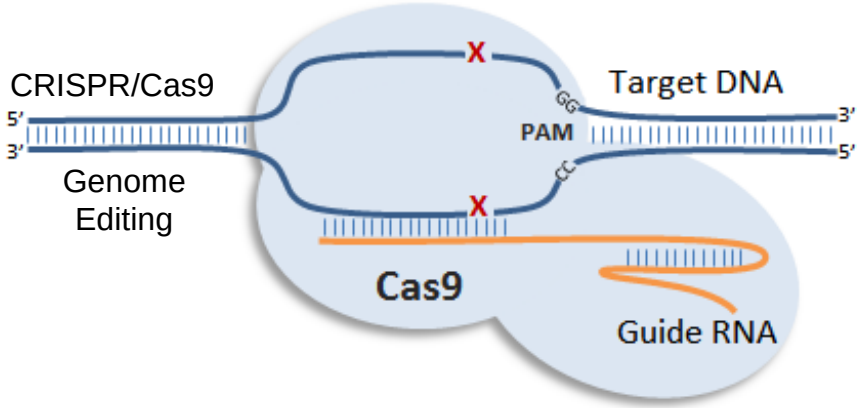
Genetically Engineered Primate Models for Brain Disorder Research



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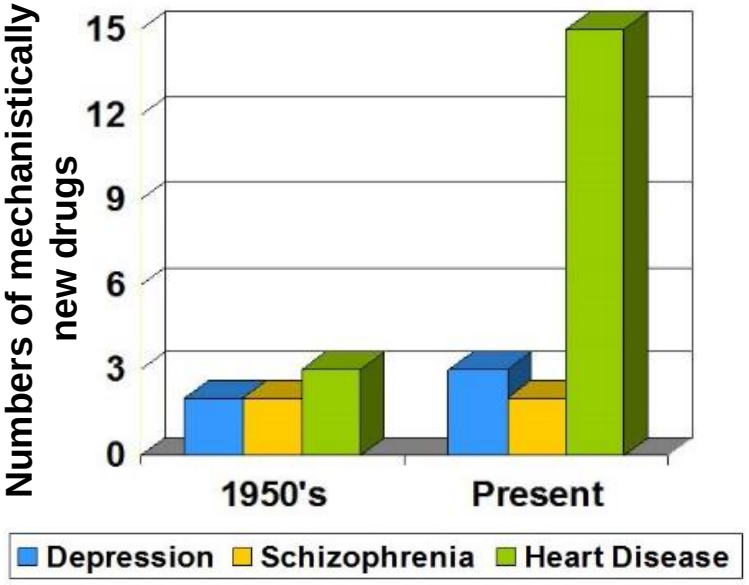
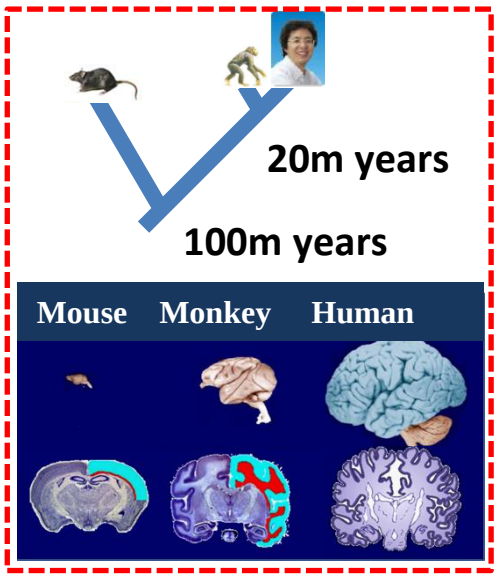
The need for better models in brain disorder research

Genetic engineering in mice has revolutionized biomedical research. However, its impact on developing treatments for brain disorders is limited.

- **Limitations of rodent models for brain disorders：**

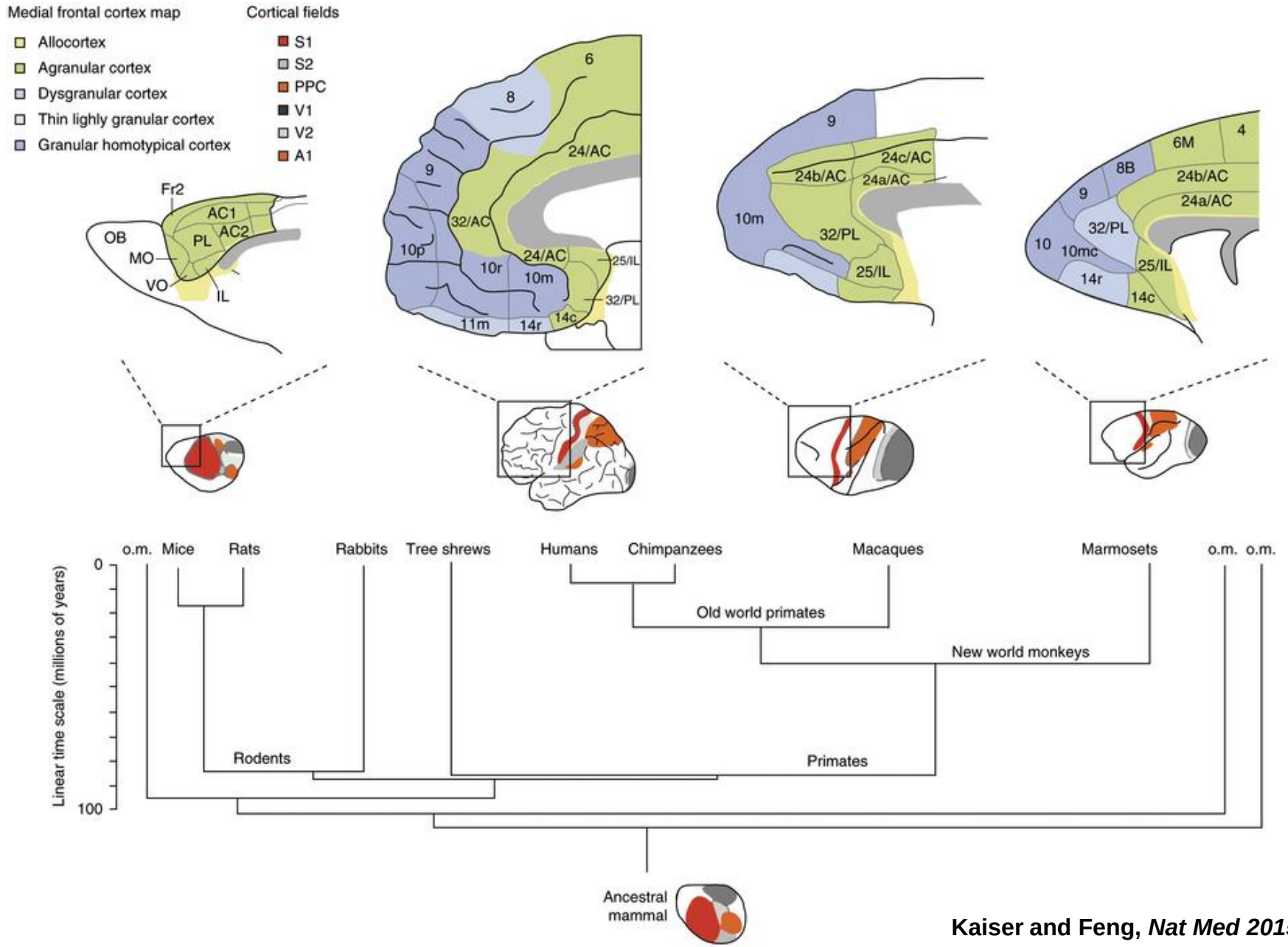
1. Huge differences in both structure and function between rodent and human brain.
2. Evolutional divergence in behaviors and underlying circuits
3. Numerous failures in translating preclinical success in rodents to clinical trials in humans





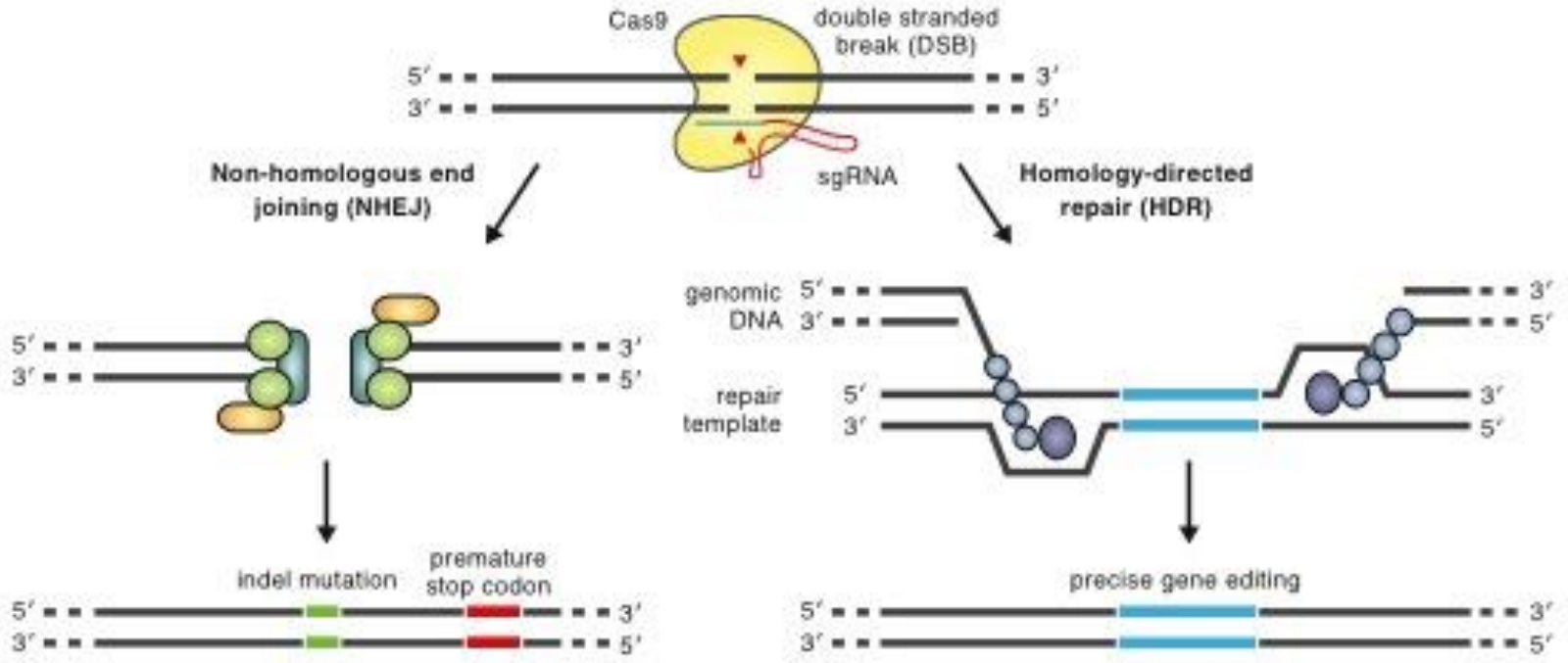
The lack of good animal models is considered one of bottlenecks to the development of new drugs for brain disorders

Better animal models for prefrontal cortex and developmental studies



Kaiser and Feng, *Nat Med 2015*

Feasibility: New genome-editing technology—CRISPR/Cas9 and TALEN



1. **Highly efficient (heterozygous and homozygous mutations)**
2. **Can make multiple mutations in parallel**
3. **Direct embryo injection leads to genetically engineered animals**

Cell

Resource

Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos

Yuyu Niu,[1,5,7] Bin Shen,[2,7] Yiqiang Cui,[3,7] Yongchang Chen,[1,5,7] Jianying Wang,[2] Lei Wang,[3] Yu Kang,[1,5] Xiaoyang Zhao,[4] Wei Si,[1,5] Wei Li,[4] Andy Peng Xiang,[6] Jiankui Zhou,[2] Xuejiang Guo,[3] Ye Bi,[3] Chenyang Si,[1,5] Bian Hu,[2] Guoying Dong,[3] Hong Wang,[1,5] Zuomin Zhou,[3] Tianqing Li,[1,5] Tao Tan,[1,5] Xiuqiong Pu,[1,5] Fang Wang,[1,5] Shaohui Ji,[1,5] Qi Zhou,[4] Xingxu Huang,[2,*] Weizhi Ji,[1,5,*] and Jiahao Sha[3,*]
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Cell Stem Cell
Brief Report

Cell PRESS

TALEN-Mediated Gene Mutagenesis in Rhesus and Cynomolgus Monkeys

Hailiang Liu,[1,2,9] Yongchang Chen,[1,3,4,9] Yuyu Niu,[1,3,4,9] Kunshan Zhang,[2,9] Yu Kang,[1,3] Weihong Ge,[6] Xiaojing Liu,[2] Enfeng Zhao,[2,8] Chencheng Wang,[2] Shaoyun Lin,[2] Bo Jing,[2] Chenyang Si,[1,3] Quan Lin,[2,6] Xiaoying Chen,[2,6] Haijun Lin,[2] Xiuqiong Pu,[1,3] Yingying Wang,[2] Binlian Qin,[2] Fang Wang,[1,3] Hong Wang,[1,3,4] Wei Si,[1,3,4] Jing Zhou,[2] Tao Tan,[1,3,4] Tianqing Li,[1,3,4] Shaohui Ji,[1,3,4] Zhigang Xue,[2] Yuping Luo,[2] Liming Cheng,[2] Qi Zhou,[5] Siguang Li,[2,*] Yi Eve Sun,[1,2,6,*] and Weizhi Ji[1,3,7,*]
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Cell Stem Cell
Resource

CellPress

Generation of a Nonhuman Primate Model of Severe Combined Immunodeficiency Using Highly Efficient Genome Editing

Kenya Sato,[1] Ryo Oiwa,[1] Wakako Kumita,[1] Rachel Henry,[2] Tetsushi Sakuma,[3] Ryoji Ito,[1] Ryoko Nozu,[1] Takashi Inoue,[1] Ikumi Katano,[1] Kengo Sato,[4] Norio Okahara,[1] Junko Okahara,[1] Yoshihisa Shimizu,[1] Masafumi Yamamoto,[1] Kisaburo Hanazawa,[5] Takao Kawakami,[6] Yoshie Kametani,[7] Ryuji Suzuki,[8] Takeshi Takahashi,[1] Edward J. Weinstein,[2] Takashi Yamamoto,[3] Yasubumi Sakakibara,[4] Sonoko Habu,[9] Jun-ichi Hata,[1] Hideyuki Okano,[10,*] and Erika Sasaki[1,11,*]

Genome-editing in primates to generate better disease models?

Macaque (Cynomolgus)

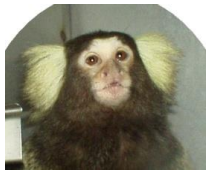
Large (7-20lb), 30 years of life span, native to Southeast Asia
Mature in 4 years, 180 days gestation.
Very similar to rhesus monkey
Similar brain structure to humans
Great for study of higher brain function

Common marmoset

Small (~350g), 12-16 years of life span, New World monkey
Short reproduction cycle, mature in 1 year, 150 days gestation
Give birth twice a year and produce twins each birth.
Very social and communicative

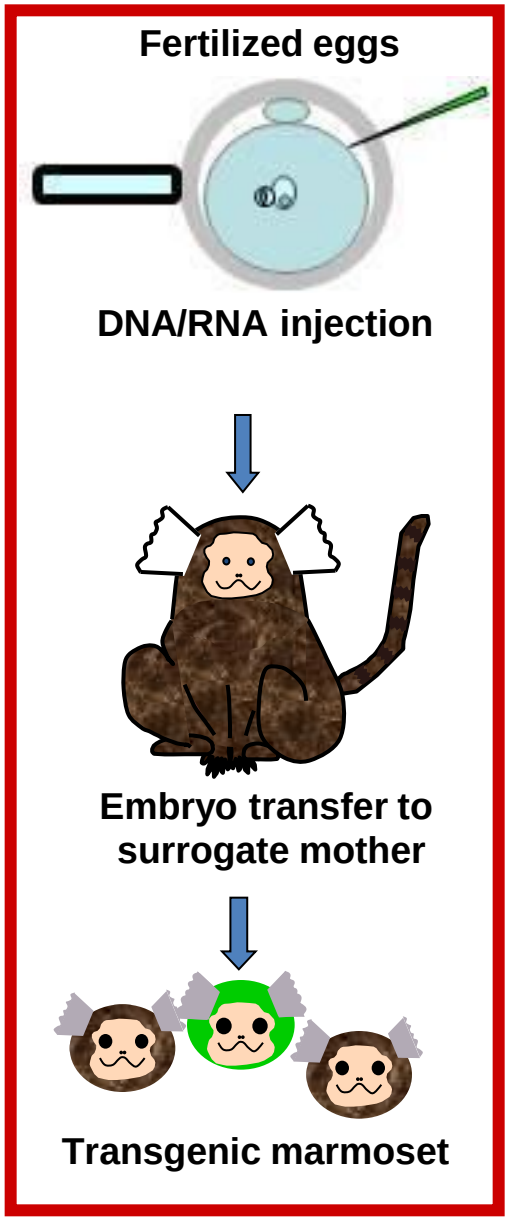
Comparison of reproductive characteristics

	Mouse	Marmoset	Macaque
Age of puberty	8 weeks	1 year old	3-4 years
Litter size	6-8	2-3	1
Gestation period	18 days	~140 days	~160 days
Delivery interval	20-28 days	~150 days	~550 days
offspring/year	36-48	4-6	<1



Fast reproduction: Faster to germline; large numbers of animals for behavioral testing; large numbers of animals for brain tissues.

Precise engineering of human genetic mutations in primates



Transgenic primates

Disease models (knockouts and knockins):

- Gene deletions
- Gene insertions
- Point mutations
- Conditional mutations

Genetic tools (knockins and transgenes):

- Cell type-specific Cre lines
- Cell type-specific indicators
- Cell type-specific actuators

Genetically engineered marmosets as a primate model for studying brain disorders—a collaborative project at MIT and Broad Institute

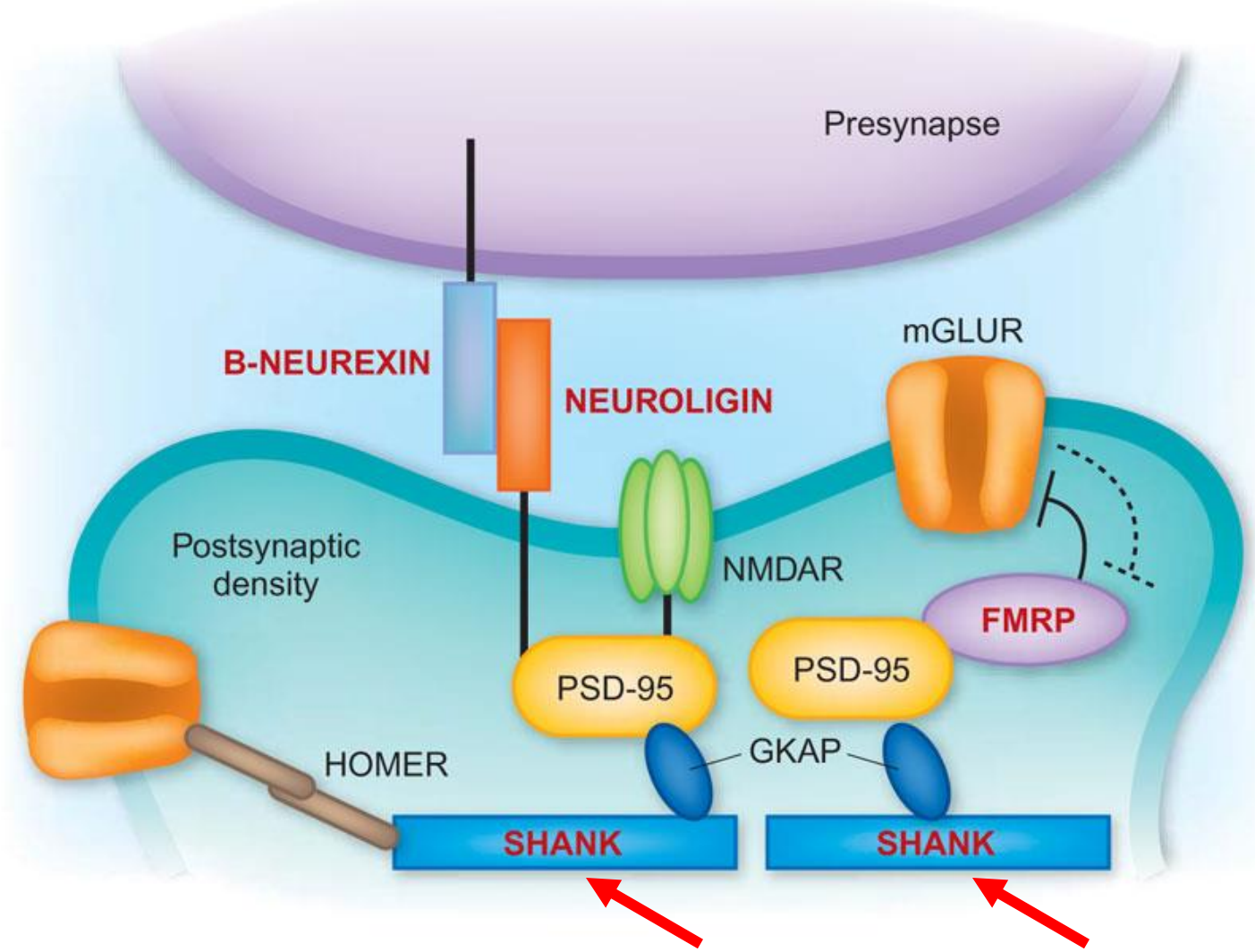
Long term goal:

Establish genetically engineered primates as a genetic model for brain disorder research and new drug discovery, using CRISPR system for targeted genetic mutations. Program will serve as a hub for academic and commercial research activities nationwide.

Current Status:

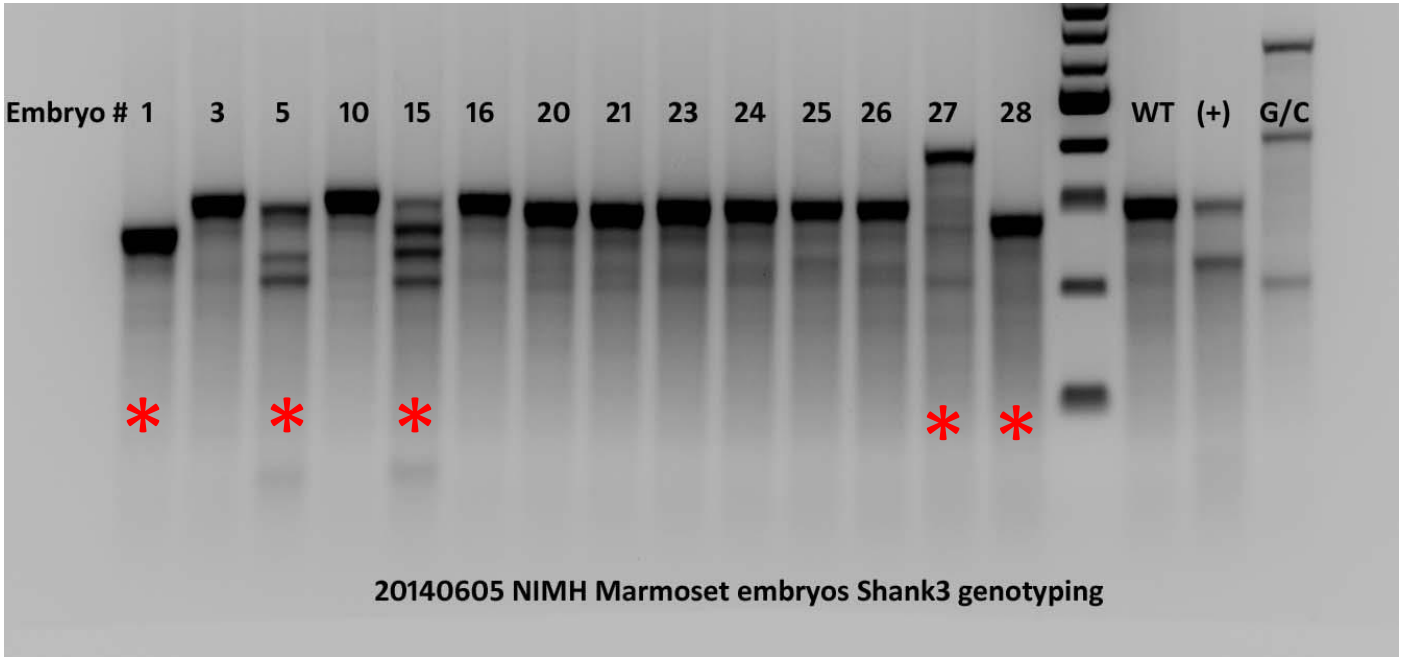
1. Established marmoset colonies
2. Establishing a genetic engineering platform—in collaboration with Jim Pickel and Afonso Silva at NIMH and Hideyuki Okano and Erika Sasaki at Keio University and CIEA.
3. Generated a better marmoset reference sequence.
4. Developed hardware and software for automated and quantitative vocal communication analysis—with MIT Lincoln Laboratory
5. Developing hardware and software for automated and quantitative behavioral analysis—with MIT Computer Science and Artificial Intelligence Laboratory (CSAIL).
6. Establishing brain imaging methods—in collaboration with Martino Center for Biomedical Imaging at MGH
7. Single ell transcriptome analysis using Dropseq—with Steve McCarroll at Harvard.

Shank3 mutations cause autism spectrum disorder



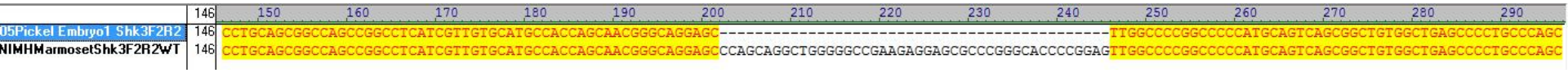
Marmoset embryos injected with Shank3 Cas9/sgRNA

(In collaboration with Jim Pickel at NIMH)

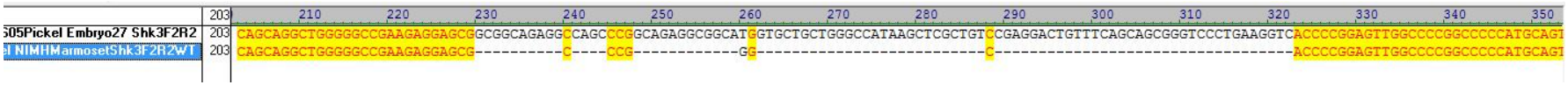


Targeting rate: ~50%

Embryo # 1: at least one allele carrying large deletion (43bp), will induce frameshift and loss of shank3 protein, as revealed by direct sequencing from PCR product



Embryo # 27: at least one allele carrying insertion (85bp), will induce frameshift and loss of shank3 protein, as revealed by direct sequencing from PCR product

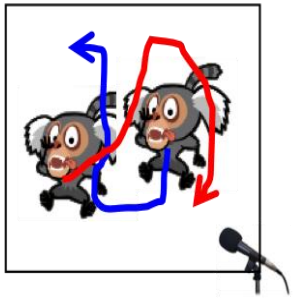


Developing behavioral testing and monitoring systems

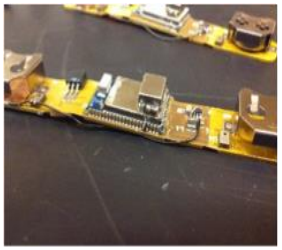
Vocal behavior

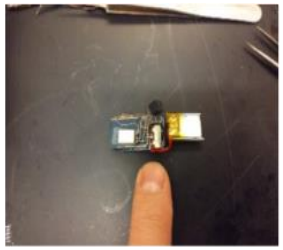
A

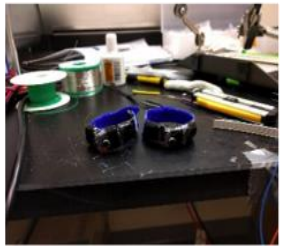
B



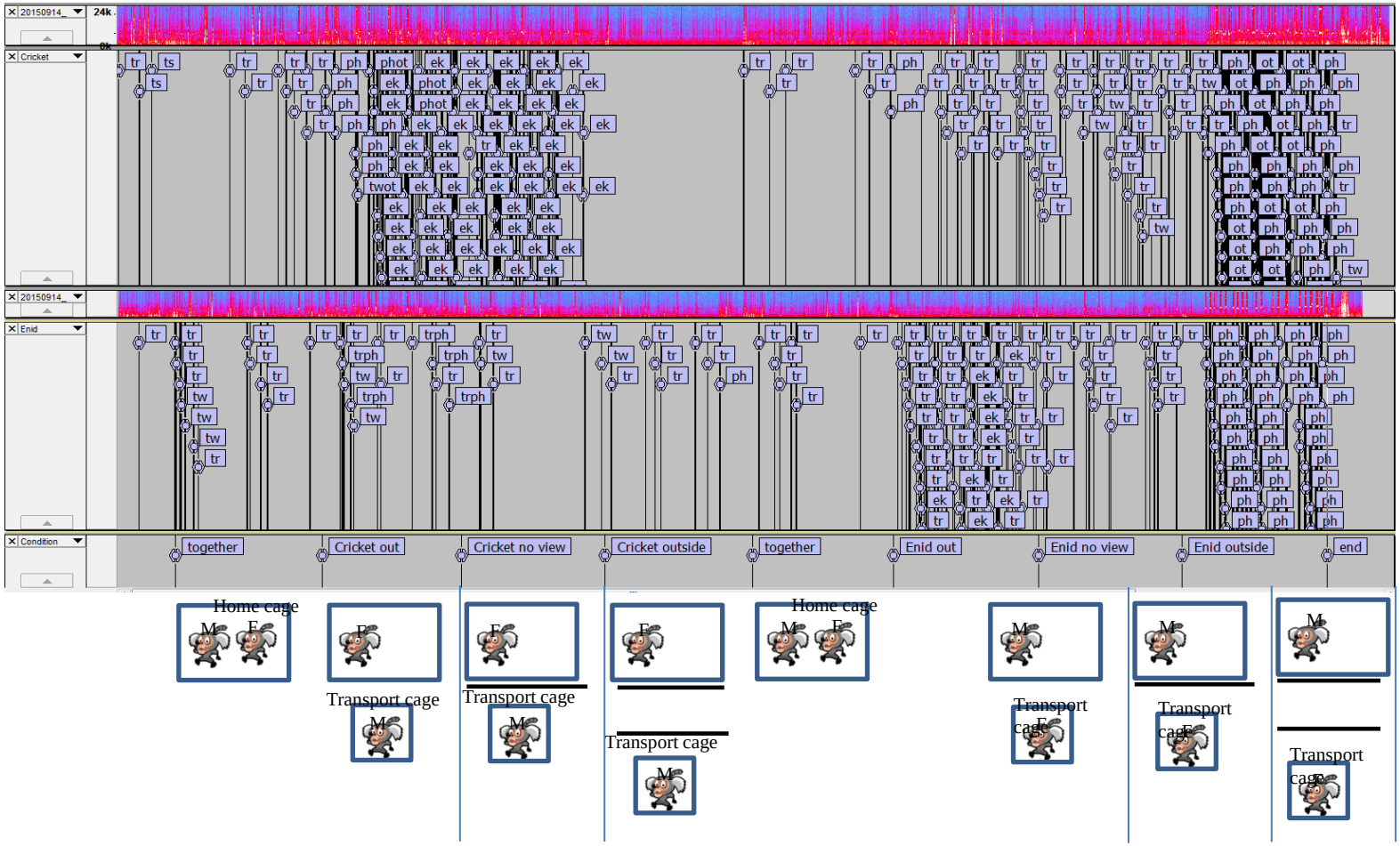
C





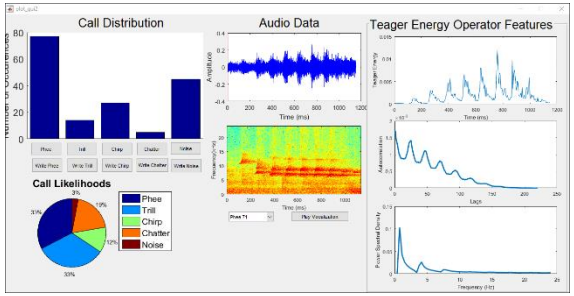


Vocal behavior of adults

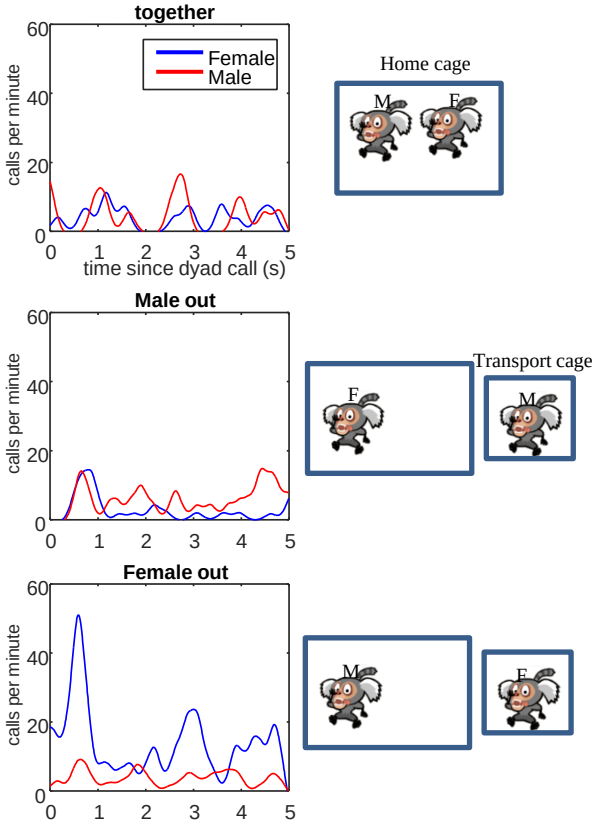


Vocal behavior

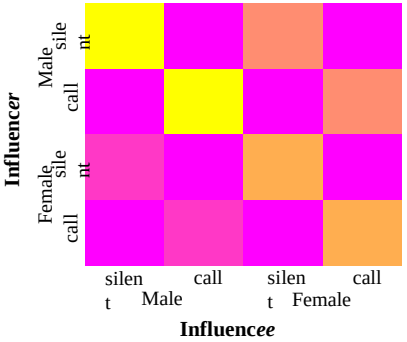
Automatic call detection



Vocal interactions

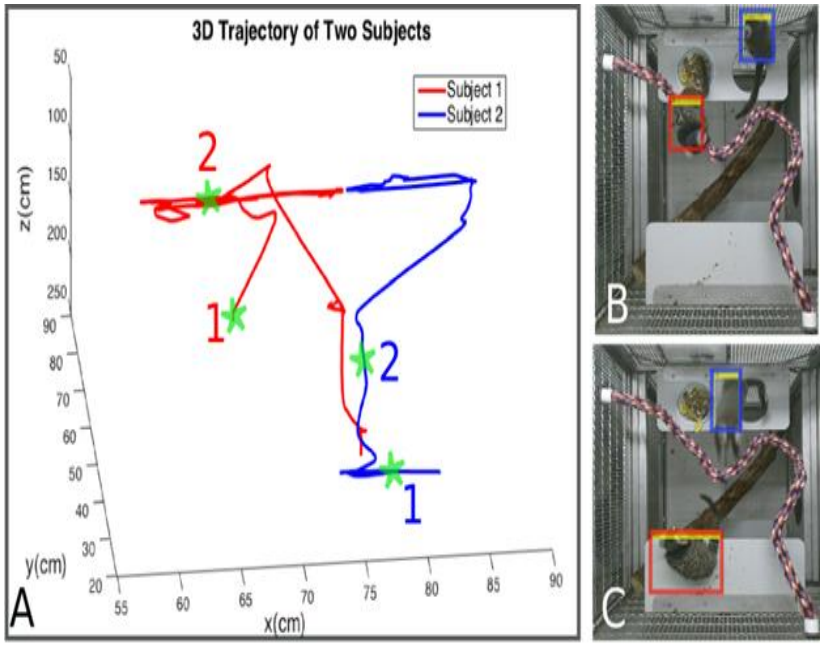


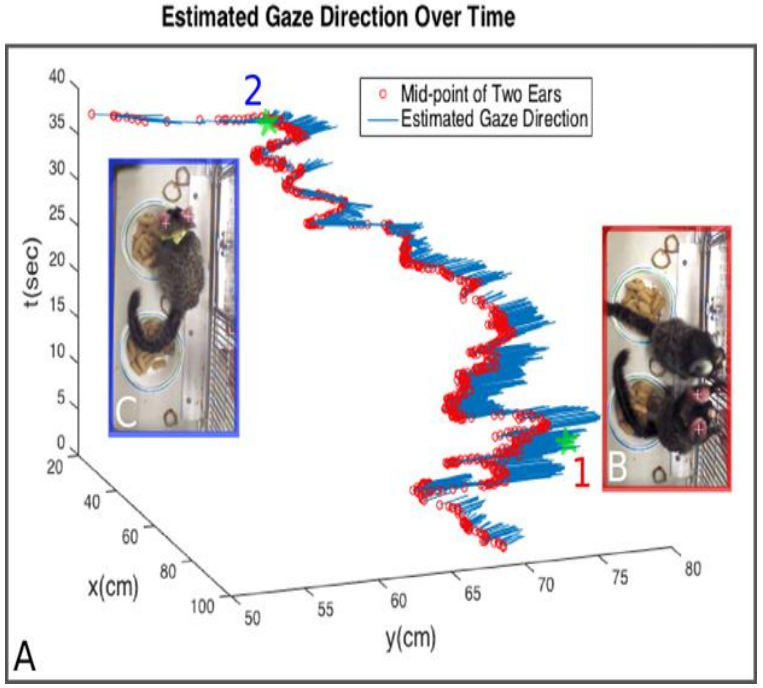
Influence modeling



Developing behavioral testing and monitoring systems

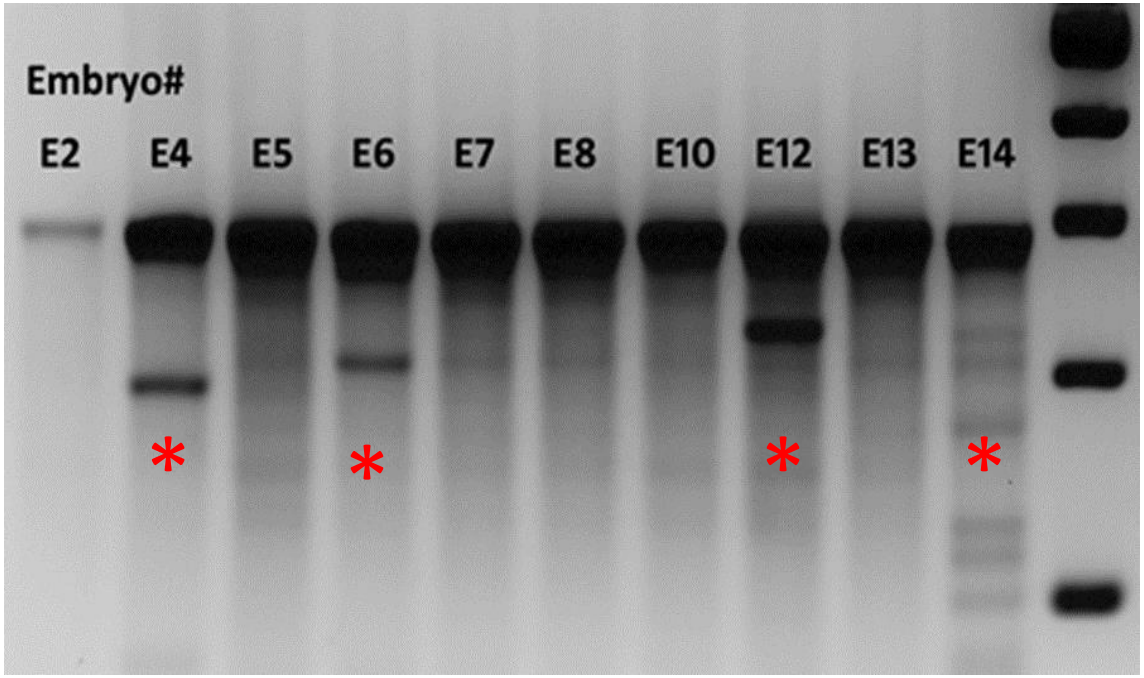
Automated tracking and behavior recognition using depth cameras and machine learning





Cynomolgus macaque embryos injected with Shank3 Cas9/sgRNA

(In collaboration with Shihua Yang and Xiang Peng in Guangzhou, China)



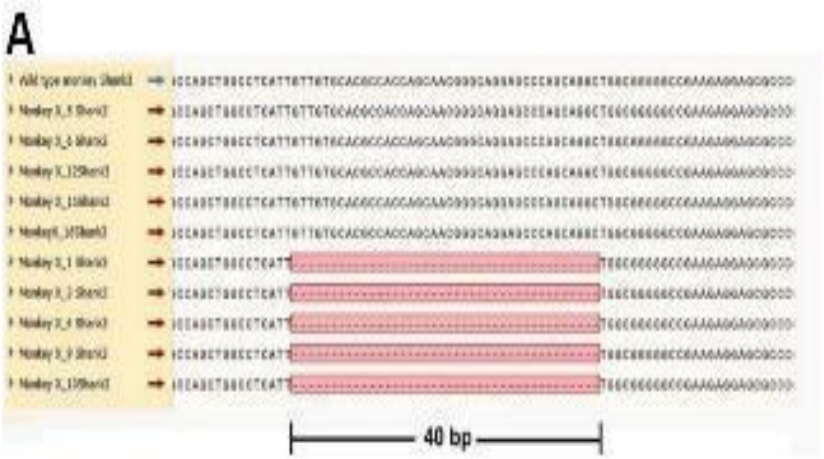
Modified embryos: #4, #5, #12, #14

Targeting rate: ~30%

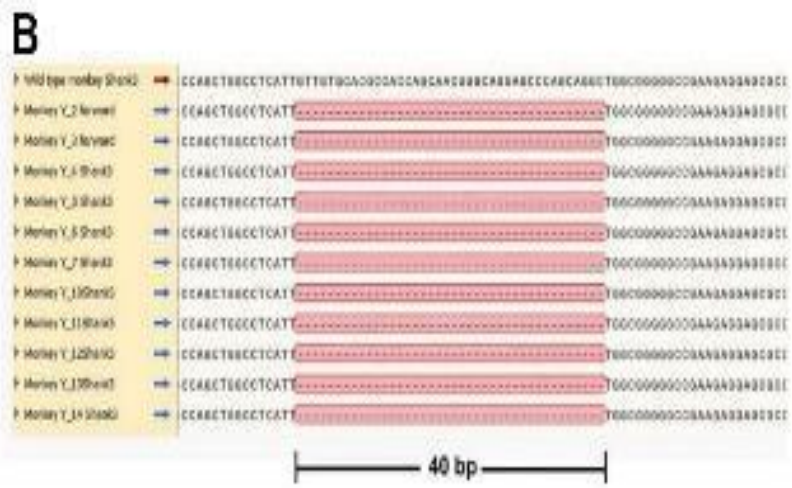
Toward development of macaque models of Shank3 mutations

Macaque model

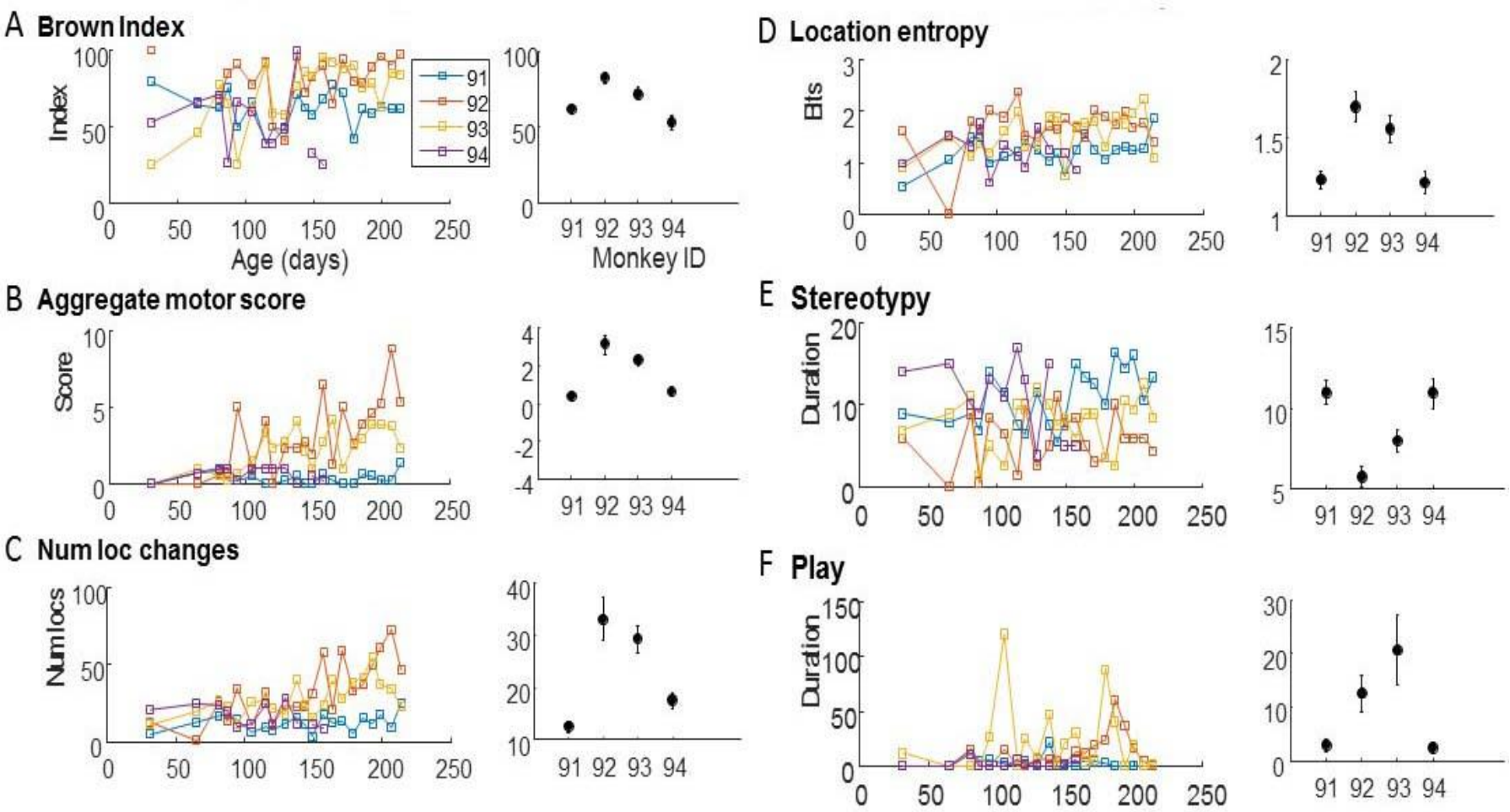
- **Established a collaborative project with Shenzhen Institute of Advanced Technology: Liping Wang, Huihui Zhou and the team.**
- **Active collaboration with Andy (Peng) Xiang at Sun Yat-sen University and Shihua Yang at Southern China Agricultural University to generate Shank3 mutant Cynomolgus macaques**
- **Generated several Shank3 mutant macaque monkeys**
- **Behavioral testing is in progress**



Heterozygous Shank3 deletion



Homozygous Shank3 deletion


A Brown Index
Index
Age (days)
91
92
93
94
Monkey ID
B Aggregate motor score
Score
C Num loc changes
Num locs
D Location entropy
Bits
E Stereotypy
Duration
F Play
Duration

Looking Forward

1. **Generating multiple monogenic disease models for studying neurobiological mechanisms.**
2. **Focus on prefrontal cortex, developmental process and longitudinal molecular and cellular changes.**
3. **Improve CRISPR/Cas9-mediated homologous recombination efficiency.**
4. **Develop cell type-specific Cre lines**
5. **Mapping disease relevant cell type-specific molecular changes.**
6. **Identify cell type-specific targets for correcting circuit dysfunction.**

Our Primate Genetic Engineering Team

Guoping Feng, (MIT and Stanley Center for Psychiatric Research at Broad Institute)

Feng Zhang, (MIT and Broad Institute)

Robert Desimone and McGovern Institute for Brain Research

Steve Hyman and the Stanley Center for Psychiatric Research

Steve McCarroll, (Harvard Medical School and Stanley Center for Psychiatric Research)

Laura Brattain, Thomas Quatieri, Edward Wack and MIT Lincoln Lab

Yang Zhou, Rogier Landman, Julia Hyman, Jitendra Sharma, Charles Jennings, Martin Wienisch Alan Wisler, Karthik Srinivasan, Ricardo del Rosario (MIT and Broad)

James Fox, ***Robert Marini and Monika Burns,*** (Division of Comparative Medicine, MIT)

Many others at MIT and Stanley Center/Broad Institute (Jacquelyn Stathopoulos***)***

Key Collaborators:

Jim Pickel, (Director of NIMH Transgenic Core)

Afonso Silva, (Investigator, NIMH)

Hideyuki Okano and Erika Sasaki (Professors at Keio University and CIEA.

Shihua Yang (Professor, South China Agricultural University)

Peng Xiang (Professor, Sun Yat-sen University)

Jon Hennebold (Director of Reproduction lab, Oregon Primate Center)

Liping Wang (Professor, Shenzhen Institute of Advanced Technology, CAS)

Huihui Zhou (Professor, Shenzhen Institute of Advanced Technology, CAS)

SIAT Peacock Team**:** Xingtian Wu, Fuqiang Xu, Yu Chen, Yang Zhan

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Poitras Center for Affective Disorder Research at MIT
Stanley Center for Psychiatric Research at Broad
Massachusetts Life Science Center
Broad *Next10*
Brain Research Foundation
Cure Huntington Disease Initiative (CHDI)