Phenotyping animal social behaviors

1st ISBS Summer School

St. Petersburg, Russia

May 9th -15th,2008

Social behavior patterns

1. Initial contact-social investigation
2. Contact promoting and sexual behavior
3. Approach, flight, attack, and other agonistic patterns
4. Miscellaneous behaviors seen in a social context
5. Parental behavior

Mus musculus

In their natural habitat:

- Social species
- Establish group territories (size based on food availability)
- The social group shares the nest
- Both parents retrieve pups to the nest
- Adult offspring leave the group to establish new groups in new territories

Familial organization

- Different from rat colonies in some ways
- Colony defended by a dominant male

http://mouseworksonline.com/images/mice.jpg

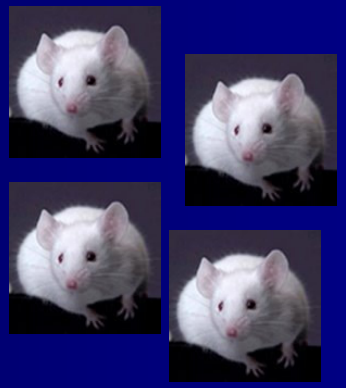
Housing considerations

In laboratory cage:

- Fighting is common
- Unable to colonize new territories
- Aggressive dominance hierarchy
- Can be overcrowded or socially isolated
- Poor housing can affect all behavioral phenotypes

Housing considerations

Social isolation



Group Housing

Crowding stress

Behavioral differences from long-term individual housing

	B6 single vs. group	DBA single vs. group
Body weight	↓	-------
Anxiety-like behavior		
▪ Elevated plus maze	↓	↓
▪ Light-dark exploration	-------	↑
▪ Novel cage	-------	↑
▪ Hyponeophagia	↑	↑
Spontaneous locomotion and exploration	↑	↑
Habituation of activity	↓	↓
Behavioral Despair	-------	↓
Nociception	-------	-------
Coordination	↑	↓
Learning and memory		
▪ Novel object discrimination	↓	↓
▪ Contextual fear	↓	↓
▪ Cued fear	↓	↓
▪ Spatial memory	-------	-------

Voikar, 2006

Effects of overcrowding stress

- ↑ infant mortality
- ↓ full-term pregnancies and mother survival
- ↓ female nursing, nesting behaviors
- Abnormally high male aggression levels
- ↑ territoriality

Social deprivation

Induces aberrant social behaviors:

- Isolated pups more reactive to handling (measured by ↑ cardiac acceleration)
- Self-manipulation (tail-biting, convulsive activities)
- High levels of aggressive behavior

Social Recognition

Ability to one animal to recognize another animal that it has already encountered.

In mice, involves:

- Olfactory processing
- Social interest
- Learning and memory

Huddling

The inclination for mice to exactly to sleep near each other

- Usually observed during the light phase of the circadian cycle
- This can be videotaped to avoid disturbing the animals

Nesting

- Usually measured in the home cage
- May be measured in the novel cage in some strains
- Uses nestlet or cotton as nest-building material.

Nesting protocol

- Mice were housed individually for 3 weeks in small plastic cages
- A standard piece of paper towel was provided 3 days prior to inspection. Nests were assessed and scored:

0) - no nests
1) - primitive flat nests (pad-shaped, consist of a flat paper tissue which slightly elevates a mouse above the bedding)
2) - more complex nests (including warping and biting the paper towel)
3) - complex accurate cup-shaped nests (with shredded paper interwoven to form the walls of the cup),
4) - complex hooded nests, with walls forming a ceiling so the nest becomes a hollow sphere with one opening

Mouse nests

Nesting "paperwork" protocol

1. Each nest was unwrapped and glued to a black paper
2. The amount of paperwork was assessed using the following scoring system:

   0—intact paper of little damage (<5% paper destroyed)

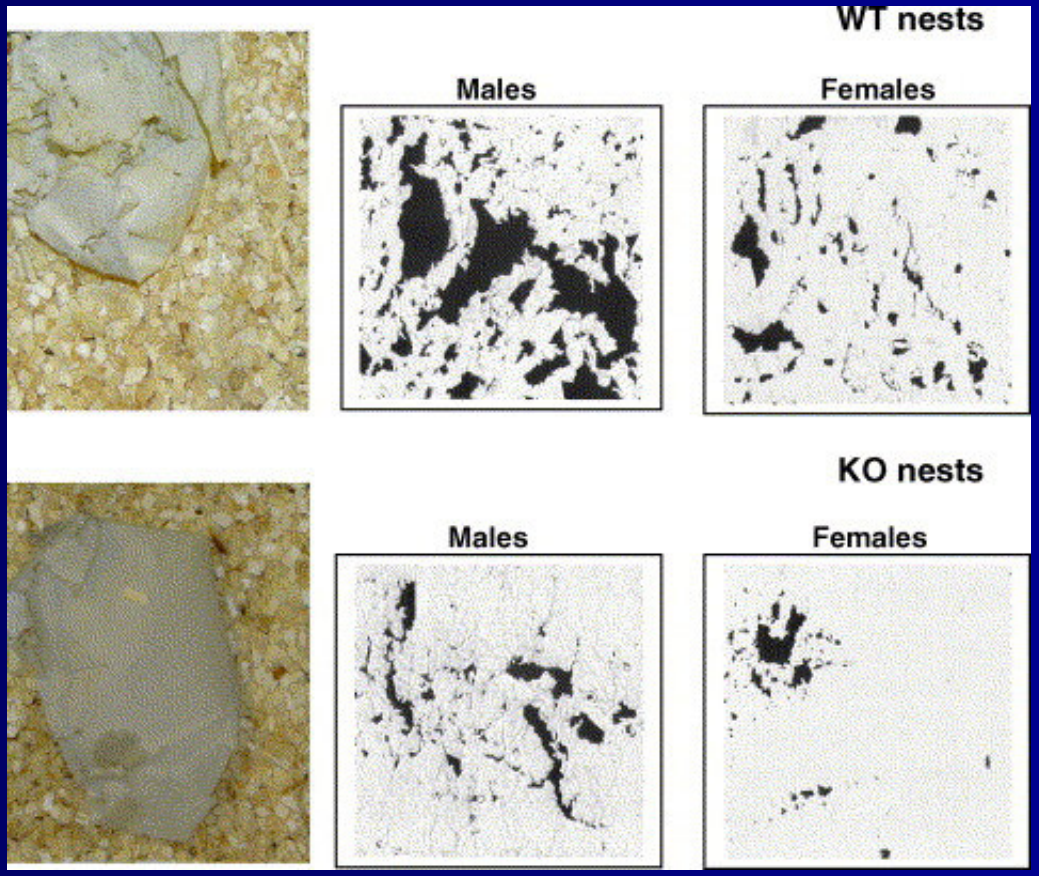
   1—some paper damage (5–20%)

   2—pronounced paper damage (20–40%)

   3—severe paper damage (>40%)

3. All nests were photographed and assessed by two raters blind to the genotypes, to avoid any possible biases in scoring the nest completeness and "paperwork"

Impaired nest building behavior in the VDR knockout (KO) mice



Kalueff et al., 2005

Juvenile play behavior

- Observed in 25-31 day-old rat pups
- Low, red-light illumination is used
- The test animal is introduced to a “stimulus” animal for 10 minutes
- Test animal scored on: 1) number of crossovers 2) number of play grooms 3) darts

Parental Care

Observations of normal maternal behaviors using:

- Nesting cotton/Nestlet squares (height and shape)
- retrieving the pups to the nest
- licking/grooming of the pups
- nursing pups
- Time spent by dam inside vs. outside the nest

Ultrasonic Vocalization

- Induced by isolation from conspecifics
- Often used as measure of anxiety
- More sociable species emit more vocalizations

Aggression

There are many ways to test animal aggression. Some experimental paradigms:

- Round-robin approach
- Standard opponent method
- Isolation-induced
- Resident-intruder
- Social dominance
- Social confrontation test

Round-robin approach

- Pairs of male mice are tested
- Each pair is scored on dominance versus submissive postures/attacks
- The scores rank the mice along a dominance hierarchy

Standard opponent

- Simpler approach than round-robin
- Mice are selected for their highly-replicable behavior

Isolation-induced

- A modified standard opponent test for aggressive traits
- Male mice are housed in isolation for 1 month
- This increases likelihood of attack behaviors when reintroduced to conspecifics

Resident-intruder

- A modification of the resident-intruder test
- Test is conducted in the test mouse's home cage
- The "intruder" is the standard opponent
- This intrusion prompts attacks from the "resident" mouse in his home cage

Social dominance

- Utilizes a special chamber to avoid any physical injuries to either mouse

Photo: Reber et al., 2008

Social recognition

- *Test*: a female partner is introduced in the home cage of the isolated resident for 3 min and after that returned to its home cage
- *Retest Same*: 45 min after the test, the same female partner is re-introduced in the home cage of the resident for 3 min
- *Retest Different*: 45 min after the Retest Same, a novel partner is introduced for 3 min in the cage of resident

Venerosi et al., 2006

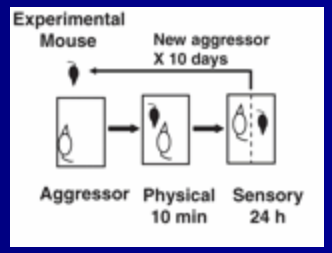
Social defeat model

Social stress-based model (Kudryavtseva, 1991)

- social winners or losers in male mice
- daily social confrontations
- daily non-contact exposures to winners
- anxiety (10 days) and depression (20 days)
- sensitivity to antidepressants or anxiolytic drugs

Pathophysiology

- ↑ DA and ↓ 5HT (winners), ↑opioid system (losers)
- ↑ immune deficiency
- ↑ susceptibility to transplanted tumor growth



Bridging behavior and genetics

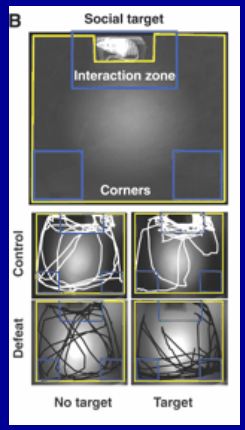
Aggressive mice:

↓ mRNA of catechol-O-methyltransferase

↑ mRNA of DAT and tyrosine hydroxylase

Submissive mice:

Repeated defeats ↑ mRNA of SERT, MAOA



Complex mouse behavior

- *Berton et al. Science, 2006*
- *Kalueff et al. Science, 2006*

Applications to humans

Aberrant social behaviors

Common symptom of many neuropsychiatric disorders:

- Anxiety, social anxiety
- Autism
- Williams syndrome
- Schizophrenia

The growing number of mutant or transgenic animals with abnormal social behaviors:

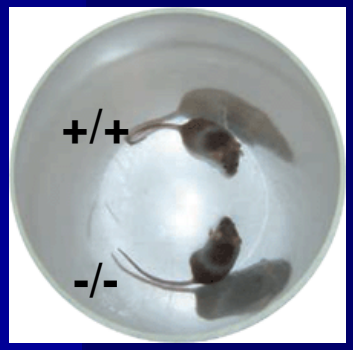
- >230 genotypes in the Mouse Genome Informatics database (Nov 2007)

Socio-cognitive dysfunctions:

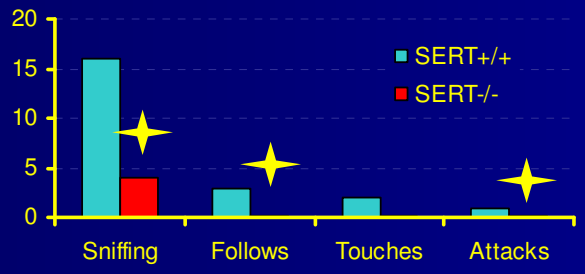
- Alzheimer's
- Parkinson's
- Stroke

The importance of examining social deficits in animal models of various brain disorders

Social confrontation test

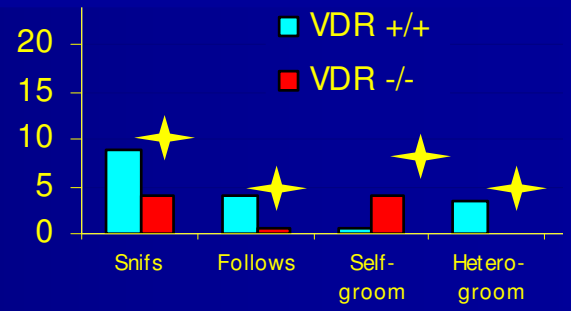


Social deficits in SERT-/- mice



Kalueff et al., Genes Brain Behav., 2007

Social deficits in VDR-/- mice

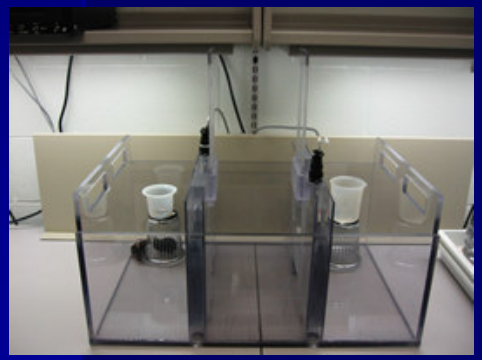


Kalueff et al., J. Neurosci. Res., 2006

Dissecting social interaction phenotypes

Other important approaches:

- Isolation-induced USV
- Social recognition test



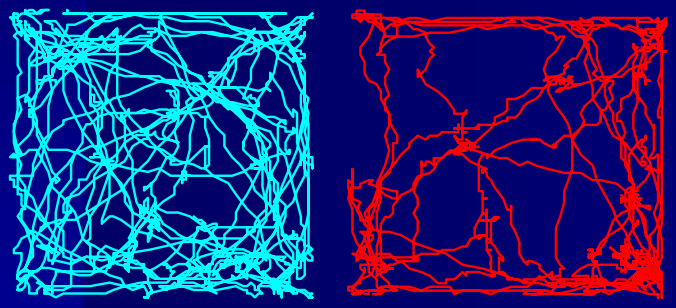
Sociability test: mice are given a choice between spending time with another mouse vs. staying alone

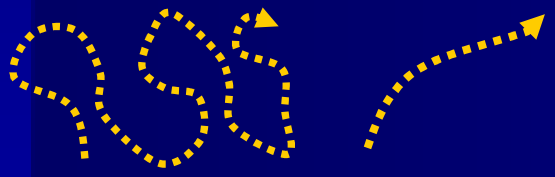
Preference for social novelty: mice are given a choice between spending time with an unfamiliar mouse vs. an already-explored mouse

Modeling autism: beyond social deficits

Clinical symptoms of autism in mice?	Can be modeled	SERT-/- mice
• Social deficits	Yes	Yes
• Language problems	No	N/A
• Altered cognitive processing	Yes	?
• Behavioral perseverations	Yes	Yes

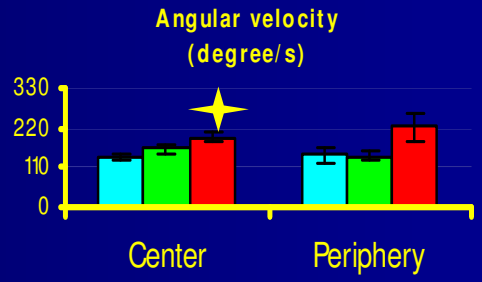
SERT+/+ SERT-/-

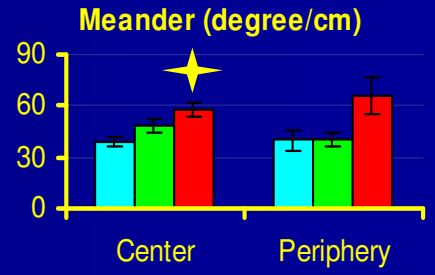




High meander Low meander

Behavioral perseverations: increased turning/meandering



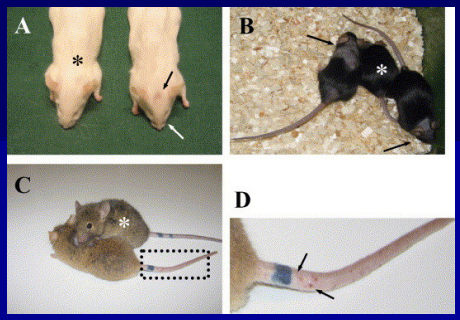


Kalueff et al., Brain Research, 2007

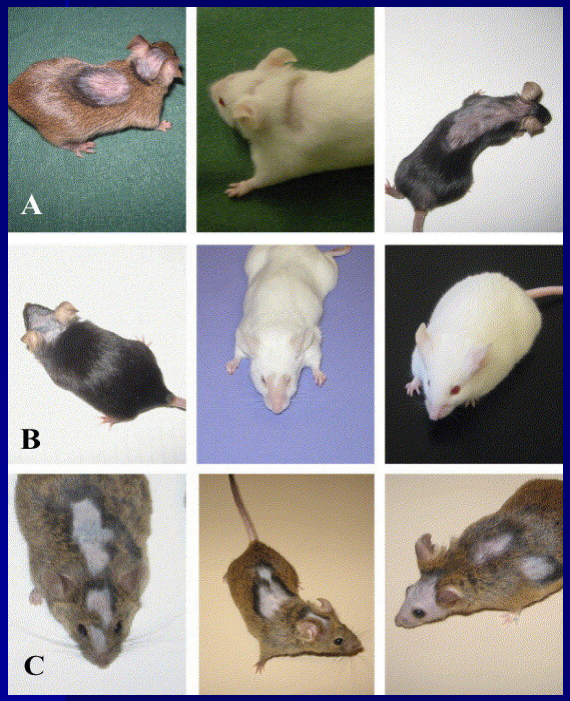
SERT-/- mice as a genetic model of ASD? Consistent with clinical data on the role of SERT in autism

Other relevant phenotype: barbering

A number of mutant mice with abnormal barbering phenotypes in the Mouse Genome Informatics database (Nov 2007)



- Genetically-determined behavior
- Relevant to social dominance, aggression and OCD
- High barbering = low aggression (a “social stress”-coping strategy ?)



Kalueff et al., Behav Processes, 2006

Genetics of barbering behavior in F1 mice: strong parental influences

Behaviors	S1BC	S1N	S1B6	S1	*F*(3,23)	*P*
Barbering activity						
% Cages with barbering	0 ± 0ab	100 ± 0a	83 ± 1 b	50 ± 22	10.04	<0.0003
% Barbered animals	0 ± 0abc	70 ± 0 a	75 ± 10b	85 ± 8 c	36.78	<0.0001
% Barbers	0 ± 0 ab	30±0abc	25 ± 10b	15 ± 8 c	48.40	<0.00001
Aggressiveness						
% Cages with scarring	100 ± 0a	0 ± 0 a	67 ± 21	50 ± 22	7.52	<0.0015
% Animals with scars	100±0ab	0 ± 0acd	45±11bc	40±11bc	27.93	<0.00001

Kalueff et al., Behav Brain Res, 2007

Pheromones

- specific communication strategies to identify and attract mates, and to discern the social status of conspecifics
- involves the emission and detection of species- and gender-specific chemical cues that provide information about social and sexual status
- neuronal processing of pheromone signals leads to marked changes in animal behaviors and endocrine status
- The highly reproducible and species-specific character of the response to pheromones offers a unique opportunity to uncover the neural basis of genetically pre-programmed behaviors

Dulac and Torello, 2003

Pheromones

Table 1 | **Small organic compounds with pheromonal activity**

Compound	Source	Effects	Synthetic compound active in water, CMU or both?	Binds to MUPs?	Other organisms in which pheromone previously identified	Refs
2-sec-butyl-4,5-dihydrothiazole (SBT) (1)	Male mouse urine. Production is testosterone dependent	Oestrous induction, intermale aggression and female attraction	CMU. Activity requires both SBT and DHB	Yes	Similar to male territory-marking compound used by grey and red duiker and two species of African antelope	26,33, 99–101, 106
2,3-dehydro-exo-brevicomin (DHB) (2)	Male mouse urine. Production is testosterone dependent	Oestrous induction, intermale aggression and female attraction	CMU. Activity requires both SBT and DHB	Yes	Male attractant of western pine beetle	26,33, 99–101, 106
α and β farnesenes (3)	Male mouse preputial gland. Production is testosterone dependent	Oestrous induction, intermale aggression	Both CMU and water	Yes	Trail marker of red fire ants, alarm phermones of aphids, defense substance of wild potato plants against aphids	26,27, 102
6-hydroxy-6-methyl-3-heptanone (4)	Male mouse urine	Puberty acceleration	Water	Yes		26
2-heptanone (5) *trans*-5-hepten-2-one (6) *trans*-4-hepten-2-one (7) *n*-pentyl acetate (8) *cis*-2-penten-1-yl-acetate (9) 2,5-dimethylpyrazine (10)	Female mouse urine. Production is dependent on adrenal gland function	Puberty delay	Active in both water and adrenelectomized female urine, in specific ratios with the other compounds isolated in this study			103
Dodecyl propionate (11)	Rat pup preputial gland	Maternal anogenital licking	Compound diluted in dichloromethane			104
(*Z*)-7-dodecen-1-yl acetate (12)	Female elephant pre-ovulatory urine	Male elephant sexual behaviour	Active in water		Turnip and cabbage looper, at least 100 species of lepidoptera	105

Dulac and Torello, 2003

Social interaction test in cows

Photo: T. Keisala