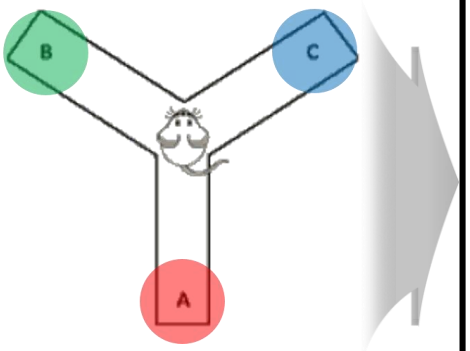


The animal's fitness is described by
- Total track length
- Average velocity
- Latency for each zone's first visit

Parameters are calculated for each maze arm
- Latency till first visit
- Total number of visits
- Summed up duration of all visits
- Percentage of duration
- Percentage of counted visits



https://youtu.be/WNx72ebsNLE



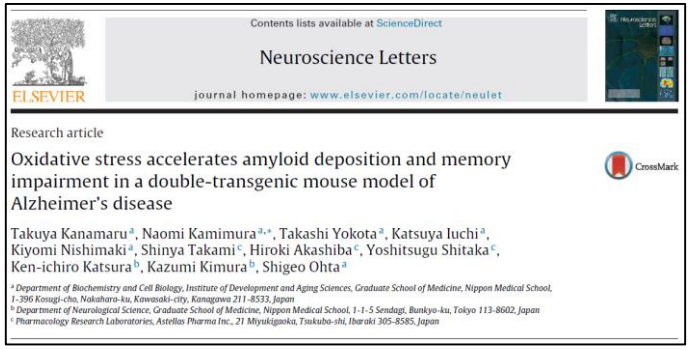
Alternate Arm Returns (AAR)

B B A B A B A C A B C B

Same Arm Returns (SAR)

Spontaneous Alteration Performance (SAP)

**AAR, SAR → Memory impairment



Contents lists available at ScienceDirect

Neuroscience Letters

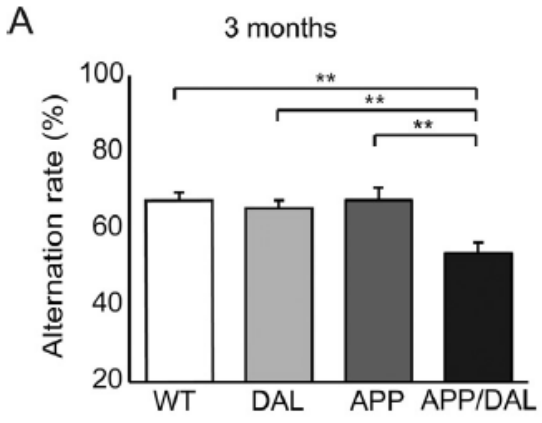
journal homepage: www.elsevier.com/locate/neulet

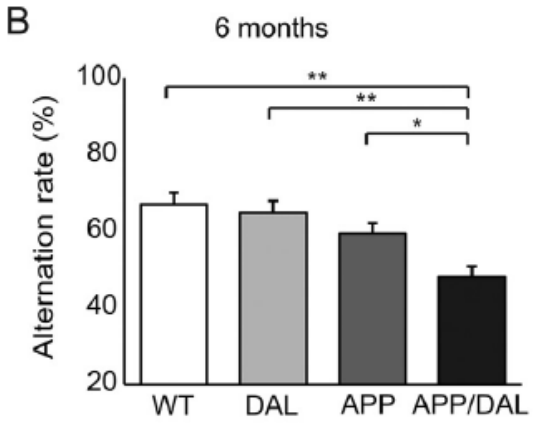
Research article

Oxidative stress accelerates amyloid deposition and memory impairment in a double-transgenic mouse model of Alzheimer's disease

Takuya Kanamaru[a], Naomi Kamimura[a,*], Takashi Yokota[a], Katsuya Iuchi[a], Kiyomi Nishimaki[a], Shinya Takami[c], Hiroki Akashiba[c], Yoshitsugu Shitaka[c], Ken-ichiro Katsura[b], Kazumi Kimura[b], Shigeo Ohta[a]

[a] Department of Biochemistry and Cell Biology, Institute of Development and Aging Sciences, Graduate School of Medicine, Nippon Medical School, 1-396 Kosugi-cho, Nakahara-ku, Kawasaki-city, Kanagawa 211-8533, Japan
[b] Department of Neurological Science, Graduate School of Medicine, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan
[c] Pharmacology Research Laboratories, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan

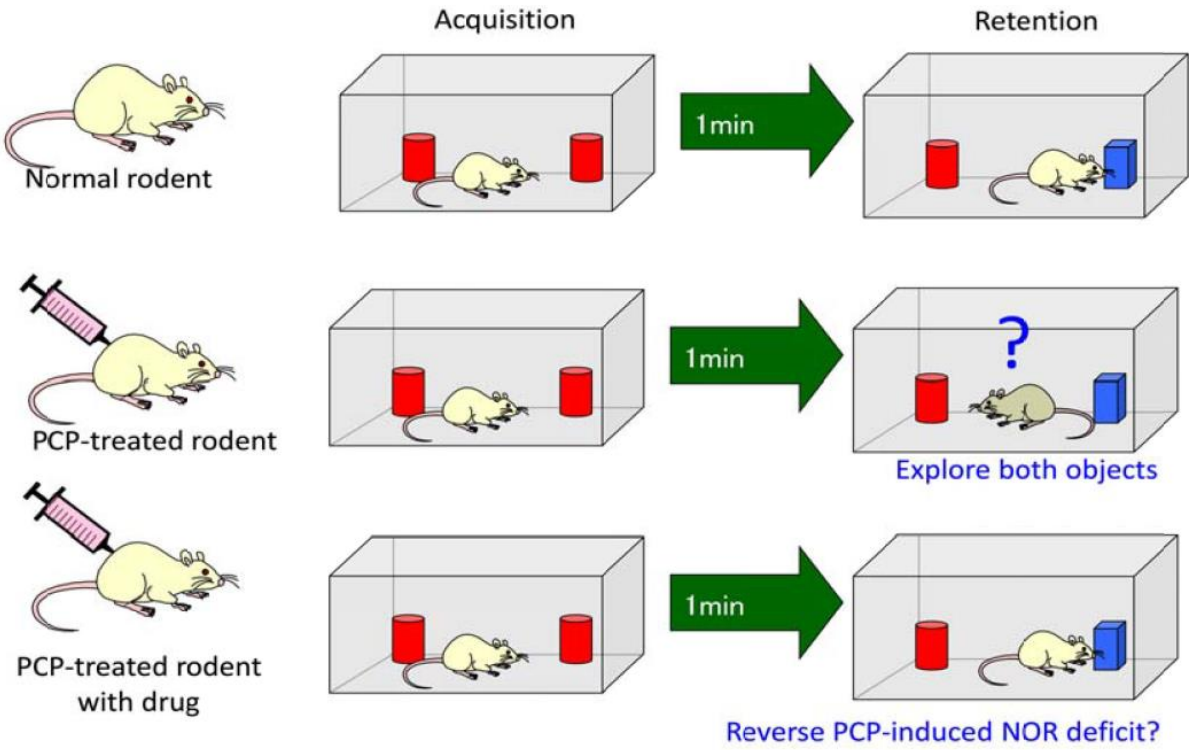




Neuroscience Letters 2015, 587, 126–131

- DAL : Dominant-negative mutant of ALDH2 (HNE detoxification)
- APP : Human amyloid precursor protein >>> AD model
- APP/DAL
- Oxidative stress → Spatial learning and memory impairments in APP mice with aging

Novel object recognition test

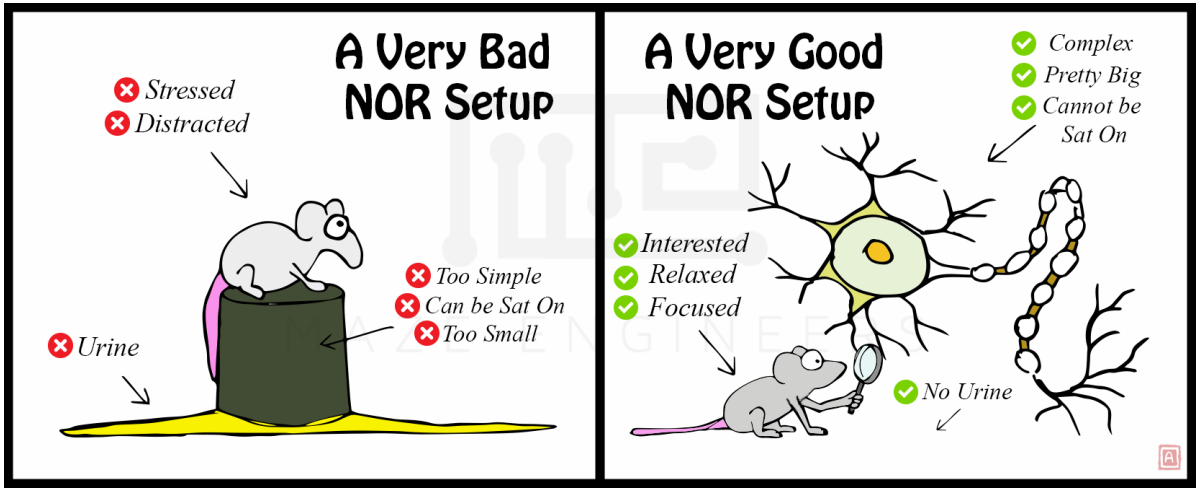


Schizophrenia
PCP(Phencyclidine)
NMDAR non-competitive antagonist

- External motivation, reward, training, punishment X
- Short-, intermediate-, long-term memory
- Familiarization session - Interval - Test session
- Based on the innate preference of rodent

  → To explore the novel object rather than the familiar one

- Rodent that remembers the familiar object will spend more time exploring the novel object



Directing the nose

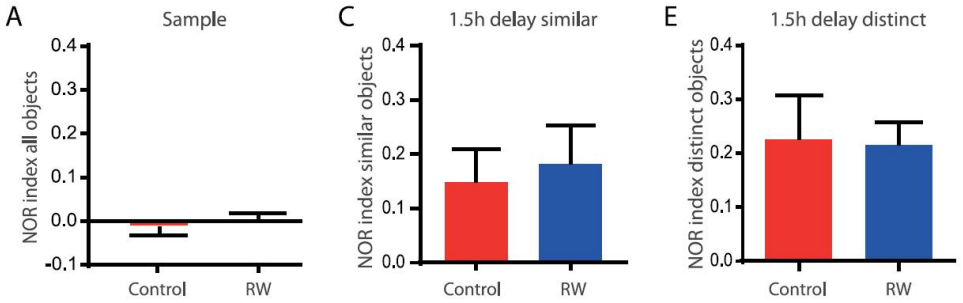
+

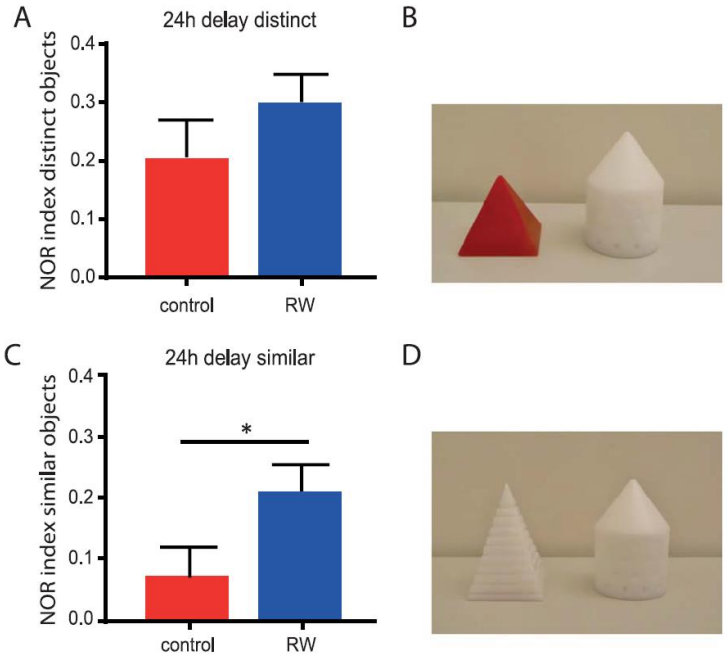
At a distance less than or equal to 2 cm

↓

Sniffed the object

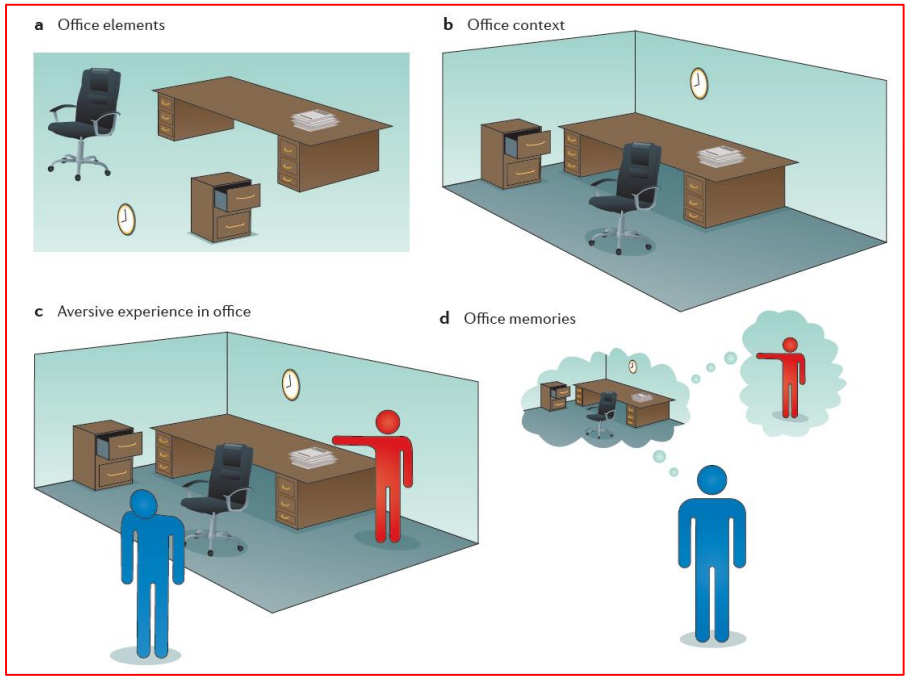
or touched the object while looking at it

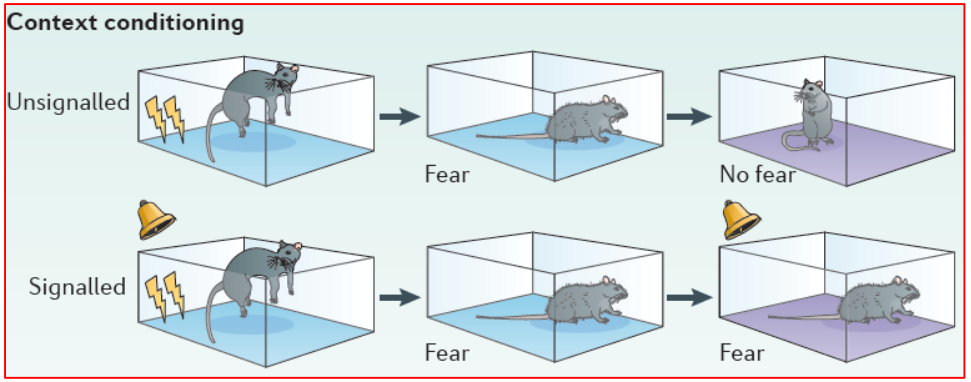




Brain Plasticity, 2015, 1, 129–141

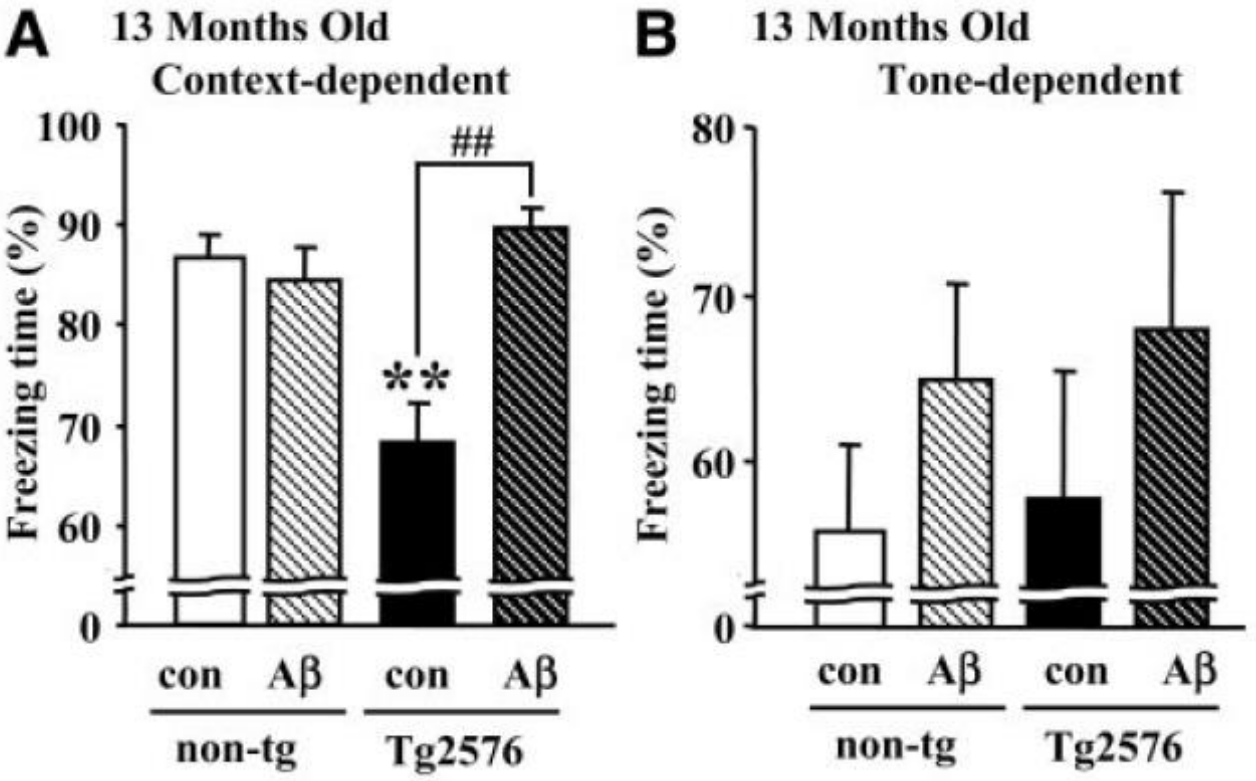
Conditioned fear learning test





Conditioned-fear learning test of Tg2576 mice

FASEB Journal, 2007, 21, 2135-2148

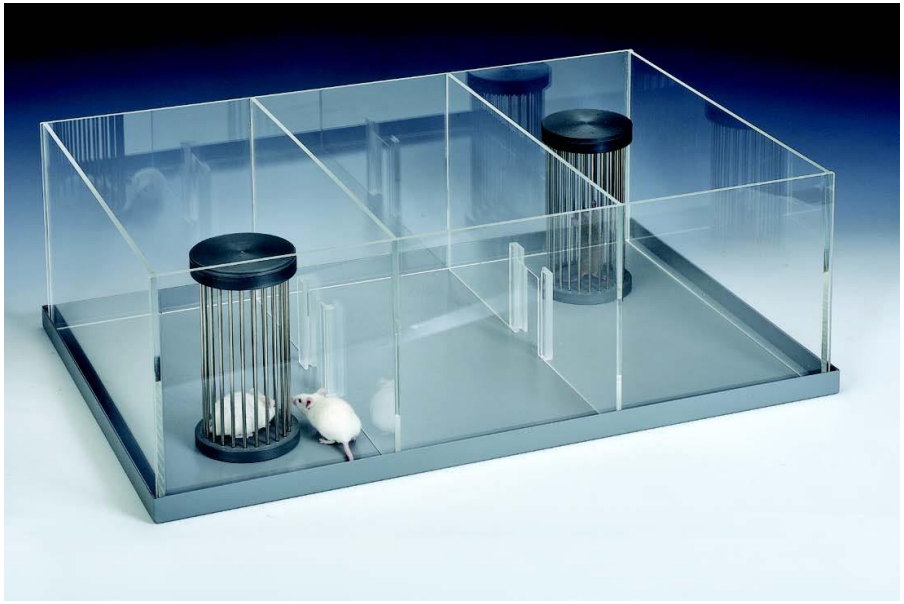


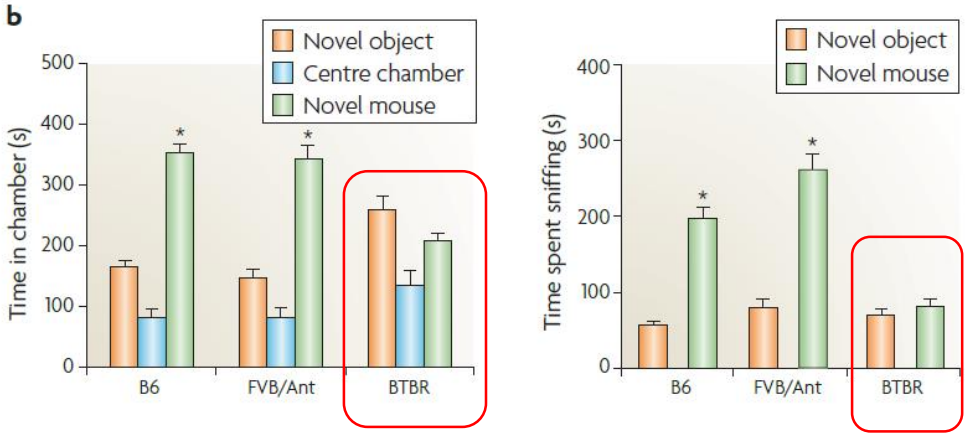
Context-dependent

Cue-dependent

Non-tg, nontransgenic mice
Tg2576, APP transgenic mice
Aβ, AAV/Aβ vaccination

Three chamber test





Nature reviews: Neuroscience, 2010, 11, 490-502

- Alterations in social behavior → symptom of several neuropsychiatric and neurologic disease
- To measure social approach behavior

  Mouse → into the middle chamber and allowed to explore the other compartments.

  Docile stimulus mouse is situated in a mesh-wire container,

  Adjacent compartment a similar container is located without stimulus mouse (object compartment)

  The tendency to approach or avoid the compartment with the stimulus

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-019-09382-9 OPEN

Abnormal mGluR-mediated synaptic plasticity and autism-like behaviours in *Gprasp2* mutant mice

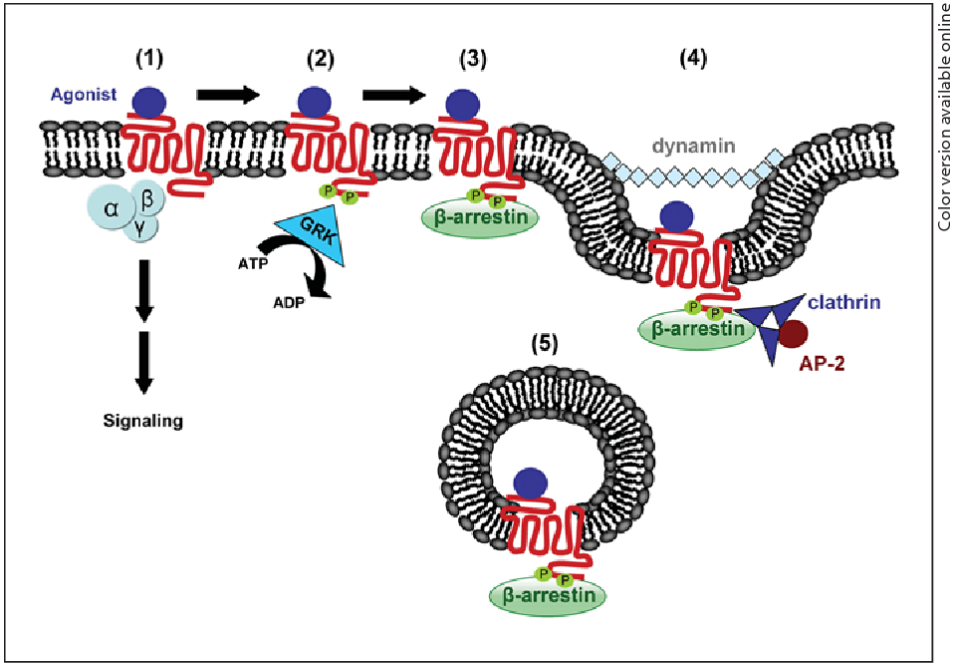
Mohamed Edfawy[1,2,3], Joana R. Guedes[1,2], Marta I. Pereira[1], Mariana Laranjo[1], Mário J. Carvalho[1], Xian Gao[4,5,6], Pedro A. Ferreira[1], Gladys Caldeira[1,2], Lara O. Franco[1,2,3], Dongqing Wang[4], Ana Luisa Cardoso[1,2], Guoping Feng[4,5,6], Ana Luisa Carvalho[1,7] & João Peça[1,2]

Gprasp : G-protein-coupled receptor associated sorting proteins

- interact and regulate trafficking of GPCRs : delta opioid receptor, D2 dopamine receptor, muscarinic receptors, and mGluR1 and mGluR5 receptors

Gprasp2

- GPRASP2 mutations in autism and schizophrenia patients
- downregulation of this gene autism patients



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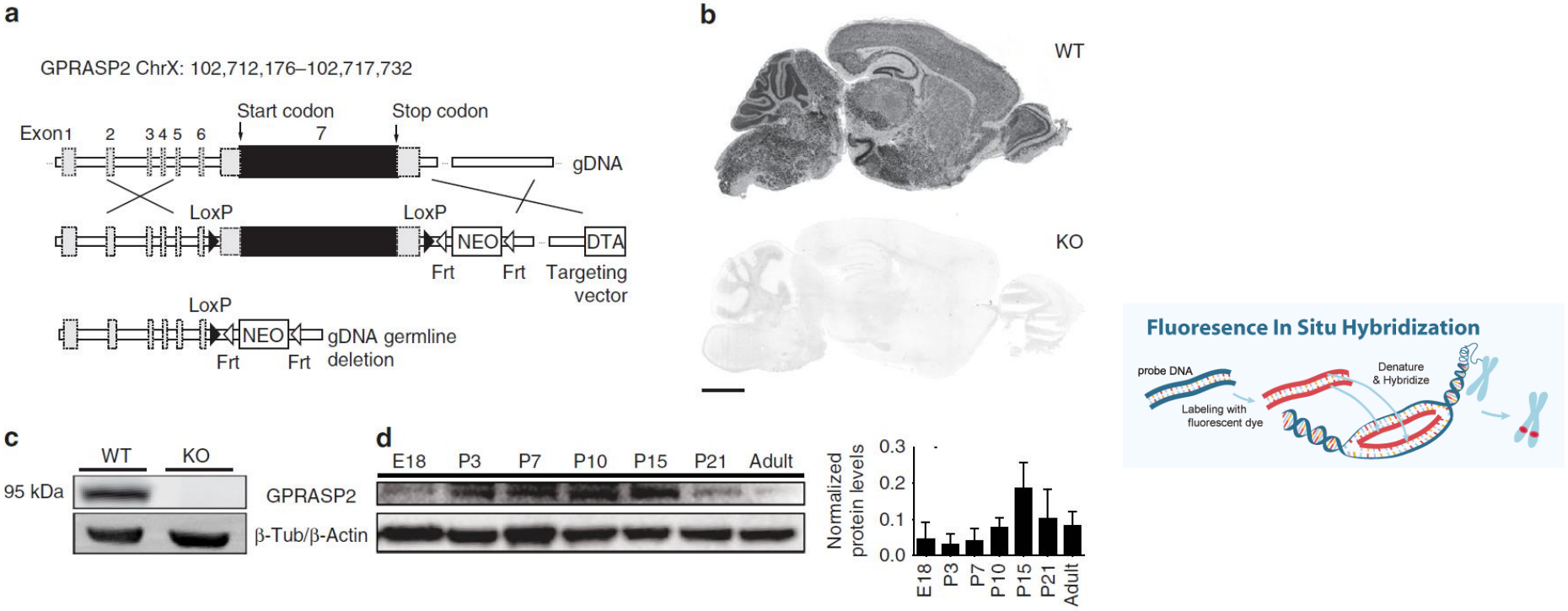
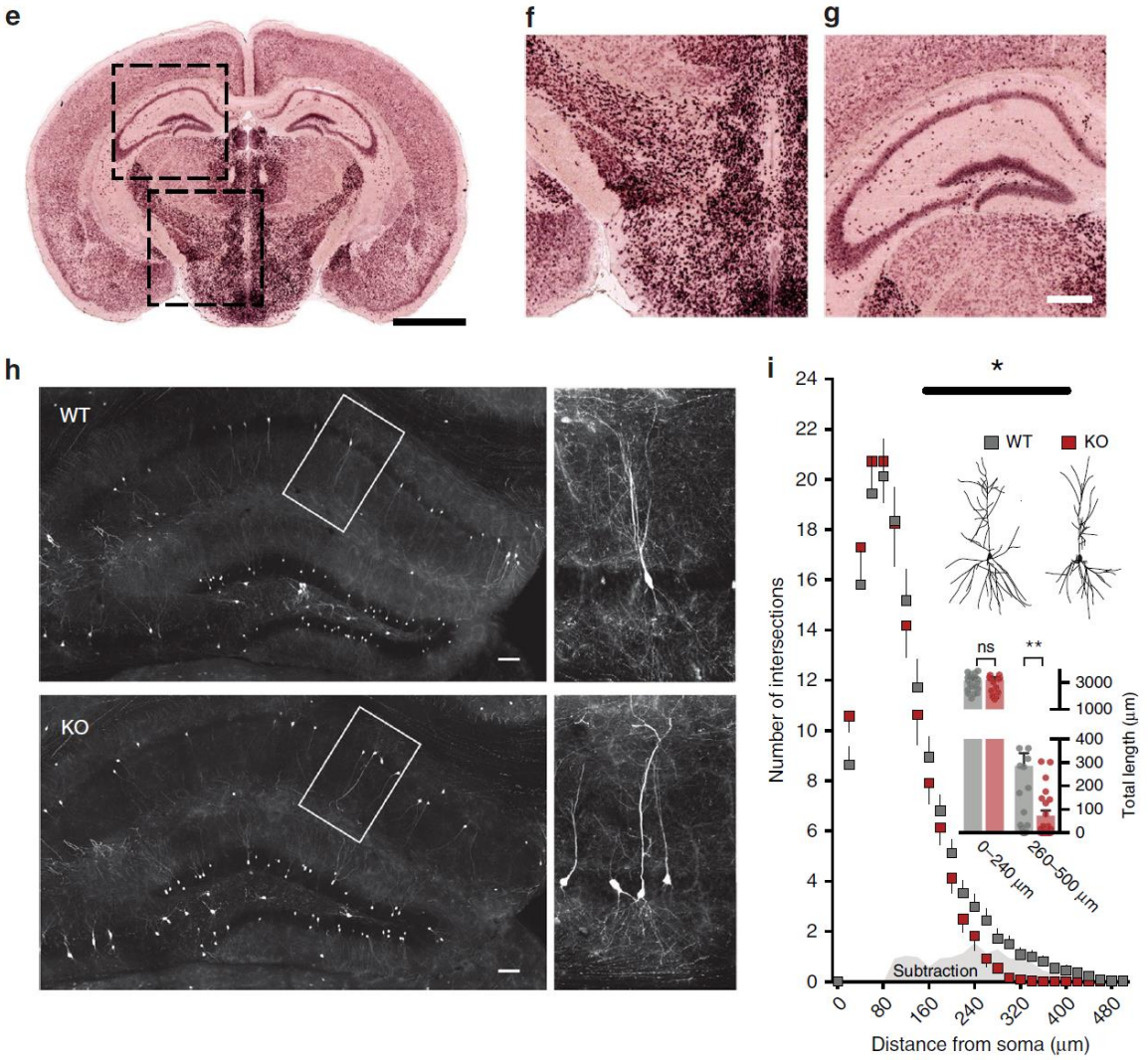


Fig. 1 Gprasp2 knockout mice display structural alterations in hippocampal neurons.



in vivo Golgi-like labelling, via peripheral injection of AAV9.hSyn.GFP

Fig. 1 Gprasp2 knockout mice display structural alterations in hippocampal neurons.

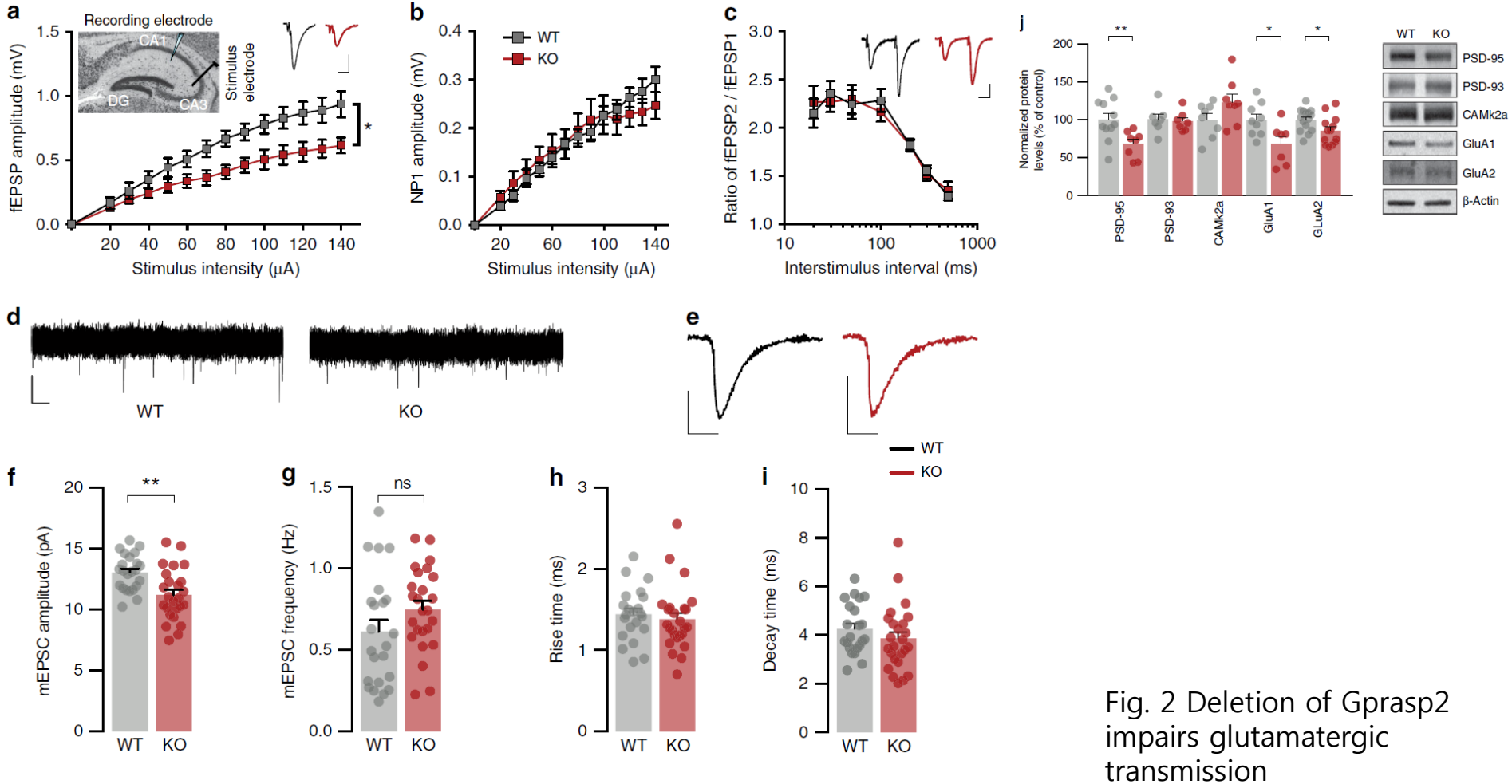


Fig. 2 Deletion of Gprasp2 impairs glutamatergic transmission

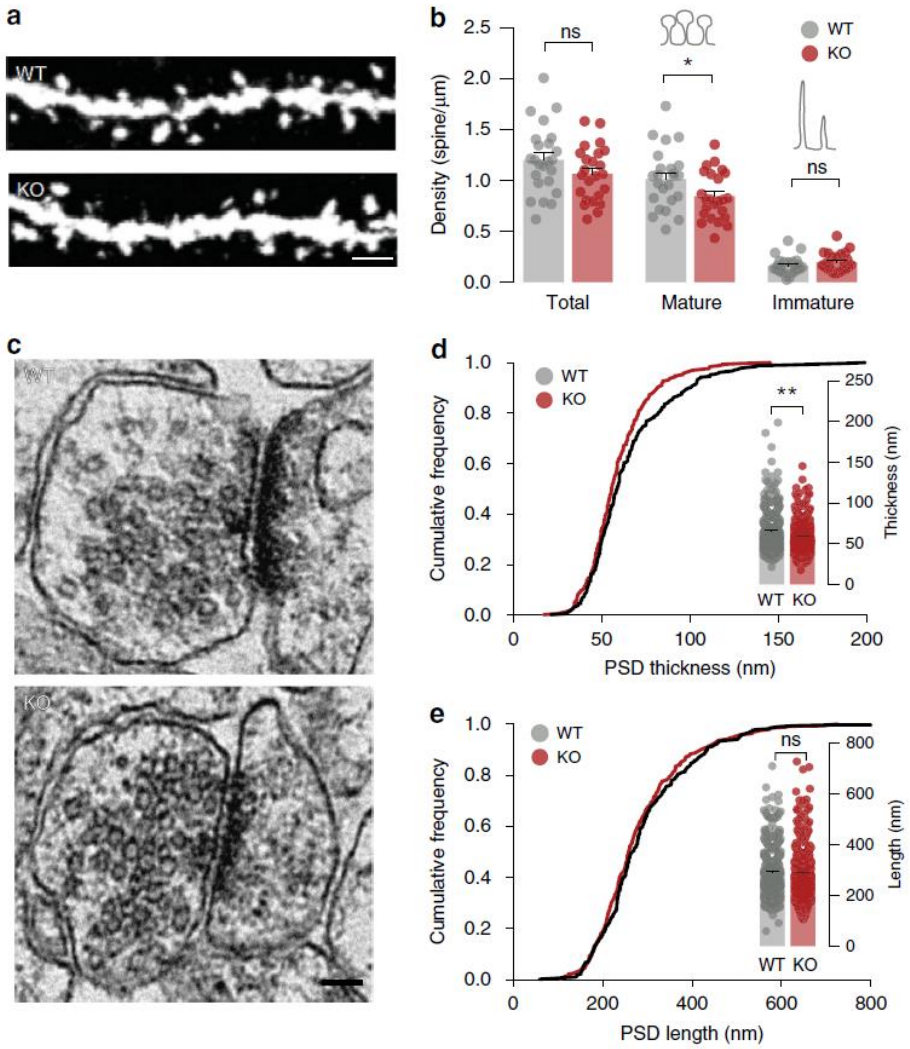


Fig. 3 Impaired spine maturation in hippocampal spines in Gprasp2 KO mice.

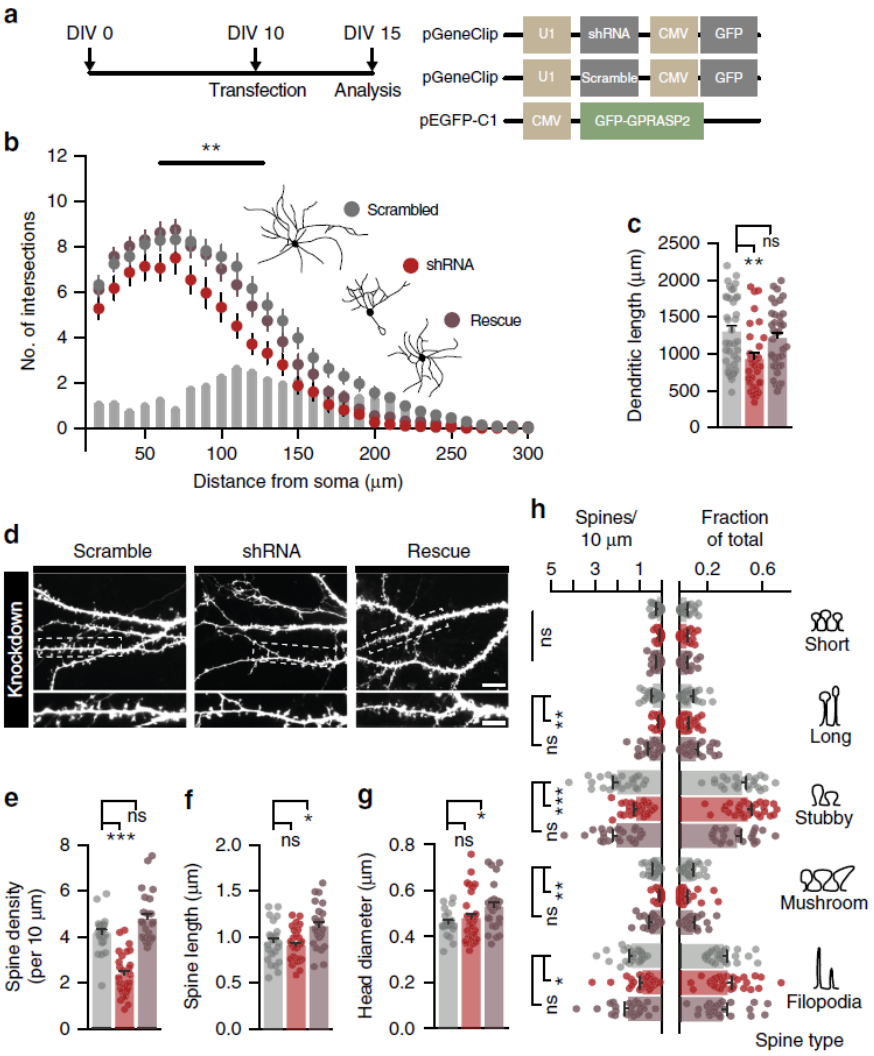


Fig. 4 Cell-autonomous reduction in dendritic complexity and spine density in acute GPRASP2 knockdown.

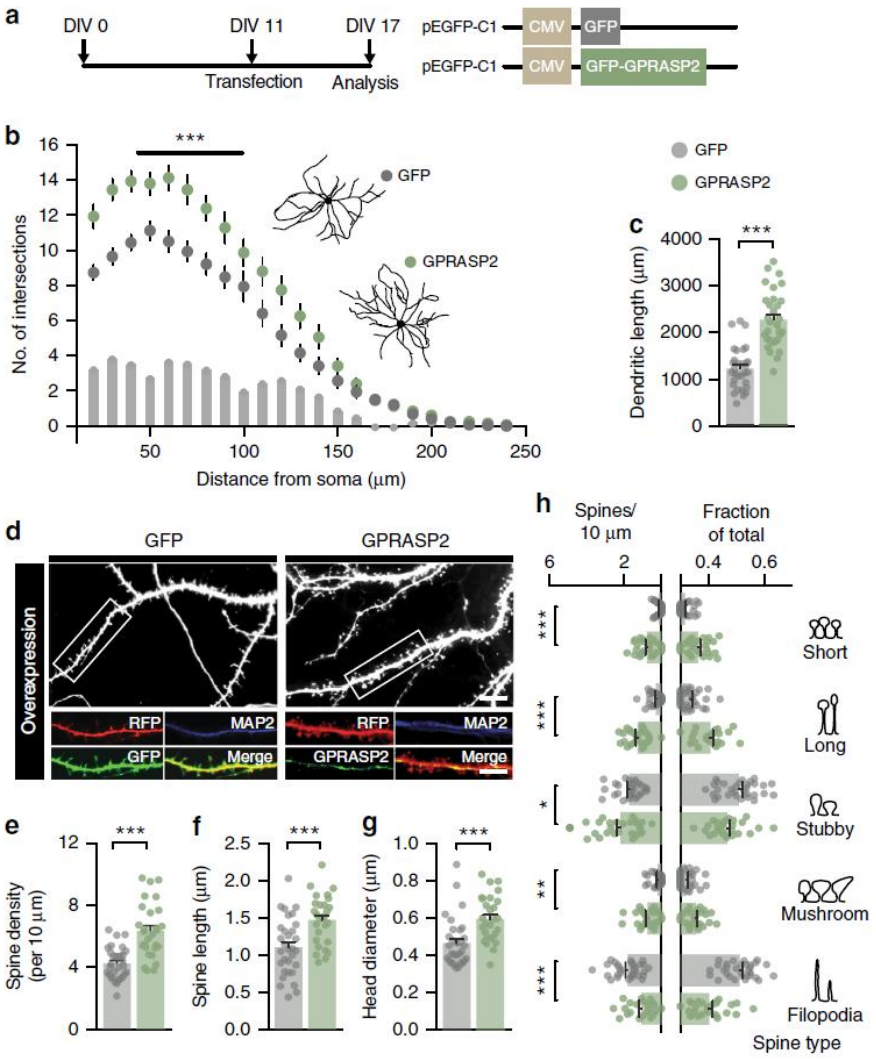
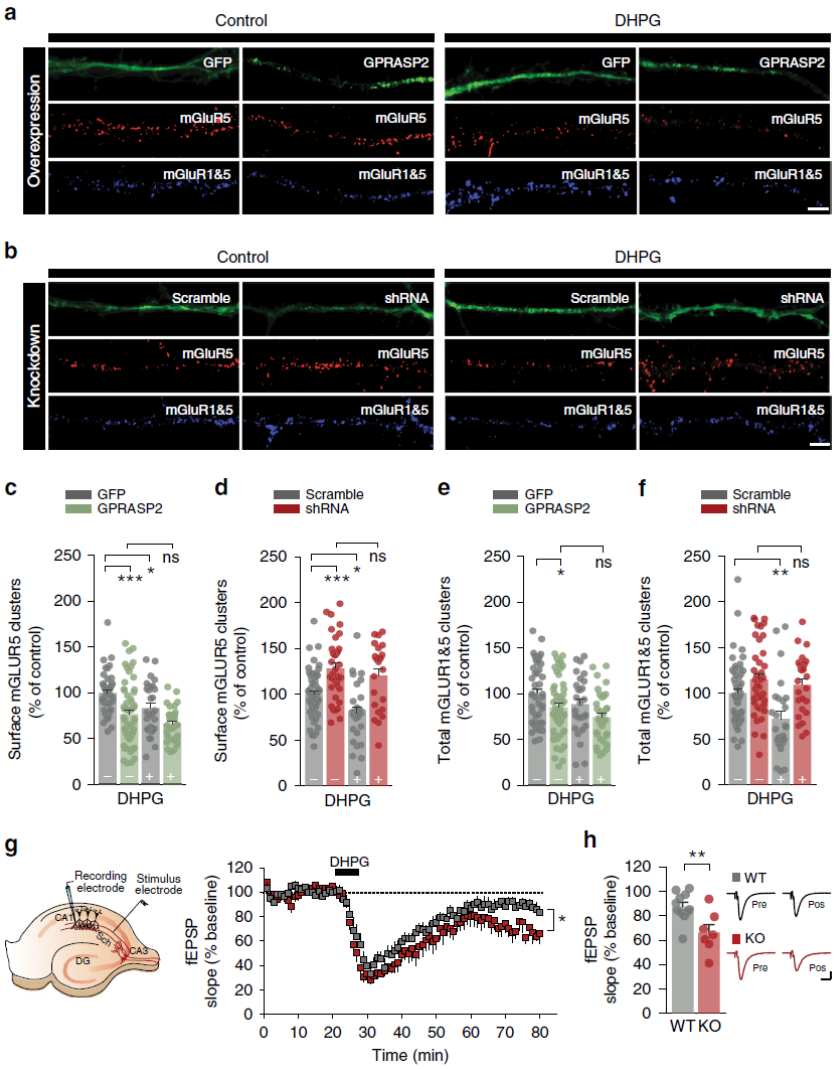


Fig. 5 GPRASP2 overexpression induces cell autonomous increased in spine density and spine maturation.



3,5-Dihydroxyphenylglycine (DHPG) : mGluR5 receptor agonist

MPEP : a selective antagonist for mGluR5

- GPRASP2 ->

Activity dependent internalization of mGluR5 (decrease on surface)->
LTD increase ->
memory impairment

Fig. 6 Bidirectional regulation of mGluR5 surface availability and enhanced mGluR-LTD in Gprasp2−/y mice.

Barnes Maze test

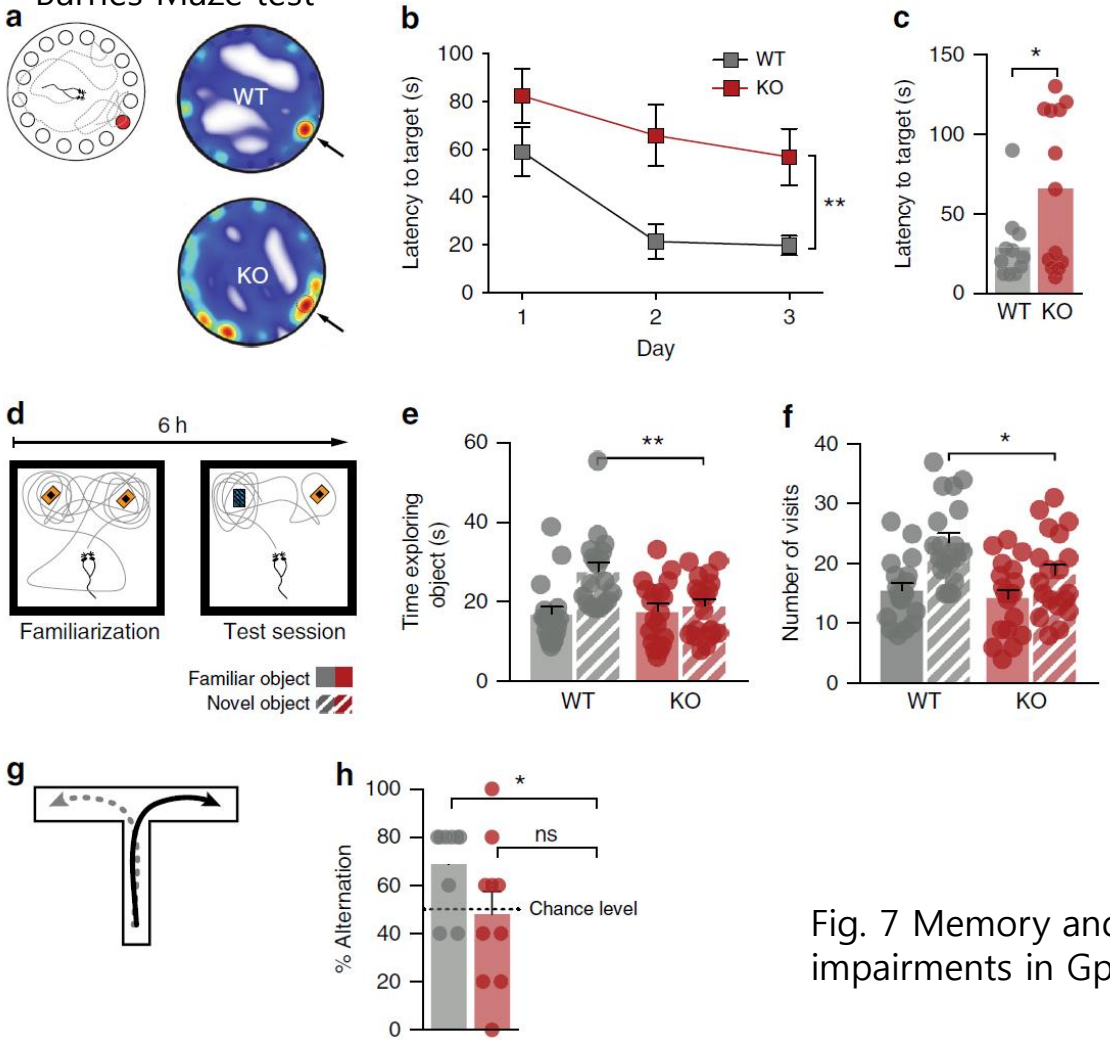


Fig. 7 Memory and learning impairments in Gprasp2−/y mice.

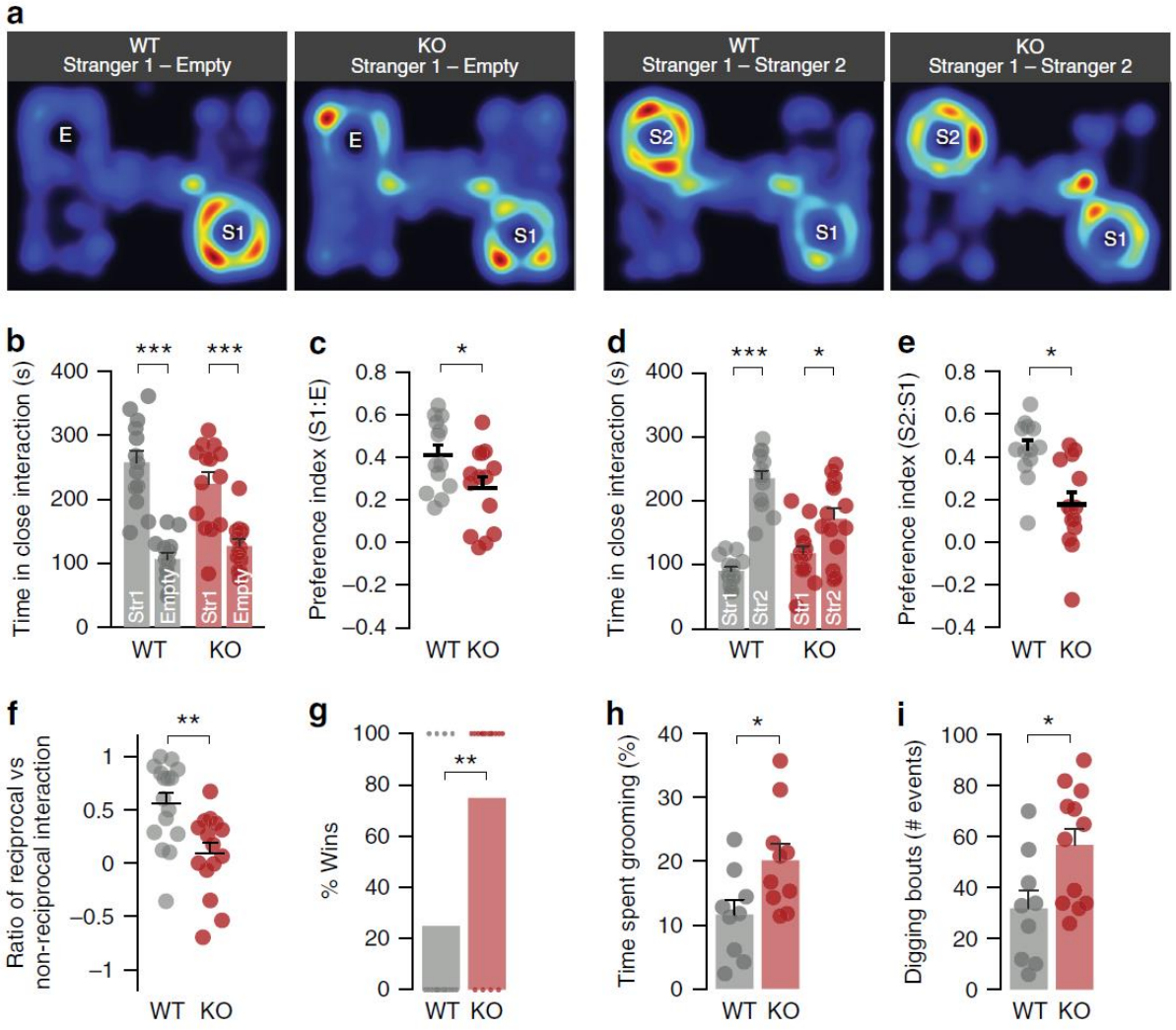


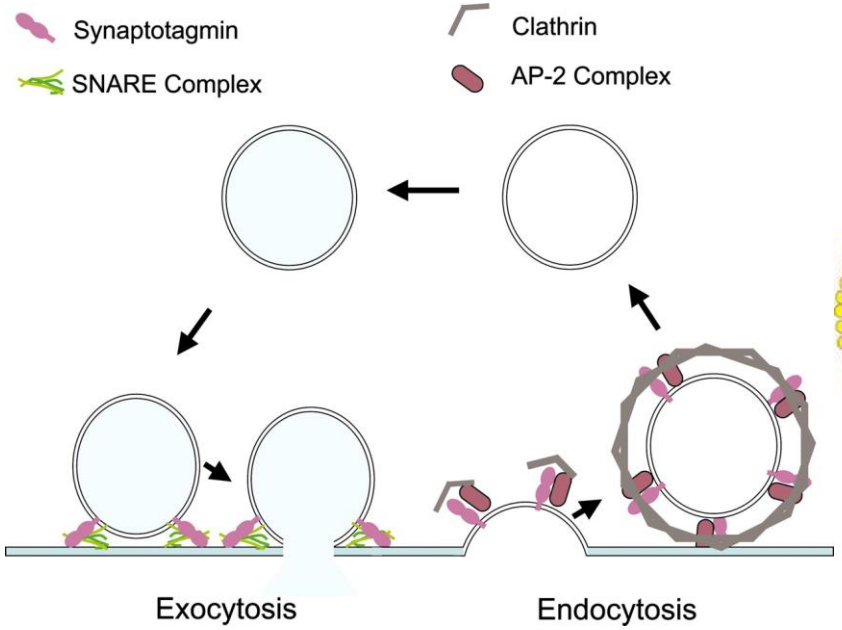
Fig. 8 Gprasp2−/y mice display social and ASD-like behavioural alterations.

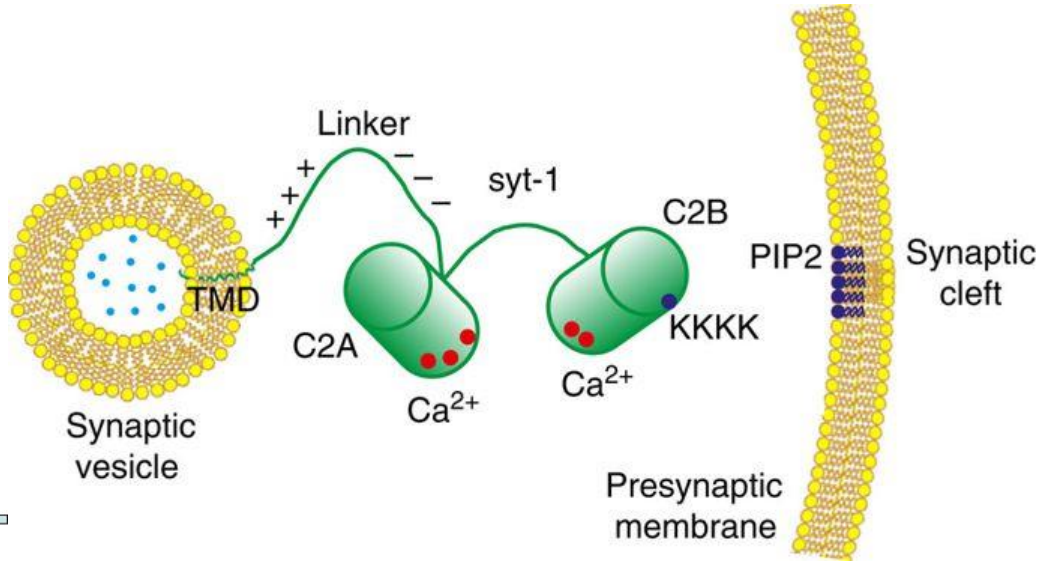
RESEARCH ARTICLE SUMMARY

NEUROSCIENCE

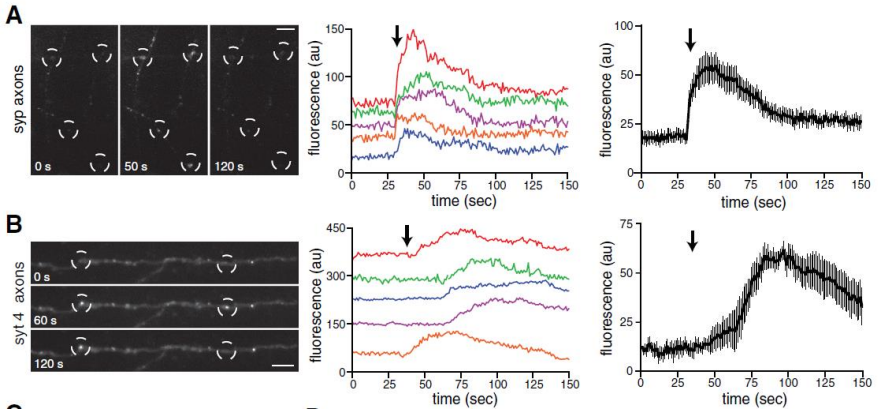
Synaptotagmin-3 drives AMPA receptor endocytosis, depression of synapse strength, and forgetting

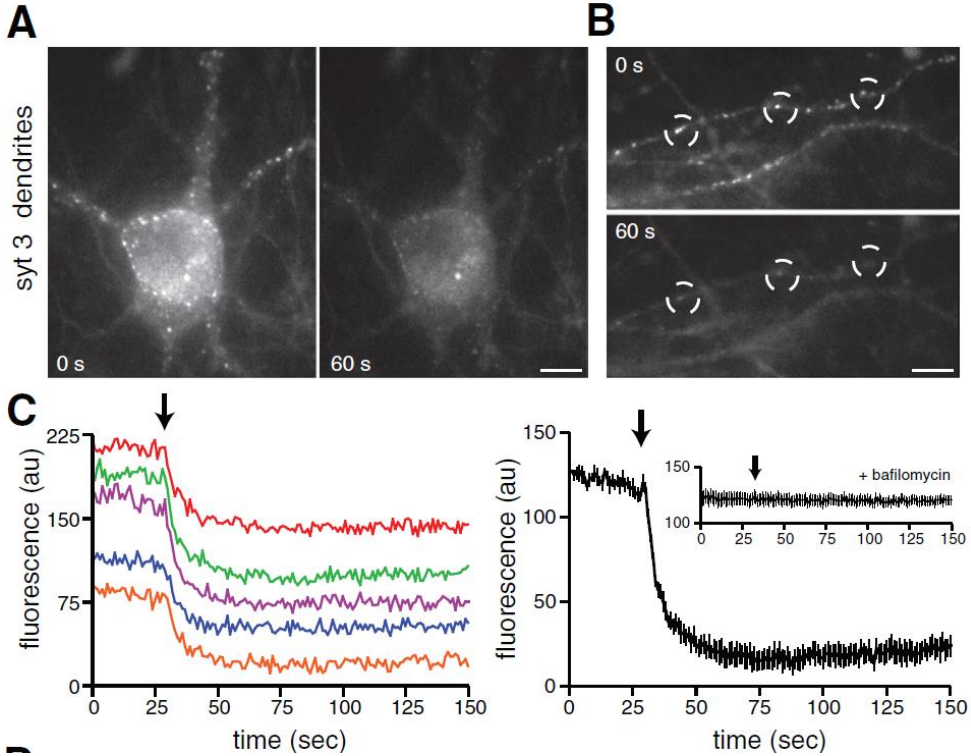
Ankit Awasthi*, Binu Ramachandran*, Saheeb Ahmed, Eva Benito, Yo Shinoda, Noam Nitzan, Alina Heukamp, Sabine Rannio, Henrik Martens, Jonas Barth, Katja Burk, Yu Tian Wang, Andre Fischer, Camin Dean†





Nat Commun. 2014 Dec 15;5:5859.





Mol Biol Cell. 2012 May;23(9):1715-27

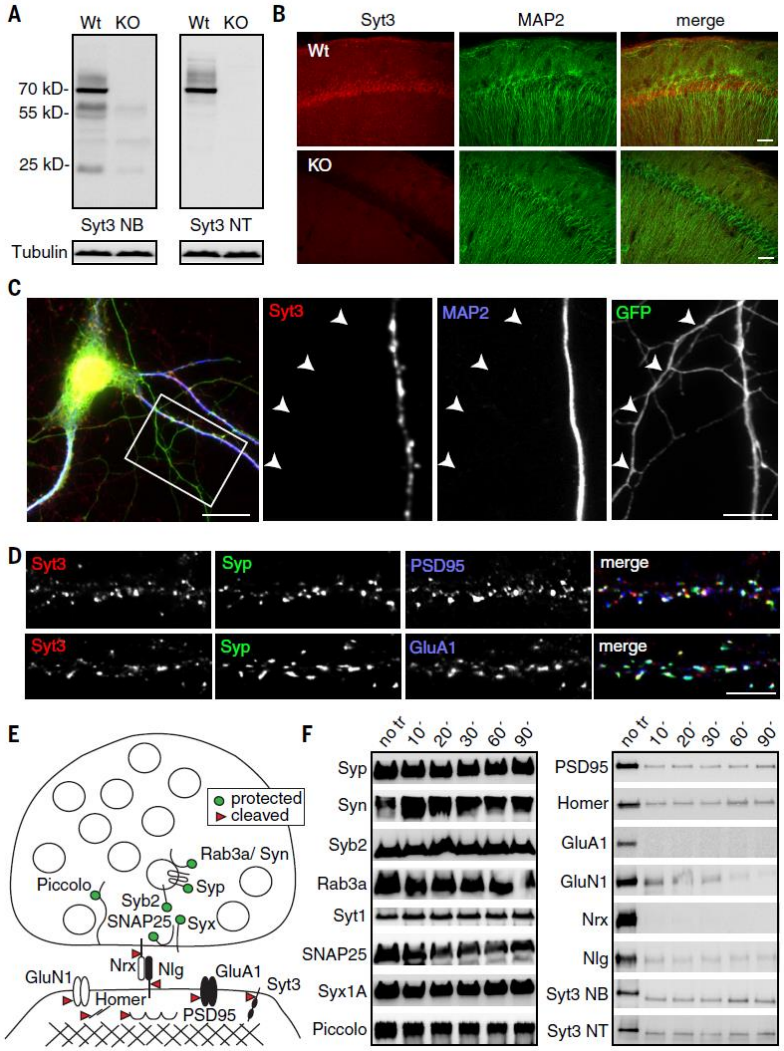


Fig. 1. Syt3 is postsynaptic.

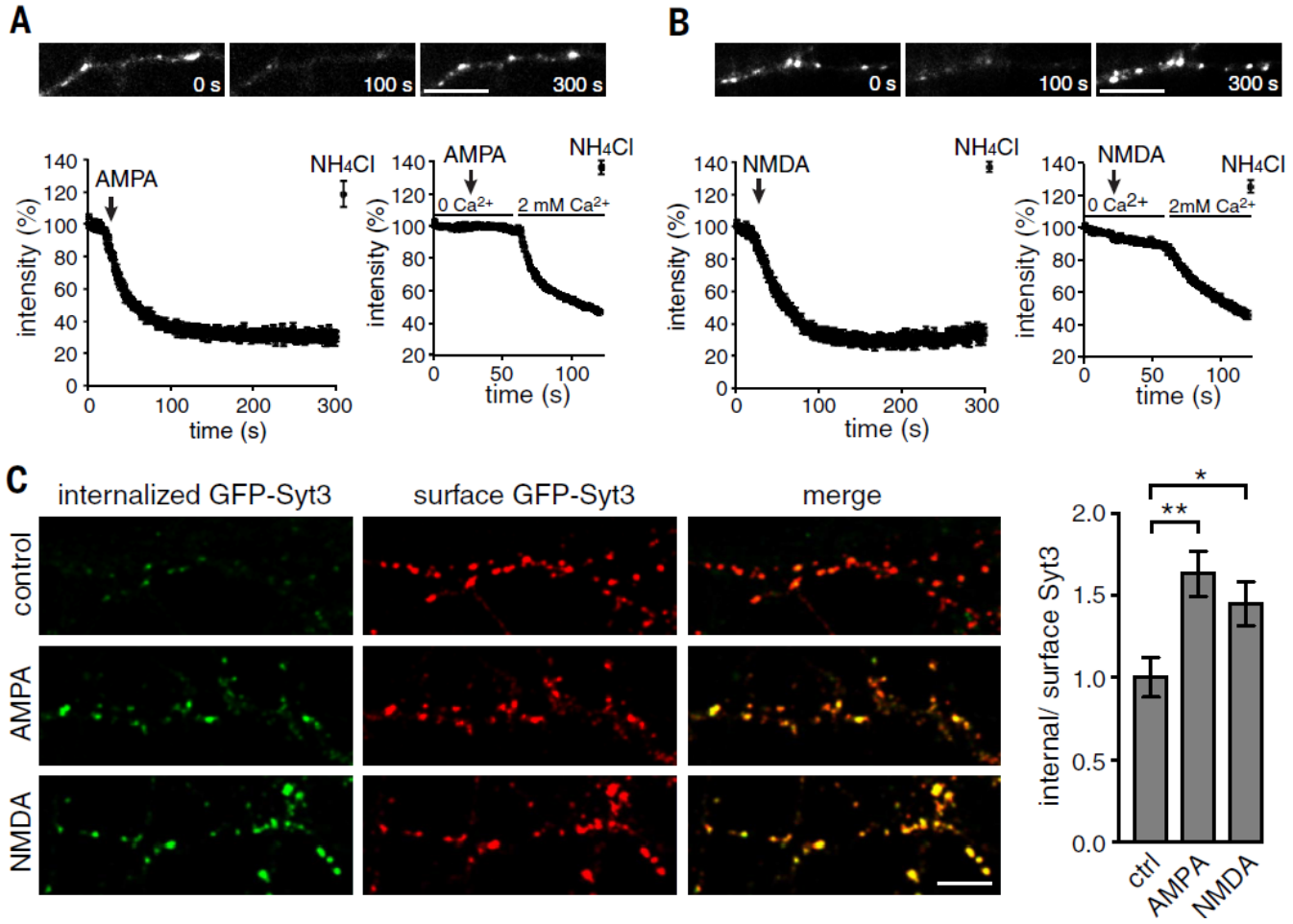
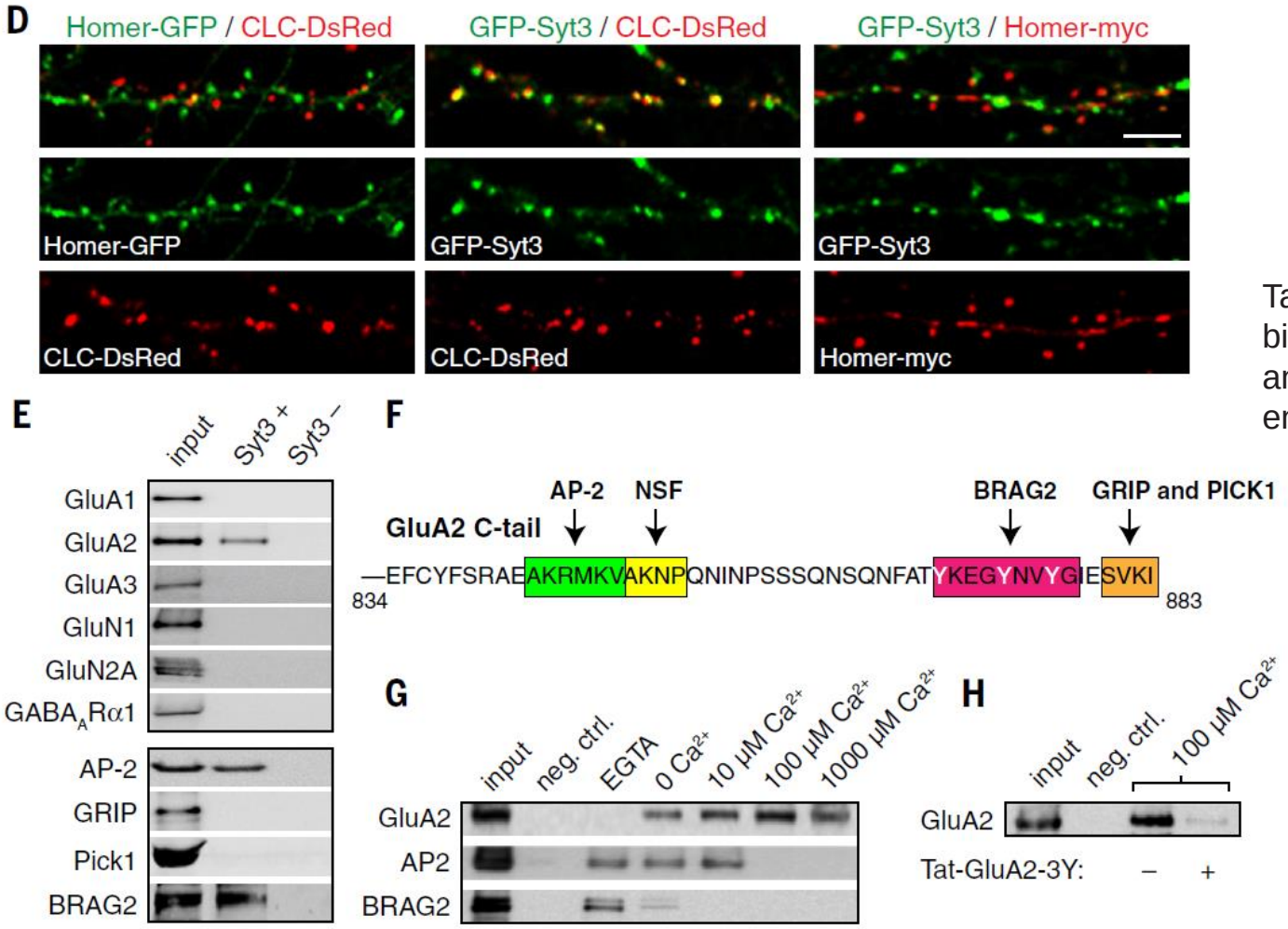


Fig. 2. Syt3 endocytoses in response to stimulation and binds GluA2, AP-2, and BRAG2



Tat-GluA2-3Y : inhibits protein binding to the GluA2 3Y region and blocks activity dependent endocytosis of receptors

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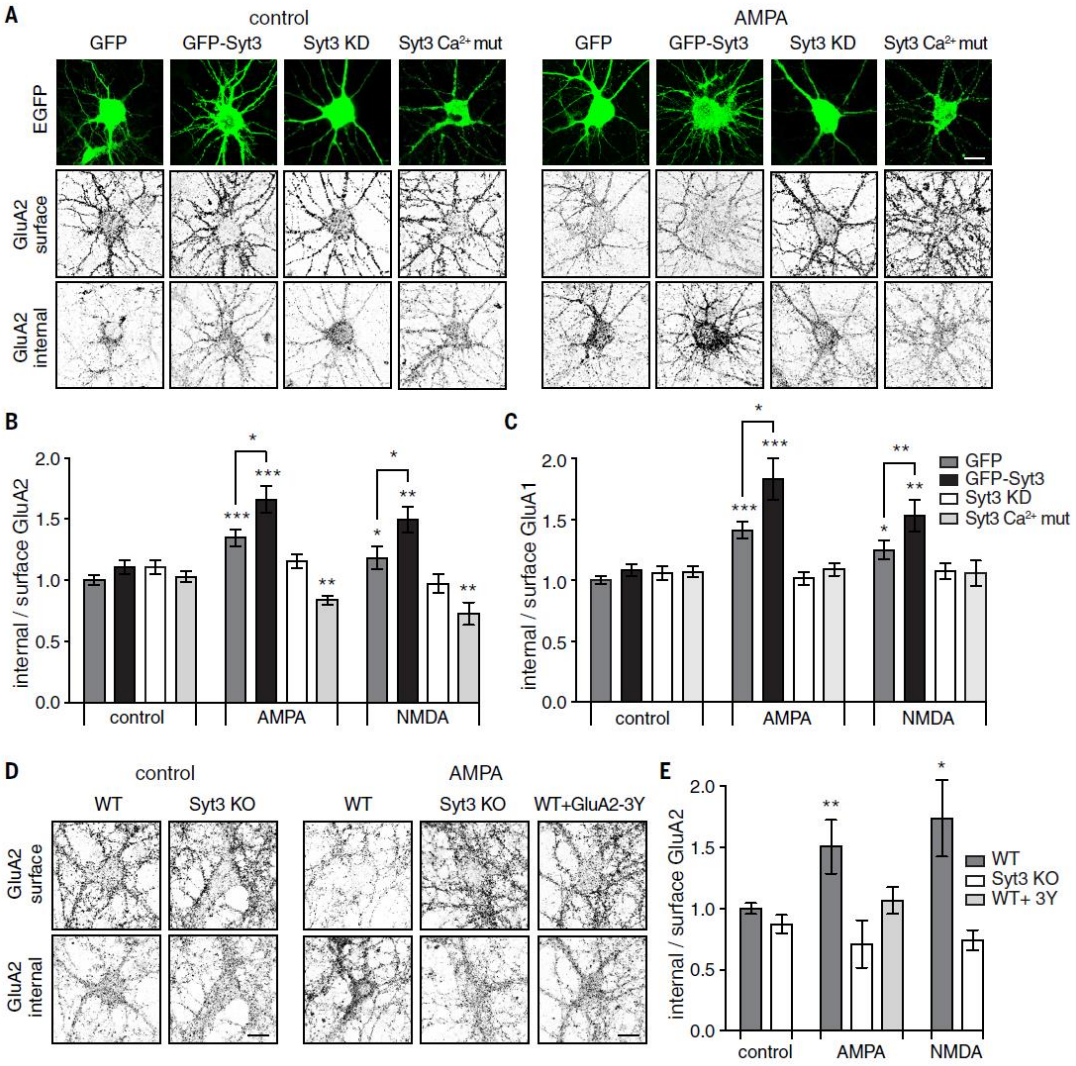
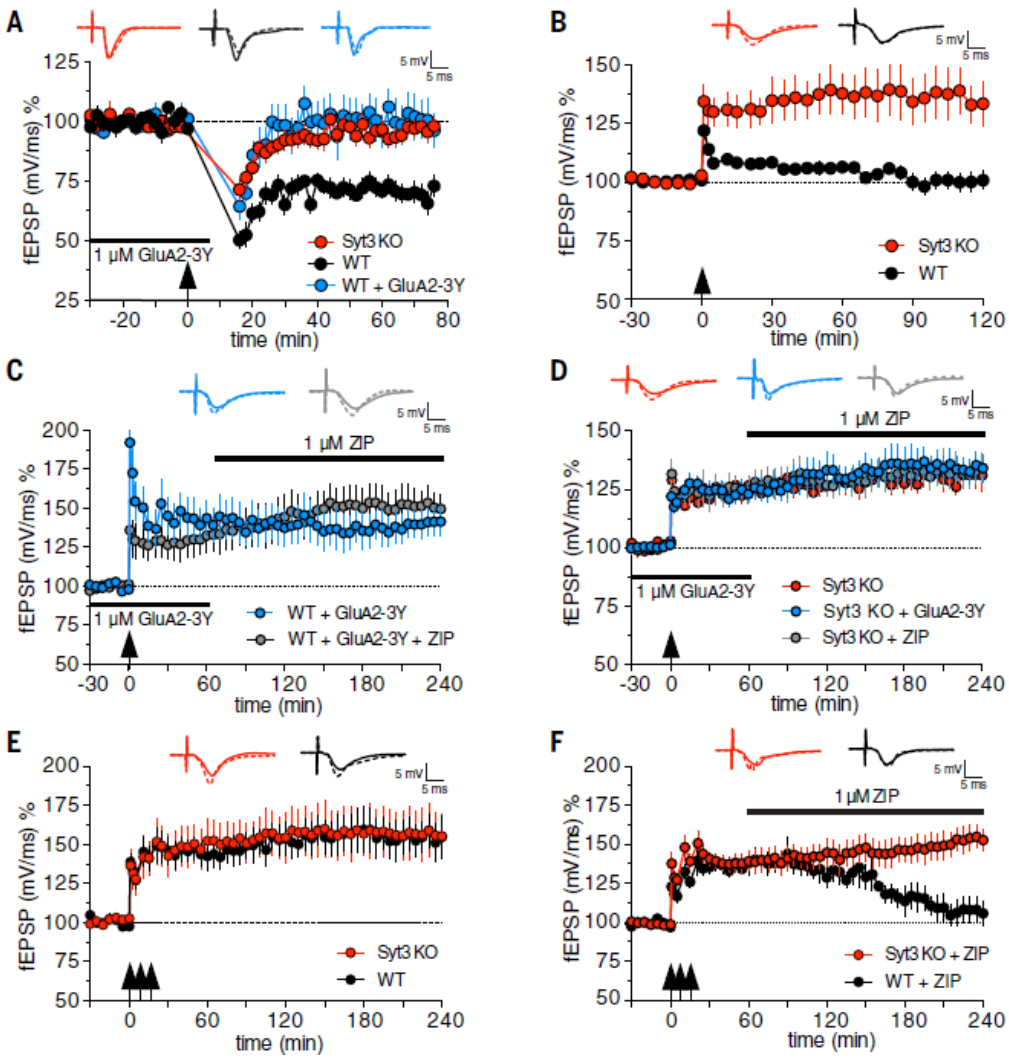
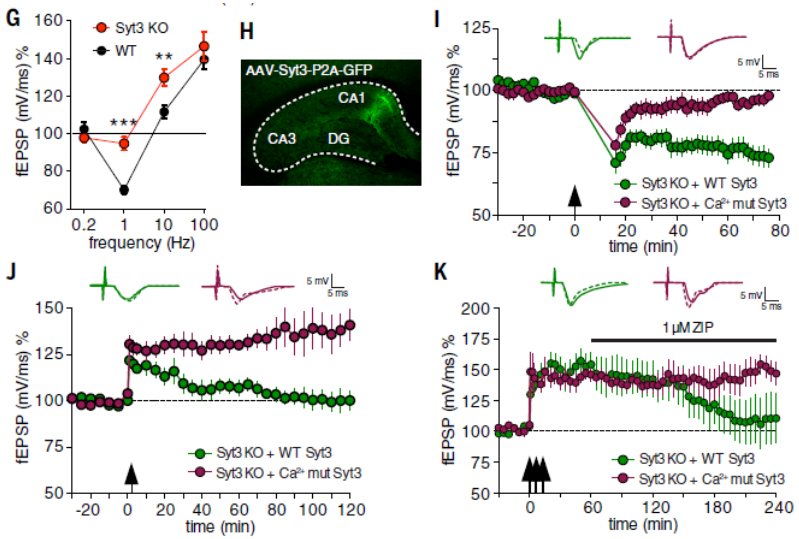


Fig. 3. Syt3 internalizes AMPA receptors in response to stimulation.





- Tat-GluA2-3Y : inhibits protein binding to the GluA2 3Y region and blocks activity dependent endocytosis of receptors
- ZIP : reversing LTP after induction, induce endocytosis of AMPA receptors

Fig. 4. LTD and decay of LTP is abolished in Syt3 knockouts.

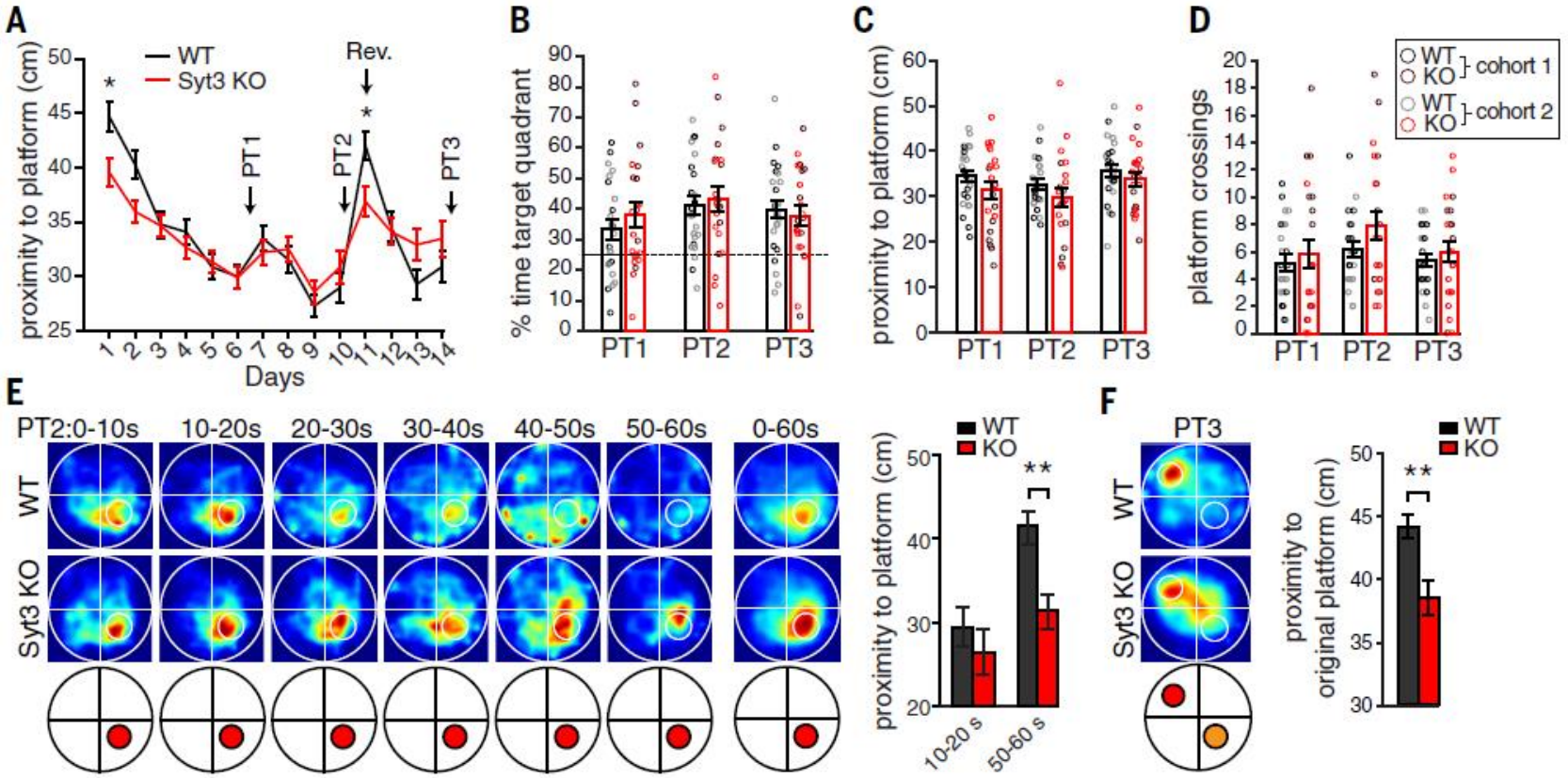
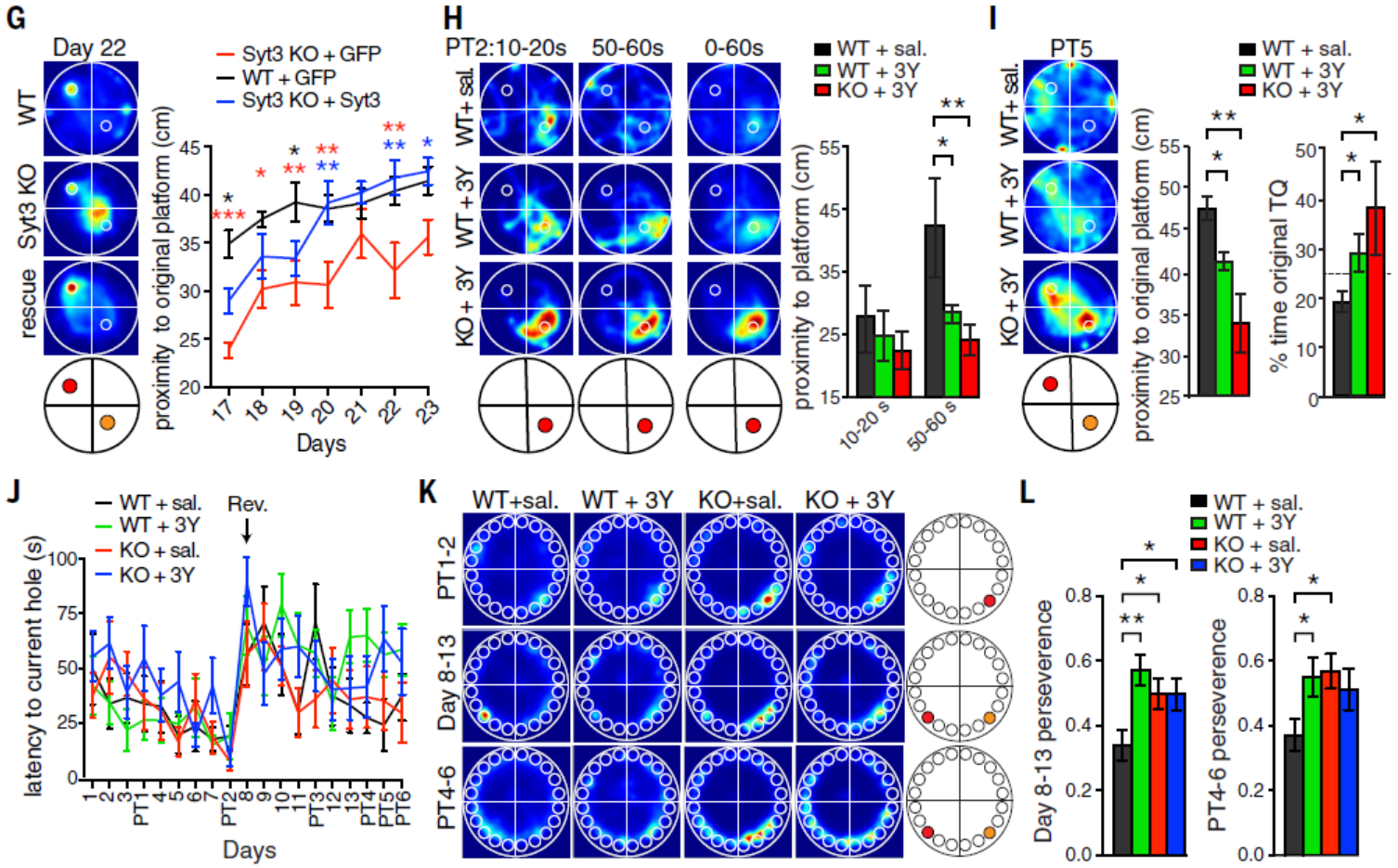


Fig. 5. Syt3 knockout mice learn as well as wild-type mice but have impaired forgetting.



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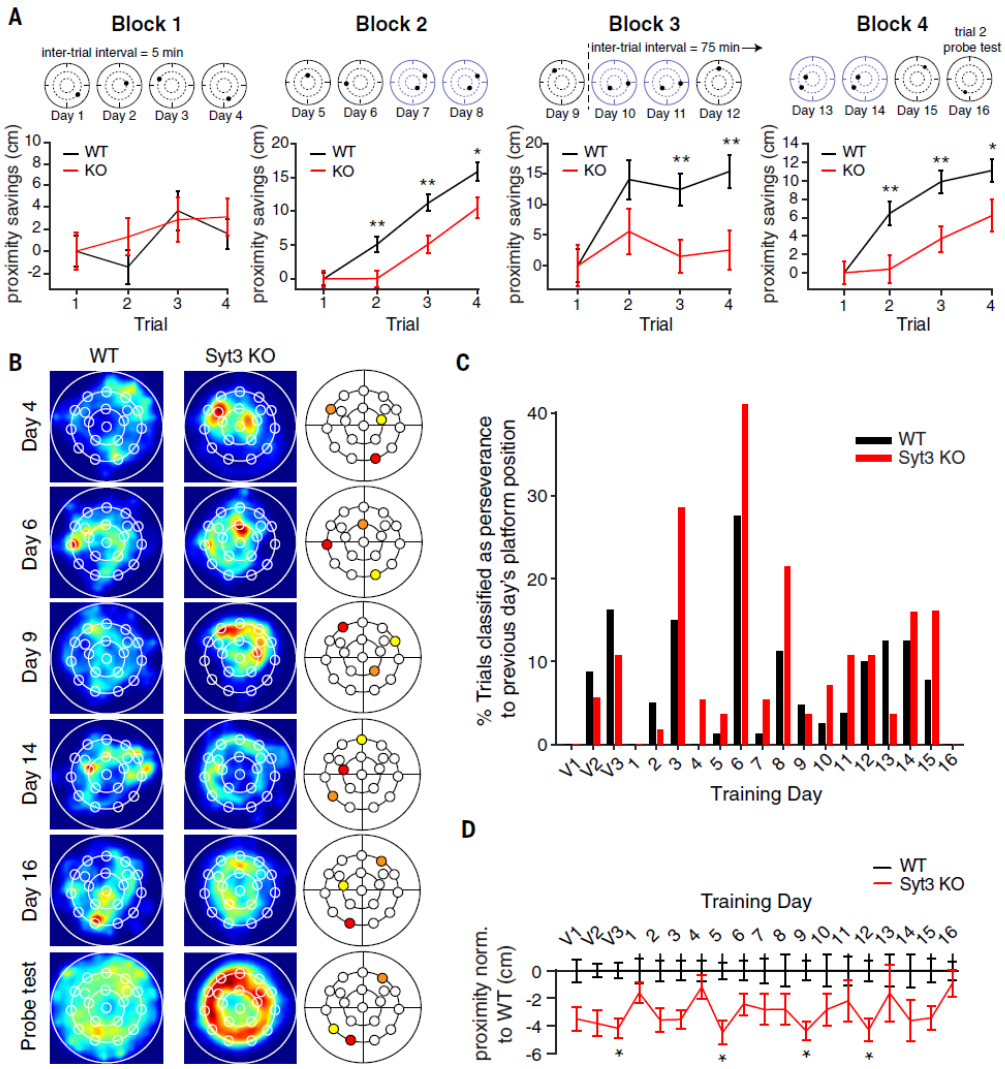
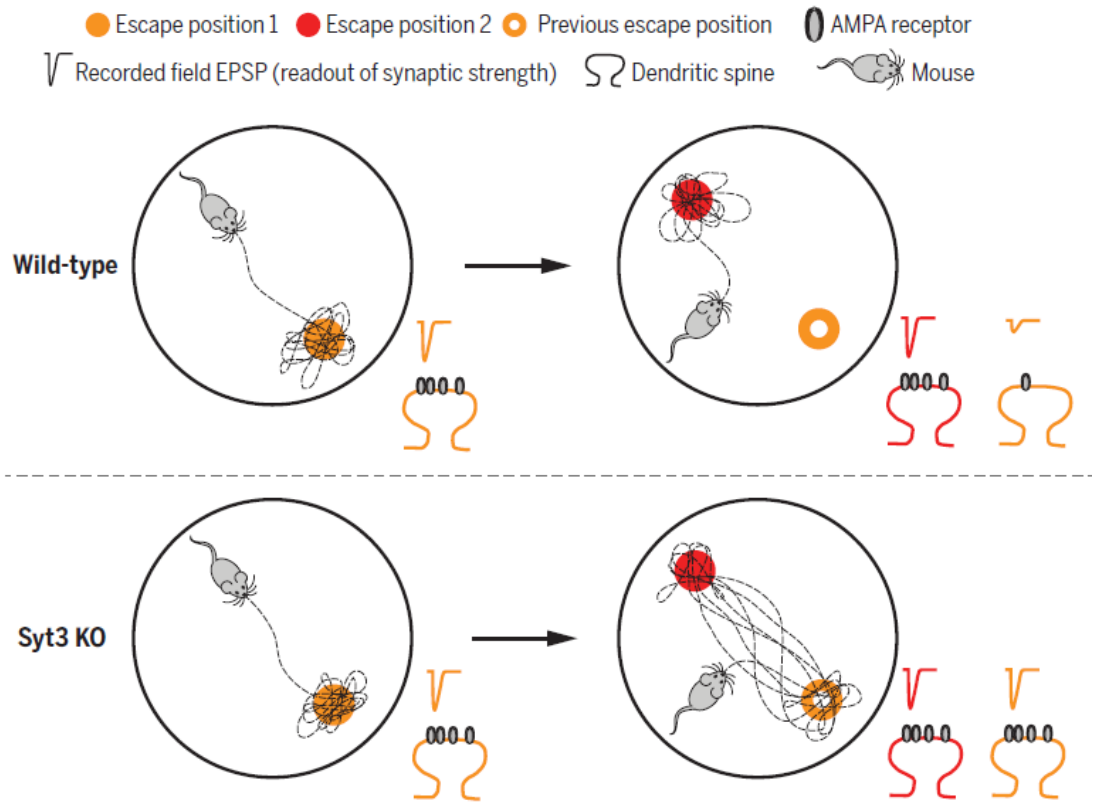


- Delayed matching to place (DMP)

Fig. 6. Syt3 knockout mice show deficits in the delayed matching to place task because of impaired forgetting.



Syt3 knockout mice do not forget. Both wild-type mice and Syt3 knockout mice can learn an escape position in the water maze, in which corresponding synapses are strengthened through the increase of AMPA receptors. These synapses are weakened by the removal of receptors if the memory is no longer needed—for example, when a new escape position is learned. Syt3 knockout mice cannot remove receptors and therefore cannot forget previous escape positions.