Animal models and mechanisms of impulsivity and gambling proneness: investigation on sex and age differences

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Impulsivity

- **A multifaceted concept defined in several ways:**
  - inability to wait or to inhibit inappropriate behaviour
  - a tendency to act without forethought about possible outcomes
  - an insensitivity to the adverse consequences of one's own actions

- **Two main categories of impulsivity are:**
  - motor impulsivity: failure to resist a strong impulse, drive, or temptation
  - cognitive impulsivity: choice without consideration of alternatives and/or consequences

- **High level of impulsive behaviour is a clinically significant symptom in a range of psychiatric disorders, characterized by inappropriate inhibitory control:**
  - attention-deficit/hyperactivity disorder (ADHD)
  - obsessive-compulsive disorders
  - substance abuse
  - pathological gambling

Brief overview of the pathology

Attention-deficit/hyperactivity disorder (ADHD)

- the most common neurobehavioural disorder in children and adolescents
- estimated prevalence is 4-7% in the USA and 1-3% in Europe
- up to 4 times more frequent in males than in females
- highly comorbid with a range of other neuropsychiatric disorders
- in approximately 80% of children with ADHD, symptoms persist into adolescence; in about 30-50% of these cases, symptoms may even persist into adulthood

ADHD children

- impaired attention
- impulsivity
- excessive motor activity
- poor school performance
- difficulties with peers
- low self-esteem due to repeated failures

ADHD adolescents and adults

- impaired attention
- impulsivity
- risk proneness
- pathological gambling
- abuse of alcohol and drugs
- antisocial personality

Brief overview of the pathology

Pathological gambling (PG)

- PG affects 0.2-5.3% of adults worldwide
- gambling is becoming a problem also among adolescents
- 3.5-8.0% of adolescents meet indeed the criteria for PG
- highly comorbid with a range of other disorders and substance abuse
- PG in DSM-III, DSM-IV and DSM-V: from *"Impulse-Control Disorders Not Elsewhere Classified"* to *"Substance-Related Disorders"*
- PG as a "no substance addiction": tolerance, withdrawal, loss of control

Psychological symptoms

- mood alterations
- self-esteem alterations
- increased impulsivity
- superstitious behaviours

Physiological symptoms

- anxiety related physical symptoms
- insomnia
- headache
- heart attacks (due to the stress and overexcitement of gambling)

Impact on society

- financial, social, and legal problems (including bankruptcy, divorce, job loss, imprisonment)
- suicide attempts
- substance abuse

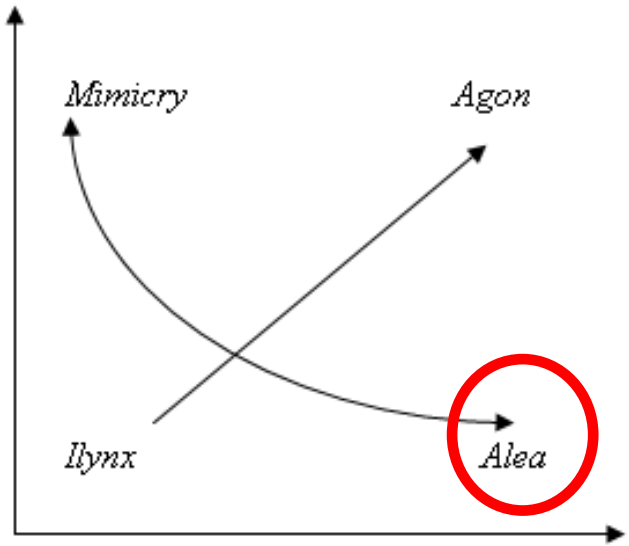
Gambling proneness

❖ A definition of gambling

◆ Betting money or other equivalent goods upon the future outcome of an event which presents a degree of uncertainty, with a view to winning a prize; winning is mainly (or exclusively) due to chance and not much (or not at all) to individual abilities.

e.g. mimesis or role playing

competition e.g. chess



vertigo e.g. roller coasters

chance e.g. slot machine

Games combine these elements in various ways:

◆ *Poker* = *Alea* + *Agon*

◆ *Russian roulette* = *Alea* + *Ilynx*

Assessment of impulsivity

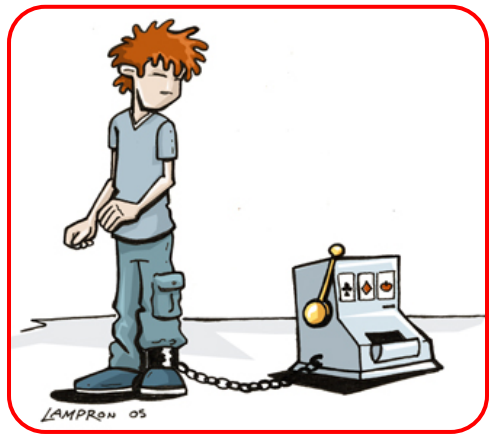
- **Personality tests and reports:**
  - Barratt Impulsiveness Scale (BIS)
  - Eysenck Impulsiveness Scale (EIS)
  - UPPS Impulsive Behaviour Scale

- **Behavioural paradigms (in both clinical and experimental settings, including animal models):**
  - Marshmallow Test (only humans) } delay of gratification
  - Delay Discounting } delay of gratification
  - Go/No-Go Task } response inhibition
  - Stop-Signal Reaction Time (SSRT) Task } response inhibition
  - Differential Reinforcement of Low Response Rate Task (DRL) } response inhibition

Assessment of gambling proneness

- **Personality tests and reports:**
  - South Oaks Gambling Screen (SOGS)
  - South Oaks Gambling Screen Revised for Adolescents (SOGSRA)
  - Gambling Attitudes and Beliefs Survey (GABS)
  - Canadian Problem Gambling Index (CPGI)

- **Behavioural paradigms (in both clinical and experimental settings, including animal models):**
  - Iowa Gambling Task (IGT)
  - Balloon Analogue Risk Task (BART) (only humans)
  - Probability Discounting Task (PDT)



Cerebral areas involved in impulsivity and gambling proneness

❖ Prefrontal cortex

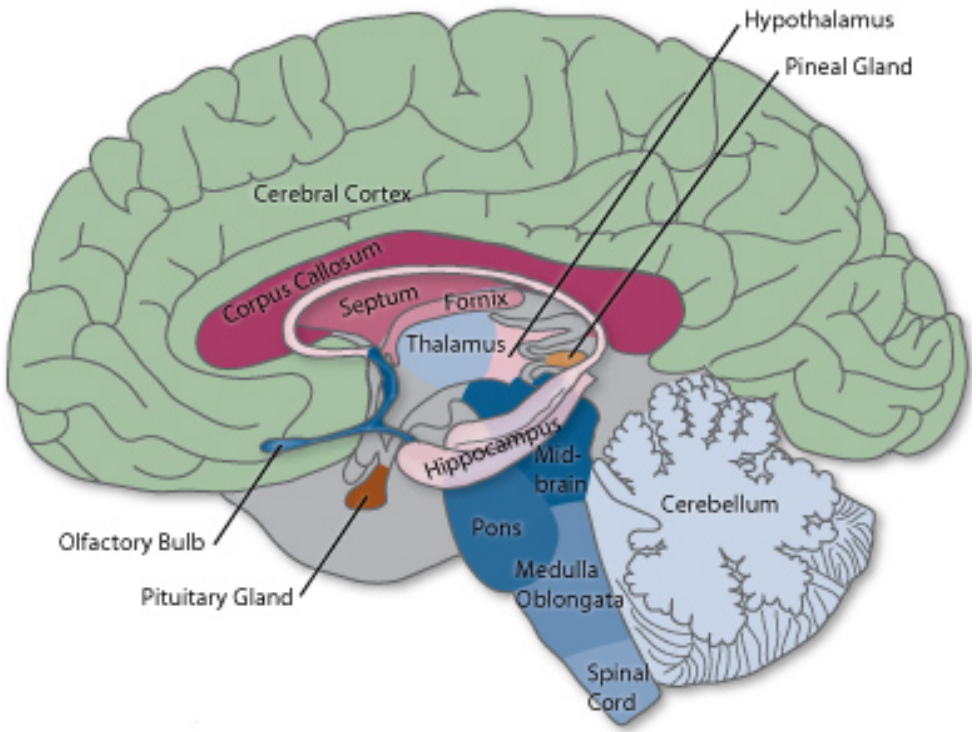
- elaboration and actuation of more evolved behavioural responses
- active inhibition of subcortical responses

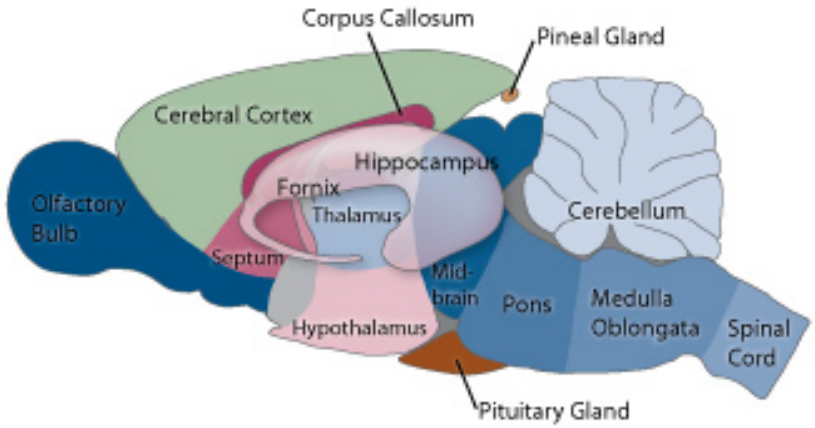
❖ Dorsal striatum

- learning of novel procedures and strategies
- formation and establishment of new habits
- innate/acquired automatic behaviours

❖ Ventral striatum (nucleus accumbens)

- reaction (attraction/avoidance) to reward-related, salient cues
- subcortical evaluation of reward value
- sustaining the efforts towards reward in goal-directed behaviour





Cerebral areas involved in impulsivity and gambling proneness

❖ Prefrontal cortex

- involved in resolution of conflicting decisions through planning, feedback regulation, and inhibition of competing behaviour *(Dalley et al. 2004)*
- subserves cognitive attention to both stimulus features and task contingencies, supporting attentional shifts and cognitive behavioural flexibility

❖ Dorsal striatum

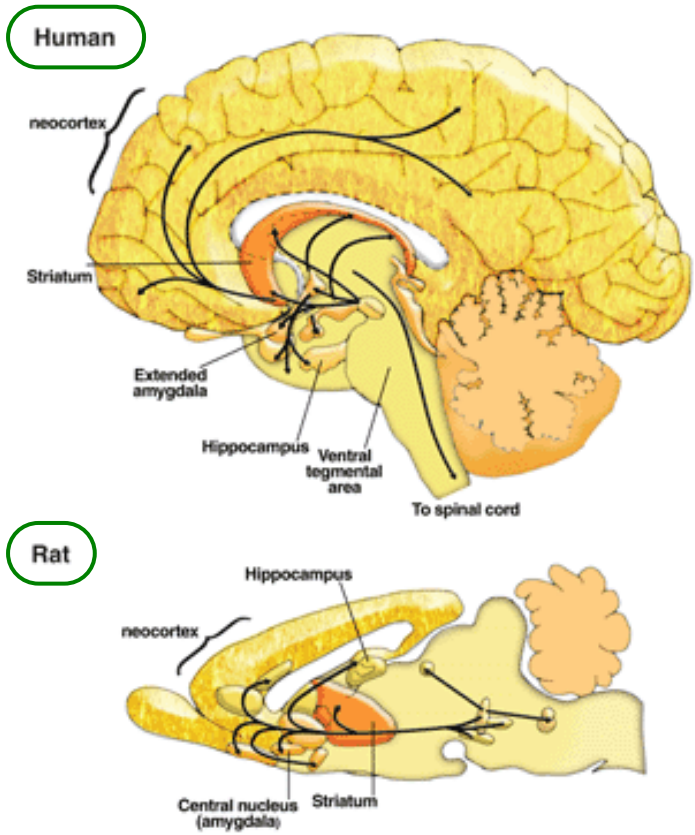
- classically thought to subserve innate or acquired, habit-based behaviour
- while the dorso-lateral striatum is involved in habit formation and expression, dorso-medial striatum plays a crucial role in supporting behavioural flexibility, hence allowing subjects to behave differently from what suggested by their instinct in the first instance *(Ragozzino 2003; Yin et al. 2004)*

❖ Ventral striatum

- consists of the nucleus accumbens (NAcc) and the olfactory tubercle
- involved in reinforcement processes: it does account for the affective evaluation of outcome features, and for feedback modulation of future choice *(Cardinal et al. 2004; Christakou et al. 2004)*
- the NAcc plays a pivotal role in making efforts towards goals, determining the maximal affordable effort *(Salamone et al. 2005; Salamone et al. 2007)*

Neurochemical systems

Dopaminergic system

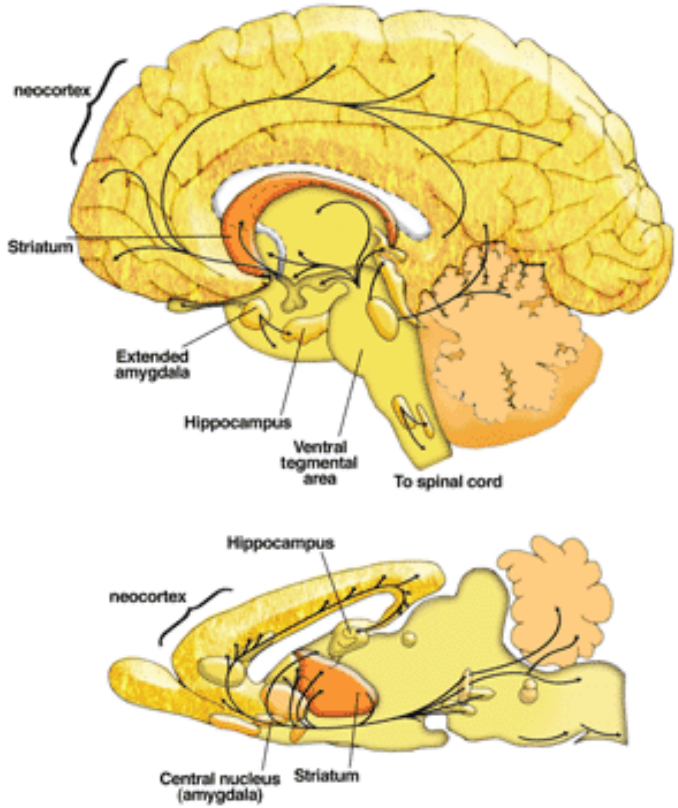


Neurotransmitter: Dopamine

Origin: Ventral tegmentum; Substantia nigra

Target: Nucleus accumbens; Dorsal striatum

Serotonergic system

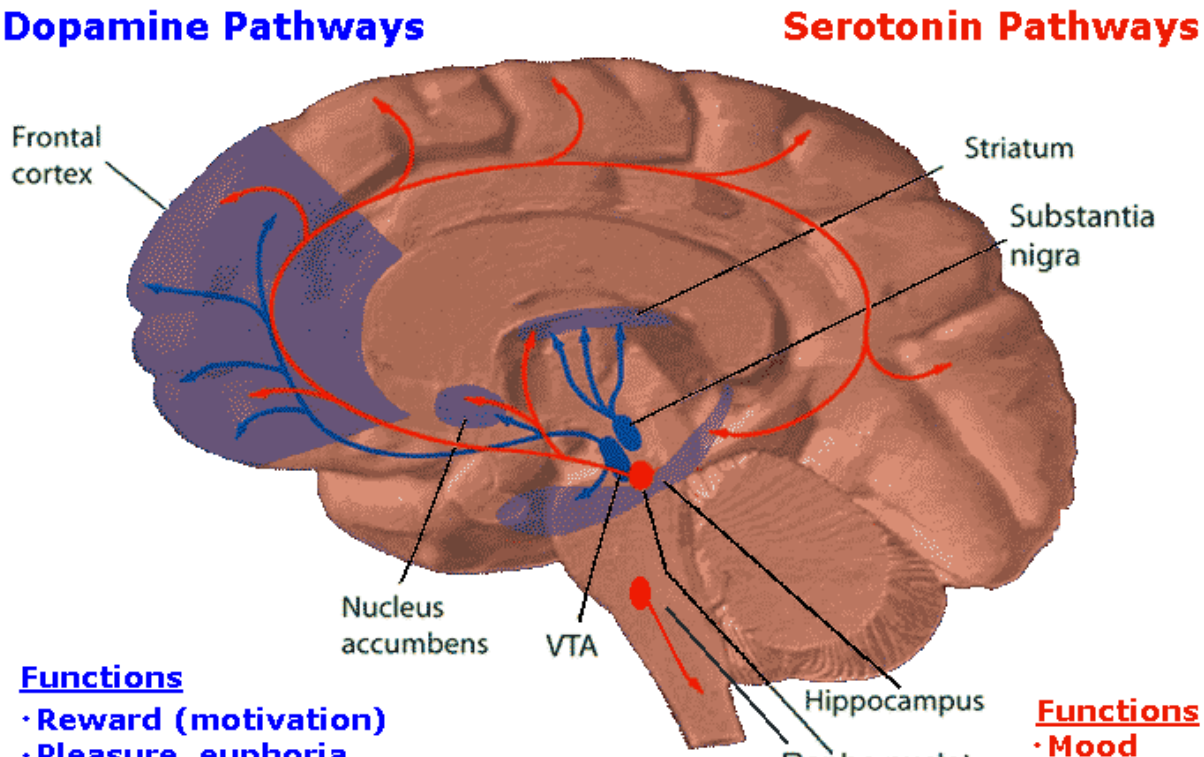


Neurotransmitter: Serotonin

Origin: Raphe nuclei

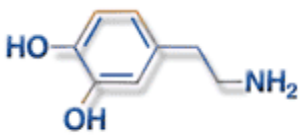
Target: Prefrontal cortex

Neurochemical systems



Functions
- Reward (motivation)
- Pleasure, euphoria
- Motor function (fine tuning)
- Compulsion
- Perseveration

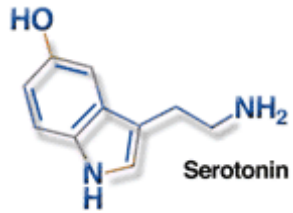
Functions
- Mood
- Memory processing
- Sleep
- Cognition



Dopamine

Neurotransmitter: **Dopamine**

Neurotransmitter: **Serotonin**

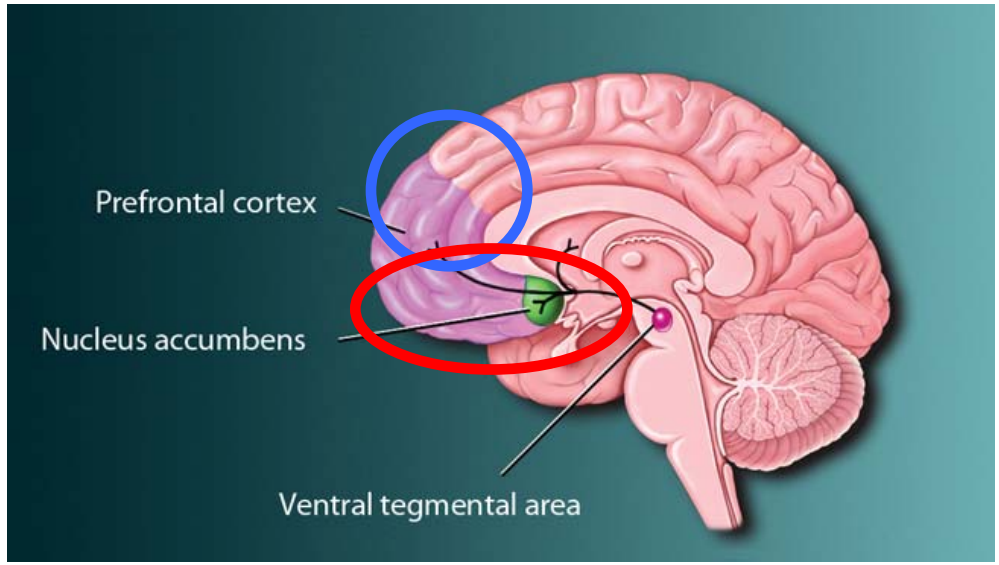


Serotonin

The reward system in impulse control disorders

Reward and reinforcement systems

- the circuit includes the dopamine-containing neurons of the ventral tegmental area (VTA), which projects to the nucleus accumbens and to part of the prefrontal cortex
- this major neurochemical pathway is termed mesolimbic system, paralleled by the meso-cortical branch - i.e., meso-cortico-limbic!
- another system, which contributes to the reinforcement, is the nigro-striatal pathway



Altered reward perception vs impaired reinforcement sensitivity

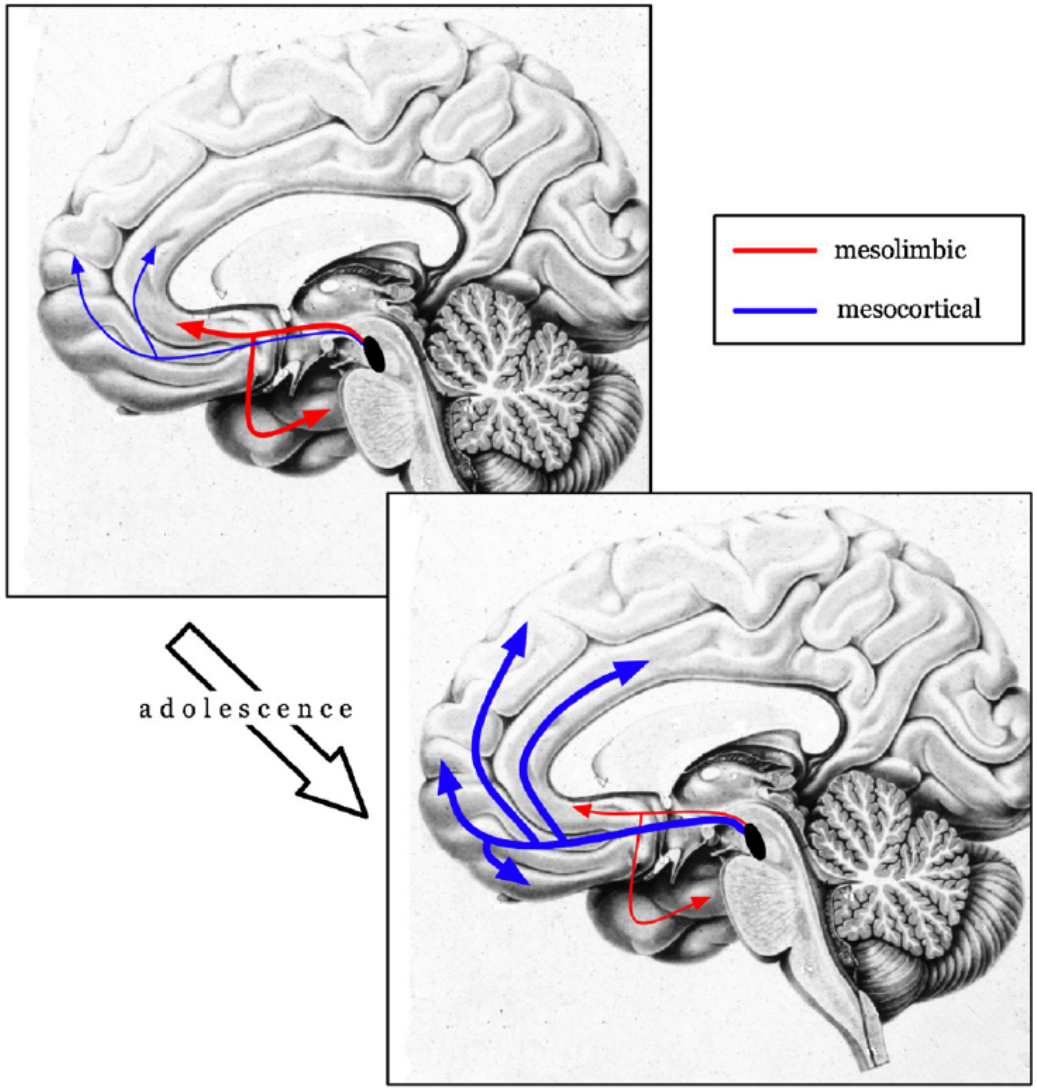
- limbic cortex (medial-prefrontal and orbitofrontal) and ventral-striatal circuitry (nucleus accumbens) subserve motivational processes (e.g. goal-directed behaviour, “appetitive” drives)
- dorsomedial and dorsolateral cortico-striatal circuitry subserve semi-automatic executive processes
- both pathways are driven by dopamine activity

The reward system during adolescence

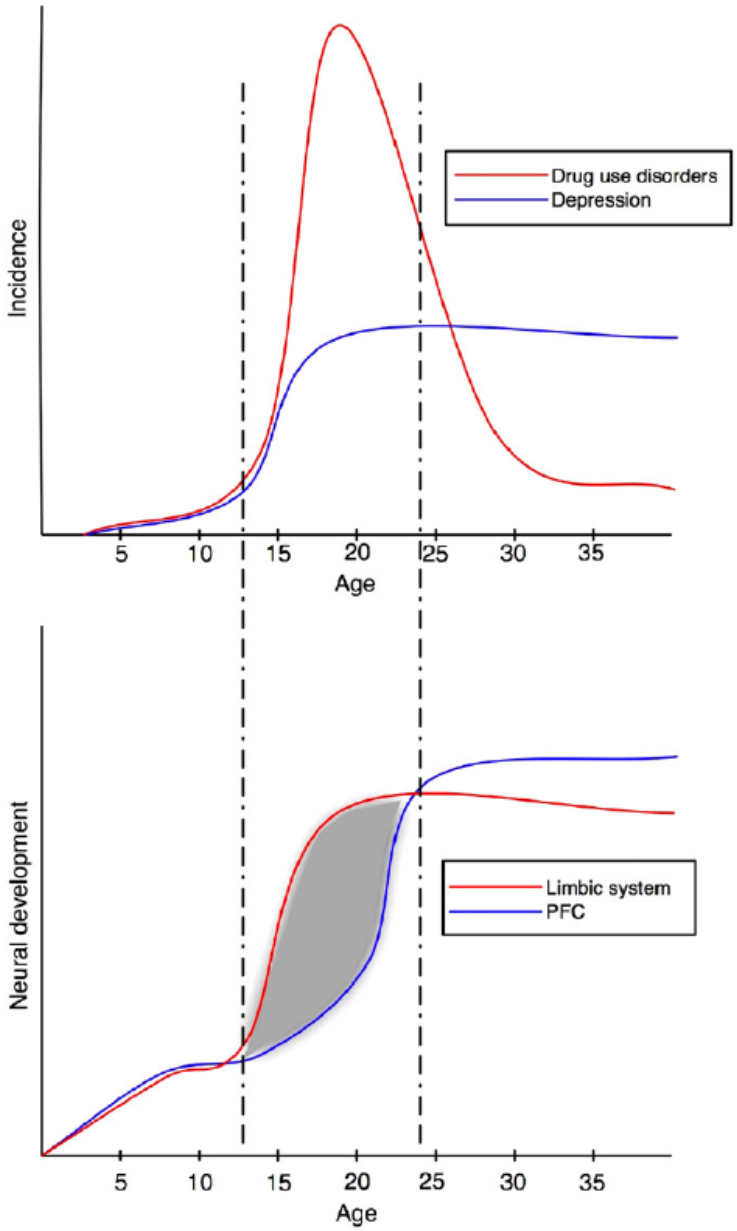
The mesolimbic and mesocortical dopaminergic projections from VTA

- increase in the number of dopamine fibers projecting to the prefrontal cortex and overproduction of dopamine receptors followed by pruning
- receptor pruning more pronounced in limbic (subcortical) than in cortical regions
- shift in the relative balance between subcortical and cortical dopaminergic systems; dominance, following adolescence, of the mesocortical dopaminergic system

(Davey et al., 2008)



Explanations for vulnerability to mental disorder in adolescence



- The top graph describes the ages of onset for depression and drug use disorders across the first four decades of life, and the bottom is a representation of the development of the limbic system and prefrontal cortex (PFC) over the same time span.
- Recent theories have implicated a mismatch between development of the limbic system and PFC as being responsible for a heightened vulnerability to mental disorder in adolescence *(Davey et al., 2008)*.

INSIDE THE ADOLESCENT BRAIN

The brain undergoes two major developmental spurts, one in the womb and the second from childhood through the teen years, when the organ matures by fits and starts in a sequence that moves from the back of the brain to the front

Nerve Proliferation ...

By age 11 for girls and 12½ for boys, the neurons in the front of the brain have formed thousands of new connections. Over the next few years, most of these links will be pruned

... and Pruning

Those that are used and reinforced—the pathways involved in language, for example—will be strengthened, while the ones that aren't used will die out

Corpus Callosum

Thought to be involved in problem solving and creativity, this **bundle of nerve fibers** connects the left and right hemispheres of the brain. During adolescence, the nerve fibers thicken and process information more and more efficiently

Prefrontal Cortex

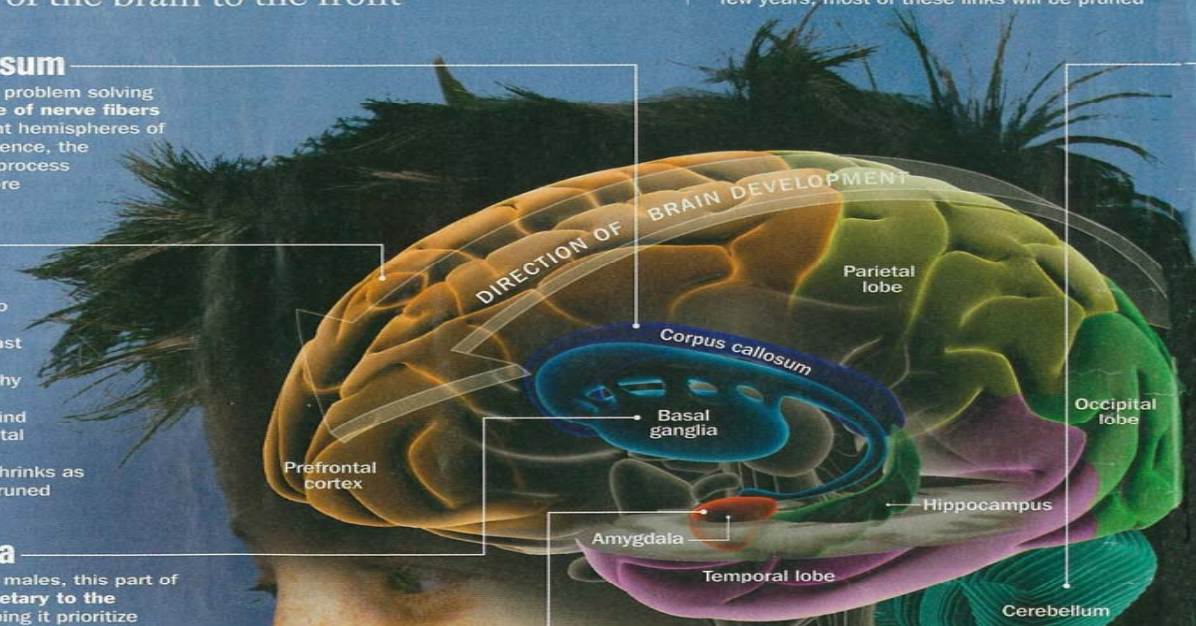
The CEO of the brain, also called the **area of sober second thought,** is the last part of the brain to mature—which may be why teens get into so much trouble. Located just behind the forehead, the prefrontal cortex grows during the preteen years and then shrinks as neural connections are pruned during adolescence

Basal Ganglia

Larger in females than in males, this part of the brain acts like a **secretary to the prefrontal cortex** by helping it prioritize information. The basal ganglia and prefrontal cortex are tightly connected: at nearly the same time, they grow neuron connections and then prune them. This area of the brain is also active in **small and large motor movements,** so it may be important to expose preteens to music and sports while it is growing

Amygdala

This is the emotional center of the brain, home to **such primal feelings as fear and rage.** In processing emotional information, teens tend to rely more heavily on the amygdala. Adults depend more on the rational prefrontal cortex, a part of the brain that is underdeveloped in teens. That may explain why adolescents often react more impulsively than adults

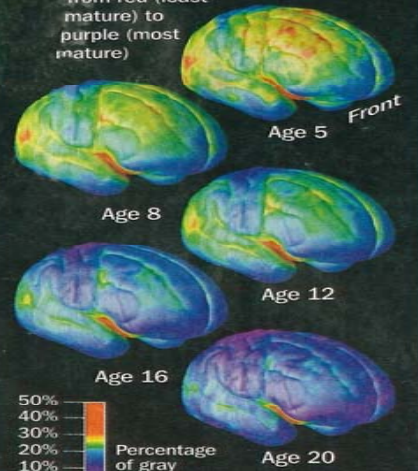


Cerebellum

Long thought to play a role in physical coordination, this area may also regulate certain thought processes. More sensitive to environment than to heredity, the cerebellum supports activities of higher learning like **mathematics, music and advanced social skills.** New research shows that it changes dramatically during adolescence, increasing the number of neurons and the complexity of their connections. The cerebellum is the only part of the brain that continues growing well into the early 20s

Time-Lapse Brain

Gray matter wanes as the brain matures. Here, 15 years of brain development are compressed into five images showing a shift from red (least mature) to purple (most mature)

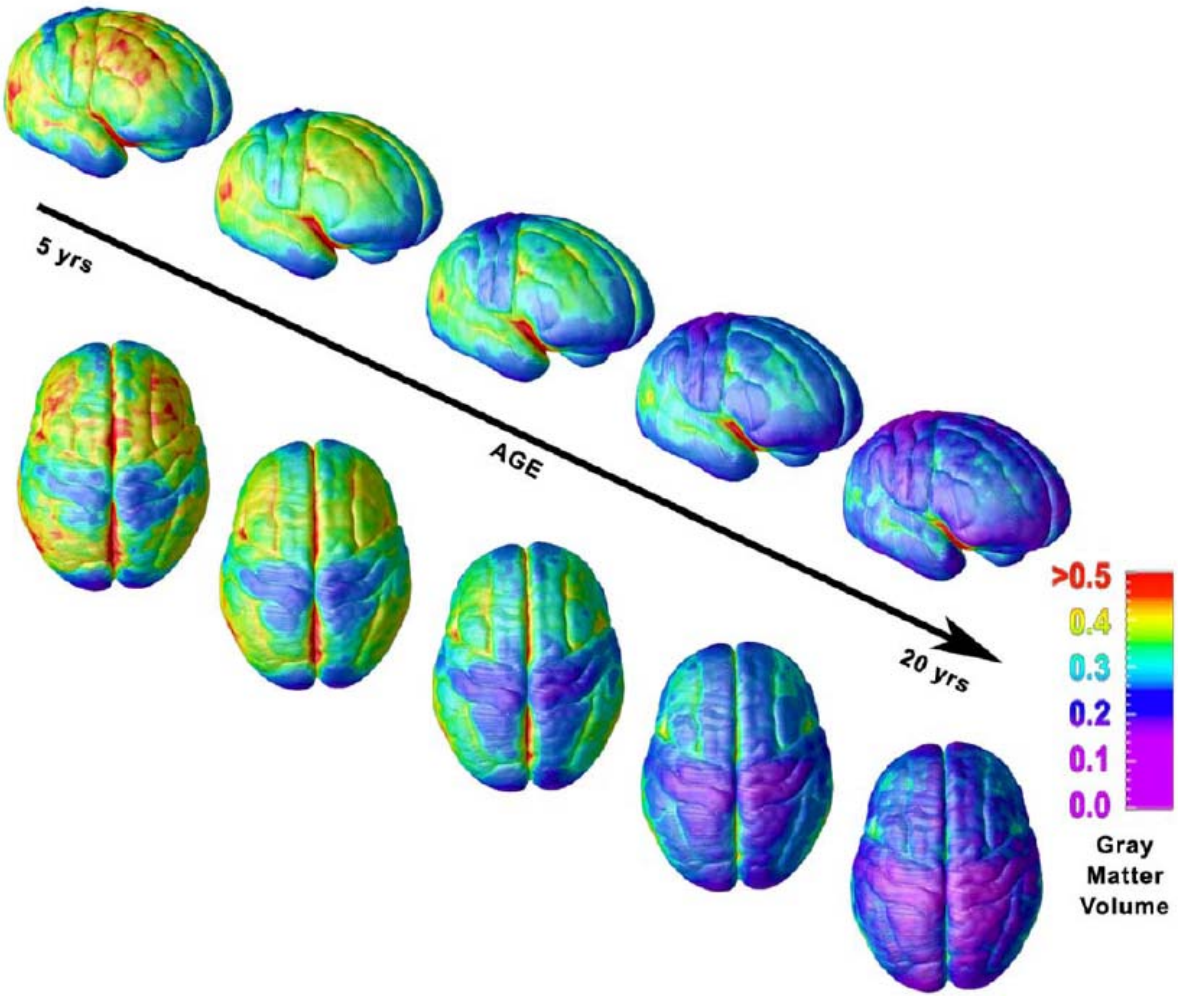


Sources: Dr. Jay Giedd, chief of brain imaging, child psychiatry branch, NIMH; Paul Thompson, Andrew Lee, Kiralee Hayashi and Arthur Toga, UCLA Lab of Neuro Imaging; Nitin Gogtay and Judy Rapoport, child psychiatry branch, NIMH

TIME Diagram by Joe Lertola; text by Kristina Dell

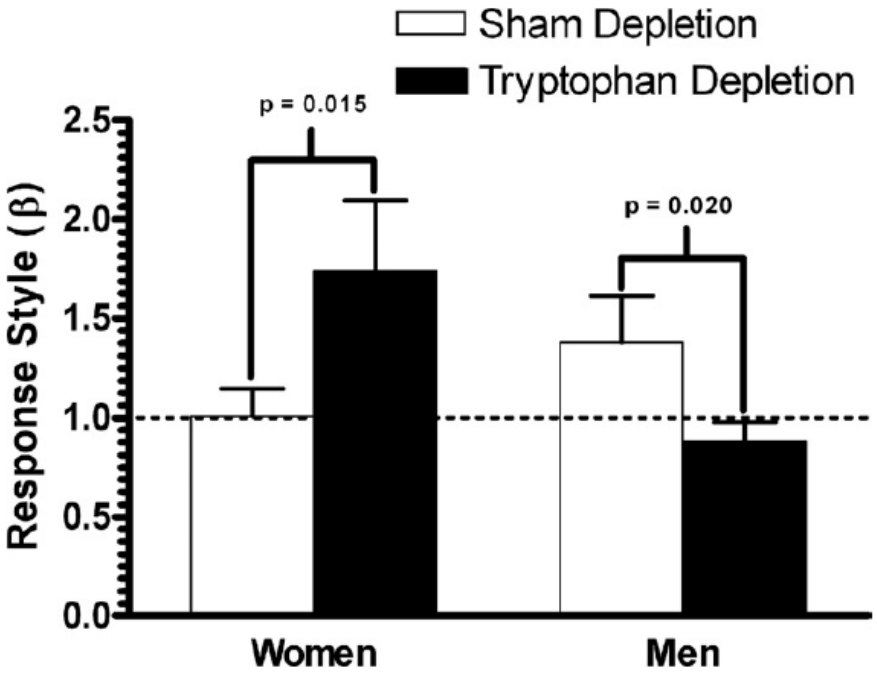
THE IMAGE BANK—GETTY IMAGES

Age differences in humans: anatomical MRI



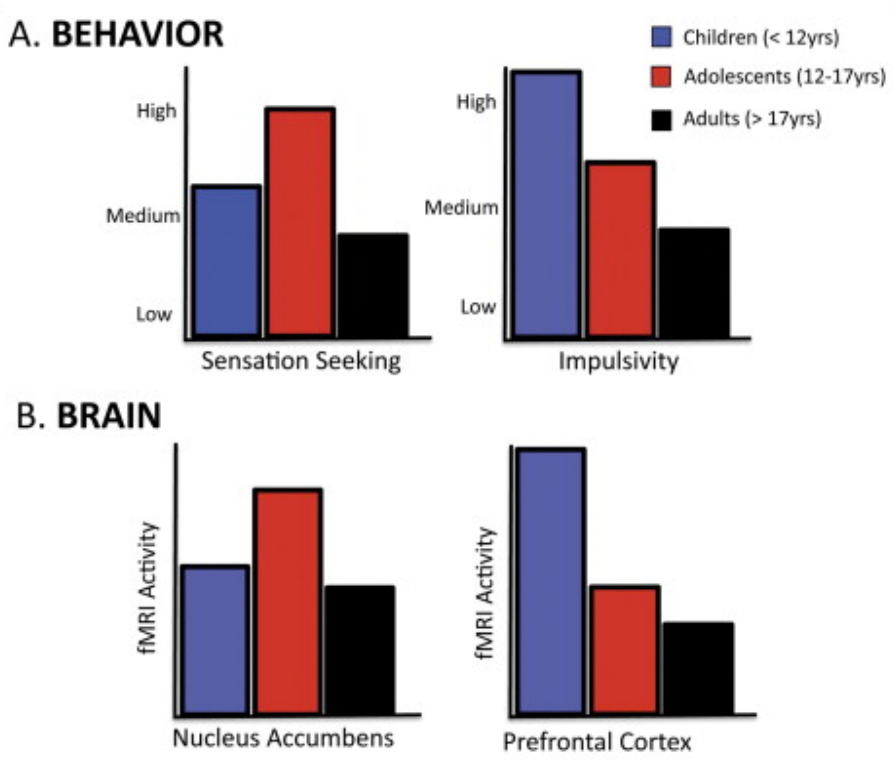
The developmental trajectory of cortical gray matter followed a regionally specific pattern. Gray matters wanes as the brain matures. Here, 15 years of brain development are compressed into five images showing a shift from red = least mature to purple = most mature *(Lenroot & Giedd, 2006)*.

Sex and age differences in impulsivity in humans



The effects of acute tryptophan depletion (ATD) and sham depletion on response style in women and men.

A response style score < 1 indicates an impulsive response style, > 1 indicates a cautious response style *(Walderhaug et al., 2007)*.



(A) Plot of sensation-seeking and impulsivity as a function of age. **(B)** Plot of patterns of activity in two key forebrain regions, sensitive to reward outcomes during a cognitive control task across development *(Casey & Jones, 2010)*.

Age and sex differences in gambling proneness in humans

Table 1 Descriptive statistics for young adults aged 15–24 years

	Age 15–24							
	No-problem gambling ($n = 921$)		Low-risk gambling ($n = 239$)		Moderate-risk or problem gambling ($n = 81$)		Chi-squared or Fisher's exact test	
	n	%	*n*	%	*n*	%	*P*-value	
Gender								
Men	475	51.6	152	63.6	70	86.4	***	$P < 0.001$
Women	445	48.4	87	36.4	11	13.6		

Young adults

(15-24 years)

Table 2 Descriptive statistics for adults aged 25–64 years

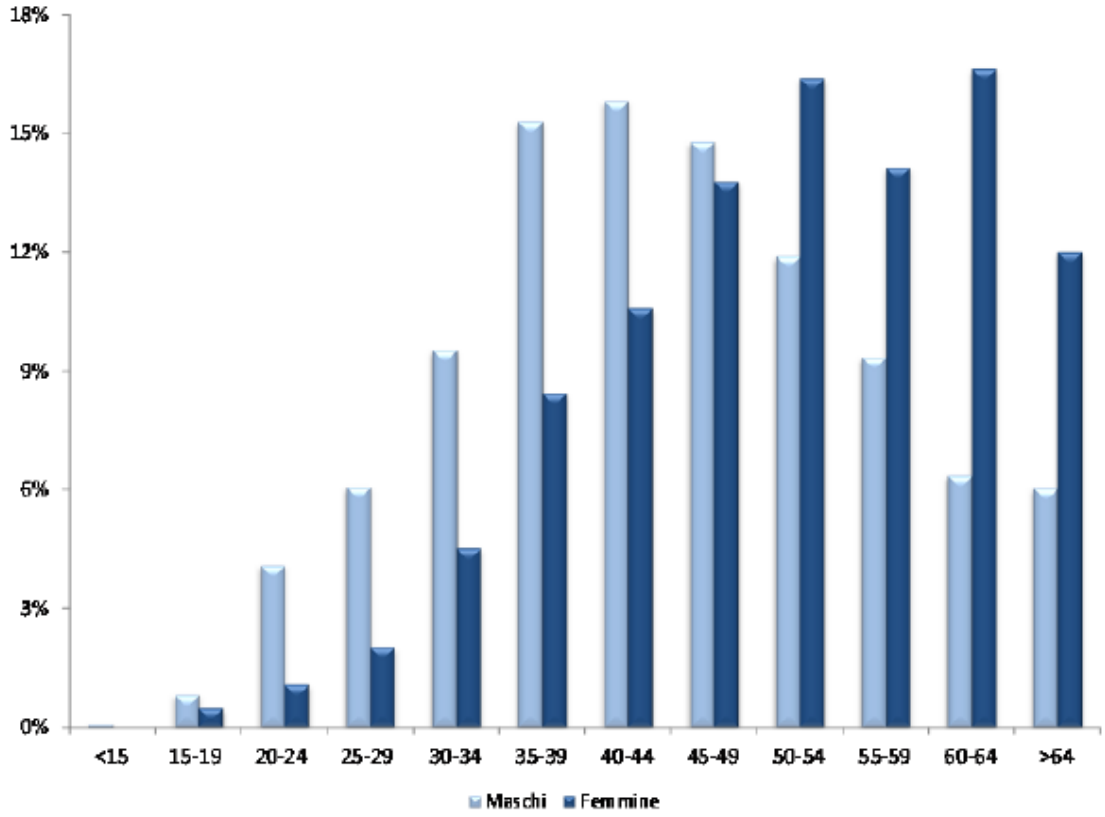
	Age 25–64							
	No-problem gambling ($n = 2683$)		Low-risk gambling ($n = 415$)		Moderate-risk or problem gambling ($n = 155$)		Chi-squared or Fisher's exact test	
	n	%	*n*	%	*n*	%	*P*-value	
Gender								
Men	1359	50.8	278	67.1	113	73.4	***	$P < 0.001$
Women	1318	49.2	136	32.9	41	26.6		

Adults

(25-64 years)

A sample of 4494 gamblers was drawn from IPSAD-Italia 2007-2008 (Italian Population Survey on Alcohol and Drugs) in order to examine different gambling patterns, assessed using the Canadian Problem Gambling Index Short form scale *(Bastiani et al., 2013)*.

Age and sex differences in gambling proneness in humans



Distribution of Italian subjects who were registered for therapy against pathological gambling in 2011, according to sex and age group (a total of 4544 subjects, of which 82% were male and 18% female).

Summary

- Human adolescents are associated with patterns of temporary deviance and the expression of risky behaviours, including the search for and use of psychoactive agents.
- The delay in maturation of prefrontal cortex compared to earlier maturing sub-cortical (limbic) areas has most often been emphasised; this may help to explain the increased rates of dysregulated behaviour, especially drug use and risk taking during adolescence.

- The contribution of puberty, with all its important developmental rearrangements in neurobiological and neuroendocrinological processes, has received a growing attention in experimental investigation during the last two decades.
- It is thus emphasized the importance of characterizing novelty-seeking and risk-prone behaviour in the rodent, during adolescence and/or using specific models.

Animal models in behavioural neuroscience

Animal models

- enable the investigation of brain-behaviour relations, with the aim of gaining insight into normal and abnormal human behaviour and its underlying neuronal and neuro-endocrinological processes *(van der Staay, 2006)*
- allow to analyse these relations under controlled conditions (e.g. standardized housing and testing)

Why are animal models necessary?

- obtaining information that cannot be gained in other ways (genetic and environmental manipulations, pharmacological treatments, psychoactive agents, etc.)
- in-vitro approaches cannot model the interactions in complex systems, such as the brain-behaviour relations
- the only possibility to model behavioural disorders is to study the animal that behaves

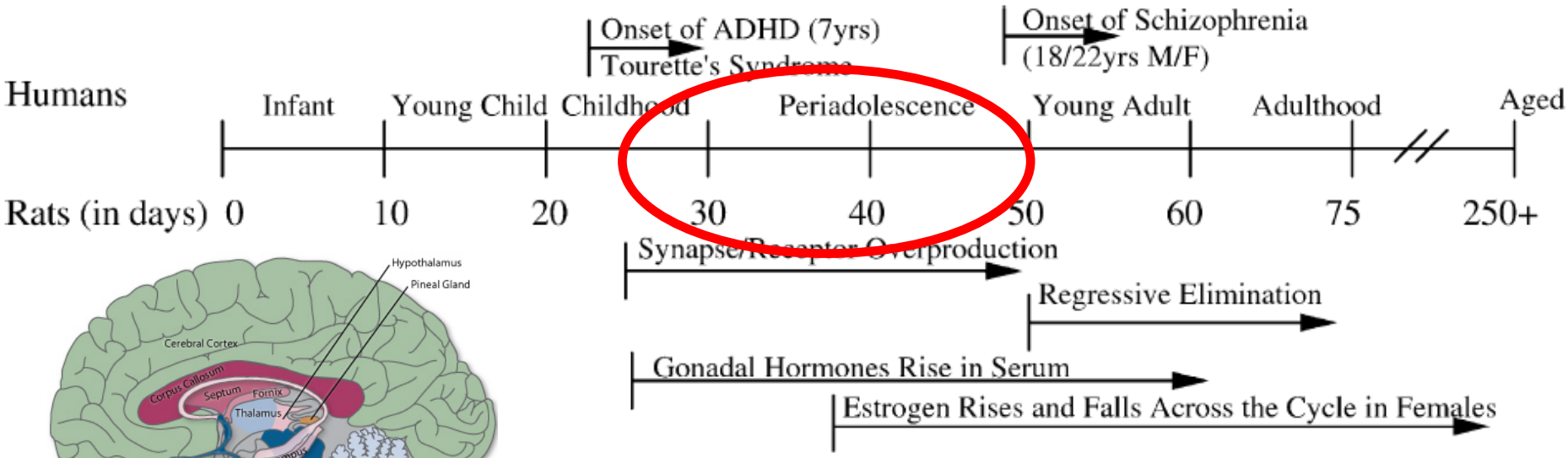
Validity of animal models

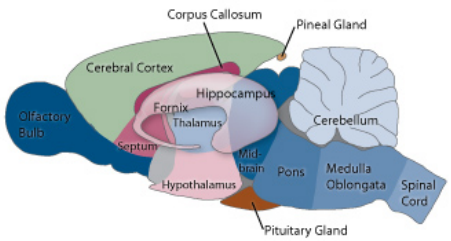
The distinction between different forms of validity is useful to identify the weaknesses and limitations of a model: poor validity determines an increase in the number of false positives and false negatives in basic research, thereby limiting the possibility of developing new therapeutic strategies *(Willner 1984, 1995, 2002; van der Staay 2006, 2009)*.

Categories of validity

- **Internal validity (reliability and replicability)** refers to the technical and methodological quality of the experimental evaluation of the animal model.
- **Face validity** is the degree of descriptive similarity between the behavioral dysfunction seen in an animal model and in humans affected by a given neurobehavioral disorder (similarity of symptoms).
- An animal model with high **predictive validity** allows extrapolation of the effect exerted by a particular experimental manipulation (e.g. therapeutic compounds) from one species to other species.
- **Construct validity** points to the degree of similarity between the mechanisms underlying behaviour in the model and those underlying behavior in the pathological condition which is being modeled.
- **External validity (generalizability)** is the extent to which the results obtained using a particular animal model can be applied to and across populations (strains, species, etc.) and lab environments.

The human versus rat development





A relative comparison of ages and stages of human versus rat development *(Andersen, 2003)*.

The study of impulsivity in animal models

- "Motor impulsivity" (impulsive action) can be evaluated with non-choice-based paradigms, which resemble the classical "Go/No-Go Task" tasks in humans.

- Assessment of "cognitive impulsivity" (impulsive choice) requires by definition a two-choice paradigm; one of the most successfully utilised is the "delay-discounting paradigm".

  → impulsive subjects are highly intolerant to situations where reward is delayed: smaller but immediate reinforcers are preferred to larger ones, which come only after a delay *(Thiebot et al., 1985; Logue, 1988; Evenden & Ryan, 1996; 1999; Bizot et al., 1999)*

- The "five-choice serial reaction time task (5CSRTT)", initially developed to study attention, also implies aspects of behavioural inhibition such as premature/perseverative responding *(Carli et al., 1983)*.

- The "unpredictable operant conditioning schedule (variable interval-15, VI-15)" for food reinforcement, allowing to measure "free" responses emitted during a variable refractory period *(Coppens et al., 2012)*.

- The "conditioned locomotor activity to food", a non-operant paradigm allowing to measure behavioural disinhibition/expectation *(Matthews et al., 1996; Winstanley et al., 2004)*.

The study of gambling proneness in animal models

- Many operant paradigms have been developed to study (in)tolerance to uncertainty and/or risk proneness *(Mobini et al., 2000; Cardinal & Howes, 2005; Adriani et al., 2006; Wilhelm & Mitchell, 2008)*.

- "Probabilistic Delivery Task (PDT)": choice between either a certain, small amount of food or larger amounts of food delivered (or not) depending on a given (and progressively decreasing) probability *(Adriani & Laviola, 2006; Adriani et al., 2006)*.

- "Iowa Gambling Task (IGT)": choice between a low probability of a large reward or a high probability of a small reward *(van den Bos et al., 2006)*.

- "Risky Decision-Making Task (RDT)": choice between a small, "safe" food reward or a larger food reward associated with the risk of punishment (e.g. footshock) *(Simon et al., 2009)*.

“Uncertainty”, “Risk”, “Loss”

“uncertainty” and “risk” are not synonymous

“risk of losing” is distinct from “failing to win”

- “risk”: a construct implying a potential for overtly adverse consequences (e.g. foot-shock)
- “risk of losing”: the resources staked on a favourable outcome are lost when a wager is unsuccessful
- “failing to win”: the absence of any additional gain, causing “frustration”

→ most paradigms of risky decision-making *(Adriani and Laviola, Cardinal and Howes, 2005; Mobini et al., 2000 van den Bos et al., 2006)* deal exclusively with “failing to win” (i.e. omission of reward or delivery of an unpalatable reward): no risk of finishing the session at a disadvantage compared with the start

→ every case of unsuccess is an “unlucky event” causing “frustration” but not necessarily a “risk”

→ while the attraction for uncertain reward may resemble the features of a “gambling proneness”, it is not necessarily fitting with the construct of “risk proneness”

The study of impulsivity and gambling proneness in our lab

- operant paradigms for the study of
  - impulsivity: **Intolerance to Delay (ID) task**
  - gambling proneness: **Probabilistic Delivery (PD) task**

- classical operant cages (Skinner-box) or operant panels inside standard home-cages

❖ **Classical operant cages (Skinner-box)**

❖ **Operant panels inside standard home-cages**

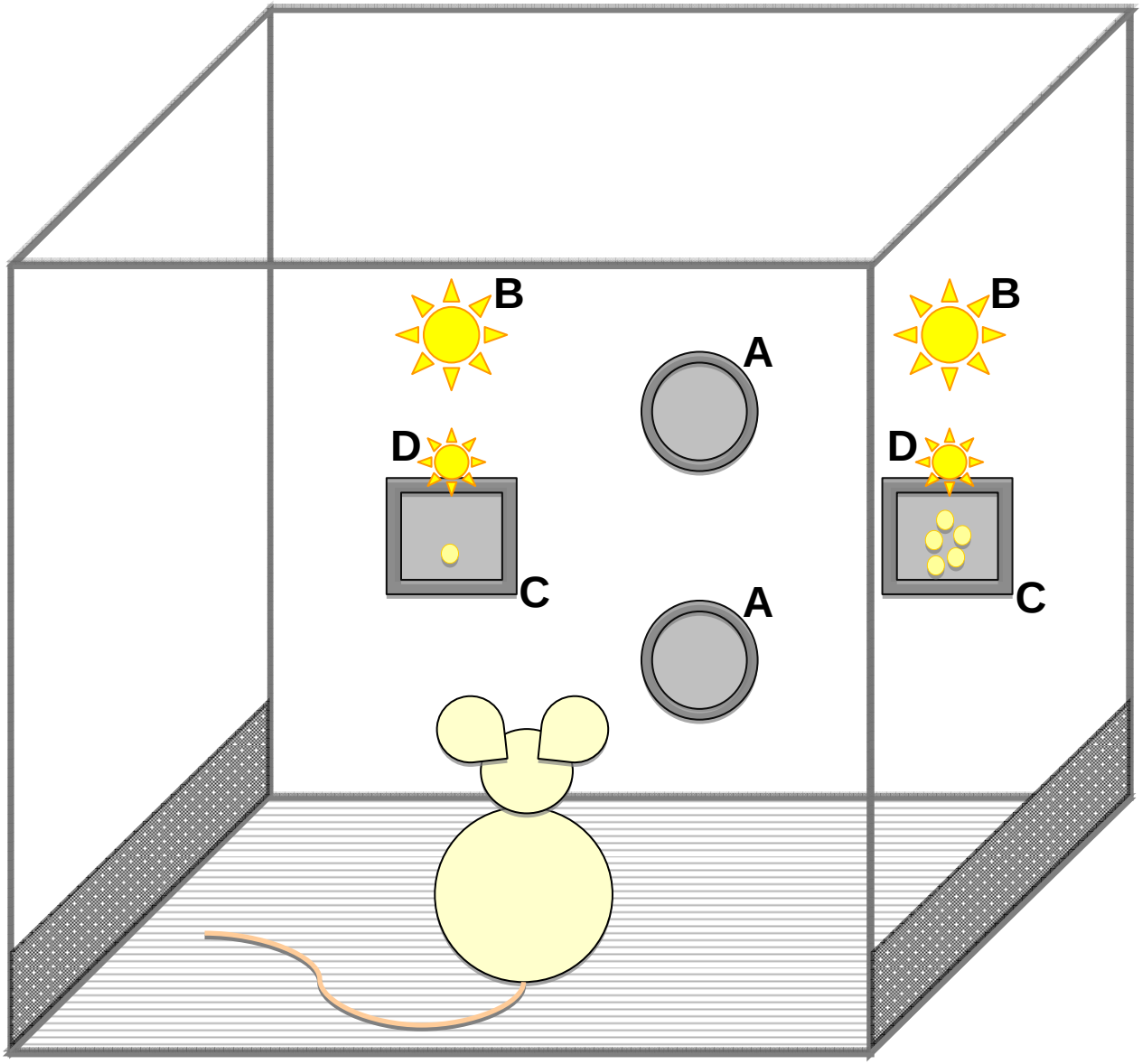
Schematic representation of an apparatus

A: nose-poking hole

B: chamber light, signalling:
- duration of delay (ID task)
- flash (4 s) between nose-poke and reinforcer delivery (PD task)

C: feeding magazine

D: magazine light, signalling the duration of **timeout (TO)**



Timeout (TO): the brief period (about 30 s) following food delivery during which nose-poking is recorded but is without scheduled consequences (i.e. inadequate nose-pokes)

Paradigm for the measure of impulsivity

Intolerance to Delay (ID) task

→ impulsivity

nose-poking in "Small & Soon" hole **(SS)**

⇩

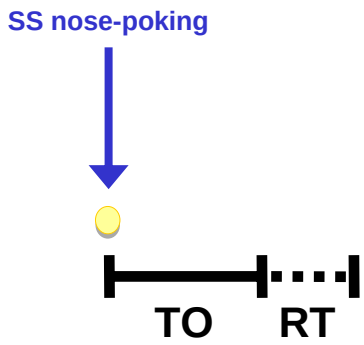
immediate delivery of 1-2 pellets

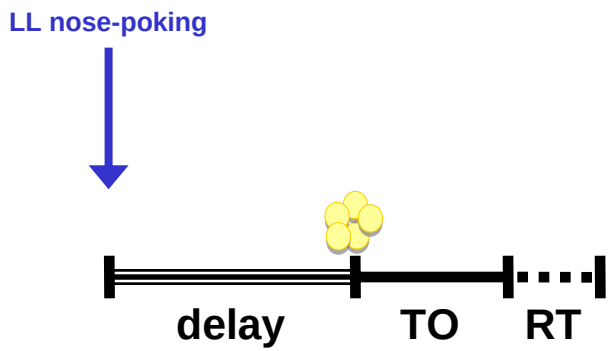
nose-poking in "Large & Late" hole **(LL)**

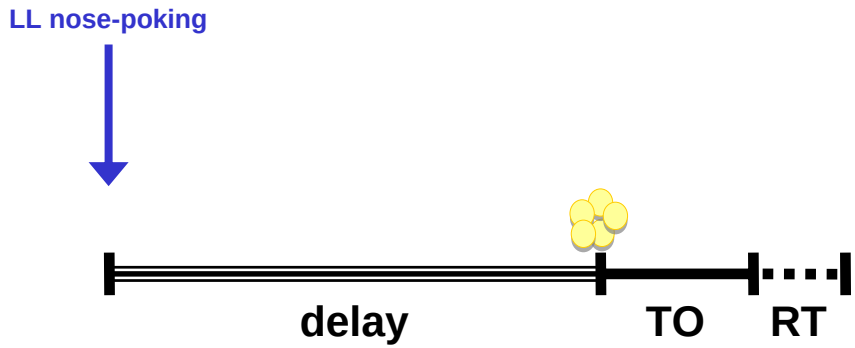
⇩

delivery of 5-6 pellets after a delay, which is increased progressively each day

8 testing sessions, from delay 0 s to 90 s
(preceded by 3 training sessions at delay 0 s)

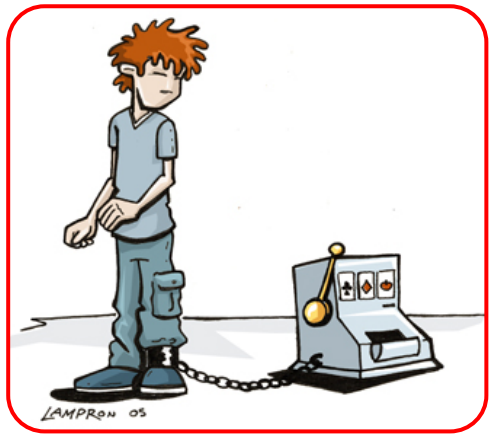






TO: timeout
RT: response time

Paradigm for the measure of gambling proneness



Probabilistic Delivery (PD) task

→ gambling proneness

nose-poking in "Small & Sure" hole **(SS)**

⇩

certain delivery of 1-2 pellets

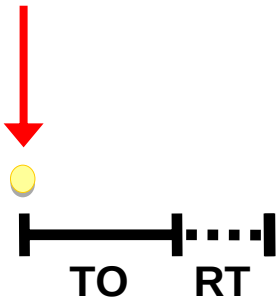
nose-poking in "Large & Luck-Linked" hole **(LLL)**

⇩

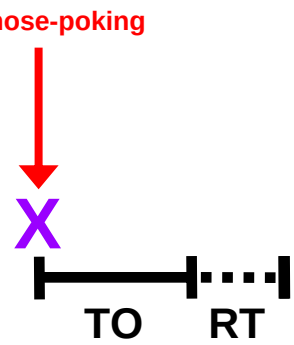
delivery (or not) of 5-6 pellets, according to the level of probability "p", which is decreased progressively each day

11 testing sessions, from probability 99% to 9%
(preceded by 2 training sessions at probability 99%)

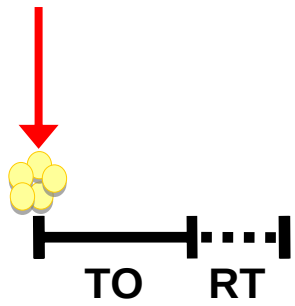
SS nose-poking



LLL nose-poking



LLL nose-poking



TO: timeout
RT: response time

The indifferent point

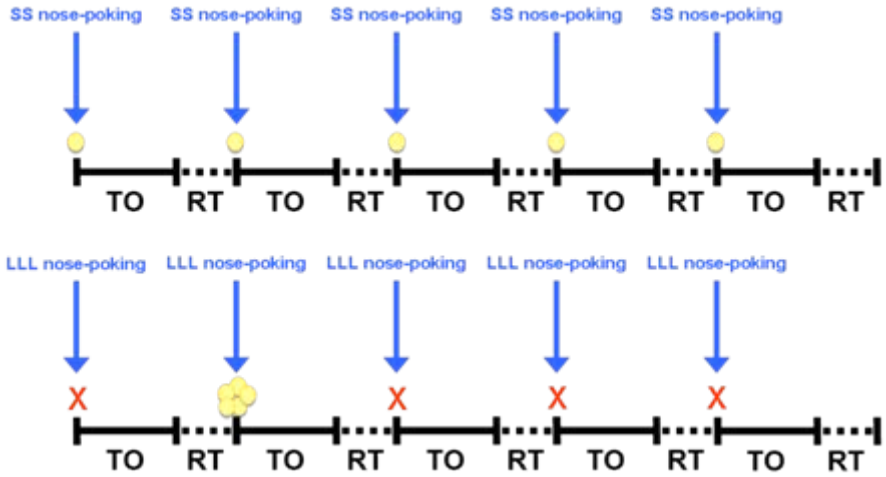
Intolerance to Delay (ID) task

either choice mathematically identical in terms of total foraging

TO: timeout

RT: response time

Probabilistic Delivery (PD) task



Sex differences in impulsivity

Behavioural Brain Research 200 (2009) 134–143

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Gender differences in delay-discounting under mild food restriction

Susanne Koot[a,b], Ruud van den Bos[a,*], Walter Adriani[b], Giovanni Laviola[b]

Environmental Health Perspectives • VOLUME 111 | NUMBER 4 | April 2003

Research | Articles

Altered Profiles of Spontaneous Novelty Seeking, Impulsive Behavior, and Response to D-Amphetamine in Rats Perinatally Exposed to Bisphenol A

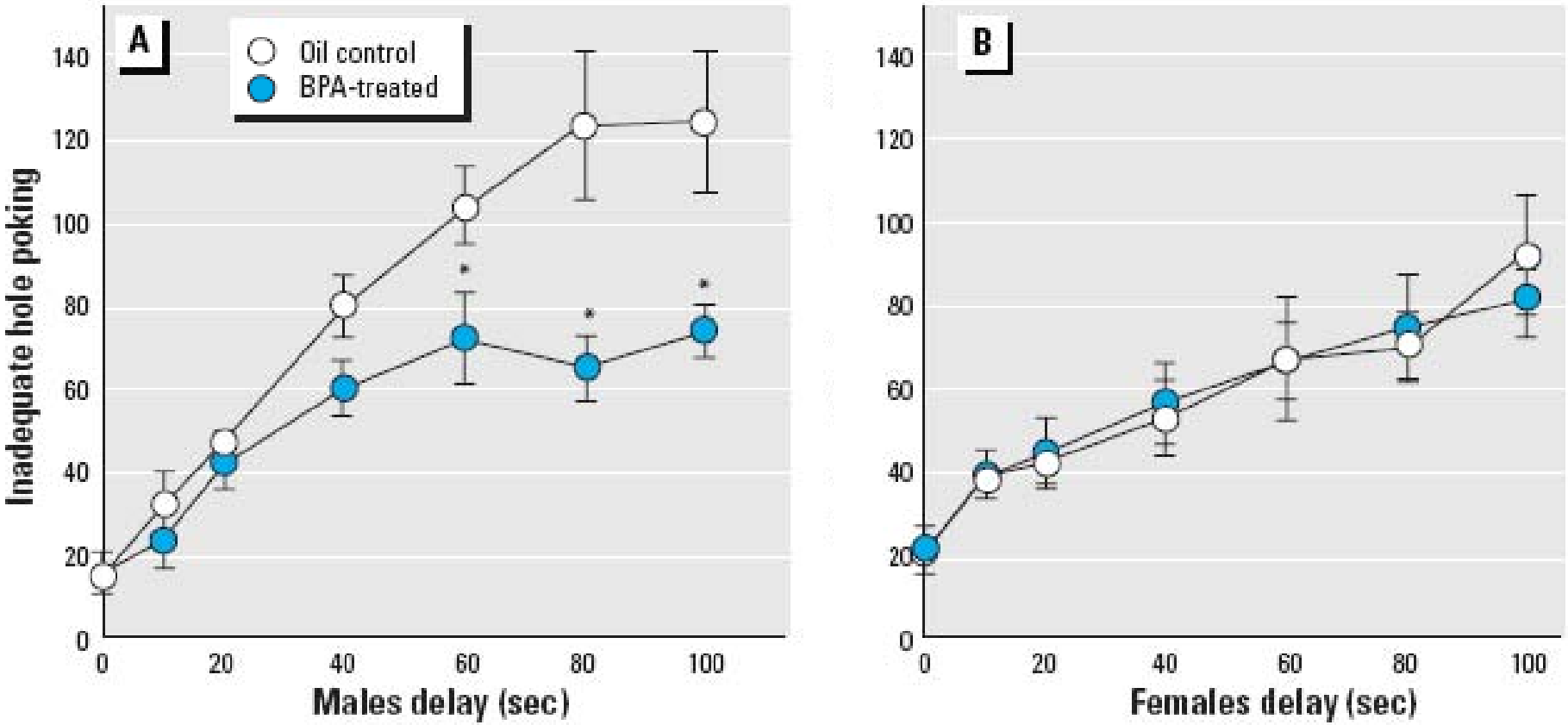
Walter Adriani,[1] Daniele Della Seta,[2] Francesco Dessì-Fulgheri,[3] Francesca Farabollini,[2] and Giovanni Laviola[1]

Environmental Health Perspectives • VOLUME 111 | NUMBER 4 | April 2003

Altered Profiles of Spontaneous Novelty Seeking, Impulsive Behavior, and Response to D-Amphetamine in Rats Perinatally Exposed to Bisphenol A

Walter Adriani,[1] Daniele Della Seta,[2] Francesco Dessì-Fulgheri,[3] Francesca Farabollini,[2] and Giovanni Laviola[1]

Levels of motor impulsivity



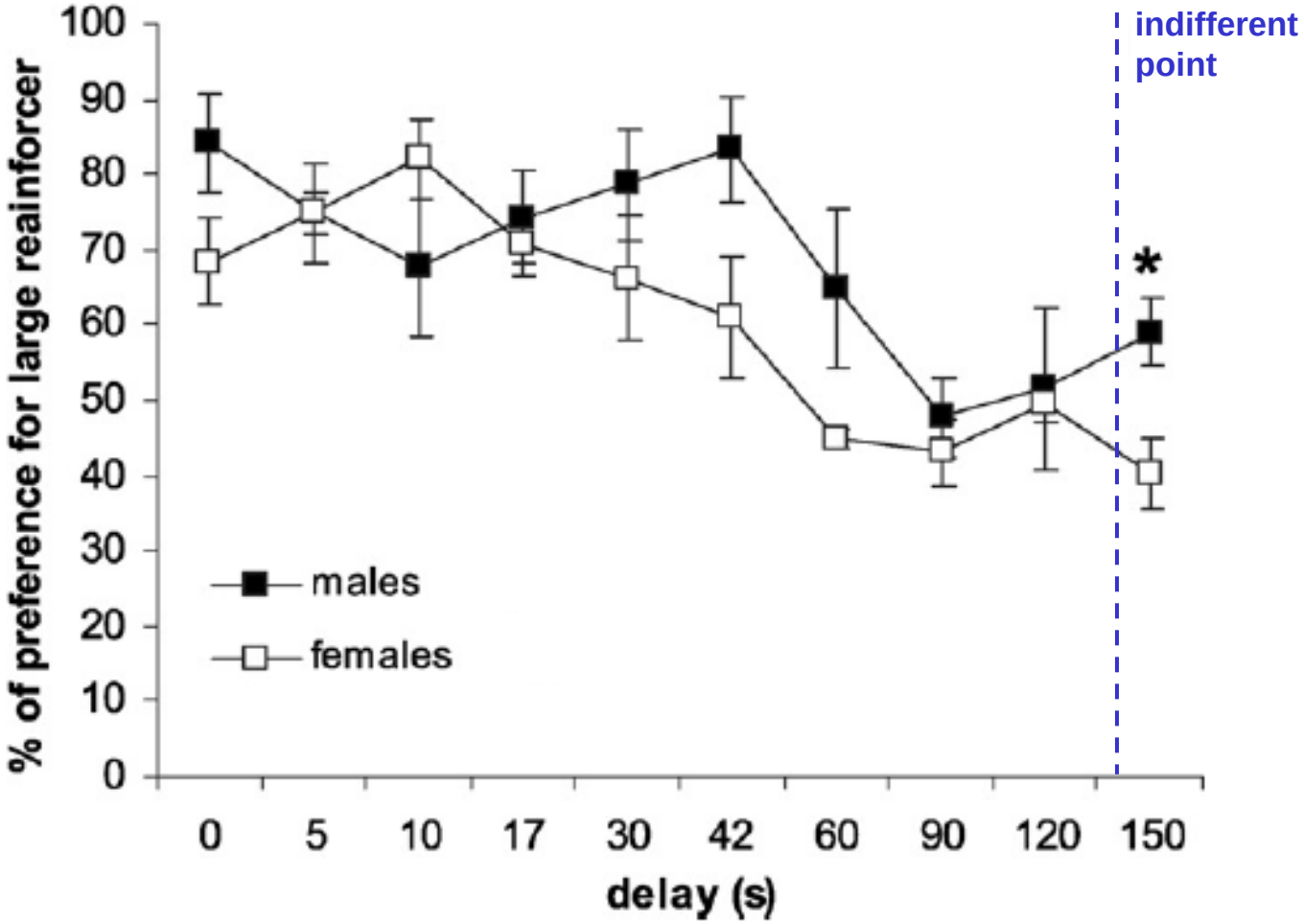
Oil control males are less able than females to inhibit nose poking behaviour during the delay. Interestingly, the profile shown by BPA-treated males is comparable with that expressed by females, suggesting a demasculinization for this measure *(Adriani et al., 2003)*.

Behavioural Brain Research 200 (2009) 134–143

Gender differences in delay-discounting under mild food restriction

Susanne Koot[a,b], Ruud van den Bos[a,*], Walter Adriani[b], Giovanni Laviola[b]

Levels of cognitive impulsivity



Female mice shift towards the economically advantageous option when delays increase earlier than male mice do *(Koot et al., 2009)*.

Sex differences in gambling proneness

Behavioural Brain Research 234 (2012) 375–379

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Short communication

Male and female Wistar rats differ in decision-making performance in a rodent version of the Iowa Gambling Task

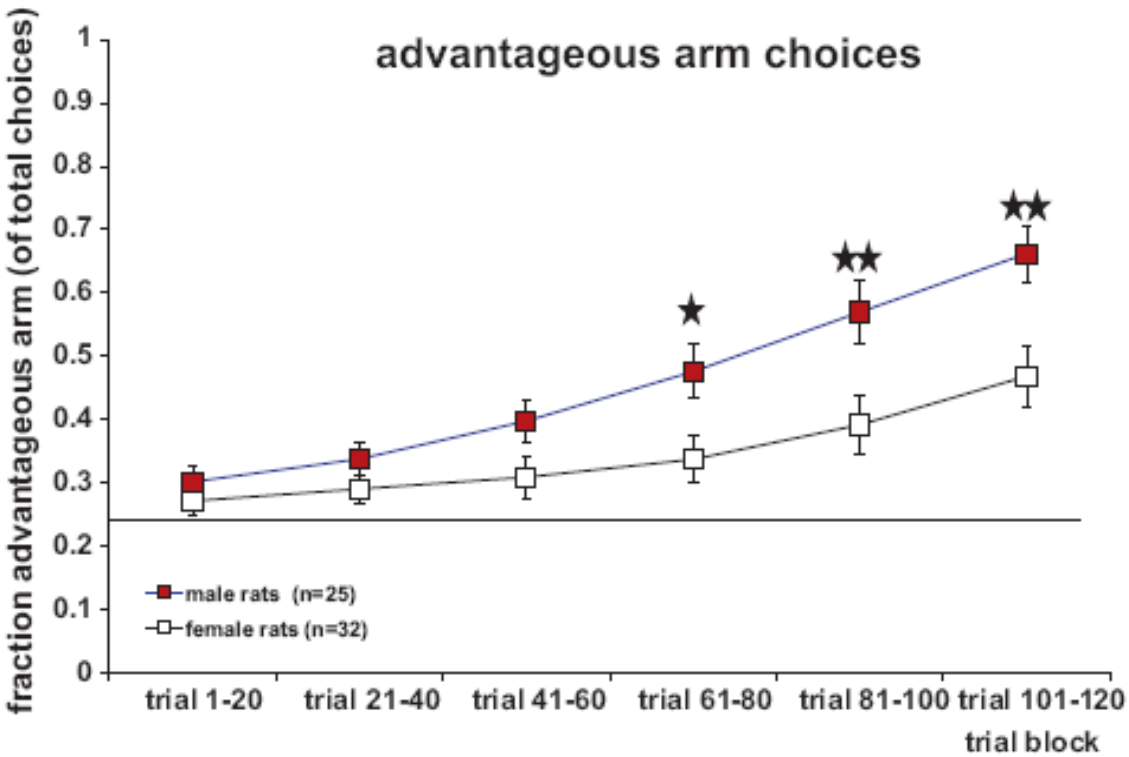
Ruud van den Bos[a,b,*], Jolle Jolles[a,c], Lisette van der Knaap[a], Annemarie Baars[a,d], Leonie de Visser[a,b]

Behavioural Brain Research 234 (2012) 375–379

Male and female Wistar rats differ in decision-making performance in a rodent version of the Iowa Gambling Task

Ruud van den Bos[a,b,*], Jolle Jolles[a,c], Lisette van der Knaap[a], Annemarie Baars[a,d], Leonie de Visser[a,b]

Decision-making performance



As the task progresses male subjects more rapidly chose the best long-term option than female subjects *(van den Bos et al., 2012)*.

Age differences in impulsivity

Behavioral Neuroscience
2003, Vol. 117, No. 4, 695–703

0735-7044/03/$12.00 DOI: 10.1037/0735-7044.117.4.695

Elevated Levels of Impulsivity and Reduced Place Conditioning With *d*-Amphetamine: Two Behavioral Features of Adolescence in Mice

Walter Adriani and Giovanni Laviola

Behavioral Neuroscience
2003, Vol. 117, No. 4, 695–703

Elevated Levels of Impulsivity and Reduced Place Conditioning With *d*-Amphetamine: Two Behavioral Features of Adolescence in Mice

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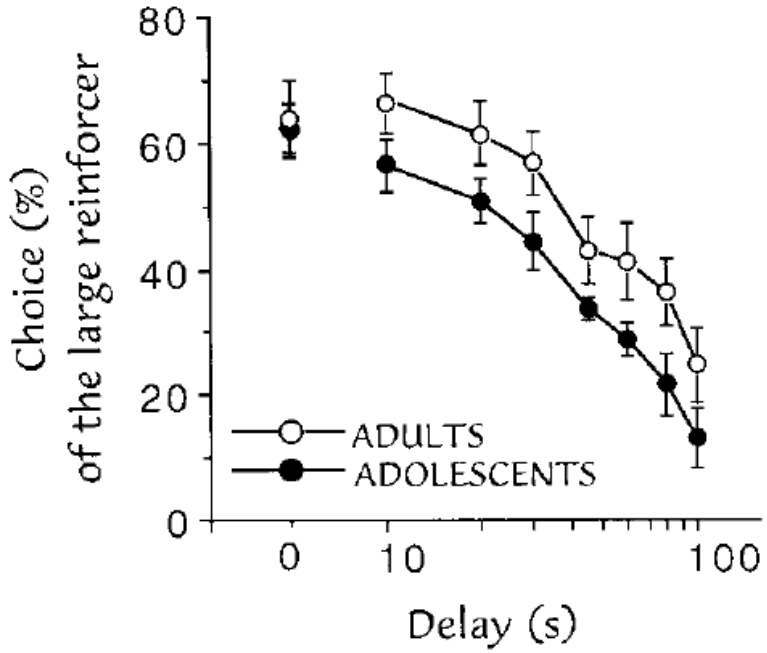
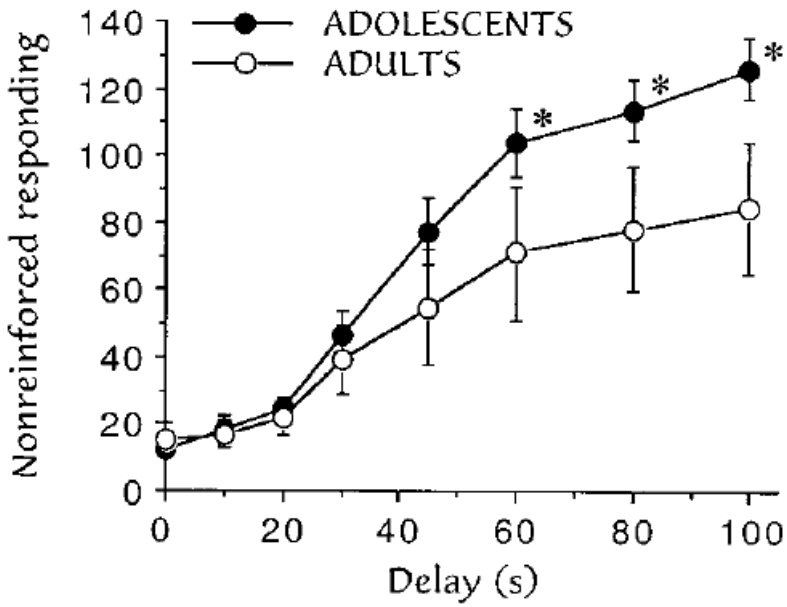
Levels of cognitive impulsivity



Levels of motor impulsivity



A more marked impulsivity profile was evident in adolescent mice when compared with adults. A more marked restlessness was evident in adolescent mice compared with adults *(Adriani & Laviola, 2003)*.

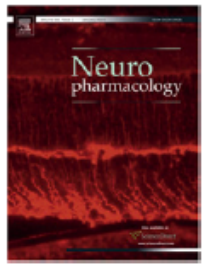
Age differences in gambling proneness

Neuropharmacology 67 (2013) 444–454

Contents lists available at SciVerse ScienceDirect

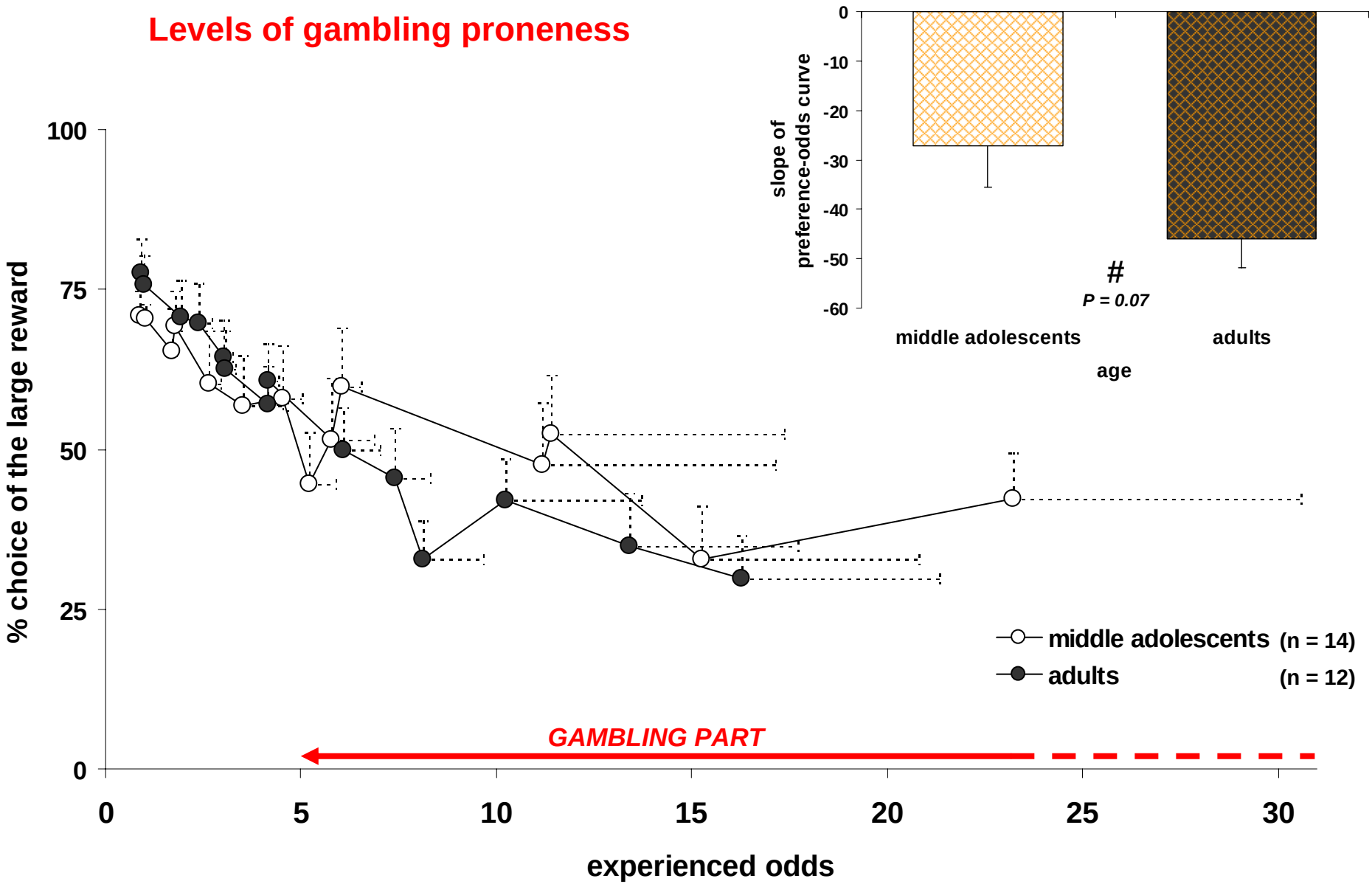
Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm



Gambling proneness in rats during the transition from adolescence to young adulthood: A home-cage method

Francesca Zoratto, Giovanni Laviola, Walter Adriani*



Slightly enhanced gambling behaviour in adolescent (pnd 36-49) than adult (pnd 67-80) rats.

Frequency of inadequate responding, an index of "frustration"

Neuropharmacology 67 (2013) 444–454

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Gambling proneness in rats during the transition from adolescence to young adulthood: A home-cage method

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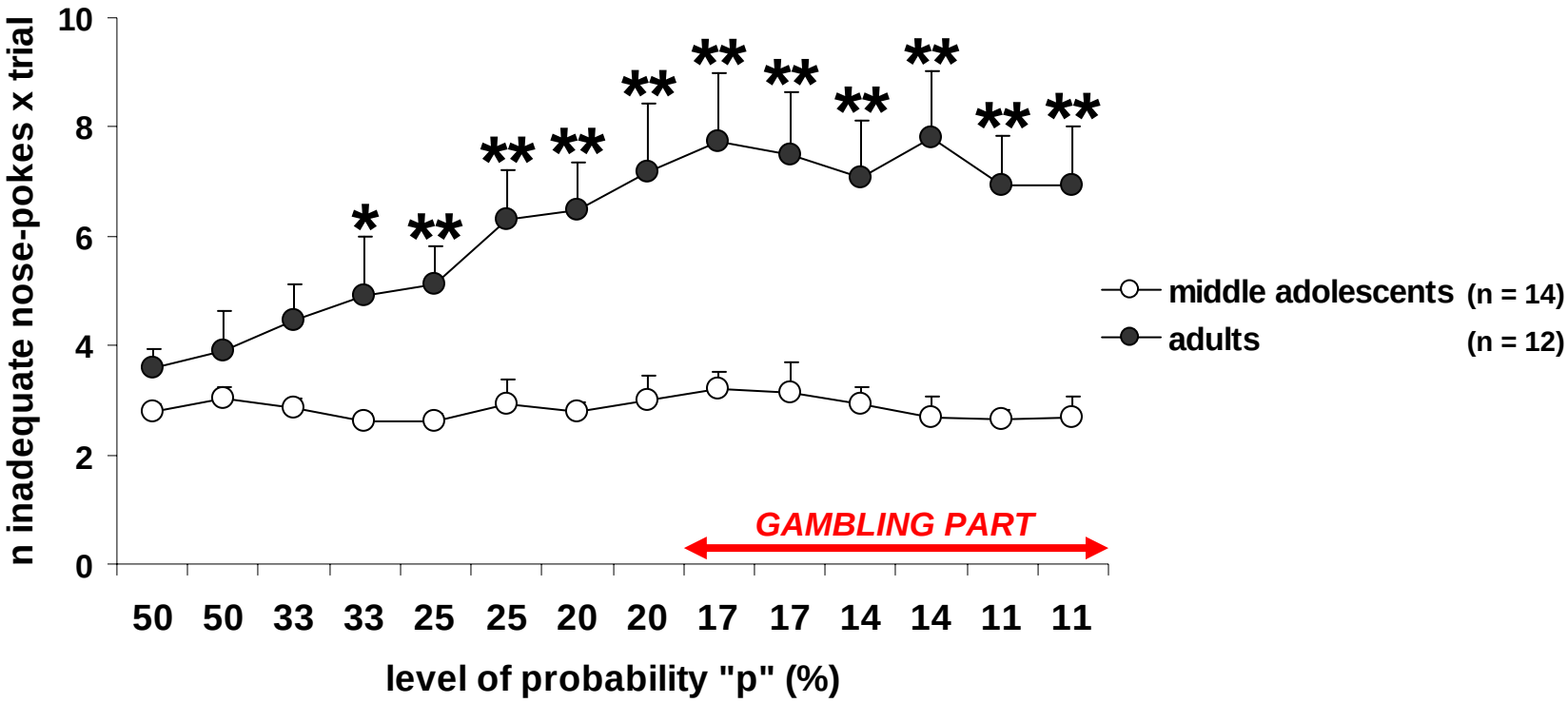


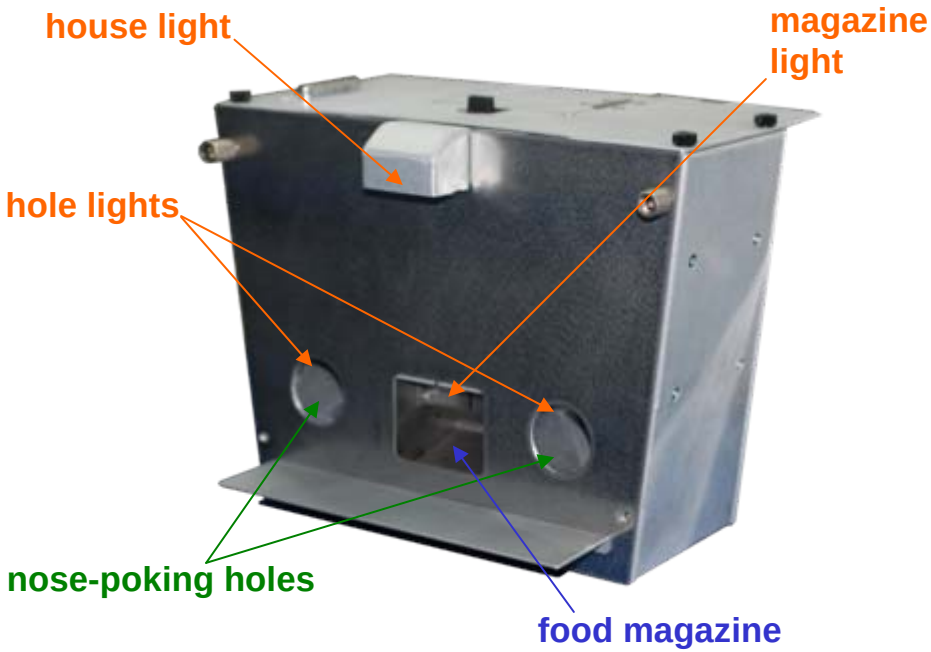
Frequency in adults comparable to what found previously in adult rats tested individually *(Zoratto et al., 2012)*; frequency in adolescents surprisingly low.

- Considering behavioural features of adolescence, we expected adolescent rats to maintain an even stronger attraction for highly uncertain reward in the gambling part of the task

- Inadequate nose-pokes in reaction to reward omission are an index of frustration; adolescents could be insensitive to reward loss

  → decreased behavioural and brain responsiveness to punishment? unlikely!

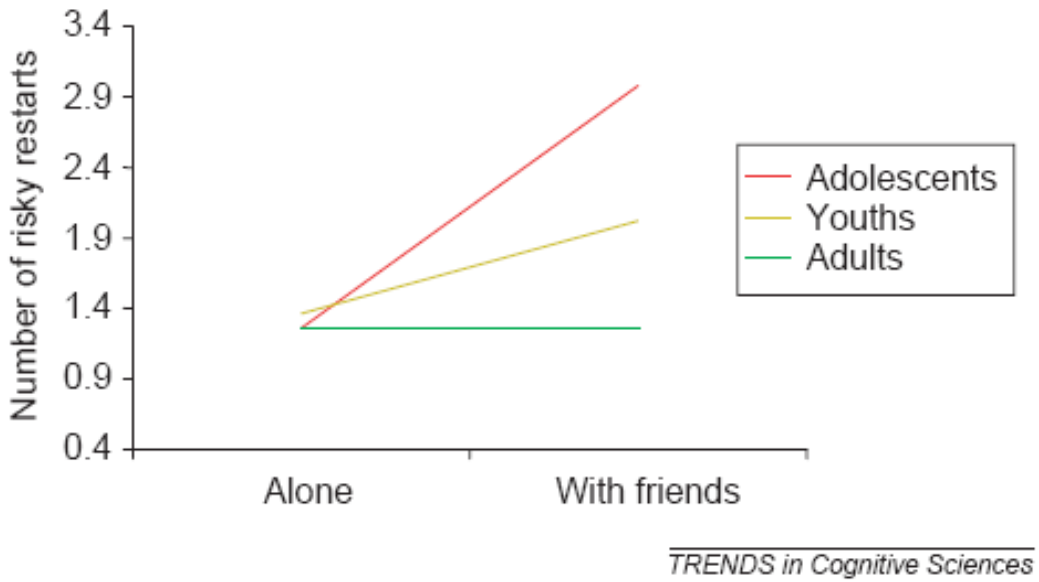
  → increased subjective value of the secondary reinforcer? a role for hole/magazine lights …



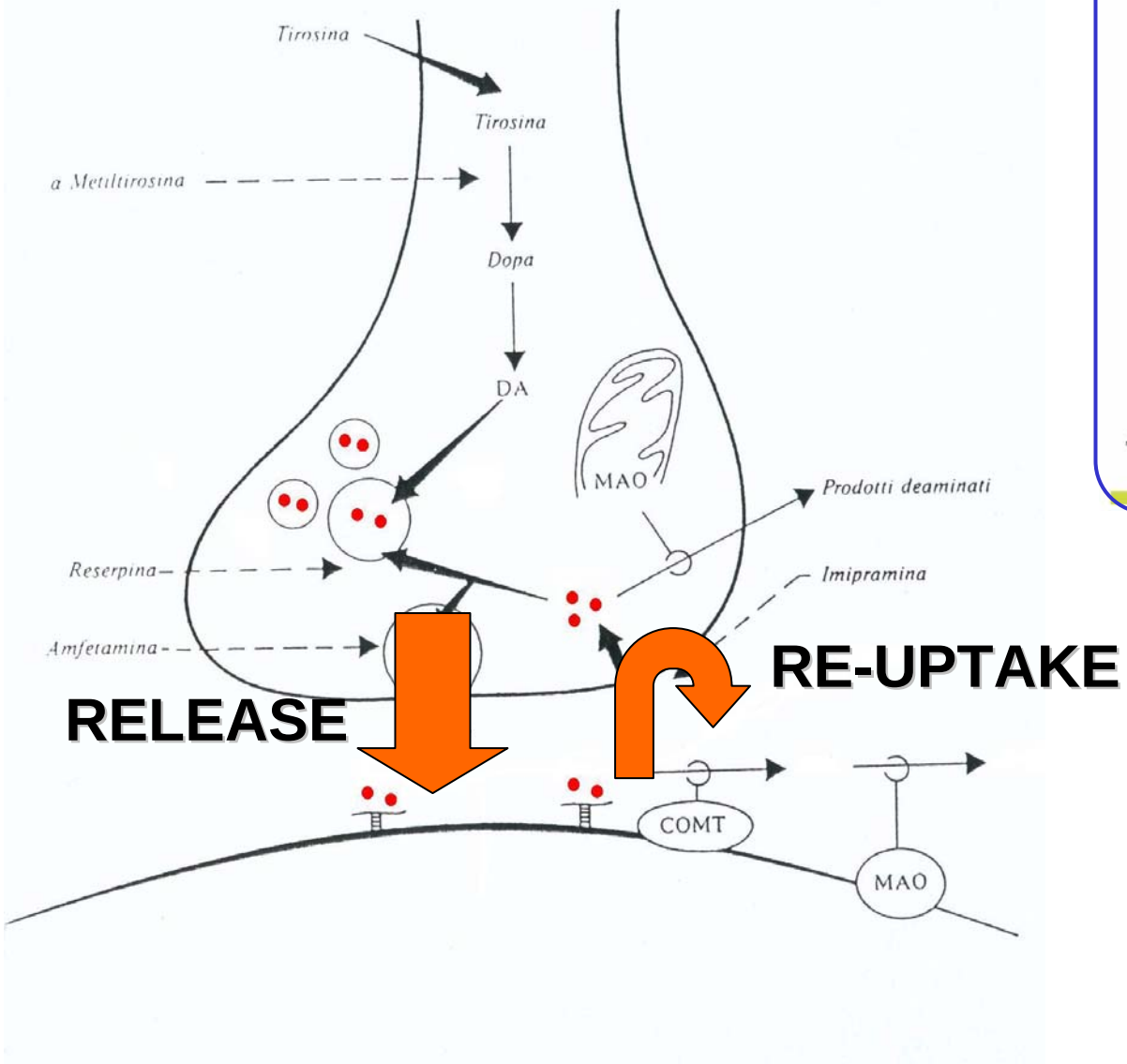
Slightly enhanced gambling behaviour in adolescents

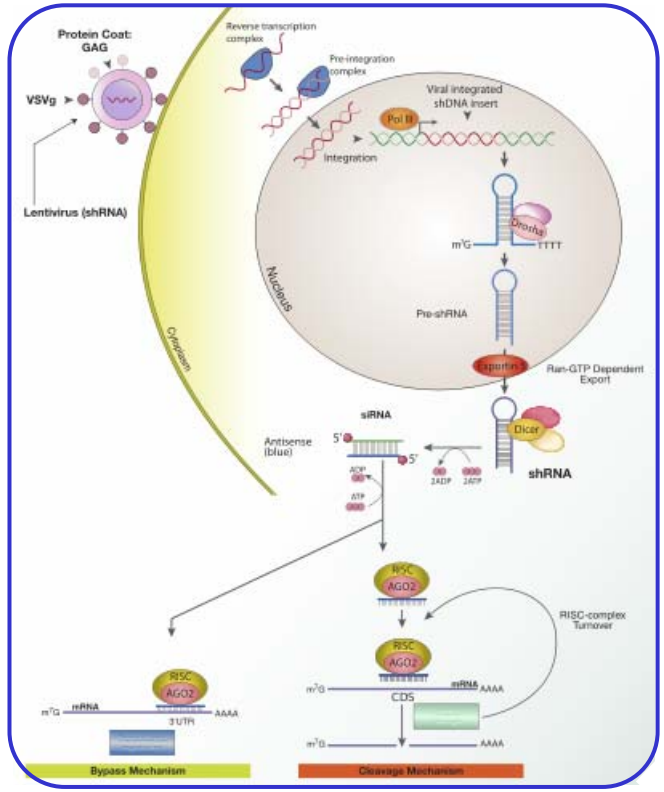
- existing evidences in clinical experimental research are contrasting and do not support very clear age differences *(Scheres et al., 2006; Crone & van der Molen, 2004)*

- the presence of peers may actually render potentially risky activities far more rewarding *(Steinberg, 2005; Chein et al., 2011)*



(Steinberg, 2005)





Lentiviral delivery of shRNAs and the mechanism of RNAi interference in mammalian cells (Cojocari D., 2010)

Medications used for ADHD treatment: methylphenidate (Ritalin®)

BMC Neuroscience

BioMed Central

Research article

Open Access

Delay aversion but preference for large and rare rewards in two choice tasks: implications for the measurement of self-control parameters

Walter Adriani* and Giovanni Laviola

Neuropsychopharmacology (2006) 31, 1946–1956

www.neuropsychopharmacology.org

Methylphenidate Administration to Adolescent Rats Determines Plastic Changes on Reward-Related Behavior and Striatal Gene Expression

Walter Adriani[1,5], Damiana Leo[2,5], Dario Greco[3], Monica Rea[1], Umberto di Porzio[2], Giovanni Laviola[1] and Carla Perrone-Capano*[,2,4]

[5]Both authors have equally contributed to this work.

ENDURING EFFECT OF ADOLESCENT MPH ADMINISTRATION

Administration of **methylphenidate** (MPH, Ritalin®) may produce its beneficial modulation of impulsive behavior through:

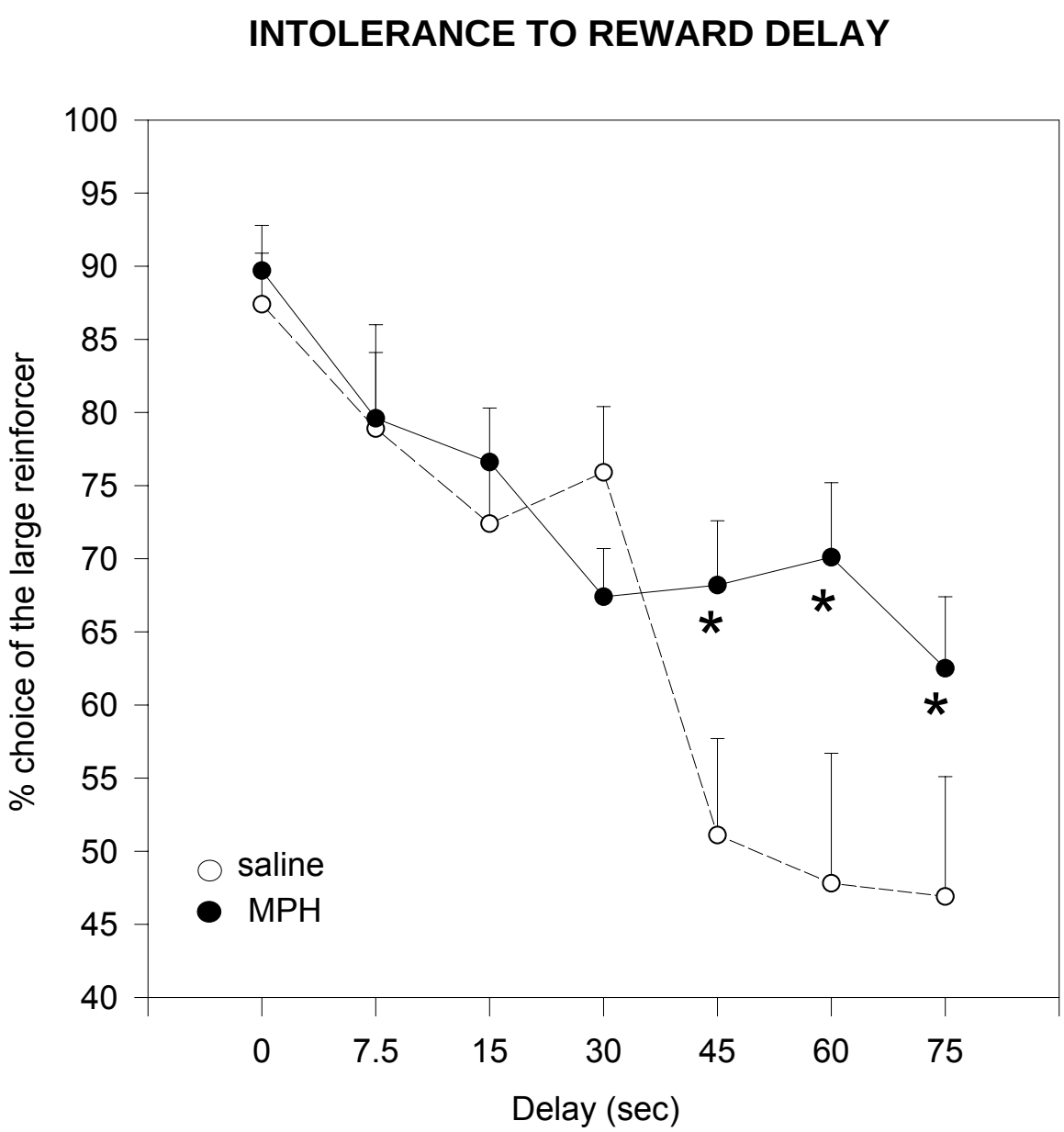
- an enduring **increase** of 5-HT(7) neurotransmission.

- enduring functional changes within **fronto-striatal circuits**.

EXPERIMENTAL DESIGN

Adolescent rats (30- to 44-day-old) were administered MPH (2 mg/kg i.p.) or saline for 14 days, and were tested when adult for :

1) MPH-induced changes in impulsivity (intolerance to delay)
2) blockade of MPH effects with a 5-HT(7) antagonist
3) reproduction of MPH-like effects with a 5-HT(7) agonist (ongoing)
4) magnetic resonance: spectrometry, DTI, fMRI connectivity (ongoing)



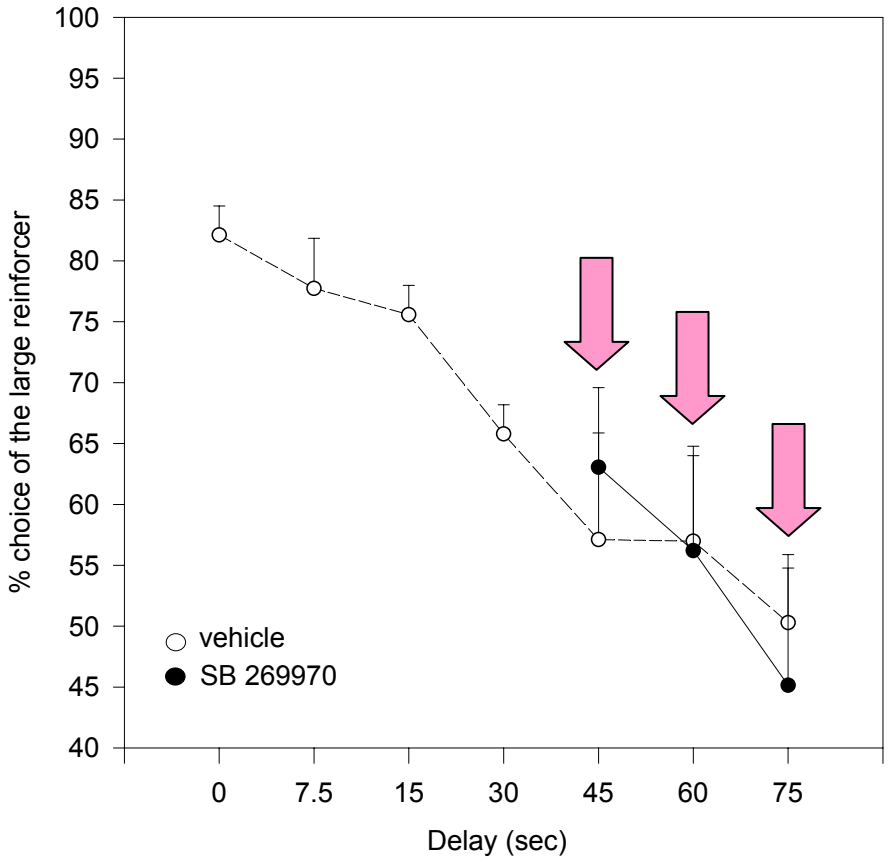
LONG-TERM EFFECT of MPH

RATS TESTED DURING ADULTHOOD, ONE MONTH AFTER MPH PRETREATMENT

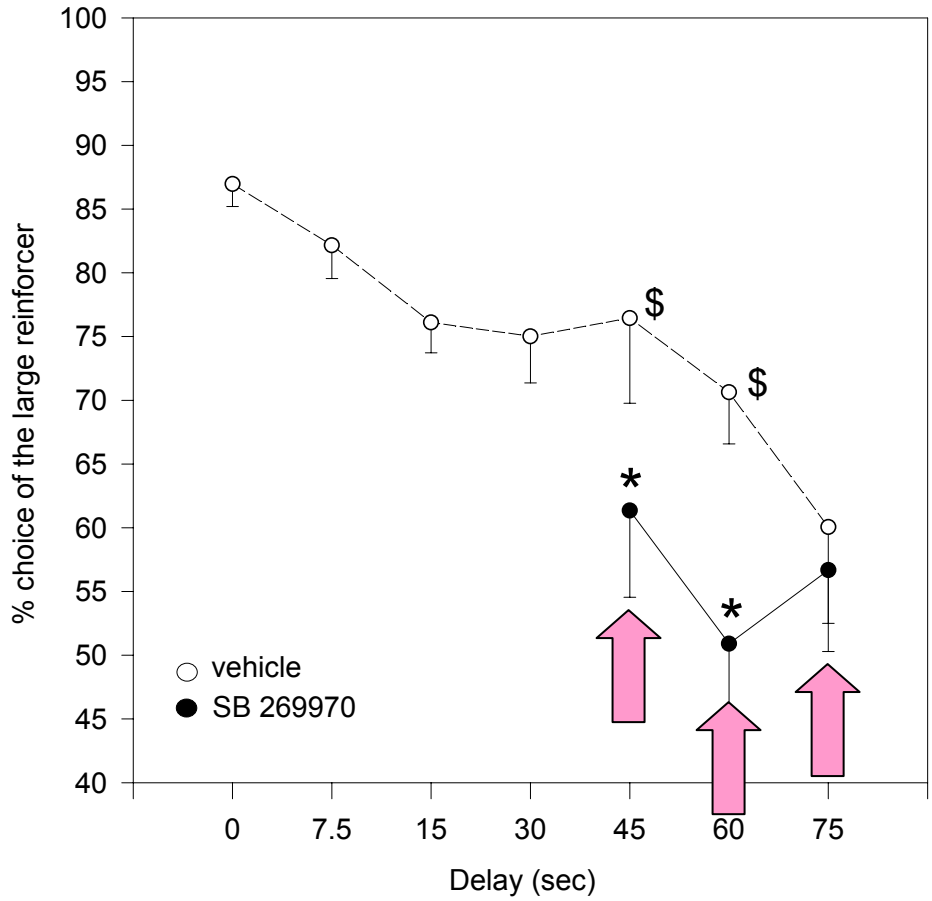
tolerance to delay
= lower impulsivity

the BLOCKADE of MPH-INDUCED LONG-TERM EFFECT with acute administration of the 5-HT(7) selective antagonist

PRE-EXPOSED TO SALINE DURING ADOLESCENCE



PRE-EXPOSED TO MPH DURING ADOLESCENCE



Lentiviral manipulation of DAT expression

Neuroscience 159 (2009) 47–58

INCREASED IMPULSIVE BEHAVIOR AND RISK PRONENESS FOLLOWING LENTIVIRUS-MEDIATED DOPAMINE TRANSPORTER OVER-EXPRESSION IN RATS' NUCLEUS ACCUMBENS

W. ADRIANI,[a1] F. BOYER,[b1] L. GIOIOSA,[a] S. MACRÌ,[a]
J.-L. DREYER[b] AND G. LAVIOLA[a]*

[1] Both authors contributed equally to this work.

International Journal of Neuropsychopharmacology (2010), 13, 1329–1342.
doi:10.1017/S1461145709991210

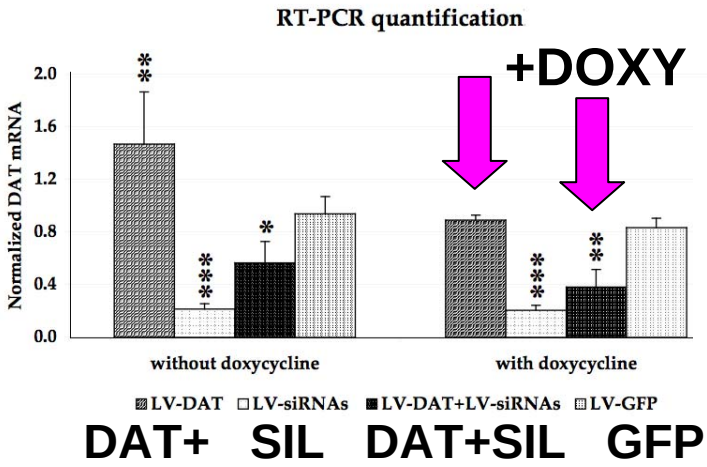
Social withdrawal and gambling-like profile after lentiviral manipulation of DAT expression in the rat accumbens

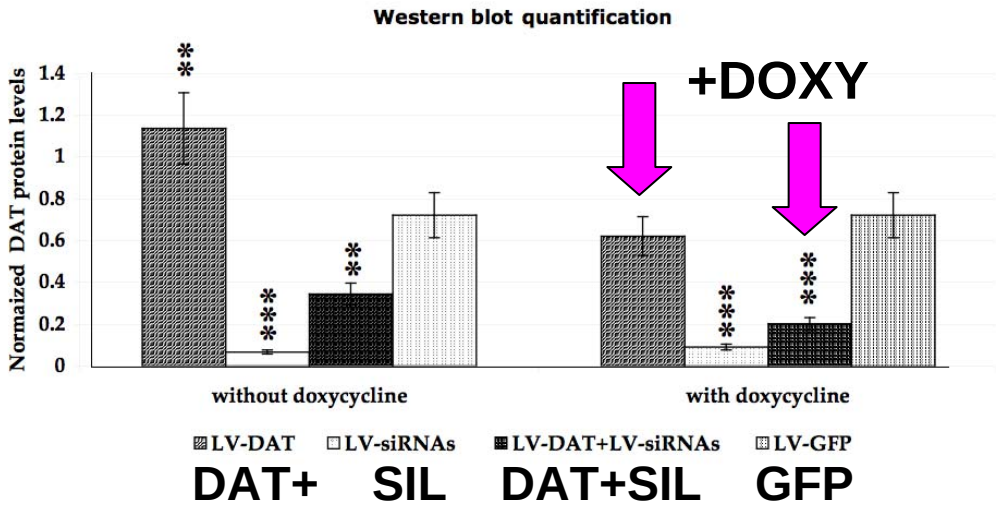
Walter Adriani[1]*, Frederic Boyer[2]*, Damiana Leo[3], Rossella Canese[1], Franca Podo[1], Carla Perrone-Capano[3,4], Jean-Luc Dreyer[2] and Giovanni Laviola[1]

* These authors contributed equally to this work.

MODEL of IMPULSIVITY / GAMBLING

DAT mRNA and protein levels, extracted from the NAcc of rats inoculated with lentivirus vectors.

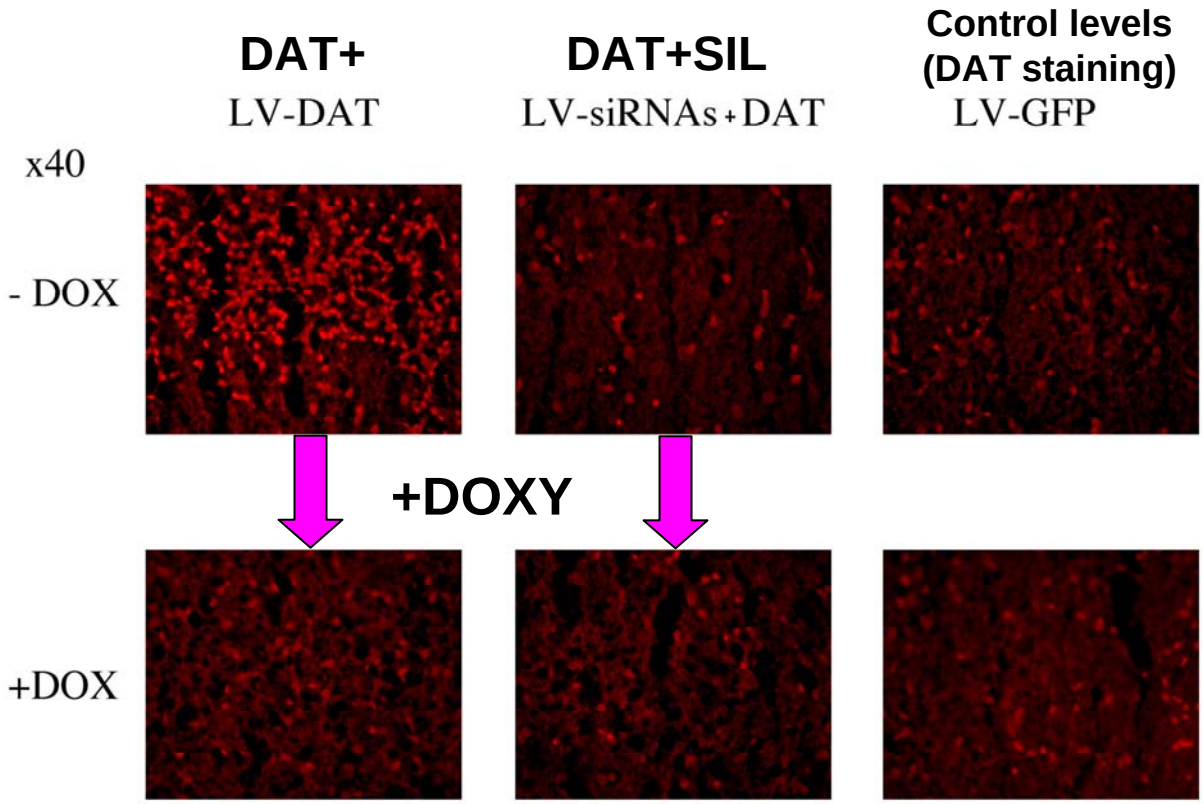




Doxy switches-off DAT enhancer :

DAT+SIL rats face **strong** silencing

MODEL of IMPULSIVITY / GAMBLING



Efficacy of lentiviruses inoculated in the NAcc of rats: Lenti-DAT with or without Lenti-DAT-siRNAs.

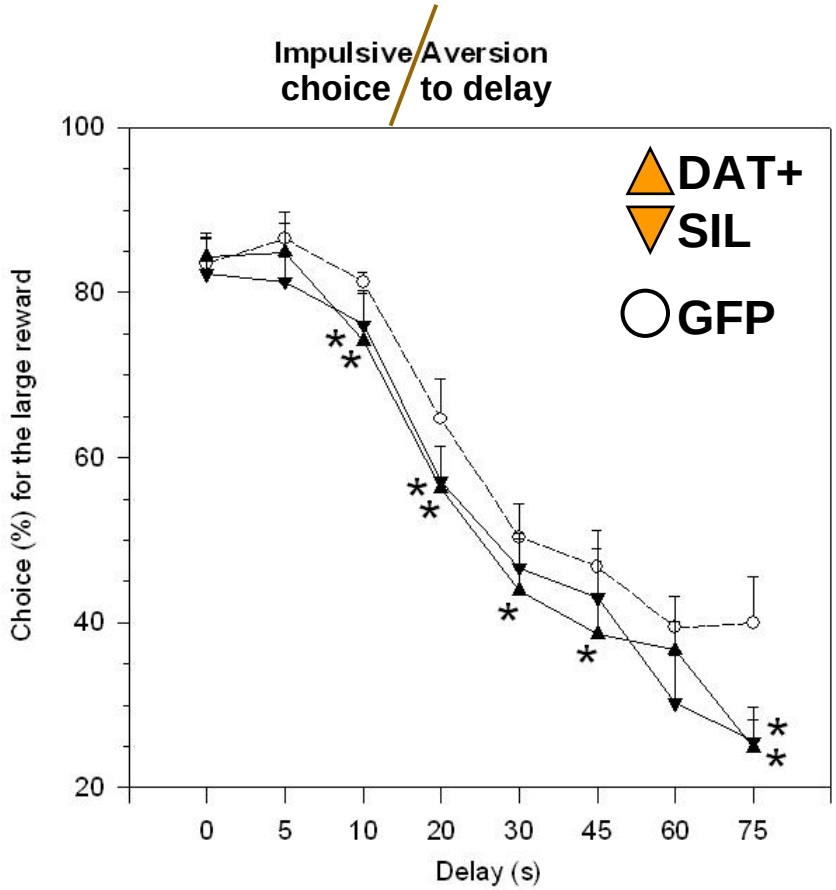
Adriani W, Boyer F, Gioiosa L, Macrì S, Dreyer J-L, Laviola G (2009) Neuroscience, 159: 47-58.

MODEL of IMPULSIVITY / GAMBLING

Levels of cognitive impulsivity

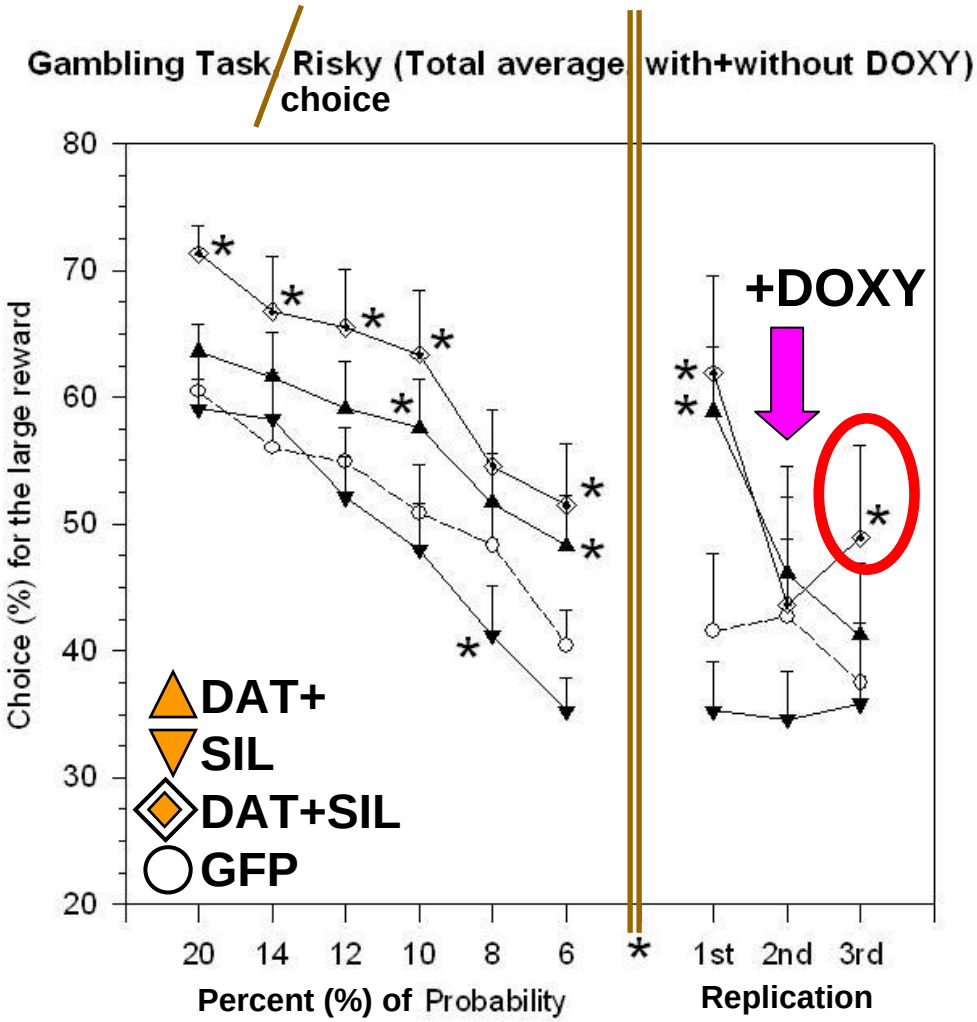
Delay-intolerance task. Rats have free choice between:

- **one** food pellet delivered **immediately**, or
- **five** food pellets delivered with a given **delay** that increases progressively.



Adriani W, Boyer F, Gioiosa L, Macrì S, Dreyer J-L, Laviola G (2009) Neuroscience, 159: 47-58.

MODEL of IMPULSIVITY / GAMBLING

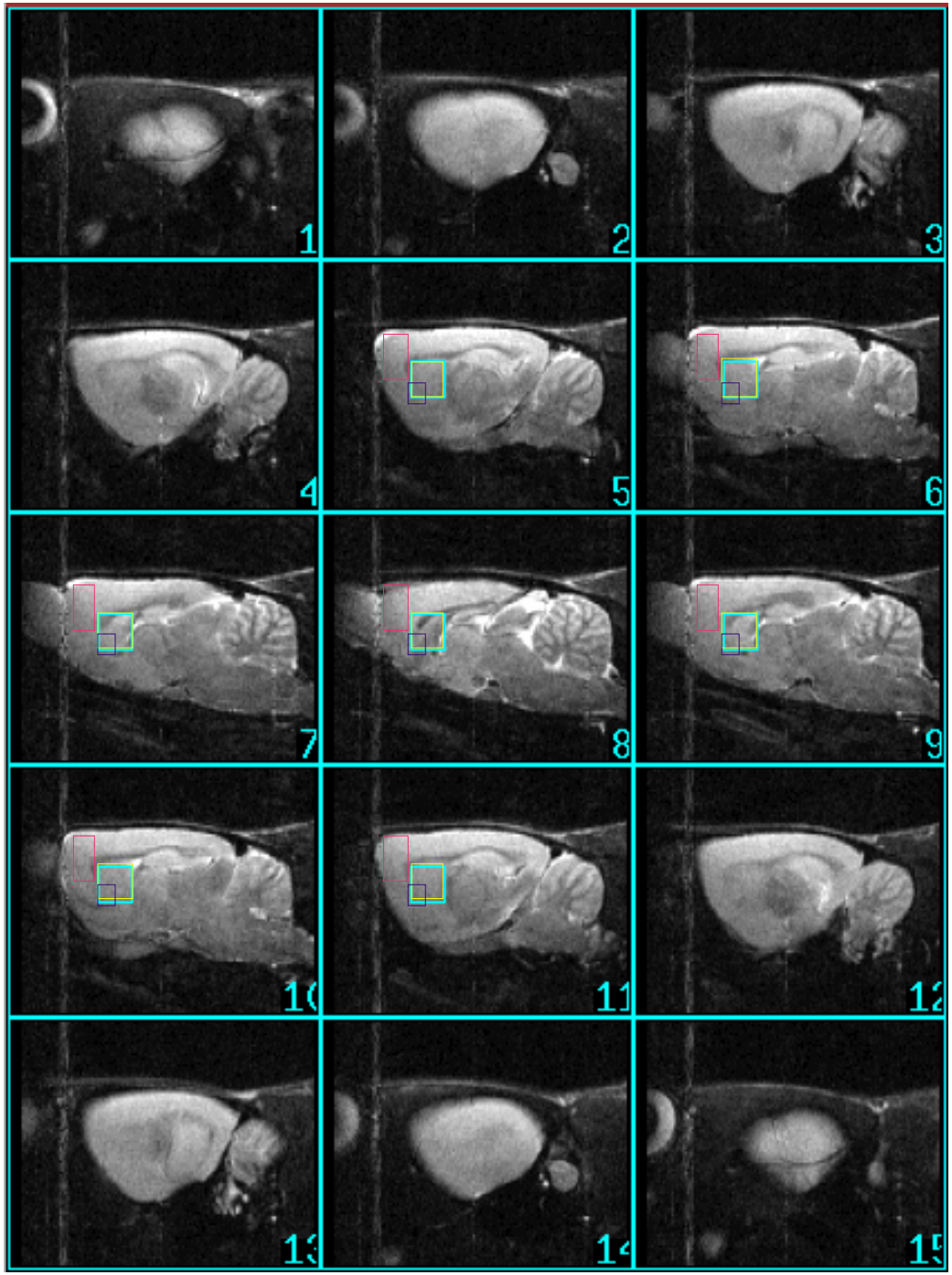


Levels of gambling proneness

Choice (%) of the large but uncertain reward (i.e. one food pellet for sure vs five food pellets delivered, or not, with a progressively decreasing probability).

Task was replicated thrice: rats were exposed to doxycycline (**doxy**) on 2nd replication, to switch off any exogenous DAT over-expression.

Left panel: average of the 3 replications. Right panel: the last point (at p=6% only) is presented for first (no doxy), second (**under doxy**) and third (no doxy) replication.



MAGNETIC RESONANCE SPECTROSCOPY

Data obtained at:
Molecular and Cellular Imaging division at ISS (dr R. Canese)

Dorsal Striatum
Nucleus Accumbens
Prefrontal Cortex

MAGNETIC RESONANCE SPECTROSCOPY

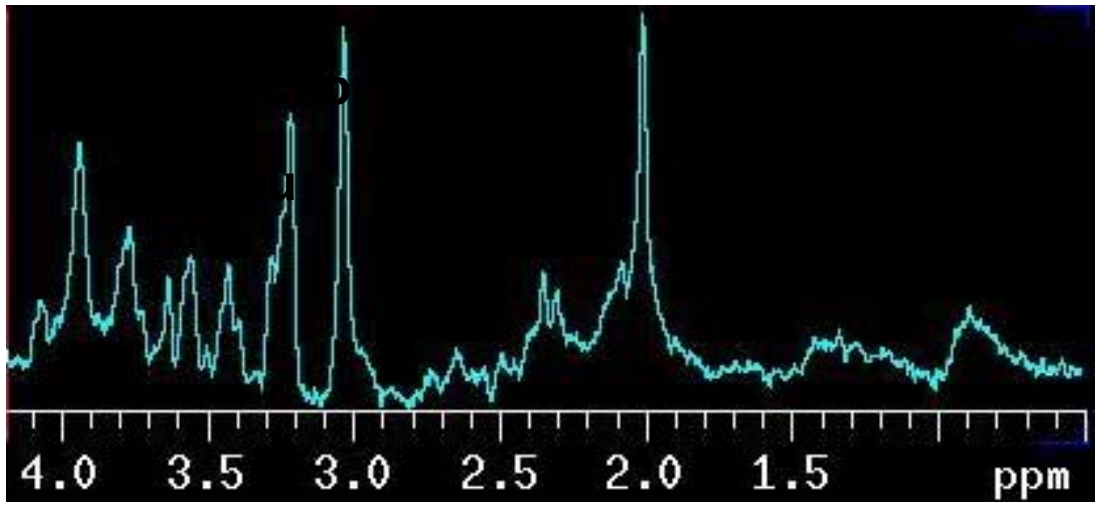
in: striatum, n. accumbens, prefrontal cortex

The major metabolites can be reliably detected in selected voxels.

tCr/PCr= total / phospho- creatine ***tCho = total choline***

NAA = N-acetyl-aspartate ***Glx = glutamine + glutamate***

Tau = taurine ***Ins = inositols phosphates***



MAGNETIC RESONANCE SPECTROSCOPY

in: striatum, n. accumbens, prefrontal cortex

Metabolic parameters in the dStr and the NAcc of adult male rats (n=6) with previous inoculation of lentiviral vectors (DAT+ and/or SIL) into the NAcc.

Dorsal Striatum	**PCr/tot (%)**	**Phospho-creatine**	**Total creatine**
GFP control	**65.4±3.4 %**	**6.29±0.54**	**9.86±0.41**
DAT+	**63.0±2.7 %**	**6.60±0.30 ***	**10.46±0.10 ***
SIL	**64.8±2.6 %**	**6.24±0.52**	**9.57±0.46**
DAT+SIL	**65.5±2.1 %**	**6.97±0.35 ***	**10.62±0.29 ***
N. Accumbens	**PCr/tot (%)**	**Phospho-creatine**	**Total creatine**
GFP control	**64.3±1.5 %**	**6.49±0.17**	**10.10±0.26**
DAT+	**67.5±2.3 %**	**6.72±0.27**	**9.96±0.22**
SIL	**59.7±3.4 % ***	**5.63±0.44 ***	**9.56±0.26**
DAT+SIL	**59.1±3.6 % ***	**5.84±0.32 ***	**9.80±0.26**

Levels of metabolites given in arbitrary units, referred to the unsuppressed water signal. (*) $p < .05$ compared to GFP control rats. Only DAT+SIL rats display changes in both areas.

OPERANT BEHAVIOUR and MR SPECTROSCOPY

Role of dorsal striatum *vs* n. accumbens in self-control

Interestingly, **total creatine** and/or **phospho-creatine** (*bioenergetic metabolites*) were **up-regulated** in the **dStr** and conversely **down-regulated** in the **NAcc** of DAT+SIL rats. The unbalanced influence by these two areas on behavioural output does generate **impulsivity and/or risk-proneness**.

Consistent with the functional role of these two forebrain areas, DAT+SIL animals may display *an enhancement in their coping* ability via **novel behavioural strategies** (**dStr**), and to be *less driven by attractiveness of* reward, or by salient contingencies like *immediacy or binging* - i.e. by **instinct** (**NAcc**).

Lentiviral manipulation of SERT expression

Impulsivity and home-cage activity are decreased by lentivirus-mediated silencing of serotonin transporter in the rat hippocampus

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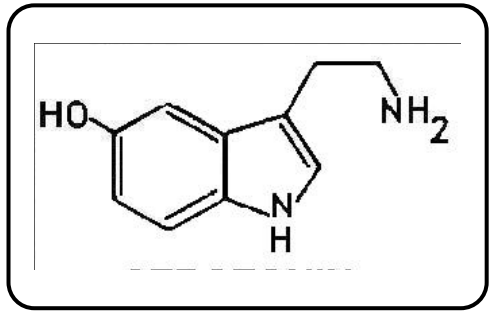
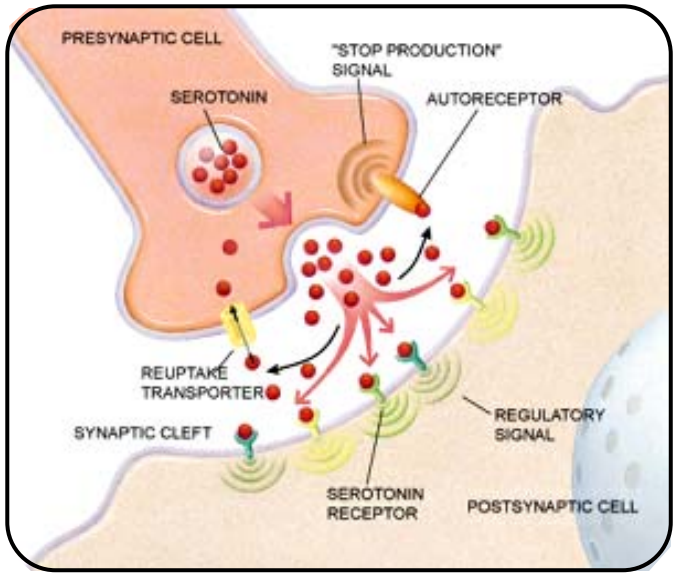
Zoratto F., Laviola G., Adriani W.

Neuroscience Letters (submitted)

Serotonin (5-HT)



- The serotonergic system is well known for modulation of emotional, cognitive and motivational processes
- Serotonin (5-HT) has a key role in the top-down inhibitory control over behavioural initiation, which is important for withholding of instinctive reactions and for an appropriate feedback regulation of behaviour
- Deficits in the homeostasis of this system play a crucial role in many psychiatric disorders, including affective and impulse-control disorders



Serotonin transporter (SERT)

- SERT, which selectively removes 5-HT out of the synaptic cleft, is a major determinant of serotonergic signalling efficiency
- mutations in the SERT gene promoter do influence the rate of 5-HT reuptake and have been associated with susceptibility towards the development of several psychiatric disorders
- the s-variant of the 5-HTTLPR is associated with reduced SERT gene transcription efficiency, resulting in reduced SERT levels and reduced 5-HT uptake; consequently, the extracellular levels of 5-HT are higher compared to the l-variant

Experimental subjects

❖ spontaneous locomotor activity:

- adult male rats
- inside the home-cage, 50 days after inoculation
- continuous automatic registration (15 days) using infrared sensors (20 Hz)
- mean day calculated on 5-days intervals

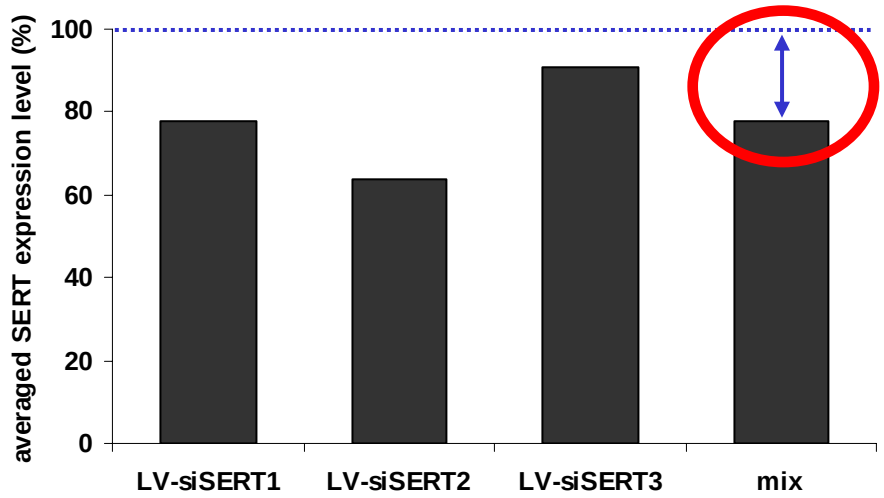
❖ Intolerance to Delay (ID) task:

- tested in classical operant chambers during the dark phase of the cycle
- mild food restriction
- testing: 8 days, 1 session per day (40 min), timeout 30 s

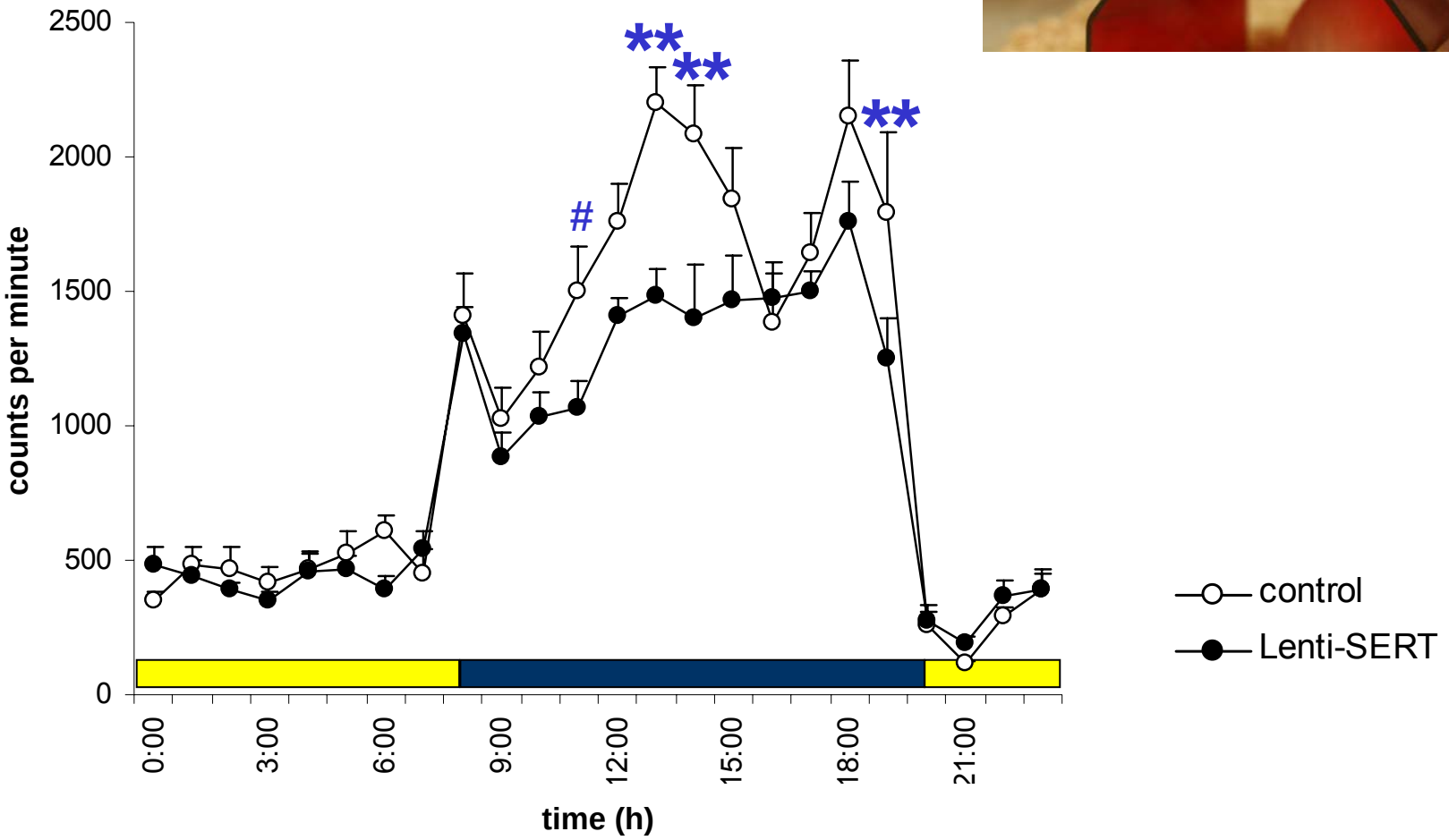
Experimental groups

- **Lenti-SERT** (n=11): inoculation of active lentiviruses
- **control** (n=6): inoculation of heat-inactivated lentiviruses
- bilateral inoculation (volume 1 µl) in the hippocampus; coordinates AP −3.3, ML ±2.0, DV −4.0

In-vitro quantification of SERT silencing

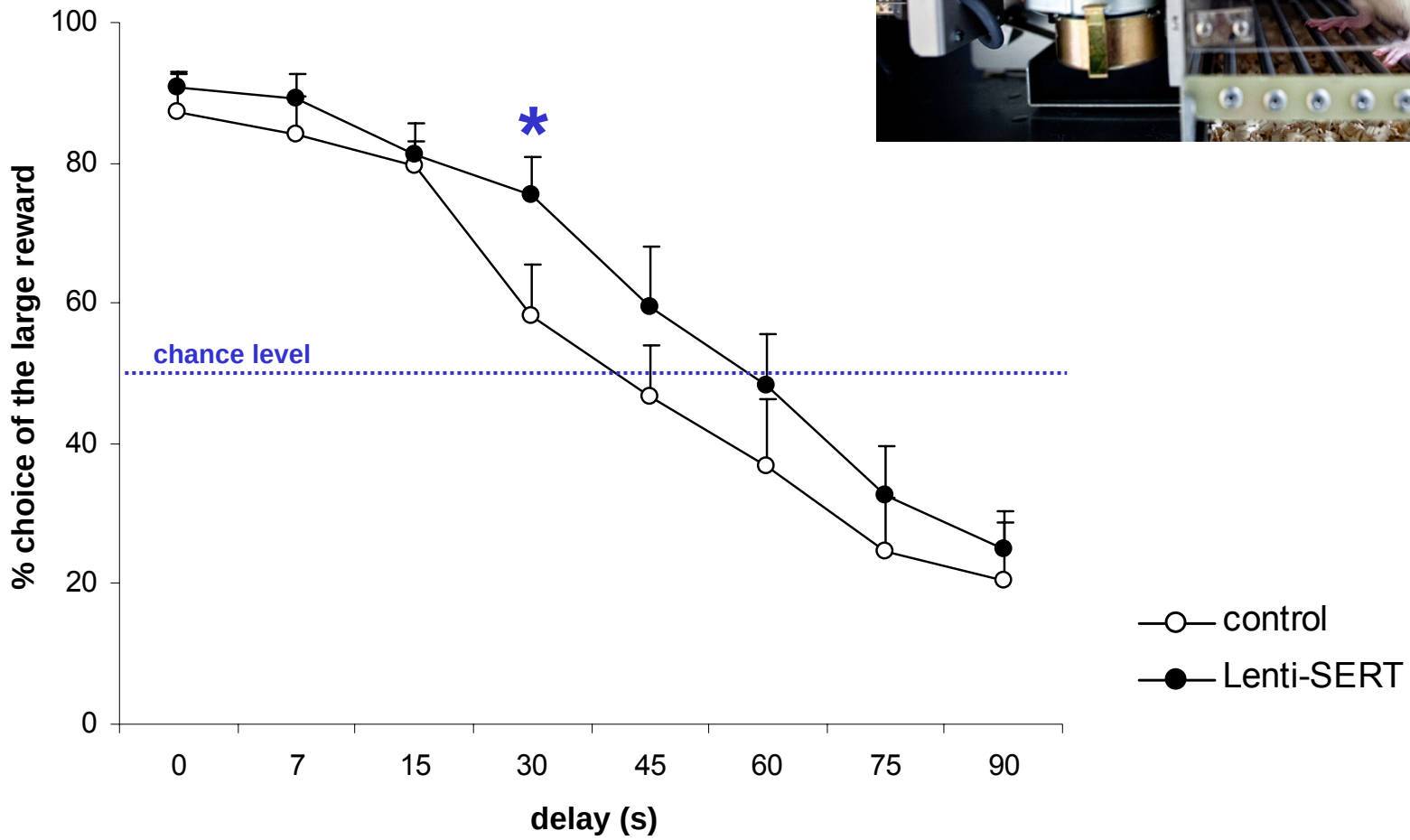


Spontaneous locomotor activity inside the home-cage



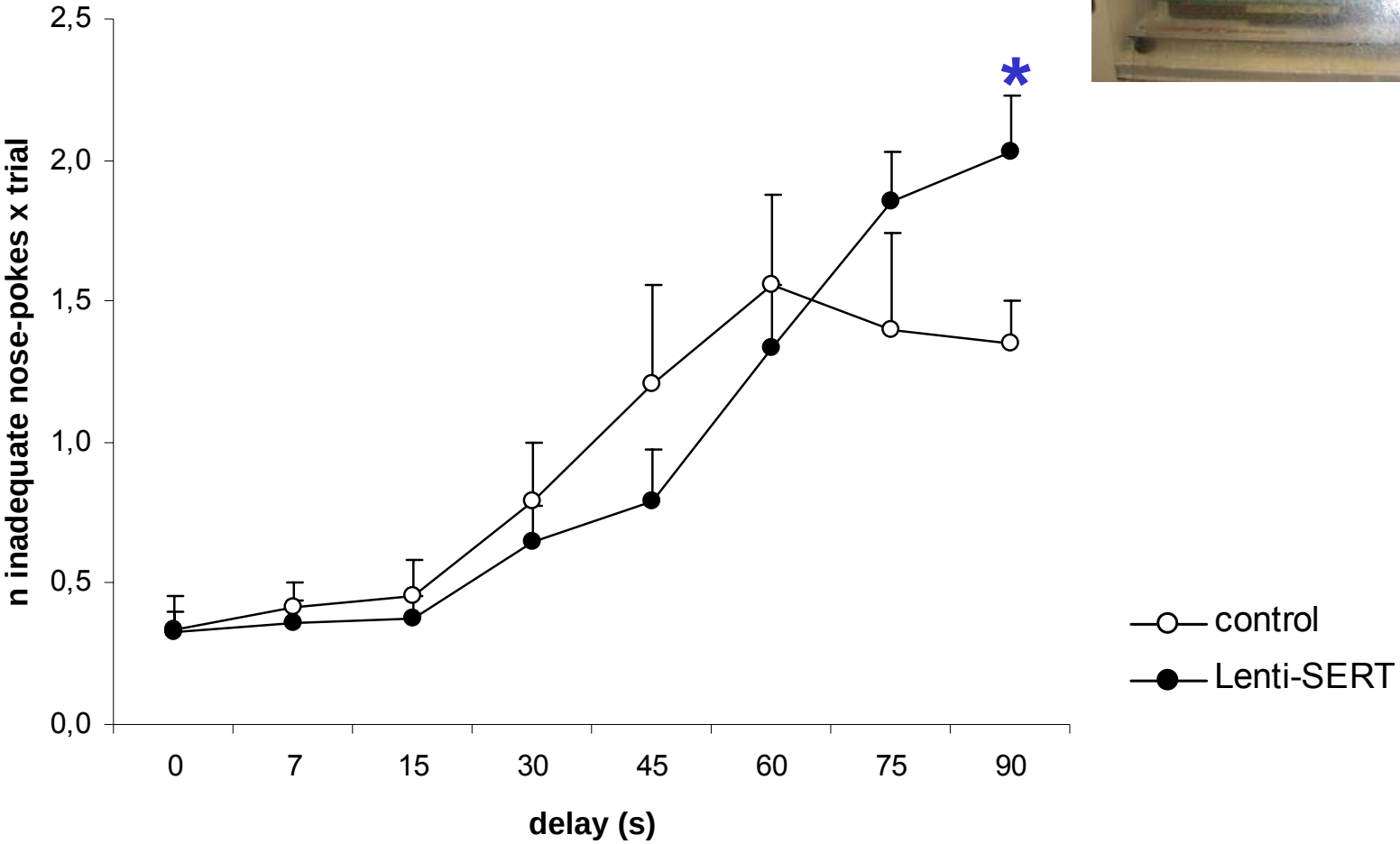
** P < 0.01; # 0.05 < P < 0.1 when comparing Lenti-SERT vs control rats in post-hoc test

Levels of cognitive impulsivity



*** P < 0.05 when comparing Lenti-SERT vs control rats in post-hoc test**

Levels of motor impulsivity

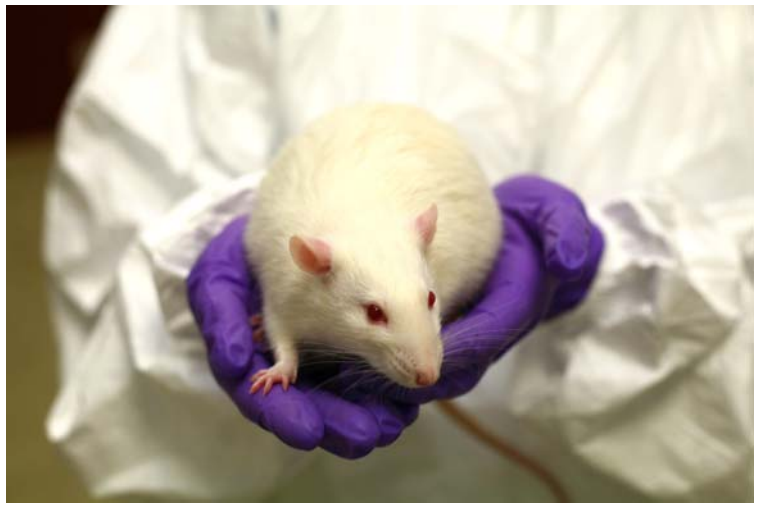


*** P < 0.05 when comparing Lenti-SERT vs control rats in post-hoc test**

A reduced SERT function significantly impact on activity and impulsivity profiles

- These profiles confirm the inverse relationship between 5-HT and impulsivity reported by studies carried out with various manipulations of the serotonergic system.

- Considering the complexity of the serotoninergic system, it may not be so surprising the association between the short allelic variant (s) and the increase in impulsivity found in clinical research.
- The association between the s/s genotype and the decrease in SERT expression in the human central nervous system has not yet been proven definitively.



Conclusion

- In general, the refinement of ID and PD tasks and the development of innovative animal models are essential to increase knowledge about the neurobiological mechanisms underlying neuropsychiatric disorders and for the development of new therapeutic approaches.
- Impulsivity and gambling proneness are modulated by age, gender, genetics (polymorphisms), environmental changes (e.g. stress, dietary manipulations, drug treatments), which act through key modifications in specific markers of dopamine and serotonin systems.

Future perspectives

- Preclinical studies on medications currently used to treat
  - pathological gambling: opioid antagonists (naltrexone, nalmefene); selective serotonin re-uptake inhibitors, SSRI (paroxetine, fluvoxamine)
  - Parkinson's disease: levodopa; dopamine replacement therapy (dopamine receptor agonists)
- The development of paradigms that can be carried out directly in rats' home-cages may allow to improve both animal welfare and the quality of experimental data.

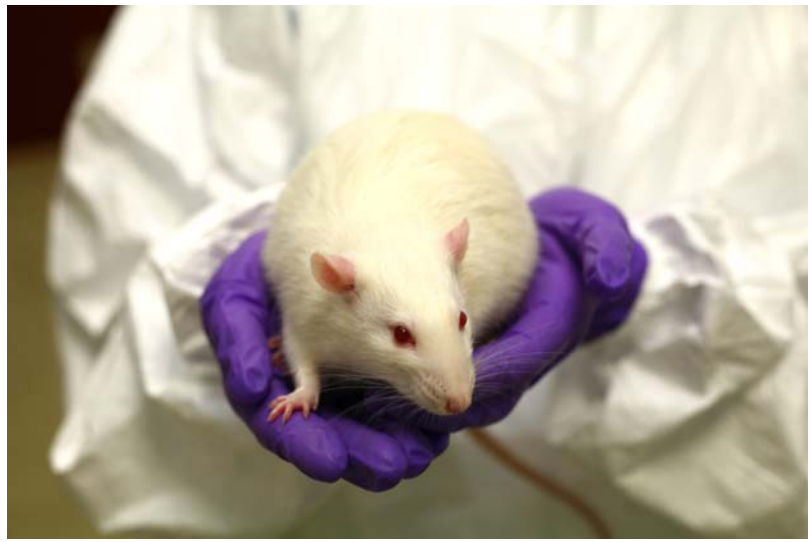
Hikikomori

- extreme form of social withdrawal identified in Japan in the early 1990s
- young people secluding themselves in their homes for months or even years
- some researchers believe it may become a worldwide phenomenon
- lack of a robust body of clinical research

"… they often stay awake all night using the Internet, playing computer games, or watching TV and sleep throughout the day …"

by Maggie Jones, The New York Times (January 15, 2006)

Thank you for your attention



Pictures of laboratory rats from the Understanding Animal Research image library

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