Ube3a reinstatement identifies distinct developmental windows in a murine Angelman syndrome model

Sara Silva Santos[1,2,3], Geeske M. van Woerden[1,2,#], Caroline F. Bruinsma[1,2,#], Edwin Mientjes[1,2,#], Mehrnoush Aghadavoud Jolfaei[1,2], Ben Distel[4], Steven A. Kushner[2,5], Ype Elgersma[1,2]

[1]Department of Neuroscience, Erasmus Medical Center, Rotterdam, Wytemaweg 80, 3015 CN, The Netherlands.

[2]*ENCORE* center for neurodevelopmental disorders, Erasmus Medical Center, Rotterdam, Wytemaweg 80, 3015 CN, The Netherlands.

[3]Graduate Program in Areas of Basic and Applied Biology, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4050-313 Porto, Portugal.

[4]Department of Medical Biochemistry, Academic Medical Center, Amsterdam, Meibergdreef 9, 1105 AZ, The Netherlands

[5]Department of Psychiatry, Erasmus Medical Center, Rotterdam, ‘s-Gravendijkwal 230, 3015 CE, The Netherlands.

Supplemental Data Index

Supplemental Methods

Generation and breeding of the Ube3a$^{stop/+}$ line. The *Ube3a*$^{stop/p+}$ mouse was generated as follows: the *Ube3a* genomic sequence (ENSMUSG00000025326) was obtained from Ensembl and used to design the primers for the targeting constructs. PCR fragments encompassing exon 3 using 5′ primer: 5′-CCGCGGGCTCCACTAGTCAATTTC-3′ and 3′ primer: 5′-GCGGCCGCACCACAGTCCCTGGAGTTC-3′ (4.9 kb; exon denotation according to ENSMUSG00000025326) and exon 4 using 5′ primer: 5′-GGCCGGCCGGAACTACCATATCCTGTTTTAC-3′ and 3′ primer: 5′-GCGGCCGCAGCCGATCTAGGTATTC′ (4.6 kb) were amplified using High Fidelity Taq Polymerase (Roche) on ES cell genomic DNA and cloned on either side of a Neomycin-stop cassette flanked by loxP sites (1). Exon 3 and 4 were sequenced to verify that no other mutations were introduced. For counter selection, the diphtheria toxin chain A (DTA) gene was inserted at the 5′ of the targeting construct. The targeting construct was linearized and electroporated into embryonic day 14 (E14) ES cells (derived from 129P2 mice). Cells were cultured in BRL cell-conditioned medium in the presence of leukemia inhibitory factor. After selection with G418 (200 μg/ml), targeted clones were identified by PCR (long-range PCR from neomycin resistance gene to the region flanking the targeted sequence). A clone with verified karyotype was injected into blastocysts of C57BL/6 mice. Male chimeras were crossed with female 129S2/SvPasCrl mice. The resulting heterozygous offspring was used for subsequent breedings. The *Ube3a*$^{stop/p+}$ was maintained by breeding heterozygous males with wild-type 129S2/SvPasCrl mice (Charles River). For all behavioural experiments except epilepsy tests, we crossed female *Ube3a*$^{stop/p+}$ with either *TgCAG-cre mice* (2) *(hereafter referred as Cre*$^{+}$ mice*)* or with *Tg(CAG-cre/Esr1*)5Amc/J* (Jackson) (3) (here after referred as *Cre*$^{ERT+}$) kept in the in C57BL/6J background (Charles River) to generate F1 heterozygous *Ube3a*$^{stop/p+}$ *;Cre*$^{+}$ and *Ube3a*$^{stop/p+}$ *:Cre*$^{ERT+}$ mutants and littermate controls in the F1 hybrid 129S2-C57BL/6 background. For the epilepsy test, both Cre lines were crossed 5 times into 129S2/SvPasCrl and subsequently crossed with *Ube3a*$^{stop/p+}$ mice to obtain *Ube3a*$^{stop/p+}$*;Cre*$^{+}$ and *Ube3a*$^{stop/p+}$ *;Cre*$^{ERT+}$ mutants and littermate controls in the 129S2 background.

Mice were genotyped when they were 7-10 days, and re-genotyped at the moment the mice were sacrificed. Genotyping records were obtained and kept by a technician not involved in the

experimental design, performance and analysis. All animals were kept at 22±2^{0}C with 12 hours dark and light cycle and were provided with food and water *ad libitum*. Mice were preferably group-housed (2-4) cage, unless when fighting between males was observed.

Tamoxifen treatment and randomization. Cages were semi-randomly (alternatingly) assigned to either treat all mice within the cage with vehicle or with tamoxifen. Both male and female mice were used. The alternating randomization was adjusted if there was an imbalance of genotype or sex.

One day to eight-month-old *Ube3a*$^{Stop/p+}$ mice and their WT littermates (both males and females) were used in this study. The group of *Ube3a*$^{Stop/p+}$ and WT mice crossed with *Cre*$^{ERT+}$ transgenic mice were divided into 4 different experimental groups (classified as Newborn, Juvenile, Adolescent and Adult) based on the age of Tamoxifen administration to induce Cre-mediated deletion of the Stop-cassette. Tamoxifen (Sigma-Aldrich) was diluted in sunflower oil at a concentration of 20mg/ml. Each mouse received 0.10 mg Tamoxifen per gram body weight, by daily intraperitoneal (i.p.) injection. The control groups were treated with daily i.p. injections of sunflower oil (vehicle). The Newborn group received Tamoxifen through the milk of the mother, who received daily intraperitoneal (i.p.) injections of Tamoxifen for 5 consecutive days starting at the day of delivery. Tamoxifen treatment was initiated between 21-23 days of age in the Juvenile group; at 6 weeks of age in the Adolescent group; and at 14 weeks in the Adult group. These last 3 groups received 7 daily i.p. injections of Tamoxifen. The group of *Ube3a*$^{Stop/p+}$ and WT mice crossed with the embryonic active *Cre*$^{+}$ transgenic mice ('Embryonic') received 3 vehicle injections when they were 6-8 weeks old.

Behavioral analysis. All behavioral experiments were performed during the light period of the cycle. All animal experiments were approved by the Dutch Ethical Committee and in accordance with Dutch animal care and use laws. The experimenter remained blind to the genotype and treatment until final statistical analysis. Both male and female mice were used for the experiments. All behavioural assays and scoring were done by an experimenter blind to genotype and treatment. Behavioral tests were typically run in the order as presented below. However the

marble burying and nest building test were added later to the battery, and a new cohort of adolescent and adult mice were used for these tests. Since the mice for the epilepsy test required a different genetic background (see above) we used a separate cohort for this test.

Accelerating Rotarod. Motor function was tested using the accelerating rotarod (4-40 rpm, in 5 minutes; model 7650, Ugo Basile Biological Research Apparatus, Varese, Italy). Mice were given two trials per day with a 45-60 min inter-trial interval for 5 consecutive days. For each day we calculated the average of the time spent on the rotarod, or the time until the mouse made 3 consecutive rotations on the rotarod. Maximum duration of a trial was 5 min. Number of mice used in this task: Number of mice used: Embryonic: Wt;Cre+ (n=14), Ube3a$^{Stop/p+}$;Cre– (n=8), Ube3a$^{Stop/p+}$;Cre+ (n=17), Inducible: Adult – WT;Cre^{ERT+} Veh. (n=11), WT;Cre^{ERT+} Tamox. (n=9), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=12), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=13); Adolescent – WT;Cre^{ERT+} Veh. (n=11), WT;Cre^{ERT+} Tamox. (n=11), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=10), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=11); Juvenile – WT;Cre^{ERT+} Veh. (n=22), WT;Cre^{ERT+} Tamox. (n=20), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=22), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=22); Newborn – WT;Cre^{ERT+} Veh. (n=14), WT;Cre^{ERT+} Tamox. (n=12), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=14), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=11).

Marble burying test. Clean open makrolon (polycarbonate) cages (50x26x18 cm) were filled with 4 cm of bedding material. On top of the bedding material 20 blue glass marbles were arranged in an equidistant 5 x 4 grid and the animals were given access to the marbles for 30 minutes. After the test the mice were gently removed from the cage. Marbles which were covered for more than 50% by bedding were scored as buried. Occasionally, a mouse managed to escape out of the cage during the test, and was excluded. Number of mice used in this task: Embryonic: Wt;Cre+ (n=24), Ube3a$^{Stop/p+}$;Cre– (n=18), Ube3a$^{Stop/p+}$;Cre+ (n=28), Inducible: Adult – WT;Cre^{ERT+} Veh. (n=13), WT;Cre^{ERT+} Tamox. (n=11), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=14), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=15); Adolescent – WT;Cre^{ERT+} Veh. (n=20), WT;Cre^{ERT+} Tamox. (n=20), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=21), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=23); Juvenile – WT;Cre^{ERT+} Veh. (n=21), WT;Cre^{ERT+} Tamox. (n=20), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=20), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=20); Newborn – WT;Cre^{ERT+} Veh. (n=14), WT;Cre^{ERT+} Tamox. (n=12), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=14), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=11).

Open Field test. To test locomotor activity and anxiety, the mice were individually placed in a brightly lit 120 cm diameter circular open field. The total distance travelled was recorded for 10 minutes (SMART software, Panlab, Barcelona). Number of mice used in this task: Embryonic: Wt;Cre+ (n=14), Ube3a$^{Stop/p+}$;Cre– (n=8), Ube3a$^{Stop/p+}$;Cre+ (n=17), Inducible: Adult – WT;Cre^{ERT+} Veh. (n=10), WT;Cre^{ERT+} Tamox. (n=8), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=9), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=10); Adolescent – WT;Cre^{ERT+} Veh. (n=11), WT;Cre^{ERT+} Tamox. (n=11), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=10), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=11); Juvenile – WT;Cre^{ERT+} Veh. (n=21), WT;Cre^{ERT+} Tamox. (n=21), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=22), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=22); Newborn – WT;Cre^{ERT+} Veh. (n=14), WT;Cre^{ERT+} Tamox. (n=12), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=14), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=11).

Nest Building test. To measure nest building, mice were single housed for a period of 5 to 7 days before the starting the experiment. Subsequently 12 grams (12±1) of compressed extra-thick blot filter paper (Bio-rad©) was added to the cage and cages were put back in the rack and undisturbed for 24h. For 5 consecutive days and approximately at the same time of the day, the unused nesting material was carefully cleaned, dried and weighed to determine the amount used for nestbuilding. Number of mice used in this task: Embryonic: Wt;Cre+ (n=7), Ube3a$^{Stop/p+}$;Cre– (n=7), Ube3a$^{Stop/p+}$;Cre+ (n=7), Inducible: Adult – WT;Cre^{ERT+} Veh. (n=9), WT;Cre^{ERT+} Tamox. (n=8), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=9), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=8); Adolescent – WT;Cre^{ERT+} Veh. (n=14), WT;Cre^{ERT+} Tamox. (n=13), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=16), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=17); Juvenile – WT;Cre^{ERT+} Veh. (n=12), WT;Cre^{ERT+} Tamox. (n=13), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=14), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=13); Newborn – WT;Cre^{ERT+} Veh. (n=12), WT;Cre^{ERT+} Tamox. (n=12), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=12), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=11).

Forced swim test. Mice were placed for 6 min in a cylindrical transparent tank (27cm high and 18cm diameter), filled with water (kept at 26±1 degrees Celsius) 15 cm deep. Since little or no immobility is observed during the first 2 min after the mouse has been placed in the water, the duration of immobility was only assessed during the last 4 min of the test. The mouse was considered to be immobile when he ceased to move altogether, making only movements necessary to keep its head above water. Number of mice used in this task: Embryonic: Wt;Cre+ (n=14), Ube3a$^{Stop/p+}$;Cre– (n=8), Ube3a$^{Stop/p+}$;Cre+ (n=17), Inducible: Adult – WT;Cre^{ERT+} Veh.

(n=11), WT;Cre^{ERT+} Tamox. (n=8), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=9), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=9); Adolescent – WT;Cre^{ERT+} Veh. (n=11), WT;Cre^{ERT+} Tamox. (n=11), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=10), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=11); Juvenile – WT;Cre^{ERT+} Veh. (n=17), WT;Cre^{ERT+} Tamox. (n=19), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=18), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=19); Newborn – WT;Cre^{ERT+} Veh. (n=14), WT;Cre^{ERT+} Tamox. (n=12), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=14), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=11).

Epilepsy test. Since epilepsy susceptibility in AS mice is dependent on the genetic background, these experiments were performed in a 129/sv background. For this test, mice were taken from their home cage and placed in a clean cage. To assess seizure susceptibility, audiogenic seizures were induced by producing a loud and continuous noise, achieved by vigorously screeching scissors across the metal grating of the cage lid. This was done for 20s or shorter if a tonic-clonic seizure developed before that time. Number of mice used in this task: Embryonic: Wt;Cre+ (n=7), Ube3a$^{Stop/p+}$;Cre– (n=10), Ube3a$^{Stop/p+}$;Cre+ (n=8), Inducible: Juvenile – WT;Cre^{ERT+} Veh. (n=8), WT;Cre^{ERT+} Tamox. (n=8), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=8), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=8).

Mean age of the mice used in the epilepsy susceptibility test was the following: Embryonic, 10 weeks; Juvenile, 9 weeks.

Antiepileptic drug (AED) treatment. Mutant Ube3aStop/p+;Cre– (n=2), Ube3am-/p+ (n=12) and WT littermates (n=3) in the 129sv background were used for these experiments.

Tonic-clonic seizures were verified in all mutant mice upon audiogenic stimulation. No seizues were observed in WT mice. Mutant mice were randomized into two treatment groups (sodium valproate and clonazepam), and drug administration started 24hrs after the first epilepsy test.

1 Ube3a$^{Stop/p+}$;Cre^{-}, 7 Ube3a$^{m-/p+}$ and 2 WT mice were treated with 5 daily i.p. injections of sodium valproate (Alliance Healthcare™) at a concentration of 200mg/kg. The valproate was dispensed in sterilized water through agitation and syringes were filled while stirring. Valproate was freshly prepared daily.

The second treatment group composed of 1 *Ube3a*$^{Stop/p+}$*;Cre–*, *5 Ube3a*$^{m-/p+}$ and 1 WT mouse, which were treated with 5 daily i.p. injections of 0.05mg/kg Clonazepam (Roche) dispensed in PBS-methylcellulose. Clonazepam was prepared fresh every two days.

Two re-tests experiments were performed in both treatment groups; the first re-test was performed 30 min. after the last injection, on the fifth day of treatment, and the second re-test was done 3 days after treatment cessation.

Electrophysiology. After the behavioural tests, animals were been sacrificed, sagittal slices (400 µm) were made and submerged in ice-cold artificial CSF (ACSF) using a vibratome, and hippocampi were dissected out. These sagittal hippocampal slices were maintained at room temperature for at least 1.5 h to recover before experiments were initiated. Then they were placed in a submerged recording chamber and perfused continuously at a rate of 2 ml/min with ACSF equilibrated with 95% O_2, 5% CO_2 at 31°C. ACSF contained the following (in mM): 120 NaCl, 3.5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 D-glucose. Extracellular recording of field EPSP (fEPSPs) were made in CA1 stratum radiatum with platinum/iridium electrodes (Frederick Haer). A bipolar Pt/Ir was used to stimulate Schaffer-collateral/commissural afferents with a stimulus duration of 100 µs. LTP was evoked using the 10 Theta burst protocol (10 trains of 4 stimuli at 100Hz, 200ms apart), performed at two-third of the maximum fEPSP. fEPSP measurements were done once per minute. Potentiation was measured as the normalized increase of the mean fEPSP slope for the duration of the baseline. Only stable recordings were included, and this judgment was made blind to genotype. Average LTP was defined as the mean last 10 min of each protocol. Recordings showing unstable baselines were excluded from the experiment.

Number of slices/mice used: Juvenile- WT;Cre^{ERT+} Veh. (n=16/4), WT;Cre^{ERT+} Tamox. (n=25/4), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=22/4), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=22/5); Adult - WT;Cre^{ERT+} Veh. (n=18/6), WT;Cre^{ERT+} Tamox. (n=37/8), Ube3a$^{Stop/p+}$;Cre^{ERT+} Veh. (n=23/4), Ube3a$^{Stop/p+}$;Cre^{ERT+} Tamox. (n=15/4).

Western Blots. To collect tissue for Western blot analysis, hippocampus, cortex and cerebellum were dissected and immediately frozen in liquid nitrogen. The lysates were prepared by adding lysis buffer (10mM Tris-HCL pH 6.8, 2.5% SDS) supplemented with protease inhibitor cocktail (Sigma-Aldrich®) to the tissue and homogenization was achieved by sonication. After centrifugation (6000 rpm for 5min.) supernatants were collected. The protein concentration of the supernatants was determined using a BCA kit (Pierce, Thermo Scientific). A total of 20µg of each sample was loaded on the gel and a wet transfer was performed. The blotted nitrocellulose membrane was probed with antibodies directed against E6AP (E8655 Sigma-Aldrich®; 1:1,000) and Actin (MAB1501R Millipore©; 1:20,000). A fluorophore-conjugated secondary Goat anti-mouse antibody (Westburg©, IRDye 800CW 1:15,000) was used and the protein was detected using Li-cor® Odyssey Scanner system. Quantification was done using Odyssey 3.0 software (Li-cor® Biosciences). Number of samples used for immunoblot analysis range from 2 to 5 per genotype/brain area.

Immunohistochemistry. Brains from adult mice were fixed by transcardial perfusion with 4% paraformaldehyde. Immunocytochemistry was performed on 40µm thick frozen sections. The sections were subjected to a hydrogen peroxidase (H_2O_2) treatment, placed in blocking solution (10% normal horse serum (NHS), 0.5% Triton X-100) for 1h and incubated overnight with the primary antibody (mouse α-E6AP (E8655 Sigma-Aldrich, 1:2000) in 2% of NHS, 0.5% Triton X-100). The next day the slices were incubated with the secondary antibody (α-mouse HRP (Dako©; 1:200), which was detected by 3,3'-diaminobenzidine (DAB) as the chromogen. DAB sections were analysed and photographed using a Leica© DM-RB microscope and a Leica DFC450 digital camera. For overview pictures of the slices a Zeiss Stemi SV6 was used.

Data analysis and statistics. Values are represented as means ± S.E.M. All the data was statistically analyzed using the IBM® SPSS software and P-values of <0.05 were considered significant. Statistical analysis was performed using a one-way analysis of variance (ANOVA) or a two-way ANOVA with Bonferroni's post hoc comparison.

Supplemental References

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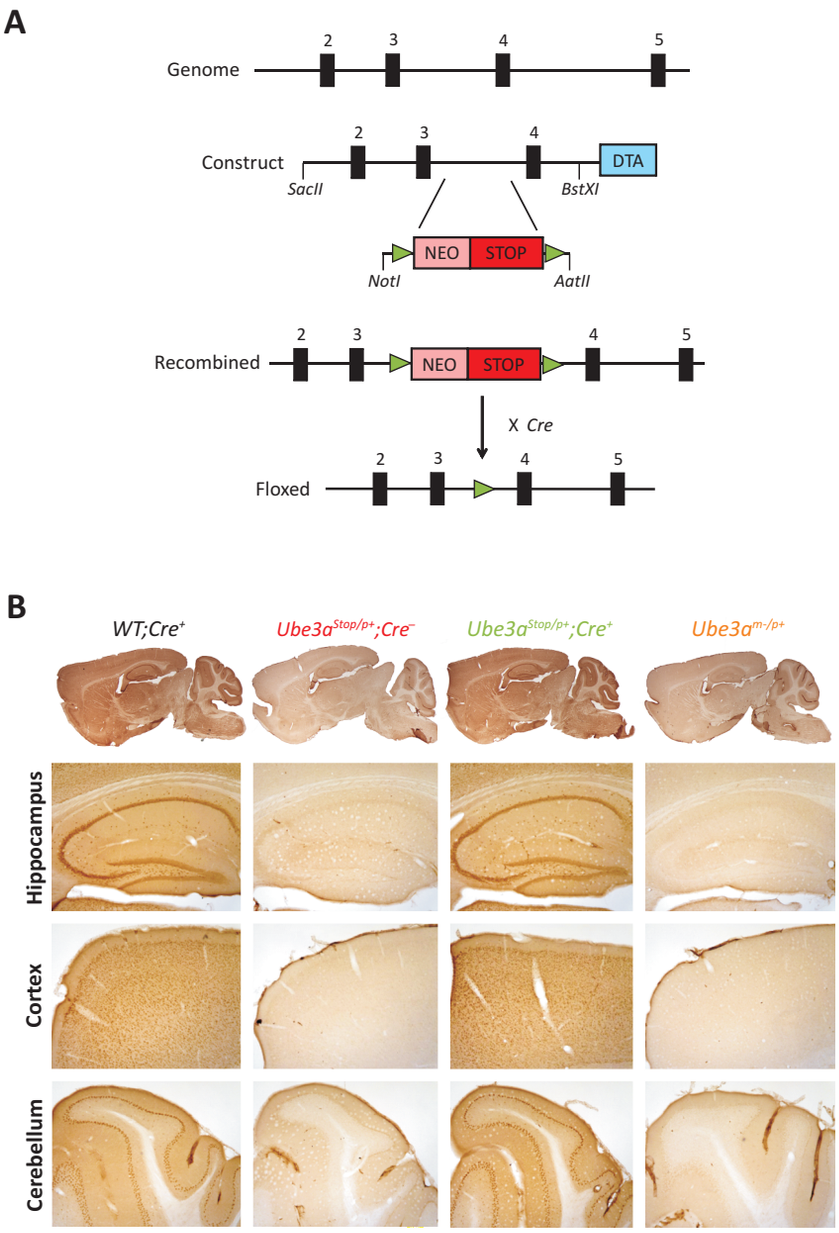
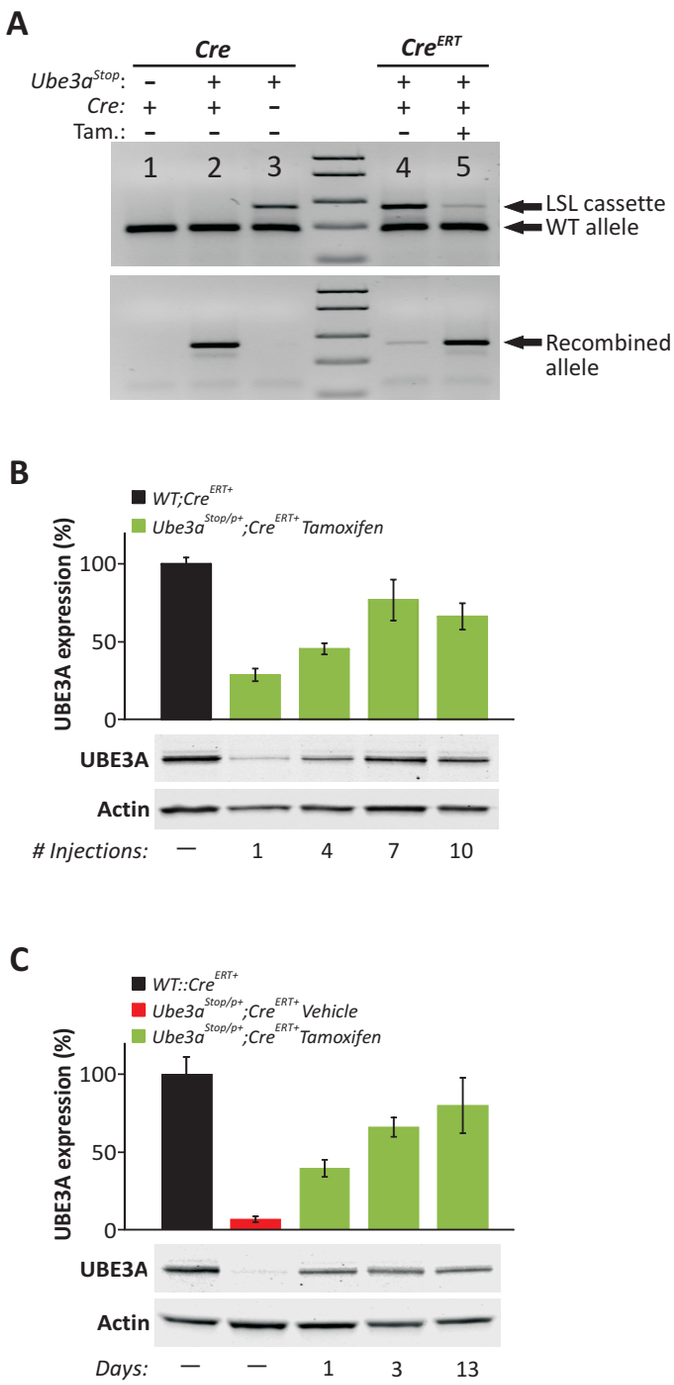
Supplemental Figures

Supplemental Figure 1.



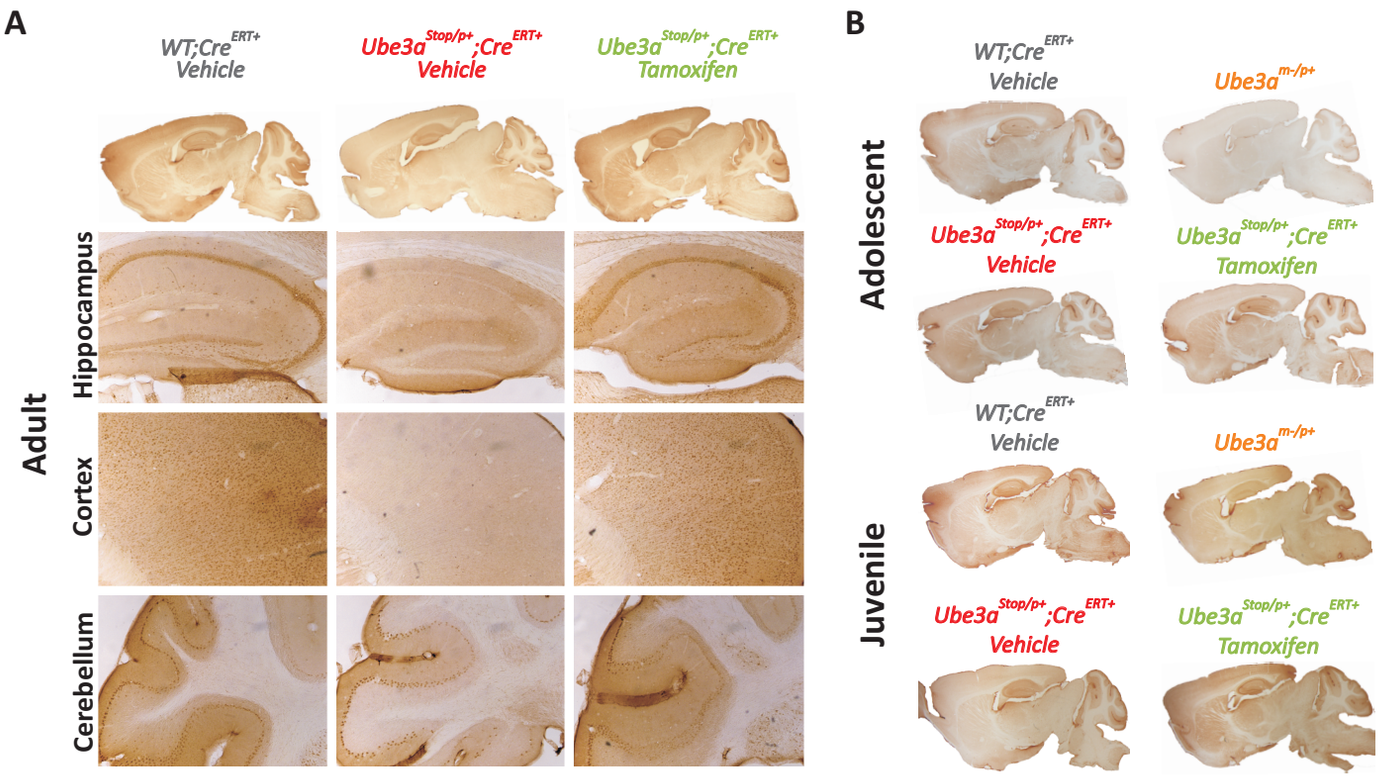
Supplemental Figure 1. Generation and histological validation of *Ube3a*$^{Stop/p+}$ knock-in mice. (**A**) Schematics depicting the generation of the inducible mouse model by the insertion of a floxed stop cassette into intron 3 of *Ube3a*, for which cre-mediated recombination leads to reinstatement of *Ube3a* gene expression. Black boxes correspond to *Ube3a* coding exons and green triangles represent the LoxP sites. (**B**) Immunohistochemical UBE3A stainings from *Ube3a*$^{m-/p+}$ knock-out mice and *Ube3a*$^{Stop/p+}$ and WT littermates crossed with a embryonically active cre-line. Brain overviews magnification = 1.6x (upper); zoomed-in pictures magnification = 5x (lower).

Supplemental Figure 2.



Supplemental Figure 2. High level of recombination is achieved by embryonically expressed cre ('Cre') and tamoxifen inducible cre expression ('Cre^{ERT}'). (**A**) Polymerase chain reaction (PCR) of hippocampal tissue reveals successful recombination in mutant mice expressing Cag-Cre and in *Ube3a*$^{Stop/p+}$;Cre^{ERT+} mutant mice injected 7 times with tamoxifen (Tam.). (**B**) Representative Western blot and corresponding quantification illustrates hippocampal UBE3A reinstatement achieved by successive tamoxifen injections in the *Ube3a*$^{Stop/p+}$;Cre^{ERT+} mutant mice. (**C**) Time-course of UBE3A expression following gene reinstatement (days following the last seventh injection of tamoxifen). All data are represented as mean ± S.E.M.

Supplemental Figure 3.



Supplemental Figure 3. Histological analysis of *Ube3A*$^{Stop/p+}$;*Cre*$^{ERT+}$ mice reveals successful reactivation of the maternal *Ube3a* gene upon tamoxifen induction. (**A-B**) Hippocampal, cortical, and cerebellar sections demonstrate brain-wide reactivation of *Ube3a* expression in tamoxifen-treated *Ube3A*$^{Stop/p+}$;*Cre*$^{ERT+}$ mice. Brain overviews magnification = 1.6x; zoomed-in pictures magnification = 5x.

Supplemental Tables

Supplemental Table S1.

	Paradigm	Dependent variable	Statistical test	Independent variable	p	Post-hoc test		p
Embryonic *Ube3a* gene reinstatement	**RR**	Latency to fall (s)	Repeated measures one-way ANOVA	Genotype	p<0.01	$Ube3a^{Stop/p+};Cre^{+}$	$WT;Cre^{+}$	p= 1.000
							$Ube3a^{Stop/p+};Cre^{-}$	p<0.01
	Marble	Number unburied marbles	Univariate one-way ANOVA	Genotype	p<0.01	$Ube3a^{Stop/p+};Cre^{+}$	$WT;Cre^{+}$	p= 1.000
							$Ube3a^{Stop/p+};Cre^{-}$	p<0.01
	OF	Path length (m)	Univariate one-way ANOVA	Genotype	p<0.01	$Ube3a^{Stop/p+};Cre^{+}$	$WT;Cre^{+}$	p= 1.000
							$Ube3a^{Stop/p+};Cre^{-}$	p<0.05
	NB	Used nesting material (%)	Repeated measures one-way ANOVA	Genotype	p<0.01	$Ube3a^{Stop/p+};Cre^{+}$	$WT;Cre^{+}$	p= 1.000
							$Ube3a^{Stop/p+};Cre^{-}$	p<0.01
	FST	Floating time (%)	Univariate one-way ANOVA	Genotype	p<0.01	$Ube3a^{Stop/p+};Cre^{+}$	$WT;Cre^{+}$	p= 1.000
							$Ube3a^{Stop/p+};Cre^{-}$	p<0.01
	Epilepsy	Presence of seizures	Univariate one-way ANOVA	Genotype	p<0.01	$Ube3a^{Stop/p+};Cre^{+}$	$WT;Cre^{+}$	p= 1.000
							$Ube3a^{Stop/p+};Cre^{-}$	p<0.01

Supplemental Table S1 Summary of the statistical tests used for each behavioral paradigm performed on the early embryonic reactivation group and statistical outcomes obtained. Statistical significance ($P < 0.05$) is indicated by green shading.

Supplemental Table S2.

		Paradigm	Dependent variable	Statistical test	Independent variable	p
Adult *Ube3a* gene reinstatement	Induction at 14 weeks	**RR**	Latency to fall (s)	Repeated measures two-way ANOVA	Genotype	p<0.01
					Treatment	p= 0.602
					Genotype*Treatment	p= 0.613
		Marble	Number unburied marbles	Univariate two-way ANOVA	Genotype	p<0.01
					Treatment	p= 0.751
					Genotype*Treatment	p= 0.722
		OF	Path lenght (m)	Univariate two-way ANOVA	Genotype	p<0.01
					Treatment	p= 0.203
					Genotype*Treatment	p= 0.411
		NB	Used nesting material (%)	Repeated measures two-way ANOVA	Genotype	p<0.01
					Treatment	p<0.05
					Genotype*Treatment	p= 0.377
		FST	Floating time (%)	Univariate two-way ANOVA	Genotype	p<0.01
					Treatment	p= 0.499
					Genotype*Treatment	p= 0.154
		LTP	fEPSP slope (% from baseline)	Univariate two-way ANOVA	Genotype	p= 0.849
					Treatment	p= 0.071
					Genotype*Treatment	p<0.01
Adolescent *Ube3a* gene reinstatement	Induction at 6 weeks	**RR**	Latency to fall (s)	Repeated measures two-way ANOVA	Genotype	p<0.01
					Treatment	p= 0.284
					Genotype*Treatment	p= 0.284
		Marble	Number unburied marbles	Univariate two-way ANOVA	Genotype	p<0.01
					Treatment	p= 0.249
					Genotype*Treatment	p= 0.221
		OF	Path lenght (m)	Univariate two-way ANOVA	Genotype	p<0.01
					Treatment	p= 0.240
					Genotype*Treatment	p= 0.413
		NB	Used nesting material (%)	Repeated measures two-way ANOVA	Genotype	p<0.01
					Treatment	p= 0.308
					Genotype*Treatment	p= 0.530
		FST	Floating time (%)	Univariate two-way ANOVA	Genotype	p<0.01
					Treatment	p= 0.374
					Genotype*Treatment	p= 0.822
Juvenile *Ube3a* gene reinstatement	Induction at P21-23	**RR**	Latency to fall (s)	Repeated measures two-way ANOVA	Genotype	p<0.01
					Treatment	p<0.01
					Genotype*Treatment	p<0.01
		Marble	Number unburied marbles	Univariate two-way ANOVA	Genotype	p<0.01
					Treatment	p= 0.080
					Genotype*Treatment	p= 0.537
		OF	Path lenght (m)	Univariate two-way ANOVA	Genotype	p<0.01
					Treatment	p<0.01
					Genotype*Treatment	p= 0.948
		NB	Used nesting material (%)	Repeated measures two-way ANOVA	Genotype	p<0.01
					Treatment	p= 0.173
					Genotype*Treatment	p= 0.708
		FST	Floating time (%)	Univariate two-way ANOVA	Genotype	p<0.01
					Treatment	p<0.01
					Genotype*Treatment	p= 0.135
		LTP	fEPSP slope (% from baseline)	Univariate two-way ANOVA	Genotype	p= 0.088
					Treatment	p<0.01
					Genotype*Treatment	p=0.055
Newborn *Ube3a* gene reinstatement	Induction at P1	**RR**	Latency to fall (s)	Repeated measures two-way ANOVA	Genotype	p<0.01
					Treatment	p<0.01
					Genotype*Treatment	p<0.05
		Marble	Number unburied marbles	Univariate two-way ANOVA	Genotype	p<0.01
					Treatment	p<0.05
					Genotype*Treatment	p= 0.124
		OF	Path length (m)	Univariate two-way ANOVA	Genotype	p<0.01
					Treatment	p= 0.365
					Genotype*Treatment	p<0.05
		NB	Used nesting material (%)	Repeated measures two-way ANOVA	Genotype	p<0.01
					Treatment	p<0.05
					Genotype*Treatment	p= 0.208
		FST	Floating time (%)	Univariate two-way ANOVA	Genotype	p<0.01
					Treatment	p= 0.987
					Genotype*Treatment	p= 0.663

Supplemental Table S2 Summary of the statistical tests applied for each behavioral paradigm performed on the postnatal reactivation groups. Statistical significance ($P < 0.05$) is indicated by green shading.