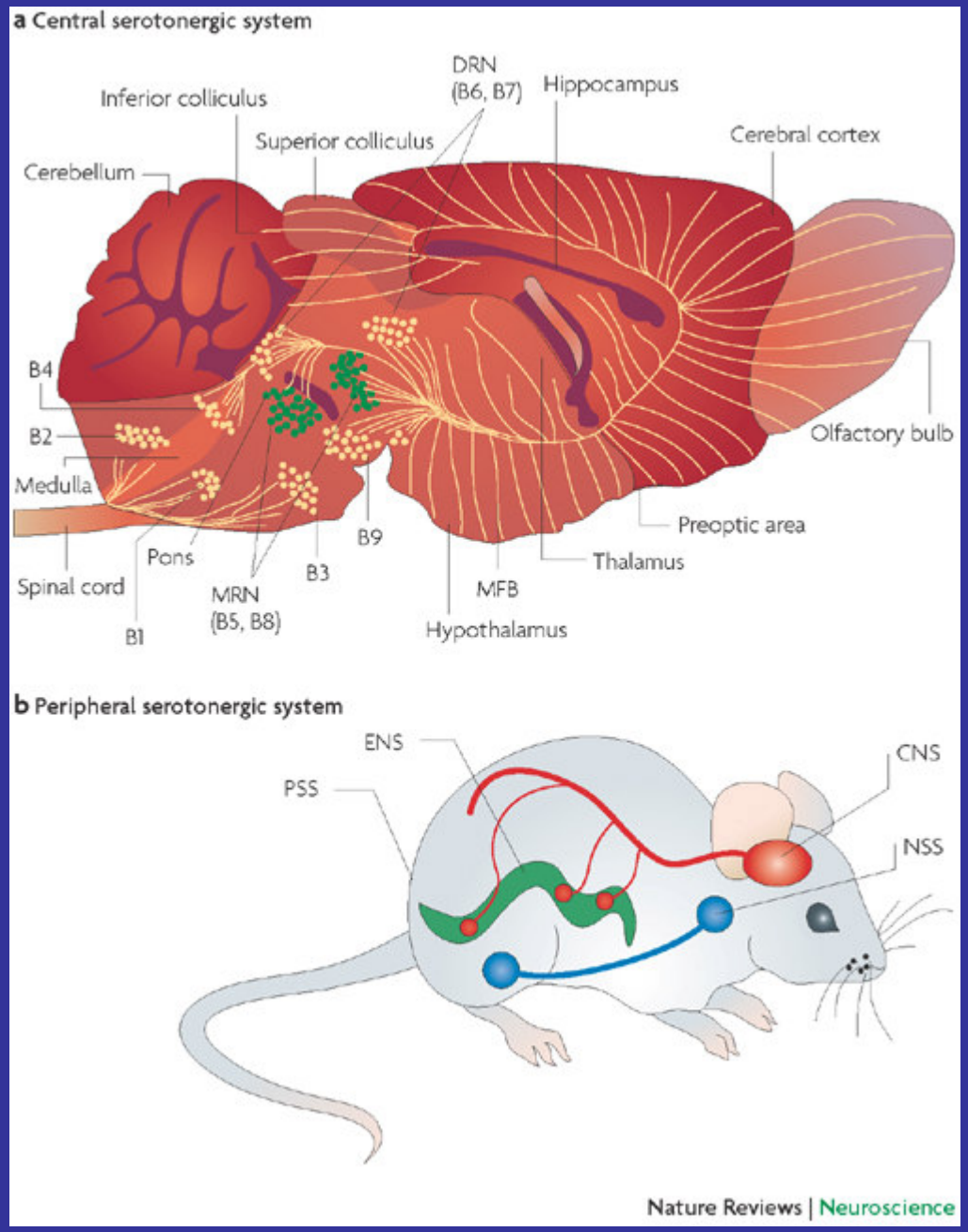
Neuropharmacology
Part 2

1st ISBS Summer School

St. Petersburg, Russia

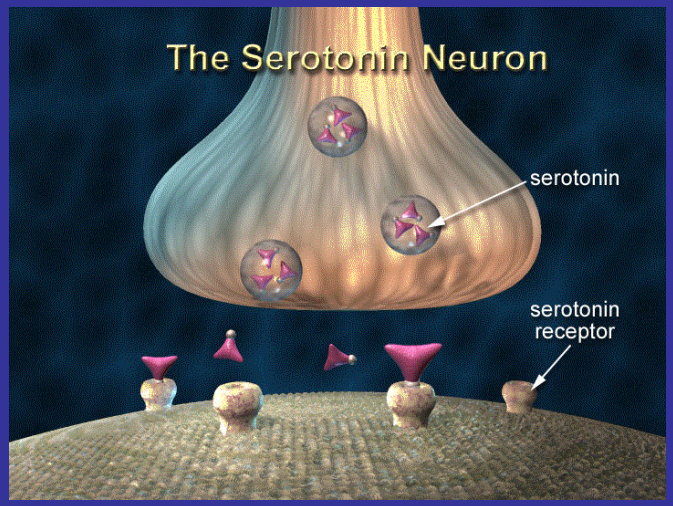
May 9th -15th,2008

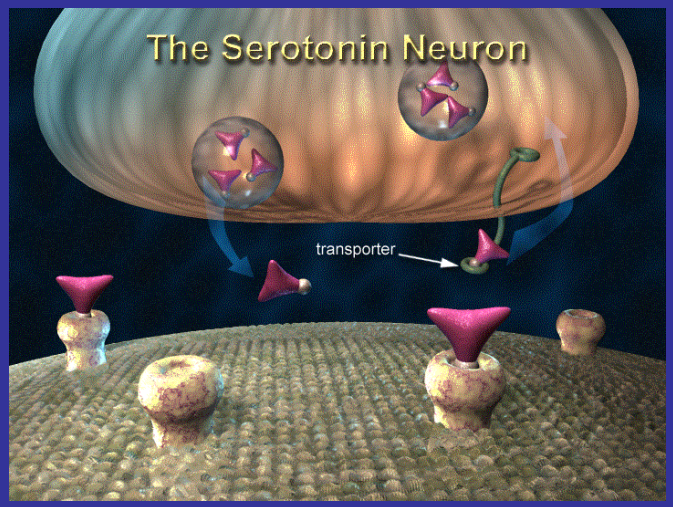
5-HT systems



Murphy and Lesch, 2008

SS and serotonin transporter (SERT)



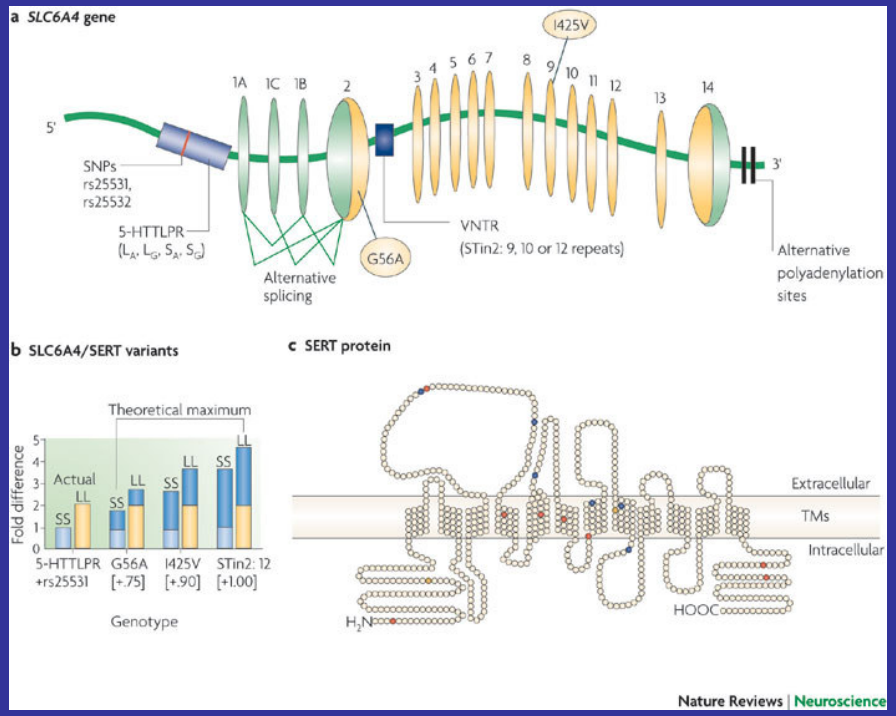


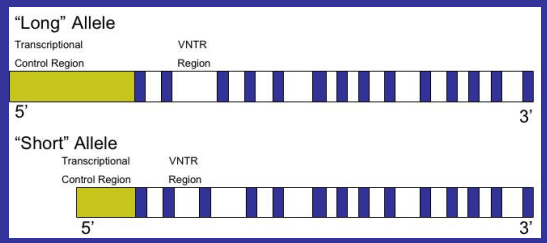
Serotonin transporter (SERT) is the key regulator of serotonergic neurotransmission

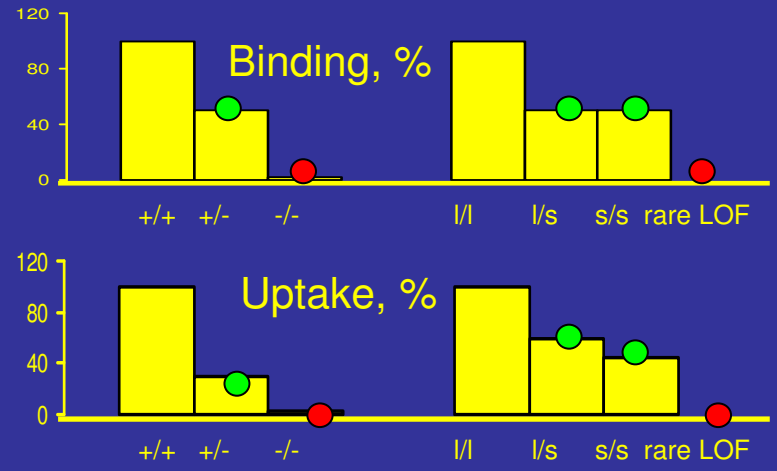
SERT is a target of many psychotropic drugs [SSRIs]

Mice with reduced SERT function may be an interesting model of brain disorders

SERT

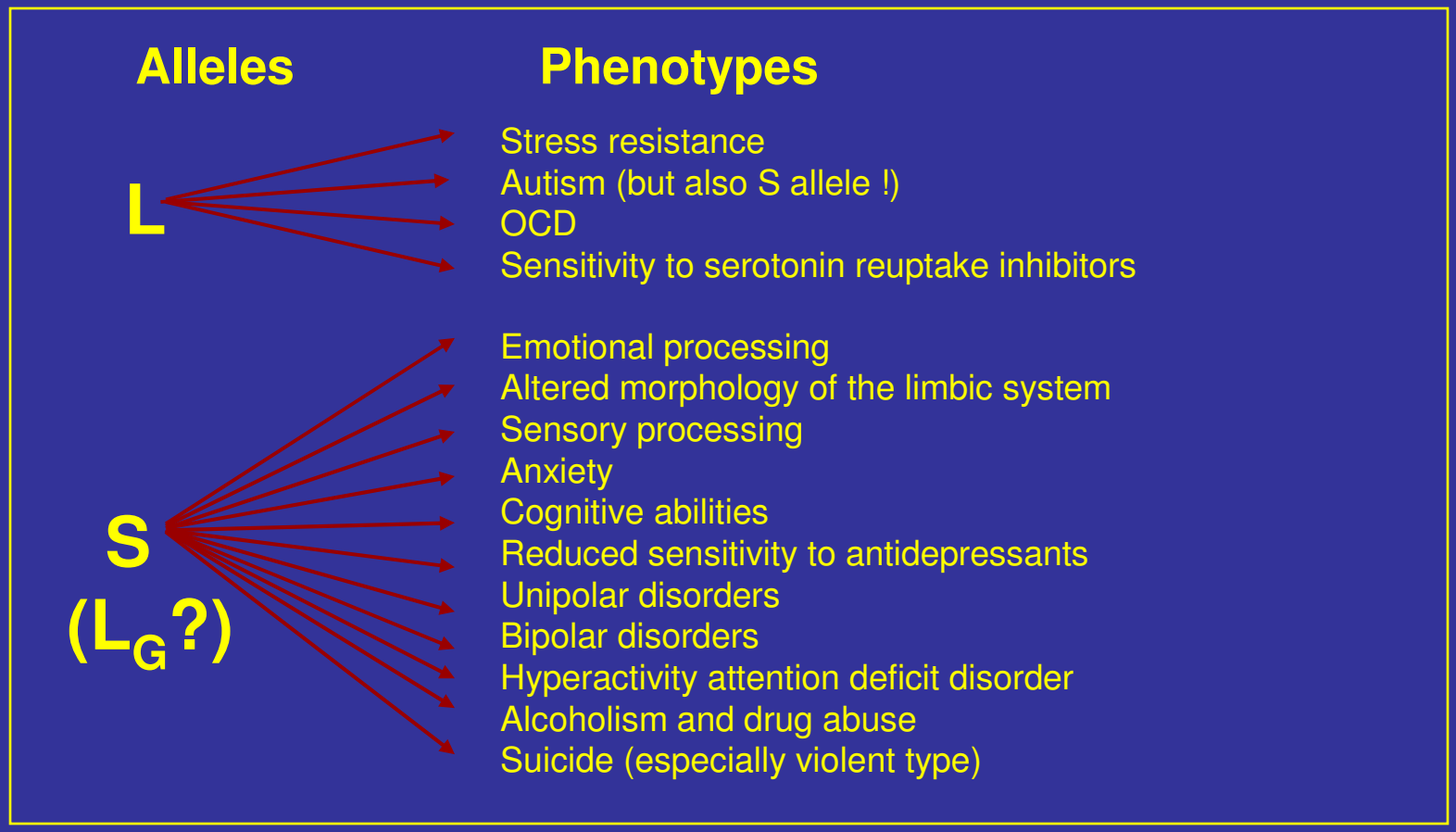






Murphy and Lesch, 2008

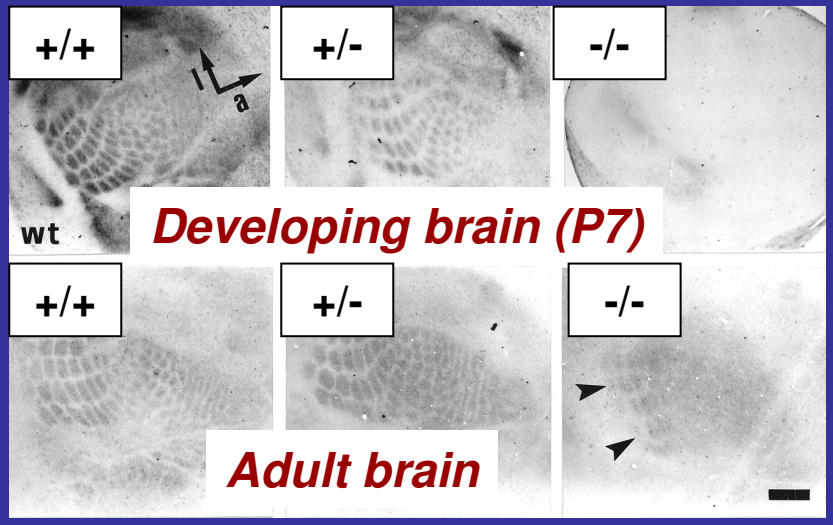
SERT alleles and psychiatric disorders



Kalueff et al., Trends Pharm Sci, 2007

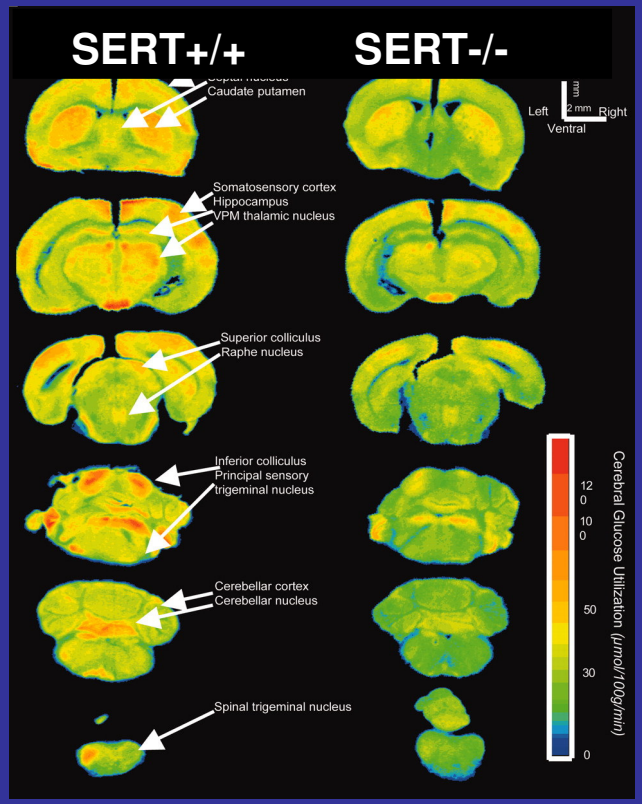
SERT-/- mice: developmental brain anomalies

Barrel pattern in the primary somatosensory cortex of SERT mice



Persico et al., 2001, J. Neurosci.

Metabolic activation (glucose utilization) of whisker-to-somatosensory cortex pathway by whisker stimulation



Esaki et al., 2005, PNAS

SERT-/- mice: developmental brain anomalies

Other findings:

- Altered visual cortex (Salichon et al., 2001)
- Altered cortex thickness (Altamura et al., 2006)
- Abnormal phenotype is rescued by prenatal serotonin depleting drugs

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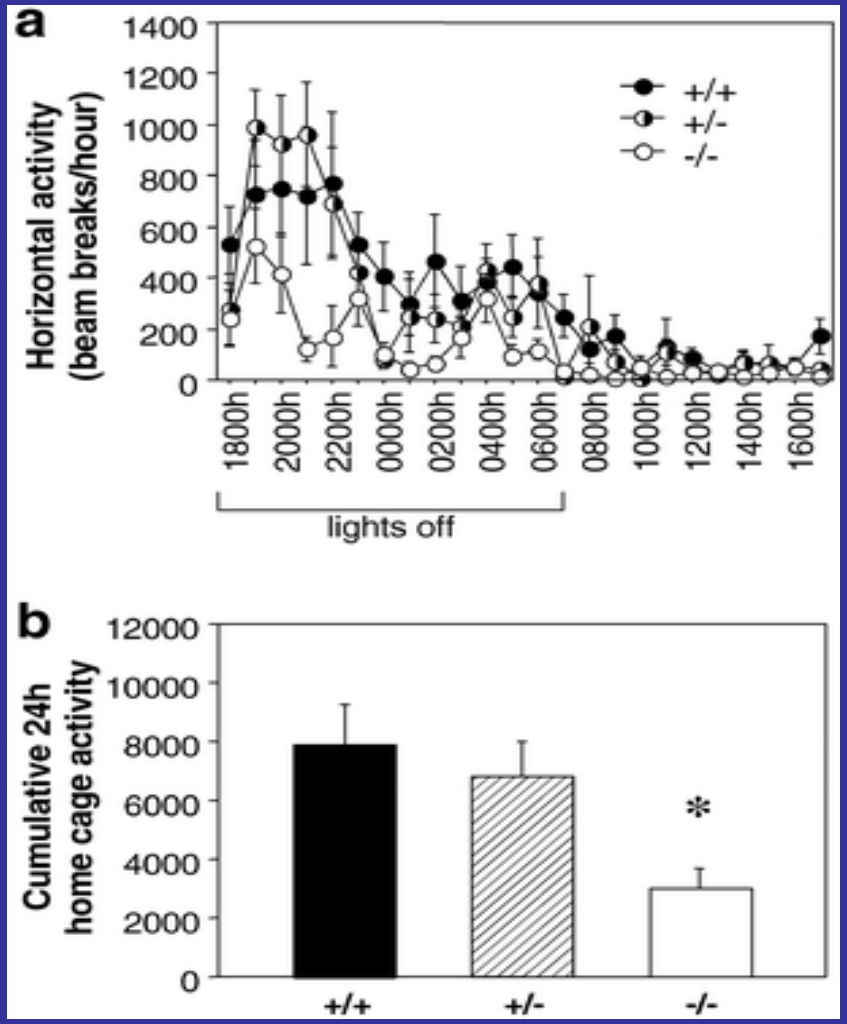
- Serotonin plays a key developmental role in the brain

- SERT-/- mice as a genetic model of serotonin-related developmental anomalies?

General hypoactivity



Dramatic reduction of 24-h motor activity in SERT-/- mice

Holmes et al., 2002, Psychopharmacology

Anxiety

- Reduced open field, elevated plus/zero maze exploration in SERTR-/- mice
- Interpreted as an increased anxiety-like phenotype
- Consistent with clinical data linking SERT to anxiety-related traits

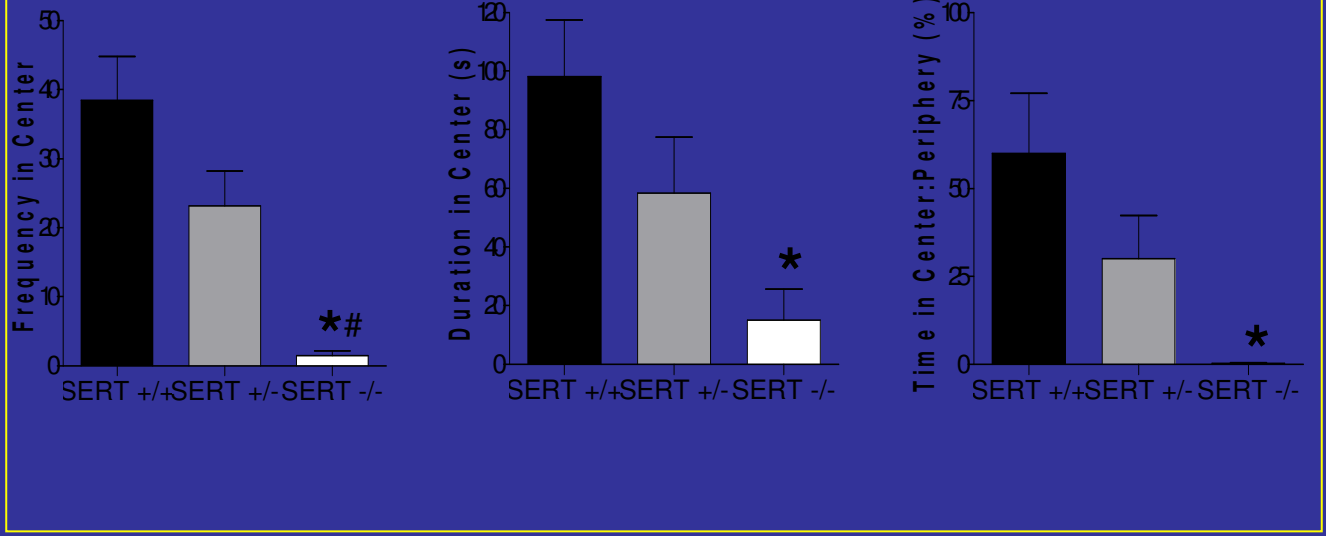
Holmes et al., 2002, 2003

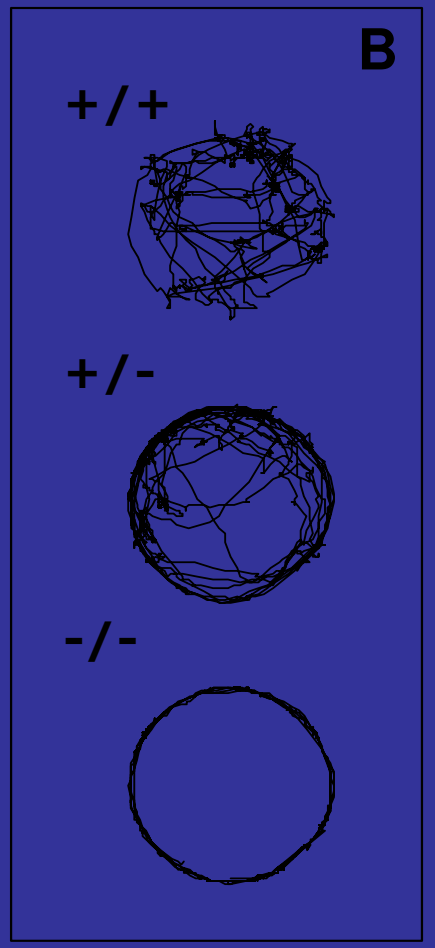
Can we rely on activity-based data in light of hypolocomotion in SERT-/- mice?

Anxiety

Increased thigmotaxis (peripheral vs. central activity) in SERT-/- mice

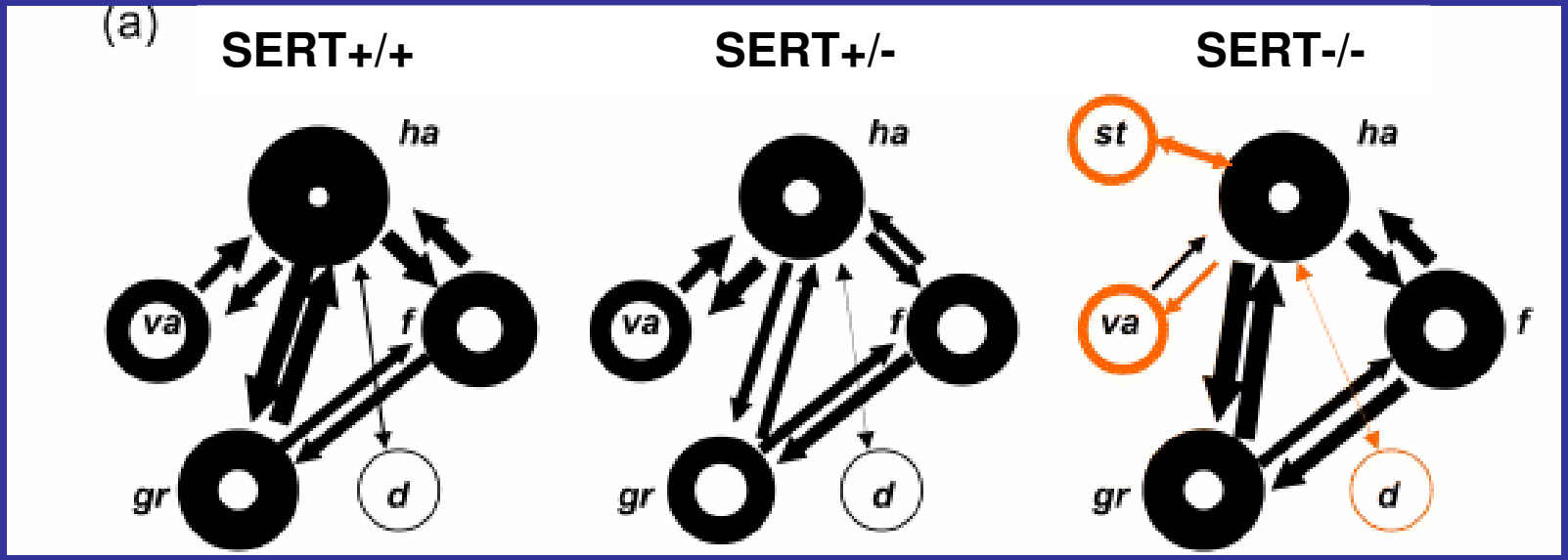
Reduced exploration activity in SERT-/- mice





Kalueff et al., Genes Brain Behav, 2007

SERT -/- mice: behavioral microstructure (patterning)

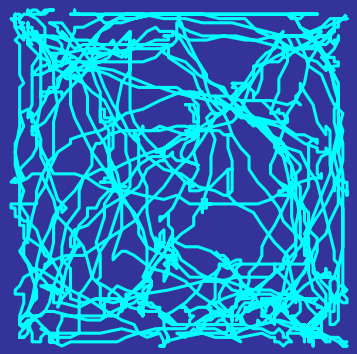


Ethograms (5 min, observation cylinder): ha, horizontal activity (number of ha episodes); va, vertical activity (protected rears); f, freezing episodes; g, grooming bouts; d, defecation; st, Straub tail. Line width reflects frequency of behaviors (circles) or their transitions (arrows). SERT –/– mouse behaviors/transitions significantly different from those of SERT +/+ and +/– mice are marked by a different color.

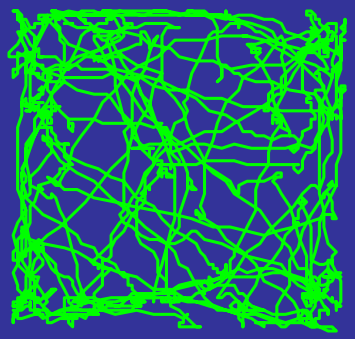
Kalueff et al., Genes Brain Behav, 2007

Exploratory strategies

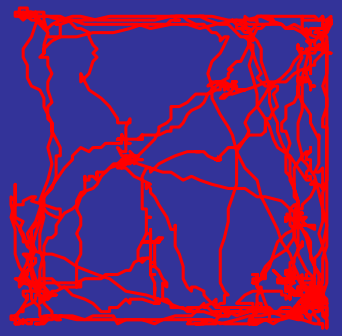
SERT+/+



SERT+/-

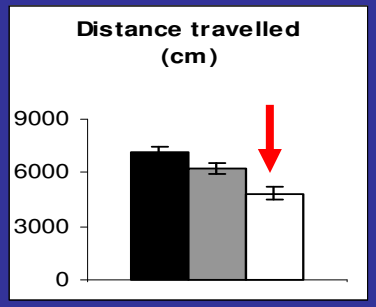


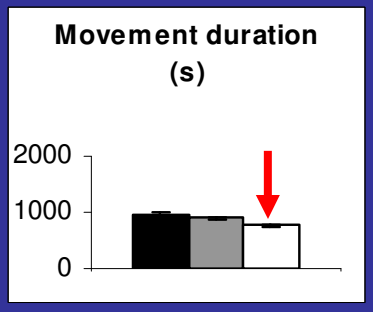
SERT-/-

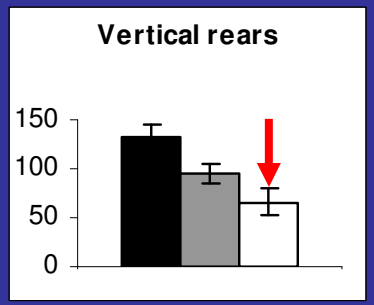


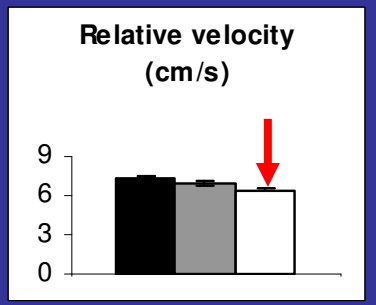
Exploratory strategies are relevant to mimicking human personality traits

Q: Do SERT-/- mice employ different strategies of novelty exploration?









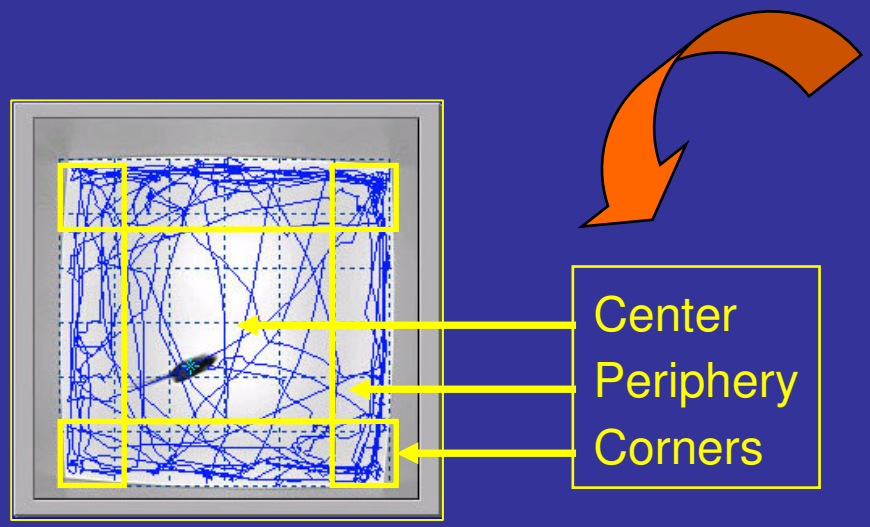
Kalueff et al., 2007, Brain Research

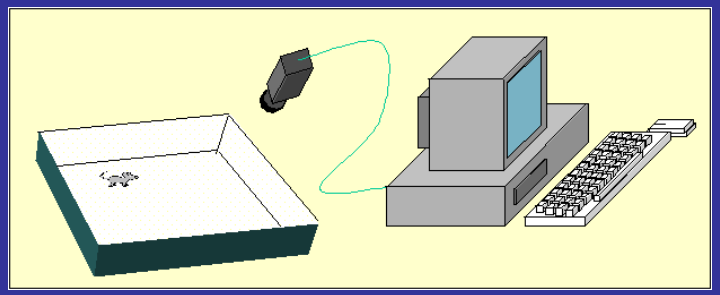
SERT-/- mice and exploratory strategies

In addition to assessment of the ***"amount"*** of behavior (i.e., frequency and duration measures), analyses of ***"quality"*** of behavioral represent an important part of behavioral phenotyping:

- spatial
- temporal
- spatio-temporal characteristics

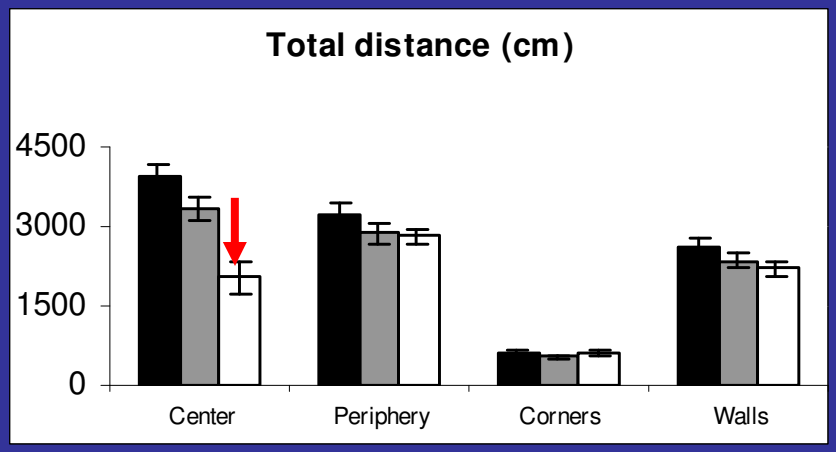
What? When? Where?

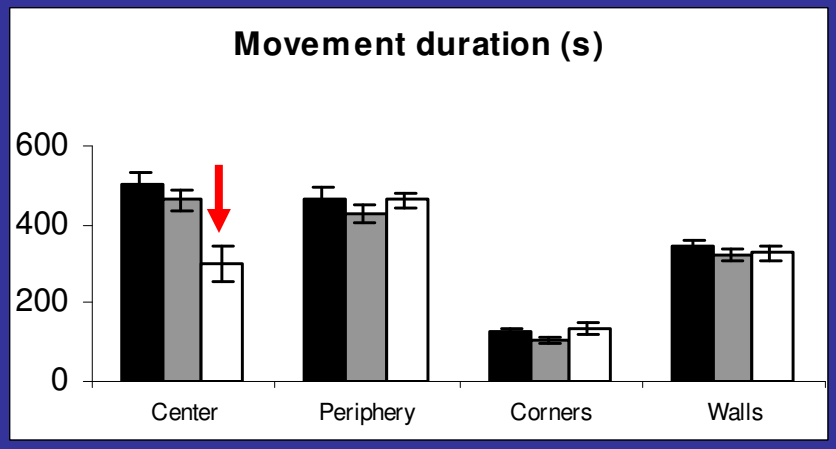




We analyzed spatial and temporal distribution of activity in SERT mice

SERT-/- mice and exploratory strategies





SERT-/- horizontal activity is reduced only in the aversive center

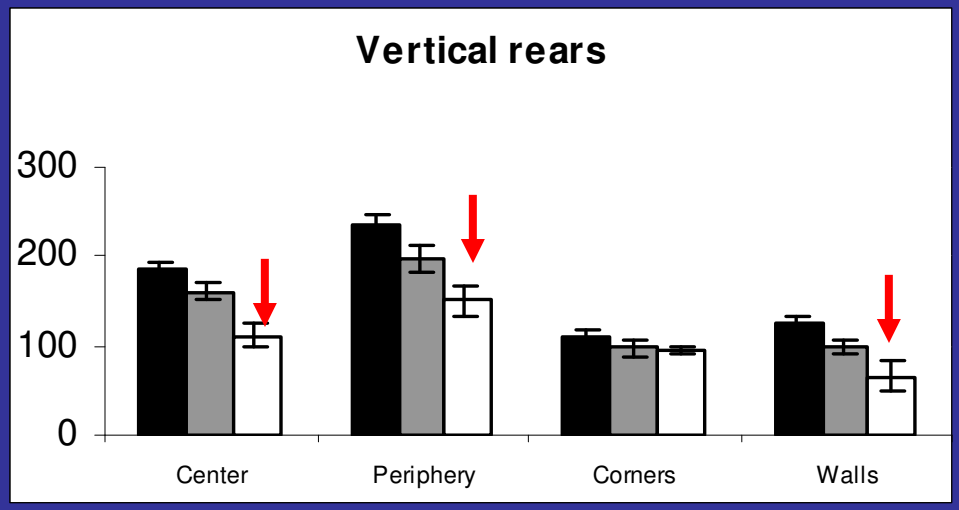
SERT-/- mice maintain normal activity at the periphery

SERT-/- mice respond to stress by spatially re-organizing their activity (rather than by overall reduction of their activity)

Anxiety

Kalueff et al., 2007, Brain Research

SERT-/- mice and exploratory strategies



Unlike horizontal activity, SERT-/- mouse vertical activity is reduced ***throughout*** the arena

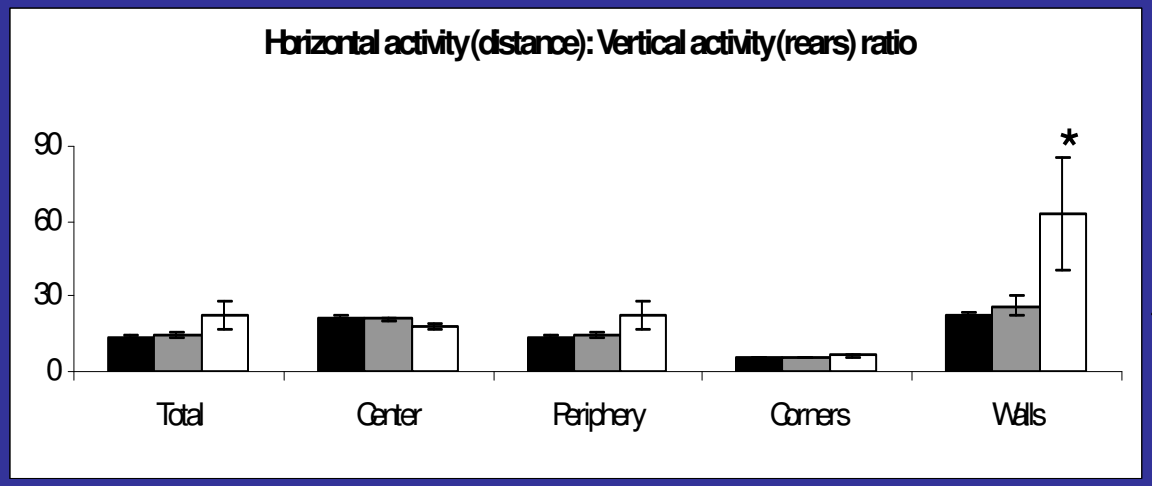
SERT-/- mice employ different "vertical" strategies of exploration

Vertical activity is more sensitive to anxiety (e.g, Lapin, 1998)

Vertical and horizontal activity represents two distinct domains differentially regulated in SERT-/- mice

Kalueff et al., 2007, Brain Research

Horizontal vs. vertical dimensions



Elevated plus maze: Horizontal:vertical activity indices				
Center	5.9 + 2	6.7 + 1	6.9 + 1	NS
Open arms	45 + 5	44 + 4	41 + 5	NS
Closed arms	34 + 3a	41 + 3b	73 + 13ab	18.9 (0.004)
Total arena	41 ± 4	55 ± 14	53 ± 7	NS

In safe, protected areas (such as close to walls), SERT-/- mice more actively use horizontal locomotion, traveling more between two consecutive vertical rears

Spatial working and long-term memory

Serotonin is involved in the regulation of memory and other cognitive functions

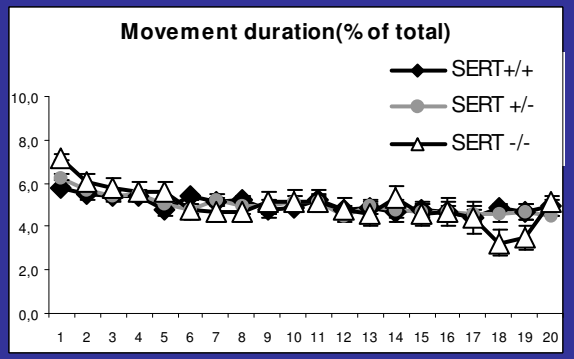
SSRIs have effects on memory in habituation tasks

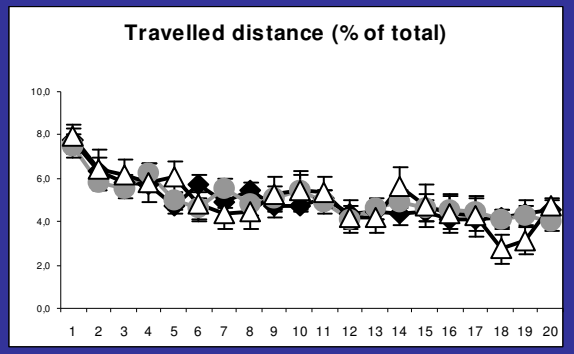
We used OF and EPM within- and between-trial habituation to assess spatial memory in SERT-/- mice

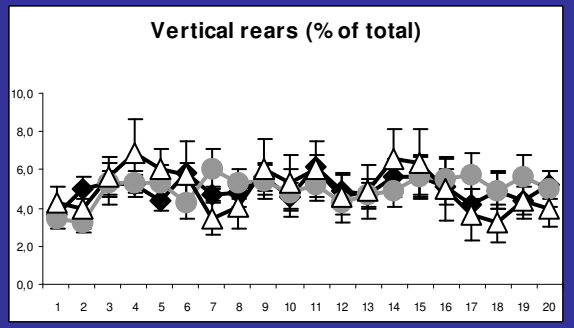
“Normalized” mouse activity (% of total)

Spatial working memory is not affected in SERT-/- mice

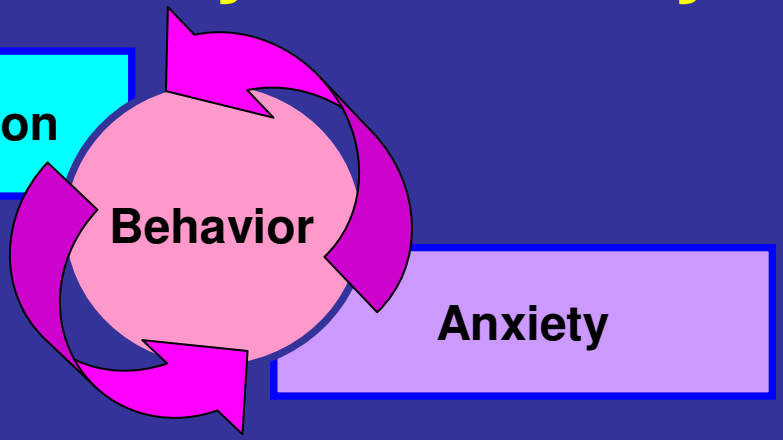
Long-term spatial memory (data not shown) is also unaffected in SERT-/- mice





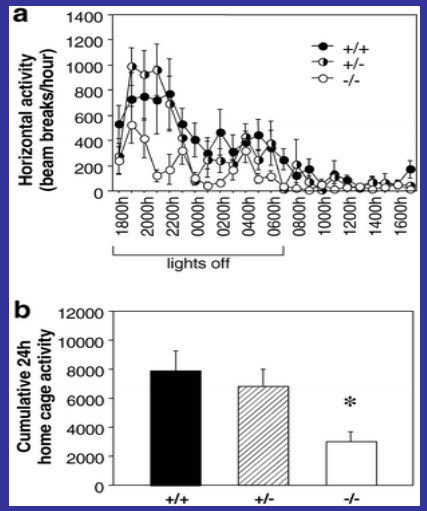


Domain interplay: inactivity vs. anxiety



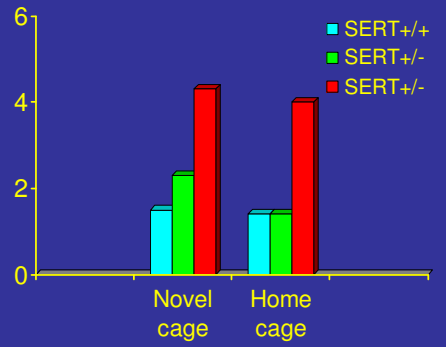
Q: Can some SERT-/- behaviors be due to hypolocomotion?

Marble burying test
- Anxiety
- Activity
- OCD-like behavior



Hypoactivity may dominate all other behavioral domains in SERT-/- mice

Number of non-buried marbles

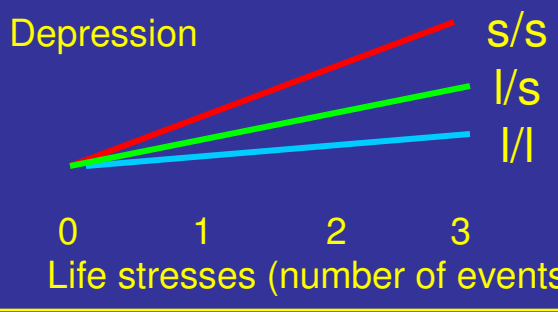


Holmes et al., 2002 Psychopharmacology

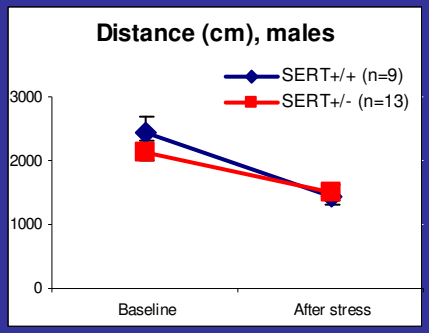
Kalueff et al., 2006, NeuroReport

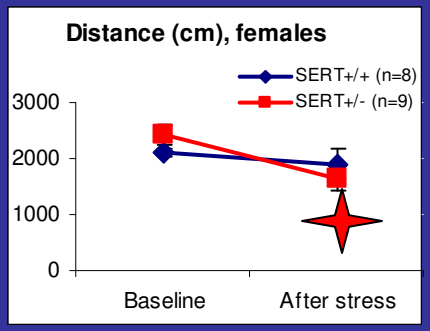
Depression: pilot "Caspi-like" study

Caspi et al., Science, 2002



SERT gene x Environment

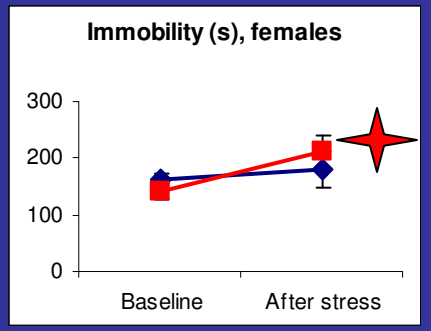




We wanted to replicate this study using SERT+/- mice

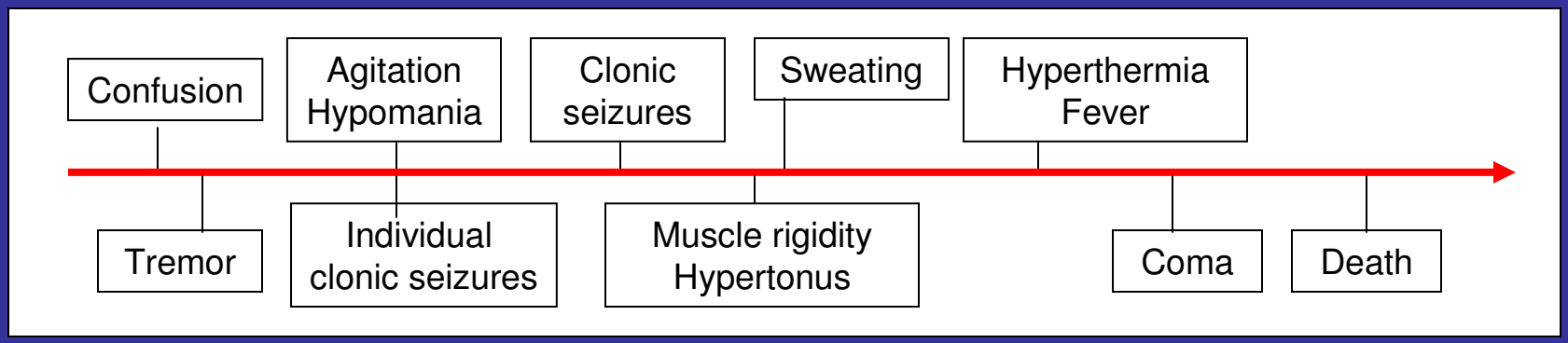
Used 2-week rat exposure (10-15 min daily)

Female SERT+/+ mice were protected from chronic stress



Phenotyping serotonin syndrome (SS)

A serious disorder associated with increased serotonergic tone
On the rise globally, due to the growing intake of serotonergic drugs

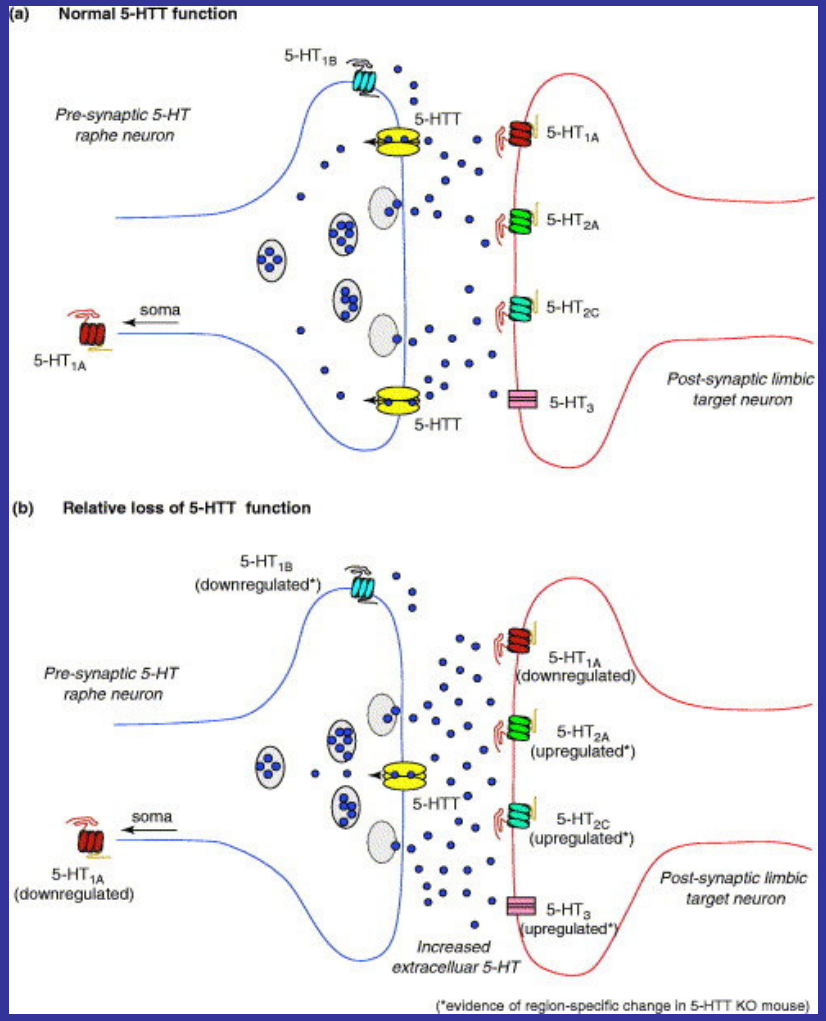


Animal SS-like behavior can be induced by various serotonergic drugs

Symptoms: Tremor, hind leg abduction, low/flat body position, Straub tail, head weaving, head twitches, hyperthermia, backward gait

The growing number of mutant or transgenic animals display various serotonergic abnormalities

The importance of modeling SS-related phenotypes in animals



SERT and SS ?

Normal brain serotonin
in SERT+/+ mice

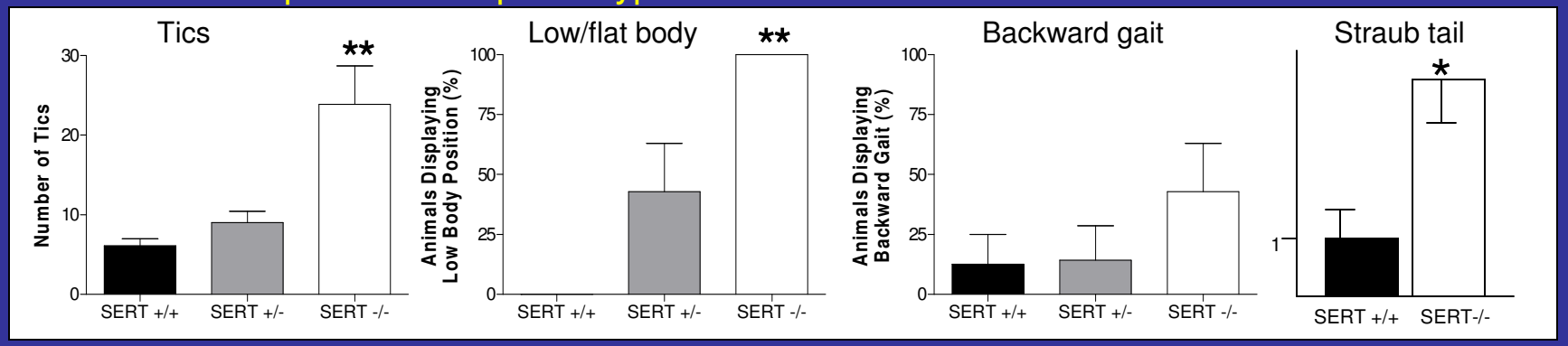
Excess of brain serotonin
in SERT-/- and SERT+/- mice

HYPOTHESIS: Mice with reduced SERT expression and function may be relevant to SS

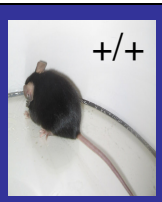
Pro: 10-fold elevation of brain serotonin in SERT-/- mice (Li et al., 2003)

Genetic model of SS ?

SERT-/- mice: spontaneous phenotype

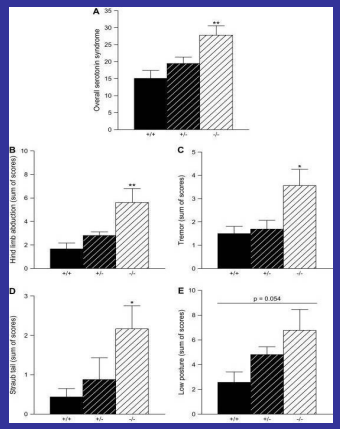


Kalueff et al., 2007



Further pharmacological validation

MAO Inhibitor tranyl-cypromine



Fox et al., 2007, Neuropharmacology

SS-like behaviors	Spontaneous SERT-/-	Drug-evoked SERT-/-
• Muscle rigidity	+	+
• Tremor	+	+
• Forepaw treading		+
• Head weaving		+
• Myoclonus (seizures)		
• Ticing, back muscle contraction	+	+
• Flat/low body posture	+	+
• Incoordination	+	+
• Hind limb abduction	+	+
• Backward gait		+
• Hyperthermia	+	+
• Straub tail	+	+

Neurophenotype → Hypothesis → Model

SERT-/- mice: the first genetic animal model of SS

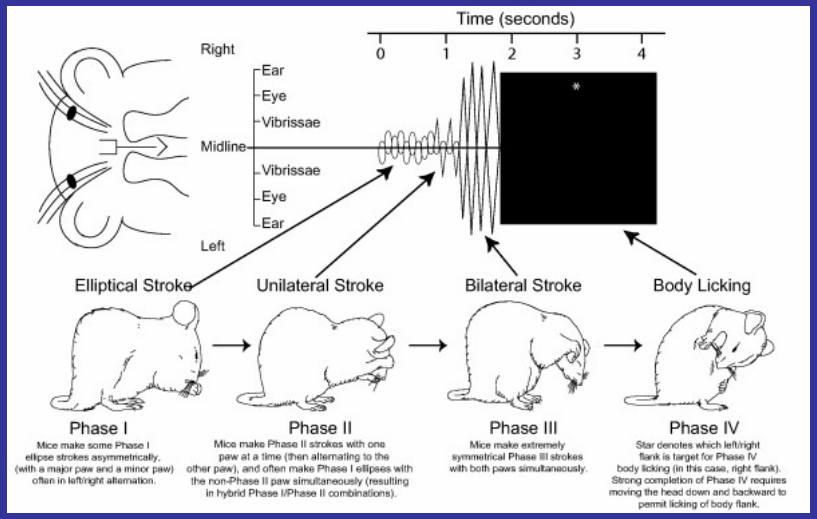
Kalueff et al., 2007

SERT-/- mice and OCD-like behaviors?

SERT has long been implicated in pathogenesis of OCD

- Reduced SERT in OCD patients (Hesse et al, 2005)
- SERT mutation in OCD families (Ozaki et al., 2003)
- Association of “s” allele with OCD (Hasler et al., 2006)

Q: Can SERT mutant mice be a model of OCD-spectrum disorders?



Grooming behavior?

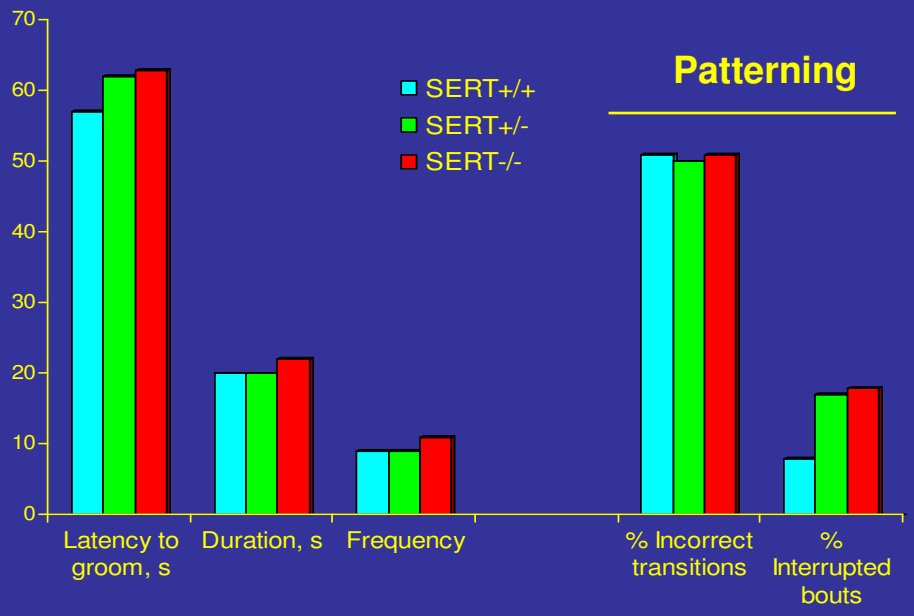
Self-grooming is an excellent example of animal OCD-related behavior (e.g., Greer and Capecci, 2002)

Berridge et al., 2005, BMC Biology

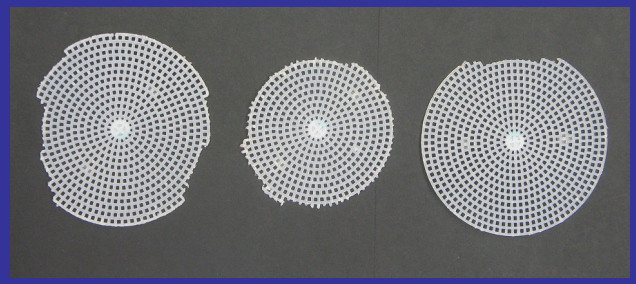
SERT-/- mice and OCD-like behaviors?

However, grooming was NOT affected in SERT-/- mice:

- Normal frequency and duration
- Unaltered patterning/organization



In plastic canvas chewing (Chow-Green et al., 2005) test stereotypic chewing was NOT affected in SERT-/- mice



SERT+/+ SERT+/- SERT-/-

Q: Are other OCD-like behaviors affected?

Kalueff et al., 2007, Genes Brain Behavior

SERT and autism

Genetic factors play a key role in pathogenesis of autism and autism spectrum disorders (ASD)

SERT gene and the 17q chromosomal area have been consistently associated with autism (Yonan et al., 2003; McCauley et al., 2004; 2005) and “Autism +” (ASD with a strong OCD component; Ozaki et al., 2003)

Several studies have shown association of SERT short “s” and long “l” alleles with autism (rev: Sutcliffe et al., 2005)

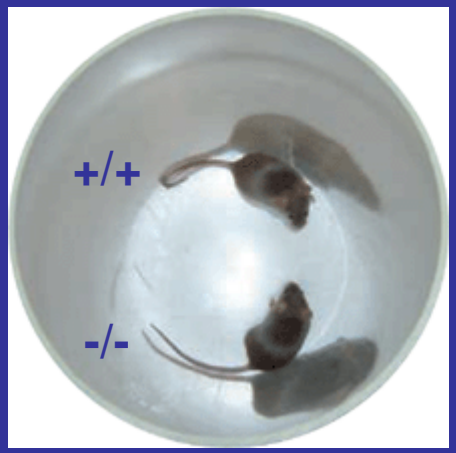
Bruno et al. (2006) reported that autistic “s” allele carriers were more severe on the subdomain "failure to use nonverbal communication to regulate social interaction"

Animal genetic models emerge as an important tool to explore the neurobiology and neurogenetics of autism (Moy et al., 2006)

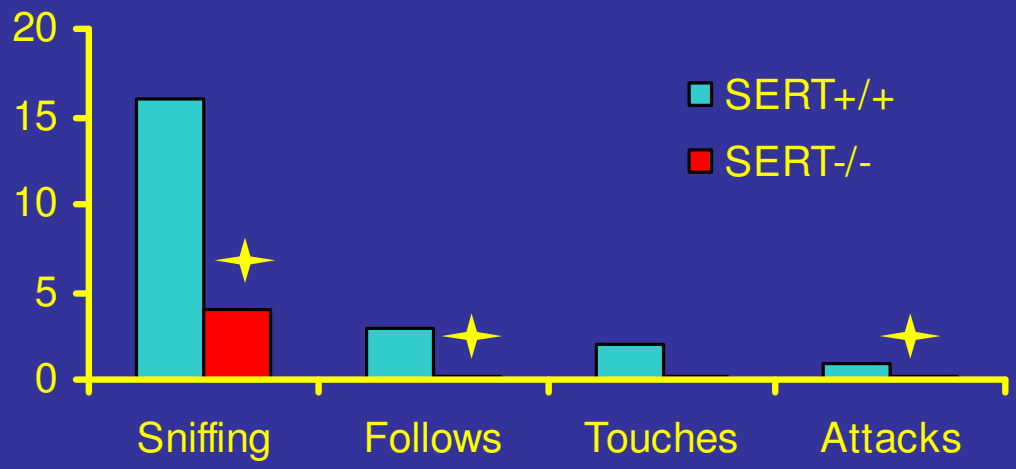
Q: Can our SERT-/- mice be a genetic model of autism and ASD?

SERT-/- mice: social interaction

Q: Is social interaction domain affected in SERT -/- mice?



Initiated behaviors (social interaction test)



Potential rationale:

- Anxiety (e.g., social anxiety) ?
- Hypolocomotion?
- Reduced aggression?
- Autism-like behaviors?

Kalueff et al., Genes Brain Behav, 2007

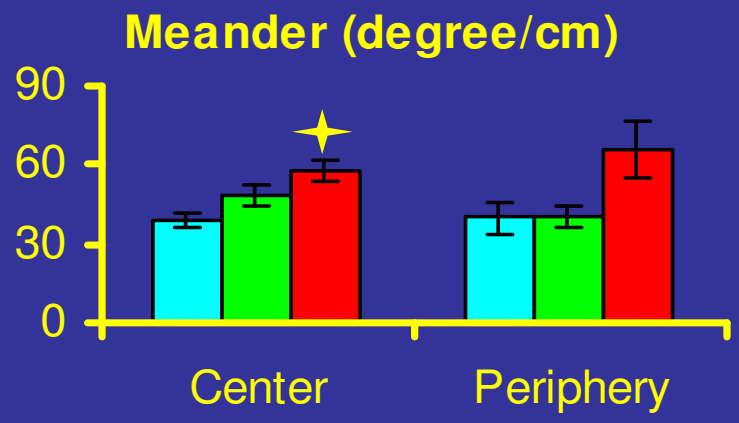
SERT-/- mice and autism?

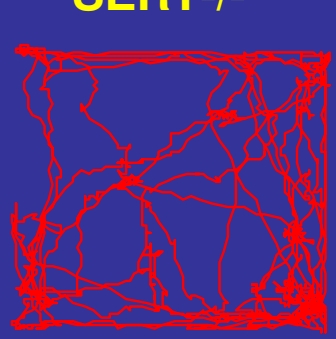
Clinical symptoms of autism

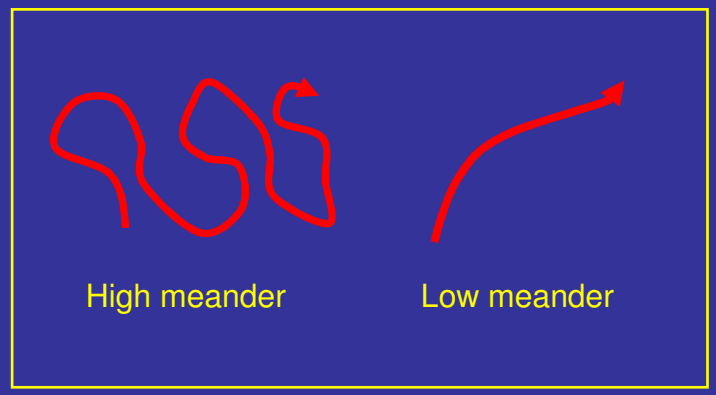
- Social deficits
- Language problems
- Altered cognitive processing
- Behavioral perseverations

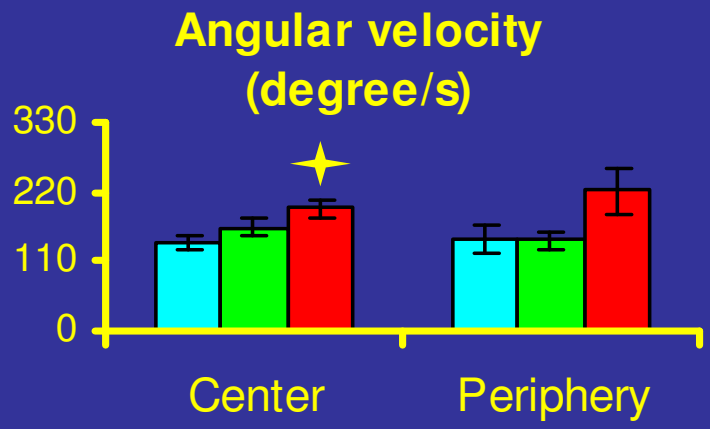
Q: Do other, non-social, behaviors in SERT-/- mice resemble symptoms of autism?

SERT-/- mice and autism? Behavioral perseverations









Kalueff et al., 2007, Brain Research

SERT-/- mice and autism?

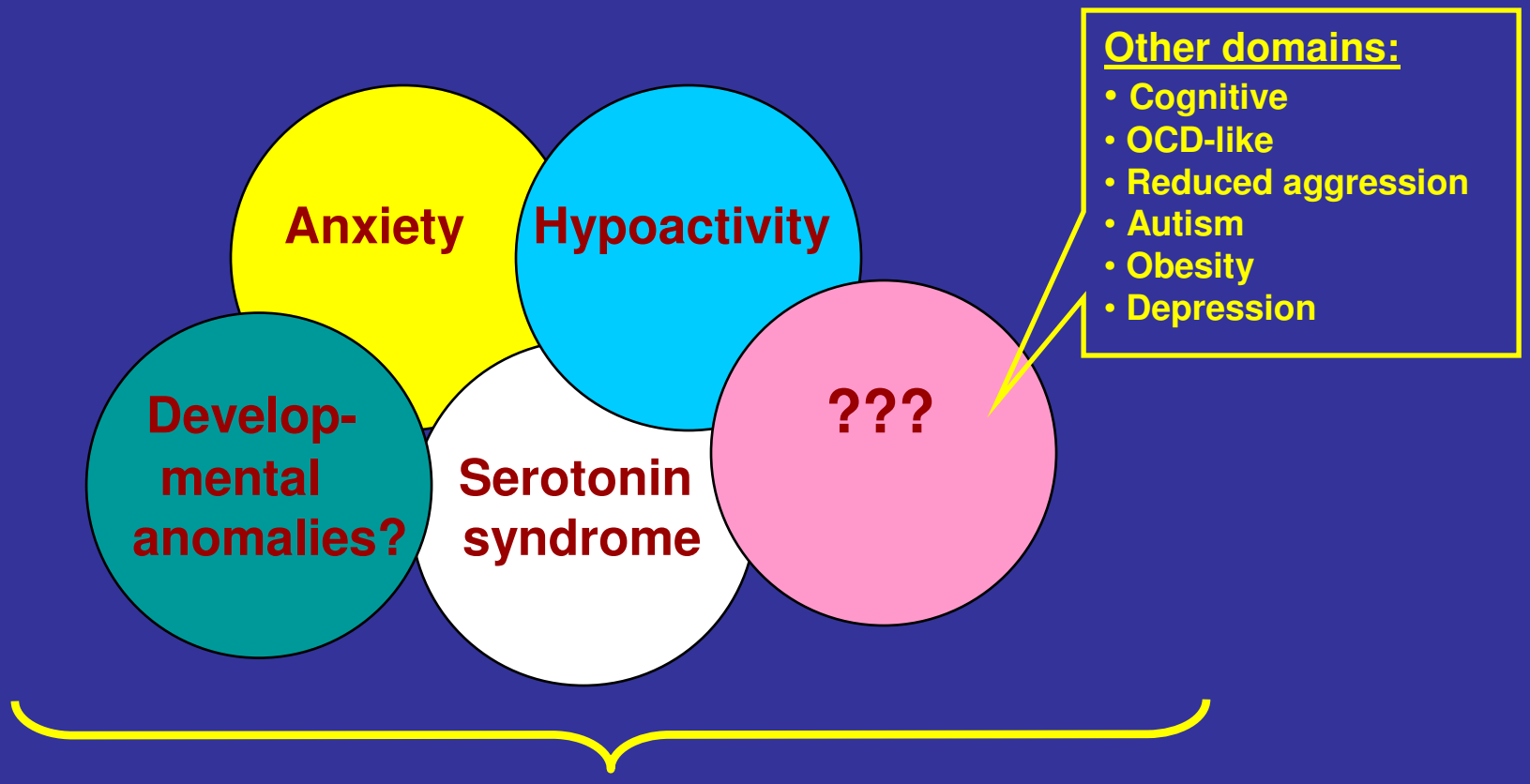
Symptoms of autism that can be modeled in animals:

- Social deficits ✓ **SERT-/- mice**
- Altered cognitive processing
- Behavioral perseverations ✓ **SERT-/- mice**

Additional evidence:
SERT -/- rats display “reduced social play” (Homberg et al., 2006)

SERT mutant rodents may lead to potentially interesting genetic models related to ASD

Complex behavioral phenotype in SERT-/- mice



SERT-/- mice as a genetic model of depression?

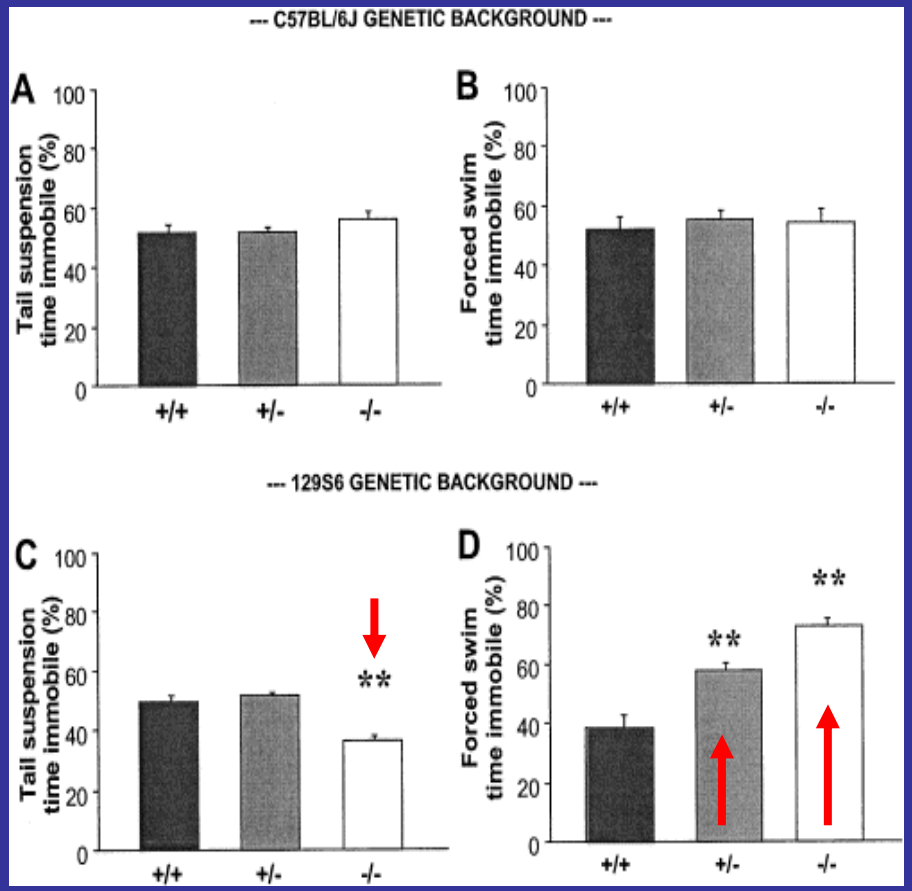
• Conflicting clinical data on the role of human SERT gene in the pathogenesis of depression (comments in:

Kaufman et al., 2006; Biol. Psychiatry
Kalueff et al., 2006, Biol. Psychiatry
Berton et al., 2006, Science
Kalueff et al., 2006, Science

• Unclear rationale (why SERT-/- mice shall be depressed when they have SERT inhibited since birth?)

Further studies are needed to assess depression-like behavior in SERT-/- mice

Conflicting data on depression-like behavior in SERT-/- mice



- opposite depression profiles in two different "despair" models: TST and FST
- genetic background-dependent effects (C57 vs. 129S6)

Holmes et al., 2002, Neuropsychopharmacol.

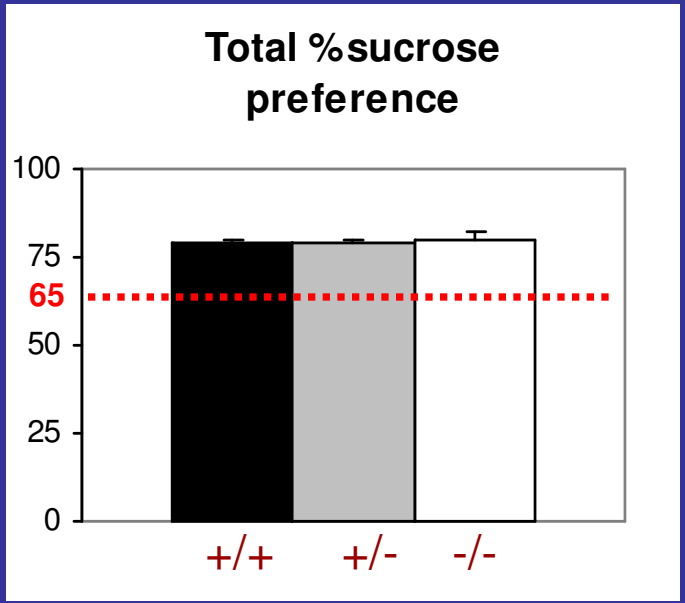
Do SERT-/- mice have a higher baseline depression?

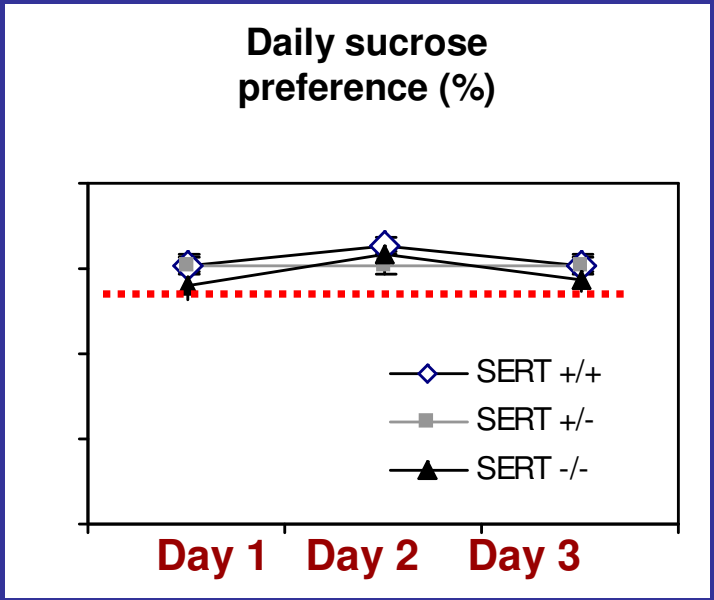
Sucrose preference test
4% sucrose, 2 bottle choice, 3 days

- Based on anhedonia – the core feature of human depression
- Independent of general motor activity levels
- Highly sensitive to factors known to induce depression
- Frequently used in C57Bl/6 mice (background of SERT-/- mice)

Do SERT-/- mice have a higher baseline depression?

- Unaltered sucrose preference in SERT-/- mice
- Lack of anhedonic depression (>>65% preference)
- SERT-/- mice are not a genetic model of depression





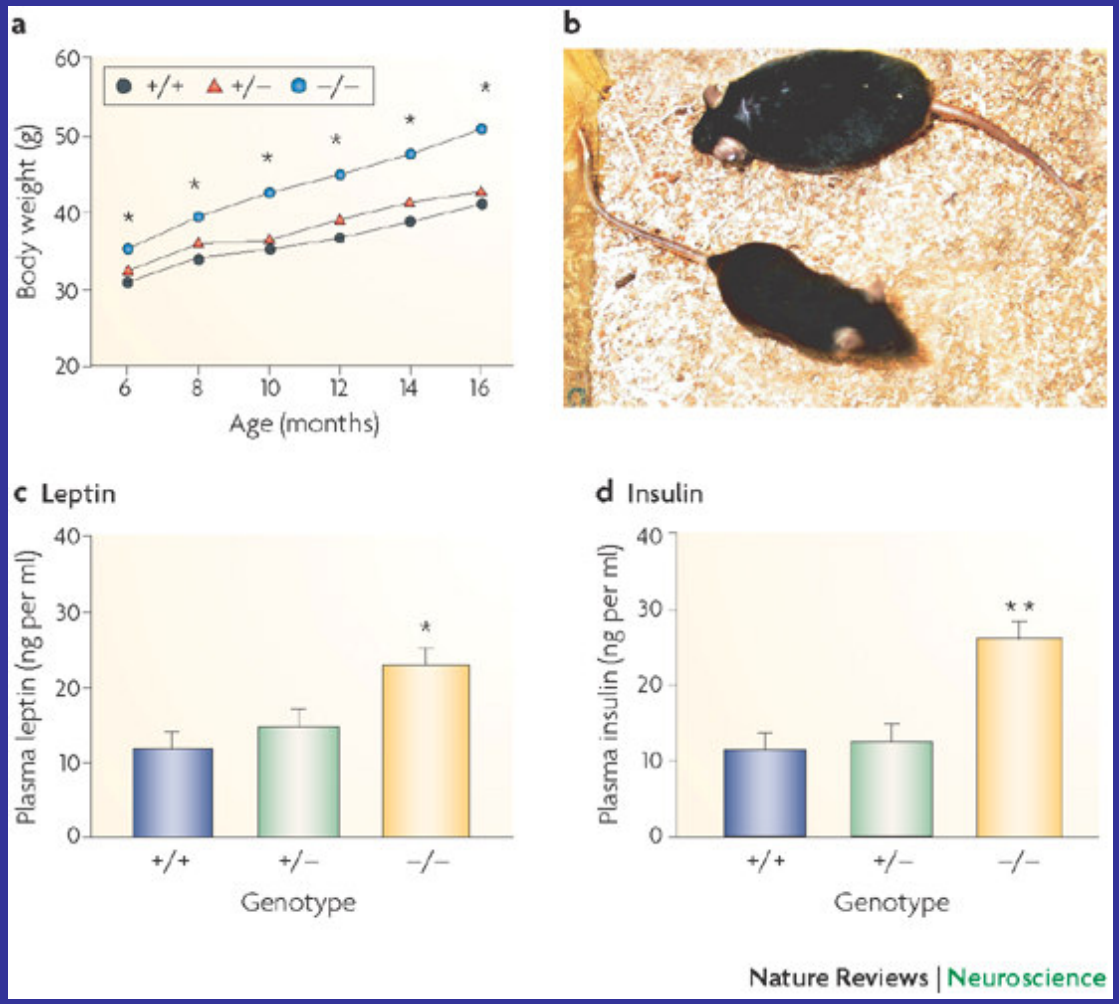
Kalueff et al., 2006, Neuroreport

Clasping phenotype: “129 problem”?

No clasping:
C57 mice
CD1 mice

Photos: M. Ansorge

SERT and obesity



Murphy and Lesch, 2008

Biochemical features	*Slc6a4*$^{-/-}$	*Slc6a4*$^{+/-}$
SERT binding sites	↓	↓
Serotonin uptake	↓	↓
Serotonin clearance	↓	↓
Serotonin content in brain and periphery	↓	NS
Extracellular fluid serotonin	↑	↑
Serotonin synthesis	↑	NS
5-HT_{1A} receptor sites and mRNA	↓	↓
5-HT_{1B} receptors	↓	↓
5-HT_{2A} and 5-HT_{2C} receptors	Δ	NS
5-HT_3 receptors	↑	Δ
Adenosine receptors	↑	NT
OCT3 mRNA	↑	NS
Glucose	↑	NS
Leptin	↑	NT
Cholesterol	↑	NT
Triglycerides	↑	NT

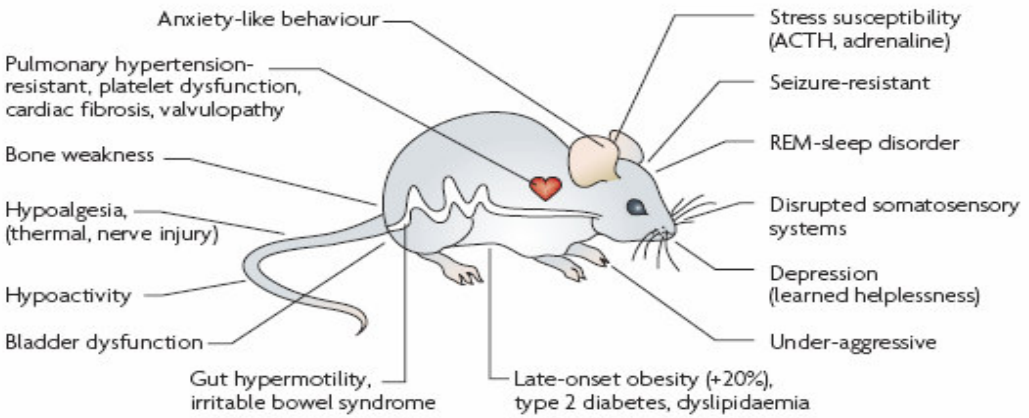
Behavioural features	*Slc6a4*$^{-/-}$	*Slc6a4*$^{+/-}$
Anxiety	↑	↑
Learned fear	↑	NS
Learned helplessness (forced-swim and tail-suspension tests)	↑	NS
Aggression	↓	↓
Acoustic startle response	↑	↑
Exploratory activity	↓	NS
Rotorod agility	↓	NS
Wire hang	↓	↓

Anatomical features	*Slc6a4*$^{-/-}$	*Slc6a4*$^{+/-}$
Somatosensory and visual cortex	Δ	Δ
Infralimbic cortex (dendrite morphology changes)	Δ	Δ
Apoptosis in neonatal brain	↓	NS
Cell density in neonatal cortex	↑	NT
Pyramidal neuron spine density in amygdala	↑	NT

Physiological features	*Slc6a4*$^{-/-}$	*Slc6a4*$^{+/-}$
Stress responses (ACTH, corticosterone, epinephrine, temperature and motor responses)	↑	↑
Gut motility (diarrhoea, constipation)	↑	NT
Body weight	↑	NS
Glucose tolerance	↓	NT
Insulin sensitivity	↓	NT
Brain glucose utilization	↓	NT
Bone mass and strength	↓	NT
Nociception (nerve injury and thermal)	↓	NT
Bladder function	↓	NS
Hypoxia-induced pulmonary hypertension	↓	NT
Raphe serotonin neuron firing rate	↓	↓
REM sleep	↑	↑
EEG power spectra, 'bursting'	Δ	NS

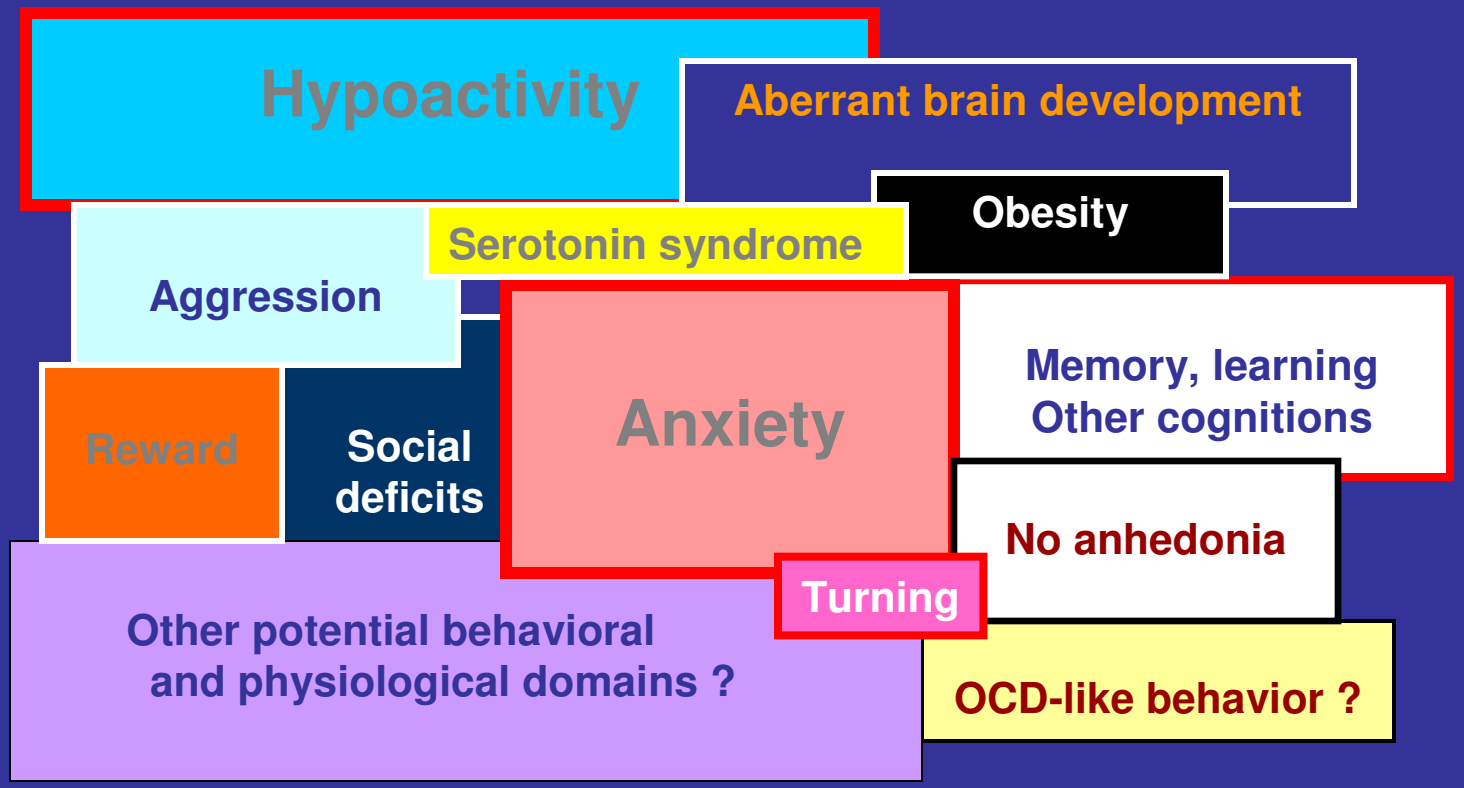
Pharmacological features	*Slc6a4*$^{-/-}$	*Slc6a4*$^{+/-}$
Effect of SSRIs (inescapable stress and 5-HT clearance)	↓	Δ
Effect of ipsapirone (serotonin synthesis and DRN firing rate)	↓	NS
Effect of RU24969 (motor function)	↓	NS
Effect of CP 93129 (serotonin clearance)	↓	NS
Effect of DOI (head twitch)	↓	↓
Effect of pentylenetetrazole (seizure)	↓	NS
Effect of MDMA (motor function and self-administration)	↓	↓
Effect of 2′-NH_2-MPTP (motor function and temperature)	↓	NS
Effect of alcohol (10-day intake)	↓	NS
Effect of 8-OH-DPAT (temperature and neuronal activity)	↓	↓
Effect of cocaine (preference)	↑	↑
Effect of alcohol (motor function)	↑	NT

***Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice: overview of major phenotypes**



Murphy and Lesch, 2008

The beauty of complexity



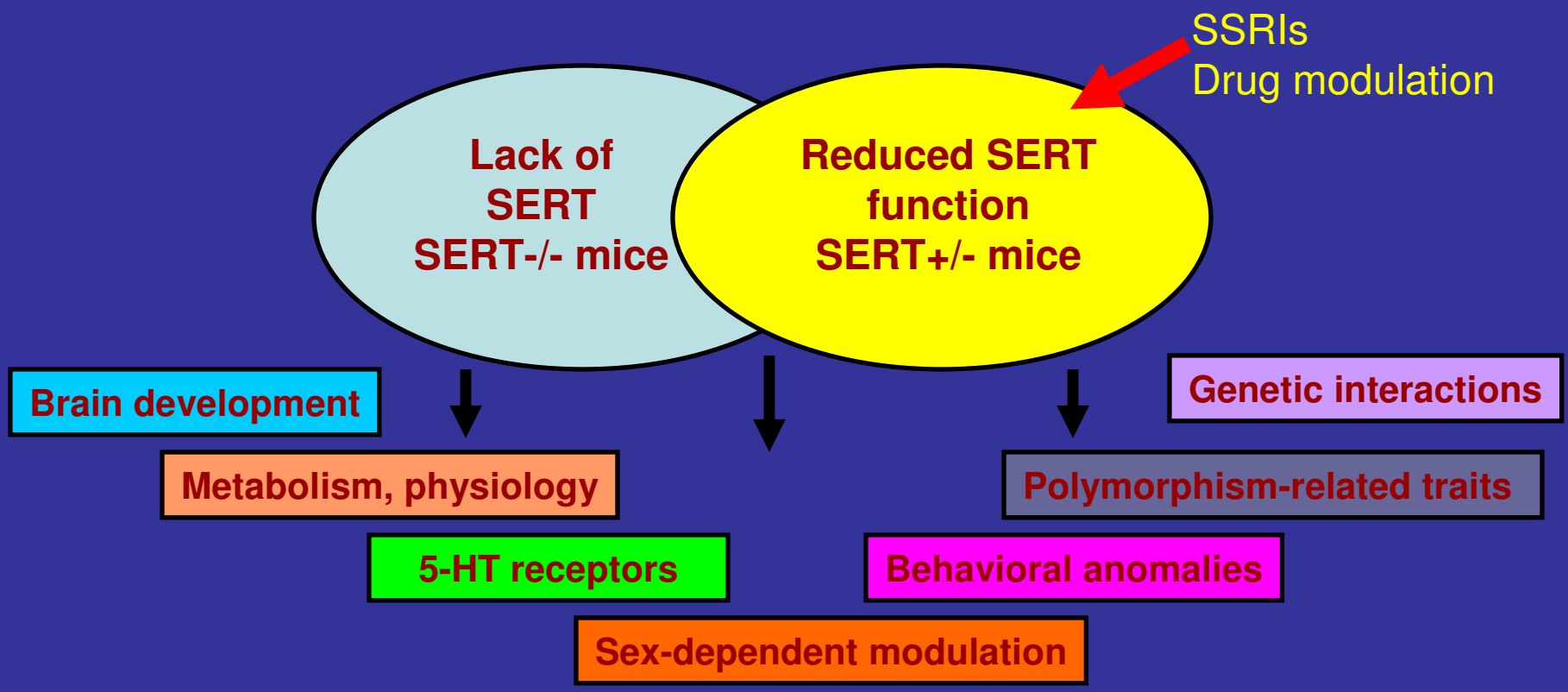
Future studies: focus on SERT+/- mice

A less confounded model of SERT-related dysfunctions?

SERT-/- mice	SERT+/- mice	Advantage
• No barrel patterns	• Present barrel pattern	+
• Hypoactivity, obesity	• No hypoactivity	+
• Hyperthermia	• No hyperthermia	+
• 10-fold elevated serotonin	• 5-fold elevated serotonin	
• Absent SERT binding	• 50% reduction in SERT	
• Insensitive to SSRIs	• Hypersensitive to SSRIs	+
• Irrelevant to human SERT polymorphisms	• Relevant to human SERT polymorphisms	+
• Some gender differences	• Robust gender differences	+

SERT mutant mouse models

Both SERT-/- and SERT+/- mutant mice may be useful in modeling a wide spectrum of different domains of SERT-related brain disorders



Conclusion

SERT-/- and +/- mice are an indispensable tool to explore various brain/behavioral disorders

We shall expect further complexities of their behavioral, physiological and pharmacological phenotypes

+/- mice are more clinically relevant

Special attention shall be paid to G-G and G-E interactions

Parallels to other models species: e.g., rats, monkeys

Acknowledgement

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