Dear Sirs,

Representing the members of Bitsios' Lab who participated in the study that I will present you, I would like to thank the members of the scientific committee who accepted our participation in "Neuroscience Days 2012".

The title of my presentation is the sequent: **The Influence of the rs1358278A/G *FOXP2* Polymorphism on Gating, Cognition, Language/Thought and Affect in Healthy Males.**

My presentation will be divided in three parts. In the first one I will present fundamental knowledge regarding the foxp2 based on previous studies. In the second part I will present the recent study of our laboratory relative with this particular foxp2 polymorphism. In the third part I will proceed with the conclusions of the above-mentioned study.

(**Διαφάνεια 1**) The *FOXP2* gene encodes a transcription factor involved in speech and language and in the control of the corticobasal ganglia circuits. It might be a human-specific gene, because speech and language is unique to humans.

Comparison of the foxp2 genes of many organisms has revealed that the Foxp2 protein is rather extraordinarily conserved among mammals .There are only two amino acids different between humans and chimpanzees and three different between humans and mice. Several groups have demonstrated the expression patterns of Foxp2mRNA or protein in rodent, non human primate and human brains. The expression patterns of foxp2 show striking similarities in the three categories of organisms. More specifically Foxp2 is expressed in several structures of the central nervous system during development, including cerebral cortex, striatum, thalamus, cerebellum and spinal cord.

Based on the studies realized in the last years we know that the foxp2 gene is correlated with autism, ADHD and schizophrenia. Language impairment, inattention, impulsivity and abnormalities of corticobasal ganglia circuitry are central features of these disorders; however the impact of *FOXP2* risk polymorphisms on relevant intermediate phenotypes has not yet been studied.

In the present study we selected this *FOXP2* non-coding polymorphism which has been associated with schizophrenia.

(**Διαφάνεια 2**) In our study we have focused on the effects of schizophrenia risk genes on intermediate phenotypes, because we consider this field of research as a promising one. It may point to the mechanisms by which genes increase the risk for the disease. The study of functional mechanisms of risk genes in healthy people is devoid confounds which strongly impact the study and interpretation of findings in patient populations.

(**Διαφάνεια 3**) 829 unrelated young healthy males entered the study. Initial assessment included IQ, Physical and Psychiatric examination.

(**Διαφάνεια 4**) Subjects were classified as AA (*437*), AG (322) and GG (*70).* The genotypic distribution was consistent with the Hardy-Weinberg expectations. There were no population stratification effects. These subjects underwent extensive phenotypic assessment for Gating, Gognition, and Affective Processing which you see briefly here on the right side. The paradigms used for each domain are well established in the literature and are shown after the dashes. The subjects were also phenotyped for Non-Affective and Affective personality traits and the relevant questionnaires are shown in brackets.

(**Διαφάνεια 5**) There were no demographic differences between the genotype groups, but because the smoking to non-smoking ratio was higher in the GG group at a trend level of significance, smoking status was taken as a covariate in all analyses.

(**Διαφάνεια 6**) From the personality measures, alexithymia i.e. the ability to recognize, process and verbalize emotions stands out as the main measure with significant differences between genotype groups. You can see that the GG homozygotes scored higher for the difficulty in identifying and describing feelings subscales and for total alexithymia score. These results suggest abnormalities in the affective processes underlying language in GG subjects

(**Διαφάνεια 7**) Gating and Cognitive measures revealed no significant differences among genotype groups except for Working Memory. GG homozygotes had worse working memory performance as they made more errors in the Cantab spatial working memory test, which is useful for testing the participant's ability to retain spatial information and to

manipulate remembered items in working memory. This test is a measure of frontal lobe and executive dysfunction.

(**Διαφάνεια 8**) Although no significant results were revealed from cognition, the results from emotional decision making are interesting. Risky or emotional decisions involve an evaluation of reward and punishment, with emotional significance attached to both outcomes. For example, the decision between financing one of several potentially excellent but risky business opportunities is a risky-decision; it is difficult to ignore emotions. This kind of decision-making process is dependent on the medial and orbital sectors of the prefrontal cortex, which connect with limbic emotion-related areas such as the amygdala. We measured these processes using the Iowa Gambling Task or IGT. Choices in this simulated gambling task are made under conditions of uncertainty. This type of decision-making is motivated by reward and has been regarded as a type of emotional decision-making. Participants were instructed to select one card at a time from four decks (A, B, C, D) displayed on the screen in order to win "pretend" money. Unknown to the subjects, decks A and B were associated with high monetary rewards but also high penalties (monetary loses) while decks C and D had lower rewards but also lower penalties. The win or loss associated with the selection of a card appeared visually on the screen. Across 100 trials, more choices from the decks C and D lead to a net gain while choosing from decks A and B results in greater loss. We can actually see in this game how people after a few initial random picks, they eventually tend to pick from the safe decks C and D and avoid the disadvantageous A and B decks. Scores were a) total numbers of cards selected from advantageous decks C and D minus total numbers of cards selected from "risky" decks A and B, with a higher score indicating superior performance b) total money won. The first of these scores is called general index of learning for each successive block of 20 trials resulting in 5 blocks. This index indicate learning to avoid the risky decks.

(**Διαφάνεια 9**) Here you see on the left that genotype groups did not present a statistically significant difference in performance either in a) total numbers of cards selected from advantageous decks C and D minus total numbers of cards selected from "risky" decks A and B (top), or b) total money won (bottom).However, it is noteworthy in the right panel

that GG subjects presented with what is known in the literature as an alexithymic profile. You can see that GG homozygotes, learned to avoid disadvantageous decks over the first half of the task. Over the later trials they showed a disruption in performance. This kind of behavior, called explore-learn-change-return strategy is a typical one for people with alexithymia and is due to an inability to fully consolidate earlier learning due to a difficulty in using previous memories associated with emotions. This may cause them to temporary reductions in the experience of loss which in turn leads to lapses in performance.

(**Διαφάνεια 10**) The startle is a defensive cross-species reflex which consists of a fast contraction of the body musculature in response to a sudden and intense stimulus. It is designed to protect the organism from the appearance of sudden threats. In humans, we typically measure the eyeblink component of startle using electromyography (EMG) of the orbicularis oculi muscle. In order to elicit the reflex, we deliver brief and intense pulses of white noise through headphones. Here we see such an EMG startle response from the orbicularis oculi muscle.

The startle reflex shows interesting forms of plasticity, which are of great relevance to psychiatric research.

(**Διαφάνεια 11**) The term "affective startle modulation" has been coined to describe the enhancement or attenuation of startle when this reflex is elicited in the presence of affectively unpleasant or pleasant pictures. This paradigm has become a prominent methodological tool in the study of human emotion and its disorders.

(**Διαφάνεια 12**) When startle is elicited during viewing of an affectively neutral picture like this or the next one, then startle is not affected.

(**Διαφάνεια 13**) Here is a startle reflex elicited during viewing of an emotionally neutral picture.

(**Διαφάνεια 14**) Affectively pleasant pictures like this one inhibit startle.

(**Διαφάνεια 15**) As for example here.

(**Διαφάνεια 16**) While the opposite is true for unpleasant pictures designed to elicit aversion, fear or horror.

(**Διαφάνεια 17**) This is an example of startle enhancement when startle is elicited during viewing of an unpleasant picture. So we followed a standard affective startle protocol whereby startle is elicited from acoustic probes delivered at 4500 ms after affective picture onset.

(**Διαφάνεια 18**) We found that GG subjects failed to inhibit their acoustic startle when viewing the pleasant pictures, suggesting abnormalities in the processing of pleasant affect.

(**Διαφάνεια 19**) In conclusion, the rs1358278G which was previously shown to be part of a risk haplotype for schizophrenia and was associated with speech incoherence in patients, shows evidence of abnormalities in working memory and the processing of emotional material in healthy male G homozygotes.

(**Διαφάνεια 20**) Our results suggest that one way for this *FOXP2* polymorphism to increase risk for schizophrenia, may be through impairments in working memory and affective processes underlying response to linguistic, reward and pictorial stimuli.