

# Alpha4 nACh Genotype and Attentional Network Dysfunction - Implications for Nicotine Dependence in Schizophrenia

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# ***Nicotine Abuse in Schizophrenia***

- **Nicotine Abuse/Dependence: approx. 60-70%\* [30-35%]\*\***
- **Heavy Smoking in Schizophrenia Patients (particularly during acute psychosis)**

*de Leon et al 1995; Lasser et al. 2000*



***Cardiovascular Risk Increase contributing to 20% diminished Life Expectancy***

\* Jahresprävalenz

\*\* Erwachsene Allgemeinbevölkerung

# ***Why are Schizophrenics smoking?***

- ***Accelerated Metabolism of Neuroleptics***
- ***Stressregulation (Self-Medication)***
- ***Stimulation of dopaminergic Reward System  
(Self-Medication of Negative Symptoms/Depression)***
- ***Self-Medication of cognitive Deficits***

# ***Nicotine Abuse & cognitive Deficits in Schizophrenia***

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- ***In Schizophrenia, Nicotine is normalising:***

***Attentional Deficits, P50-Filter, Working Memory, Smooth Pursuit Eye Movements***

*Adler et al 1998; Levin et al., 1996a; Balfour & Fagerström, 1996; Sherr et al., 2002*

- ***Smoking at Age of 18-20 y. = protective: Risk Ratio 0.8 [95% CI=0.7-0.9]***

*Zammit et al 2003 (Cohort Study from Sweden)*

## Effects of transdermal Nicotine-Application on Cognition

### Non-Smoking Study Population:

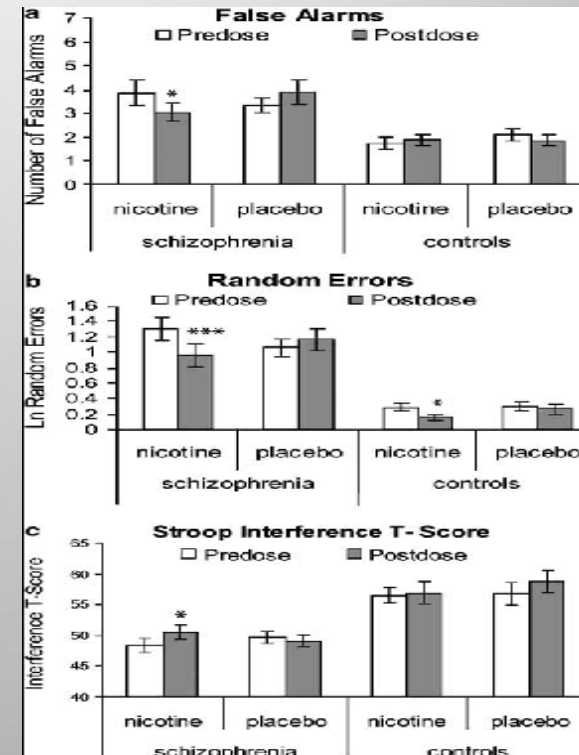
Schizophrenia (n = 28), Controls (n = 32)

### Study Design:

Transdermal Nicotin (14mg)/Placebo  
randomised, cross-over, 3h pre- und 3h post

### Dependent Variable(s):

Cognitive Battery: CPT = Primary Variable



Nicotine Effect on Cognition is stronger in Schizophrenia than in Controls

## Effects of transdermal Nicotine-Application on Cerebral Function

### Study Population:

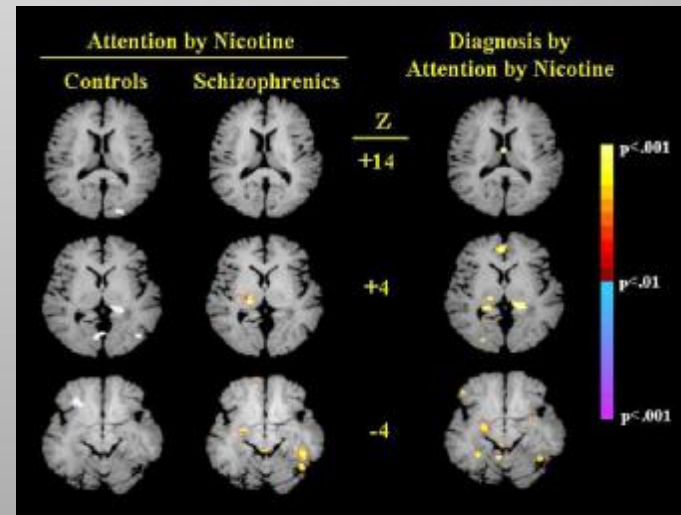
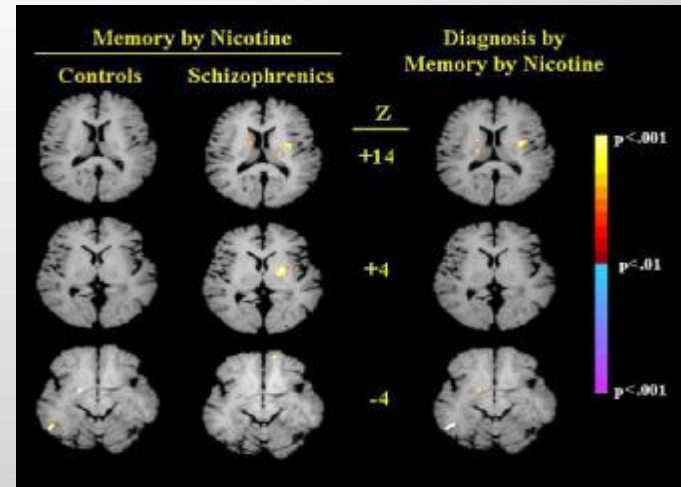
Smoking Schizophrenics (N=13)  
Smoking Controls (N=13)

### Study Design:

Nicotine transdermal (28mg)/Placebo,  
randomised, double-blind,  
2 Measurement Time Points

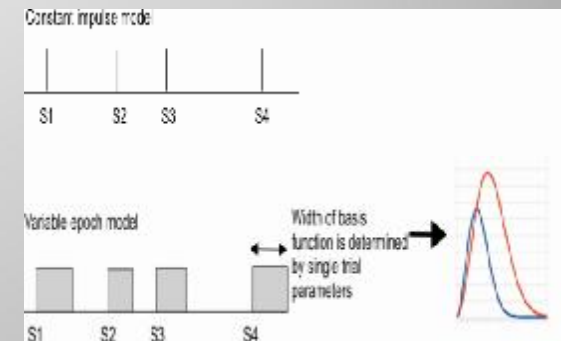
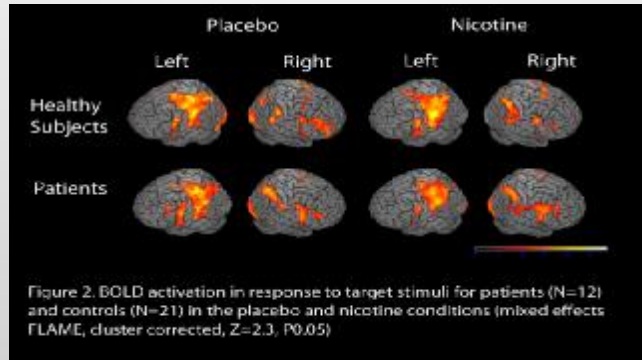
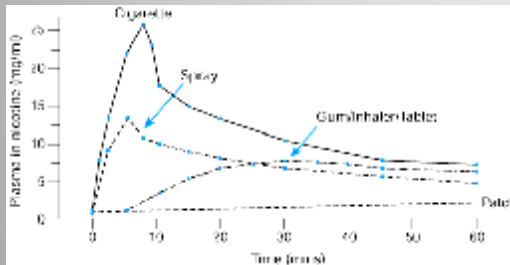
### Abhängige Variable:

BOLD-Response (fMRT) during  
Auditory N-Back Task



**Nicotine activates Attentional Network (incl. Thalamus, Anterior Cingulate) more in Schizophrenia than in Controls**

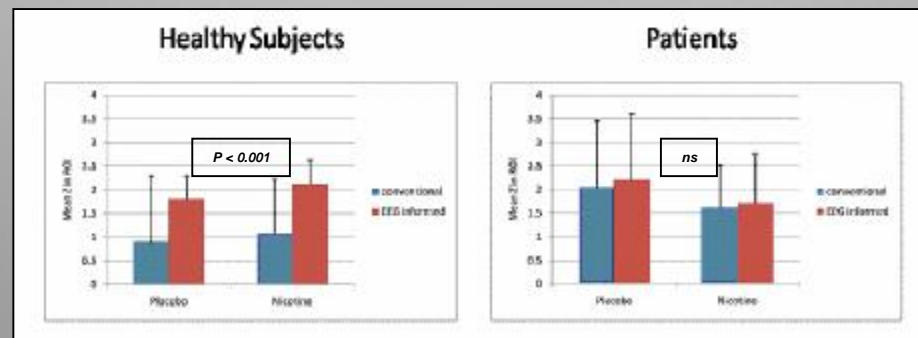
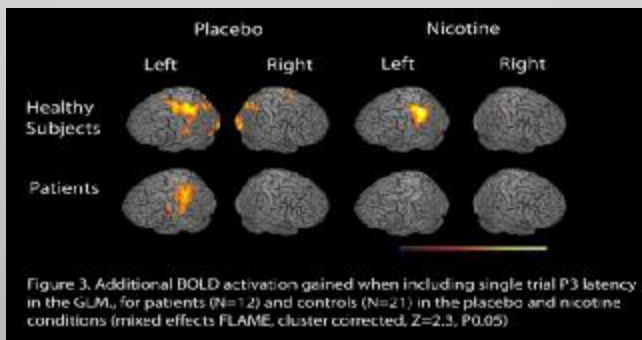
# Nicotine Effects in Controls & Schizophrenia - Simultaneous fMRI/EEG Study -



Single Trial Parameter = P300-Latency

**Nicotine Spray (1mg) vs Placebo**  
(double-blind, cross-over, randomized)  
N = 21 Controls  
N = 12 Schizophrenics

Visual Oddball Task



ROI-Analysis (Z-values) of Nicotine Effects: Conventional vs EEG-informed

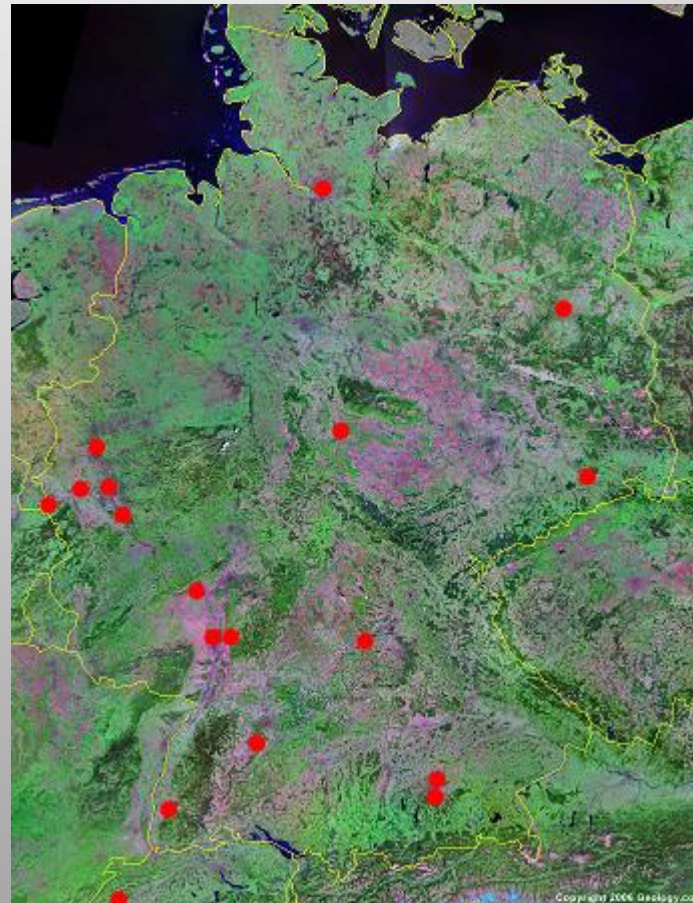
# National Priority Program of the German Research Foundation (DFG):

## Molecular and physiological Nicotine Effects in CNS

*Coordinator: Georg Winterer (Düsseldorf)*

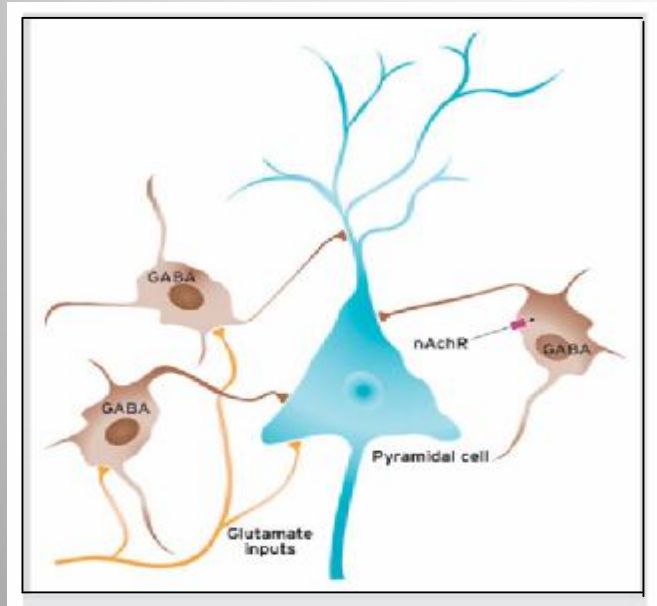
Interaction: Nicotine Dependence & Schizophrenia

- Aachen
- Berlin
- Bonn
- Cologne
- Dresden
- Duesseldorf
- Erlangen
- Freiburg
- Geneva
- Goettingen
- Hamburg
- Heidelberg
- Juelich
- Mainz
- Mannheim
- Munich
- Neuherberg
- Tuebingen

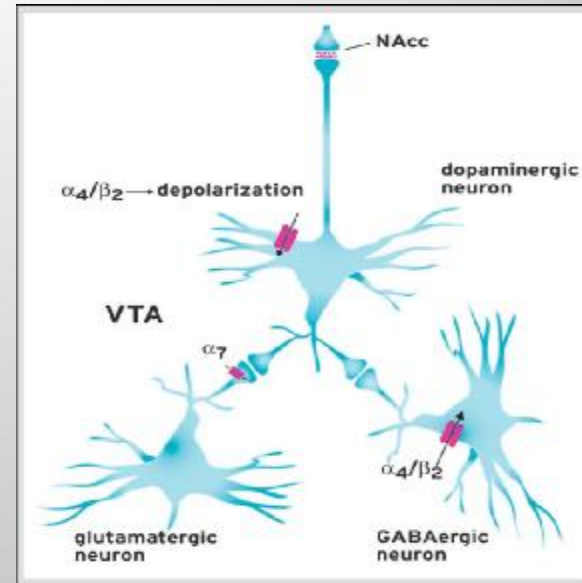


<http://www.nicotine-research.com>

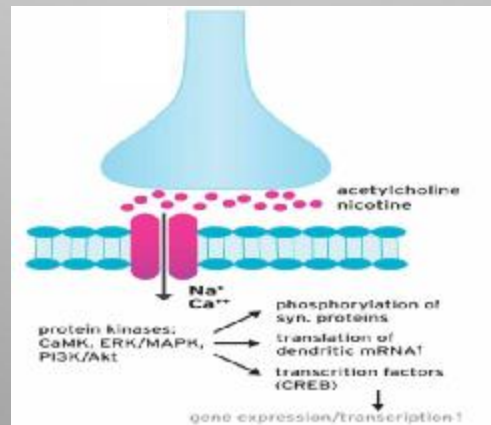
# Improving Attention & Working Memory with Nicotine



Nicotinic modulation of GABAergic input in layer 5 pyramidal cells in the prefrontal cortex

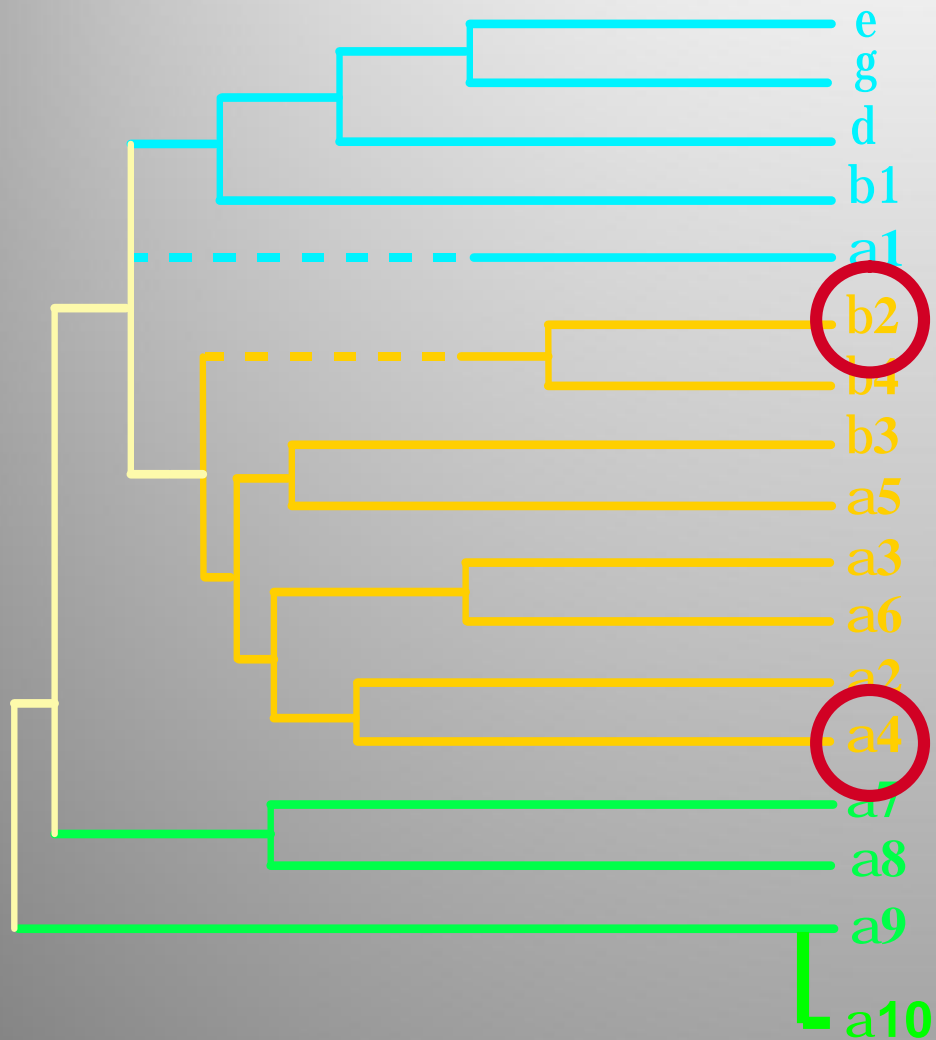


Nicotine may also impact on prefrontal functioning by modulating glutamatergic and dopaminergic input

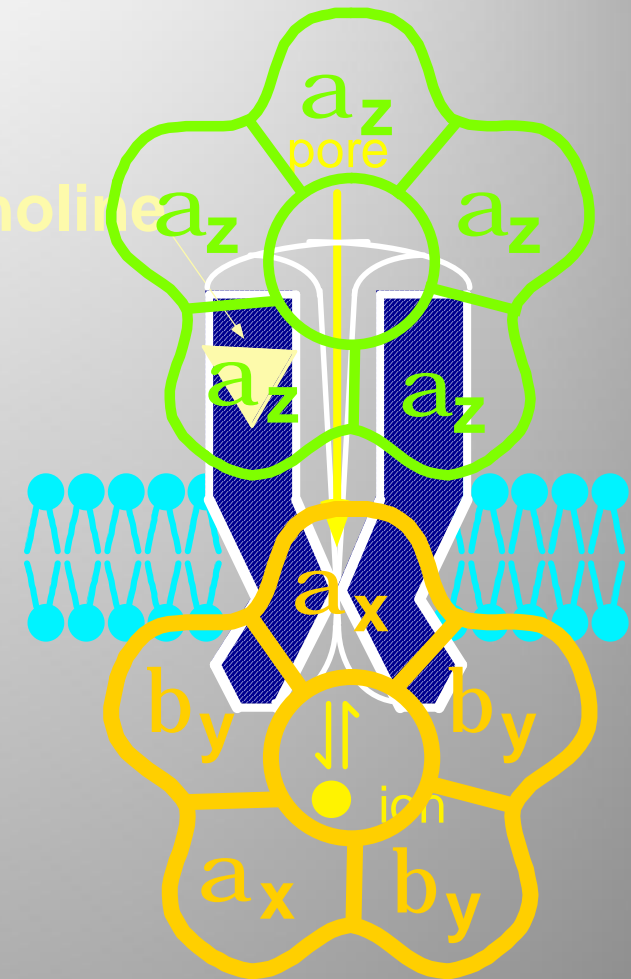


*Induction of neuroplastic changes*

# Nicotinic ACh Receptors



acetylcholine



*Lenovere und Changeux, 1995*

## ***Alpha4Beta2 Receptor Subtype: CHRNA4 Candidate Gene?***

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- **Nicotine Effects on behavioral and physiological level appear to be mediated considerably through the high-affinity  $\alpha 4\beta 2$  Subtype because they most abundant in the brain ( 90% of all high-affinity Receptor in mammalian Brain)**

***Lindstrom et al (1987); Alkondon et al (2000)***

- **Transgenic Mice with  $\alpha 4$  Subunits, which show a Point Mutation in the Ion-Channel forming M2 Domain, react hypersensitive to nicotine**

***Tapper et al Science (2004)***

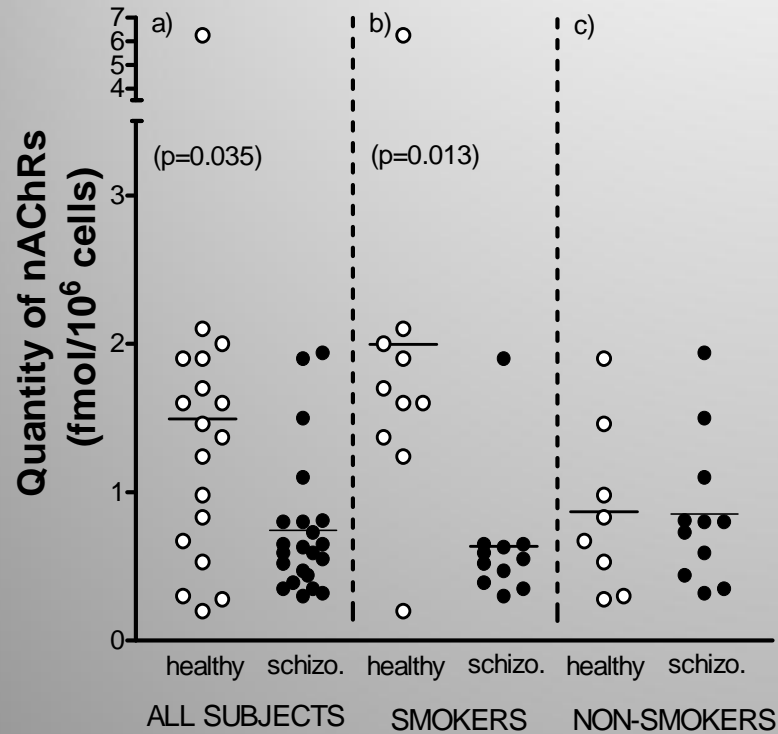
- **Normally there is a substantial increase in high affinity nicotinic receptors in smokers (Benwell et al. 1988)**

**However, high-affinity  $\alpha 4\beta 2$  nicotine binding is decreased by approximately one-half in multiple regions of post-mortem brain in schizophrenic smokers, including hippocampus, caudate and cortex (Breese et al. 2000)**

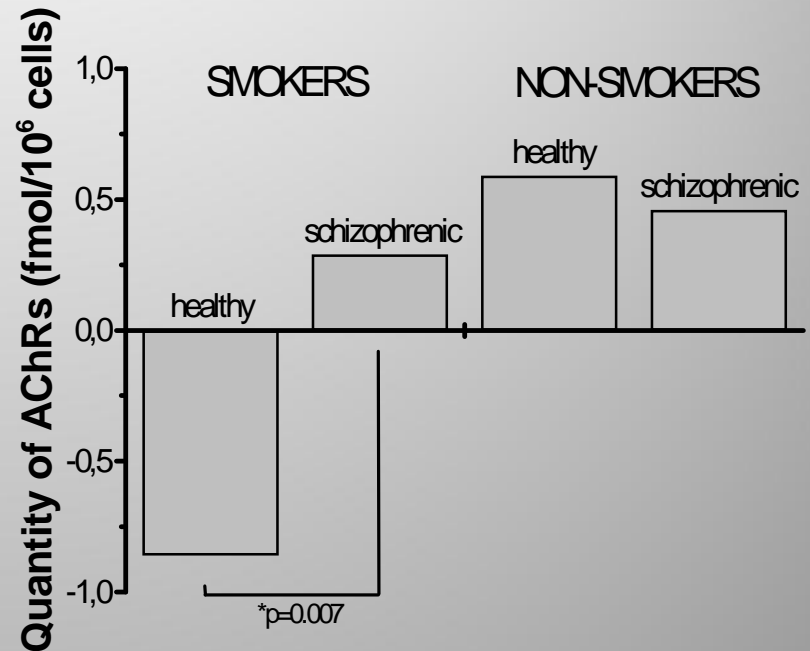
***[Finding confirmed by us in lymphocyte expression study]***

**This points out an important possibility of a gene per drug interaction**

## nACh Receptor Quantity in [3H]-nicotine labelled EBV-transformed B-Lymphoblasts



Before Nicotine Incubation



After Nicotine Incubation

*Luckhaus et al. (in preparation)*

## ***Nicotine Receptor Genetics & Schizophrenia***

### ***Alpha4Beta2 Receptor Subtype: CHRNA4 Candidate Gene***

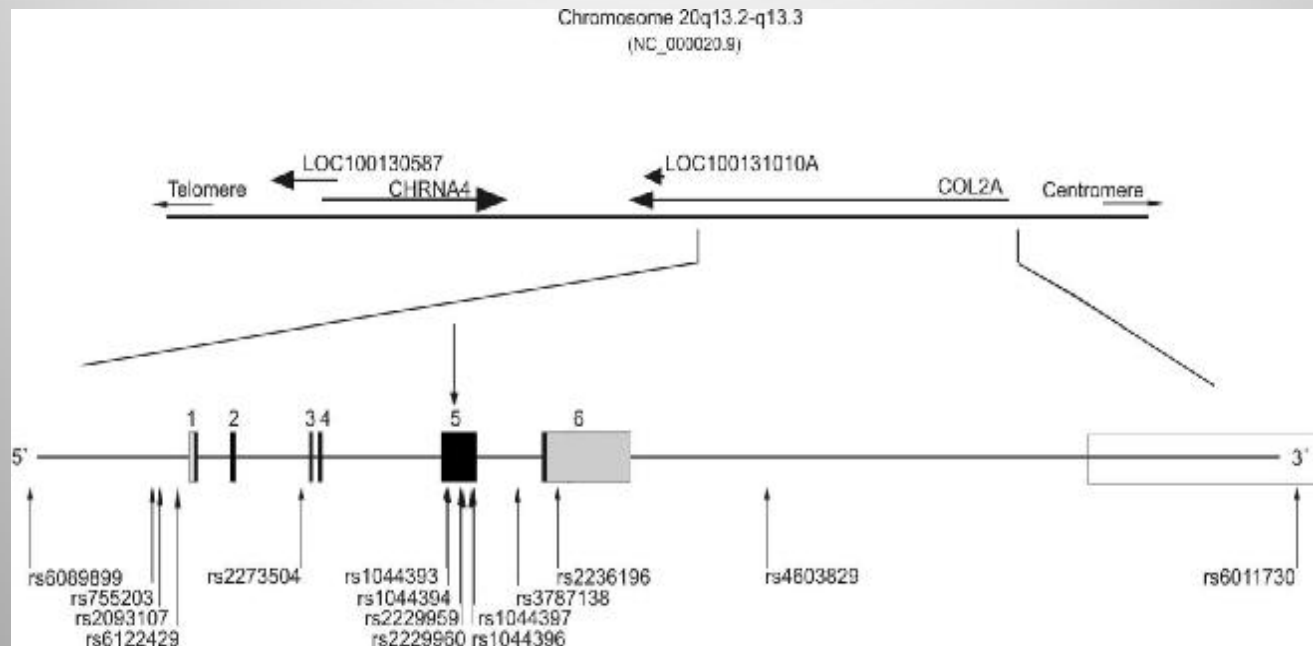
- Several mutations have been identified in *CHRNA4* (20q13.3) (Exon 5) to cause *autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)*
- Behavioral symptoms most common (43%) abnormality in ADNFLE incl. cognitive deficits and serious psychiatric disorders with mostly psychosis or schizophrenia-like clinical features\*
- Genetic variations in *CHRNA4* have been associated with *Attentional Deficit Hyperactivity Disorder (ADHD)* [Note: ADHD patients are frequently smokers (self-medication of attentional deficits)]
- Genetic variations (rs1044396) in *CHRNA4* (Exon 5) have been repeatedly associated with *Nicotine Dependence* (incl. own studies)



***Smoking in schizophrenia: self-medication of attentional deficits because of genetically altered alpha4 subunits?***

*\*analogous to velocardiofacial syndrome (Chr: 22q11)*

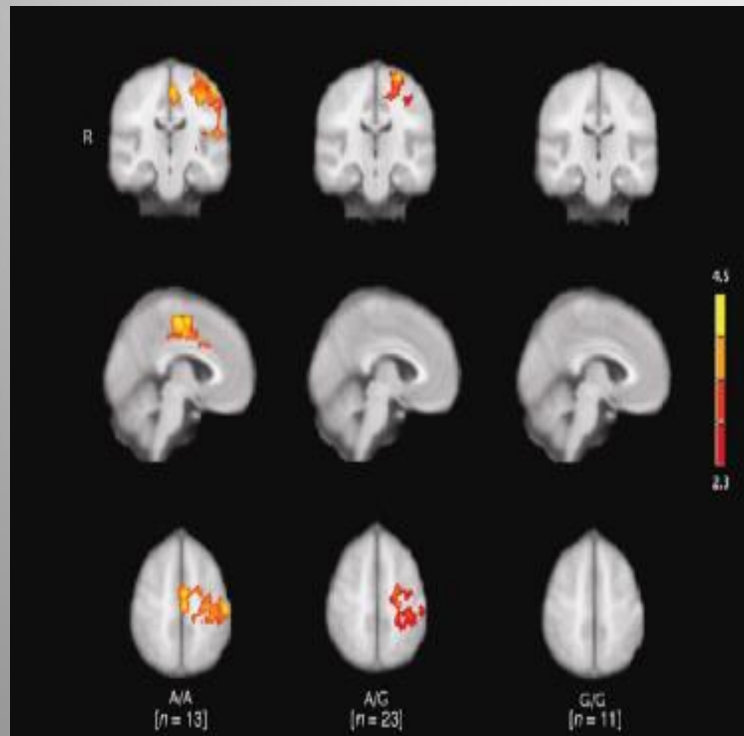
# CHRNA4 – rs1044396



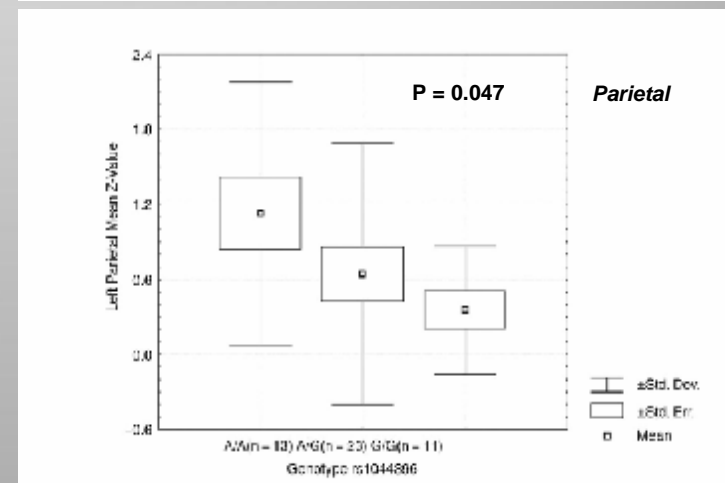
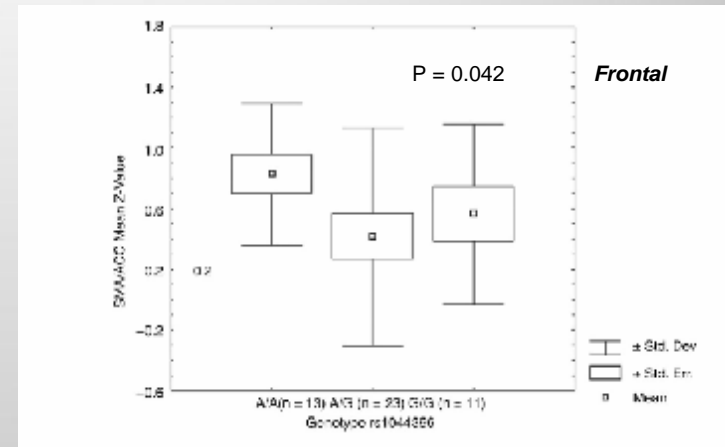
# CHRNA4 & Attentional Network

SNP: rs1044396

*N = 47 Healthy Subjects*



*fMRI-Study (Visual Oddball task)*



*ROI-Analysis*

*Winterer et al (2007) Human Molecular Genetics*

**NICOTINE RECEPTOR GENE CHR4 (rs1044396)  
MODULATES EARLY EVENT-RELATED POTENTIALS IN AUDITORY AND  
VISUAL ODDBALL TARGET DETECTION TASKS**

Event-related potentials (N1 amplitude) are modulated by genotype group  
in the same direction  
with main effects at frontal and parietal electrodes

***Independent (indirect) Confirmation!***

***Espeseth et al. Neuroscience (2007)***

# **CHRNA4 & Schizophrenia**

**1) De Luca et al. (2006) *Exp Brain Res* [family-based, Canada]:**

**4 SNPs in the CHRNA4 region – none near exon5 - and 2 SNPs in the CHRNB2 region were investigated**

**Findings:**

**No significant associations (SNPs, haplotypes)  
but interaction of one CRNA4 with one CHRNB2 SNP**

**2) Kishi et al. (2008) *J Neural Transm* [case-control, Japan]:**

**7 SNPs in the CHRNA4 region and 5 SNPs in the CHRNB2 region were investigated**

**No significant associations (SNPs, haplotypes)  
but *post hoc* association with schizophrenia in males (Exon 5 SNP: rs1044397)**

## ***CHRNA4* & Schizophrenia – Own Results**

**N = 310 Schizophrenia Patients**

**vs**

**N = 272 Controls (Population-Representative)**

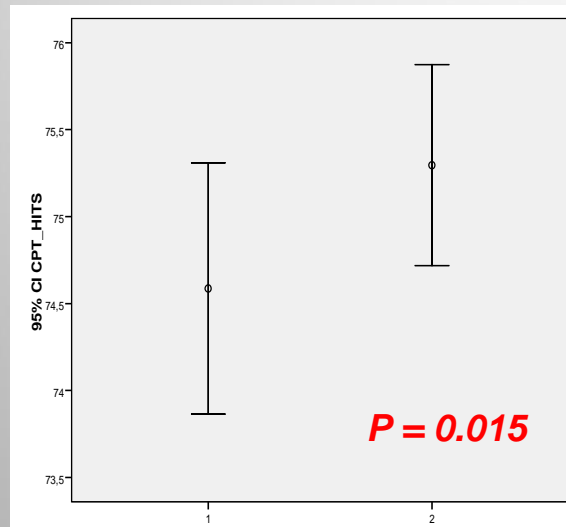
**SNP: rs1044396 – Allele Frequency**

***Chi*<sup>2</sup>=3.680      *df*=1      *p*=0.055**

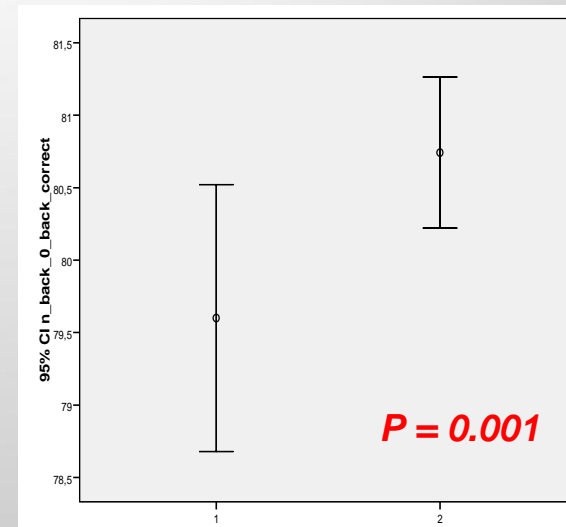
**\*Munich Sample (Rujescu)**

# Attention & SNP: rs1044396

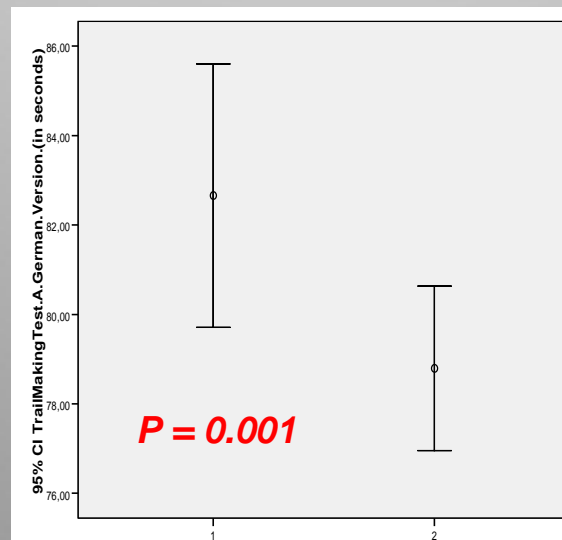
## Continuous Performance Task



## N-Back Task (0-Back)



## Trail Making-A Test

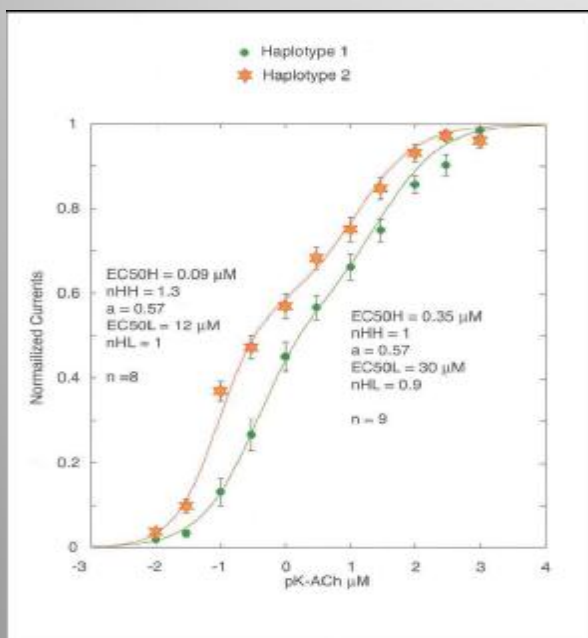


**1-Way ANOVA**

# SNP: rs1044396: Loss-of-Function

## Investigation of Receptor Function

Risk vs non-risk haplotypes with cDNAs carrying complementary haplotypes (*incl. rs1044396*) were coinjected into *Xenopus oocytes* with *CHRNA2* cDNA in equal amounts to study receptor function (with patch clamp)



Given that all six SNPs in the *CHRNA4* haplotype (*incl. rs1044396*) are silent, possible explanations for this finding would include:

- mechanisms of splicing
- altered RNA stability
- introduction of RNA editing sites
- codon-dependent differences in the efficiency and accuracy of tRNA transport during translation

A marked difference on the concentration-activation curve with a right shift indicating that presence of the rarer risk haplotype causes a 2-3 fold decrease in ACh receptor sensitivity (loss of function)

Steinlein, in preparation

# Summary

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Among other reasons, schizophrenia patients may smoke to improve cognitive deficits („self-medication“) which in part may result from loss-of-function of the nACh alpha4 receptor subunit which in turn may account for the differential pattern of gene expression and brain activation

***Thank you for your attention!***

<http://www.nicotine-research.de>

<http://www.neuropsychiatric-research-laboratory.de>

**Georg Winterer**