La ricerca di base nel trattamento delle psicosi con metodiche di *gene imaging*

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Overview

- Cosa è la psichiatria traslazionale?
- Cosa è il trascrittoma? In che modo la schizofrenia è una patologia della espressione genica?
- Come si studia l'espressione genica?
- In che modo la ricerca traslazionale può essere di aiuto nella pratica psichiatrica clinica?

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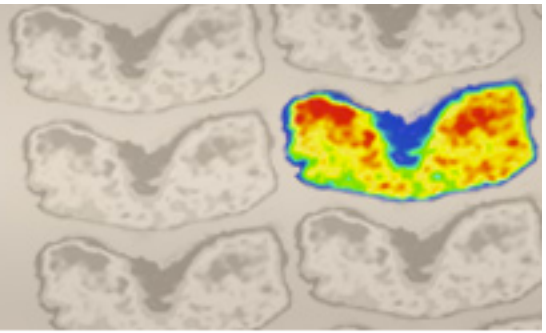
Translational Psychiatry



Aims and Scope

Top

Psychiatry has suffered tremendously by the limited translational pipeline. Nobel laureate Julius Axelrod's discovery in 1961 of monoamine reuptake by pre-synaptic neurons still forms the basis of contemporary antidepressant treatment. There is a grievous gap between the explosion of knowledge in neuroscience and conceptually novel treatments for our patients. *Translational Psychiatry* bridges this gap by fostering and highlighting the pathway from discovery to clinical applications, healthcare and global health. We view translation broadly as the full spectrum of work that marks the pathway from discovery to global health, inclusive. The steps of translation that are within the scope of *Translational Psychiatry* include (i) fundamental discovery, (ii) bench to bedside, (iii) bedside to clinical applications (clinical trials), (iv) translation to policy and health care guidelines, (v) assessment of health policy and usage, and (vi) global health. All areas of medical research, including — but not restricted to — molecular biology, genetics, pharmacology, imaging and epidemiology are welcome as they contribute to enhance the field of translational psychiatry.

Bench to Bedside

1. Translate basic scientific findings into therapeutic interventions

2. Increase understanding of basic disease processes

Targeting glutamate system for novel antipsychotic approaches: Relevance for residual psychotic symptoms and treatment resistant schizophrenia

Andrea de Bartolomeis [a], Chiara Sarappa [a], Salvatore Magara [a,b], Felice Iasevoli [a,*]

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[b] Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

Table 1
Overview of the most studied glutamate receptors' modulators.

Drugs	Mechanism of action	Doses	Therapeutic indication (FDA)	Clinical studies
Glycine	NMDA-Rs co-agonist	800 mg/kg/day	Solution for endoscopic irrigation and intravenous injection; antioxidant drugs	Potkin et al., 1999; Tsai et al., 1999; Javitt et al., 2001; Heresco-Levy and Javitt, 2004; Diaz et al., 2005; Tuominen et al., 2005; Buchanan et al., 2007.
D-serine	NMDA-Rs co-agonist	30 mg/kg/day	Food integrator	Tanii et al., 1994; Lanza et al., 1997; Tsai et al., 1998; Heresco-Levy and Javitt, 2004; Heresco-Levy et al., 2005; Tuominen et al., 2005; Lane et al., 2005; Olsen et al., 2006; Buchanan et al., 2007; Chiusaroli et al., 2010.
D-cycloserine	NMDA-Rs co-agonist	50 mg/day	Anti-tubercular drugs	Fletcher and MacDonald, 1993; Banerjee et al., 1995; McCoy and Richfield, 1996; Goff et al., 1999a,b; Heresco-Levy et al., 2002; Evins et al., 2002; Tuominen et al., 2005; Goff et al., 2008a,b;
Sarcosine	Gly-Transporter inhibitor	2 g/day	Manufacturing biodegradable surfactants and toothpastes	Tsai et al., 2004a,b; Lane et al., 2005,2006;
CX516	AMPAkine	–	–	Nishikawa et al., 1983; Deakin et al., 1989; Johnson et al., 1999; Gao et al., 2000; Goff et al., 2001.
ADX47273	mGluR5 agonist	–	–	Liu et al., 2008.
Memantine	NMDA-Rs partial trapping blocker	5 mg/kg/week	Alzheimer Disease (FDA)	Thomas et al., 2005; Carpenter et al., 2006; Krivoy et al., 2008; de Lucena et al., 2009

Table 2
Augmentation strategies involving glutamate receptors' modulators subdivided for the class of antipsychotics.

Drugs	Add-on to Clozapine	Add-on to Atypical Antipsychotics	Add-on to Typical Antipsychotics
Glycine	No augmentation effect	Improvement in negative and cognitive symptoms	Improvement in negative and cognitive symptoms
D-serine	No augmentation effect	Reduction of positive, negative symptoms. Improvement in depressive and cognitive symptoms	Reduction of positive, negative symptoms. Improvement of depressive and cognitive symptoms
D-cycloserine	Worsening of negative and positive symptoms	Reduction of negative symptom	Reduction of negative symptom; improvement in global performance
Sarcosine	No augmentation effect	Improvement in positive, negative and cognitive symptoms	Improvement in positive, negative and cognitive symptoms
CX516	Improvement in negative symptoms and in cognitive and memory tasks	Non specific improvement	Non specific improvement
Memantine	Clinical Improvement	Non specific improvement. Higher incidence of adverse effects	Non specific improvement

Targeting glutamate system for novel antipsychotic approaches: Relevance for residual psychotic symptoms and treatment resistant schizophrenia

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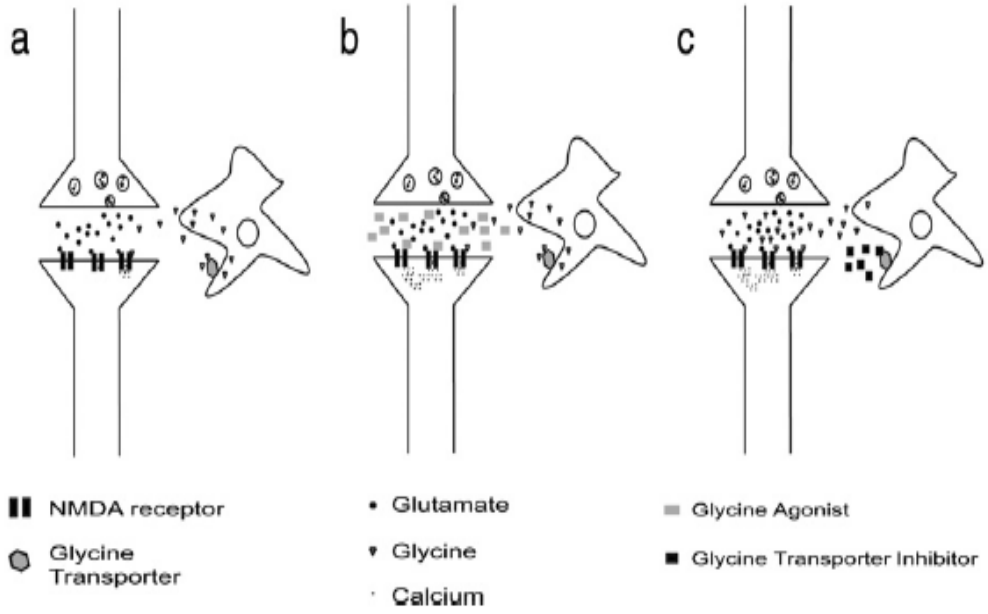
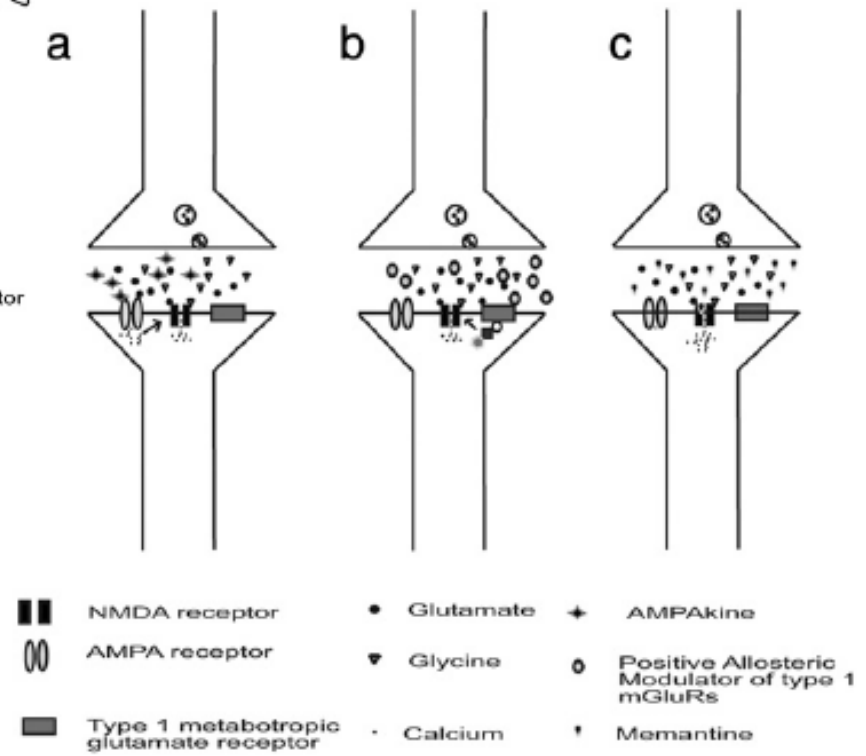


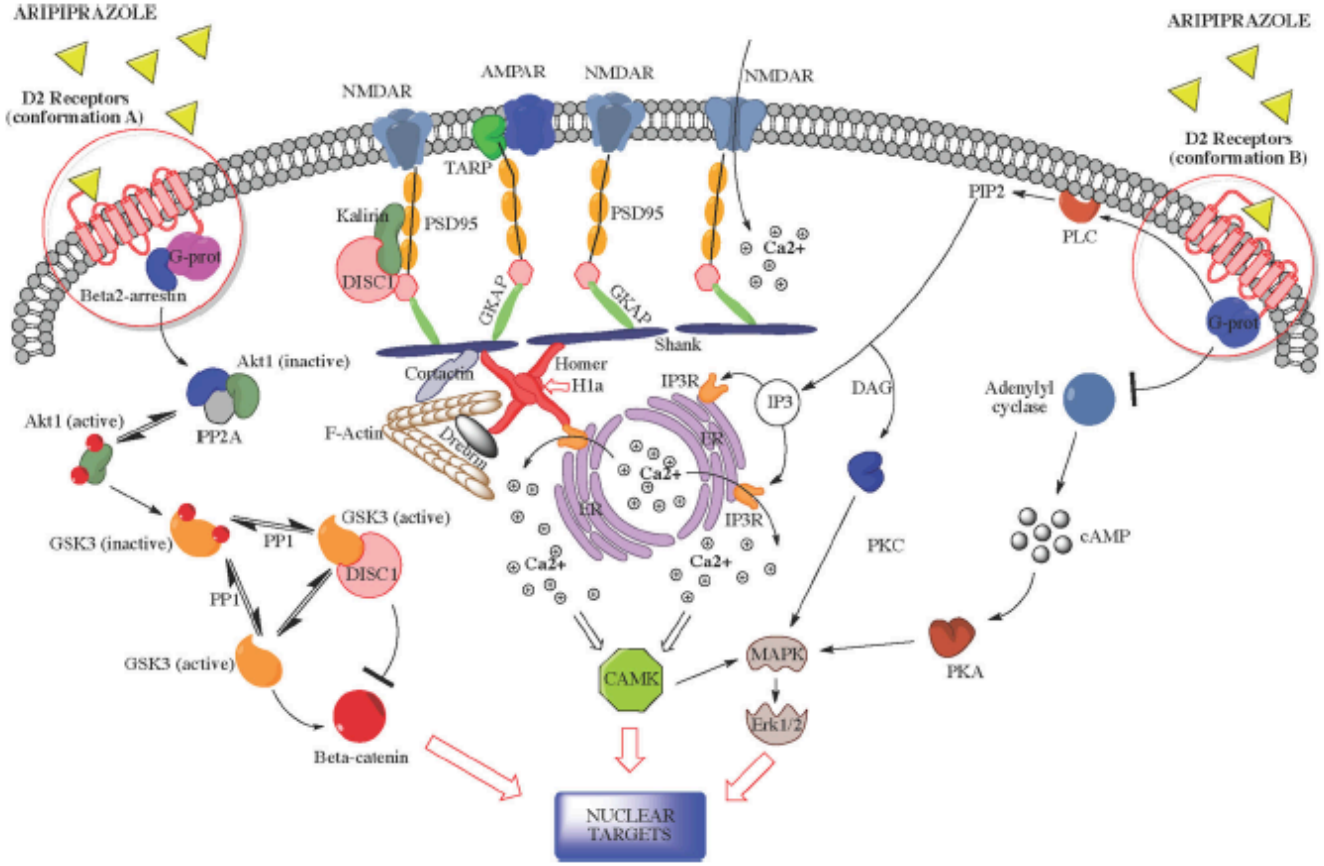


CNS Drugs (2015) 29:773–799
DOI 10.1007/s40263-015-0278-3

REVIEW ARTICLE

Update on the Mechanism of Action of Aripiprazole: Translational Insights into Antipsychotic Strategies Beyond Dopamine Receptor Antagonism

Andrea de Bartolomeis[1] · Carmine Tomasetti[1] · Felice Iasevoli[1]



Puzzling over schizophrenia

Effective treatment for schizophrenia is still an unmet clinical need. Alleviating problems associated with cognitive impairment and finding the root of the disease remain priorities for clinicians and scientists. The incomplete understanding of the basis of this pathology has urged for research that will unravel the genetic origin of schizophrenia. But studies involving environmental exposure and social impact have also hinted at extrinsic factors as players in the pathogenesis of schizophrenia, which may be exploited to prevent the development of the disease. In 'Bench to Bedside', Patrick Sullivan proposes a model putting forward how genetic variants may confer risk by functioning together within the same pathway. This disease pathway hypothesis would imply a polygenetic variation affecting the same pathway and the alteration of a transcriptional network as a root for increasing schizophrenia risk. In 'Bedside to Bench', Andreas Meyer-Linderberg and Heike Tost discuss human-based population studies that suggest that environmental factors linked to development of schizophrenia can affect brain regions involved in the human social-emotional processing network. Genetic risk variants for schizophrenia can also influence similar regions in the brain, suggesting that environmental and intrinsic factors may converge in the same neural circuit.

■ BENCH TO BEDSIDE

Schizophrenia as a pathway disease

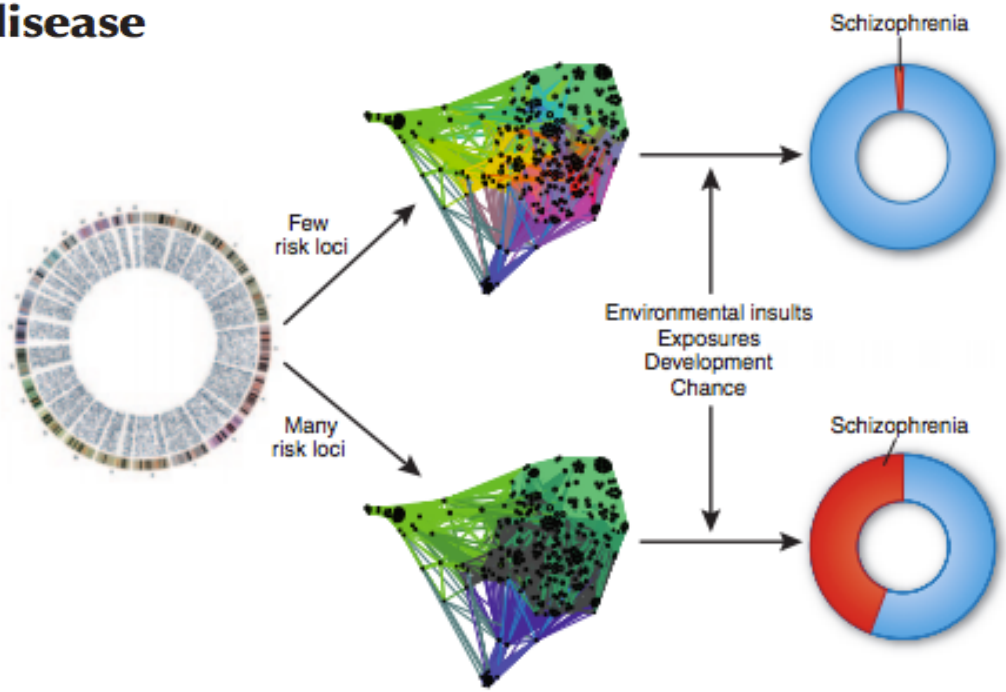


Figure 1 Schematic of schizophrenia as a pathway disease. Genetic variants at many loci encode the components of a pathway or pathways. Many risk loci can be affected, resulting in a disease pathway greatly modified owing to polygenetic variation. When a few risk loci are affected, there may be limited impact on the disease pathway. This pathway itself—in conjunction with environmental risk factors or other factors—mediates risk of schizophrenia.

Bedside to Bench (Reverse Translation)

- Start from unmet issues of clinical practice that cannot be resolved by clinical research

- Use preclinical (translational) research to make inferences on clinical problems

Lamotrigine augmentation in patients with schizophrenia who show partial response to clozapine treatment

Sefa Vayısoğlu [a,1], A. Elif Anıl Yağcıoğlu [b,*], Süha Yağcıoğlu [c], Sevilay Karahan [d], Oğuzhan Karcı [e,1], Ş. Can Gürel [f,1], M. Kâzım Yazıcı [b]

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[f] Birecik State Hospital, Psychiatry Clinic, Şanlıurfa, Turkey

Table 3
Change in psychopathology: Repeated Measures of ANOVA results for the Positive and Negative Syndrome, Clinical Global Impression-Severity and Calgary Depression Scales.

Assessments		Mean ± SD		Mean difference (SE)		Effect					
						Treatment group (df = 1)		Time (df = 1)		Treatment group × time (df = 1)	
		Lamotrigine	Placebo	Difference	p	F	p	F	p	F	p
PANSS total	Baseline	71.6 ± 3.9	71.8 ± 2.2	0.19 (1.11)	0.86	1.37	0.25	9.11	**<0.001**	2.73	**0.04**
	12-week	71.8 ± 5.1	69.1 ± 2.4	−2.75 (1.39)	0.05						
PANSS positive	Baseline	14.8 ± 2.7	14.8 ± 3.1	−0.51 (1.01)	0.96	0.01	0.9	19.45	**<0.001**	0.38	0.76
	12-week	13.1 ± 2.8	13.06 ± 2.9	−0.66 (1.01)	0.94						
PANSS negative	Baseline	19.0 ± 2.3	19.7 ± 3.1	0.76 (0.98)	0.44	0.003	0.95	4.87	**<0.001**	3.16	**0.02**
	12-week	19.7 ± 3.04	19.06 ± 2.8	−0.69 (1.02)	0.5						
PANSS general psychopathology	Baseline	37.8 ± 2.9	37.2 ± 2.4	−0.51 (0.93)	0.58	1.86	0.18	4.56	**0.006**	1.97	0.13
	12-week	39.0 ± 3.6	37.0 ± 2.9	−2.0 (1.14)	0.09						
Clinical Global Impression-Severity Scale	Baseline	3.3 ± 0.6	3.5 ± 0.6	−0.004 (0.08)	0.96	0.021	0.89	0.48	0.50	6.17	**0.02**
	12-week	3.6 ± 0.7	3.4 ± 0.5	−0.18 (0.13)	0.19						
Calgary Depression Scale	Baseline	3.6 ± 1.5	4.2 ± 4.3	0.61 (1.14)	0.59	0.02	0.88	0.49	0.49	2.48	0.12
	12-week	3.9 ± 2.2	3.6 ± 4.4	−0.28 (1.22)	0.81						

PANSS: Positive and Negative Syndrome Scale; p values ≤ 0.05 are shown bold.

Attenuation of the Neuropsychiatric Effects of Ketamine With Lamotrigine

Support for Hyperglutamatergic Effects of N-methyl-D-aspartate Receptor Antagonists

Amit Anand, MD; Dennis S. Charney, MD; Dan A. Oren, MD; Robert M. Berman, MD; X. Sylvia Hu, PhD; Angela Cappiello, MD, PhD; John H. Krystal, MD

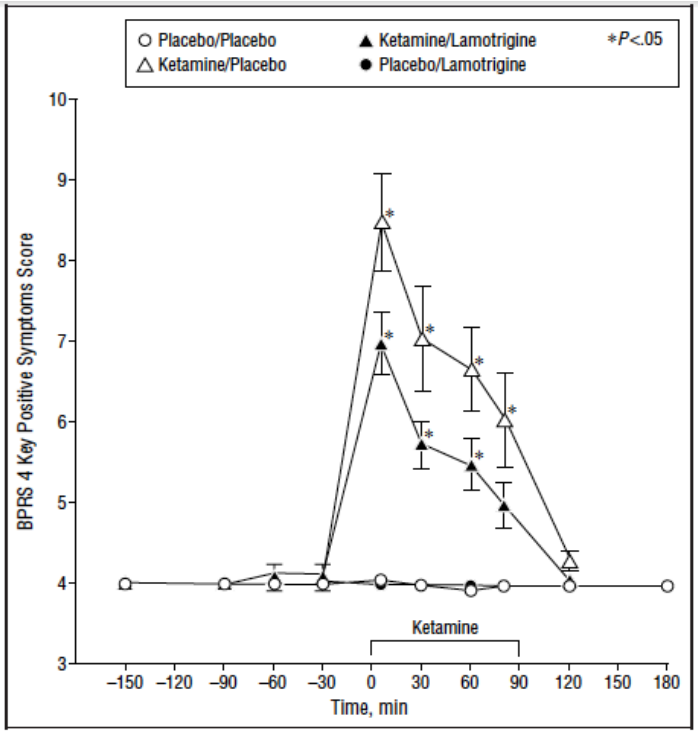


Figure 3. *Effects of ketamine and lamotrigine on Brief Psychiatric Rating Scale (BPRS) 4 key positive symptoms scores. The ketamine × lamotrigine × time effect was significant (P<.001). Individual time-point differences between ketamine/lamotrigine and ketamine/placebo days were examined using the Dunnett t test after Bonferroni adjustment. T-shaped bars indicate SEMs.*

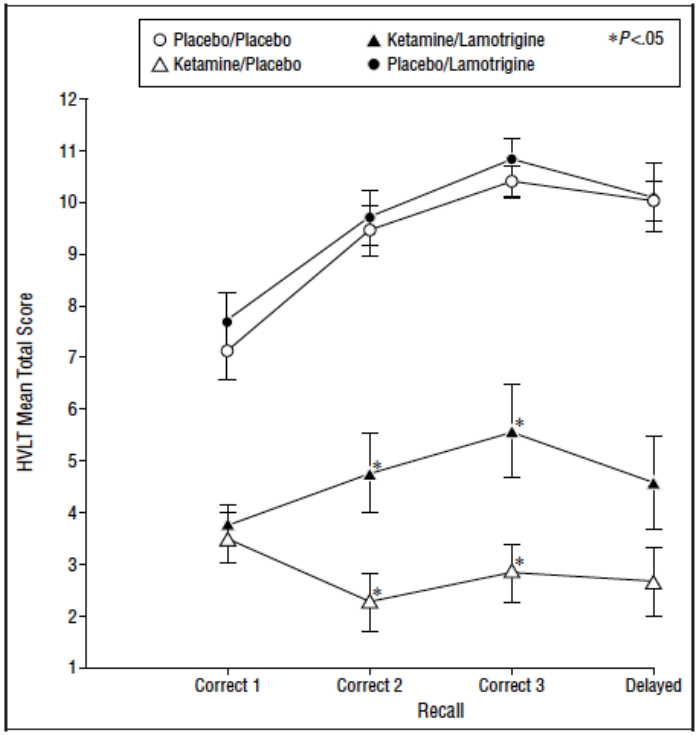


Figure 6. *Effects of ketamine and lamotrigine on Hopkins Verbal Learning Test (HVLT) scores. Correct 1 indicates correct in trial 1; correct 2, correct in trial 2; correct 3, correct in trial 3; and delayed, delayed recall after 30 minutes. Individual trial differences between ketamine/lamotrigine and ketamine/placebo days were examined using the Dunnett t test after Bonferroni adjustment. T-shaped bars indicate SEMs.*

Arch Gen Psychiatry. 2000;57:270-276

Effects of clozapine plus lamotrigine on phencyclidine-induced hyperactivity

Harriet J. Williams [b,1], Christina R. Zamzow [b,1], Harold Robertson [b], Serdar M. Dursun [a,b,c,*]

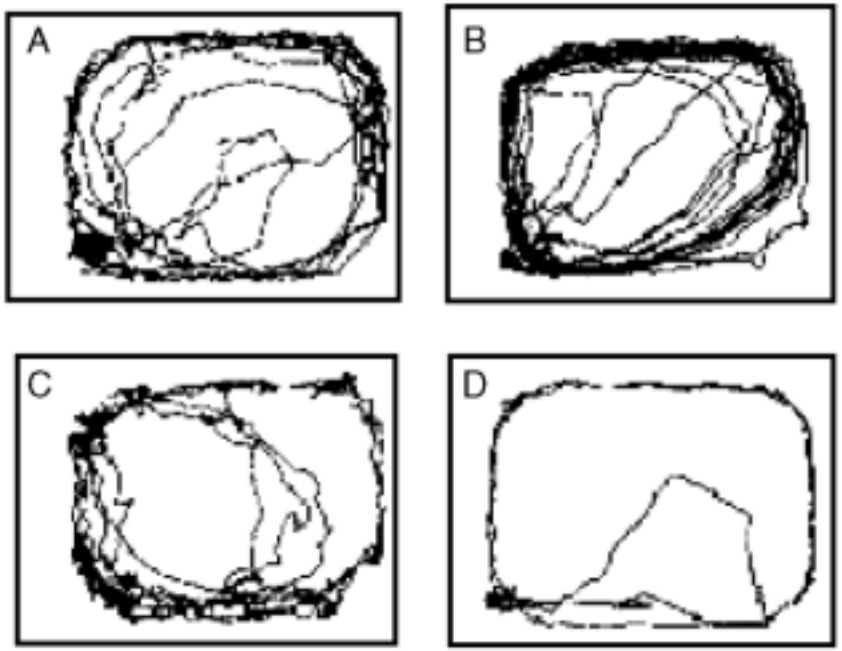
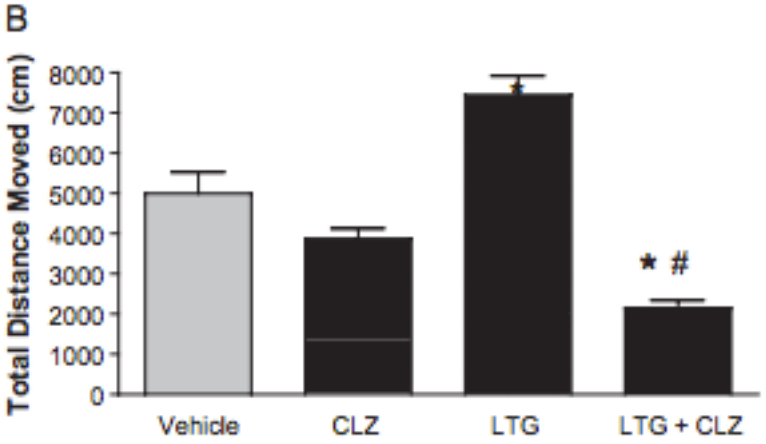


Fig. 1. Effect of pre-treatment with CLZ (5 mg/kg) and LTG (10 mg/kg) on PCP induced hyper-locomotion as illustrated by these Etho Vision generated tracks. Rats were pre-treated with drug or vehicle 30 min prior to PCP. Each track represents the movement of a single rat in the 10-min period directly after the PCP. Tracks are labelled as A: Vehicle; B: LTG; C: CLZ; D: LTG plus CLZ.



Progress in Neuro-Psychopharmacology & Biological Psychiatry 30 (2006) 239 – 243

Suzanne A. Brody · Mark A. Geyer · Charles H. Large

Lamotrigine prevents ketamine but not amphetamine-induced deficits in prepulse inhibition in mice

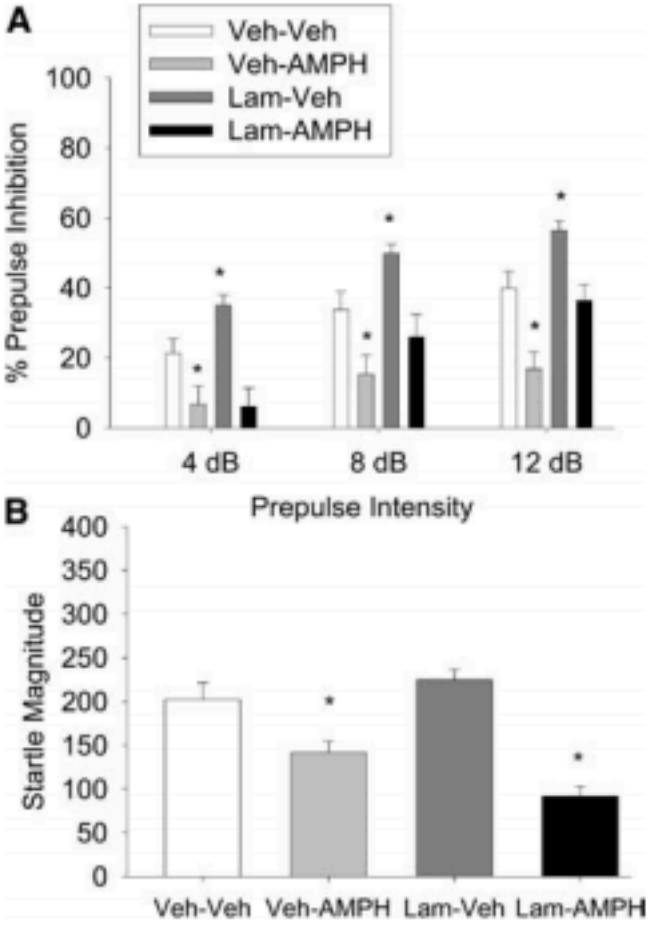


Fig. 2A, B Lamotrigine does not ameliorate amphetamine-induced

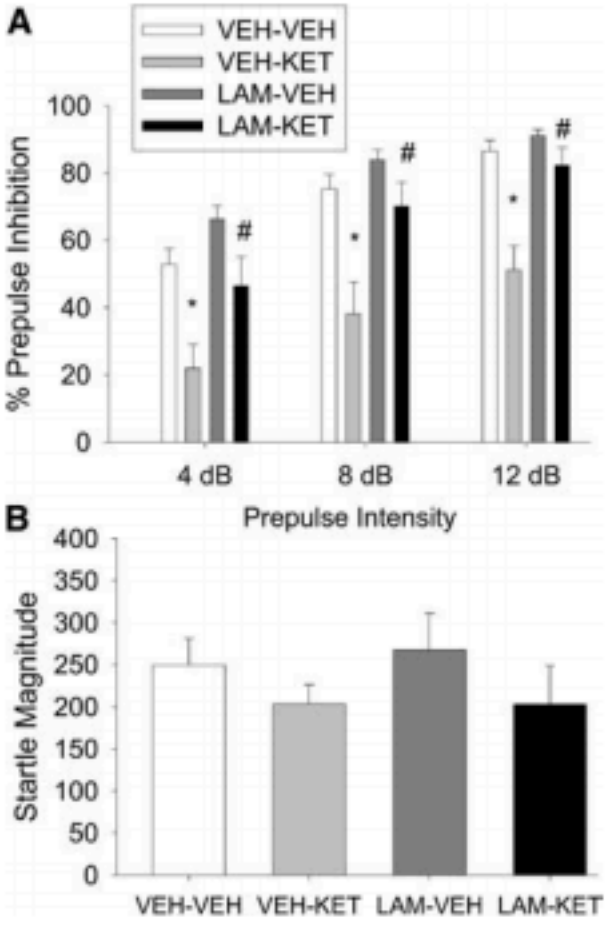


Fig. 3A, B Lamotrigine reverses ketamine-induced prepulse inhi-

Psychopharmacology (2003) 169:240–246
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Overview

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  - Studio Traslazionale 1
  - Studio Traslazionale 2

DEFINITION

transcriptome

A transcriptome is the full range of messenger RNA, or mRNA, molecules expressed by an organism. The term "transcriptome" can also be used to describe the array of mRNA transcripts produced in a particular cell or tissue type. In contrast with the genome, which is characterized by its stability, the transcriptome actively changes. In fact, an organism's transcriptome varies depending on many factors, including stage of development and environmental conditions.

A laboratory technique called the microarray can be used to examine changes in the transcriptome. Microarrays can be used to measure the expression of thousands of genes at the same time, as well as to provide gene expression profiles, which describe changes in the transcriptome in response to a particular condition or treatment.

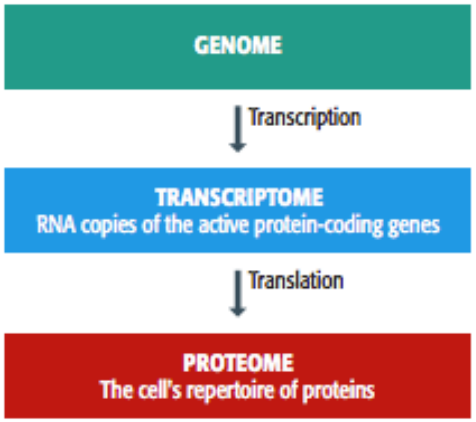


Figure 1.2 The genome, transcriptome, and proteome.

Genetic architectures of psychiatric disorders: the emerging picture and its implications

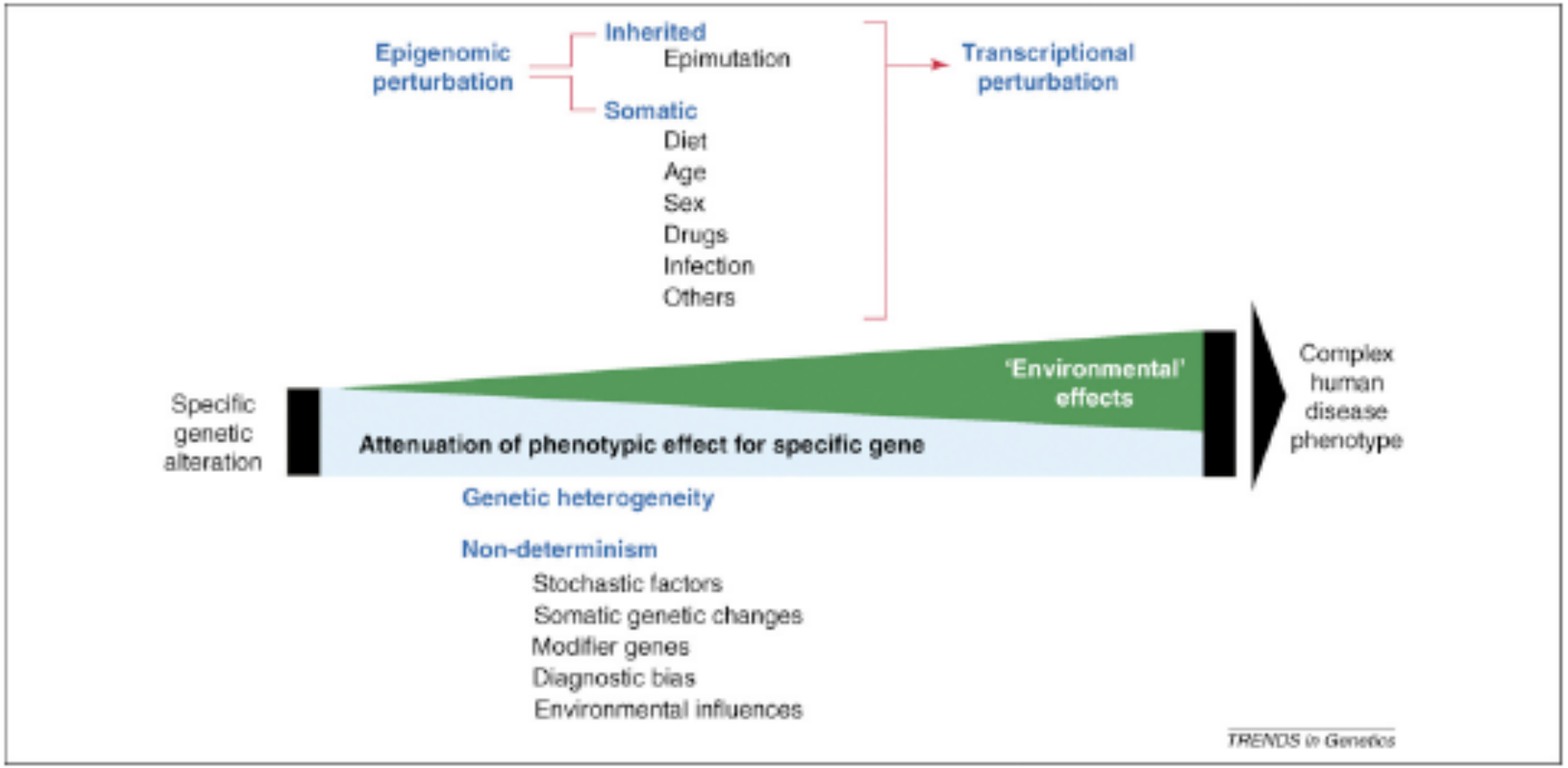
Patrick F. Sullivan[1], Mark J. Daly[2] and Michael O'Donovan[3]

Table 1 | **Defining features of nine psychiatric disorders***

Name	Life prevalence	Heritability	Essential characteristics	Notable feature
Alzheimer's disease	0.132	0.58	Dementia, defining neuropathology	Of the top ten causes of death in the United States, Alzheimer's disease alone has increasing mortality
Attention-deficit hyperactivity disorder (ADHD)	0.053	0.75	Persistent inattention, hyperactivity, impulsivity	Costs estimated at ~$US100 × 10^9 per year
Alcohol dependence (ALC)	0.178	0.57	Persistent ethanol use despite tolerance, withdrawal, dysfunction	Most expensive psychiatric disorder (total costs exceed US$225 × 10^9 per year)
Anorexia nervosa	0.006	0.56	Dangerously low weight from self-starvation	Notably high standardized mortality ratio
Autism spectrum disorder (ASD)	0.001	0.80	Markedly abnormal social interaction and communication beginning before age 3	Huge range of function, from people requiring complete daily care to exceptional occupational achievement
Bipolar disorder (BIP)	0.007	0.75	Manic-depressive illness, episodes of mania, usually with major depressive disorder	As a group, nearly as disabling as schizophrenia
Major depressive disorder (MDD)	0.130	0.37	Unipolar depression, marked and persistent dysphoria with physical and cognitive symptoms	Ranks number one in the burden of disease in the world
Nicotine dependence (NIC)	0.240	0.67	Persistent nicotine use with physical dependence (usually cigarettes)	Major preventable risk factor for many diseases
Schizophrenia (SCZ)	0.004	0.81	Long-standing delusions and hallucinations	Life expectancy decreased by 12–15 years

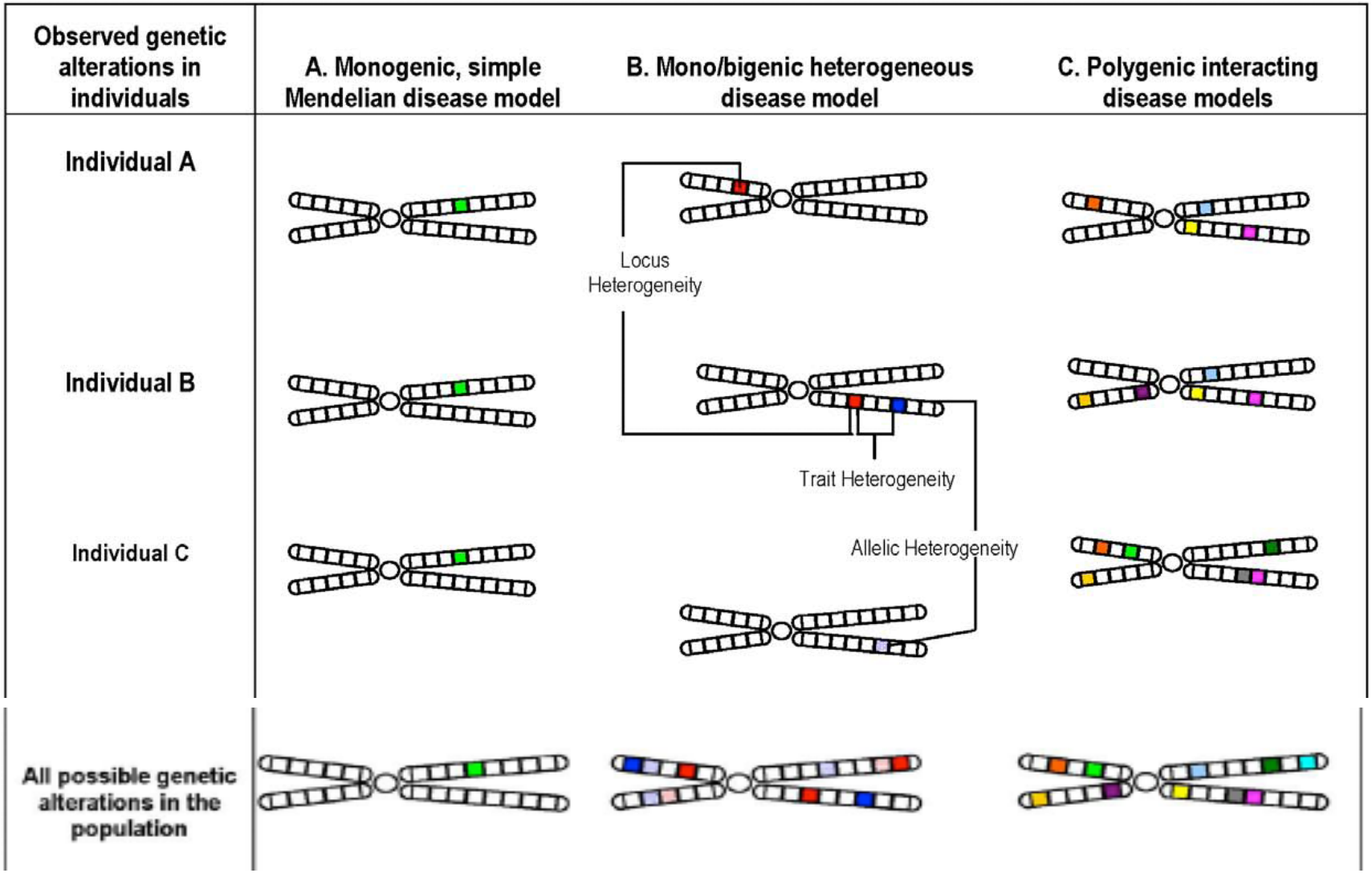
*Most of these definitions are made more restrictive by requiring persistence over time (for example, the criteria for SCZ require ≥6 months of symptoms), substantial impairment and presence across multiple different contexts. See Supplementary information S1 (table) for more detail. Additional sources are REFS 1,2,181–183).

Mental disorders are complex diseases



HATCHWELL, 2007

"Predisposition to complex disorders is determined by a complex interaction of multiple genetic and enviromental factors" Venken, 2007



ASKLAND, 2006

La componente genetica della gran parte dei complex (common) diseases è da attribuire ad un limitato numero di varianti genetiche presenti in più dell' 1-5% della popolazione.

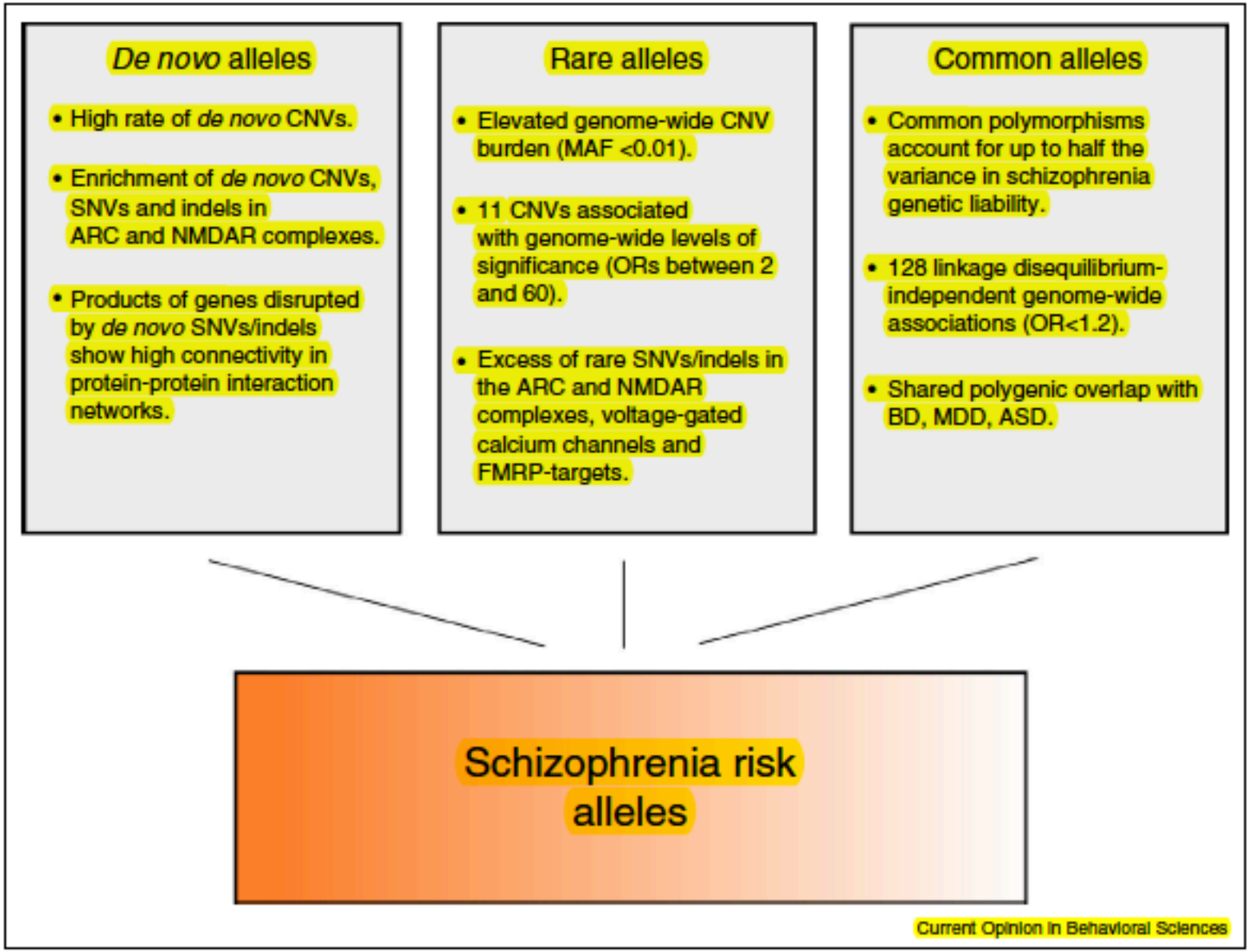
Risk Variants

- Research has allowed the identification of a large set of “risk variants” that are defined as genetic elements that have been associated to an increased risk (i.e. susceptibility) to develop the pathology

- These risk variants occur across the entire allelic frequency spectrum, and are in many cases also associated to susceptibility for other neuropsychiatric diseases

Risk Variants

1. Common Alleles, i.e. Single Nucleotide Polymorphisms (SNP)
2. Rare Alleles
   a. Copy Number Variants (CNV)
   b. Single Nucleotide Variants (SNV)
   c. Insertion/Deletion mutations (Ins/Del)
3. De novo Mutations, i.e. CNV, SNV or Ins/Del which arise de novo in the germline



Schizophrenia risk alleles. Bullet points summarise some of the key findings covered in this review. MAF = minor allele frequency, OR = odds ratio, CNV = copy number variation, SNV = single nucleotide variant, indel = insertion/deletion, ARC = activity-regulated cytoskeleton-associated protein, NMDAR = *N*-methyl-D-aspartate receptor, FMRP = fragile X mental retardation protein, BD = bipolar disorder, MDD = major depressive disorder, ASD = autism spectrum disorder.

ARTICLE

doi:10.1038/nature13595

Biological insights from 108 schizophrenia-associated genetic loci

Schizophrenia Working Group of the Psychiatric Genomics Consortium*

only in 10 instances the associated SNP was credibly attributable to a known non-synonymous exonic polymorphism [11], i.e. determining a variation in the protein sequence of the gene's protein product. The vast majority of polymorphisms appear to exert their effects through the modulation of gene expression

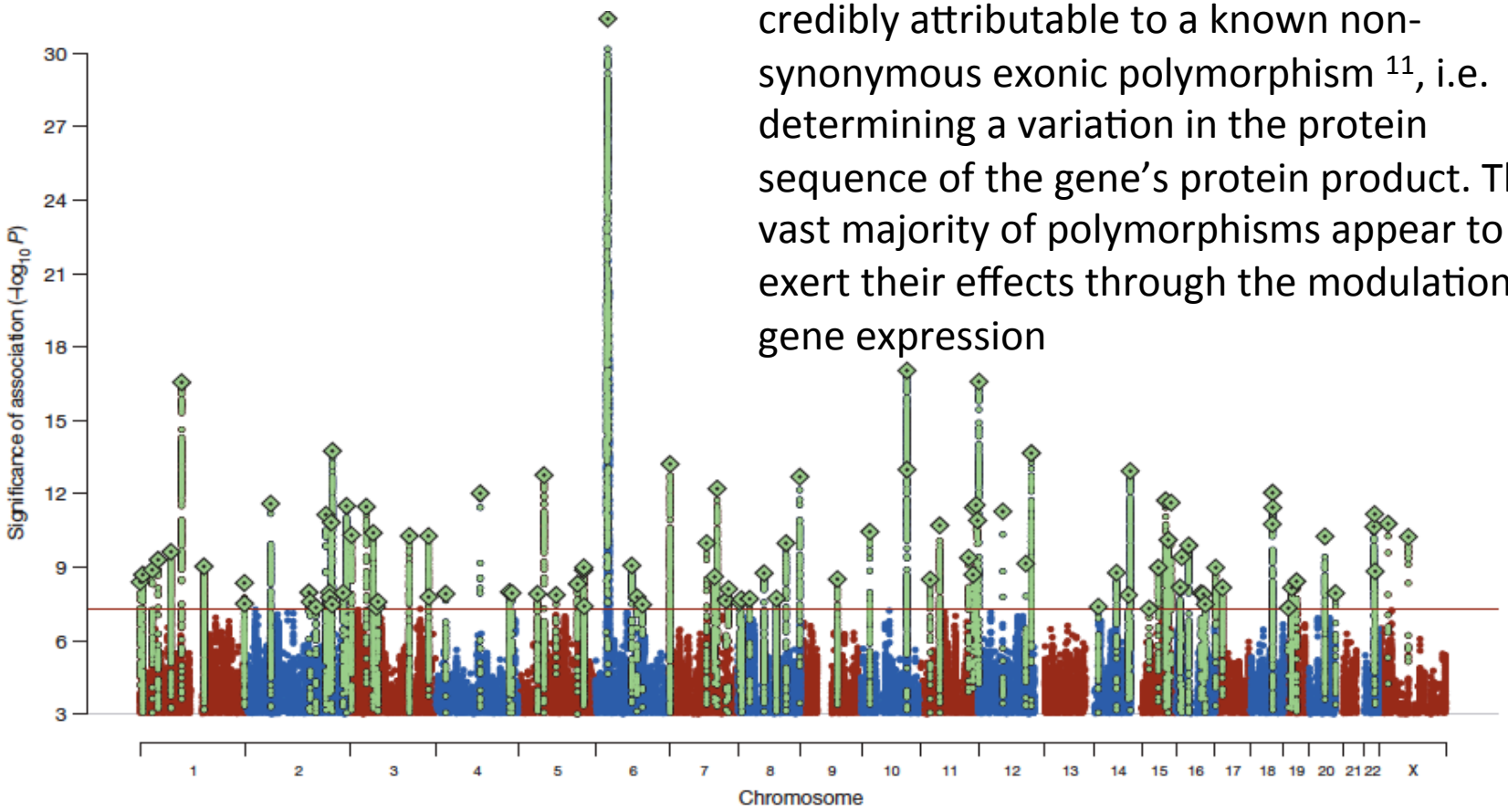


Figure 1 | Manhattan plot showing schizophrenia associations. Manhattan plot of the discovery genome-wide association meta-analysis of 49 case control samples (34,241 cases and 45,604 controls) and 3 family based association studies (1,235 parent affected-offspring trios). The *x* axis is chromosomal position and the *y* axis is the significance ($-\log_{10} P$; 2-tailed) of association derived by logistic regression. The red line shows the genome-wide significance level (5×10^{-8}). SNPs in green are in linkage disequilibrium with the index SNPs (diamonds) which represent independent genome-wide significant associations.

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PRS explained about 7% of liability variance in the sample [11].

When individuals in three independent samples from those constituting the PGC mega-analysis were grouped into PRS deciles (that is: with increasing number of common risk alleles with high-to-moderate statistical association with schizophrenia), the estimated OR of being affected consistently increased in each independent sample with greater number of schizophrenia risk alleles

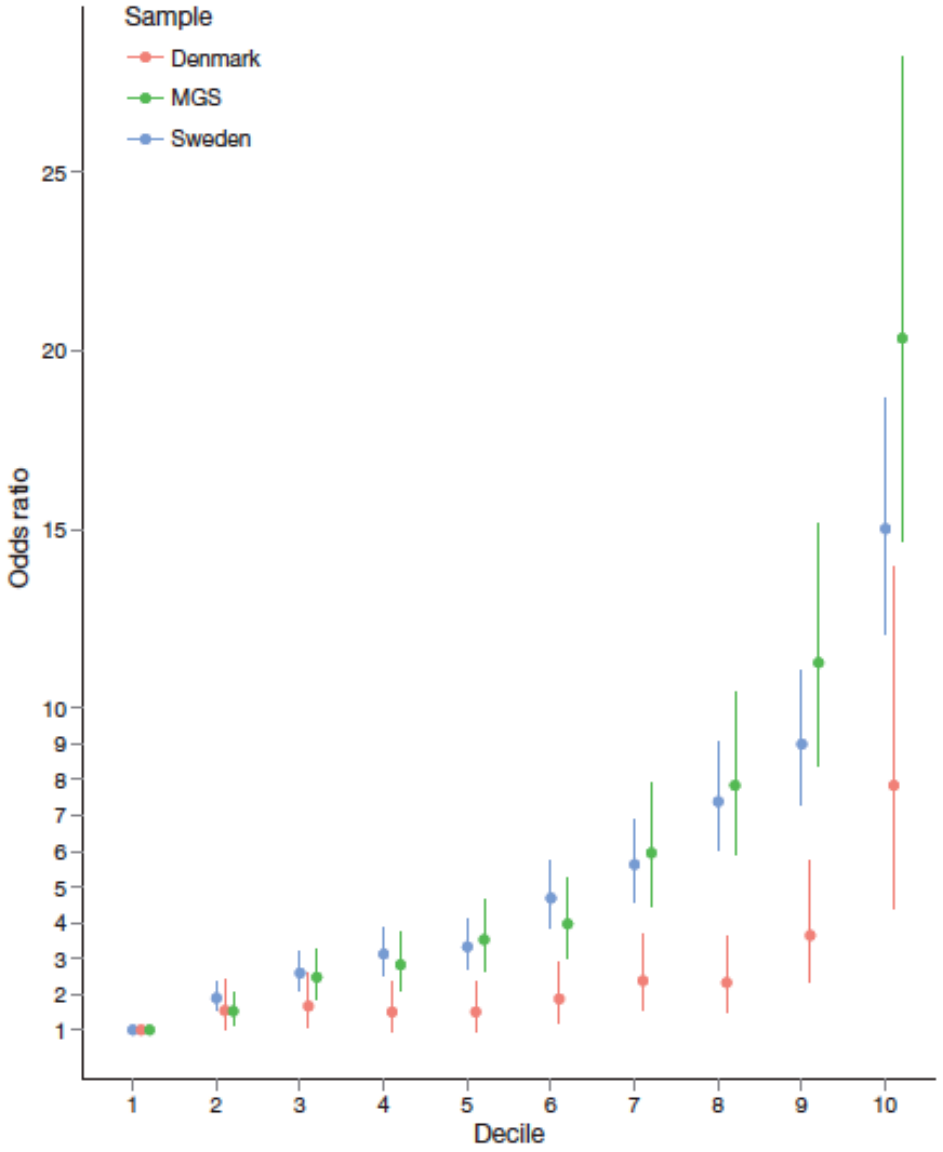

Figure 3 | Odds ratio by risk score profile. Odds ratio for schizophrenia by

ARTICLE

doi:10.1038/nature12975

A polygenic burden of rare disruptive mutations in schizophrenia

Shaun M. Purcell[1,2,3,4,5], Jennifer L. Moran[1]*, Menachem Fromer[1,2,3,4]*, Douglas Ruderfer[2,3]*, Nadia Solovieff[4], Panos Roussos[2,3], Colm O'Dushlaine[1], Kimberly Chambert[1], Sarah E. Bergen[1,6], Anna Kähler[6], Laramie Duncan[1,4,5], Eli Stahl[2,3], Giulio Genovese[1], Esperanza Fernández[7,8], Mark O. Collins[9], Noboru H. Komiyama[9], Jyoti S. Choudhary[9], Patrik K. E. Magnusson[6], Eric Banks[5], Khalid Shakir[5], Kiran Garimella[5], Tim Fennell[5], Mark DePristo[5], Seth G. N. Grant[10], Stephen J. Haggarty[1,4,11], Stacey Gabriel[5], Edward M. Scolnick[1], Eric S. Lander[5], Christina M. Hultman[6], Patrick F. Sullivan[12], Steven A. McCarroll[1,5,13] & Pamela Sklar[2,3,14]

Table 1 | Gene set analysis of primary schizophrenia candidate gene sets

Variant type	Gene set/subset	*n* genes	Singletons	MAF < 0.1%	MAF < 0.5%
Disruptive	Primary	2,546	**0.0008**	**0.0001**	**0.0002**
NS_{strict}			0.0059	**0.0015**	0.0110
NS_{broad}			0.0986	0.1295	0.1126
Disruptive	SCZ *de novo* genes				
	Exome sequencing (disruptive)[18,19,30]	87	0.0319	**0.0007**	**0.0003**
	Exome sequencing (nonsyn.)[18,19,30]	611	0.0053	**0.0011**	0.0055
	Copy number variants				
	de novo CNV genes[13]	234	0.0234	0.0039	0.0124
	SCZ-associated CNV genes[5]	345	0.3308	0.4596	0.4376
	GWAS				
	Voltage-gated calcium channel genes[12]	26	**0.0019**	0.0214	0.0212
	Common SNPs ($P < 10^{-4}$ intervals)[3]	479	0.1794	0.0368	0.0037
	miR-137 targets[32]	446	0.6573	0.5609	0.4747
	Synaptic genes				
	PSD (human core)[13]	685	0.0808	0.1154	0.1256
	ARC[13]	28	**0.0016**	**0.0014**	**0.0014**
	NMDAR network[13]	61	0.0158	0.0251	0.0252
	PSD-95[13]	65	**0.0017**	**0.0009**	**0.0010**
	mGluR5[13]	39	0.1327	0.0900	0.0902

Molecular Psychiatry (2012) 17, 142–153

OPEN

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IMMEDIATE COMMUNICATION

De novo CNV analysis implicates specific abnormalities of postsynaptic signalling complexes in the pathogenesis of schizophrenia

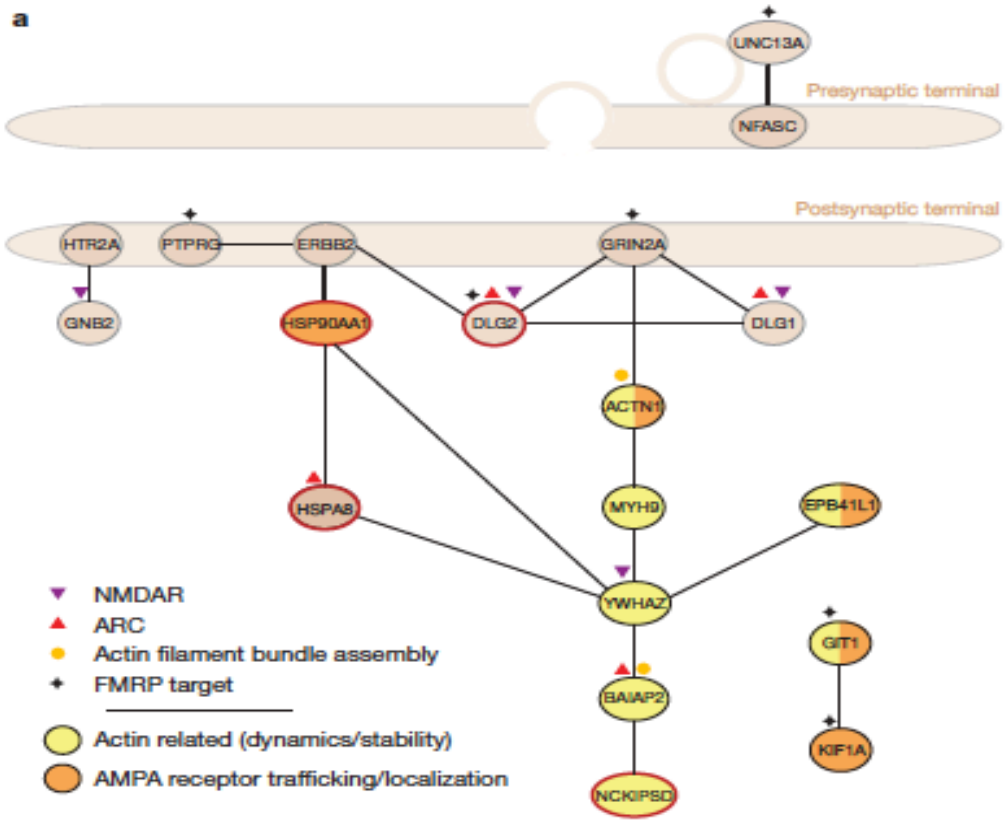
Table 2 Enrichment of gene sets for *de novo* CNV hits in comparison with control CNVs

Gene set	N *genes*	N *genes hit by CNVs* P-*value*				*Genes hit by SCZ* de novos
		SCZ de novo *(34)*	*Bulgarian control (1367)*	*Icelandic control* de novo *(59)*	*Autism control* de novo *(14)*	
PSD	664	19	49 (**1.72×10^{-6}**)	13 (**0.045**)	4 (0.11)	*DLG1, DLG2, DLGAP1, RYR2, SND1,* ***STX1A, MDH2****, HSPB1,* ***YWHAG, RPH3A, CYFIP1, TJP1, ALDOA****, TAOK2, MAPK3*
ARC complex	25	8	7 (**3.78×10^{-8}**)	1 (**2.51×10^{-4}**)	0 (**0.0049**)	*DLG1, DLG2, DLGAP1, CYFIP1*
NMDAR complex	59	8	6 (**4.24×10^{-6}**)	2 (**0.0061**)	0 (**0.01**)	*DLG1, DLG2, DLGAP1, STX1A, YWHAG, TJP1, MAPK3*
PSD-95 complex	58	4	3 (**1.17×10^{-5}**)	1 (**0.017**)	0 (**0.033**)	*DLG1, DLG2, DLGAP1*
mGluR5 complex	37	3	4 (**0.026**)	2 (0.45)	0 (0.15)	*YWHAG, RPH3A, ALDOA*
Presynapse	426	8	25 (**0.033**)	8 (0.32)	2 (0.28)	***STX1A, RPH3A, CYFIP1, ALDOA, MDH2***
Synaptic vesicle	333	7	20 (**0.014**)	8 (0.39)	2 (0.31)	*STX1A, RPH3A, CYFIP1, ALDOA*
Active zone	176	2	6 (0.29)	3 (0.91)	0 (0.26)	*ALDOA, MDH2*
Nucleus	160	5	10 (**0.0024**)	2 (**0.026**)	0 (**0.018**)	***CYFIP1, TJP1***
Mitochondrion	189	3	9 (0.41)	1 (0.11)	0 (0.093)	***MDH2****, BDH1, KIAA0564*
Cytoplasm	263	4	11 (0.68)	3 (0.55)	0 (0.15)	*EIF4H,* ***YWHAG****, MSRA, MVP*
Endoplasmic reticulum	94	1	3 (0.75)	0 (0.18)	0 (0.31)	*POR*
Endoplasmic reticulum/ Golgi-derived vesicles	94	0	0	0	0	
Recycling endosomes	65	0	2 (0.83)	0	0	
Early endosomes	17	0	1 (0.82)	0	0	
Golgi	31	0	1 (0.82)	0	0	
Plasma membrane	50	0	2 (0.61)	0	0	

ARTICLE

doi:10.1038/nature12929

De novo mutations in schizophrenia implicate synaptic networks



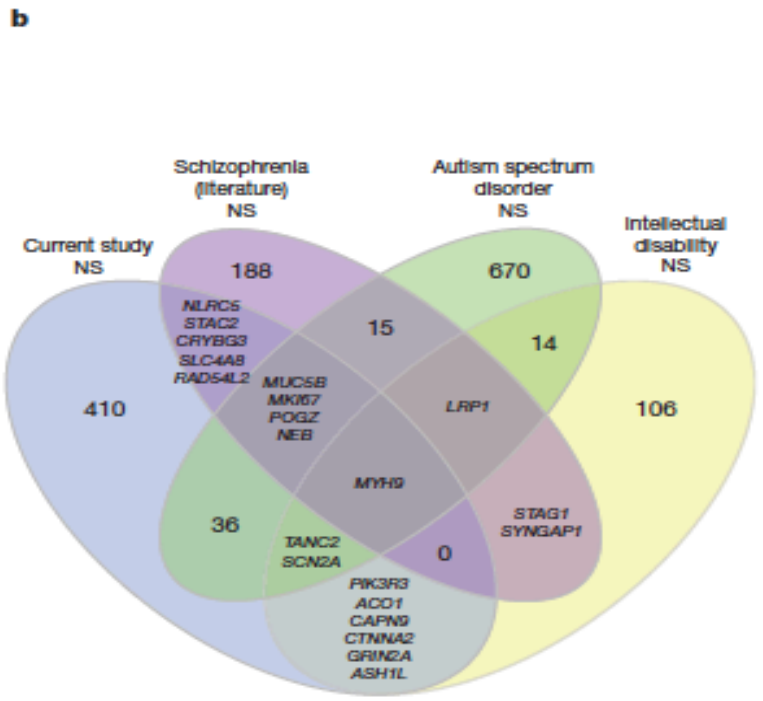


Figure 1 | *De novo* mutations from schizophrenia affect genes coding for synaptic proteins and genes affected in other neuropsychiatric diseases. **a**, Synaptic protein–protein interactions between proteins affected by nonsynonymous *de novo* mutations in schizophrenia. Interactions were retrieved from the expert-curated lists in the SynSysNet database (http://bioinformatics.charite.de/synsysnet/) and plotted to show their general pre/postsynaptic localization. Proteins belonging to various functional sets

mutations are noted with a red outline. Proteins with nonsynonymous *de novo* mutations had more than expected direct interconnections ($P = 0.008$), which was consistent with more overall connectivity to synaptic proteins as a whole ($P = 0.005$). **b**, Overlap of genes bearing nonsynonymous (NS) *de novo* mutations in schizophrenia (refs 12–14), autism spectrum disorder (refs 6–9) and intellectual disability (refs 10, 11). Overlaps of six or fewer genes are listed by name. See Extended Data Table 5 for statistical significance of

EXPERT REVIEW

Integrating the roles of long and small non-coding RNA in brain function and disease

G Barry

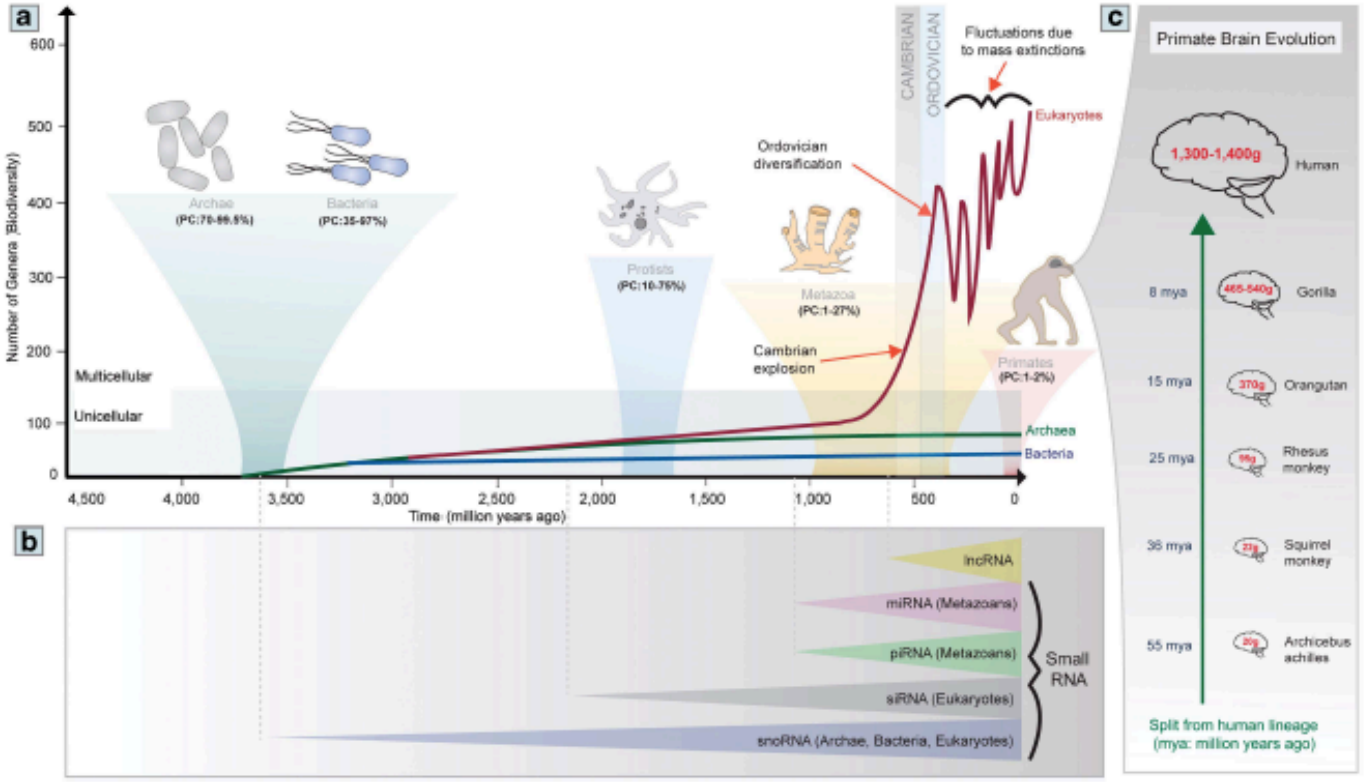


Figure 1. Evolution of organismal complexity scales with the increase in non-protein-coding size of the genome. (**a**) Life originated on earth between 3.5 and 4 billion years ago with the appearance of archae and bacteria. Their respective genomes consisted of a high percentage of protein-coding (PC) transcripts (archae 70–99.5%; bacteria 35–97%).[2] The emergence of protists between 1.5 and 2 billion years ago saw a significant reduction in PC percentage of the genome (10–75%) whereas metazoans first emerging around 500 million years later showed an even more striking decrease (1–27%). Primates possess only around 1–2% PC capacity in their genome. (**b**) The increase in non-protein-coding regions of the genome has seen an expansion in classes on non-coding RNA, including both small (for example, piRNA, miRNA) and long (for example, lncRNA) families. (**c**) The development of protein regulatory systems via non-coding RNA may have contributed to the considerable and rapid growth during primate brain evolution and the acquisition of higher-order cognition.[3]

MicroRNAs in Schizophrenia: Implications for Synaptic Plasticity and Dopamine–Glutamate Interaction at the Postsynaptic Density. New Avenues for Antipsychotic Treatment Under a Theranostic Perspective

Andrea de Bartolomeis · Felice Iasevoli · Carmine Tomasetti · Elisabetta F. Buonaguro

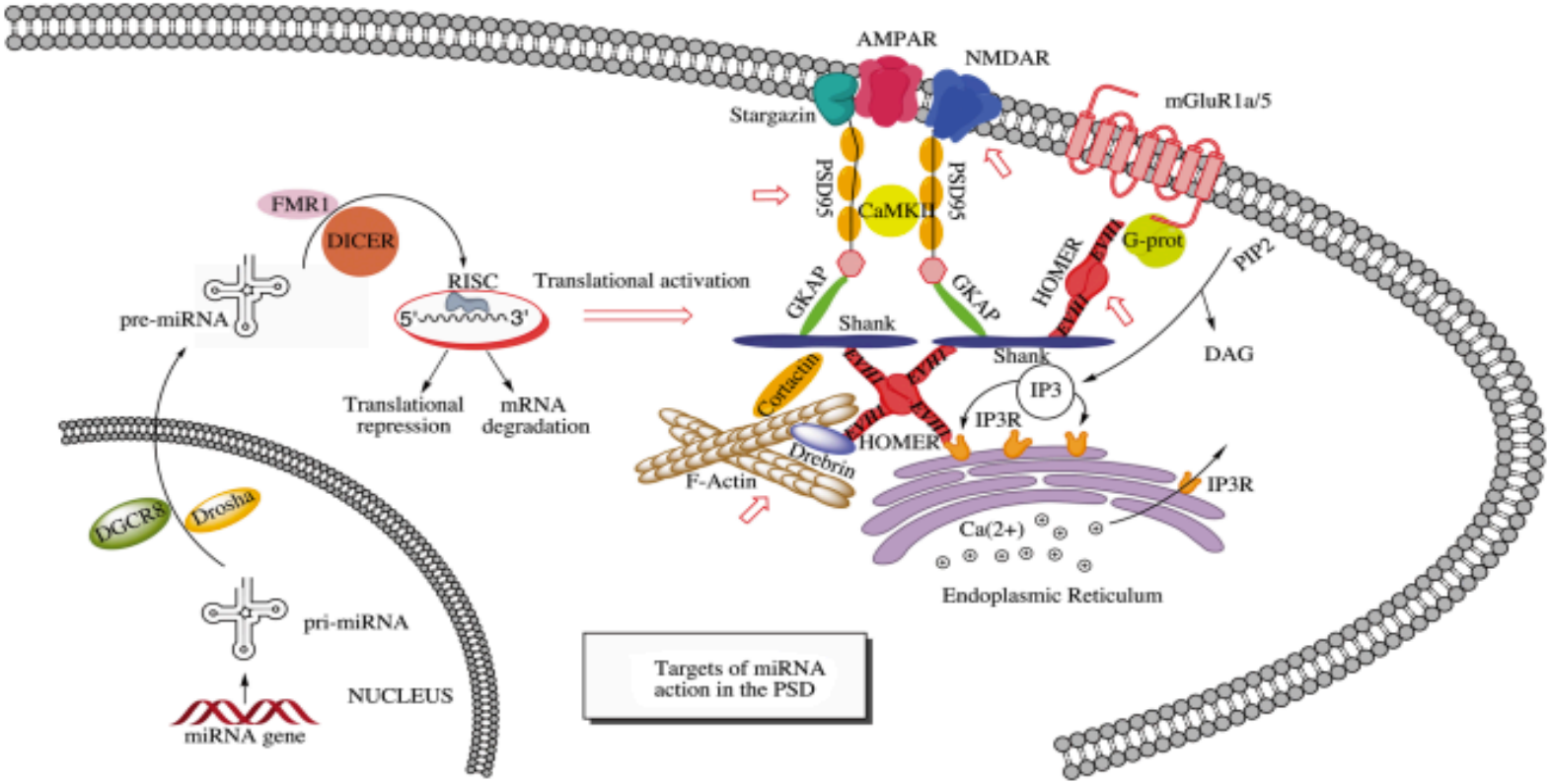
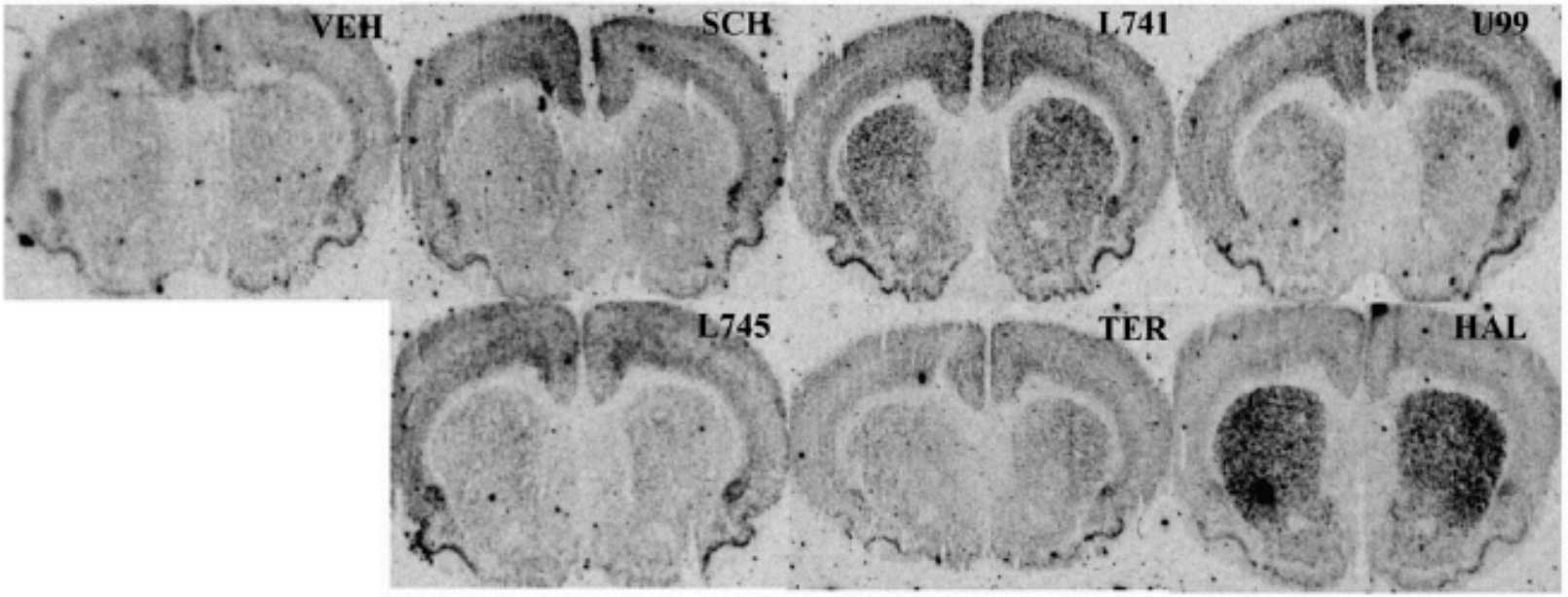


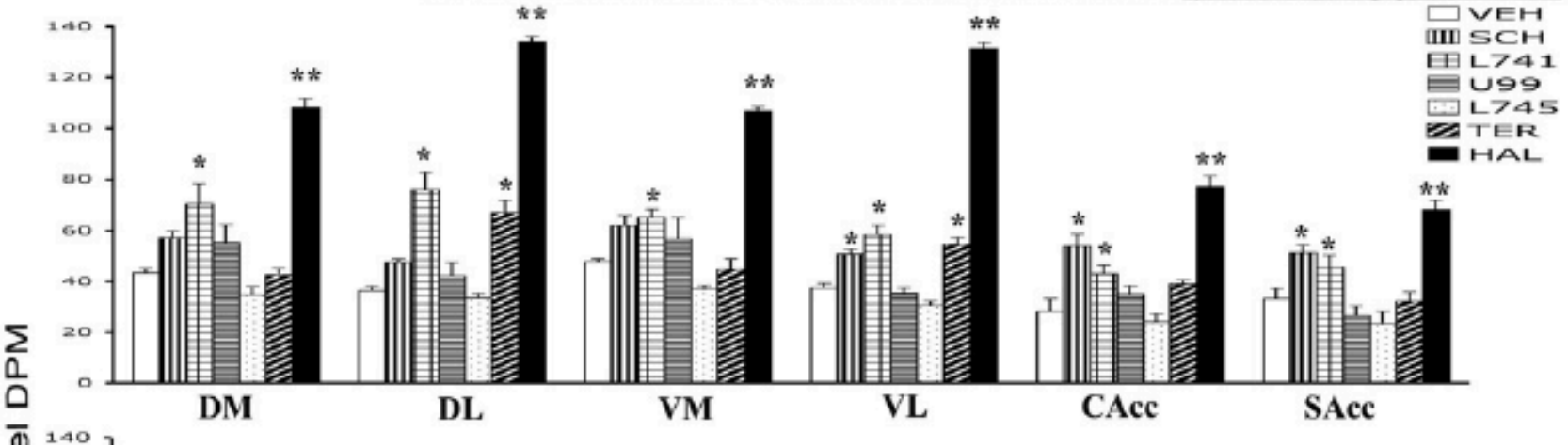
Fig. 1 Overview of miRNA processing from transcription to the formation of the effector complex, and location of the mature miRNA at postsynaptic density (PSD). PSD components are also shown. *miRNA* microRNA, *pri-miRNA* primary microRNA transcript, *pre-miRNA* precursor microRNA, *DGCR8* DiGeorge syndrome critical region gene 8 microprocessor complex subunit, *FMR1* fragile X mental retardation protein, *RISC* RNA-induced silencing complex, *IP3* inositol trisphosphate, *IP3R* inositol trisphosphate receptor, *DAG* diacyl-glycerol, *PIP2* phosphatidylinositol biphosphate, *EVH1* enabled/VASP homology 1 domain, *GKAP* guanylate kinase-associated protein, *CaMKII* Ca(2+)/calmodulin-dependent protein kinase II, *AMPAR* α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, *NMDAR* *N*-methyl-D-aspartate receptor, *mGluR1a/5* metabotropic glutamate receptor 1a/5

Overview

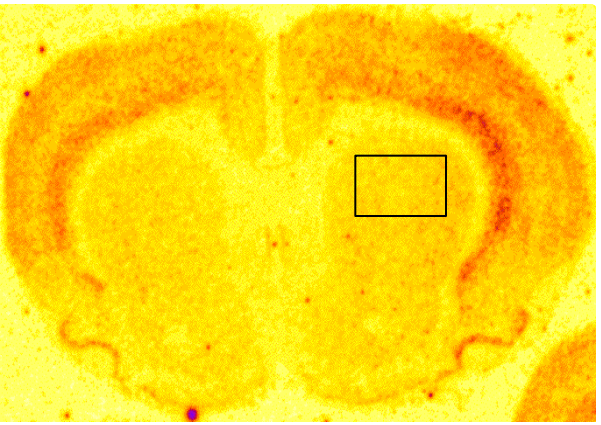
- Cosa è la psichiatria traslazionale?
- Cosa è il trascrittoma? In che modo la schizofrenia è una patologia della espressione genica?
- **Come si studia l'espressione genica?**
- In che modo la ricerca traslazionale può essere di aiuto nella pratica psichiatrica clinica?

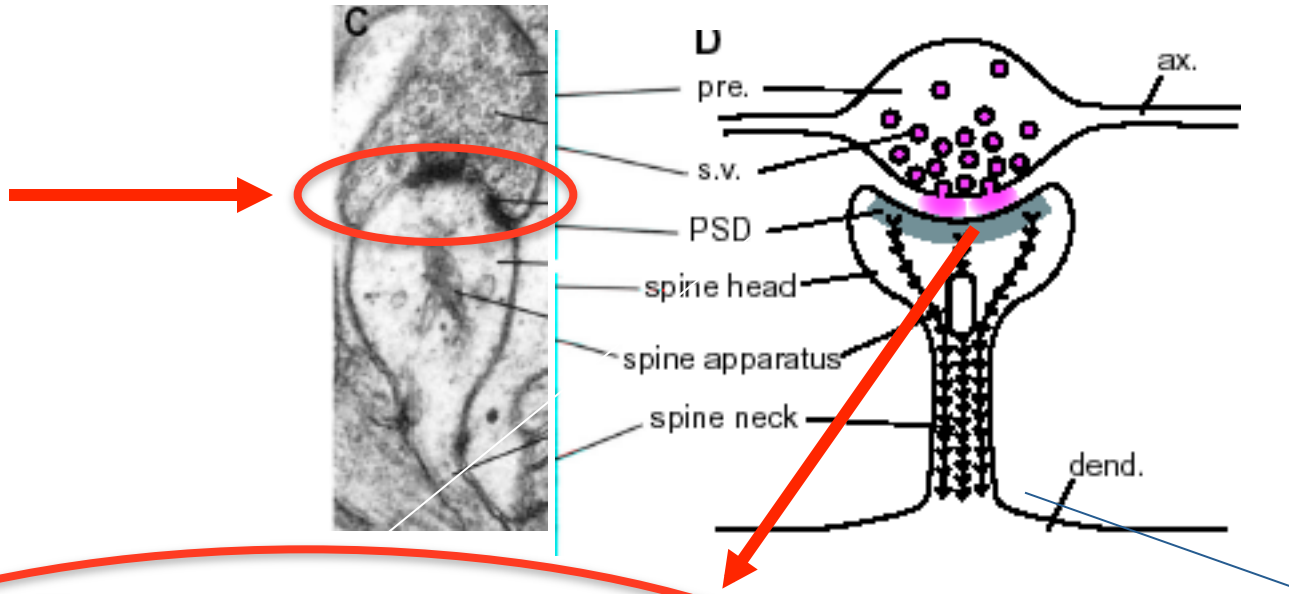
Gene Expression Lab Exp

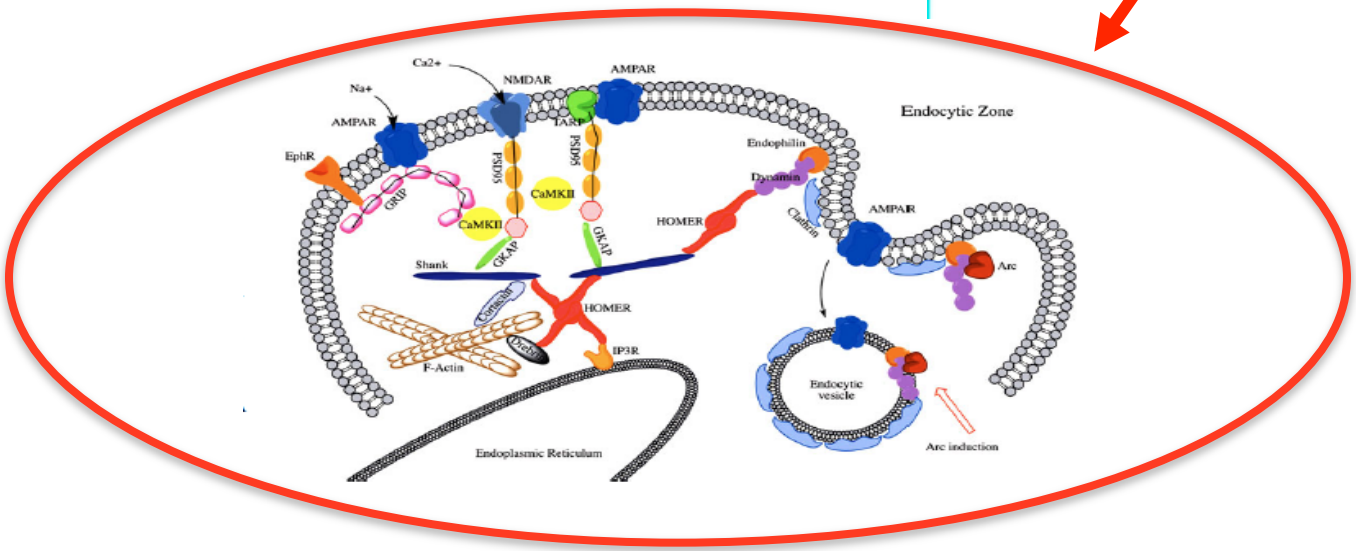




The post-synaptic density (PSD)







Modified from: *Kennnedy et al., 2000; de Bartolomeis et al., 2013.*

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Protocol

Method for quantitative in situ hybridization histochemistry and image analysis applied for Homer1a gene expression in rat brain

Alberto Ambesi-Impiombato, Giordano D'Urso, Giovanni Muscettola, Andrea de Bartolomeis*

Laboratory of Molecular Psychiatry and Psychopharmacotherapy, Section of Psychiatry, Department of Neuroscience and Behavioral Science University School of Medicine "Federico II", Edificio 18, Via Pansini 5, 80131 Naples, Italy

Accepted 29 April 2003

Abstract

Here we describe the detailed method for quantitative in situ hybridization histochemistry adopted in our previously published short communication on differential gene expression of the postsynaptic density proteins Homer and PSD-95 in rat forebrain after acute treatment with antipsychotic drugs [de Bartolomeis et al., Mol. Brain Res. 98 (2002) 124–129]. Specific ^{35}S radiolabeled oligodeoxyribonucleotides were used to hybridize rat forebrain sections and data analysis was carried out on digitalized images acquired by means of a CCD camera. Special emphasis has been posed on data preprocessing options applied to a dataset obtained using a transparency scanner as an alternative image capturing method.

Theme: Neurotransmitters, modulators, transporters and receptors

Topic: Signal transduction: gene expression

Keywords: In situ hybridization histochemistry; Preprocessing; Gene expression; Quantitative analysis; Image analysis

Protocol

Method for quantitative in situ hybridization histochemistry and image analysis applied for Homer1a gene expression in rat brain

Alberto Ambesi-Impiombato, Giordano D'Urso, Giovanni Muscettola, Andrea de Bartolomeis*

1. Type of research

- In situ hybridization histochemistry for comparative analysis of rat brain gene expression under different pharmacological challenges, using specific oligodeoxyribonucleotide probes. The procedure for in situ hybridization histochemistry was adapted from several standard published protocols [1,4,12].
- Measurement of regional gene expression on digitalized autoradiograms using image analysis software.

2. Time required

- Drug treatment and tissue preparation: 1 day.
- Tissue sectioning: 3–5 days.
- Radiolabeling and purification of oligonucleotide probes: 2 h.
- Preparation of tissue sections before hybridization: 2 h.
- Hybridization: 20–24 h (overnight incubation).
- Washing: 4–5 h.
- Exposure time: 3–30 days (depending on probe).
- Acquisition of digitized autoradiograms: 1–2 h.
- Quantification of radioactive signal: 1 day.
- Whole protocol: 1 month.

Protocol

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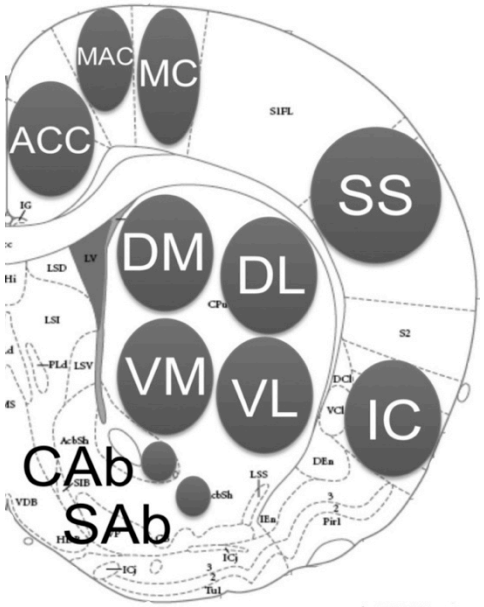

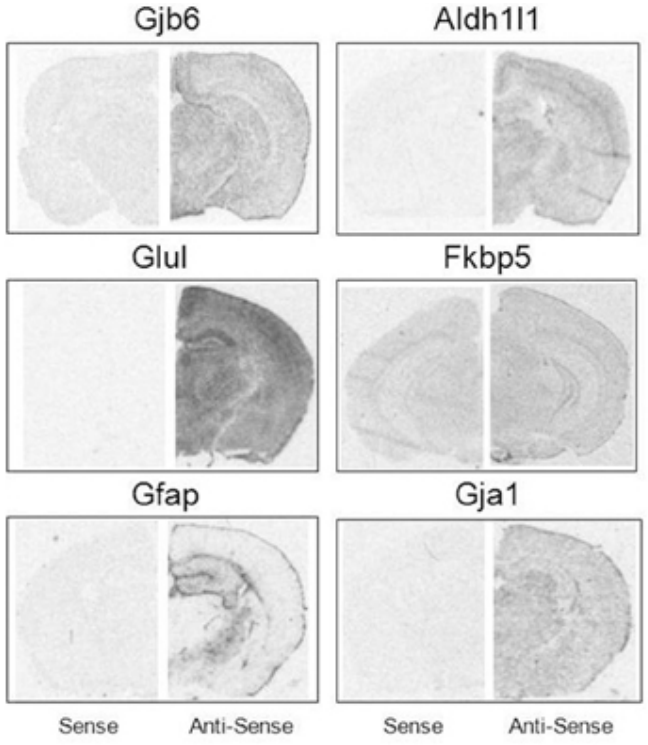

Abbreviations: ACC: anterior cingulate cortex;
MAC: medial agranular cortex; MC: motor cortex;
SS: somatosensory cortex; IC: insular cortex;
DM, DL, VL, VM: dorsomedial, dorsolateral, ventrolateral and ventromedialregions of caudate-putamen,respectively;
CAb, SAb: core and shell of nucleus accumbens.

Protocol

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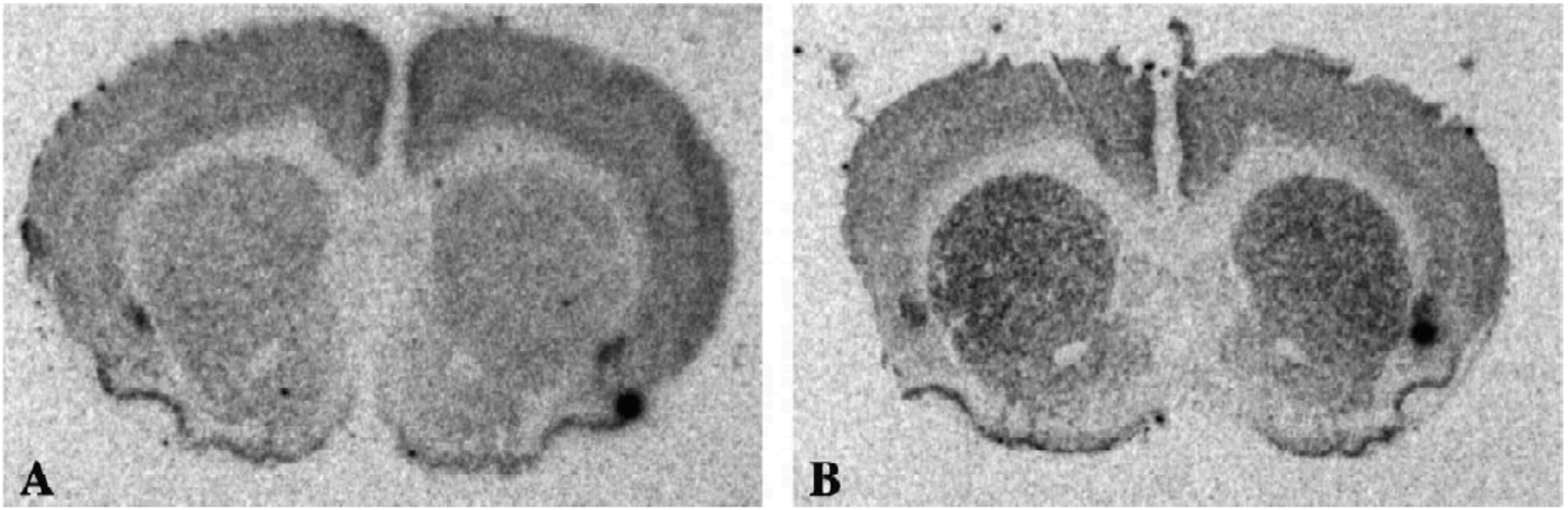
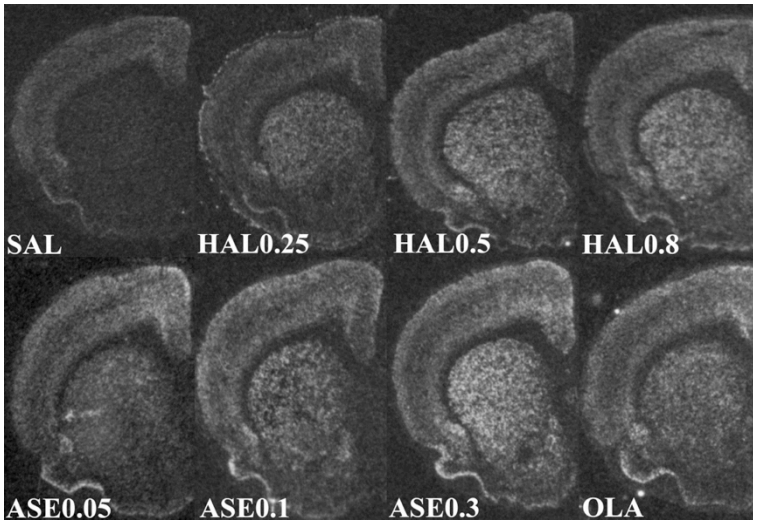
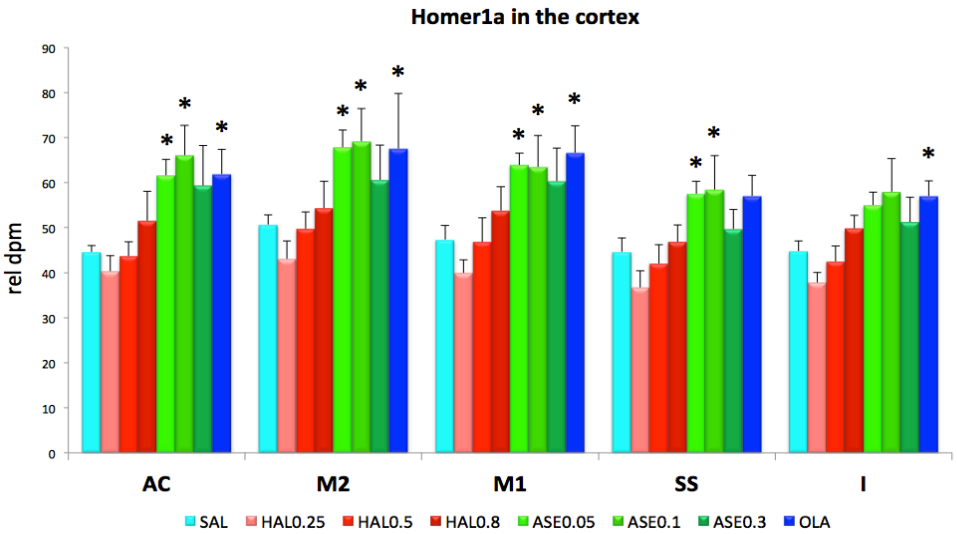
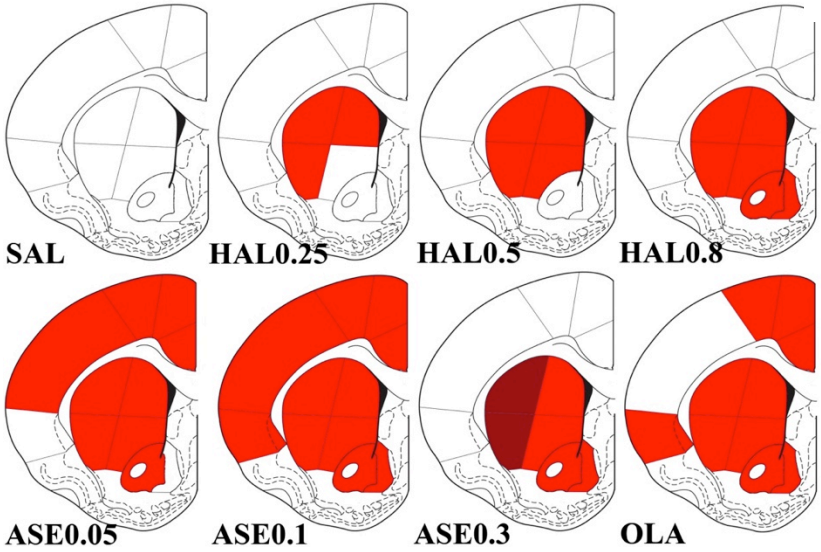


Fig. 2. Autoradiographic film images of Homer mRNA detected by means of in situ hybridization histochemistry (ISHH) in coronal brain sections from rats treated with saline (A), or haloperidol (B).

Dose and D1R/D2R ratio dependent Homer topography





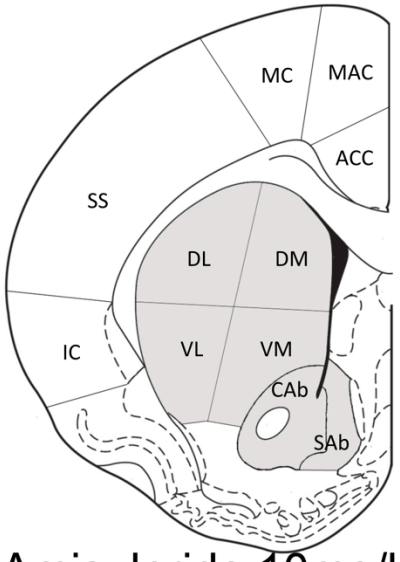


Increased expression vs SAL

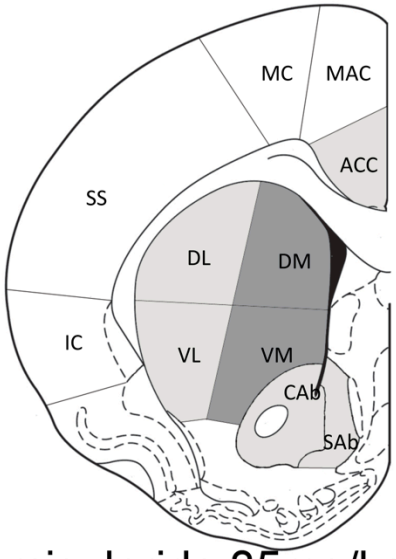
Increased expression vs all treatments

- Tukey post hoc test $p<0.05$

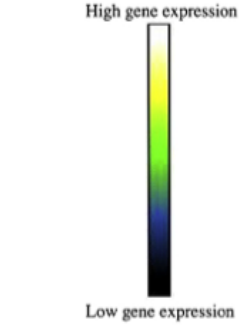
Intra-group differences of *Homer1a* expression pattern.
Not all D2R antagonists were created equal: the case of benzamides



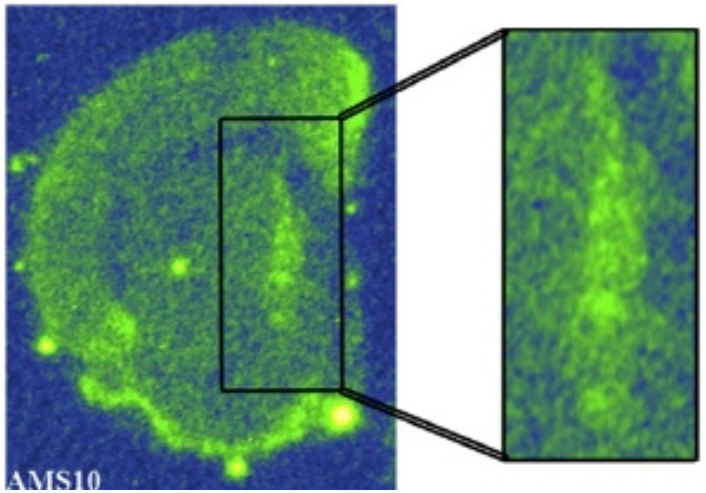
Amisulpride 10mg/kg

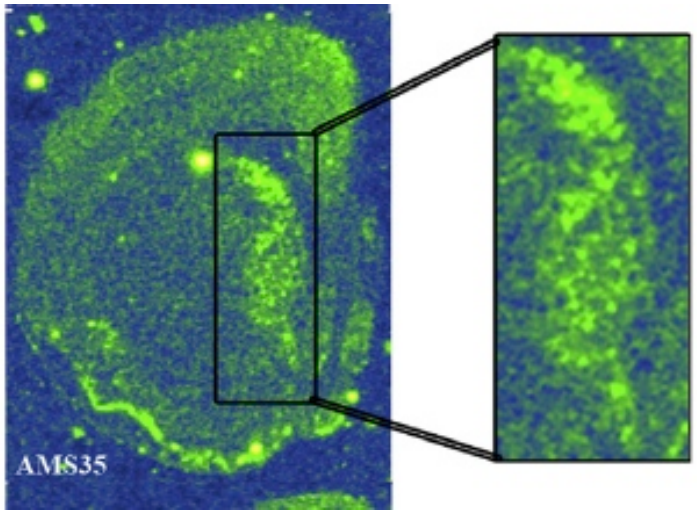


Amisulpride 35mg/kg

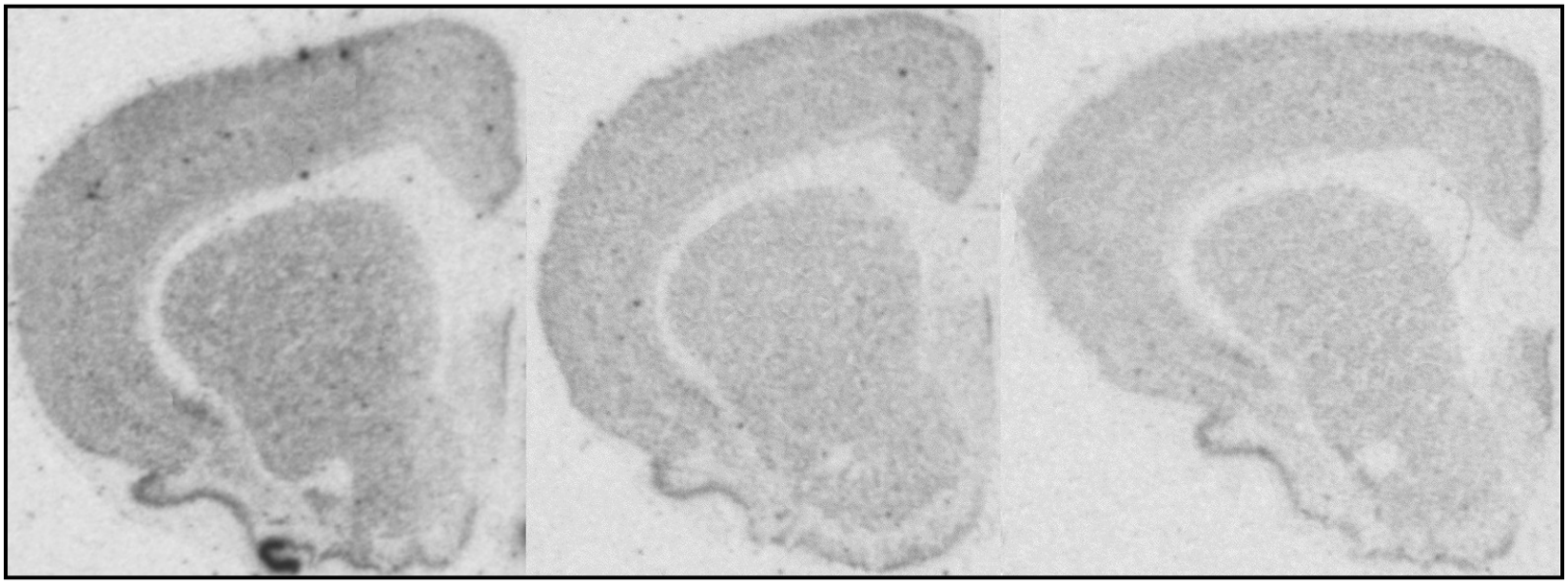


- no induction
- light induction
- moderate induction
- high induction



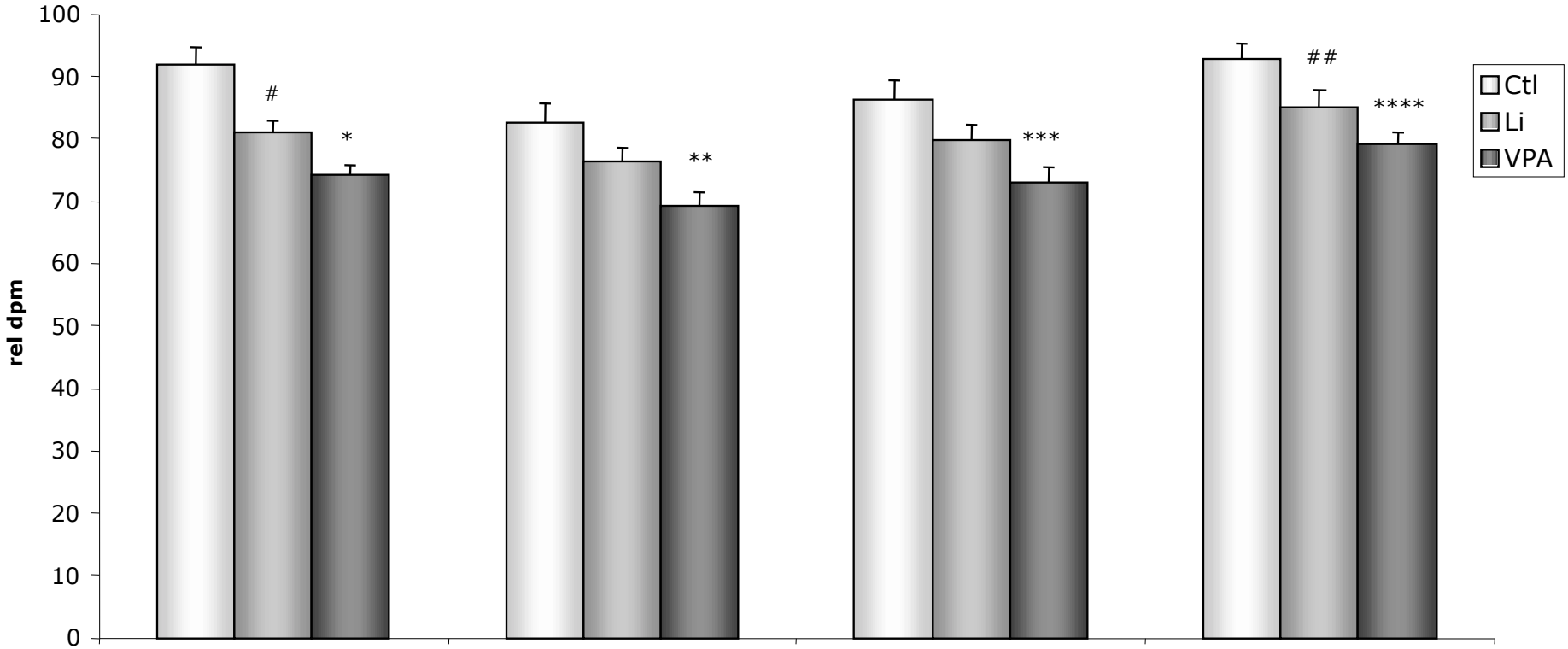


Espressione genica di *Homer 1b*: autoradiogramma



controllo litio valproato

Homer 1b mRNA in Caudato-Putamen



CP DL p =0.0001

CP DM p = 0.0045

CP VM p =0.0094

CP VL p =0.0025

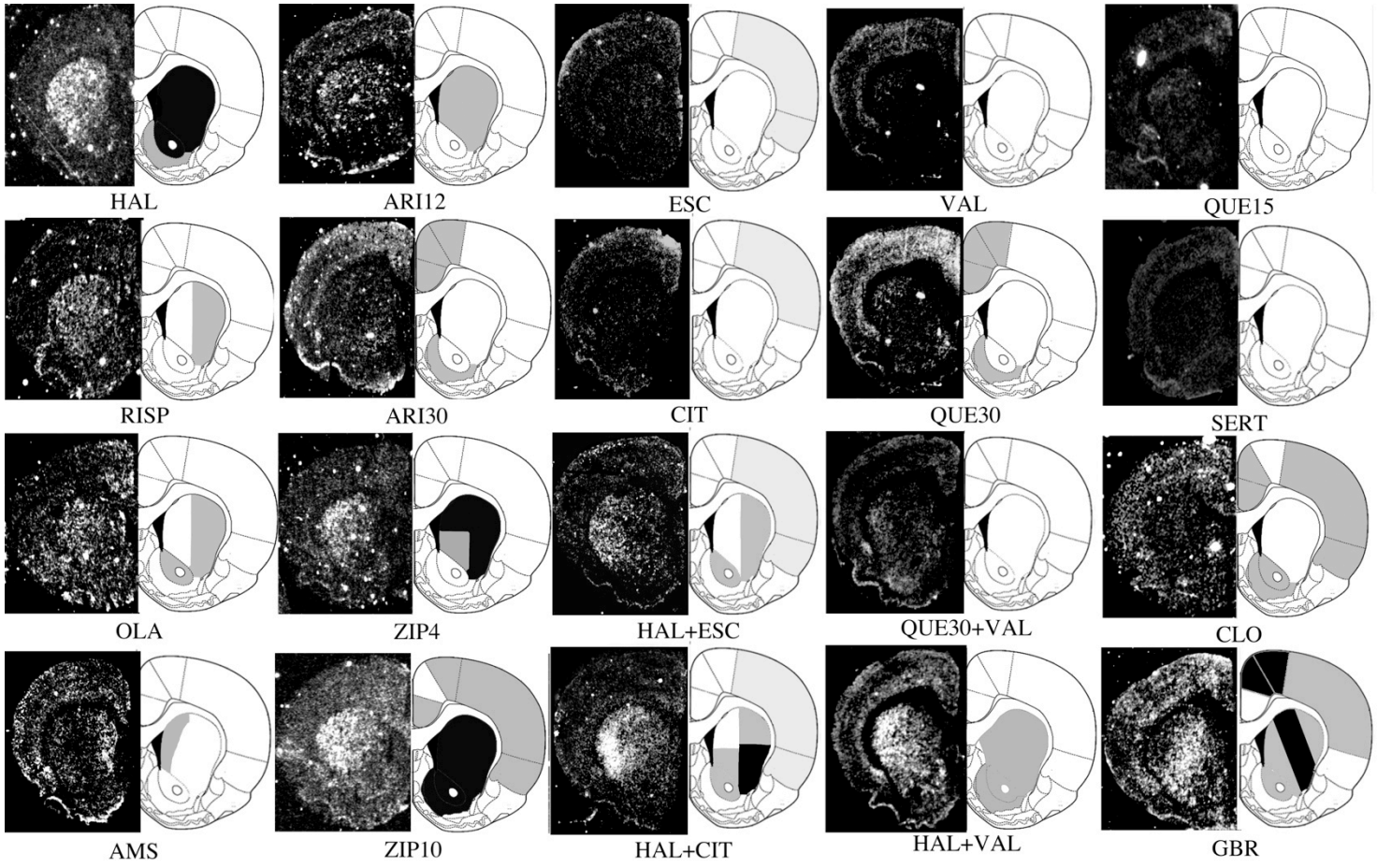
* vs. Ctl, Li
\# vs. Ctl

** vs. Ctl

*** vs. Ctl

**** vs. Ctl
vs. Ctl

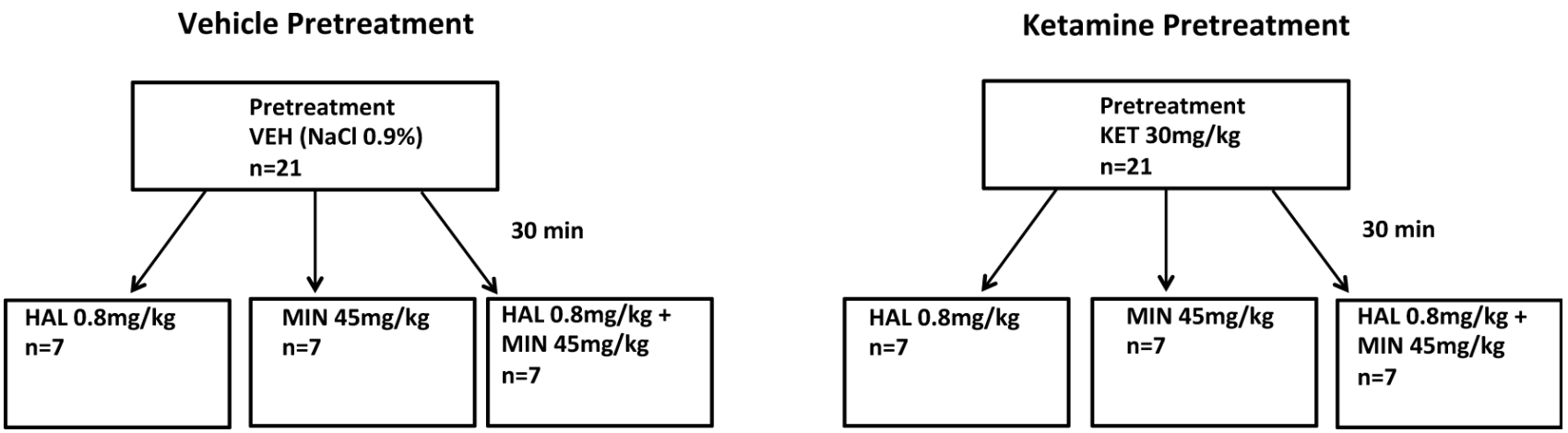
Overview


HAL
ARI12
ESC
VAL
QUE15
RISP
ARI30
CIT
QUE30
SERT
OLA
ZIP4
HAL+ESC
QUE30+VAL
CLO
AMS
ZIP10
HAL+CIT
HAL+VAL
GBR

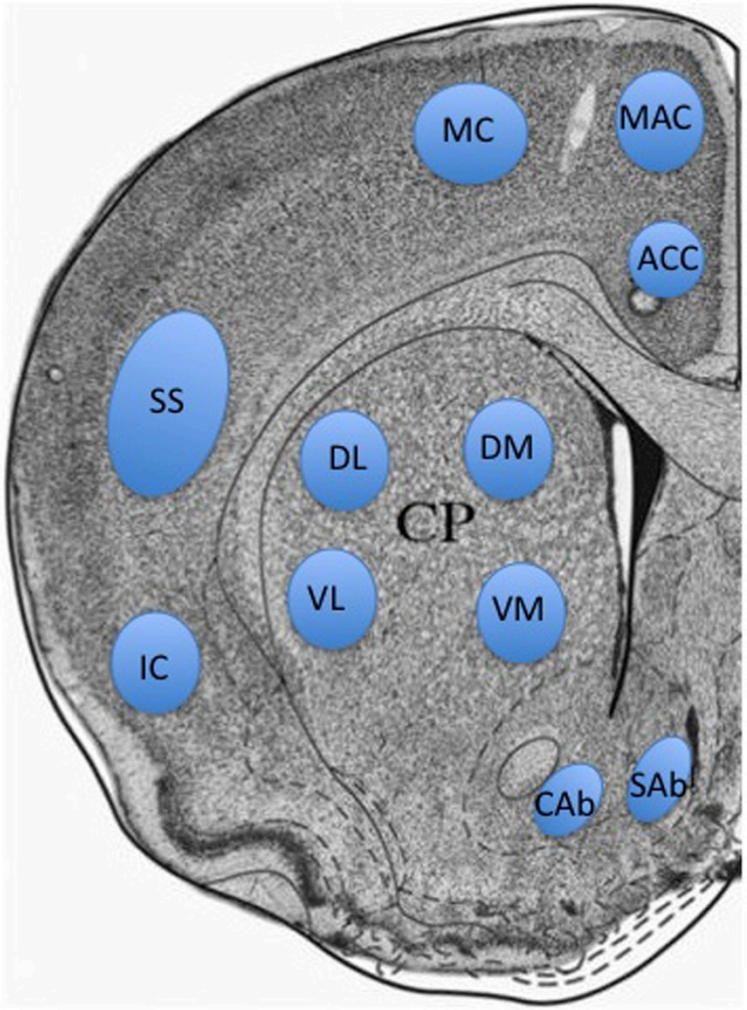
Studio Traslazionale 1

- Background
  - La minociclina è stata proposta come add-on in pazienti schizofrenici non responsivi al trattamento
  - In questi pazienti, la minociclina sembrerebbe migliorare soprattutto i sintomi negativi e depressivi

Studio Traslazionale 1

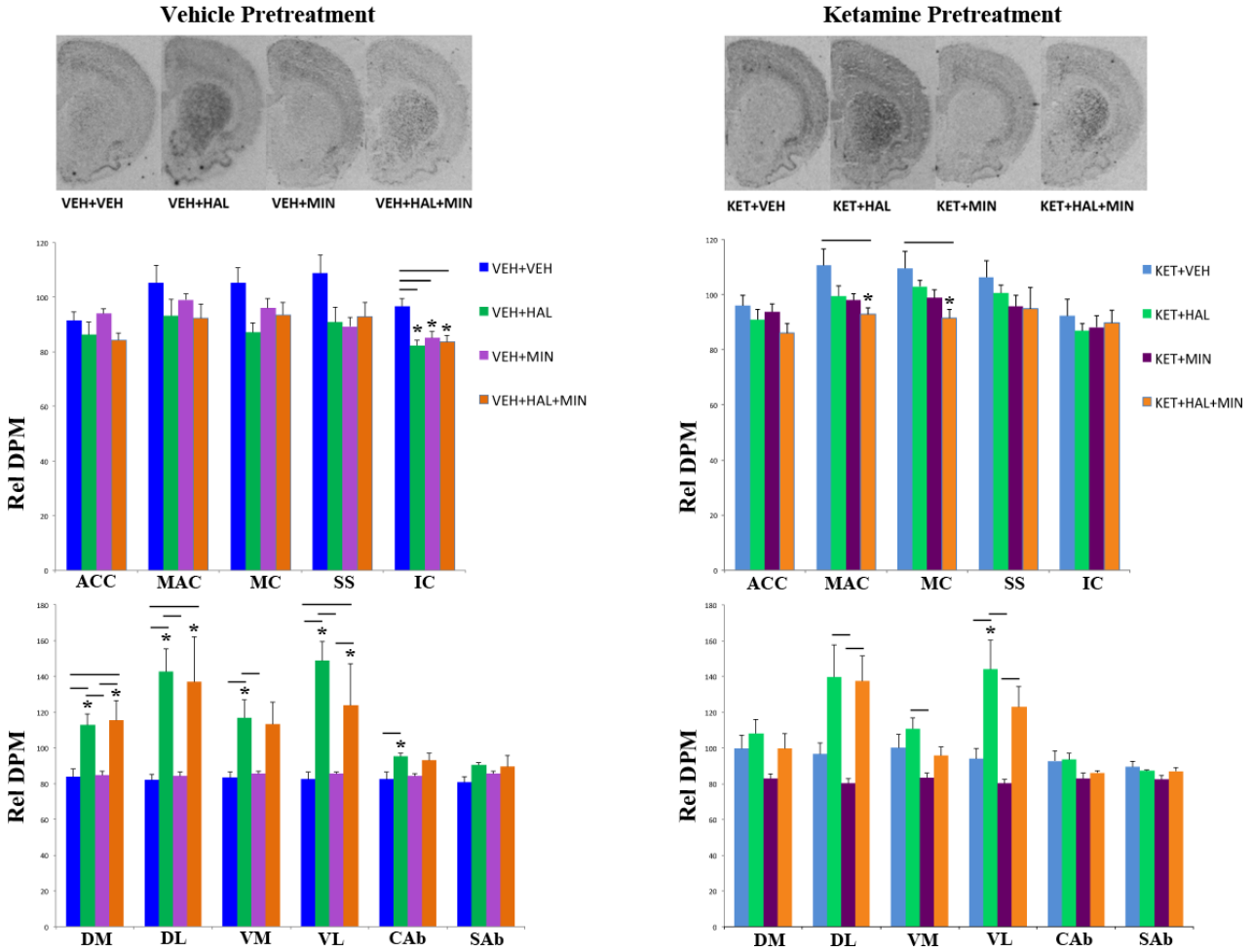


Studio Traslazionale 1



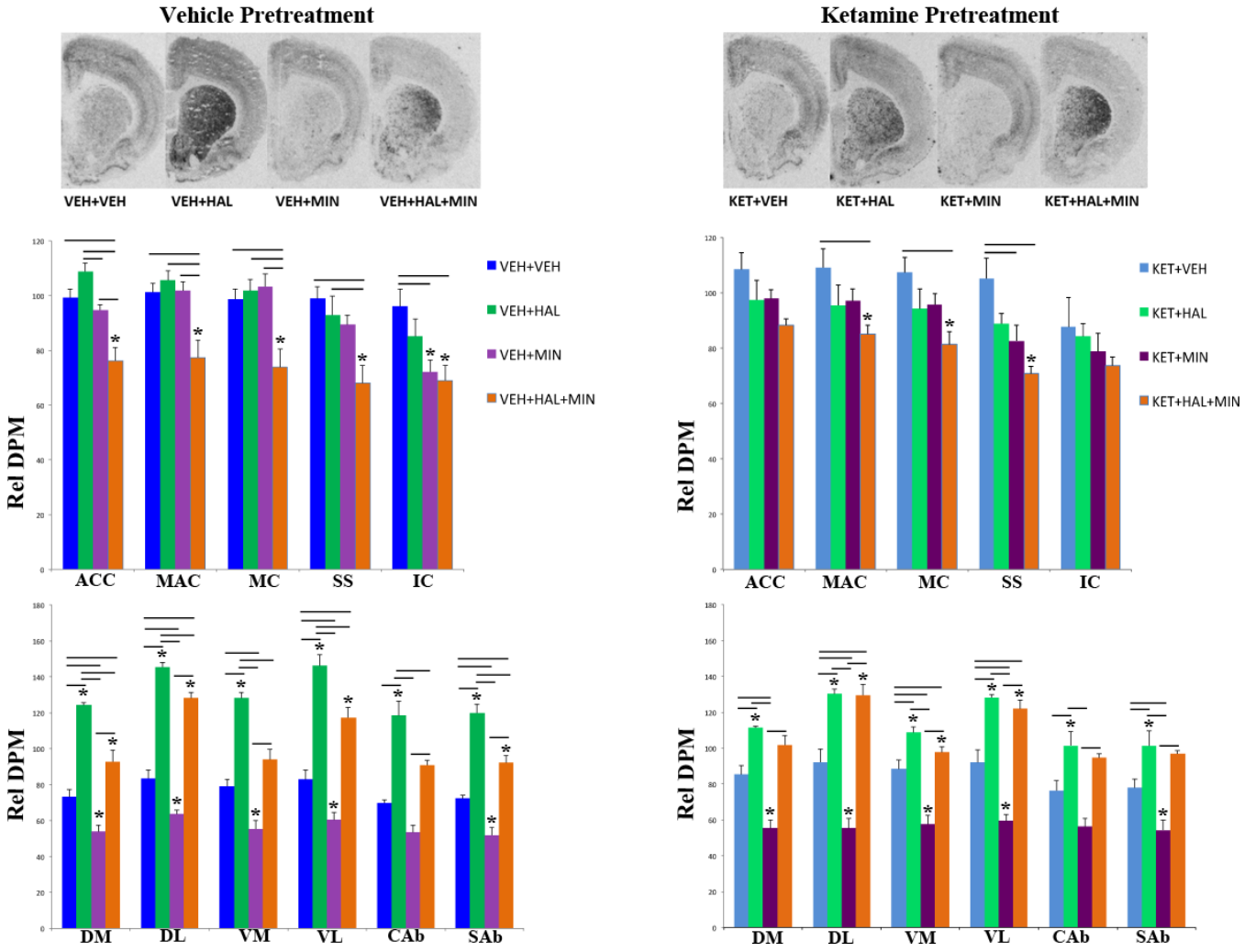
Studio Traslazionale 1

Homer1a



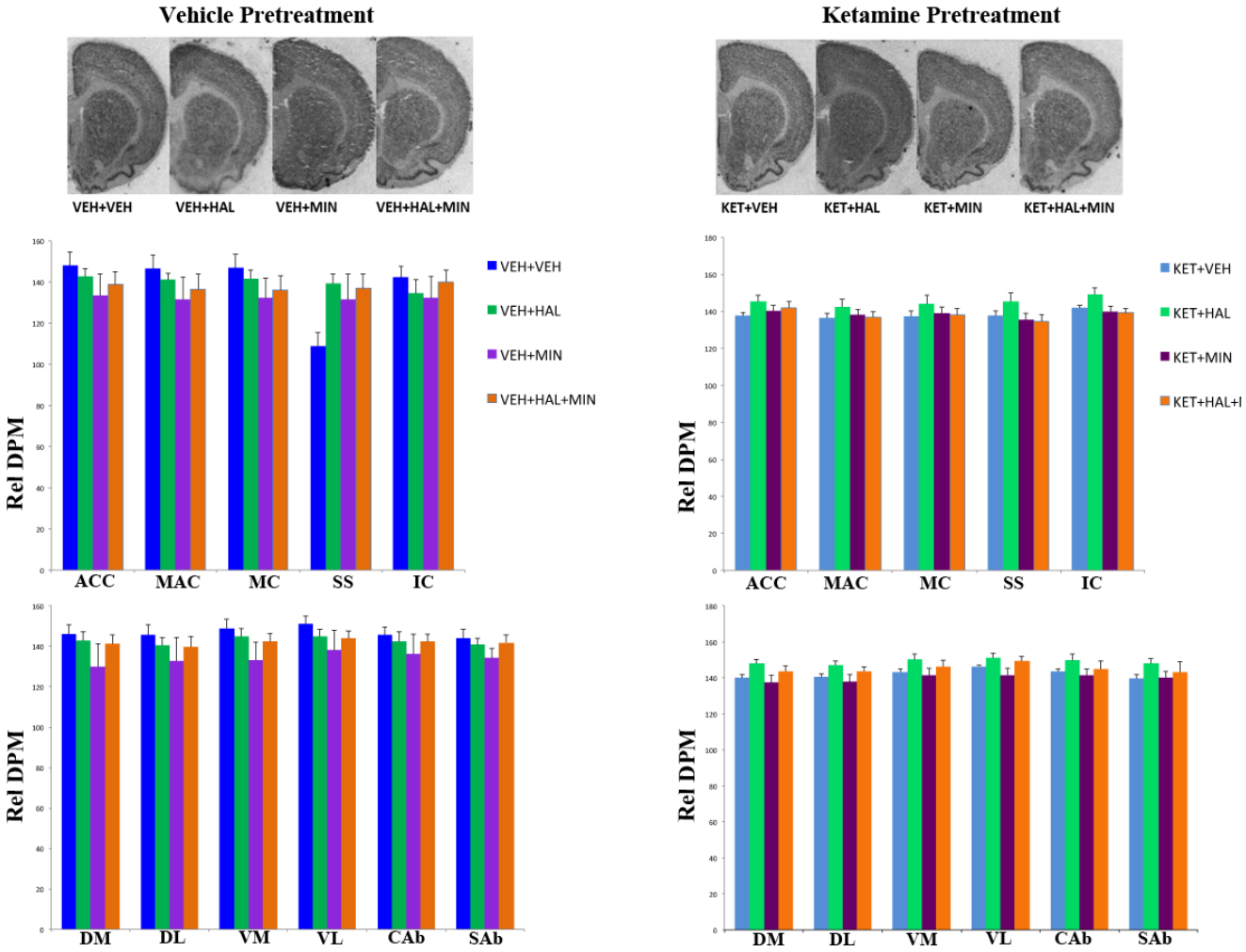
Studio Traslazionale 1

Arc



Studio Traslazionale 1

Homer1b/c



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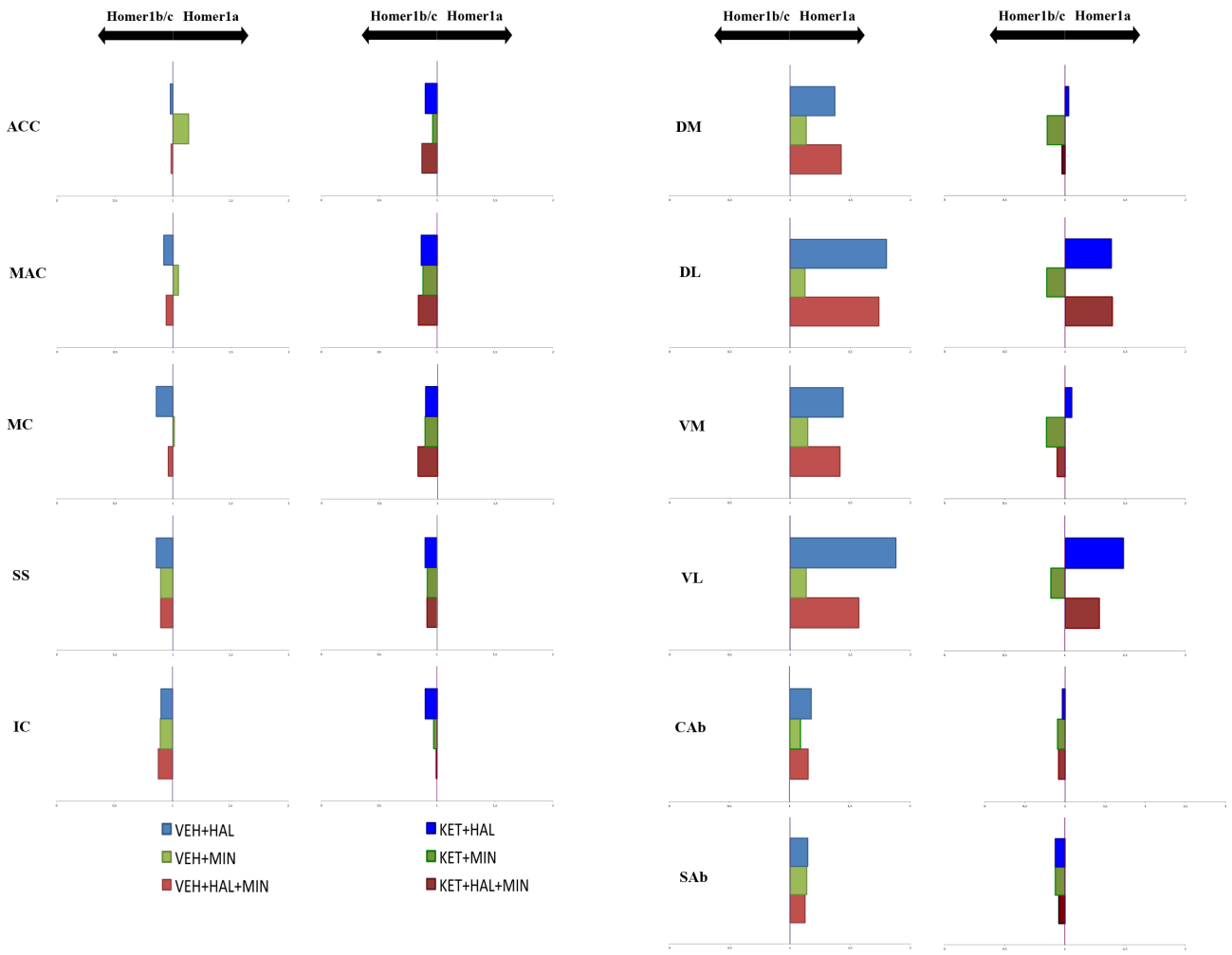


- Conclusioni:
  - La minociclina sembra ridurre l'espressione genica indotta da aloperidolo

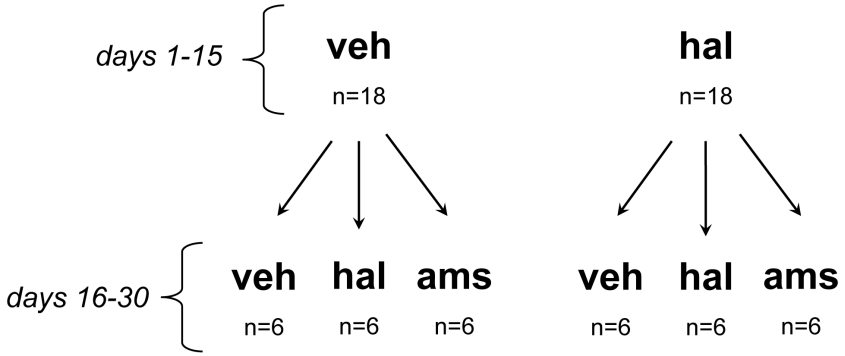
- Inferenze traslazionali:
  - La minociclina potrebbe ridurre gli effetti collaterali indotti da aloperidolo, ivi inclusi i sintomi negativi ed affettivi iatrogeni

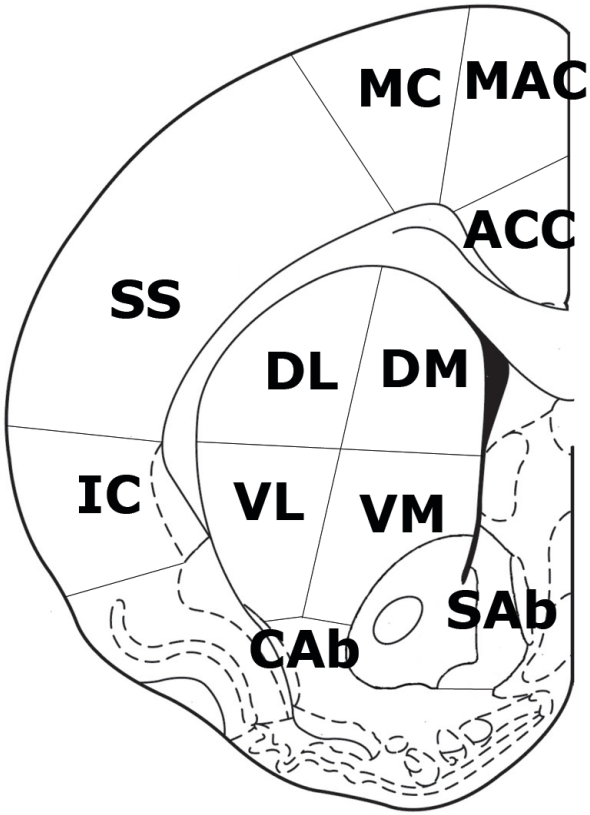
Studio Traslazionale 2

- Background
  - I trattamenti con antipsicotici sembrano modificare la propria risposta clinica (efficacia e tollerabilità) in funzione dei trattamenti precedenti

Studio Traslazionale 2

A. Experimental Design





B. Groups' Comparisons

Inter-group analysis.

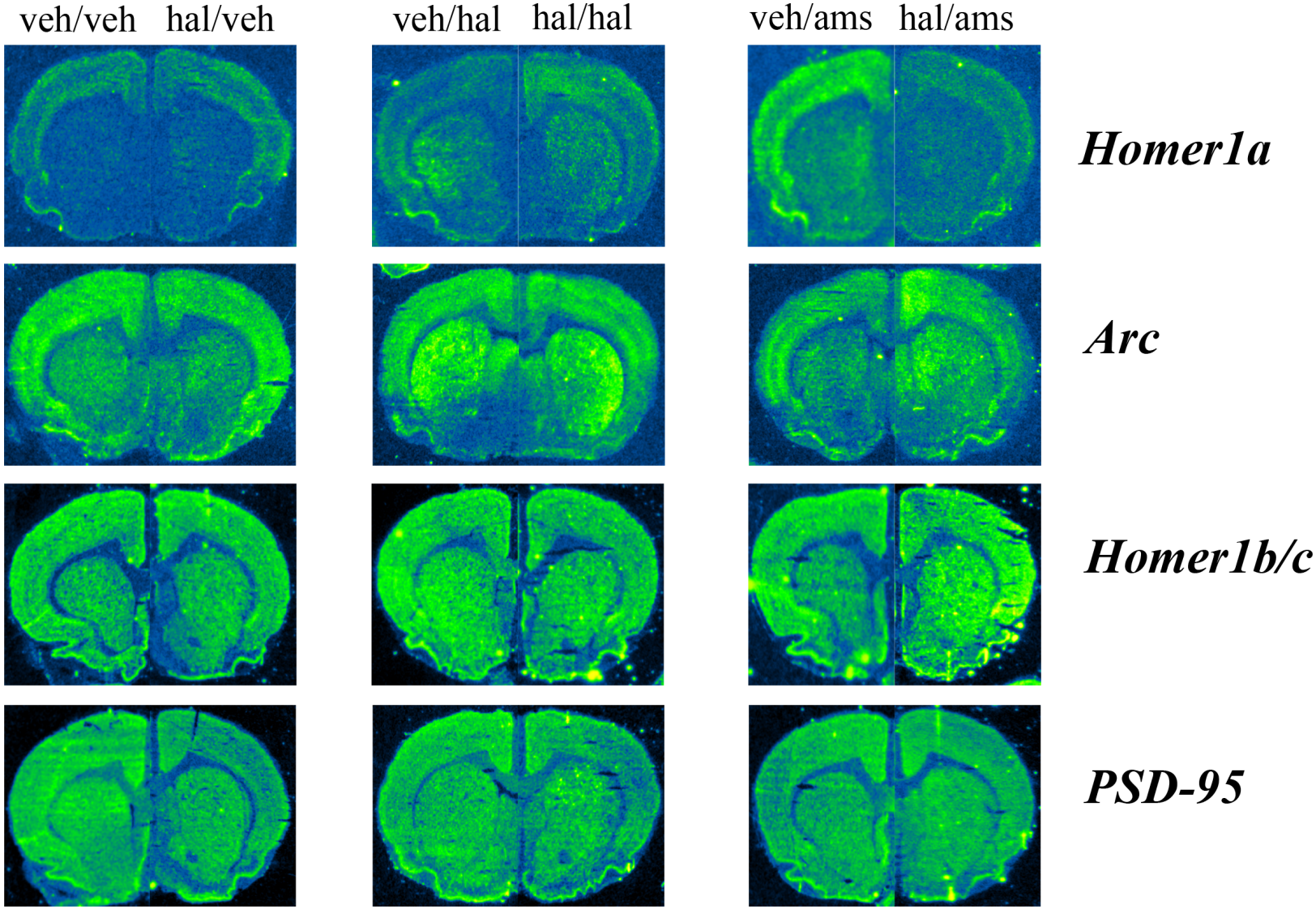
veh/veh, **veh/hal**, **veh/ams** — *ANOVA*, *Tukey's Post-hoc*

hal/veh, **hal/hal**, **hal/ams** — *ANOVA*, *Tukey's Post-hoc*

Head-to-head analysis.

veh/veh vs. hal/veh
veh/hal vs. hal/hal
veh/ams vs. hal/ams
Student's t

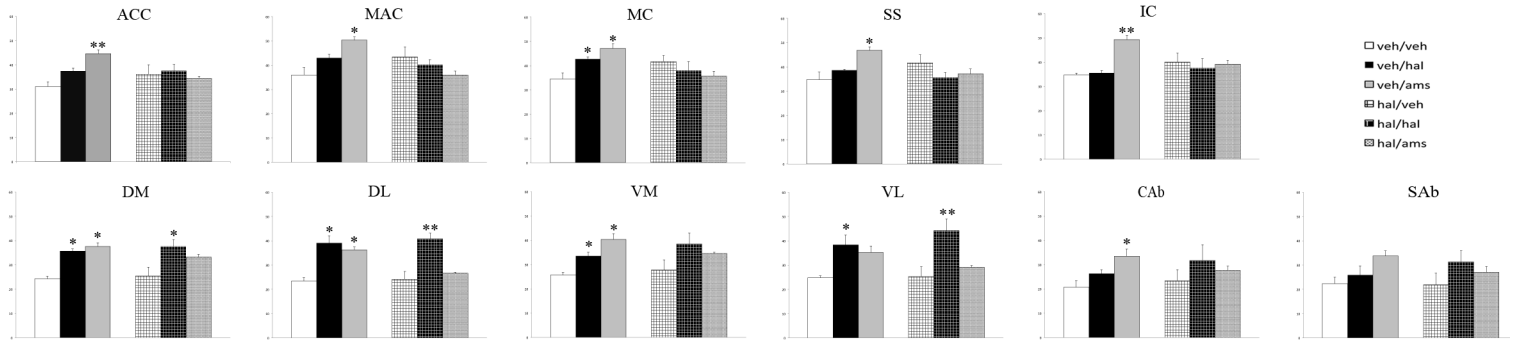
Studio Traslazionale 2



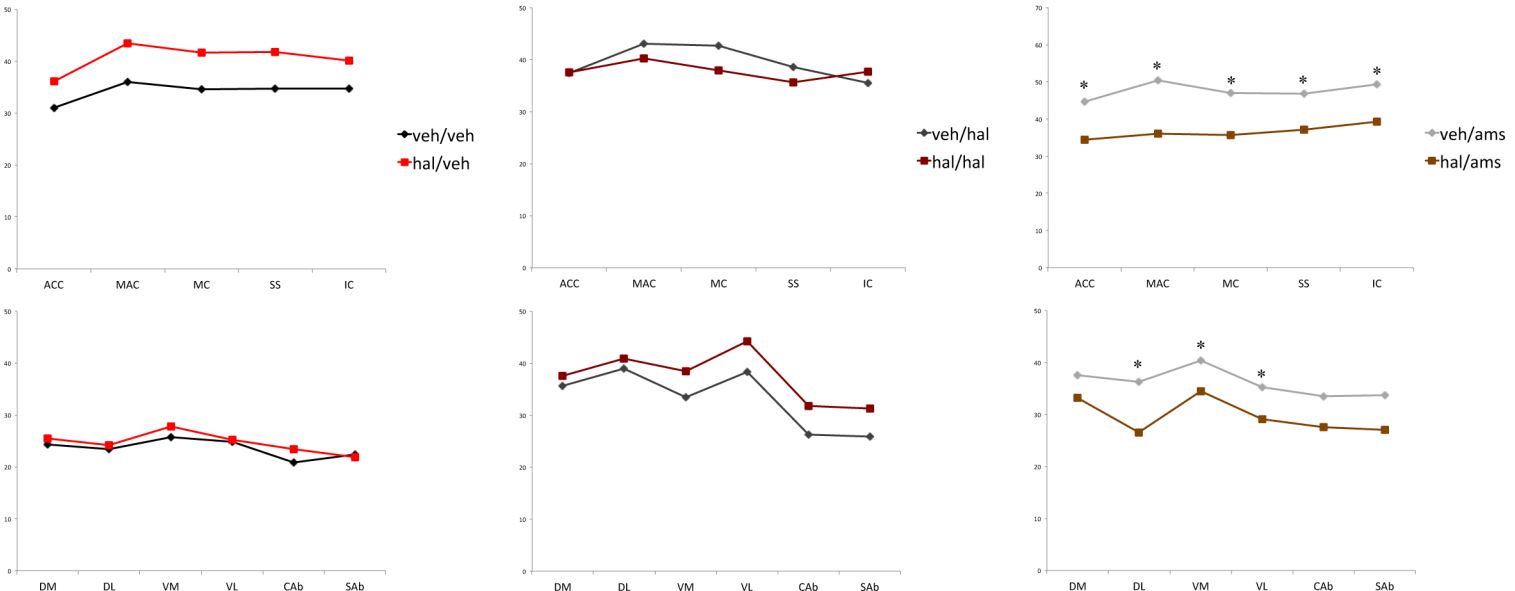
Studio Traslazionale 2

Homer1a

A



B



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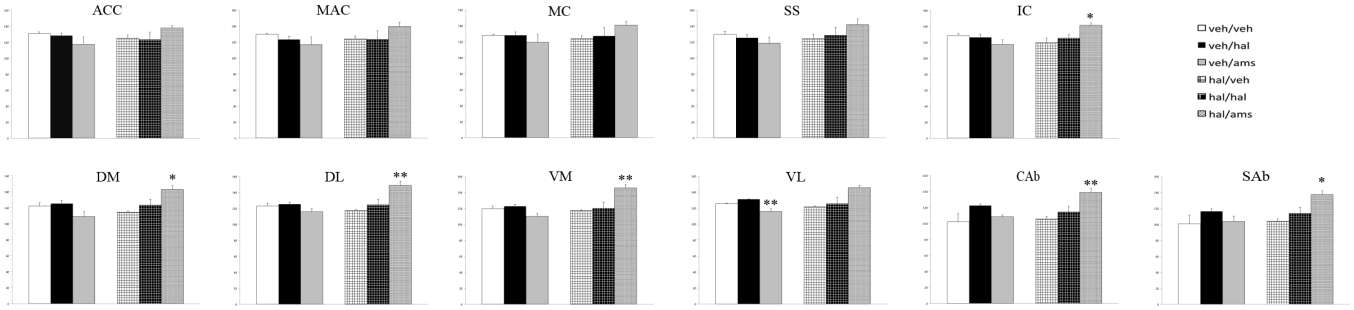
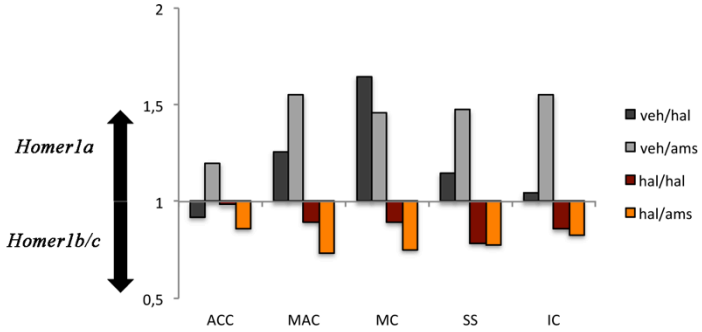
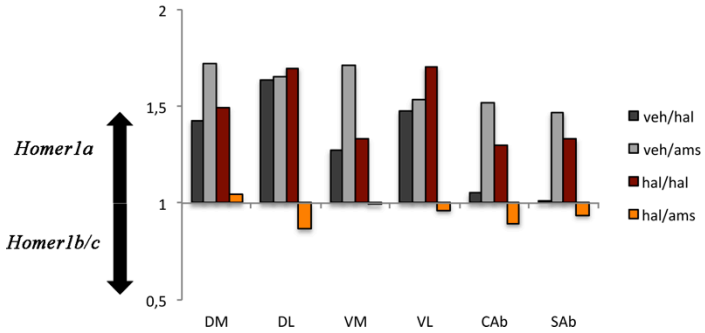


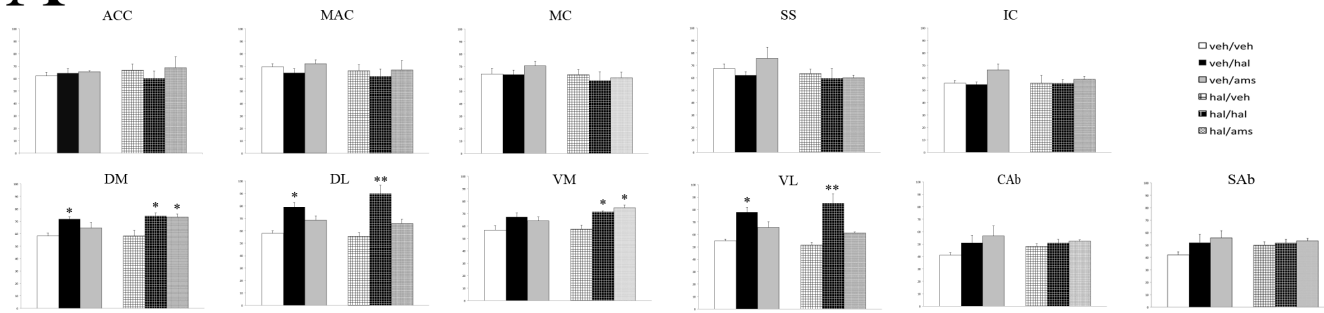




Studio Traslazionale 2

Arc

A



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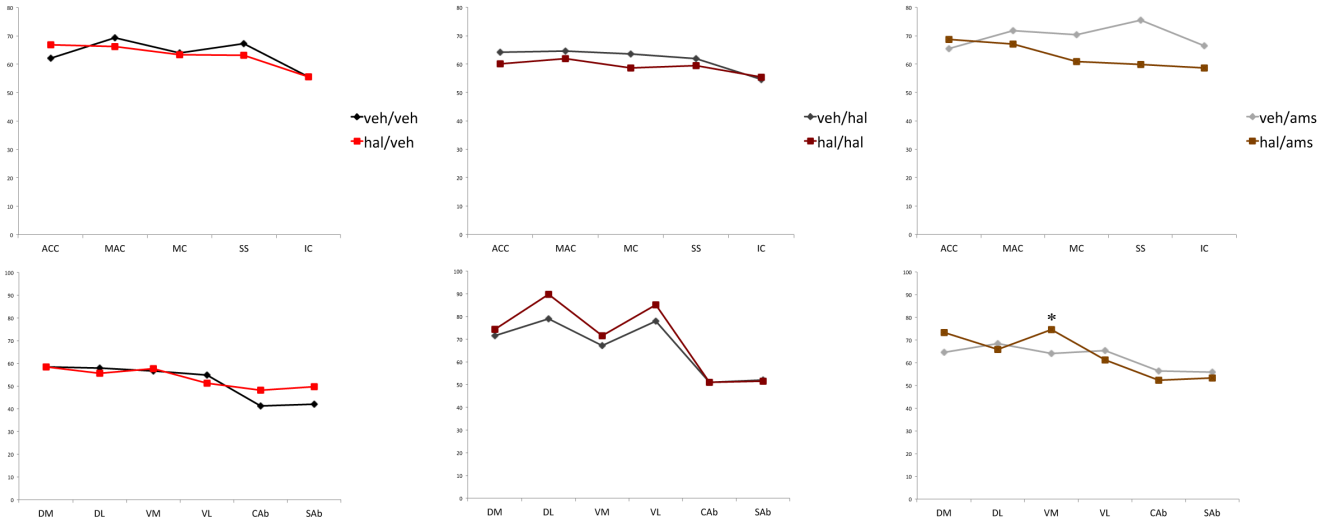

Studio Traslazionale 2

PSD-95

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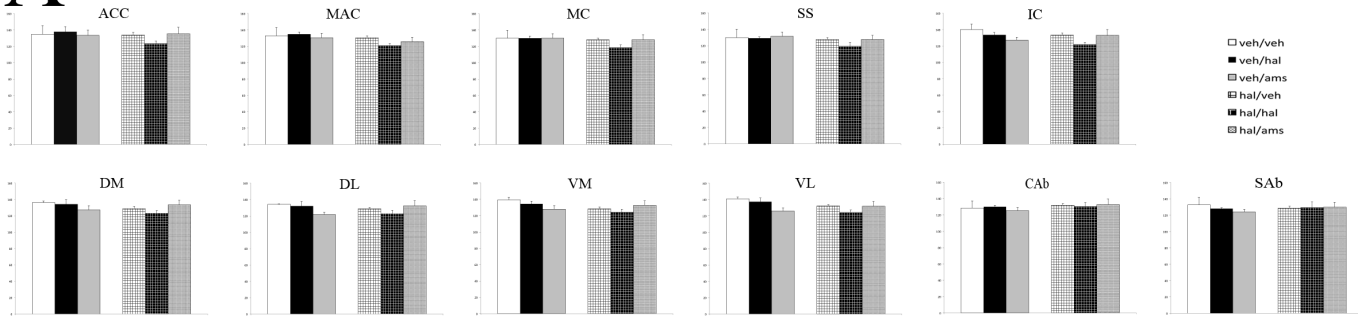

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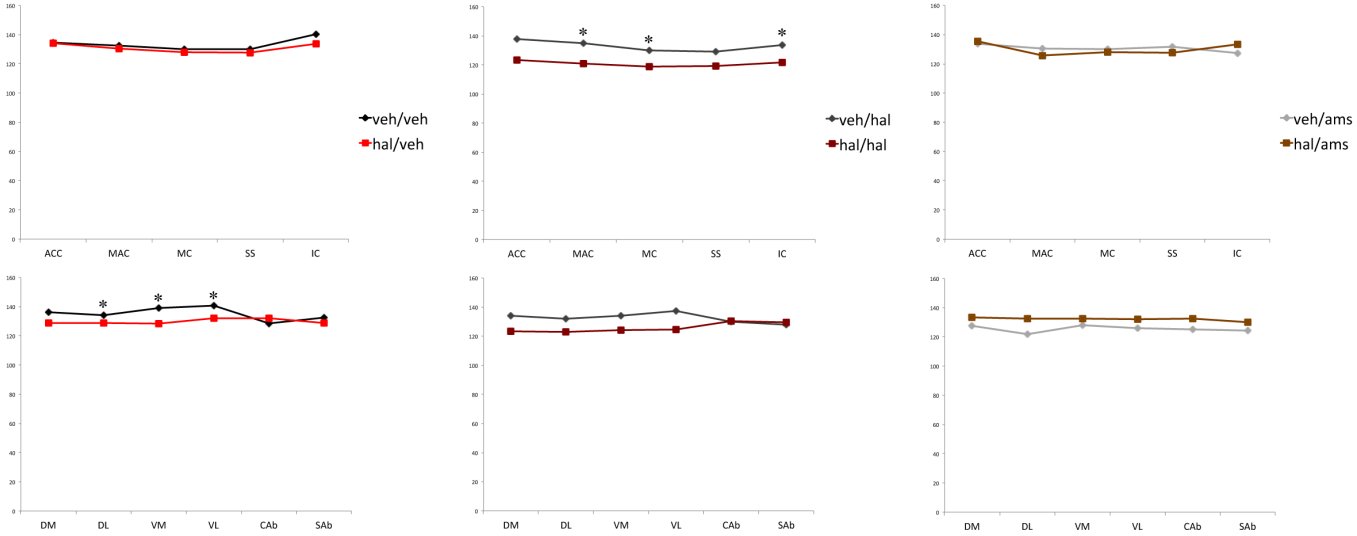
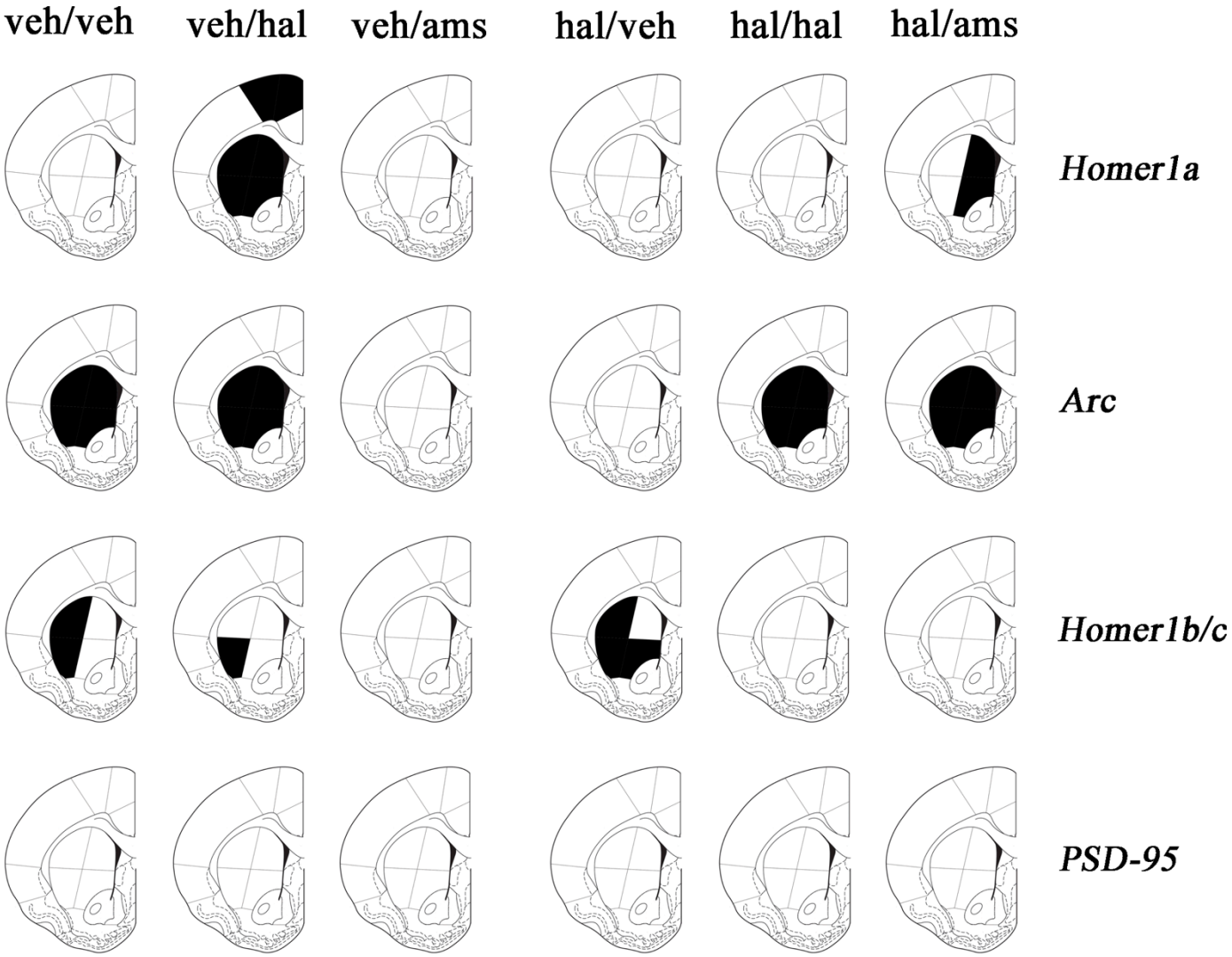


Studio Traslazionale 2



Studio Traslazionale 2

- Conclusioni
  - L'espressione genica indotta da amisulpride è notevolmente differente negli animali non pretrattati con antipsicotico rispetto a quelli pretrattati

- Inferenze Traslazionali
  - I trattamenti antipsicotici determinano modifiche neurobiologiche che potrebbero essere differenti in funzione dei trattamenti precedenti