ALLEN INSTITUTE for BRAIN SCIENCE

Allen Brain Observatory

TECHNICAL WHITE PAPER: PHENOTYPIC CHARACTERIZATION OF TRANSGENIC MOUSE LINES

OVERVIEW

The Allen Brain Observatory contains data collected via two-photon calcium imaging to quantify neuron activity in the mouse neocortex in response to visual stimuli. The use of a fluorescent calcium indicator, GCaMP6f, was used to register neural activity in the visual cortex of transgenic mice exposed to various visual stimuli. Calcium influx associated with neural activity results in transient increases in fluorescence of GCaMP6-GFP. These experiments use the transgenic mouse line Ai93, in which GCaMP6f expression is dependent on the activity of both Cre recombinase and the tetracycline-controlled transactivator protein (tTA). Triple transgenic mice (Ai93, tTA, Cre) were generated by first crossing Ai93 mice with Camk2a-tTA mice, which preferentially express tTA in forebrain excitatory neurons. Double transgenic mice were then crossed with a Cre driver line to generate mice in which GCaMP6f expression is induced in the specific populations of neurons that express both Cre and tTA.

Under the *Transgenic Characterization* tab of the Allen Brain Observatory, data is available to view the native GCaMP6 fluorescence for each line using 2-photon serial tomography as performed on animals that have not undergone surgery (described in the "Visual Coding Overview" whitepaper in Documentation).

This document provides a description of the following:

1. An overview of the transgenic strategy used for creating each line.
2. Images of GCaMP6 expression based on 2-photon serial tomography, to detect the native baseline presence of cells harboring GCaMP6-GFP.
3. Phenotypic characteristics of transgenic animals, including summaries of growth.
4. Technical considerations for animal breeding and health.

The following transgenic mouse lines are described:

- Cux2-CreERT2;Camk2a-tTA;Ai93(TITL-GCaMP6f)
- Emx1-IRES-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f)
- Nr5a1-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f)
- Rbp4-Cre_KL100;Camk2a-tTA;Ai93(TITL-GCaMP6f)
- Rorb-IRES2-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f)
- Scnn1a-Tg3-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f)

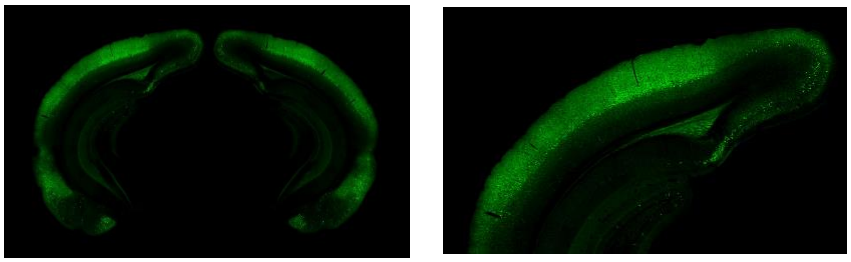
CUX2-CREERT2;CAMK2A-TTA;AI93(TITL-GCAMP6F)

Overview

Cux2-CreERT2;Camk2a-tTA;Ai93(TITL-GCaMP6f) transgenic mice express a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. Further specificity is regulated by the tamoxifen-inducible Cux2 promoter, induction of which results in Cre-mediated expression of GCaMP6f in excitatory neurons. Predominant expression is observed in cortical layers 2, 3 and 4 as well as in thalamus, midbrain, pons, medulla, and cerebellum. Calcium influx associated with neural activity results in transient increases in fluorescence of GCaMP6-GFP.

Transgene Expression

Native GCaMP6-GFP expression (green), visualized by 2P tomography:



Expressed Gene: GCaMP6f, Genetically encoded calcium indicator
Site of Expression: Expressed under the control of tetO in cells where cre recombinase is expressed

Expressed Gene: tTA, tetracycline-controlled transactivator
Site of Expression: Expressed in excitatory forebrain progenitors and neurons.

Expressed Gene: cre, cre recombinase
Site of Expression: Tamoxifen-inducible expression in cortical layers 2/3/4, thalamus, midbrain, pons, medulla and cerebellum.

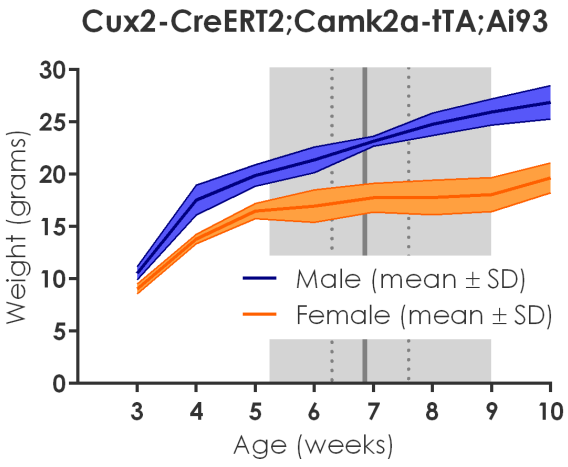
Native gene expression:

in situ hybridization of *Cux2* (NCBI Accession: NM_007804.2) in C57BL/6J
http://mouse.brain-map.org/experiment/show/72128748

in situ hybridization of *Camk2a* (NCBI Accession: NM_009792.3) in C57BL/6J
http://mouse.brain-map.org/experiment/show/79490122

Growth and Development

Mean (±SD) Weight at weaning (~p21):
Male: 8.39 ± 1.56
Female: 8.13 ± 1.46
Mean (±SD) Weight at surgery (~p40):
Male: 18.65 ± 2.31
Female: 16.38 ± 1.28
Coat Color: Black or Agouti
Mean (±SD) litter size: 5.3 ± 2.45 (4 breeding sets)
Post-weaning viability: 99.2%



Technical

Breeding Considerations: Breeding sets (pairs and trios) included only tTA heterozygous crosses as homozygous crosses for tTA caused undesirable and/or lethal phenotypes. Although the desirable genotype for experimental animals was heterozygous for all three transgenes, in some instances, animals homozygous for Cre and/or GCaMP6f were included. Despite the fact that the Cux2-CreERT2 is a "knock-in" allele that replaces the first coding ATG of *Cux2*, heterozygotes and homozygotes have been used for breeding with no reported gross defects, although sustained inbreeding of homozygous Cux2-CreERT2 may be contraindicated (Gil-Sanz C, 2015).

Inducible transgene induction procedure: Transgene induction with Tamoxifen is required. Adult mice (>p21) received 5 daily doses of Tamoxifen (200mg/kg, oral gavage) followed by a week of recovery prior to surgery.

Dietary needs: No special dietary needs. Standard chow regimen included breeder chow (PicoLab High Energy Mouse Diet (5LJ5), LabDiet) during the two weeks post-weaning followed by regular chow (PicoLab Laboratory Rodent Diet (5L0D), LabDiet). Additionally, during recovery from surgery mice received a flavor-enhanced hydration/nutrition gel (DietGel (76A), ClearH2O).

General observations: No difference was observed between heterozygous and homozygous animals for GCaMP6 expression pattern or physiology. One mouse out of five tested exhibited interictal events (Steinmetz et al., 2017).

References

Cux2-CreERT2
Source: https://www.mmrrc.org/catalog/sds.php?mmrrc_id=32779
Originating Lab: Ulrich Mueller
Franco SJ, Gil-Sanz C, Martinez-Garay I, Espinosa A, Harkins-Perry SR, Ramos C, Müller U. (2012) Fate-restricted neural progenitors in the mammalian cerebral cortex. *Science* 337(6095):746-9.
Gil-Sanz C, Espinosa A, Fregoso SP, Bluske KK, Cunningham CL, Martinez-Garay I, Zeng H, Franco SJ, Müller U. (2015) Matters Arising: Lineage tracing using Cux2-Cre and Cux2-CreERT2 mice. *Neuron* 86 (4): 1091-1099.

Camk2a-tTA
Source: https://www.jax.org/strain/007004
Originating Lab: Mark Mayford
Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER. (1996) Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274(5293):1678-83. PubMed: 8939850MGI: J:37107

Ai93(TITL-GCaMP6f)
Source: https://www.jax.org/strain/024103
Originating Lab: Allen Institute for Brain Science
Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, Lein ES, Zeng H. (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133-40.

Cux2-CreERT2; Camk2a-tTA; Ai93(TITL-GCaMP6f)
Steinmetz NA, Buetfering C, Lecoq J, Lee CR, Peters AJ, et al. 2017. Aberrant cortical activity in multiple GCaMP6-expressing transgenic mouse lines. *bioRxiv* 138511; doi: https://doi.org/10.1101/138511

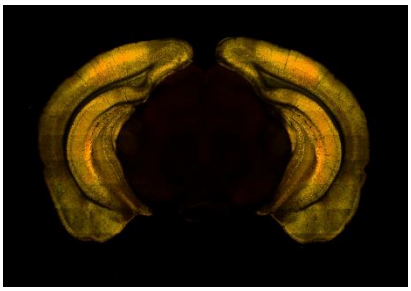
EMX1-IRES-CRE;CAMK2A-TTA;AI93(TITL-GCAMP6F)

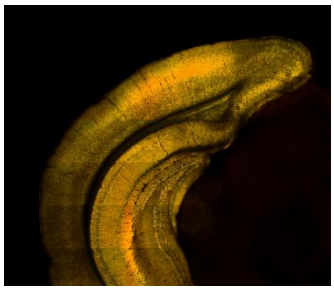
Overview

Emx1-IRES-Cre;Camk2a-tTA;Ai93 mice have a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. Further specificity is achieved by the *Emx1* promoter, resulting in mice that exhibit GCaMP6f in excitatory neurons across layers of cortex as well as in hippocampus. Calcium influx associated with neural activity results in transient increases in fluorescence of CGaMP6-GFP.

Transgene Expression

Native GCaMP6-GFP expression (yellow), visualized by 2P tomography:





Expressed Gene: GCaMP6f, Genetically encoded calcium indicator
Site of Expression: Expressed under the control of tetO in cells where cre recombinase is expressed

Expressed Gene: tTA, tetracycline-controlled transactivator
Site of Expression: Expressed in excitatory forebrain neurons that express *Camk2a*.

Expressed Gene: cre, cre recombinase
Site of Expression: Expressed in cortex (all layers) and hippocampus.

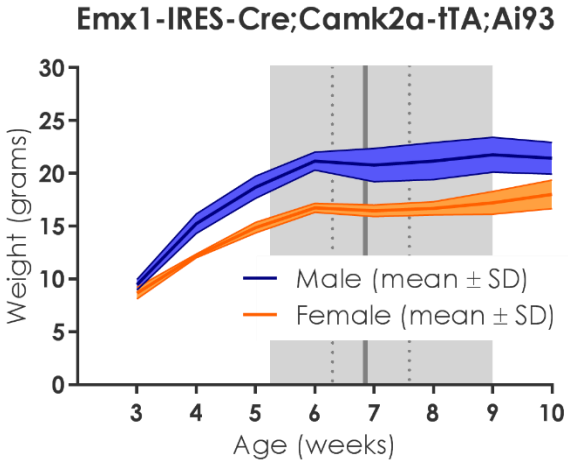
Native reporter expression:

in situ hybridization of *Emx1* (NCBI Accession: XM_132640.3) in C57BL/6J
http://mouse.brain-map.org/experiment/show/100145374

Ai93: *in situ* hybridization of *Camk2a* (NCBI Accession: NM_009792.3) in C57BL/6J
http://mouse.brain-map.org/experiment/show/79490122

Growth and Development

Mean (±SD) Weight at weening (~p21):
Male: 8.53 ± 1.83
Female: 9.09 ± 1.66
Mean (±SD) Weight at surgery (~p40):
Male: 19.68 ± 3.24
Female: 17.00 ± 1.56
Coat Color: Black or Agouti
Mean (±SD) litter size: 4.14 ± 2.96 (5 breeding sets)
Post-weaning viability: 99.7%



Technical

Breeding Considerations: Breeding sets (pairs and trios) included only tTA heterozygous animals as animals homozygous for the tTA transgenes caused undesirable and/or lethal phenotypes. It is important to note that Emx1-IRES-Cre is expressed in mouse germline. Therefore, the mating scheme should avoid combining this Cre transgene and the Cre-deopendent reporter (containing LoxP-STOP-LoxP cassette) until the last cross, which creates the experimental animal. That scheme prevents the exposure of the Cre-dependent reporter to the Cre transgene in the mouse germline. The exposure of the Cre reporter to Cre in the germline results in STOP deletion from the LoxP-STOP-LoxP cassette, and subsequent expression of the reporter that is not Cre-specific.

Dietary needs: No special dietary needs. Standard chow regimen included breeder chow (PicoLab High Energy Mouse Diet (5LJ5), LabDiet) during the two weeks post-weaning followed by regular chow (PicoLab Laboratory Rodent Diet (5L0D), LabDiet). Additionally, during recovery from surgery mice received a flavor-enhanced hydration/nutrition gel (DietGel (76A), ClearH2O).

General observations: The occurrence of interictal events were observed in ~42% of mice as measured at the Allen Institute (Steinmetz et al., 2017).

References

Emx1-IRES-Cre
Source: https://www.jax.org/strain/005628
Originating Lab: Kevin Jones
Gorski JA, Talley T, Qiu M, Puelles L, Rubenstein JL, Jones KR. (2002) Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. *J Neurosci* 22(15):6309-14.

Camk2a-tTA
Source: https://www.mmrrc.org/catalog/sds.php?mmrrc_id=31781
Originating Lab: Mark Mayford & Ullrich Mueller
Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER. (1996) Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274(5293):1678-83.

Ai93(TITL-GCaMP6f)
Source: https://www.jax.org/strain/024103
Originating Lab: Allen Institute for Brain Science
Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, Lein ES, Zeng H. (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133-40.

Emx1-IRES-Cre; Camk2a-tTA; Ai93(TITL-GCaMP6f)
Steinmetz, NA, Buetfering C, Lecoq J, Lee CR, Peters AJ, et al. 2017. Aberrant cortical activity in multiple GCaMP6-expressing transgenic mouse lines. *bioRxiv* 138511; doi: https://doi.org/10.1101/138511

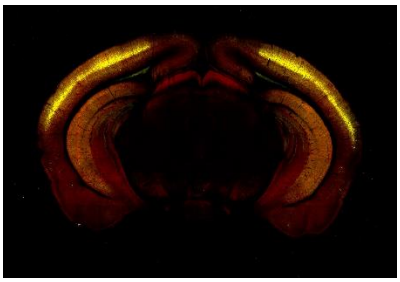
NR5A1-CRE;CAMK2A-TTA;AI93(TITL-GCAMP6F)

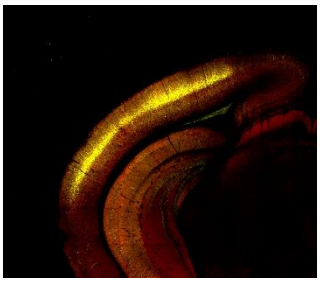
Overview

Nr5a1-Cre;CaMK2a-tTA;Ai93 mice have a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. Further specificity is achieved by the *Nr5a1* promoter, resulting in mice that exhibit GCaMP6f in excitatory neurons in cortical layer 4 as well as in the ventromedial hypothalamus. Calcium influx associated with neural activity results in transient increases in fluorescence of CGaMP6-GFP.

Transgene Expression

Native GCaMP6-GFP expression (yellow), visualized by 2P tomography:





Expressed Gene: GCaMP6f, Genetically encoded calcium indicator
Site of Expression: Expressed under the control of tetO in cells where cre recombinase is expressed

Expressed Gene: tTA, tetracycline-controlled transactivator
Site of Expression: Expressed in excitatory forebrain neurons that express *Camk2a*.

Expressed Gene: cre, cre recombinase
Site of Expression: Expressed in cortical layer 4 as well as in restricted populations within the hypothalamus (ventromedial hypothalamus).

Native reporter expression:

in situ hybridization of *Nr5a1* (NCBI Accession: NM_139051.2) in C57BL/6J
http://mouse.brain-map.org/experiment/show/734

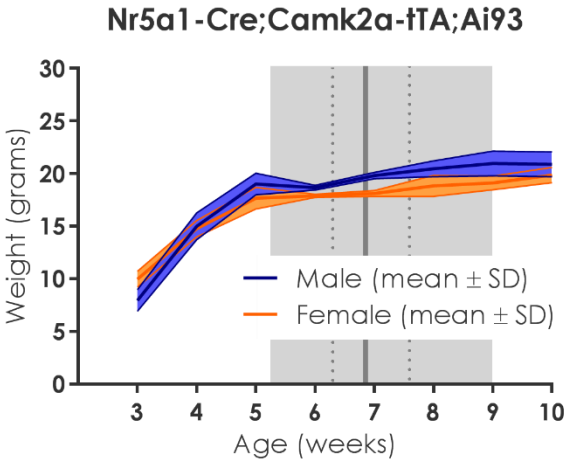
Ai93: *in situ* hybridization of *Camk2a* (NCBI Accession: NM_009792.3) in C57BL/6J
http://mouse.brain-map.org/experiment/show/79490122

Growth and Development

Mean (±SD) Weight at weening (~p21):
- **Male:** Data not available
- **Female:** Data not available

Mean (±SD) Weight at surgery (~p40):
- **Male:** 22.3 ± 2.79
- **Female:** 18.58 ± 1.14

Coat Color: Black or Agouti
Mean (±SD) litter size: 6.44 ± 2.83 (5 breeding sets)
Post-weaning viability: 97.8%



Technical

Breeding Considerations: Breeding sets (pairs and trios) included only heterozygous crosses of Cre-/tTA+ and Cre+/tTA-, as homozygous crosses for either Cre or tTA caused undesirable and/or lethal phenotypes. Both parent mice were homozygous for GCaMP6f, although heterozygous GCaMP6f mice were used when available.

Dietary needs: No special dietary needs. Standard chow regimen included breeder chow (PicoLab High Energy Mouse Diet (5LJ5), LabDiet) during the two weeks post-weaning followed by regular chow (PicoLab Laboratory Rodent Diet (5L0D), LabDiet). Additionally, during recovery from surgery mice received a flavor-enhanced hydration/nutrition gel (DietGel (76A), ClearH2O).

General observations: No abnormal phenotype or behavior.

References

Nr5a1-Cre
Source: https://www.mmrrc.org/catalog/sds.php?mmrrc_id=34276
Originating Lab: Bradford B. Lowell
Dhillon H, Zigman JM, Ye C, Lee CE, McGovern RA, Tang V, Kenny CD, Christiansen LM, White RD, Edelstein EA, Coppari R, Balthasar N, Cowley MA, Chua S Jr, Elmquist JK, Lowell BB. (2006) Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron* 49(2):191-203.

Camk2a-tTA
Source: https://www.mmrrc.org/catalog/sds.php?mmrrc_id=31781
Originating Lab: Mark Mayford & Ullrich Mueller
Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER. (1996) Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274(5293):1678-83.

Ai93(TITL-GCaMP6f)
Source: https://www.jax.org/strain/024103
Originating Lab: Allen Institute for Brain Science
Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, Lein ES, Zeng H. (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat Neurosci 13(1):133-40.

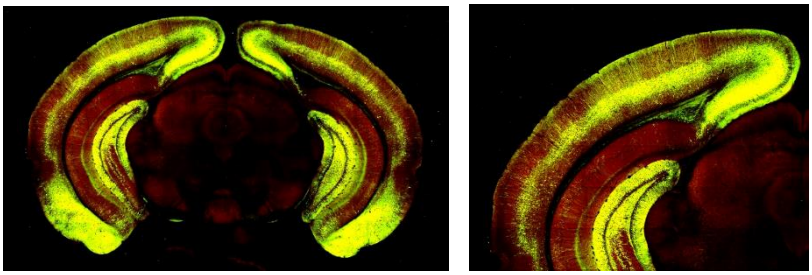
RBP4-CRE_KL100;CAMK2A-TTA;AI93 (TITL-GCAMP6F)

Overview

Rbp4-Cre_KL100;CaMK2a-tTA;Ai93 mice have a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. Further specificity is achieved by the *Rbp4* promotor that results in Cre-mediated expression of GCaMP6f in excitatory neurons in cortical layer 5 as well as dentate gyrus. Following subsequent calcium binding (such as neuronal activation), transient EGFP fluorescence is observed.

Transgene Expression

Native GCaMP6-GFP expression (green), visualized by 2P tomography:



Expressed Gene: GCaMP6f, Genetically encoded calcium indicator
Site of Expression: Expressed under the control of tetO in cells where cre recombinase is expressed

Expressed Gene: tTA, tetracycline-controlled transactivator
Site of Expression: Expressed in excitatory forebrain neurons that express *Camk2a*.

Expressed Gene: cre, cre recombinase
Site of Expression: Expressed in cortical layer 5 and dentate gyrus.

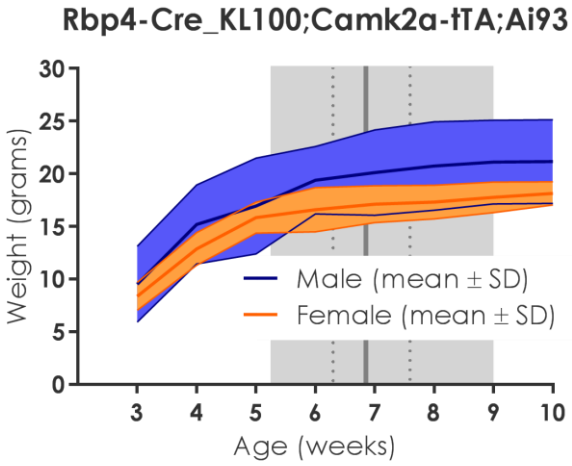
Native gene expression:

in situ hybridization of *Rbp4* (NCBI Accession: NM_011255.1) in C57BL/6J
http://mouse.brain-map.org/experiment/show/71016583

in situ hybridization of *Camk2a* (NCBI Accession: NM_009792.3) in C57BL/6J
http://mouse.brain-map.org/experiment/show/79490122

Growth and Development

Mean (±SD) Weight at weening (~p21):
Male: 8.39 ± 1.56
Female: 8.13 ± 1.46
Mean (±SD) Weight at surgery (~p40):
Male: 18.65 ± 2.31
Female: 16.38 ± 1.28
Coat Color: Black or Agouti
Mean (±SD) litter size: 5.71 ± 1.58 (9 breeding sets)
Post-weaning viability: 98.9%



Technical

Breeding Considerations: Breeding sets (pairs and trios) included only heterozygous crosses of Cre-/tTA+ and Cre+/tTA-, as homozygous crosses for either Cre or tTA caused undesirable and/or lethal phenotypes. It is important to note that Rbp4-Cre_KL100 is expressed in mouse germline. Therefore, the mating scheme should avoid combining this Cre transgene and the Cre-deopendent reporter (containing LoxP-STOP-LoxP cassette) until the last cross, which creates the experimental animal. That scheme prevents the exposure of the Cre-dependent reporter to the Cre transgene in the mouse germline. The exposure of the Cre reporter to Cre in the germline results in STOP deletion from the LoxP-STOP-LoxP cassette, and subsequent expression of the reporter that is not Cre-specific.

Dietary needs: No special dietary needs. Standard chow regimen included breeder chow (PicoLab High Energy Mouse Diet (5LJ5), LabDiet) during the two weeks post-weaning followed by regular chow (PicoLab Laboratory Rodent Diet (5L0D), LabDiet). Additionally, during recovery from surgery mice received a flavor-enhanced hydration/nutrition gel (DietGel (76A), ClearH2O).

General observations: A small fraction (7.2%) of Rbp4-Cre;CaMK2a-tTA;Ai93 mice exhibited a tremulous phenotype and were therefore excluded from the experimental pipeline. No interictal events were observed.

References

Rbp4-Cre_KL100
Source: https://www.mmrrc.org/catalog/sds.php?mmrrc_id=31125
Originating Lab: Nathaniel Heintz & Charles Gerfen
Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N, Gerfen CR. (2007) Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J Neurosci* 27(37):9817-23.
Gerfen CR, Paletzki R, Heintz N. (2013) GENSAT BAC cre-recombinase driver lines to study the functional organization of cerebral cortical and basal ganglia circuits. *Neuron* Dec 18;80(6):1368-83.

Camk2a-tTA
Source: https://www.jax.org/strain/007004
Originating Lab: Mark Mayford
Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER. (1996)_ Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274(5293):1678-83.

Ai93 (TITL-GCaMP6f)
Source: https://www.jax.org/strain/024103
Originating Lab: Allen Institute for Brain Science
Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, Lein ES, Zeng H. (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133-40.

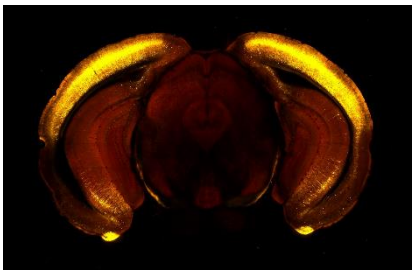
RORB-IRES2-CRE;CAMK2A-TTA;AI93(TITL-GCAMP6F)

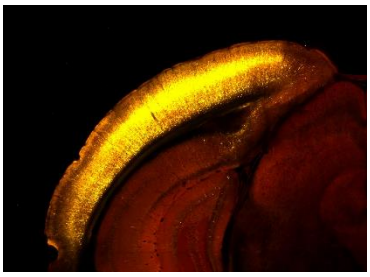
Overview

Rorb-IRES2-Cre;CaMK2a-tTA;Ai93 mice have a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. Further specificity is achieved by the *Rorb* promotor, which results in Cre-mediated expression of GCaMP6f in excitatory neurons in cortical layer 4 (dense patches) and layers 5,6 (sparse) as well as in superior colliculus and thalamus. Following subsequent calcium binding (such as neuronal activation), transient EGFP fluorescence is observed.

Transgene Expression

Native GCaMP6-GFP expression (green), visualized by 2P tomography:





Expressed Gene: GCaMP6f, Genetically encoded calcium indicator
Site of Expression: Expressed under the control of tetO in cells where cre recombinase is expressed

Expressed Gene: tTA, tetracycline-controlled transactivator
Site of Expression: Expressed in excitatory forebrain neurons that express *Camk2a*.

Expressed Gene: cre, cre recombinase
Site of Expression: Dense, patchy expression in cortical layer 4 and sparse expression in layers 5,6. Sub-cortical expression in superior colliculus and thalamus.

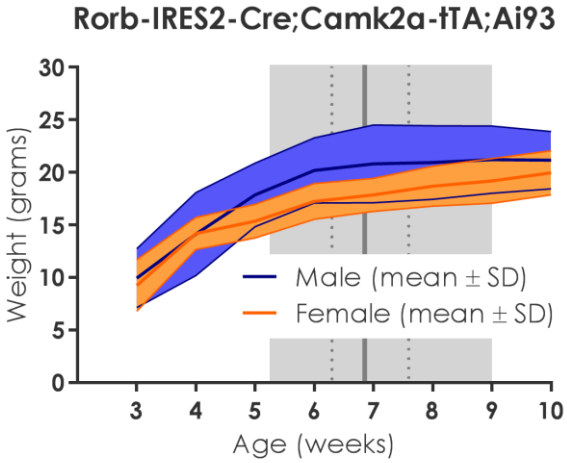
Native gene expression:

in situ hybridization of *Rorb* (NCBI Accession: NM_146095.1) in C57BL/6J
http://mouse.brain-map.org/experiment/show/79556597

in situ hybridization of *Camk2a* (NCBI Accession: NM_009792.3) in C57BL/6J
http://mouse.brain-map.org/experiment/show/79490122

Growth and Development

Mean (±SD) Weight at weening (~p21):
Male: 7.89 ± 1.52
Female: 8.92 ± 1.44
Mean (±SD) Weight at surgery (~p40):
Male: 19.49 ± 1.67
Female: 16.7 ± 1.56
Coat Color: Black
Mean (±SD) litter size: 7.7 ± 3.47 (6 breeding sets)
Post-weaning viability: 99.4%



Technical

Breeding Considerations: Breeding sets (pairs and trios) included only heterozygous crosses of Cre-/tTA+ and Cre+/tTA-, as homozygous crosses for either Cre or tTA caused undesirable and/or lethal phenotypes. Either homozygous or heterozygous GCaMP6f mice were used for breeding.

Dietary needs: No special dietary needs. Standard chow regimen included breeder chow (PicoLab High Energy Mouse Diet (5LJ5), LabDiet) during the two weeks post-weaning followed by regular chow (PicoLab Laboratory Rodent Diet (5L0D), LabDiet). Additionally, during recovery from surgery mice received a flavor-enhanced hydration/nutrition gel (DietGel (76A), ClearH2O).

General observations: No interictal events or other abnormal phenotypes were observed.

References

Rorb-IRES2-Cre
Source: https://www.jax.org/strain/023526
Originating Lab: Allen Institute for Brain Science
Harris JA, Hirokawa KE, Sorensen SA, Gu H, Mills M, Ng LL, Bohn P, Mortrud M, Ouellette B, Kidney J, Smith KA, Dang C, Sunkin S, Bernard A, Oh SW, Madisen L, Zeng H. (2014) Anatomical characterization of Cre driver mice for neural circuit mapping and manipulation. *Front Neural Circuits* 8:76.

Camk2a-tTA
Source: https://www.jax.org/strain/007004
Originating Lab: Mark Mayford
Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER. (1996) Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274(5293):1678-83.

Ai93(TITL-GCaMP6f)
Source: https://www.jax.org/strain/024103
Originating Lab: Allen Institute for Brain Science
Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, Lein ES, Zeng H. (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133-40.

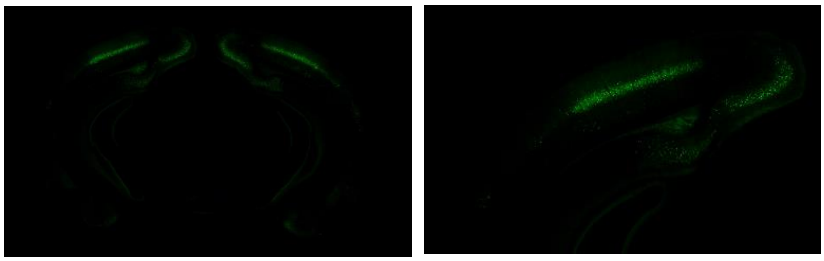
SCNN1A-TG3-CRE;CAMK2A-TTA;AI93(TITL-GCAMP6F)

Overview

Scnn1a-Tg3-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f) mice have a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, and a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. The *Scnn1a* promoter confers further specificity, resulting in mice that exhibit GCaMP6f in excitatory neurons in cortical layer 4 and in restricted populations within the cortex, thalamus, and in cerebellum. Calcium influx associated with neural activity results in transient increases in fluorescence of GCaMP6.

Transgene Expression

Native GCaMP6-GFP expression (green), visualized by 2P tomography:



Expressed Gene: GCaMP6f, Genetically encoded calcium indicator
Site of Expression: Expressed under the control of tetO in cells where cre recombinase is expressed.

Expressed Gene: tTA, tetracycline-controlled transactivator
Site of Expression: Expressed in excitatory forebrain neurons that express *Camk2a*.

Expressed Gene: cre, cre recombinase
Site of Expression: Expressed in cortical layer 4 and in restricted populations within cortex, thalamus, and in cerebellum.

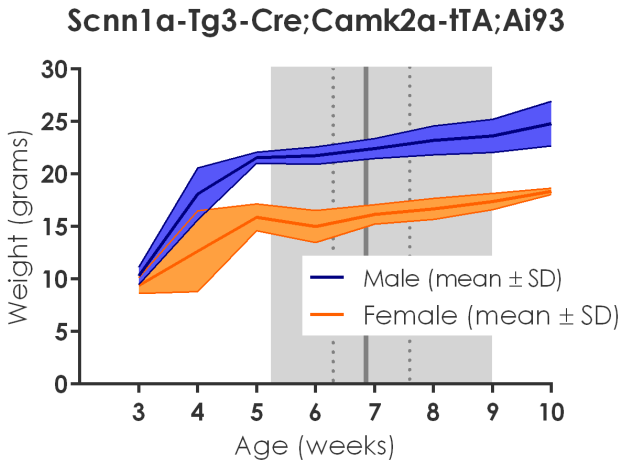
Native gene expression:

in situ hybridization of *Scnn1a* (NCBI Accession: NM_011324.1) in C57BL/6J; a subset of Scnn1a-positive neurons are represented in Tg3 strain.
http://mouse.brain-map.org/experiment/show/70562125

in situ hybridization of *Camk2a* (NCBI Accession: NM_009792.3) in C57BL/6J
http://mouse.brain-map.org/experiment/show/79490122

Growth and Development

Mean (±SD) Weight at weaning (~p21):
Male: 8.99 ± 1.27
Female: 8.76 ± 0.99
Mean (±SD) Weight at surgery (~p40):
Male: 19.85 ± 2.29
Female: 17.02 ± 1.38
Coat Color: Black
Mean (±SD) litter size: 7.33 ± 3.39 (3 breeding sets)
Post-weaning viability: 98.8%



Technical

Breeding Considerations: Breeding sets (pairs and trios) included only heterozygous crosses of Cre-/tTA+ and Cre+/tTA-, as homozygous crosses for either Cre or tTA caused undesirable and/or lethal phenotypes. Although the desirable genotype for experimental animals was heterozygous for all three transgenes, in some instances, animals homozygous for GCaMP6f were included.

Dietary needs: No special dietary needs. Standard chow regimen included breeder chow (PicoLab High Energy Mouse Diet (5LJ5), LabDiet) during the two weeks post-weaning followed by regular chow (PicoLab Laboratory Rodent Diet (5L0D), LabDiet). Additionally, during recovery from surgery mice received a flavor-enhanced hydration/nutrition gel (DietGel (76A), ClearH_2O).

General observations: No interictal events or other abnormal phenotypes were observed.

References

Scnn1a-Tg3-Cre
Source: https://www.jax.org/strain/009613
Originating Lab: Allen Institute for Brain Science
Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, Lein ES, Zeng H. (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133-40..

Camk2a-tTA
Source: https://www.jax.org/strain/007004
Originating Lab: Mark Mayford
Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER. (1996) Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274(5293):1678-83.

Ai93(TITL-GCaMP6f)
Source: https://www.jax.org/strain/024103
Originating Lab: Allen Institute for Brain Science
Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, Lein ES, Zeng H. (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133-40.