Relationship of Oxytocin and the Serotonin Transporter Single Nucleotide Polymorphisms and Antisocial Behavior

Elizabeth Chesna, BS*[a]; Charity Beherec, MS[a]; Gabriella Cansino, MS[a]; Peyton Gandy, MS[a]; Jessica Wells, MS[b]; Danielle Boisvert, PhD[b]; Todd Armstrong, PhD[b]; Sheree Hughes-Stamm, PhD[a]; and David Gangitano, PhD[a]

[a] *Department of Forensic Science*
[b] *Department of Criminal Justice and Criminology*
Sam Houston State University
Huntsville, TX, USA

Sam Houston State University

Disclosure

- There is no real or apparent conflicts of interest related to the content of this presentation

- Products used:
  - SNaPshot® Multiplex Kit
  - Ion Chef™ System
  - Ion PGM™ (Personal Genome Machine)

FSF Emerging Forensic Scientist Award
Paper Presentation

Behavior is influenced by two things:

1. Genetics

http://medgen.med.miami.edu/news/2012/02/human-genetics-and-genomics-seminar-series2

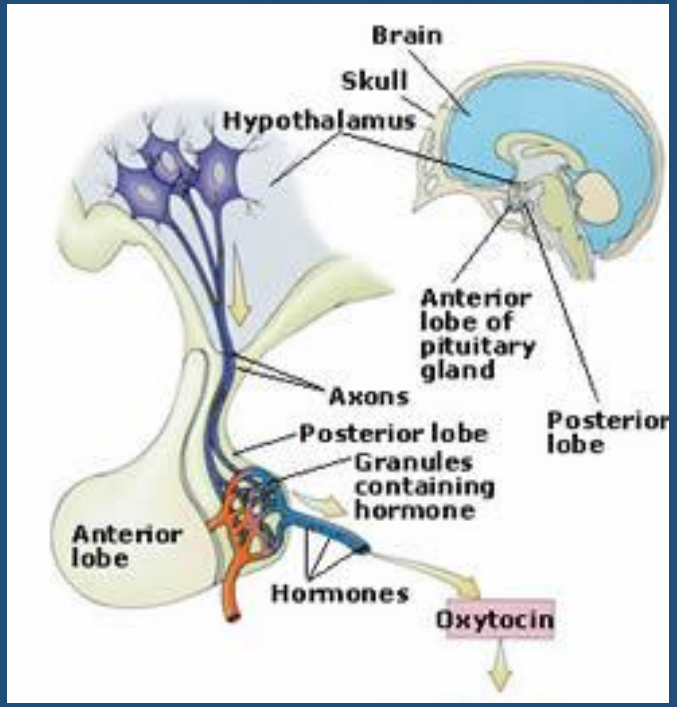
2. Environment

http://www.todayschristianwoman.com/articles/2012/mayjune-issue/meeting-god-of-nurture.html

Neurotransmitters Associated with Social Behavior

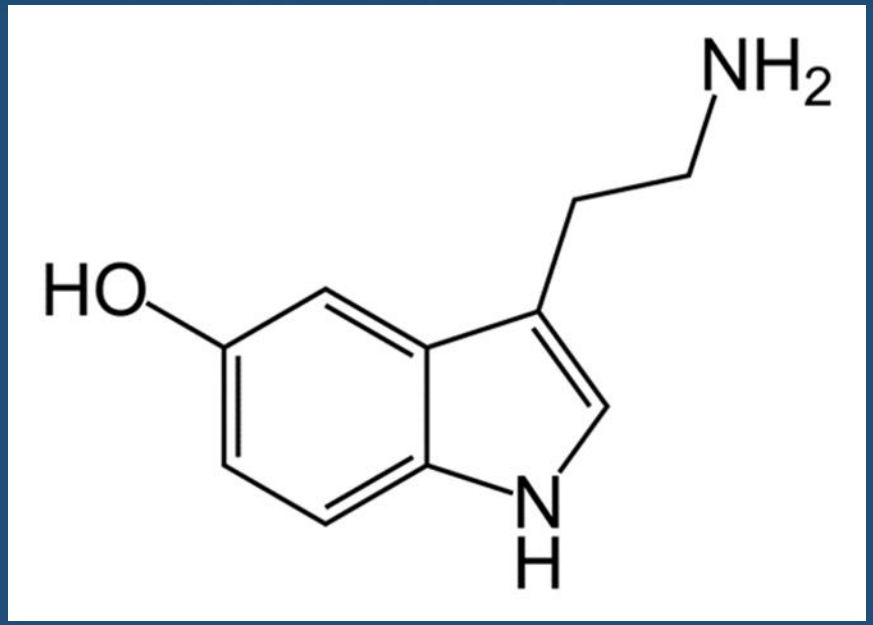
Oxytocin

- Produced in the hypothalamus
  - After secretion, is stored or circulated through the bloodstream
- Equal concentrations in men and women
  - Posterior pituitary
  - Plasma
- Oxytocin receptors are found in many parts of the brain and spinal cord
  - Including the amygdala, ventromedial hypothalamus, septum, nucleus accumbens, and brainstem
- Associated with bonding, trust, and empathy



http://www.creationofman.net/chapter3/chapter3_5.html

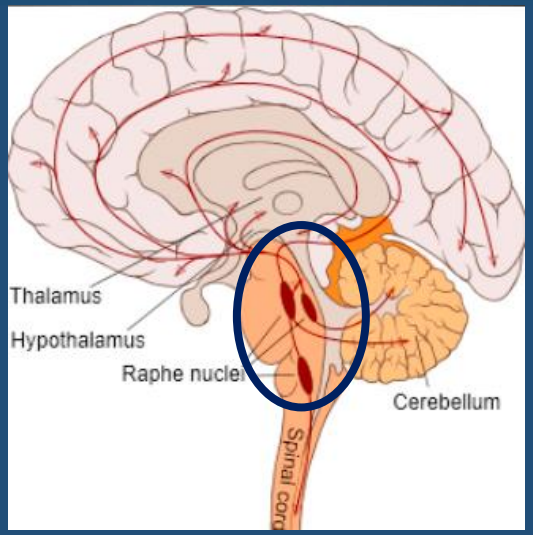
Serotonin



https://en.wikipedia.org/wiki/Serotonin

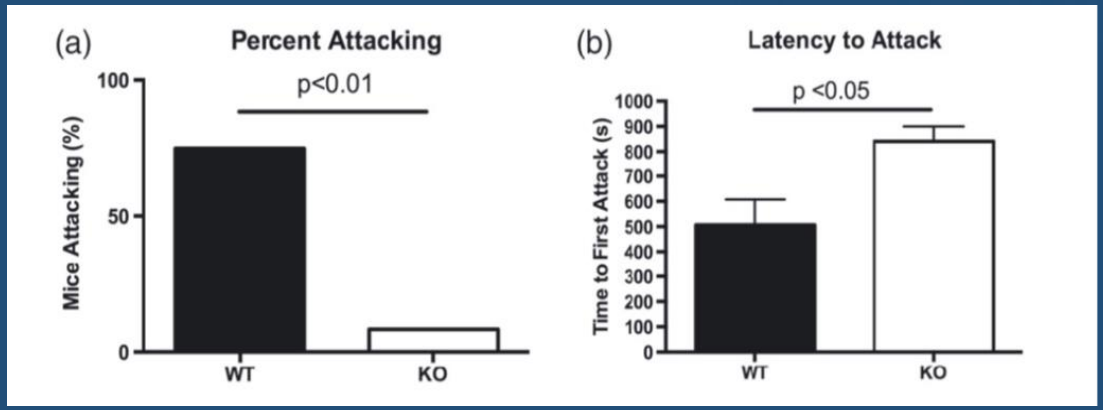
- Found in the midline of the brain stem and the gastrointestinal tract
- Important in regulation of sleep, mood, eating, attention, and appetite
- Associated with psychopathy, personality traits, depression, and social adversity

Oxytocin and Serotonin

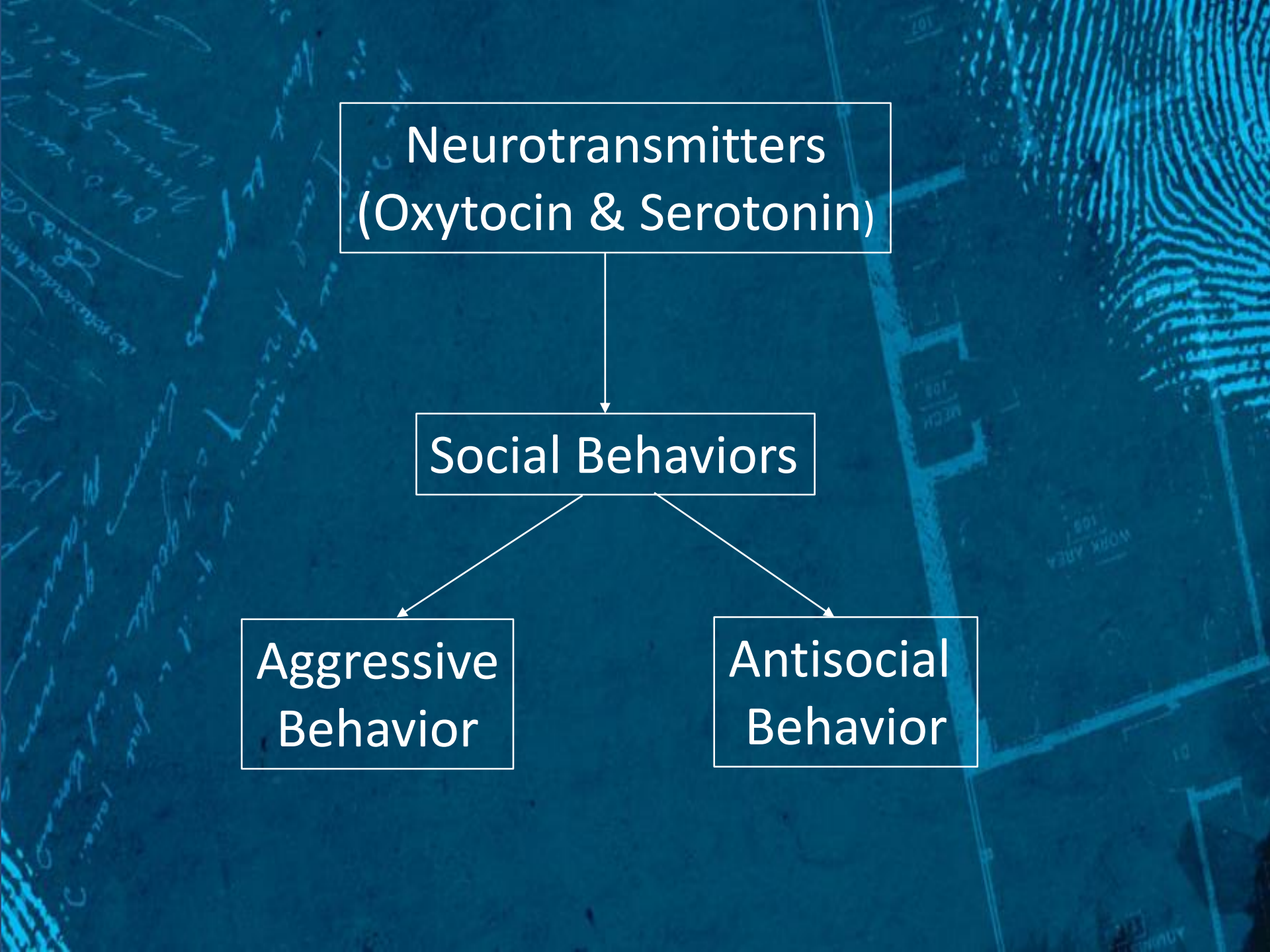


Oxytocin receptor (OXTR) expressed in the serotonergic raphe nuclei

Pagani et al.



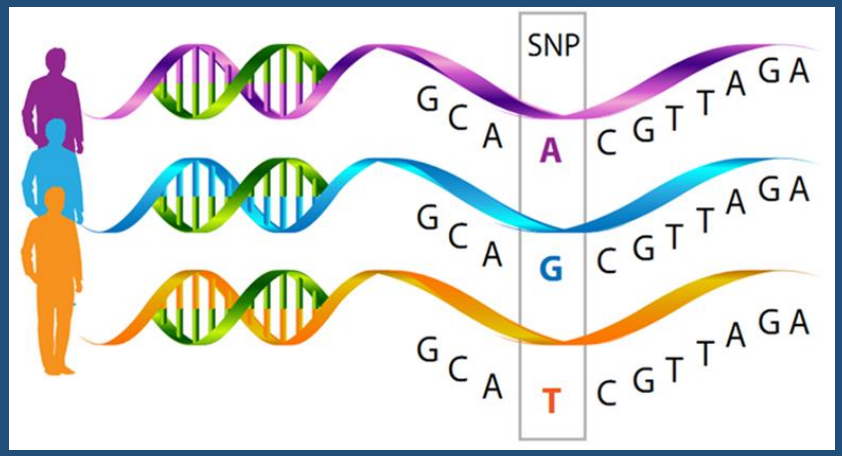
- **Raphe serotonin neuron-specific oxytocin receptor knockout**
- **Indicates there is a mechanism by which the two neurotransmitters together influence behavior**


Neurotransmitters
(Oxytocin & Serotonin)
Social Behaviors
Aggressive
Behavior
Antisocial
Behavior

Impact of Aggressive and Antisocial Behavior

- Impact on the criminal justice system
  - United States of America has the highest incarceration rate in the world
  - Over 2,000,000 people currently incarcerated
- Impact on the health field
  - Two of the leading causes of mental health referrals
  - 1 in 5 Americans experience mental illness (National Alliance on Mental Illness)
- Over 40% of individuals in prison suffer from mental health problems (Bureau of Justice Statistics)

Single Nucleotide Polymorpisms (SNPs)

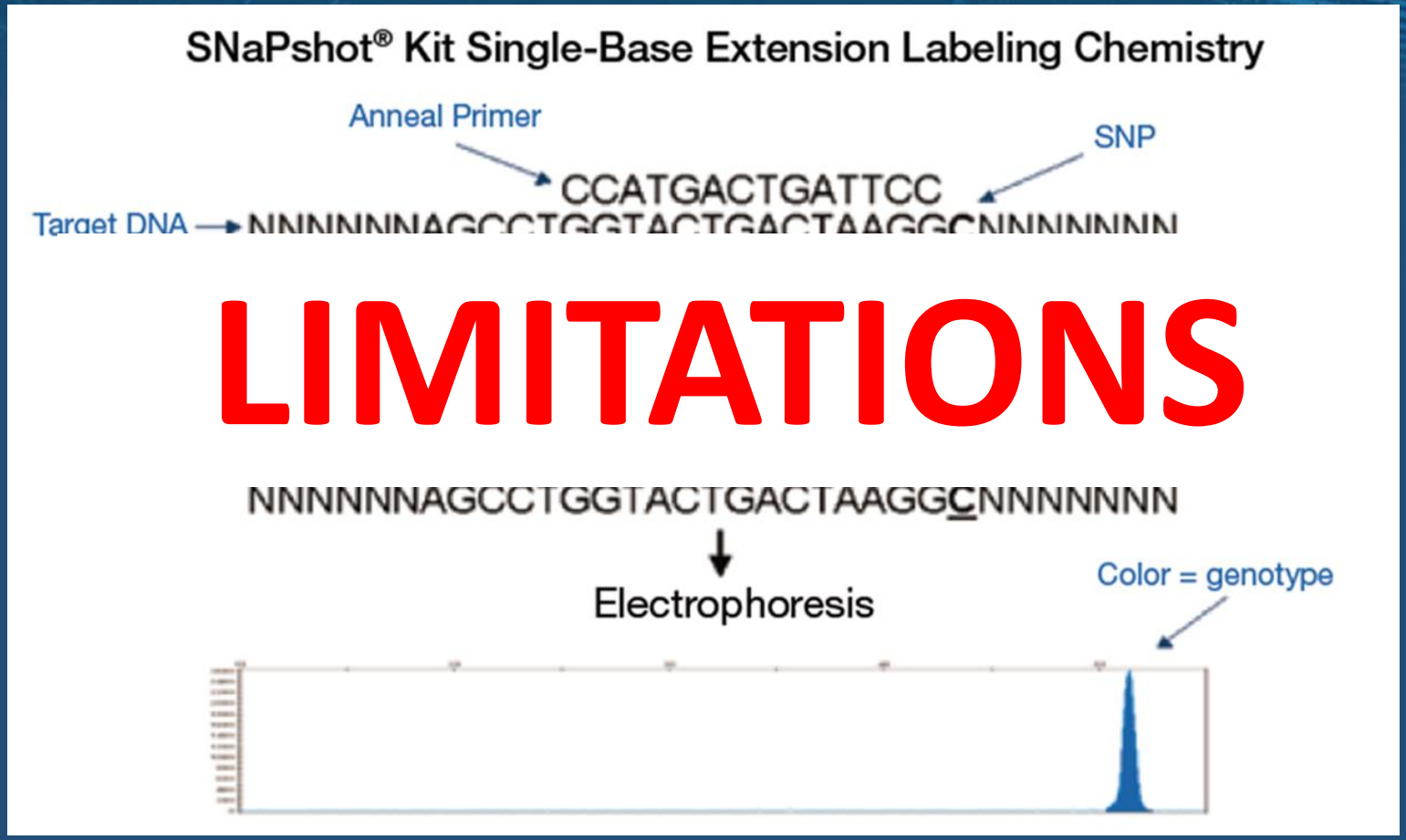


https://wp.nyu.edu/genome_diversityspring2015/?p=108

- Single base variations located at a specific location on the genome
- Most abundant type of polymorphism
- Previous studies have linked polymorphisms associated with these neurotransmitters to behavior

Methods for Analyzing SNPs

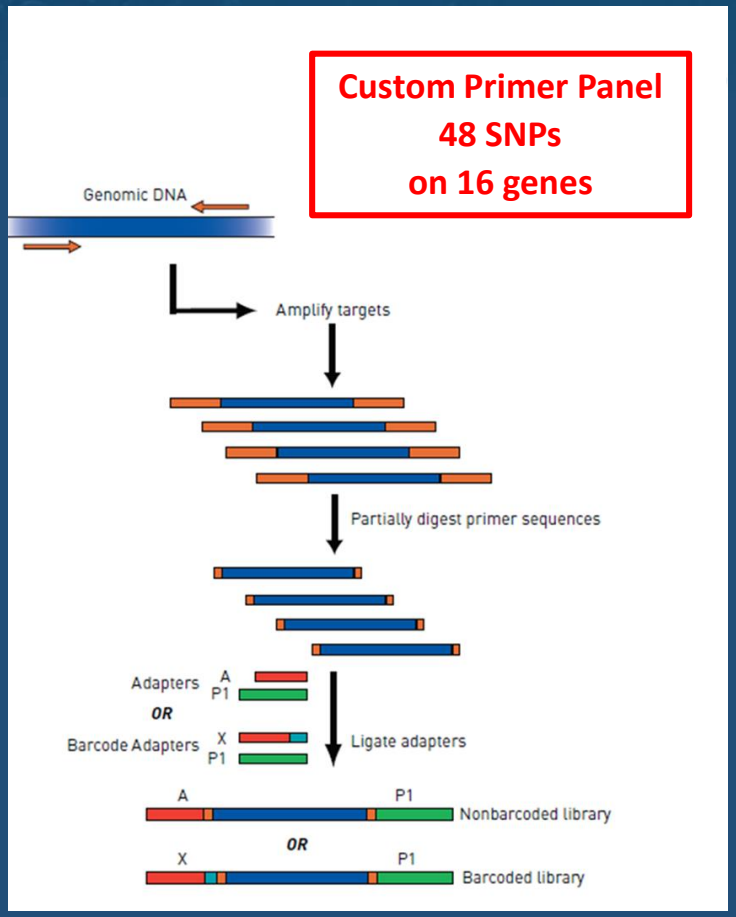
Single Base Extension (SBE)

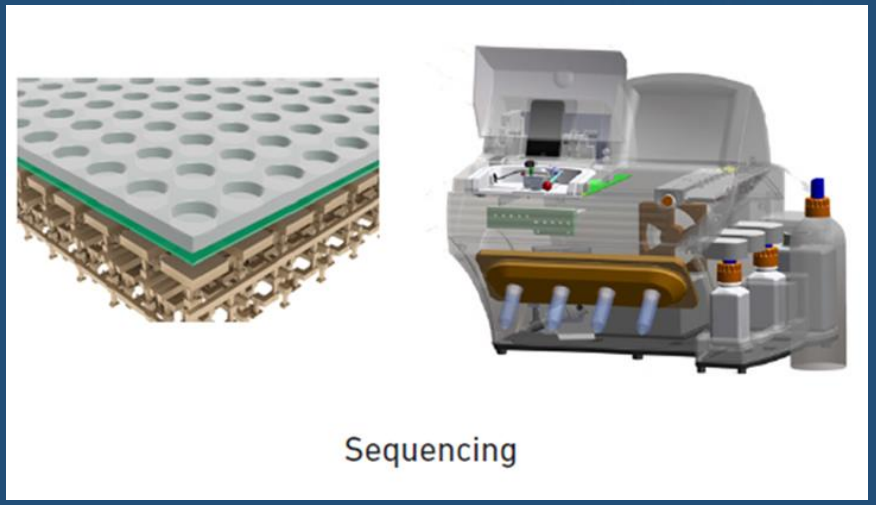


http://lifesequencing.blogspot.com/2012/09/aplicacoes-da-tecnica-de-snapshot.html

Massively Parallel Sequencing:

Ion Chef & Ion Personal Genome Machine (PGM)





https://www3.appliedbiosystems.com/cms/groups/applied_markets_marketing/documents/generaldocuments/cms_094273.pdf

Genes

CNR1: cannabinoid receptor 1

FAAH: fatty acid amide hydrolase

DRD2: dopamine receptor 2

DRD4: dopamine receptor 4

OPRM1: opioid receptor mu 1

ADH/ALDH: alcohol dehydrogenase and aldehyde dehydrogenase (alcohol metabolism)

GABRA2: gamma-aminobutyric acid type A receptor alpha2

BDNF: brain-derived neurotrophic factor

5HT2A: serotonin

COMT: catecohol-O-methyl transferase

TPH: tryptophan hydroxylase

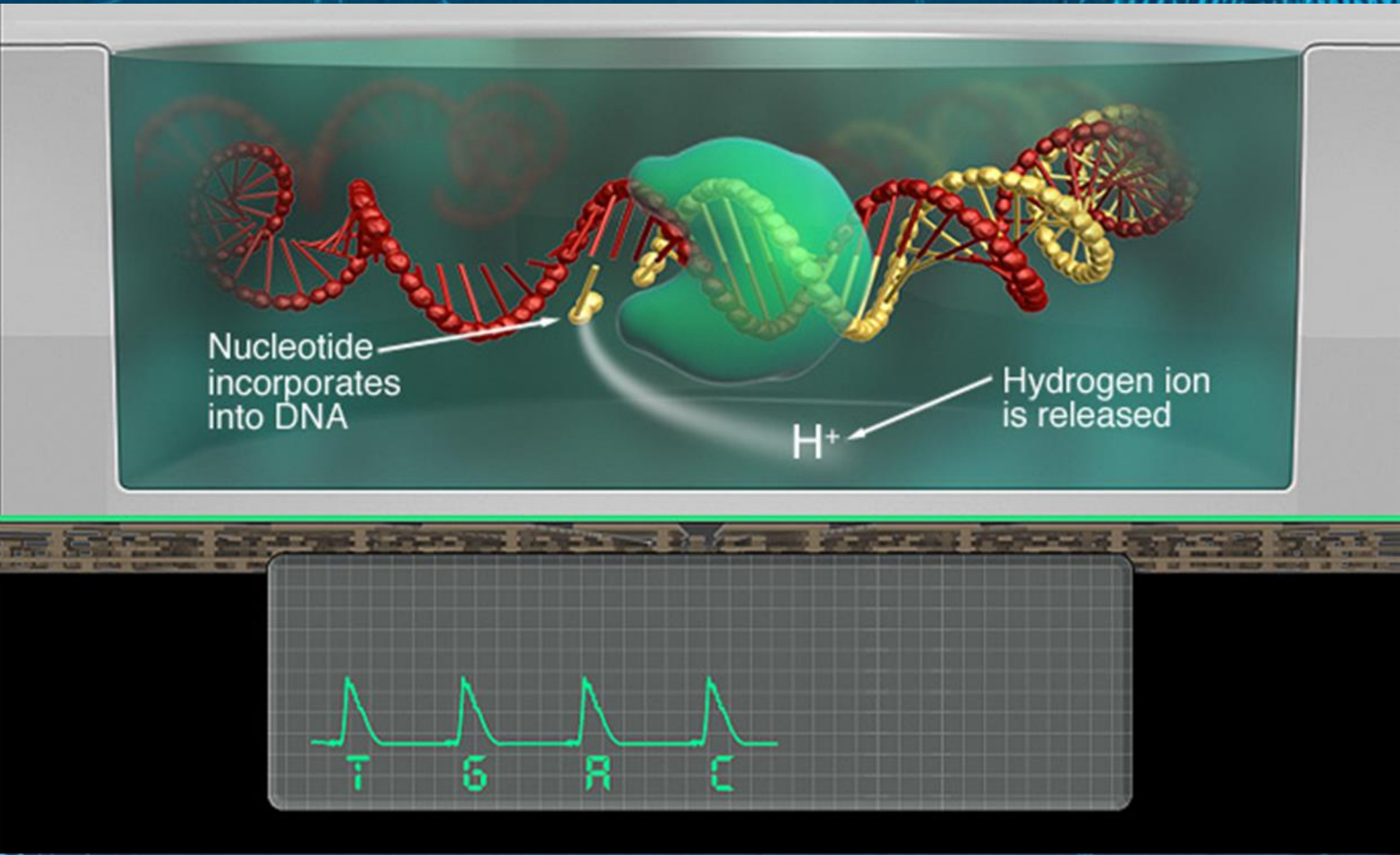
OXT: oxytocin

OXTR: oxytocin receptor

MAOB: monoamine oxidase B

MAOA: monoamine oxidase A

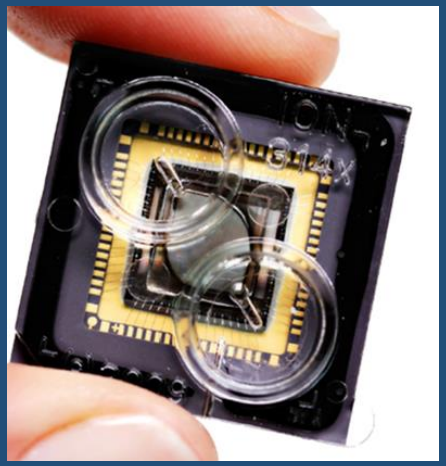
DBH: dopamine beta-hydroxylase


Nucleotide incorporates into DNA
Hydrogen ion is released
H+
T G A C

Advantages of MPS

- **Allows for the simultaneous analysis of a high number of SNPs in a large number of individuals**
  - **Introducing a barcode allows for samples to be pooled and sequenced simultaneously**

- **High throughput sequencing is more cost and time effective than previous methods**



https://www3.appliedbiosystems.com/cms/groups/applied_markets_marketing/documents/generaldocuments/cms_094273.pdf

Goals

- Explore the use of MPS in the behavioral genetics field
- To create a large primer panel of behavioral SNP markers
- Determine if it would yield the same results as conventional methods
- Analyze SNP markers associated with oxytocin and serotonin to determine if there is an association between genotype and behavior

Samples

- 100 buccal swabs from Sam Houston State University students
  - Approved by Institutional Review Board
- Each individual completed a survey designed to assess 31 behavioral categories including empathy, aggression, and psychopathy

http://www.hometrainingtools.com/swab-applicator-sterile

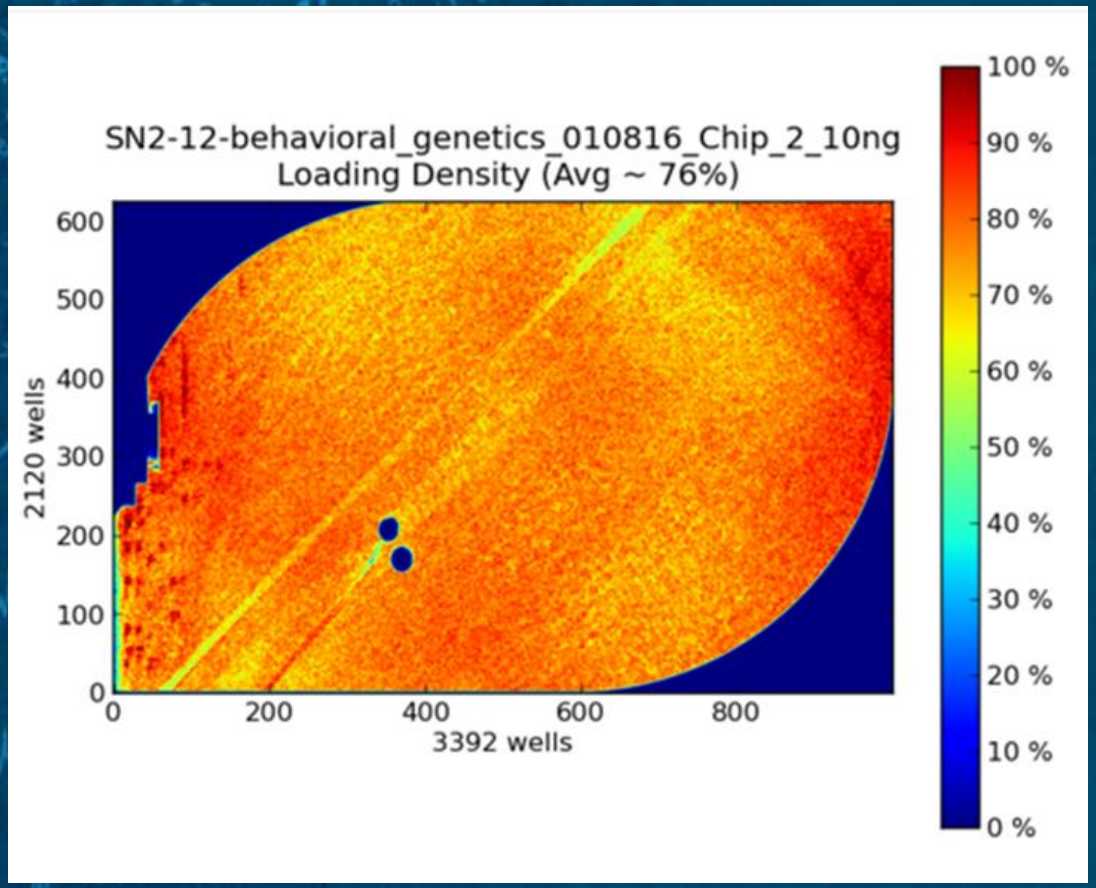
Survey

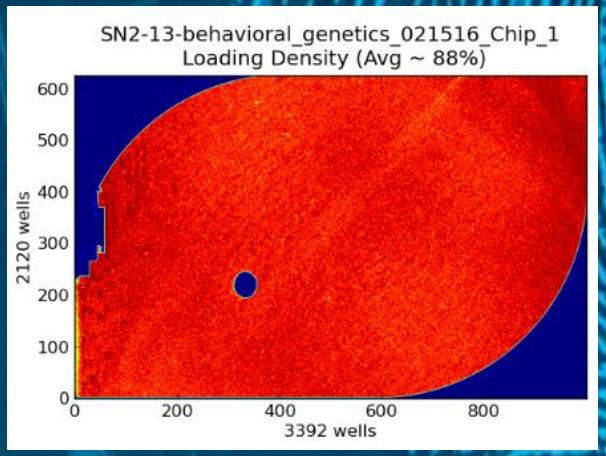
How often have you…	NEVER	HARDLY EVER	SOMETIMES	OFTEN	ALWAYS OR ALMOST NEVER
Yelled at others when they have annoyed you	❑	❑	❑	❑	❑
Had fights with others to show who was on top	❑	❑	❑	❑	❑
Reacted angrily when provoked by others	❑	❑	❑	❑	❑
Taken things from other students	❑	❑	❑	❑	❑
Had temper tantrums	❑	❑	❑	❑	❑
Vandalized something for fun	❑	❑	❑	❑	❑

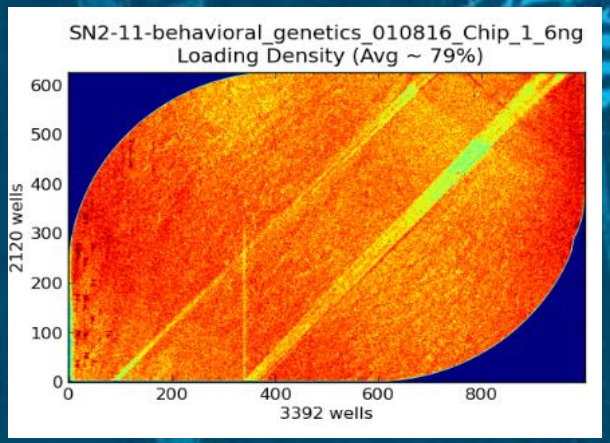
Behaviors Analyzed

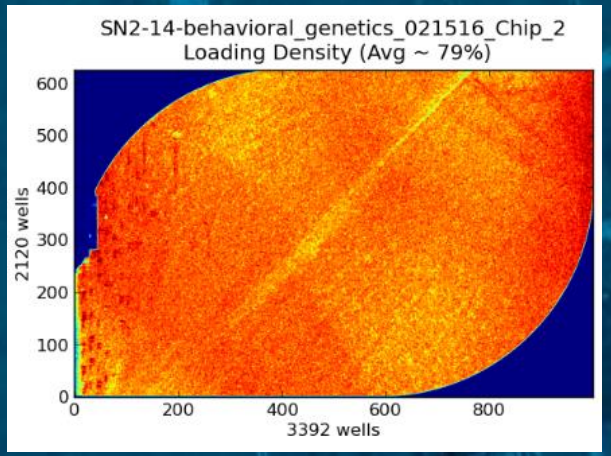
- Antisocial behavior
  - Severe 1 antisocial behavior
  - Severe 2 antisocial behavior
  - Property crimes + antisocial behavior
  - Violent antisocial behavior
  - Minor antisocial behavior
  - Drug 1 + antisocial behavior
  - Drug2 + antisocial behavior
- Sociability
- Stalking perpetrator
- Intimate partner violence
- Perception that criminal behavior is wrong
- Depression
- Stress
- Aggression
  - Reactive aggression
  - Proactive aggression
- Empathy
- Psychopathy
  - Egocentricity
  - Callous unemotional traits
  - Antisocial lifestyle

Results


SN2-12-behavioral_genetics_010816_Chip_2_10ng
Loading Density (Avg ~ 76%)
2120 wells
3392 wells
0
100
200
300
400
500
600
0
200
400
600
800
100 %
90 %
80 %
70 %
60 %
50 %
40 %
30 %
20 %
10 %
0 %


SN2-13-behavioral_genetics_021516_Chip_1
Loading Density (Avg ~ 88%)
2120 wells
3392 wells
0
100
200
300
400
500
600
0
200
400
600
800


SN2-11-behavioral_genetics_010816_Chip_1_6ng
Loading Density (Avg ~ 79%)
2120 wells
3392 wells
0
100
200
300
400
500
600
0
200
400
600
800


SN2-14-behavioral_genetics_021516_Chip_2
Loading Density (Avg ~ 79%)
2120 wells
3392 wells
0
100
200
300
400
500
600
0
200
400
600
800

Data

OXTR (oxytocin receptor)

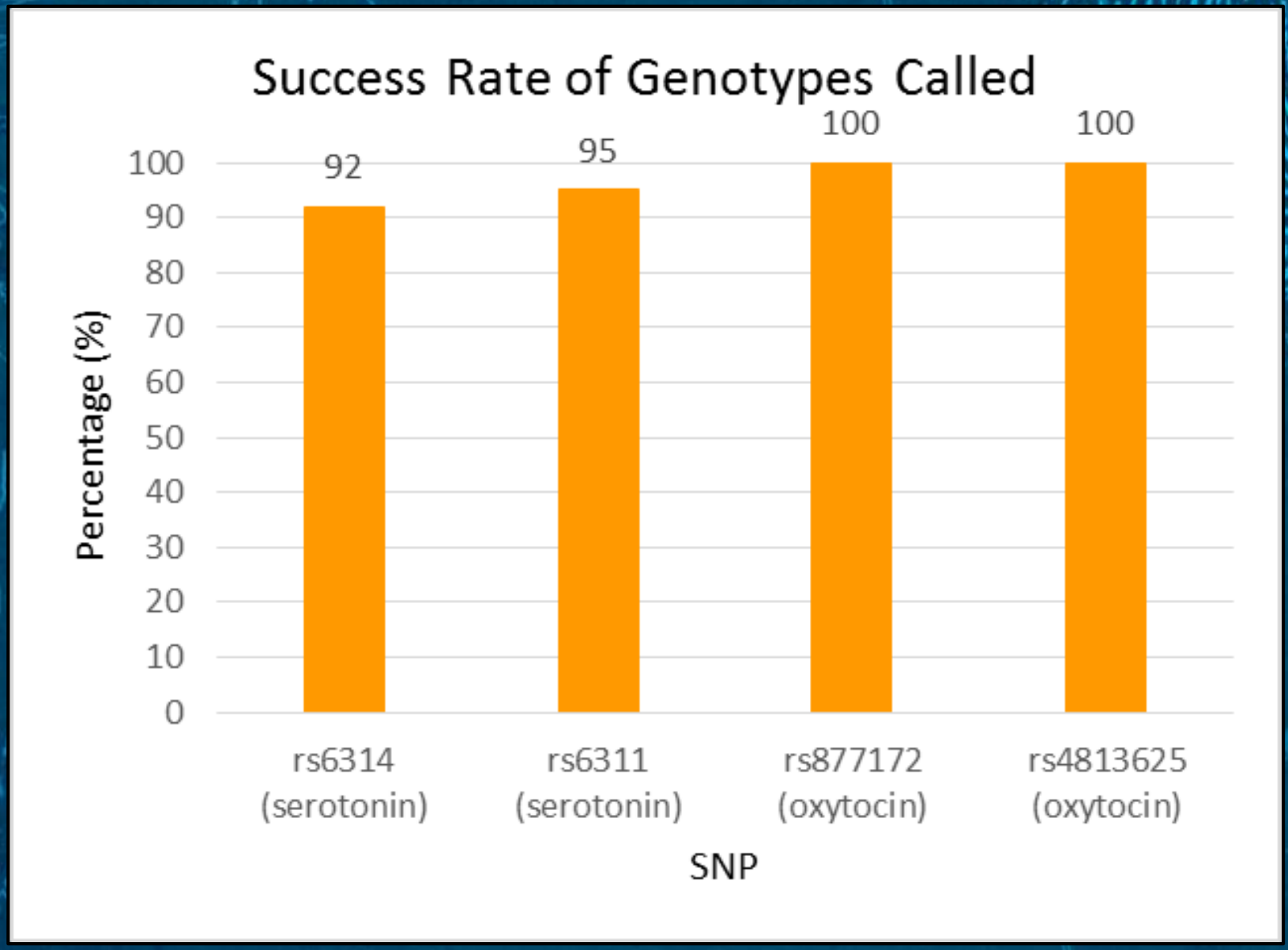
Hide Genotypes

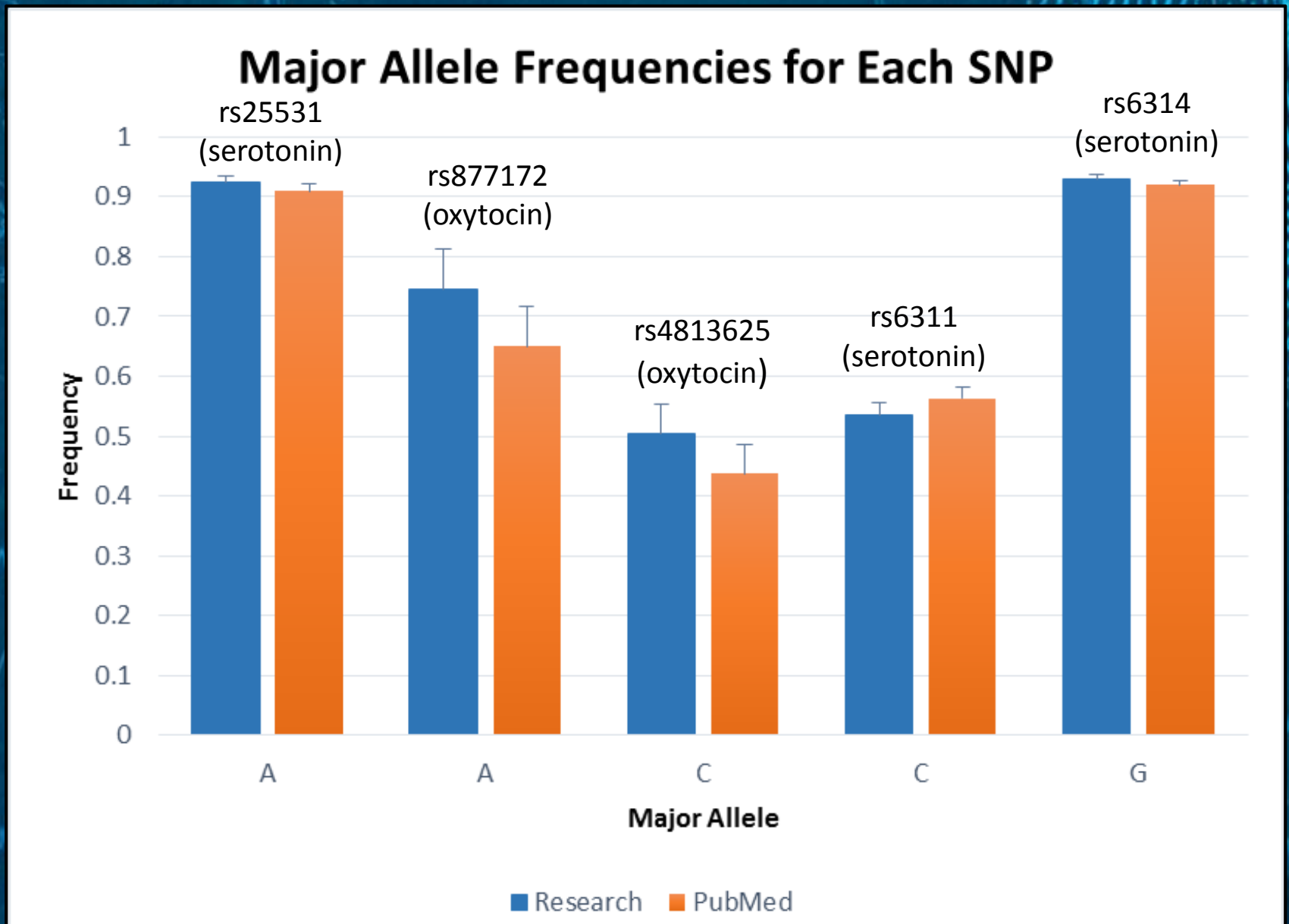
Sample Name	Barcode ID	Sample Data	rs324420	rs11476	rs6770632	rs53576
505	IonXpress_001	Download	AA	AA	CC	AG
402	IonXpress_002	Download	AC	AT	CC	AG
393	IonXpress_003	Download	CC	AT	CC	AG
397	IonXpress_004	Download	AC	AT	AC	AG

SBE vs. MPS

- Two oxytocin SNPs
  - rs877172 and rs4813625
- 92 samples compared
  - Out of those called, 100% were concordant

rs4813625 SBE	rs4813625 MPS
CC	CC
CC	CC
GC	GC
GG	GG
GC	GC


Success Rate of Genotypes Called
Percentage (%)
100
90
80
70
60
50
40
30
20
10
0
92
95
100
100
rs6314 (serotonin)
rs6311 (serotonin)
rs877172 (oxytocin)
rs4813625 (oxytocin)
SNP


Major Allele Frequencies for Each SNP
rs25531
(serotonin)
rs877172
(oxytocin)
rs4813625
(oxytocin)
rs6311
(serotonin)
rs6314
(serotonin)
Frequency
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
A
A
C
C
G
Major Allele
Research
PubMed

Significant Associations

rs25531 (serotonin)

Behavior	p value	β	SE	Exp (B)
Drug 2 + Antisocial Behavior	0.006	1.658	0.604	5.250
Drug 1 + Antisocial Behavior	0.015	1.418	0.582	4.127

Individuals with the GA genotype were more likely to show these types of behavior than individuals with the genotype AA

rs877172 (oxytocin)

Behavior	p value	β	SE	Exp (B)
Property Crime + Antisocial Behavior	0.017	-1.109	0.465	0.330

Individuals with the CC and CA genotype were more likely to exhibit this type of behavior than individuals with the genotype AA

Conclusions

- **MPS results were concordant with SBE data**
  - **Indicates this large panel of behavioral SNP markers may be used to analyze multiple types of behavior at once**
- **Significant associations were found for types of antisocial behavior in rs25531 and rs877172**
  - **Indicates that these SNPs may contribute to antisocial behavior, drug use/distribution, and property crimes**

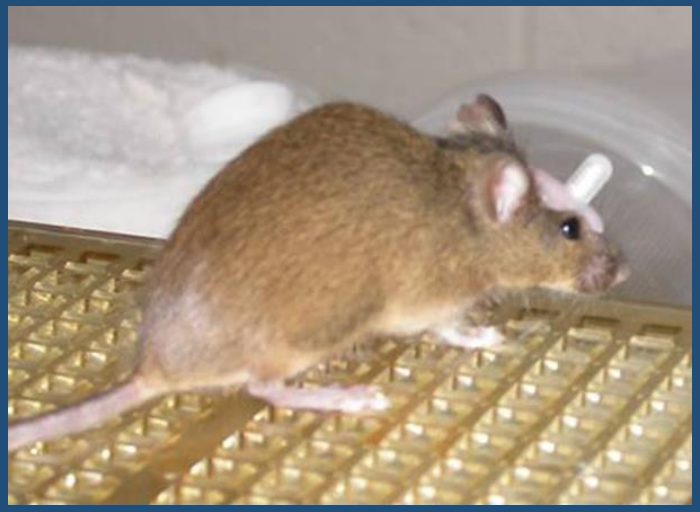
Conclusions

- Results provide some evidence that OXT and 5-HT can influence behavior
- Large panel may be used in early prevention or treatment of psychiatric disorders which have a large impact on the medical field and criminal justice field
  - Can help predict adult mental health problems including depression, anxiety, and emotional stability
  - Can help explain how to treat central imbalance of these neurotransmitters

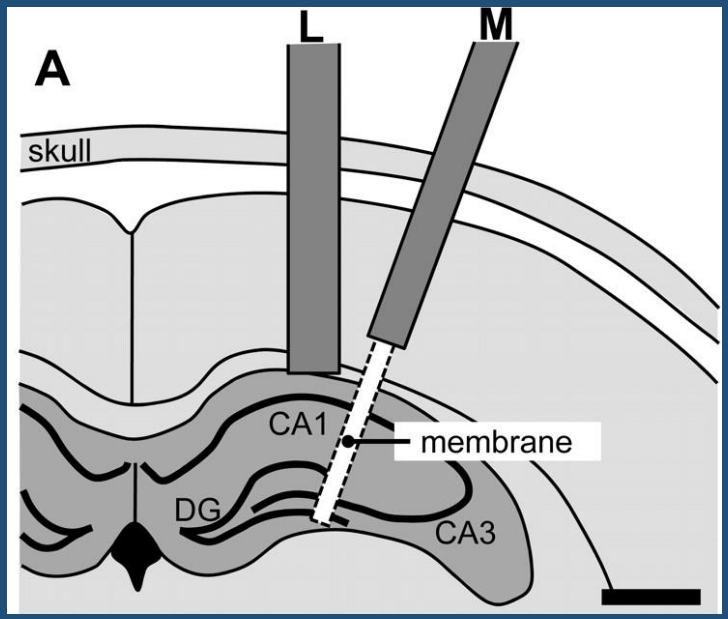
Work to be Completed

Behavioral Assessment

- Test the influence of central oxytocin on aggression and sociability using behavioral tests in mice (C57)
  - Control group: artificial cerebrospinal fluid
  - Test group: oxytocin



http://rci.rutgers.edu/~jmcgann/research.html



Resident Intruder Test

https://www.google.com/url?sa=i&rct=j&q=&esrc=s&source=images&cd=&ved=0ahUKEwiPxMLMivnLAhWEyyYKHa7ICCQQjRwIBQ&url=http%3A%2F%2Fdspace.library.uu.nl%2Fbitstream%2Fhandle%2F1874%2F259700%2FThesis%2520Lynn%2520Kraak.pdf%3Fsequence%3D1&psig=AFQjCNHRpk__czf1xyd3Ka9kQttJi3h6rw&ust=1460000158476572

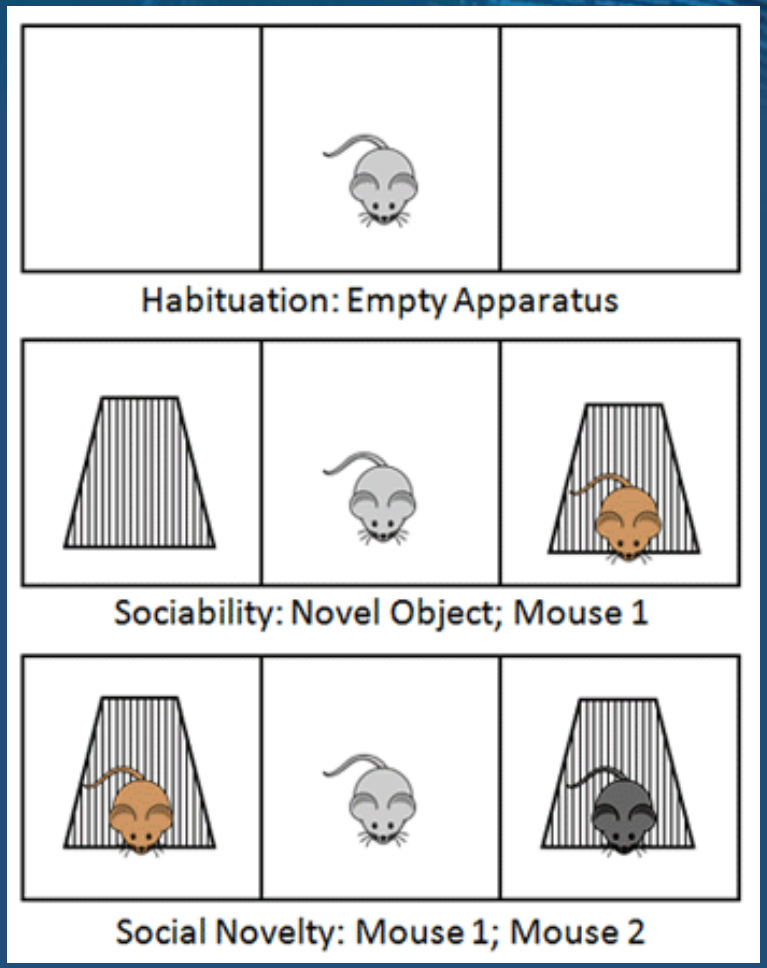
- Test for aggression

- Day 1: juvenile intruder mouse is introduced into the cage
  - Interaction is recorded for 2 min
- Day 2: experiment is repeated with same intruder mouse
- Day 3: experiment is repeated with new juvenile intruder

Three-Chambered Sociability Test

- Test for sociability

http://www.stoeltingco.com/sociability.html

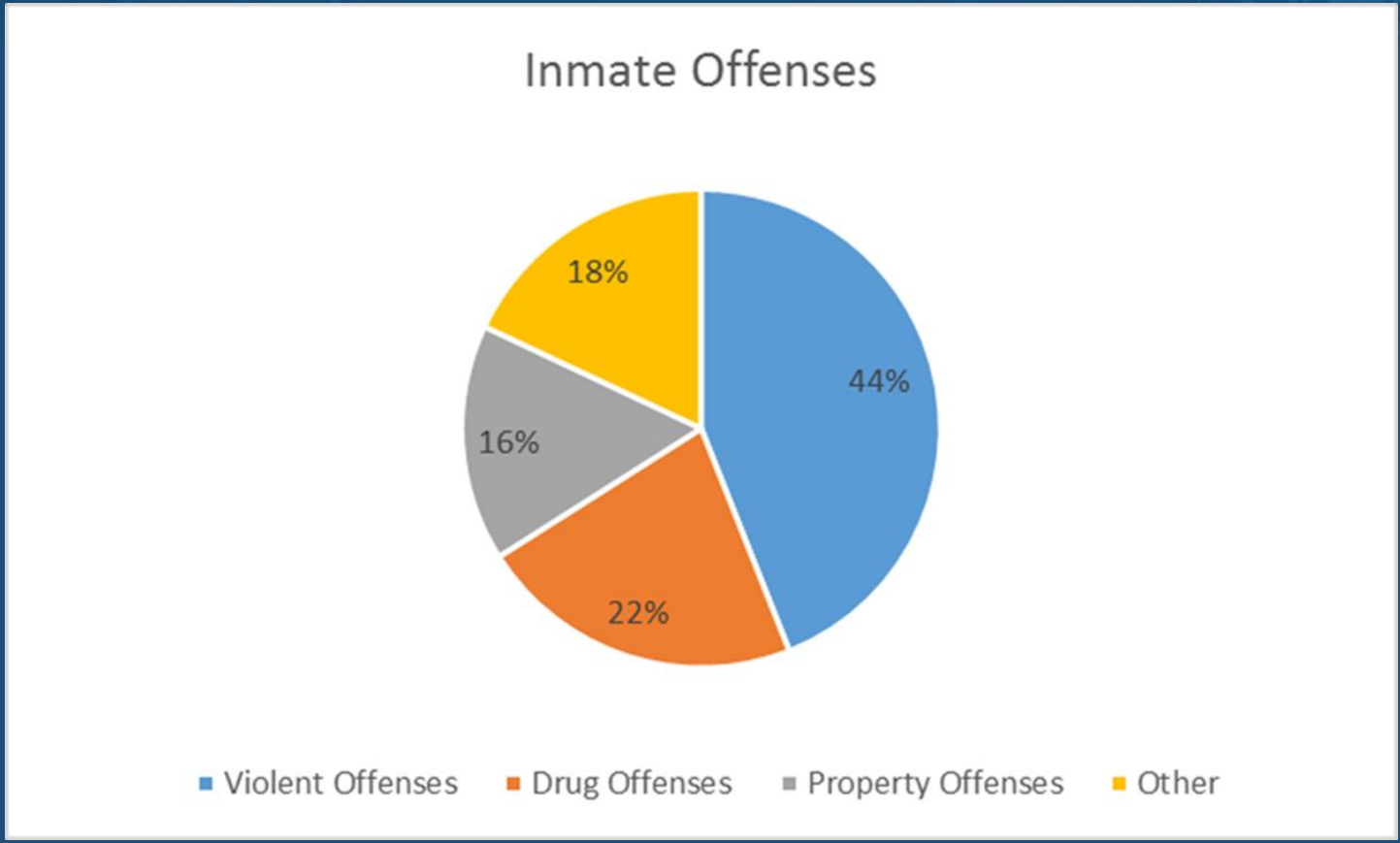


http://sbfnl.stanford.edu/cs/bm/si/bms_3chamber.html

Measuring Dopamine Response

- Gene expression:
  - DRD1
  - DRD2
- Relative expression of messenger RNA (mRNA)

Inmate Samples



References

1. Pagani J, Williams Avram S, Cui Z, Song J, Mezey É, Senerth J, et al. Raphe serotonin neuron-specific oxytocin receptor knockout reduces aggression without affecting anxiety- like behavior in male mice only. Genes, Brain and Behavior. 2015;14(2):167-76.
2. Ross HE, Young LJ. Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. Front Neuroendocrinol. 2009;30(4):534-47.
3. Brownstein MJ, Russell JT, Gainer H. Synthesis, transport, and release of posterior pituitary hormones. Science. 1980;207(4429):373-8.
4. Gimpl G, Fahrenholz F. The Oxytocin Receptor System: Structure, Function, and Regulation. Physiological Reviews. 2001;81(2):629-83.
5. Moul C, Dobson-Stone C, Brennan J, Hawes DJ, Dadds MR. Serotonin 1B Receptor Gene (HTR1B) Methylation as a Risk Factor for Callous-Unemotional Traits in Antisocial Boys. PLoS One. 2015.
6. Butler JM. Advanced Topics in Forensic DNA Typing: Methodology. Massachusetts: Waltham. 2011.
7. Beitchman JH, Zai CC, Muir K, Berall L, Nowrouzi B, Choi E, et al. Childhood aggression, callous-unemotional traits and oxytocin genes. Eur Child Adolesc Psychiatry. 2012;21(3):125-32.
8. Berglund EC, Kiialainen A, Syvanen A-C. Next-generation sequencing technologies and applications for human genetic history and forensics. Investig Genet. 2011;2:23.
9. Cai X, Sheng J, Tang C, Namdakumar V, Ye H, Ji H, et al. Frequent mutations in EGFR, KRAS and TP53 genes in human lung cancer tumors detected by ion torrent DNA sequencing. PloS One. 2014;9(4). 22. Elliot AM, Radecki J, Bellal Moghis

Acknowledgements

- Sam Houston State University:
  - Department of Forensic Science
    - Dr. David Gangitano, PhD
    - Dr. Hughes-Stamm, PhD
    - Charity Beherec, MS
  - Department of Criminal Justice
    - Dr. Todd Armstrong, PhD
    - Dr. Danielle Boisvert, PhD
    - Jessica Wells, MS
  - Department of Digital Forensics
    - Andrew Bennett, MS
- University of North Texas

Sam Houston State University

FSF Emerging Forensic Scientist Award
Paper Presentation

Questions?

Elizabeth Chesna
eac028@shsu.edu

Sam Houston State University