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9.914 Special Topics: Genetics, Neurobiology, and Pathophysiology of Psychiatric Disorders  
Fall 2008

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# The Dopamine Pathway in Psychiatric Disorders

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9.914

2008

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# Molecular genetics of cognition and intelligence



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- Given a variety of evidence implicating the prefrontal cortex and its dopaminergic circuits in cognition, most of the research conducted to date has focused on genes regulating dopaminergic function.
- Variants of COMT influence cognitive function
- specific neurocognitive processes involved continue to be a matter of debate
- Part of the difficulty is distinguishing between false positives, pleiotropy and the influence of a general intelligence factor.

# Molecular genetics of cognition and intelligence



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- Heritability estimates of standardized intelligence quotient – 50-80%
- Processing speed, working memory have heritability estimates of 30-60%
- Many genes likely involved, each with a relatively small effect
- Genetic association studies: performance in a specific domain can be statistically linked to function activity of particular proteins

# Prefrontal cortex(PFC) and DA



- Many studies: PFC is critical for executive functioning
  - Working memory (WM), response inhibition, planning, concentration, attention, perceptual organization, judgment, decision making and self-monitoring
- Previc (1999) hypothesis that Dopamine is key regulator of these cognitive skills
- 93% Parkinson's patients present WM and executive deficits, which are improved by L-DOPA (precursor of DA) (gambling as side effect)
- Deficits of WM in aging can also be improved with DA agonists
- Brain imaging studies: correlation between density of dopaminergic fibers in caudate nucleus and performance on executive and memory tests



# (some) DA drugs

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- Bromocriptine, D2R agonist
  - Improves cognition in those with low-baseline memory-capacity
  - Impairs performance in high performers
- Dextroamphetamine (potentiates DA activity)
  - improved cognitive performance in those with low-baseline levels of DA,
  - but once DA receptor stimulation reached a threshold, WM function deteriorated
- Both suboptimal and supra-optimal DA is bad for PFC!



# D1 receptor

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- D1 – signaling: in controlling the threshold of significance above which info must pass before it can be admitted to WM and processed by PFC
  - Activation stabilizes patterns of activity with PFC
  - Protects PFC from distracters until appropriate behavioral response is generated
- D1R expressed in glutamatergic pyramidal cells of pFC
- A lack of D1 signalling: poor differentiation of target from background (poor WM, attention, delusions, impulsive behaviors (schizophrenia))
- Excessive D1 signalling: perseveration, stereotypic behavior, reduced cognitive flexibility, improved memory



# D2 receptor

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- D2 – signalling in reward-based information
  - Allows PFC network to respond to new info by updating working memory system
- In GABAergic interneurons of PFC
- Depress NMDAR mediated excitatory neurotransmission
- Antagonists in healthy volunteers impair spatial WM, planning, attentional set-shifting
- Agonists improved cognition in those with low-baseline memories, and elderly





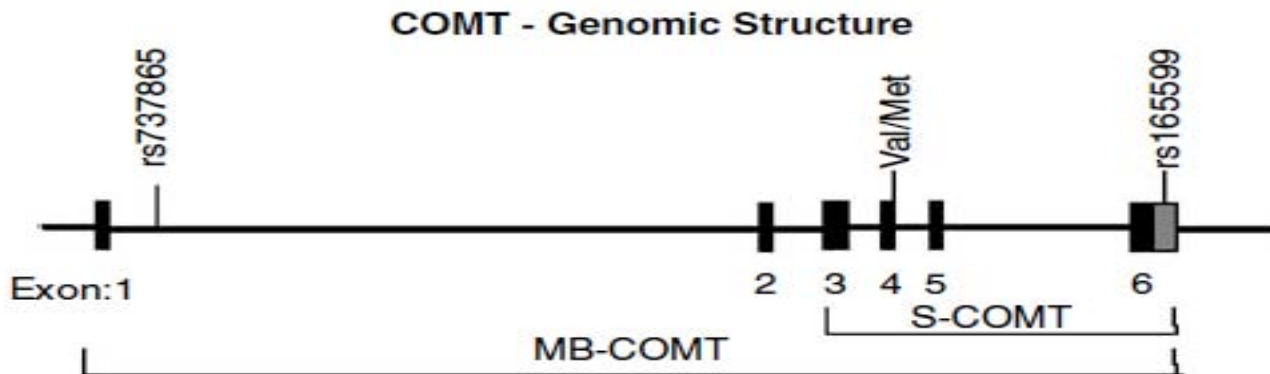
# Regulation of DA

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- DA catabolism
  - Monoamine oxidase in mitochondrial membranes of catecholamine neurons
  - COMT in extrasynaptic spaces
  - DA Transporter in dopaminergic neurons of substantia nigra and VTA (ventral tegmental area); rapid reuptake of DA from synaptic cleft

# COMT (catechol-O-methyl transferase)

- Codes for S-adenosylmethionine, which participates in the metabolic transformation of catechol compounds (catecholamines and catechol oestrogens).
- lies in a chromosomal region of interest for psychosis, schizophrenia, and bipolar spectrum disorder **22q11**
- Common (evolutionary recent) polymorphism within the gene alters the activity of the enzyme soluble (S-COMT, in many tissues) and membrane-bound (MBCOMT, in brain)



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# 22q11 deletion syndrome – Velocardio-Facial Syndrome

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- 1:2500 (vs 1% schizophrenia)
- Cardiac malformations, immunodeficiency, dysmorphic facial features, cleft palate
- Delayed motor and speech-language development, mental retardation, impaired spatial reasoning, poor attention and executive function, ASD, ADHD, Schizophrenia

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# COMT polymorphisms

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- MBCOMT appears to have higher affinity for the catecholamine neurotransmitters and plays an important role in their cortical metabolism.
- In turn, catecholamines are intimately, although not exclusively, involved in many psychological functions, learning notwithstanding.
- COMT higher in males in prefrontal cortex (Chen et al 2004)
- The male COMT knockout mouse shows a substantial increase in dopamine levels in the basal tissue, an increase not registered in females (Gogos et al., 1998)



# COMT polymorphisms

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- single nucleotide substitution at codon 158/108 in MB-COMT/S-COMT, which encodes two different amino acids, either valine (Val) or methionine (Met), and therefore is referred to as Val158Met (or G158A).
- This is associated with thermal stability of COMT
- Met158 allele enzyme is unstable at body temperature, and has only  $\frac{1}{4}$  activity of val-polypeptide
- Human Met158 homozygotes show lower activity in the prefrontal cortex than do Val158 homozygotes
- Met158 is associated with enhanced dopamine signaling



# COMT polymorphisms

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- Val158Met polymorphism has also been associated with cognitive performance in a number of studies, with individuals carrying the Met158 allele demonstrating better and more efficient processing
- This effect has been demonstrated in both adults and children
- Gender differences are once again notable, with these cognitive effects stronger in males and weaker or even inverse in females.
- However, there is also evidence in the literature that the Val158 allele can offer some advantage for specific types of cognitive performance

# Mechanism of COMT-polymorphisms



- Usually DA released diffuses and binds to extrasynaptic D1R and inactivated by COMT
- High-activity val COMT decreases extrasynaptic DA and D1R activation, shifting balance to intrasynaptic D2R activation
- Therefore (and this is supported by human studies)
- Val-allele results in lowered stability of PFC networks – decreased maintenance WM, but enhanced update of WM with new info
- Met-allele results in elevated PDF [DA] and D1R – enhanced stability of PFC networks and maintenance of information, but less cognitive flexibility



# COMT – Grigorenko study

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- Genotypes and haplotypes of four single nucleotide polymorphisms in COMT were investigated in 179 incarcerated adolescent delinquents in Russia.
- Four hierarchical logistic regression models predicting the presence/absence of reading comprehension difficulties were fitted to the data; genetic variation in COMT and the presence/absence of maternal rejection were investigated as main effects and as effects acting interactively.
- Three out of four interaction terms were found to be important predictors of individual differences in comprehension





# COMT – Grigorenko study

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- there was no evidence of comprehension difficulties being associated either with maternal rejection or with any particular COMT genotype; in addition, there were no associations between specific COMT genotypes and maternal rejection.
- four sets of logistic regressions were investigated, one for each of the studied COMT polymorphisms. Specifically, these analyses were conceived to establish (a) the contributions of the genotypes at the four COMT polymorphisms to comprehension difficulties ( present = 1, absent =0), (b) the contribution of maternal rejection ( present =1, absent = 0) to comprehension difficulties, and (c) interactive contributions of genotypes and maternal rejection to comprehension difficulties.



# DAT1

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- Previous research has generated examples of how genetic and environmental factors can interact to create risk for psychopathology.
- Using a gene-by-environment (G × E) interaction design, they tested whether three polymorphisms in the dopamine transporter gene (DAT1, also referred to as SLC6A3, located at 5p15.33) interacted with maternal parenting style to predict first onset episodes of depression.
- Participants were male adolescents (176) recruited from a juvenile detention center in northern Russia.
- As hypothesized, one of the polymorphisms (rs40184) moderated the effect of perceived maternal rejection on the onset of major depressive disorder, as well as on suicidal ideation.
- Further, this G × E interaction was specific to depression; it did not predict clinically significant anxiety.



# DA in Schizophrenia

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- Heritability of Schizophrenia = 80%!
- Cognitive Impairments (remain stable) in WM, learning, verbal fluency, motor speed, executive functions
- fMRI studies of above: abnormalities of dorsolateral prefrontal cortex (DLPFC), medial temporal lobe, hippocampus, anterior cingulate, medial frontal and posterior parietal cortex, striatum, thalamus, cerebellum



# DA in Schizophrenia

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- Schizophrenics have increased release of DA and increased density and occupancy of D2R in striatum
- Most antipsychotics = block D2R (chlorpromazine, haloperidol, perphenazine – but all cause neurologic symptoms: tremor, rigidity, dystonia, dyskinesia)
- Improve positive symptoms (hallucinations, delusions)
- Very modest effect on cognitive symptoms (?!)



# COMT polymorphisms

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- COMT polymorphisms's role is debated in this and other psychiatric disorders – lots of negative/inconclusive evidence
- longitudinal study of the Dunedin birth cohort followed to adulthood carriers of the COMT Val allele were most likely to exhibit psychotic symptoms and to develop schizophreniform disorder if they used cannabis, whereas cannabis use had no such adverse influence on individuals with two copies of the Met allele
- a family-based association study of 240 ADHD sufferers, bearers of the Val/Val genotype were substantially more susceptible to the adverse effects of prenatal risk (indexed by low birth weight) in influencing risk of early-onset antisocial behaviour.

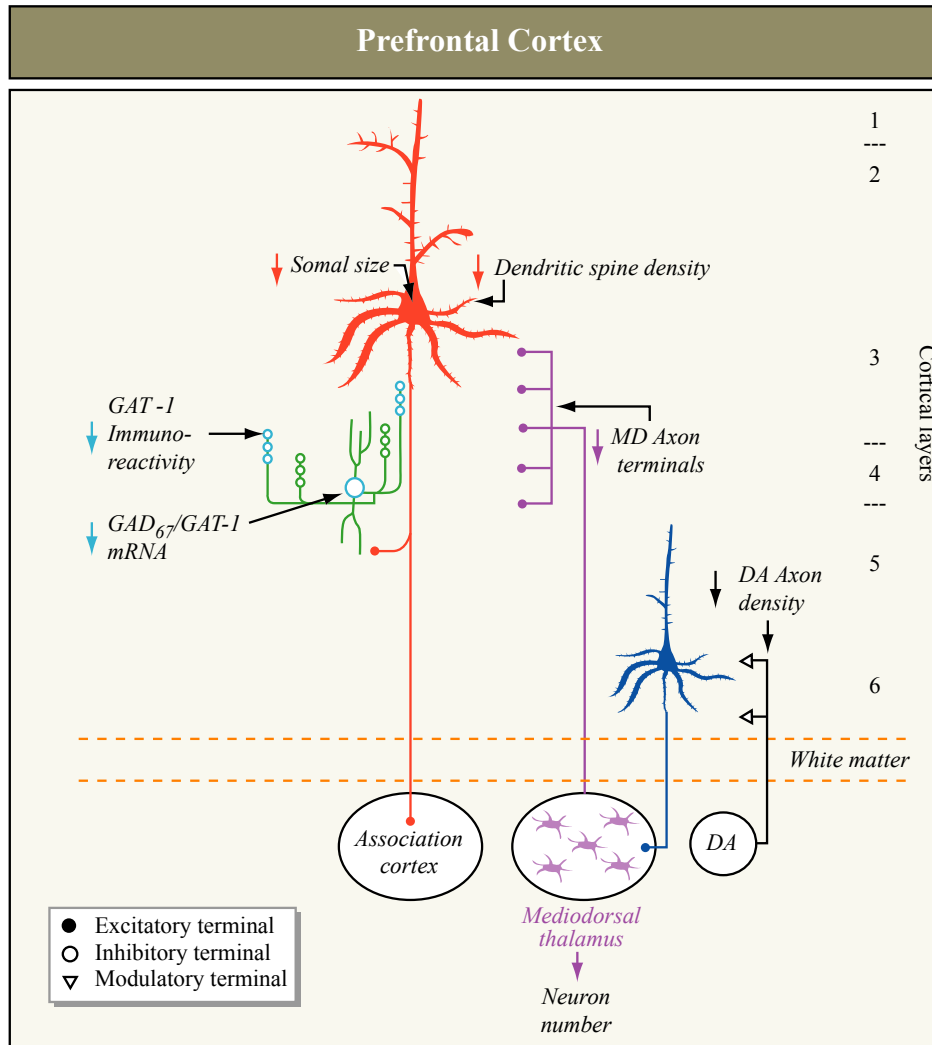
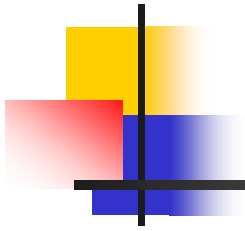
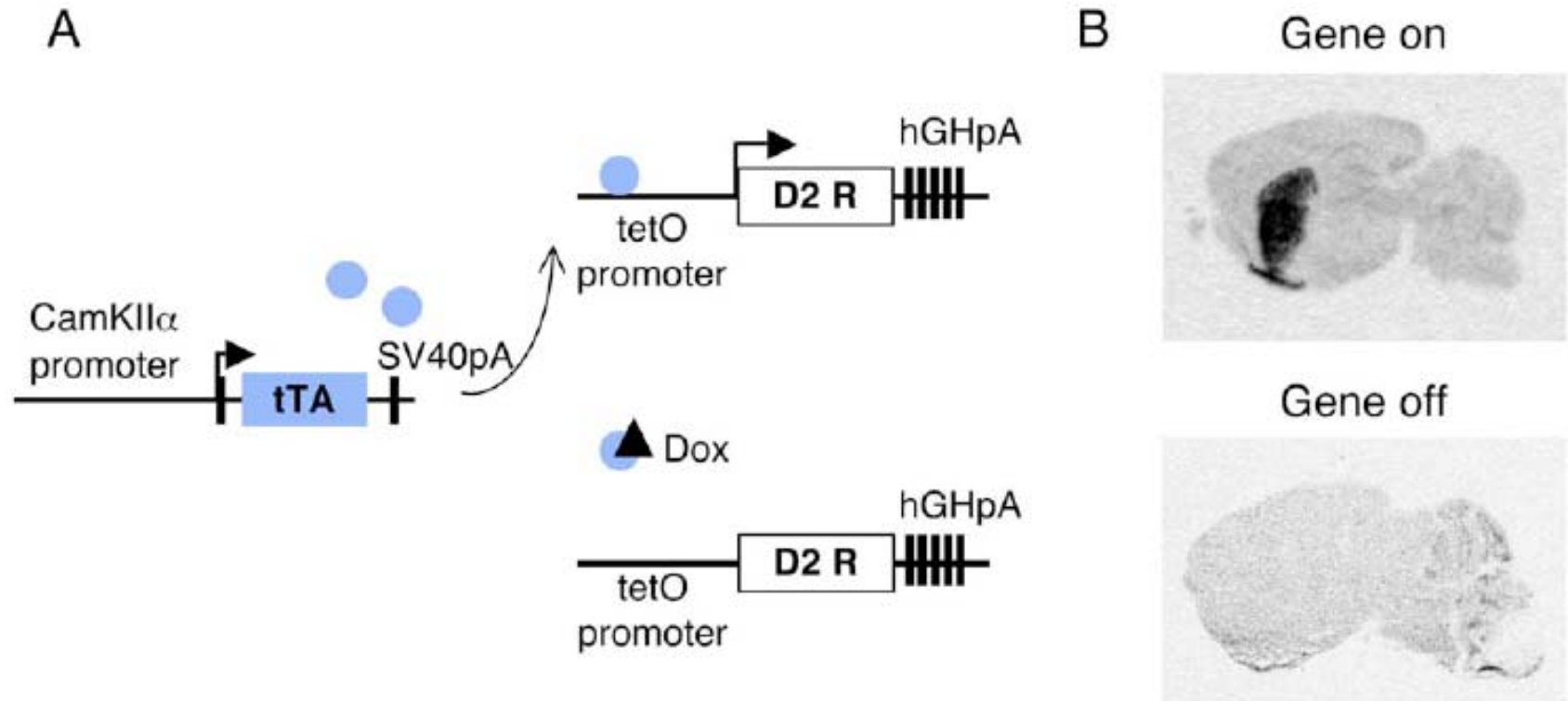


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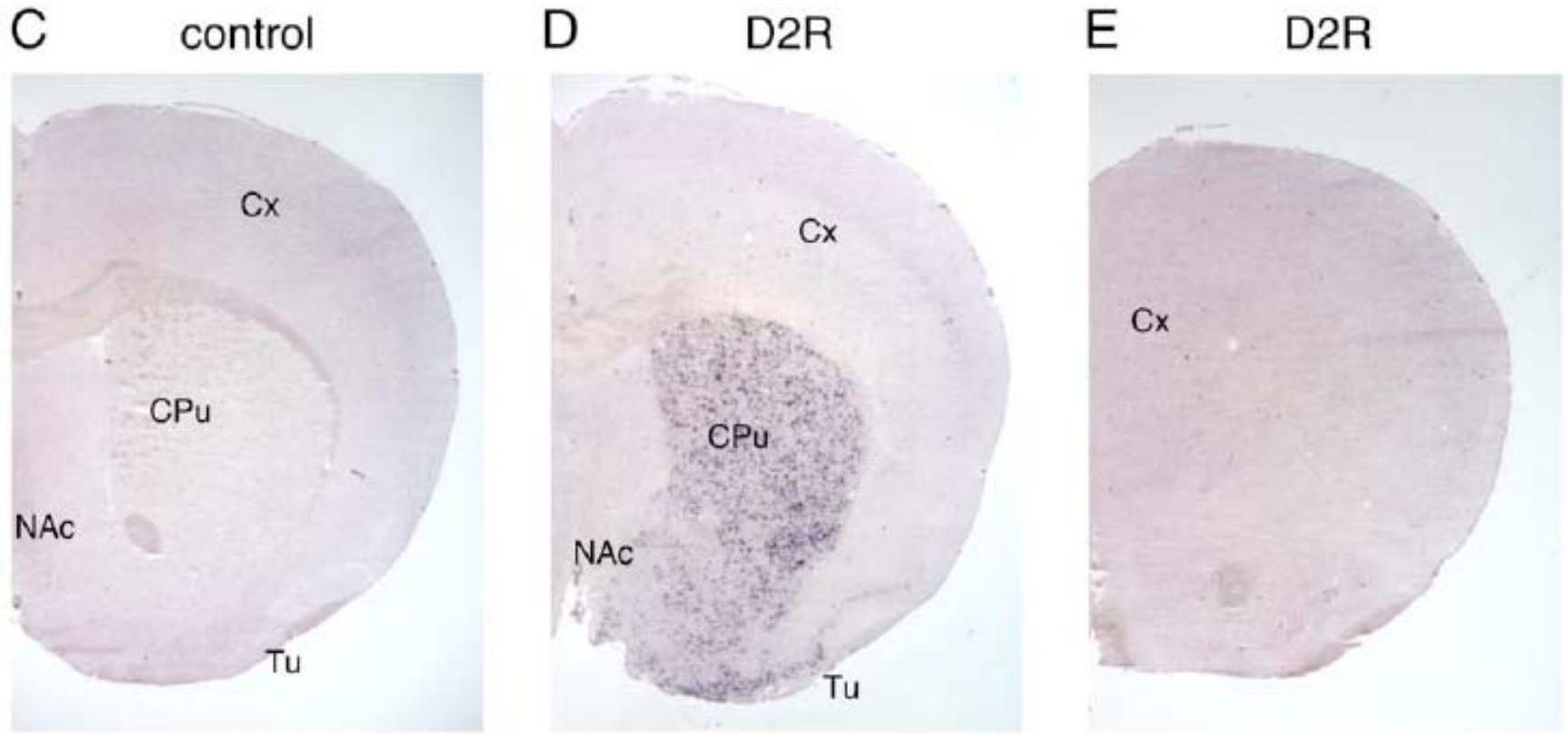
# D2R overexpression



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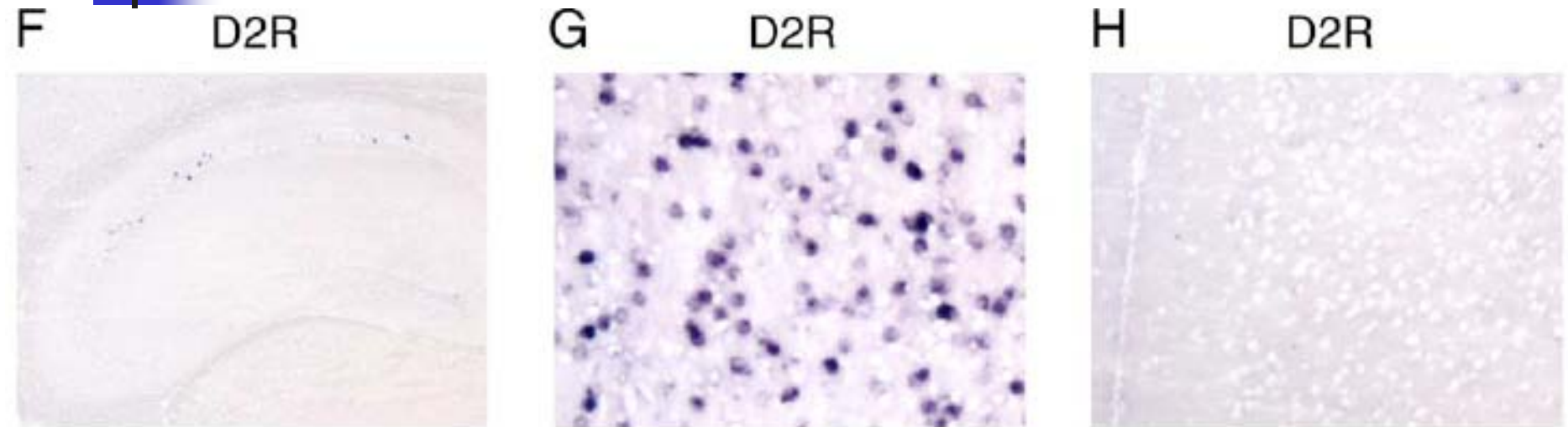
when crossed to CamKII $\alpha$ -tTA mice, about 20% of tetO responder lines give rise to tetO-driven transgene expression restricted to the striatum and olfactory tubercle

# Transgene expression in striatum





# Transgene expression



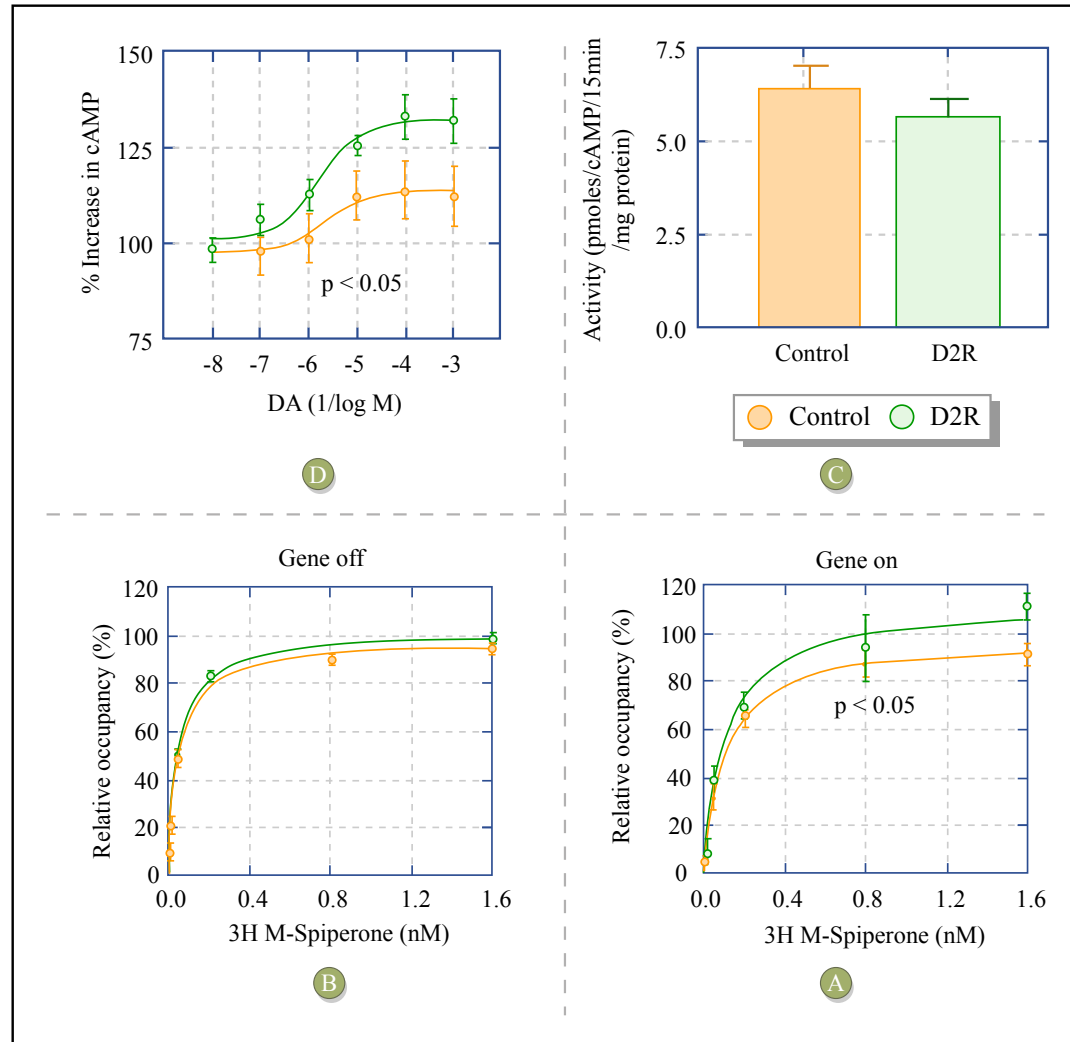
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**(F) Transgene expression in the hippocampus ().**

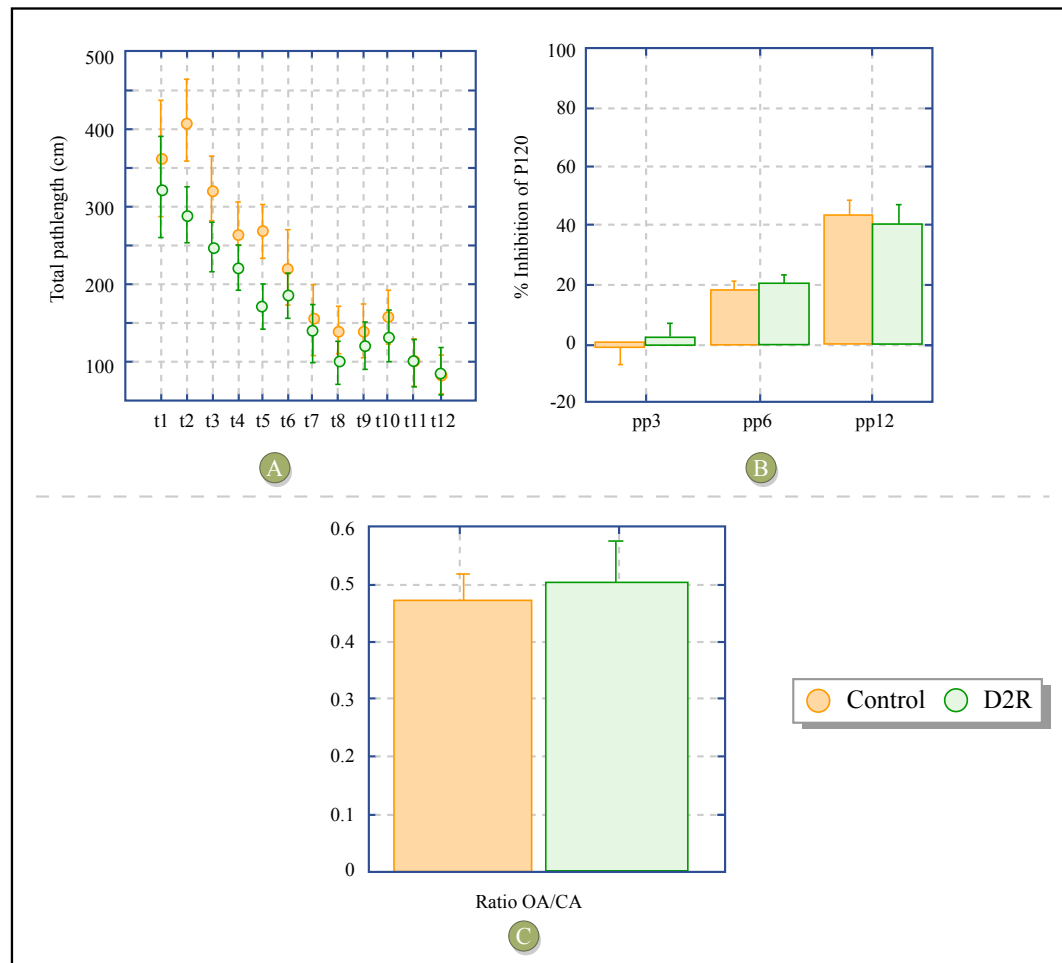
**(G) Approximately 30% of striatal cells express transgenic mRNA ().**

**(H) Transgene expression in the medial PFC ().**

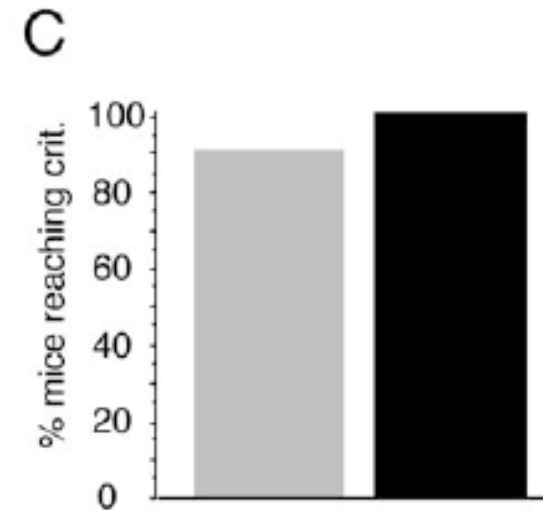
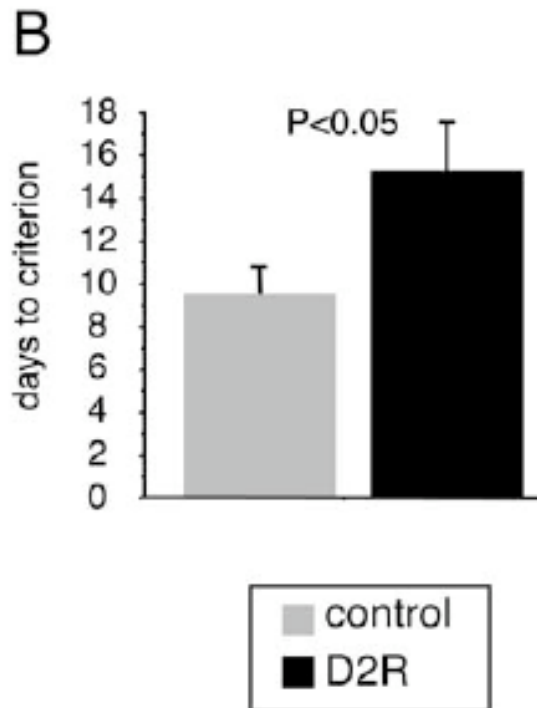
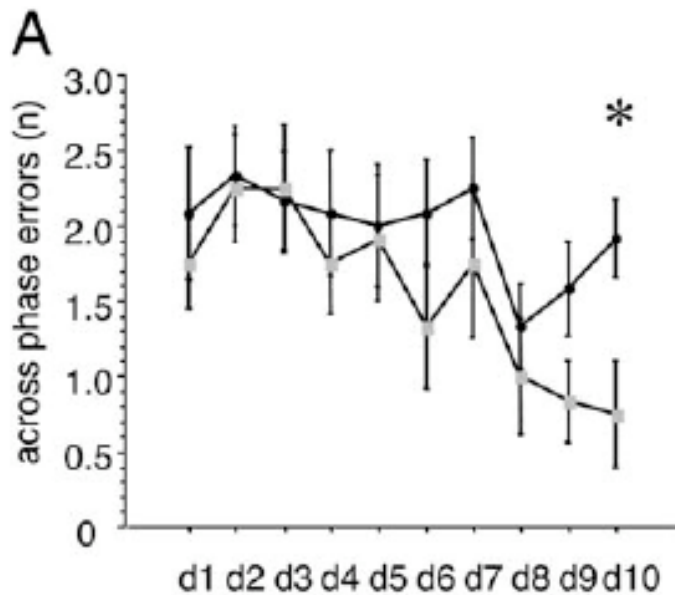
# Transgenic D2R functional



# D2R mice show unaltered locomotor activity, sensorimotor gating, generalized anxiety

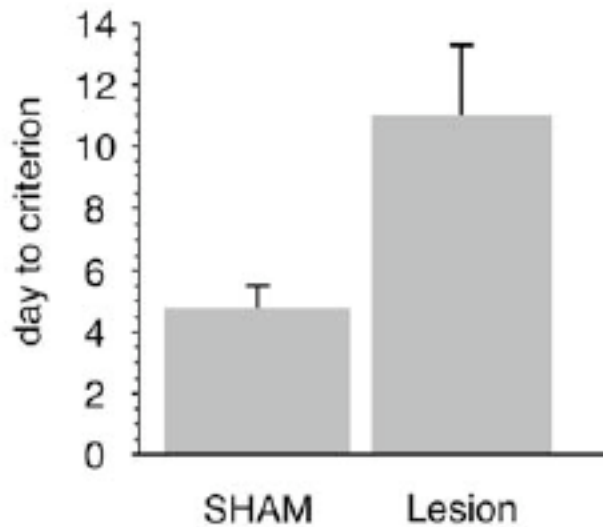


# D2R mice show deficits in WM

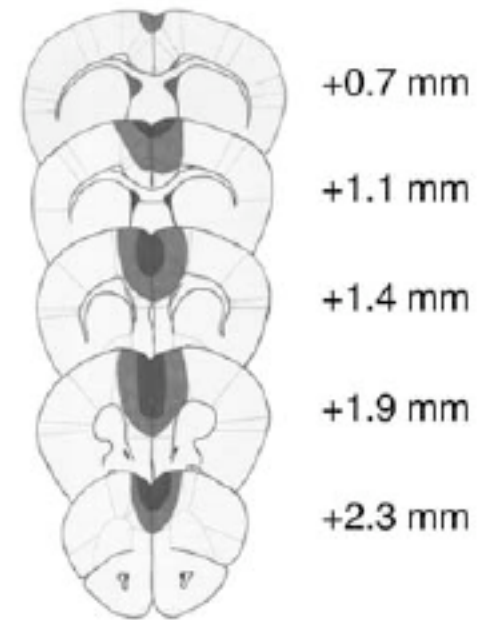


# Role of PFC?

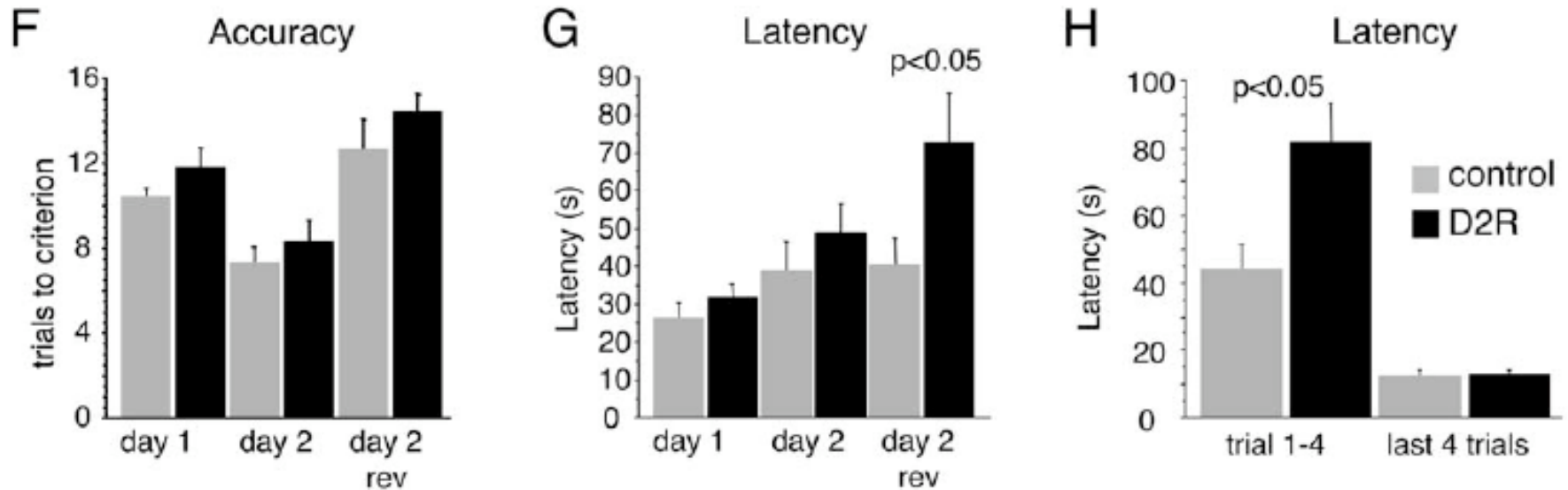
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E



# Set-shifting test – olfactory discrimination deficit in behavioral flexibility



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# Reversal of overexpression doesn't reverse deficit

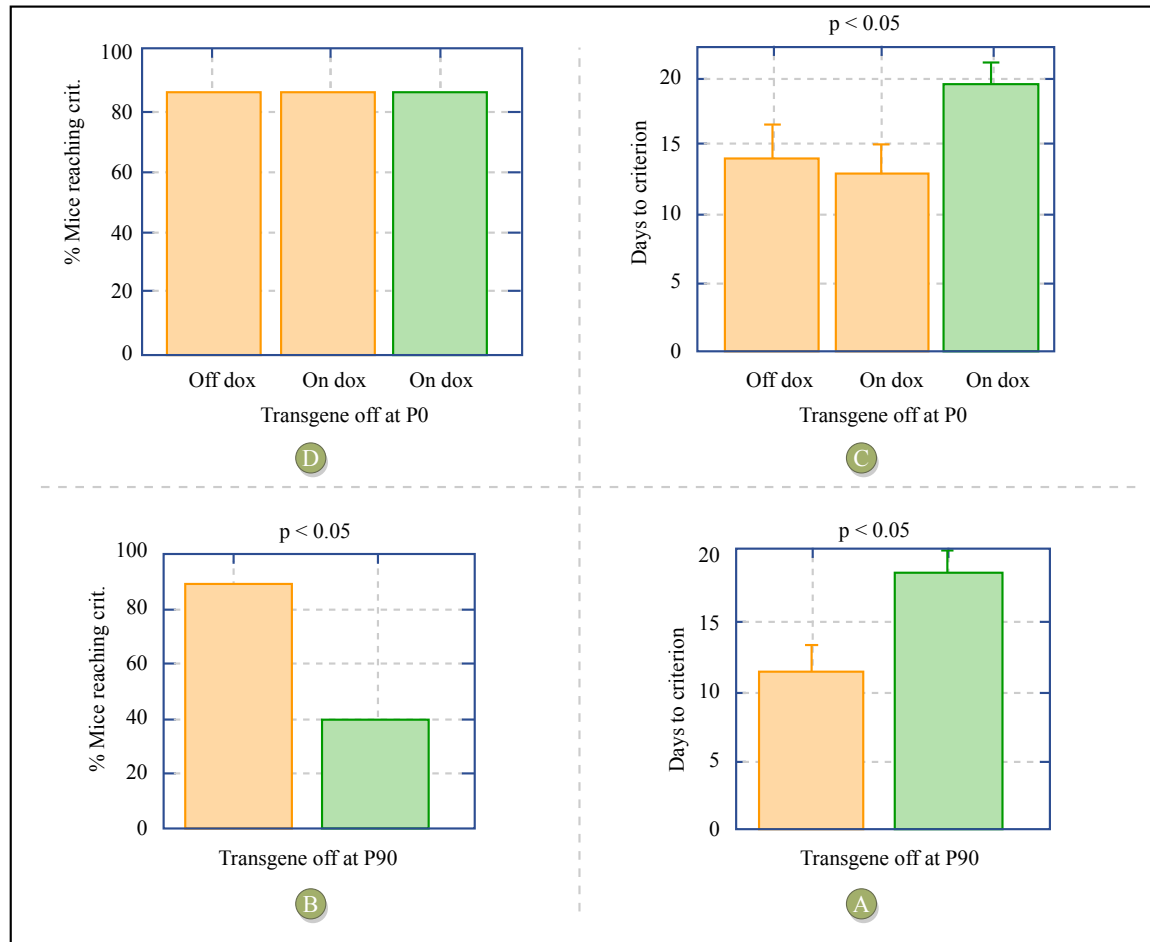
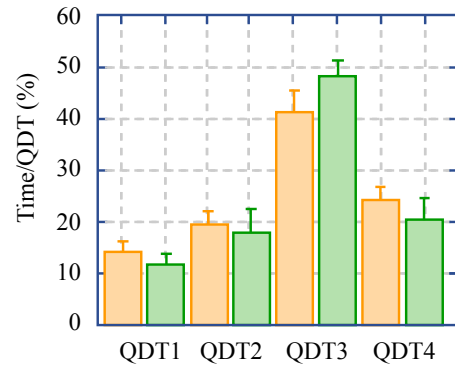
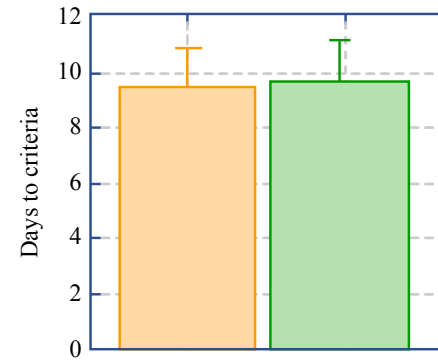


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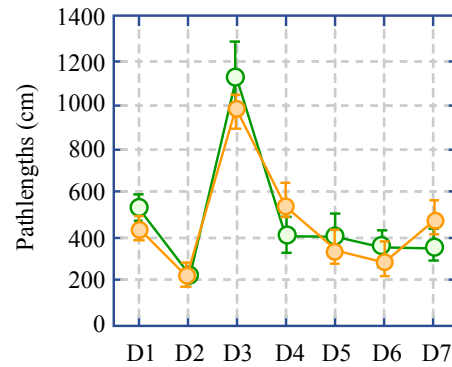
# D2R no 'general' cognitive deficit



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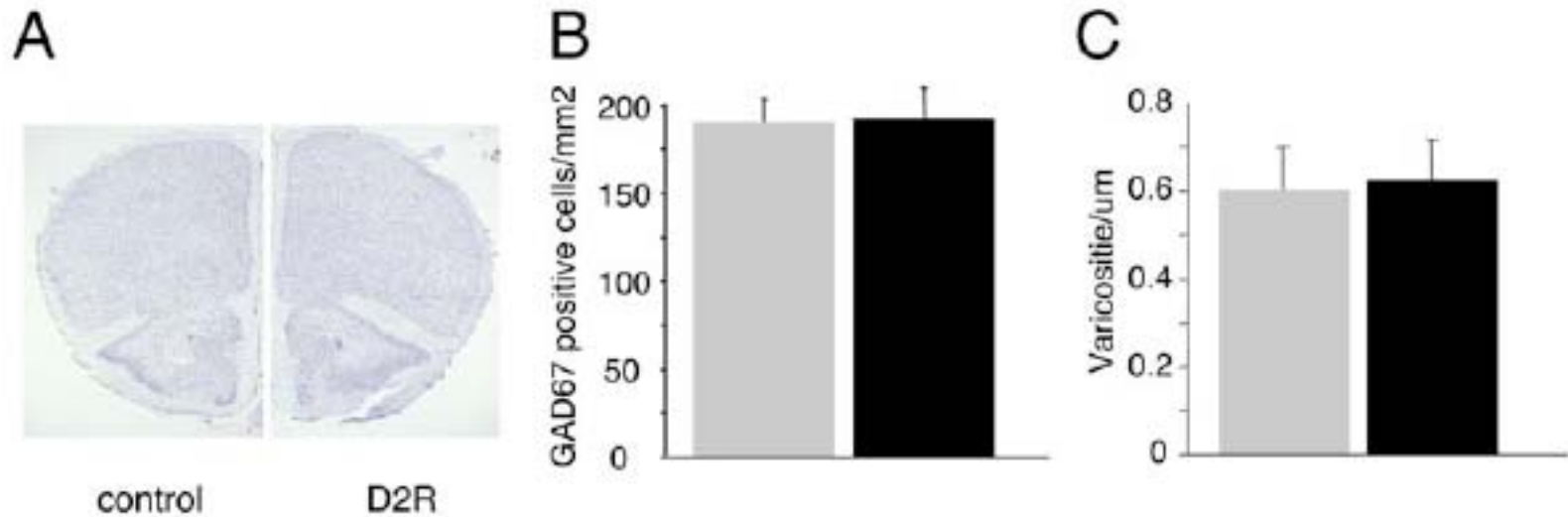


# D2R altered glucose metabolism

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- Basal metabolic activity via reupdate of radiolabeled 2-deoxy-glucose.
- Supplement figure – reduction in basal metabolic activity in caudate putamen and increases in primary motor and somatosensory cortices.
- But reversible with 2 weeks doxycycline – supplemental figure.
- So that's NOT why T maze deficits

# D2R no morphological changes, but altered DA transmission



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# Decrease in DA turnover

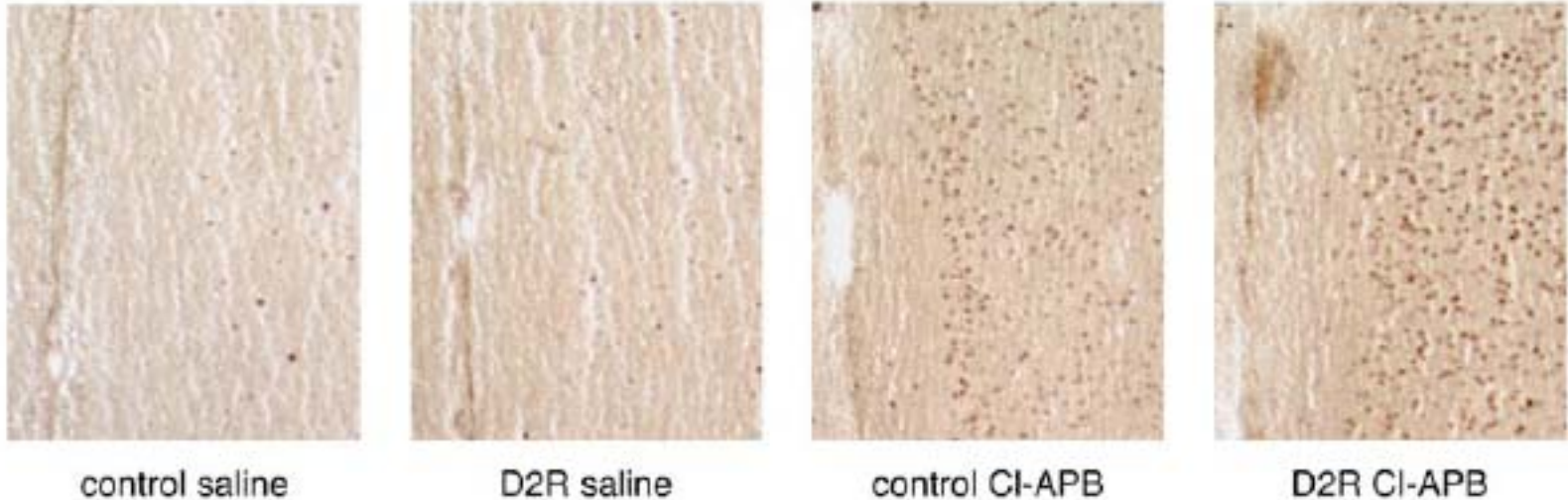
		Dopamine	HVA	DOPAC	HVA/DA	DOPAC/DA
CONTROL	Mean	3.79	2.72	0.29	0.84	0.24
	Std err	0.89	0.69	0.05	0.18	0.07
	n	17	17	14	17	
D2-R	Mean	6.68*	2.02	0.35	0.47*	0.14*
	Std err	1.78	0.35	0.08	0.08	0.06
	n	14	14	8	14	

\* Significant effect of genotype ( $p < 0.05$ )

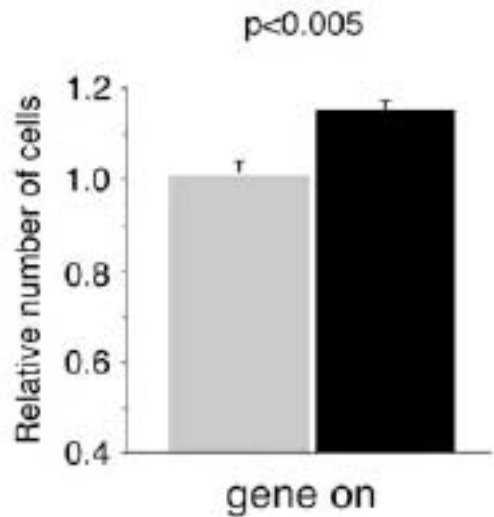
Tissue concentrations of dopamine and its metabolites in the medial frontal cortex.

# D2R affects D1R activation

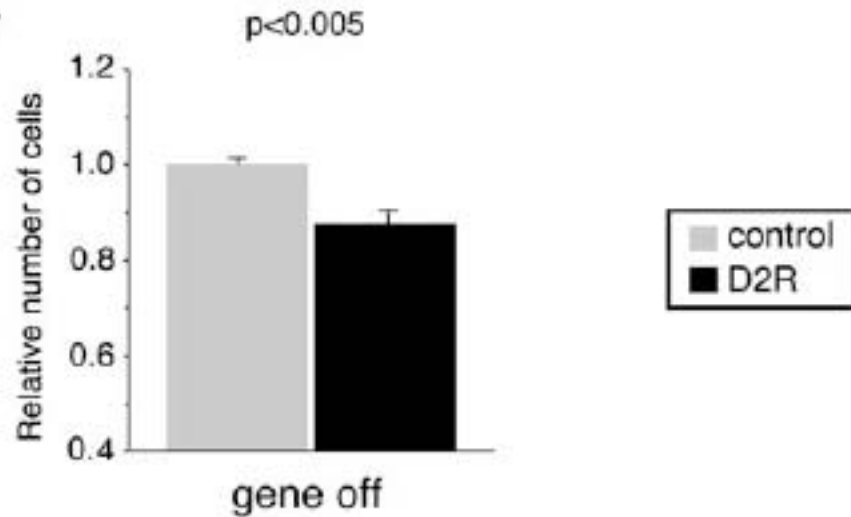
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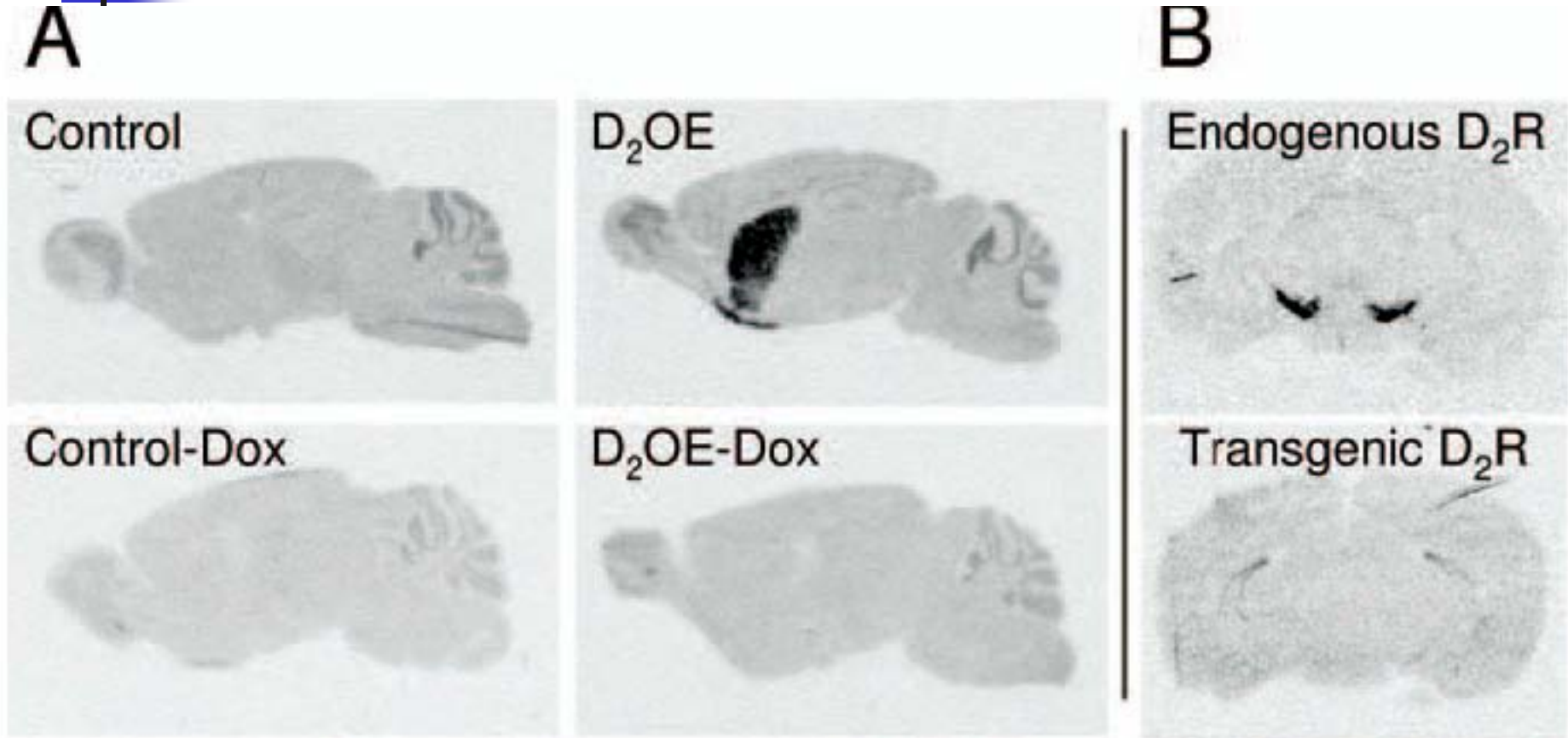


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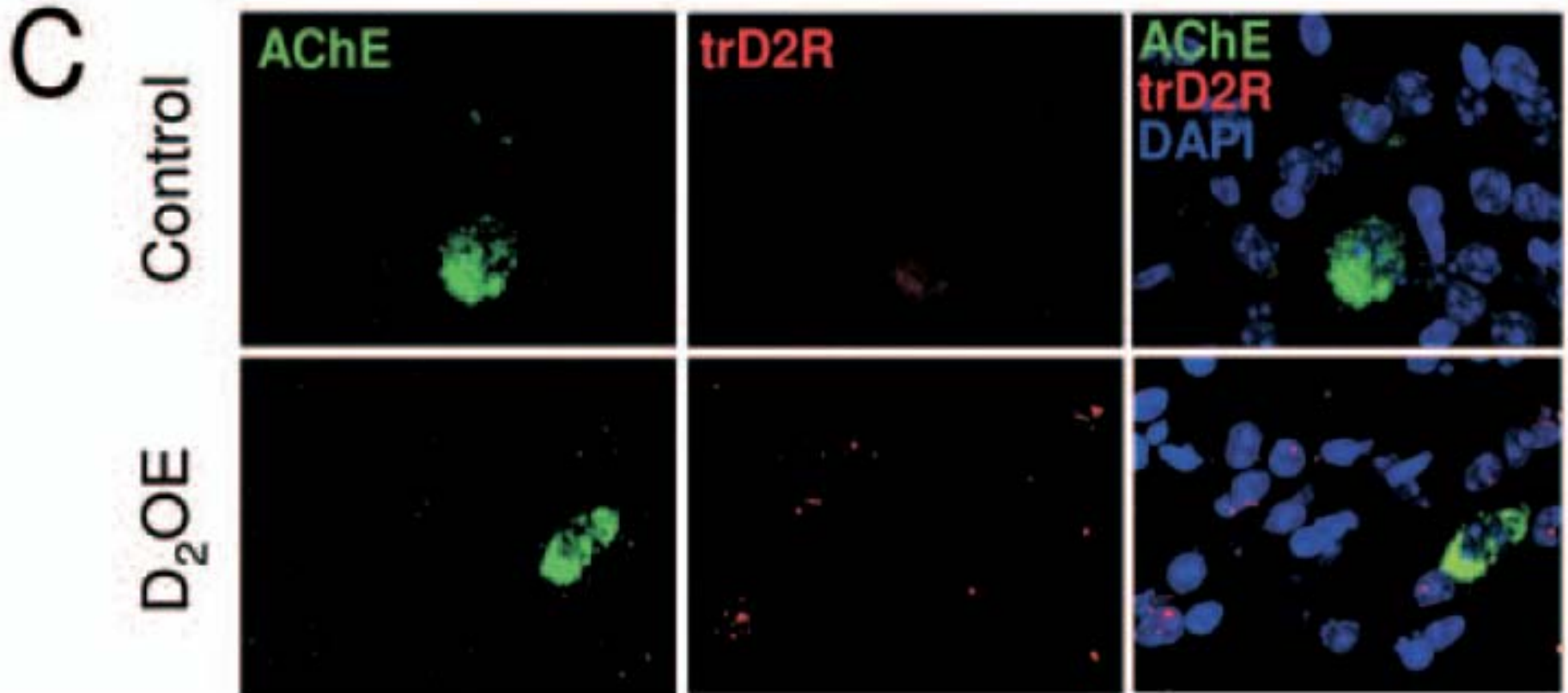
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- Transient overexpression of D2R impairs operant motivation and interval timing (internal clock)

# Transient and specific D2R overexpression



Courtesy of Journal of Neuroscience. Used with permission.

# No transgenic mRNA in AchE striatal interneurons



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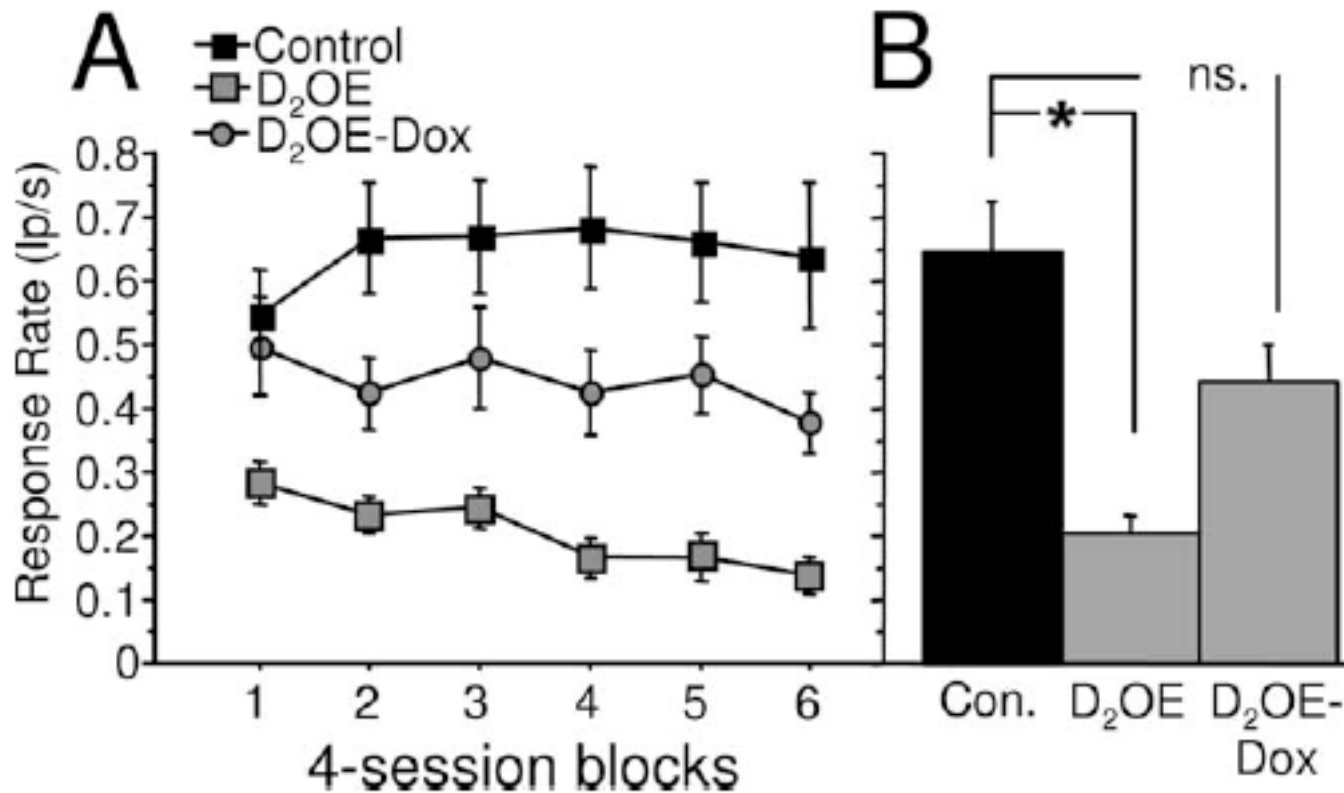
# Operant level press

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- Trained to consume liquid rewards from dipper, with trials and breaks in between of variable lengths
- Mouse learns to make lead entries during 20/30 dipper presentations
- Trained to press level to get liquid
  
- Fixed interval (FI) training – presses reinforced at fixed interval
- Peak interval training – 24s interval timing
- Progressive ration – asses amount of effort a mouse is willing to spend to get reward. Criterion doubles with trials – measure breaking point.

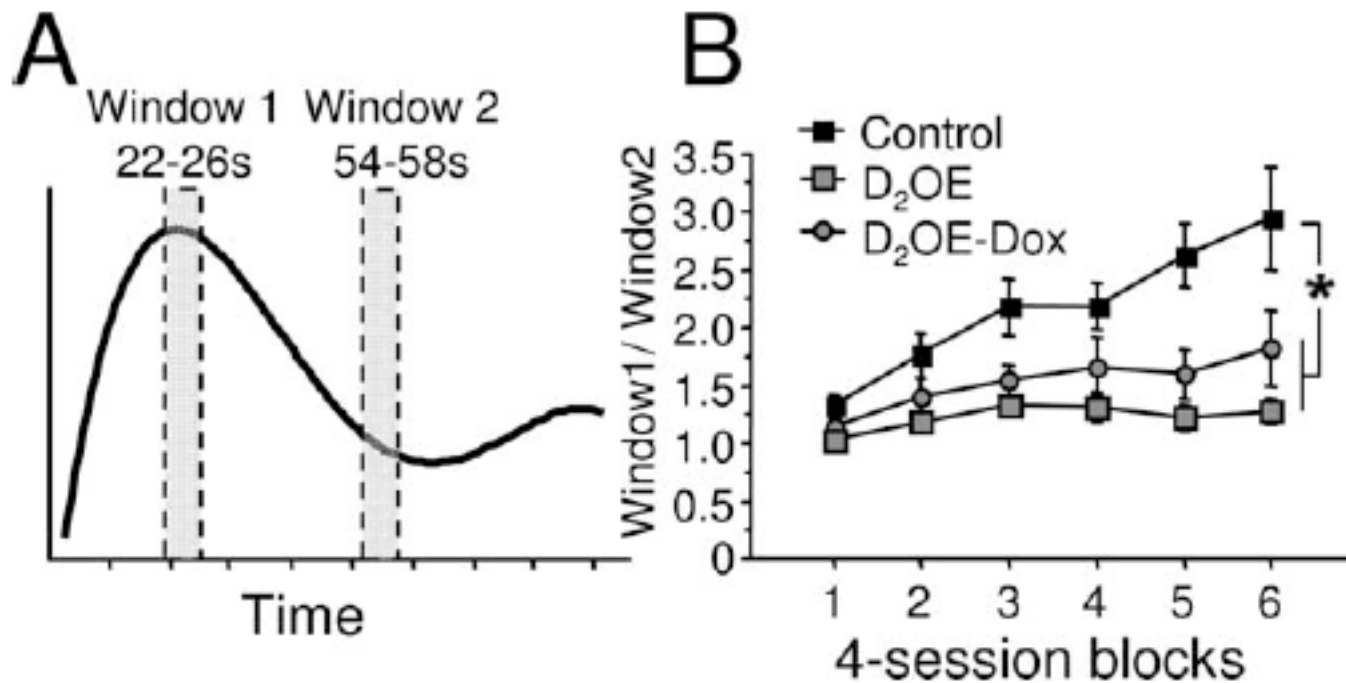


# D2R reduces operant responding



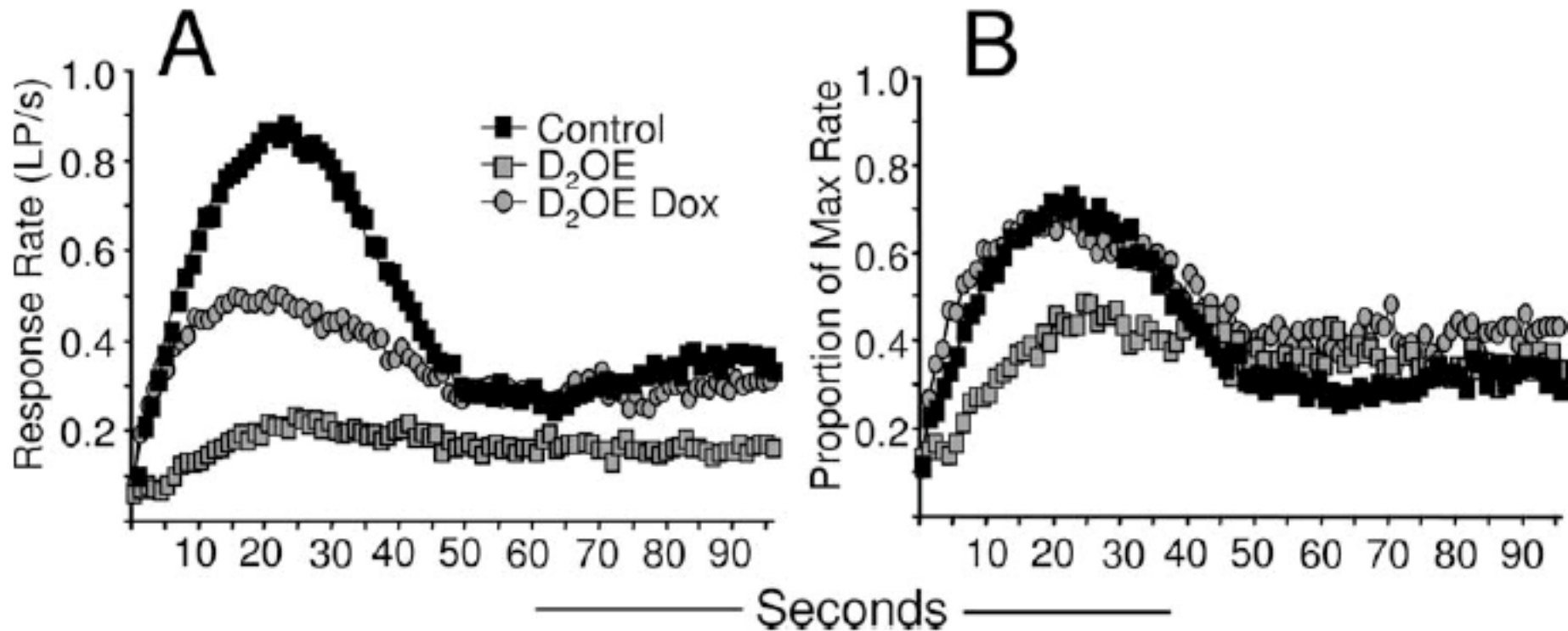
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# Ratio of response rates

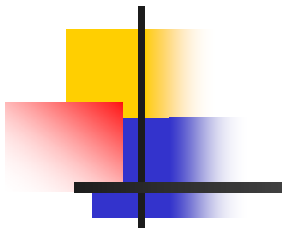


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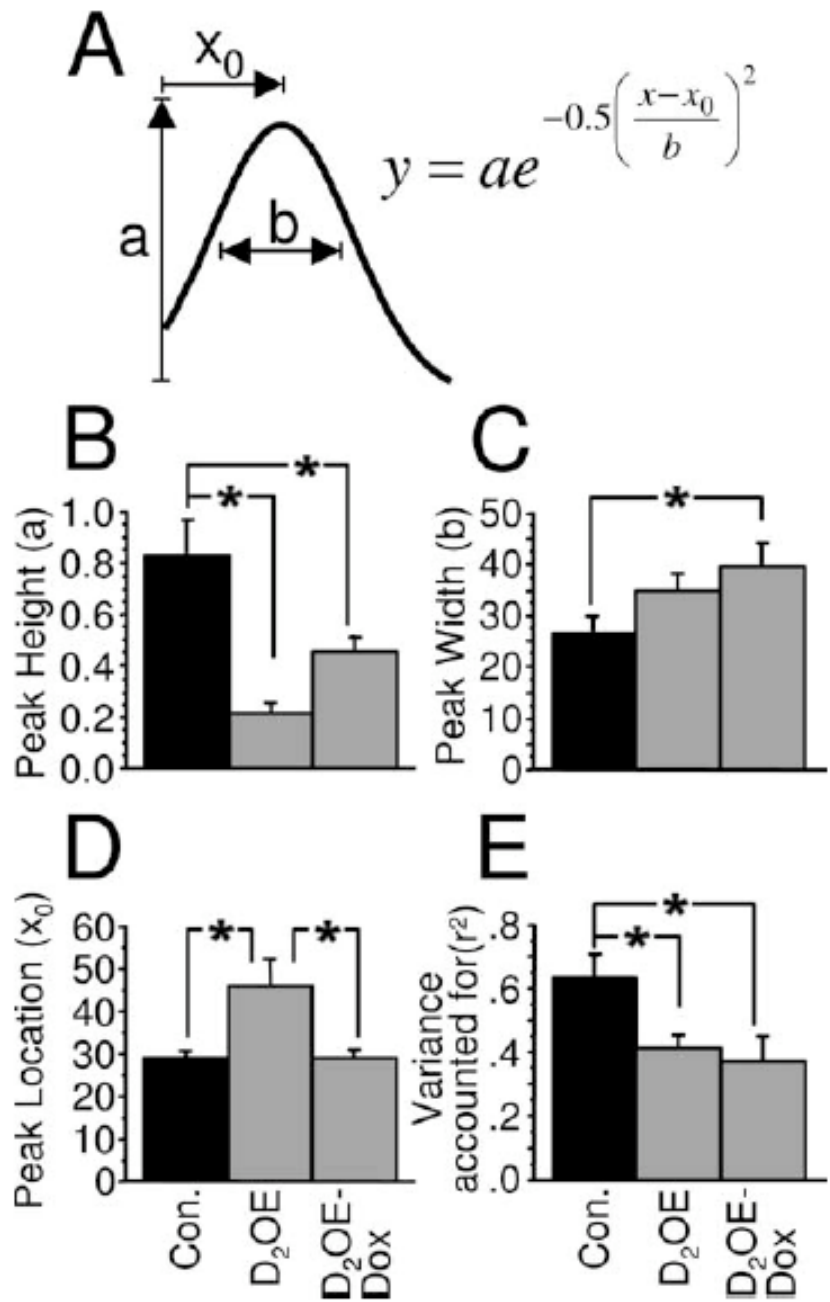
# Distribution of all level presses – peak interval training



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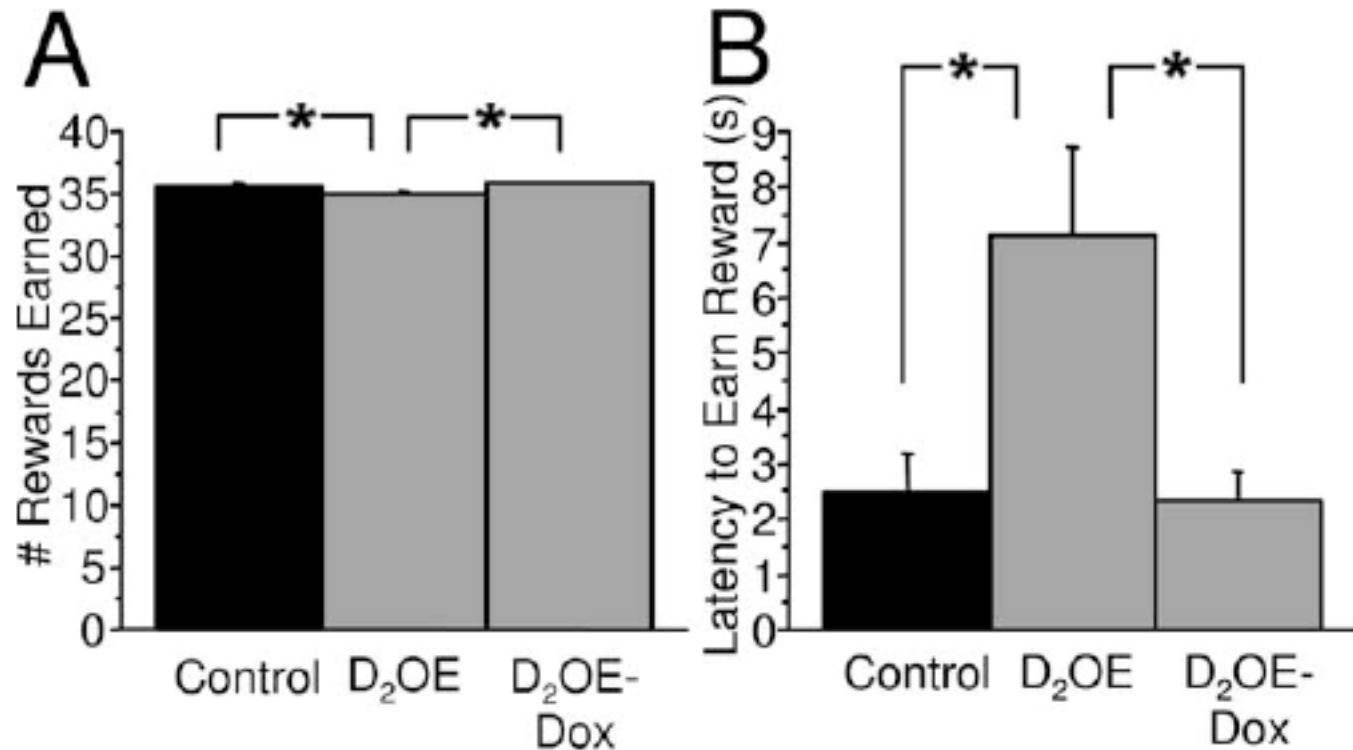


# Quantifying timing deficit



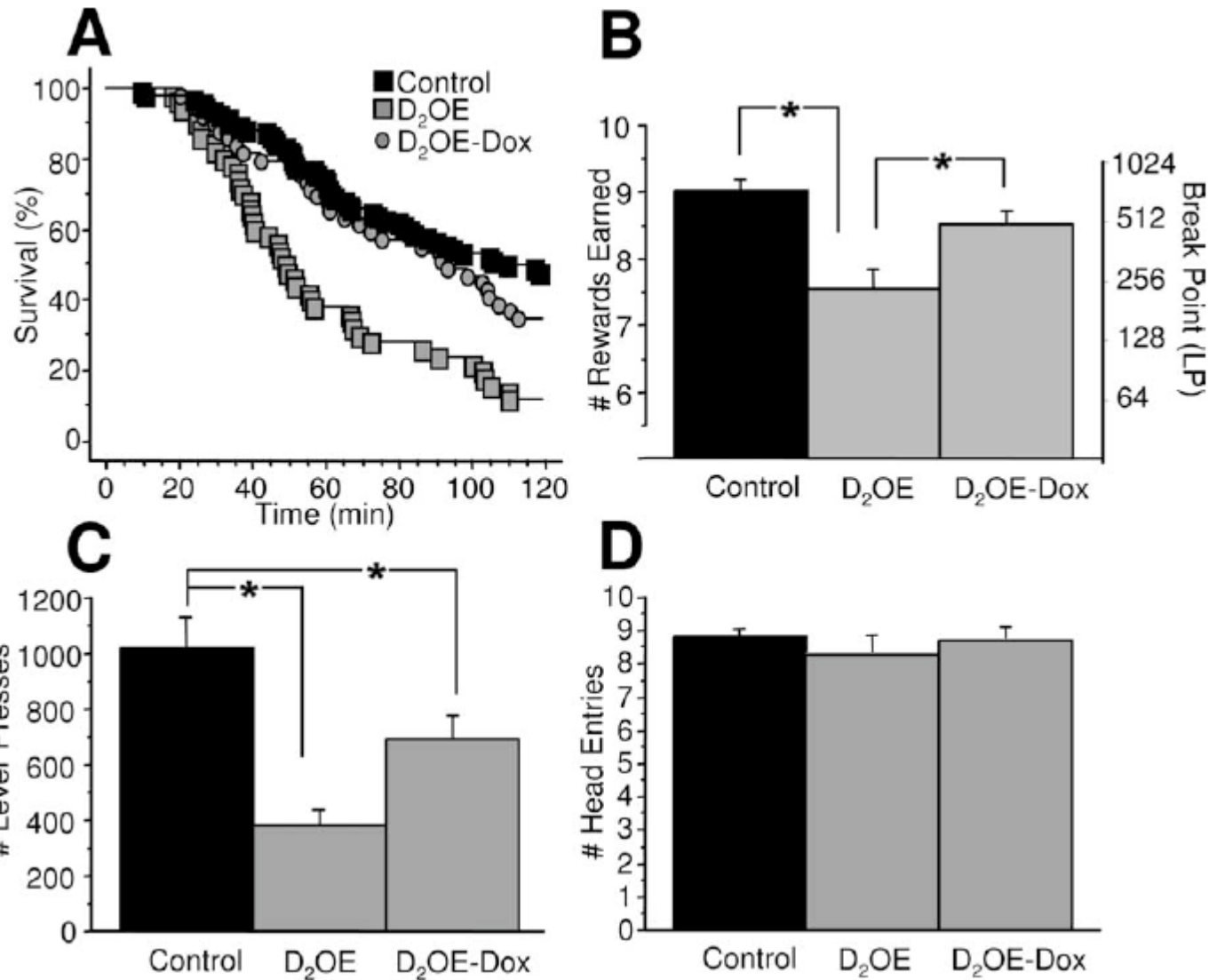
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# Timing impairments not due to failure to earn rewards!



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# D2R reversible impairment in operant motivation



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# D2R not alter sucrose preference



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# Summary

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- Reversing D2R overexpression in adult mouse did not fully rescue timing deficits – so those appear from excess expression during development
- In patients, neuroleptic drugs don't completely ameliorate those impairments either
- Motivational phenotype relevant to drug addiction (those from families with alcoholism, if higher D2R levels – less likely to be alcoholics)
- D2R overexpression – reduce incentive motivation, not hedonic





# Ed's puzzle:

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- Amphetamine increased activity
  - inhibited by D2 blockers
  - Inhibited by GSK3 beta inhibitors – increase catenin, decrease fos in striatum
- NMDA blockade (mk801)
  - Inhibited by D2 blockers – antipsychotics
  - BUT NOT inhibited by GSK3 beta inhibitors
  - Inhibited by PDE10 inhibitor (gamp, gmp, striatum, cortex)
  - Inhibited by Glu2,3 agonist



# DAT KO

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- Severe DA dysregulation (300 time increase in extracellular DA lifetime)
- But in DAT-KO mice – DA levels on 5 times higher – 95% reduction in content and 75% reduction in release
- DA synthesis rates doubled despite 90% decrease in tyrosine hydroxylase with enhanced degradation
- Hyperlocomotion (rescued by GSK3 inhibitors and lithium)
- Perseverative patterns of locomotion attenuated by D1R antagonist (not D2R antagonist)
- PPI deficit (rescued by D2R antagonist, but not D1R antagonist)
- Altered hedonic mechanisms

# Nicotine effective in normalizing hyperactivity in DAT-KO



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- Locomotion
- morris water maze



# DAT overexpression

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- On 30% upregulation, mice show spontaneous hypoactivity in novel environment (reverse phenotype)



# DARPP32

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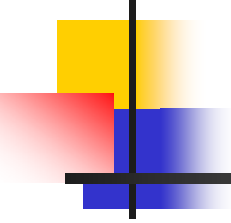
- Dopamine- and cAMP-regulated phosphoprotein, Mr 32 kDa (DARPP-32)
- major target for dopamine and protein kinase A (PKA) in striatum.
- regulation of the state of DARPP-32 phosphorylation provides a mechanism for integrating information arriving at dopaminergic neurons, in multiple brain regions, via a variety of neurotransmitters, neuromodulators, neuropeptides, and steroid hormones



# DARPP-32 regulates PP-1 and PKA

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- Activation of PKA or PKG stimulates DARPP-32 phosphorylation at Thr34 and thereby converts DARPP-32 into a potent inhibitor of protein phosphatase-1 (PP-1).
- DARPP-32 is also phosphorylated at Thr75 by Cdk5 and this converts DARPP-32 into an inhibitor of PKA.
  - Thus, DARPP-32 has the unique property of being a dual-function protein, acting either as an inhibitor of PP-1 or of PKA.
- The state of phosphorylation of DARPP-32 at Thr34 depends on the phosphorylation state of two serine residues, Ser102 and Ser137, which are phosphorylated by CK2 and CK1, respectively.

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- Used as marker for striatal spiny neurons (in birds, also marker for layer 6 in mice?)
  - That are inhibitory and use GABA



# DARPP-32 AND CDK5

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- CDK5 - Cyclin-dependent kinase
- The association of Cdk5 with one of its neuron-specific co-activators, p35 or p39, is required in processes such as neurite outgrowth, axonal migration, cortical lamination, control of cell adhesion, axonal transport, synaptic activity, neuronal adaptive changes and motor functions
- DARPP-32 is a Cdk5 substrate that plays a key role in the biology of medium spiny dopaminergic GABAergic neurons in the striatum, a nevralgic structure modulating voluntary motor movements and motivational behavior.
- phosphorylation of protein phosphatase inhibitor- 1 (PPI-1) by Cdk5 lowers the efficiency of PPI- 1 phosphorylation by PKA





# DARPP-32 AND CDK5

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- DARPP-32 and PPI-1 are thus bifunctional signal transduction elements which modulate PKA signaling in response to their phosphorylation by Cdk5.
- D1 and D2 dopamine receptors found on the GABAergic dopaminergic medium spiny neurons of the striatum regulate motor behavioral functions by initiating the mutual and antagonistic actions of Cdk5 and PKA through balancing of kinase and phosphatase activities.
- In the neo-striatum, activation of metabotropic glutamatergic receptors increases Cdk5 and casein kinase-1 activity, resulting in the phosphorylation of DARPP-32 at Thr 75 and Ser 137, respectively.
- Kinase inhibitor studies on neostriatal slices indicate that casein kinase-1 may function upstream of Cdk5