

Executive Summary:

The part played by serotonin in anxiety control

In patients suffering from depression, panic attacks, anxiety disorders or phobias, the administration of drugs that increase serotonin (5-hydroxytryptamine, 5-HT) transmission has beneficial effects. However, the underlying mechanisms are still not elucidated, and a causal role for 5-HT depletion in anxiety disorders is not established.

The difficulty arises from the fact that 5-HT is produced in several organs besides the brain, and that it affects most all brain areas with sometimes opposing effects. Moreover 5-HT has clear developmental effects so it is unclear whether effects on anxiety reflect changes in neural circuit wiring or direct effects of 5-HT depletion. To answer these questions, researchers in the DEVANX consortium decided to characterize animal models with 5-HT depletion only in the brain, that are either complete or limited to some brain regions. To determine whether changes are developmental, they used rescue strategies, or induced 5-HT depletion in adults. Different mouse models were used relying on different mechanism: 1) Mouse strains with natural variation in the activity of the enzyme controlling central serotonin synthesis, TPH2 (BalbC/C57BL/6). 2) Pet1-KO mice in which the production of a subpopulation of raphe neurons in the brain is arrested during development by the lack of a transcription factor (Pet1-KO). 3) Conditional VMAT2-KO mice in which the vesicular storage of amines is prevented specifically in the serotonin raphe neurons (VMAT2-Sert-Cre; VMAT2-Pet1-Cre;). 4) Dietary restriction of the 5-HT precursor tryptophan ; 5) Pharmacogenic silencing of the firing of raphe neurons.

The comparison of these different models (by Partners, 1, 2 , 4 and 6) taught us that severe serotonin depletion, whatever the cause, had dual effect on anxiety related behaviors, depending on the type of behaviors that are explored. On one hand serotonin depletion increased learned fear and escape responses. On the other hand serotonin depletion had anxiolytic effects in tests measuring exploration (Elevated plus maze) or conflict tests (Novelty suppressed feeding). These effects did not appear to be developmental, since they could be rescued by clorgyline in VMAT2 conditional KO mice or reproduced by dietary restriction of tryptophan. These results may best be explained in the general framework of involvement of 5-HT in behavioral inhibition, with different outcomes according to the behavioral test/ brain regions that is controlled by 5-HT.

Interestingly these studies also showed that the reduced 5-HT synthesis that is observed in the Balb/c mouse strain, due to a mutation of the TPH2 gene, does not explain their increased anxiety phenotype which could rather be due to adaptive changes in the serotonin raphe system with functional desensitization of the 5-HT_{1A} receptors, causing an increased excitability of these neurons.

Another important finding from these studies was the existence of heterogeneity of central serotonin systems, and the identification of raphe neurons genetically defined by their lack of requirement for the Pet1-transcription factor and a selective innervation of brain areas involved in stress response.

In parallel Partner 6 obtained new promising models to control selectively gene expression within the serotonin raphe neurons. The new mouse line with selective drivers (Tet- on and Cre-Ert2) that were generated will allow to drive or repress expression of genes selectively in the raphe with a tight temporal control.

Finally on a circuit level, the targets of 5-HT innervation that are important in mediating the developmental impact of 5-HT1A receptor invalidation, were clarified (Partner 2). It had been previously found that developmental ablation of 5-HT1A receptors in the forebrain was sufficient to cause anxiety phenotypes. During this project researchers demonstrated that selective rescue of 5-HT1A in selected regions of the forebrain was sufficient to restore normal responses to anxiogenic situations. This Partner also identified cellular effects of the 5-HT1A receptors on dendrite maturation in the hippocampus.

The GABA-B receptors

Dysfunction of the gamma-amino butyric acid (GABA)-ergic system has been purported to play a role in anxiety and depression. A clear link between GABA-A receptors and anxiety has long been established since most anxiolytics such as benzodiazepines are positive allosteric modulators of GABA-A receptors. However the functional role of another major signaling pathway for GABA, the GABA-B receptors, has been less explored. GABA-B receptors are expressed largely in the brain, are targeted by new molecules that work in a completely different way from conventional anxiolytics. Partners 3 and 4 of this consortium were involved in the development of a novel class of GABA-B receptor allosteric modulators, and developed a number of genetic tools to understand GABA-B receptor function. Within the framework of this project a better understanding of the structure and the function of GABA-B receptors was achieved and their interactions with the serotonergic system, were clarified.

Partner 3 demonstrated that GABA-B receptors are heterodimers (a combination of two different receptor subunits) that possess partner proteins capable of modifying their binding properties. The pharmacological properties of GABA-B receptors vary depending on how the partner proteins are organized and this Partner identified new auxiliary proteins for these receptors that contribute to the functionality of the receptors. Partner 4 showed that inhibiting GABA-B receptors can reduce depressive behavior in adults and has an effect on adult neurogenesis; they moreover were able to demonstrate a moderate anxiolytic effect of the GABA-B positive modulator. Partner 1 studied the connection between GABA-B receptors and the serotonergic system. Finally Partner 2 showed that interfering with GABA

transmission (with benzodiazepines on the GABA-A receptors) caused long term effects on anxiety-related behaviors.

Other neural circuits involved in fear control

It is becoming more and more evident that it is the normal neuron circuits specialized in dealing with fear that are pathologically affected or amplified in anxiety disorders. Thus it is very important to understand and analyze how these circuits function in "real situations" in animal models. The end purpose is to find a way of 'deconditioning' certain brain circuits that have been abnormally or over-activated.

The new approaches to physiology on the knockout animal, combined with pharmacogenetic research, have made for progress in this field. For example Partner 5 recorded different neurons from the hippocampal circuits in different fear learning situations and observed the effect of modifying the message conveyed by GABA-B and serotonin. Partner 1 used the serotonergic receptors (5-HT_{1A}) expressed in different areas of the brain in order to temporarily deactivate highly specific neuron circuits. This allowed them to identify the hippocampal and amygdala circuits involved in the generalization of fear.

Partner 4 identified the role of the rostral anterior cingulate cortex in modulating the efficiency of amygdala dependent fear learning, and demonstrated antidepressant effects of transient inactivation of the prefrontal cortex.

In parallel Partner 5 also identified the medial prefrontal cortex as having a key role in observational learning which may play significant roles in anxiety-related disorders.

Gene-Environment interactions

Risk for anxiety-related disorders is determined by a combination of genetic and environmental factors. In particular traumatic childhood experience such as maternal separation can play a role in modifying the individual's response to stress in adult life. This effect can be modulated by specific polymorphisms in genes that control neurotransmission.

Researcher in the Devanx consortium identified several important factors in this regard.

Partner 1 analyzed epigenetic changes in *bdnf* gene which occurred during anxio-depressive disorders.

Partner 2 showed with a genetic approach that the serotonin transporter (5-HTT) and neurotrophic factor, BDNF, both moderate the effects of early maternal separation: when one allele of these genes is absent the effects of maternal separation on adult anxiety behaviors such as ambiguous fear conditioning.

Partner 4 demonstrated the influence of early life stress of the brain-gut axis disorders. They showed that stress early in life alters brain-gut axis function and modifies the relative diversity of the gut microbiota, causing visceral hypersensitivity. They also showed a major effect of the genetic background on the resilience to early life stressors, as quite strikingly in some genetic backgrounds, paradoxical anxiolytic effects were observed after early prolonged maternal separation. Finally they also showed that there is an interaction between the GABA-B system and early life stress in terms of susceptibility to the effects of stress at a behavioral level and in the context of hippocampal neurogenesis.

Conclusion

Research of this consortium into anxiety disorders, combined several fields of neurosciences, combining molecular approaches with integrated approaches in live animal. The genetic tools used or produced in this project provide unequalled power for researching into a determined molecule function, or a molecular assembly within a given circuit and a precise time slot. This type of approach will continue to develop in the years to come, with the coming of tools that will allow us to activate or deactivate certain selected neuron circuits.

By solving these intertwined elementary processes step by step, progress will be made in the explanation to the mechanisms underlying pathological anxiety disorders.

Project Context and Objectives:

The project focuses on elucidating the mechanisms by which GABAergic and serotonergic systems act in the developmental programming of anxiety. New findings, brought about by members of this consortium, changed our views on the neurobiological action of these two transmitters and are the focus of the present proposal. The first original dimension is the discovery of a developmental role of serotonin (5-HT) in the genesis of anxiety disorders, and the finding of interactions between 5-HT-related genes and environmental risk factors. The second new dimension is the discovery that metabotropic GABA-B receptors play a critical role in mediating the anxiolytic effects of GABA, a starting point for the conception and design of novel therapeutic approaches.

Researchers that are at the forefront of these research domains will build on and extend new findings that involve the production of new genetic models in mice for site- and time-specific invalidation of 5-HT related genes, focusing on the hippocampus, amygdala and raphe nuclei. They will explore the role of GABA-B receptors in anxiety and the interaction of these receptors with the 5-HT system. The developmental effects of GABA-B receptors will be explored and a new generation of GABA-B modulators that produce anxiolytic effects in animal models. Finally they will investigate how exposure to adverse environments interacts with 5-HT-genes and GABA-B receptor genes to produce anxiety phenotypes.

Project Results:

WP-1-Producing/characterizing mice with different serotonin depletion

While supported by large circumstantial evidence, the causal role of 5-HT dysregulation in anxiety disorders is not yet established. It remained to be shown: 1) that the serotonergic dysfunction in anxiety is causal and not an adaptive symptom, 2) that 5-HT is deregulated in the brain rather than in the periphery, 3) that the timing of 5-HT deregulation during different life time periods plays different causal roles to generate anxiety, and 4) that 5-HT innervation to different brain parts has specific contribution to the anxiety phenotype.

Natural variants of TPH2 activity

TPH2 polymorphisms in humans have been associated with anxiety traits, yet these results have not been supported by other studies. These discrepancies might be partially attributed to the fact that different SNPs in the TPH2 gene were analyzed. In the mouse *Tph2* gene, a functional SNP has been identified (C1473G) among different inbred mouse strains, which results in the substitution of Pro447 (1473C allele) with Arg447 (1473G allele). In mouse strains homozygous for the 1473G allele (G/G), the enzymatic activity of TPH2 was reduced by 50% and correspondingly 5-HT concentrations were found to be decreased in several brain regions.

We provided a detailed comparative neurochemical, molecular, and behavioral characterization of C57BL/6N mice homozygous for either the *Tph2* 1473G or 1473C allele. We showed that this allelic variant alone leads to a reduced in vivo 5-HT synthesis rate. However, the distinct and pharmacologically reversible anxiety phenotype in 1473G/G mice is not the result of reduced 5-HT tissue content or 5-HT neurotransmission but is likely mediated via compensatory homeostatic changes involving a functional desensitization of 5-HT_{1A}-autoreceptors.

These findings suggest that functional desensitization of 5-HT_{1A} autoreceptors is a promising common denominator for increased anxiety.

Targeting the serotonin raphe neurons with new genetic tools

The generation of mice with over-expression of *Tph2* or SERT proved to be impossible, and was abandoned after the generation of 12 transgenic animals. Two alternative approaches

were followed. In the first, we investigated the behavioral and neurochemical consequences of a functional C1473G SNP in the mouse Tph2 gene affecting serotonin synthesis rate. We generated congenic C57BL/6N mice homozygous for the Tph2 1473G allele. The Arg(447) substitution in the TPH2 enzyme resulted in a significant reduction of the brain serotonin (5-HT) in vivo synthesis rate. Despite decreased 5-HT synthesis, we could detect neither a reduction of brain region-specific 5-HT concentrations nor changes in baseline and stress-induced 5-HT release. However, a functional desensitization of 5-HT(1A) autoreceptors could be identified. Furthermore, behavioral analysis revealed a distinct anxiety phenotype in homozygous Tph2 1473G mice, which could be reversed with chronic escitalopram treatment. Alterations in depressive-like behavior could not be detected under baseline conditions or after chronic mild stress. These findings provide evidence for an involvement of functional Tph2 polymorphisms in anxiety-related behaviors, which are likely not caused directly by alterations in 5-HT content or release but are rather due to compensatory changes during development involving functional desensitization of 5-HT(1A). (Berger et al., 2012).

The efforts of Partner 6 was redirected towards the production of other transgenic model such as the production of conditional rat transgenic models (Schonig K, et al., BMC Biol. 2012 Sep 3;10(1):77, Conditional Gene Expression Systems in the Transgenic Rat Brain) and models for selective manipulation in serotonergic neurons. (Weber et al., 2009; Weber et al., 2011).

The new mouse and rat driver lines targeting the serotonergic neurons provide useful molecular tools to interrogate the role of widely expressed genes (like vesicular transporter VMAT2, L-type voltage gated calcium channels Cav1.2 and Cav1.3 and glucocorticoid receptor selectively in serotonergic neurons. These tools are now used in our and other labs for such manipulations.

Characterizing the Pet1-independent serotonin pathways

-Pet1 is a transcription factor that plays a key role in the differentiation of serotonin raphe neurons. It is expressed almost exclusively in the raphe of different species and controls together with other transcription factors the serotonin identity. Partner 1a characterized a residual serotonin innervation in the Pet1-KO mice. This led to the individualization of a Pet1-independent serotonin subsystem which targets preferentially limbic areas of the forebrain implicated in stress control, and is characterized by the formation of synaptic junctions. The selective sparing of serotonin innervation in certain regions of the brain was correlated to the analysis of anxiety behaviors in these mice, showing a reduced anxiety in exploration but enhanced fear learning. This work was published: Kiyasova V, et al. J Neurosci. 2011.

-To analyze the physiological and molecular underpinnings of the heterogeneity of the serotonin raphe neurons we carried out a multiscale analysis of single raphe neurons, and anatomical tracing studies. Partner 1a validated the electrophysiological recording of raphe

neurons associated with single cell PCR of candidate genes. They also validated a genetic tracing approach using viral constructs in Sert-Cre mice using AAV-containing conditional fluorescent tags. Partner 1a is currently revisiting the anatomy of the ascending B5-B9 raphe cell groups using this approach. The completion of this task is however very time consuming and it will extend beyond the present project in order to finalize a third publication derived from this task.

-As a follow up of this work Partner 1a collaborated with a zebrafish group (Laure Bally-Cuif and Christina Lillesaar) to identify new ETS-factors involved in serotonin specification. This led to identify a new factor ETV5, which appears to be involved in the specification of hypothalamic serotonin neurons. The results of this study are in press in Development (Bosco et al. 2013).

Complete depletion of serotonin brain levels at different times during development

Partner1 generated and characterized the VMAT2^{flox}:SertCre mice, that had a complete depletion in serotonin stores, and shown that this can be rescued both during development and in adult life, using an inhibitor of monoamine oxidase, clorgyline.

Using this mouse line they showed the important role of central serotonin production in the control of anxiety like behaviors, as the mice were found to have increased flight responses that could be rescued with administration of clorgyline.

Because Sert and VMAT2 have a broad expression during development we further aimed to produce mice with a more specific targeting of VMAT2 deletion to central raphe neurons, and to delimit the timing of this invalidation. Partners 1a and 6 collaborated to further produce two additional mouse strains; The VMAT2:TpH2-Cre-ERT2T enabling a depletion of 5-HT in adult life.

Moreover Partner 1a generated a VMAT2: Pet1-Cre mice, which had not been initially planned. This produced a life-long serotonin depletion but which is limited to the central raphe neurons but did not concern all the raphe neurons. This mouse was characterized biochemically and morphologically.

The VMAT2 models overall allowed to show that central serotonin is needed for the postnatal growth spurt, but not for embryonic/brain growth. (Narboux-Name et al. in press).

That the cortical development shows only mild retardation in hyposerotoninergic mice and the barrel cortex develops normally (Narboux-Name et al. in press); 3) Adult serotonin depletion rather than developmental effects are implied in the increased escape-like responses observed in the hyposerotoninergic mice. (Narboux-Name et al 2011) 4) Adult serotonin depletion rather than developmental effects are implied in the increased impulsive-like behaviors responses observed in the hyposerotoninergic mice. Interestingly, this seems not to be a general increase in impulsivity, but rather a cue-induced change. Animals with VMAT2 knockout show addictive-like behavior which does not depend on reward. 5) Vesicular storage/release is not essential for serotonin neurotransmission. (Narboux -Name et al. 2011).

WP-2- 5-HT1A receptors in anxiety circuits

The goal of this WP was to identify the neuronal circuits that mediate the effects of the 5-HT1A receptor during the maturation of anxiety networks in hippocampus, amygdala, and raphe nuclei. 5-HT1A receptor is a clearly identified developmental modulator of anxiety (Gross et al., 2002). Anatomical, biochemical, and physiological defects downstream of this receptor have been identified in previous work by Partner 3. These findings point to a deficit in circuit maturation in the hippocampus of 5-HT1A receptor knockout mice. The consortium members will integrate novel approaches to expand and test this hypothesis, taking advantage of anatomical, morphological, and immunohistochemical expertise in the participating laboratories. This animal model will serve as a well-defined example of developmental programming of anxiety and will lead to the detailed identification of the precise neural circuits involved.

Generation of transgenic mice expressing 5-HT1A receptor in selected tissues

Partner 2 (EMBL) produced transgenic mouse lines allowing tissue-specific rescue of 5-HT1A receptors. The following transgenic lines: Htr1a-DG, Htr1a-CeA, Htr1a-RR, Htr1a-CA3, were produced and studied for their anxiety behavior.

Rescue of Htr1a in principal cortical pyramidal neurons (under control of Emx1:Cre tissue-specific driver) was associated with restored anxiety behavior, demonstrating a crucial function for the receptor in these cells for anxiety.

In addition Partner 2 showed that Htr1a autoreceptor and heteroreceptors populations interact to modulate anxiety. Partner 1b (INSERM-LL) observed that flight, but not freezing, behaviors induced by ultrasound (an ethologically relevant aversive cue) are increased in mutant mice lacking the Htr1a receptor (Htr1a knockout) compared to WT mice. Partner 1b (INSERM-LL) further analyzed the behavior of Htr1a knockout mice in the tail suspension

test (TST) and the forced swim test (FST). Htr1a knockout mice showed a marked increase of escape-like behaviors in conjunction with a decrease in tonic immobility was observed. In contrast, in the FST no effect of the 5-HT_{1A} receptor deletion was observed. The phenotype of Htr1a knockout mice in the TST was partially reproduced by blockade with blockade of 5-HT_{1A} receptors in the dorsal PAG area. Preliminary data from Partner 1b indicate that flight reactions induced by chemical stimulation of the dorsal PAG are increased in Htr1a knockout mice. Furthermore, in the aversive ultrasound paradigm, these mutant mice displayed increased flight reactions. These data suggest that Htr1a receptors in areas such as the PAG, is a crucial component of defense reactions in some, but not all of the behavioral tests used to screen antidepressant drugs.

Partners 5 (UPO) and 2 (EMBL) have been working during this period on the study of the memory recall and the persistent erasure of hippocampal memory. Following the suggestions given by the Reviewers of the submitted manuscript, both Partners carried out some new experiments. Partner 5 used selective pharmacological tools for the rapid and transient suppression of dentate gyrus granule cells activity. In addition, Partner 2 carried out in vivo electrophysiological recordings of granule cells during trace eye-blink conditioning to examine the contribution of dentate gyrus to hippocampal learning and plasticity. In previous experiments, it was concluded that the suppression of DG activity during learning was associated with rapid and persistent memory loss, and it was accompanied by long-term suppression of both conditioned responses and learning-associated synaptic plasticity. Using pharmacological tools, it was tested a possible role of adenosine A₁ receptor activation in this synaptic depotentiation. Moreover, some new experiments were designed to demonstrate the generalization of the results found using the eyeblink conditioning paradigm across hippocampus dependent memory tasks (i.e. contextual fear conditioning).

Papers published:

Gross and Canteras, The Many Paths to Fear (2012) Nat Rev Neurosci. 13:651-8.

(2012).Gozzi, Jain, et al., Neural Switch for Active and Passive Fear (2010) Neuron, 67:656-66. [Reviewed in: H.-C. Pape. (2010) Petrified or aroused with fear: the central amygdala takes the lead. Neuron 67:527-9].

Papers submitted:

Madrol et al., "Rapid erasure of hippocampal learning and plasticity following transient blockade of dentate gyrus granule cells".

Audero et al., Suppression of serotonin neuron firing increases aggression in mice

Piszczyk et al., serotonin 1A heteroreceptors and autoreceptors interact to control anxiety in mice.

Piszczyk et al., Cortical Htr1a receptors modulate anxiety in mice.

Type-I cells in the central nucleus of the amygdala (CeA) tonically bias passive and active fear responses via tonic modulation of lateral CeA projection neurons to forebrain cholinergic. Suppression of serotonin neuron firing does not alter anxiety behavior, but elicits increased aggression.

Htr1a receptors in forebrain and raphe nucleus interact to modulate anxiety, possibly via common action on serotonin homeostasis.

Dentate gyrus granule cells are not necessary for retrieval of hippocampal memory, but are necessary for its acquisition.

Non-dentate gyrus granule cell inputs (EntCtx-CA1) to hippocampus promote depotentiation of learning associated plasticity and conditioned behavior.

Adenosine A1 receptor activation in CA1 is necessary for this depotentiation.

Importantly, the reported effects can be generalized across other hippocampus-dependent memory tasks (i.e., contextual fear conditioning).

The reported findings open the possibility of the targeted erasure of hippocampal memories.

Characterization of the phenotype of tissue-specific 5-HT1A receptor rescue mice

Partner 2 has completed experiments examining hippocampal plasticity in vivo at SC and PP inputs to CA1 in Htr1a-KO mice. These studies revealed no change in plasticity. These data will be included as control experiments in a recently submitted manuscript.

Highlight of clearly significant results:

Submission of manuscript Gruart et al., Rapid erasure of hippocampal learning and plasticity following transient blockade of dentate gyrus granule cells; publication of paper describing role of serotonin in dendritic growth cone dynamics Ferreira et al., Serotonin receptor 1A modulates actin dynamics and restricts dendritic growth in hippocampal neurons Eur. J Neurosci. 2010; submission of manuscript describing structural and electrophysiological deficits in Htr1a-KO mice Klemenhausen et al., Altered CA1 hippocampal dendritic morphology and anxiety-like behavior in serotonin-receptor 1A deficient mice.

WP3- 5-HT modulation of GABA circuits

This work package was intended to identify changes in GABA receptor responses caused by loss of serotonin function. The pilot experiments conducted showed no major changes in

GABA circuits or GABA-B receptors caused by disturbed serotonin signaling. Therefore the efforts were redirected towards related questions that emerged during the course of these studies.

GABA synapse maturation in the hippocampus and raphe

Partner 5 analyzed mini-EPSC and mini-IPSC analysis of CA1 pyramidal neurons in Htr1aKO mice generated by Partner 2, to evaluate their synaptic architecture and innervation.

Partner 5 started recording of the IPSPs in the hippocampus of behaving mice during classical conditioning of the corneal reflex, with recording in the dentate gyrus, CA3 and CA1 evoked by perforant pathway, Schaffer collateral, and/or commissural inputs. The main objective is to analyse the disynaptic inhibitory synaptic field potentials (fIPSP) which are due to GABA-A and GABA-B effects, respectively. The experiments are in progress.

Morpho-functional maturation of GABA interneurons in the hippocampus and amygdale

-Partner 1a conducted morphological analyses of the GABA interneurons in the hippocampus of Vmat2 Sert/cre and Pet1 -/- mice that have respectively a 95% or an 80% depletion of central serotonin levels. Morphological analyses of the hippocampus showed no structural abnormality of the hippocampus. Markers of the GABA interneurons, such as GABA, calbindin and parvalbumin showed comparable distribution in control and KO mice, suggesting that there are no obvious structural alterations in the development of GABA interneurons.

-Partner 1a examined adult neurogenesis in the hippocampus in Pet1 and Vmat2 Sert/cre mice. This showed significantly increased survival of the newborn dentate granule cells, of both hyposerotonergic mouse strains. This an unexpected finding (given the known enhancement of neurogenesis caused by antidepressants) lead to further analyses to determine the sensitive period for this effect we used the serotonin depleting agent PCPA. This showed that chronic reduction of brain levels of 5-HT in adult- reproduces the phenotype, indicating that this is not a developmental effect

-Partner 5 designed more accurately the experiments proposed in this task, taking in account the results obtained from Partner 1.

Identification of KCTD proteins as auxiliary GABA-B receptor subunits

As an approved deviation (see 2nd periodic report Partner 3 investigated the composition of native GABA-B receptor complexes by an unbiased proteomics approach using affinity

purification and mass-spectrometry analysis. It was found that GABA-B receptors in the brain are composed of principal and auxiliary subunits (Schwenk et al., 2010, Nature 465, 231-235). The four cytosolic proteins, KCTD8, KCTD12, KCTD12b and KCTD16 tightly associate with the GABA-B core receptors at the plasma membrane and influence agonist potency and the fast kinetics of the receptor response in a KCTD subtype-specific manner. None of the KCTD proteins had been implicated in GPCR function before they were found to be part of GABA-B receptor complexes.

KCTDs are characterized by a common structural motif, the T1 tetramerization domain. In voltage-gated K⁺ channels, T1 domains are responsible for the assembly of four subunits around a central channel pore. Likewise, in KCTDs, the T1 domains of four subunits assemble into a homotetramer that tightly binds to the C-terminal domain of GABA-B2. Tyrosine 902 (Y902) in the C-terminal domain of GABA-B2 is required for binding to the T1 domains. Of note, phosphorylation of Y902 is not necessary for KCTD binding, as mutation of Y902 to phenylalanine has no effect on this process. In addition to the T1 domains, KCTD8, KCTD12, KCTD12b and KCTD16 have sequence-related H1 homology domains, with KCTD8 and KCTD16 also featuring sequence-related H2 homology domains. The H1 and H2 domains are not related to each other and exhibit no obvious sequence similarities to other proteins.

When expressed along with GABA-B1 and GABA-B2 in heterologous CHO cells or *X. laevis* oocytes, KCTDs exert a variety of effects on GABA-B receptor-mediated responses. All KCTDs markedly shorten the rise-time of the GIRK-mediated K⁺-current response by up to tenfold, which closely matches the rise-times measured in cultured hippocampal neurons. Moreover, KCTDs differentially increase the potency of GABA at the receptor. Consequently, KCTDs seem to be the missing components that confer fast activation kinetics and distinct agonist potencies to native GABA-B receptors. Additionally, KCTDs may offer an explanation for the variation in the desensitization kinetics of native GABA-B receptor-mediated responses. In the presence of KCTD12, GABA-B receptor activation elicits a strongly desensitizing K⁺ current that is characterized by two time constants of 1.5 and 8.9 seconds. Strongly desensitizing K⁺ currents are also observed in the presence of KCTD12b. By contrast, in the presence of KCTD8 or KCTD16, the activated receptors induce largely non-desensitizing K⁺ currents. Partner 3 recently identified that the KCTD subunit domains exert opposite effects on GABA-B receptor mediated desensitization (Seddik et al., 2012, J. Biol. Chem. 287, 39869-39877).

The presence of KCTD12 or KCTD12b also leads to a rapid desensitization of the GABA-B receptor mediated inhibition of voltage-gated Ca²⁺ channel (VGCC) currents. Of note, principal and auxiliary GABA-B receptor subunits can be affinity-purified together with native VGCC complexes and, vice versa, These findings show that GABA-B receptors and VGCCs form biochemically stable signaling complexes in vivo, and are in line with fluorescence resonance energy transfer (FRET) spectroscopy data demonstrating that GABA-

B receptors, G proteins and VGCCs form spatially restricted complexes in the boutons of hippocampal neurons (Laviv et al., 2011, J. Neurosci. 31, 12523-12532).

In addition, Partner 3 investigated the temporal and spatial expression patterns of the KCTD proteins in the brain (Metz et al., 2011, J. Comp. Neurol. 519, 1435-1454). The results support that most brain GABA-B receptors associate with KCTD proteins, but that the repertoire and abundance of KCTDs varies during development, among brain areas, neuronal populations, and at subcellular sites. This suggests that the distinct spatial and temporal KCTD distribution patterns underlie functional differences in native GABA-B responses.

These exciting findings opened up a new line of research which is partly summarized in a recent review (Gassmann and Bettler, 2012, Nat. Rev. Neurosci 13, 380-394). In summary, the recognition that GABA-B receptors are assembled from principal and auxiliary subunits provided a shift in the understanding of these receptors. It is now clear that molecularly distinct GABA-B receptor subtypes exist, which are distinguished by their auxiliary KCTD subunits. The functional effects of the KCTD proteins together with their distinct spatial and temporal expression patterns, indicates that these auxiliary subunits contribute to the variation in native GABA-B receptor mediated responses during development and in different neuronal populations. Finally, the discovery that KCTDs are auxiliary subunits of GABA-B receptors provides new links between these receptors and disease. In particular, KCTD12 was shown to be a molecular signature of depressive disorders.

Modulation of GABA-B signaling by 5-HT

Partner 3 studied the effects of serotonergic signaling pathways on GABA-B mediated current responses in neurons and transfected CHO cells. GABA-B receptors can be efficiently internalized from the cell surface of cultured neurons by increasing intracellular calcium through activation of either 5-HT₃ or NMDA receptors. However, Partner 3 was unable to demonstrate internalization through 5-HT₃ receptors at synaptic sites and therefore did not pursue this further. In contrast, the experiments with NMDA receptors revealed a clear internalization from dendritic spines, which Partner 3 worked out in detail and recently published (Guetg et al., 2010, Proc. Natl. Acad. Sci. USA 107, 13924-13929). In experiments that were intended to be control experiments for the above experiments Partner 3 identified by serendipity a cross-talk in the GIRK current responses of TAAR1 and dopamine D₂ G-protein coupled receptors. This finding was included in a recent publication (Bradaia et al., 2009, Proc. Natl. Acad. Sci. USA 106, 20081-20086).

WP-4- GABA-B receptors and 5-HT system

There is strong evidence that a constitutive genetic loss of GABA-B receptors produces an anxiogenic phenotype. GABA-B receptor-deficient mice are more anxious than their wild-type counterparts and show a panic-like response in the elevated zero maze, a variant of the elevated plus maze test (Mombereau et al., 2004, Eur. J. Pharmacol. 497, 119–120). Partner 3 had generated a Cre-conditional allele of the GABA-B1 gene that allowed site- and time-specific deletion of GABA-B function (Haller et al., Genesis, 40, 125-130). Given that there is a functional interaction between GABA-B and 5-HT1A receptors (Luscher et al 1997, Neuron, 19, 687-695), and 5-HT1A receptors are also able to regulate anxiety, the hypothesis tested here was that the anxiety phenotype observed in GABA-B-deficient mice was mediated by similar pathways, including the common modulation of brainstem serotonergic neurons.

Production of mice with conditional deletion of GABA-B receptors in the raphe

Partner 3 successfully generated mice that allow for a conditional deletion of GABA-B receptors in serotonergic neurons. These mice are homozygous for the floxed GABA-B1 allele (provided by Partner 3) and heterozygous for Tph2-CreERT2 (provided by Partner 6). Recombination and successful inactivation of the GABA-B1 gene following Tamoxifen injection was verified by Partner 3 in electrophysiological recordings (absence of baclofen-mediated K⁺-currents) from serotonergic neurons of the raphe nucleus.

Mice with a conditional deletion of GABA-B receptors in the raphe nucleus were generated and made available to the consortium by Partner 3.

Characterization of anxiety behaviors in mice with raphe specific deletion of GABA-B receptors

Analysis of mice with a conditional deletion of GABA-B receptors in the raphe nucleus did not reveal any significant changes in 5-HT metabolism in serotonergic neurons (Partner 1a and 1b; see below). These findings made it less likely that the conditional KO mice will exhibit an anxiety phenotype. Therefore we concentrated our efforts on the behavioral characterization of mice that allow assessing the contribution of specific GABA-B receptor subtypes and functional states of GABA-B receptors that are controlled by phosphorylation. The results of these studies are described in WP-6.

Characterization of the serotonin phenotype in mice with raphe specific deletion of GABA-B receptors

Partner 1a and 1b analyzed the 5-HT turnover in the raphe-specific GABA-B1 knockout mice by measuring 5-HT and its metabolite 5-HIAA in various brain areas (accumbens, frontal cortex, hippocampus, ventral tegmental area/substantia nigra) using HPLC. The 5-HIAA/5-HT ratio did not show any significant difference between the experimental and control groups of mice. This indicates that GABA-B receptors expressed in serotonergic neurons of the raphe nucleus do not directly contribute to the modulation of 5-HT metabolism in serotonergic neurons. However, a significant decrease in 5-HT turnover was observed in mice lacking the GABA-B1a subunit isoform, but not in mice lacking the GABA-B1b subunit isoform that are predominantly expressed at glutamatergic terminals. Therefore these results identify a GABA-B receptor-mediated modulation of feed forward inhibition of 5-HT neurons in the raphe nucleus. This most likely involves a GABA-B-mediated control of glutamatergic afferents to local GABAergic interneurons regulating the activity of serotonergic neurons. Magnetic resonance spectroscopy, which allows non-invasive measurements of glutamate and glutamine, suggested indeed an increase glutamate/glutamine ratio in the raphe, but not changes in the hippocampus. Microdialysis measurement of extracellular glutamate in living mice is ongoing to corroborate these findings.

At the behavioral level, these changes in glutamate output in the raphe and its consequences on 5-HT turnover at projection sites in the hippocampus are associated with an increase in flight reactions in the aversive ultrasound paradigm, but no increase of anxiety in the social interaction test. These data, presented at the FENS meeting in 2012 in Barcelona, are the subject of publication in preparation.

Partner 1B are also testing whether GABA-A receptors are involved in 5-HT_{2C} regulation using both genetic and pharmacological approaches. Pharmacological data indicate that the inhibitory effect of 5-HT_{2C} receptors on the stress-induced increase in 5-HT turnover is prevented by the GABA-A receptor antagonist bicuculine. Experiments are on-going to explore whether a similar effect could be mimicked by the constitutive deletion of the $\alpha 3$ subunit of GABA-A receptors, which is present mainly in monoaminergic neurons.

WP -5- GABA-B receptors and development

GABA-B receptors are implicated in the development of anxiety and the control of anxiety states. However, little is known about the developmental time-window, the neuronal systems and the subtypes of GABA-B receptors that are involved in the development of anxiety. Constitutive loss of GABA-B receptors results in increased anxiety, however, acute pharmacological antagonism of GABA-B receptors in adulthood is not anxiogenic (Mombereau et al., 2004). This behavioral profile is similar to that observed with 5-HT_{1A} receptor knockout mice (Gross et al., 2002). Thus we wanted to investigate the developmental origins of GABA-B receptor-mediated anxiety and address whether these coincide with alterations in 5-HT function.

Characterization of anxiety phenotypes in mice with transient impairment of GABA-B receptor function

Mice were given GABA-B receptor antagonists and agonist during early life and analysis of behavior will proceed in adulthood. Behavioral experiments completed. Results presented in poster format at EBPS biennial meeting in Rome, September 2009 (Sweeney FF, et al. 2009). This work was also presented as a poster at the 2009 Neuroscience Ireland conference, Trinity College Dublin. A manuscript is currently being prepared for submission to Psychopharmacology.

Partner 5 investigated the consequences of the specific inactivation of the GABA-B1a subunit (mice generated by Partner 3) on the synaptic properties of hippocampal circuits. To this aim learning dependent changes in synaptic strength at the CA3-CA1 synapse was determined by the chronic recordings of fEPSPs evoked across the acquisition and performance phase of an instrumental conditioning task in freely moving mice. The results will be published in a collaborative paper in due course.

Tissue-specific and time-specific rescue of GABA-B receptor function

The individual mouse lines to generate a mouse model allowing tissue- and time-specific rescue of GABA-B function were generated and validated. However, a functional rescue of GABA-B receptors in GABA-B knockout mice was not achieved even though the mice carried all the necessary transgenes. Hippocampal pyramidal neurons of these mice expressed the reporter transgene but not the GABA-B2 subunit necessary for the rescue of GABA-B receptor function. Accordingly, electrophysiological recordings in pyramidal neurons failed to detect functional GABA-B receptors. Therefore a behavioral reversal of the anxiety phenotype in mice with reconstituted GABA-B receptor signaling could not be analyzed.

Age-dependent GABA-B-binding characteristics

Because KCTD proteins increase agonist potency at the receptors we investigated whether these proteins possibly directly increase agonist affinity at the receptor. Using lentiviral vectors we produced stable CHO cell lines co-expressing the core GABA-B receptor subunits GABA-B1 and GABA-B2 together with KCTD8 or KCTD12 to perform 3H-CGP54626A radioligand antagonist displacement experiments with GABA. At the same time these cell lines were used in GTP γ S binding experiments. In addition, radioligand antagonist displacement and GTP γ S binding studies in transiently transfected HEK293 cells were performed. The results of these experiments will be published in due course.

WP -6- New strategies for treating anxiety

Partner 3 has generated the pharmacological and genetic tools to validate therapeutic concepts based on GABA-B drugs. While working in the pharmaceutical industry (Novartis, Basel), Partners 3 and partner 4 were involved in the identification and validation of the first generation of positive allosteric modulators at GABA-B receptors (Urwyler et al., 2001; Urwyler et al., 2003; Cryan et al., 2004).

Positive allosteric modulators possess the advantage that they discriminate between activated and non-activated receptor states, while agonists indiscriminately activate all receptors, and may therefore have a broader therapeutic window. Indeed, allosteric modulators of GABA-B receptors confer anxiolytic properties of agonists in the absence of typical side-effects of either agonists or conventional anxiolytics (Cryan et al., 2004). However, the neural circuits underlying the anxiolytic effects of GABA-B receptor modulators remain elusive. The project of this work package was designed to identify the GABA-B receptor subtypes involved in the activation of anxiety related circuits.

Despite some delay in the task due to mouse breeding and import difficulties, significant progress was made. Results were presented at meetings, and several papers are in preparation.

GABA-B receptor positive modulator-induced neuronal activation

Partner 4 (UCC): Mice were given GABA-B receptor positive modulator and exposed to open-arm stress. Brains were processed for c-Fos activation pattern. c-Fos activation pattern was assessed in a broad panel of anxiety-related brain regions. Confirmation of the role of these brain regions in the anxiolytic effects of GS39783 is currently being investigated by directly c-Fos immunohistochemistry was used to map the sites of action of the GABA-B receptor positive modulator GS39783 in the brain in control and stressed animals. Given the new and exciting data on the role of GABA-B receptor subunits in stress-mediation (Task 6.02), we decided to use this same technology to assess the impact whether GABA-B receptor subunits play a role in stress-induced neuronal activation data.

The effects of GABA-B receptor positive modulator on neuronal circuits have now been fully described.

As no data are available in relation to stress reactivity in maternally and non-separated GABA-B1a^{-/-} and GABA-B1b^{-/-} mice, we analyzed restraint-induced activation pattern of c-fos in various stress-related brain areas.

Given that the hippocampus is involved in the effects of GABA-B receptor ligands and that adult hippocampal neurogenesis is involved in the pathogenesis of stress-related psychiatric disorders we assessed the effects of acute, subchronic and chronic treatment with the GABA-B receptor antagonist CGP52432.

The effects of GS39783 on neuronal activation have been written up for publication and are under revision in Psychopharmacology- Pizzo, RC, O Leary OF and Cryan JF Elucidation of the Neural Circuits Activated by a GABA-B Receptor Positive Modulator: Relevance to Anxiety.

A Review article has been published on the general topic- Cryan JF, Sweeney FF. The age of anxiety: role of animal models of anxiolytic action in drug discovery. Br J Pharmacol. 2011 Oct;164(4):1129-61.

Regarding the relative contribution of GABA-B receptors to stress-induced neuronal activation. Our data clearly show that there is a distinct pattern of brain activation between GABA-B1a^{-/-}, GABA-B1b^{-/-} and wild type mice. Intriguingly, GABA-B1a^{-/-} and GABA-B1b^{-/-} mice displayed a similar stress-induced expression of c-fos in the cortical areas, in the paraventricular nucleus of the hypothalamus and in the amygdala but not in the hippocampal formation and in the nucleus accumbens. Specifically, GABA-B1b^{-/-} mice displayed an increase in stress-induced c-fos expression in the hippocampus and in the nucleus accumbens, two key areas that play an important role in the regulation of antidepressant-like behaviors. Moreover, early-life stress significantly affected stress-induced c-fos expression in the hippocampus in wild type and GABA-B1b^{-/-} mice but not in GABA-B1a^{-/-} mice.

These data are in preparation for publication- Felice D, O Leary OF, Bettler B, Cryan JF. GABA-B receptor subunits modulate stress-induced neuronal activation in the mouse: Impact of Early-Life Stress.

Chronic, but not acute or subchronic treatment with CGP52432 induced increased cell proliferation in the adult hippocampus. Moreover, these effects were localized to the ventral as opposed to the dorsal hippocampus.

These data have been recently published: Felice D, O'Leary OF, Pizzo RC, Cryan JF, Blockade of the GABA-B receptor increases neurogenesis in the ventral but not dorsal adult hippocampus: Relevance to antidepressant action, *Neuropharmacology* 63:1380-1388, 2012.

Receptor subtypes involved in the anxiolytic effects of positive allosteric modulators of R GABA-B

In order to study which GABA-B receptor subtype mediates the anxiolytic effects of the positive allosteric modulator GS39783, Partner 4 used mice that selectively express either the GABA-B1a or the GABA-B1b subunit. As the wildtype mice failed to show anxiolytic effects of GS39783, it was decided to investigate which GABA-B receptor subunit is responsible for the behavioral effects of cocaine. In addition the GABA-B receptor subunits involved in the effects of chronic psychosocial stress were also investigated (see explanation of the deviation part 3.2 of the Periodic Report).

GABA-B1a and GABA-B1b receptor subunit mutant mice displayed markedly different locomotor responses to both acute and repeated cocaine treatment. GABA-B1a knockout mice displayed enhanced locomotor activity relative to both wildtype and GABA-B1b knockout mice in response to acute cocaine administration. In contrast to this GABA-B1b knockout mice which failed to sensitize to the locomotor effects of cocaine, in contrast to wild type mice which display increasing levels of hyperlocomotor behavior in response to successive doses of cocaine.

Strain and protocol effects were identified that confer increased susceptibility to chronic psychosocial stress were and have been published.

Social defeat stress (SDS) reduced social interaction in wild type and GABA-B1a knockout mice but was without effect in GABA-B1b knockout mice, thus suggesting that GABA-B1b knockout mice are more resilient to the negative effects of chronic social stress on social behavior. SDS reduced the preference for saccharin in GABA-B1a but not in wild type or GABA-B1b knockout mice, thus suggesting that GABA-B1a knockout mice have increased susceptibility to the anhedonic and social effects of social defeat stress.

Taken together, the data suggest that an increased ratio of GABA-B1b receptors to GABA-B1a receptors increases susceptibility to stress-induced depression-like behaviors, while reduced GABA-B1b receptor subunit expression promotes resilience to stress-induced depression-like behaviors.

Publications in preparation:

O Leary OF, Felice D, Savignac S, Bettler GABA-B receptor subunits differentially control the susceptibility and resilience to stress-induced depression-related behaviors.

Savignac HM, Hyland NP, Dinan TG, Cryan JF. The effects of repeated social interaction stress on behavioral and physiological parameters in a stress-sensitive mouse strain. *Behav Brain Res.* 2011 Jan 20;216(2):576-84.

Savignac HM, Finger BC, Pizzo RC, O'Leary OF, Dinan TG, Cryan JF. Increased sensitivity to the effects of chronic social defeat stress in an innately anxious mouse strain. *Neuroscience.* 2011 Sep 29;192:524-36.

Sweeney F, Jacobson L., Bettler B and Cryan JF Differential role of GABA-B receptor subunits in the behavioral effects of cocaine.

Behavioral characterisation of S892A mice

Phosphorylation at the S892 residue of the GABA-B2 subunit has been shown in-vitro to modulate receptor desensitization. The effects of S892A mutants in mice (generated by P3) were assessed to determine the functional consequences of this phosphorylation in vivo. In particular animal models of anxiety and cocaine dependence were investigated.

GABA-B2-S892A mice display identical anxiety behavior to wild type littermate controls in the light-dark box, defensive marble burying and stress induced hyperthermia paradigms. In addition they displayed identical behavior to their wild type controls in a Pavlovian fear conditioning experiment with regard to the acquisition, expression and extinction of freezing behavior to a footshock associated cue. The behavior of the mice in the forced swim test, a widely used screen of antidepressant activity, was also identical to wild type controls.

GABA-B-S892A mice displayed identical preference behavior to cocaine as wild type controls in a conditioned place paradigm. Analysis of locomotor activity of mice during this experiment revealed no differences in response to cocaine between the GABA-B-S892A and wild-type mice.

A publication is in preparation - Sweeney F, Gassmann M, Bettler B and Cryan JF. The phosphorylation site of the S892 residue of the GABA-B2 subunit does not alter behavioral sensitivity to cocaine.

WP -7- Gene-environment effects of 5-HT and GABA-B

Adverse early life experiences are known risk factors for anxiety in human epidemiological studies; however, the molecular mechanisms underlying these environmental programming effects remain unknown. Several recent studies have identified specific human polymorphisms that are associated with risk for mental illness in the presence of environmental pathogens (so called GxE effects; reviewed in Caspi and Moffitt, 2005). These findings suggest that investigations of genetic susceptibility for anxiety must also consider early environmental risk factors in order to be successful and relevant to a better understanding of human mental illness.

Two paradigms for the manipulation of early environment have been developed by Partner 1b and Partner 2 and were used for testing GxE effects. The first paradigm involved the breeding of mice that have experienced either low or high levels of maternal care (Carola et al., 2006). In humans, poor maternal care is a known risk factor for anxiety (Pruessner et al., 2005). The second paradigm used the prenatal stress as a model for adverse maternal stress during pregnancy (Vallee et al., 1997).

Interactions between BDNF and maternal care on anxiety

Partner 2 completed experiments documenting the role of Bdnf in the maternal programming of anxiety in (Carola et al., GBB 2010) and as well as experiments described in the previous report in mice at postnatal day 10 (Carola et al.). This Partner has also tested for interactions between 5-HTT genotype and chronic psychosocial stress during adulthood (Bartolomucci et al., Dis. Models and Mech. 2010).

Critical period for the role of 5-HT in moderating the effects of stress on anxiety

The original plan was to overexpress the Tph2 enzyme which is the rate limiting enzyme in serotonin synthesis. Partner 6 generated the Tph2-tTA mouse driver line and obtained the tetO-Tph2 founder animals. In parallel, they carried studies using the Tph2 tTa and a reporter gene tetO-lacZ to optimize the induction course of the transgene in serotonergic neurons (Weber et. Al 2009). However the doxycycline regulated gene overexpression did not provide sufficient temporal resolution to determine the critical window so the original aim was abandoned and work efforts were redirected to analyzing the effects of stress in the C1473G SNP. This redirection involved increased efforts from the Partners to use other mouse strains in which there is constitutive depletion of serotonin, but where rescue of the phenotype can be obtained by pharmacological treatments.

-In the course of the work Partners 6 and 1b learned from analysing the C1473G SNP in Tph2, that there are strong regulatory mechanism compensating for variations in Tph2

activity in serotonergic neurons and that such changes play major role in the development and /early postnatal period.

-Partner 1 also characterized the stress responses in mice with constitutive depletion of serotonin but where pharmacological rescue can be obtained; In mice with a deletion of VMAT2 under the control of the SERT promoter. A pharmacological rescue was obtained using clorgyline and in the Pet1-KO mice a rescue of brain serotonin levels was obtained using 5-OH Tryptophan.

Assessment of the moderating effects of R GABA-B on early environmental programming of anxiety

Stress, particularly that in early life is a major predisposing factor for the development of anxiety disorders. We evaluated of the contribution of GABA-B1a and GABA-B1b receptor subunit to the effects of low maternal care on behavior.

Efforts were spent on Identifying protocols that generate robust early-life stress induced changes in the mouse Once a protocol was identified, GABA-B1a^{+/-} and GABA-B1b^{+/-} mice were bred to generate WT, GABA-B1a^{-/-} and GABA-B1b^{-/-} mice and genotype was confirmed by PCR. Male and female GABA-B1a^{-/-} and GABA-B1b^{-/-} mice underwent unpredictable maternal separation combined with unpredictable maternal stress (MSUS) from postnatal day (PND) 1 to 14. During these two weeks maternal care behaviors of the mother were also monitored. During maternal separation, the Ultrasonic vocalizations (USVs) emitted from the maternally separated pups were measured on PND1 and PND7. Upon reaching adulthood the behavior of female and male maternally-separated (MS) and non-separated (NS) animals were tested in a battery of anxiety and depression tests.

In addition given the relationship between stress anxiety and pain visceral pain responses were assessed in GABA-B1b deficient mice.

Strain and protocol effects were identified that confer increased resilience to early life stress

The data investigating the effects of early life stress in GABA-B1 subunit deficient mice revealed a clear gene x environment interaction, with adult MS GABA-B1a^{-/-} mice but not MS GABA-B1b^{-/-} mice exhibiting anhedonic-like behavior in the saccharin preference and female urine sniffing test. GABA-B1b^{-/-} mice exhibited an antidepressant-like behavior in basal conditions as assessed by the tail suspension test and forced swim test. Measurements of anxiety revealed that, GABA-B1b^{-/-} pups exhibited an increased number of USVs during MS on PND7. However, in adulthood, the stress induced hyperthermia test of anxiety revealed no differences across all experimental groups. Assessment of locomotor activity in the open field

revealed that NS GABA-B1b^{-/-} mice displayed increased activity that was attenuated by the MS paradigm, while GABA-B1a^{-/-} mice displayed no differences in locomotor activity when compared to the WT group.

These data have been presented at the ECNP meeting in Vienna 2012 as well as other local meetings and are being put together for a publication Felice D, O Leary OF, Bettler B, Cryan JF GABA-B receptor subunit isoforms differentially mediate susceptibility to early-life stress-induced depression related behavior.

Early life stress induced an increase in visceral pain responses in adults. However this was not modulated by the absence of GABA-B1b receptor subunits.

This has recently been published - Moloney RD, O'Leary OF, Felice D, Bettler B, Dinan TG, Cryan JF. Early-life stress induces visceral hypersensitivity in mice. *Neurosci Lett*. 2012 Mar 23;512:99-102.

Potential Impact:

Over 16% of individuals will experience an anxiety disorder during their lifetime (Somers et al., 2006). Recent data within the EU details that the prevalence of anxiety disorders is as high as 41.4 million at an annual cost of €41,372 million (Andlin-Sobocki and Wittchen, 2005). Anxiety disorders include generalized anxiety disorder, panic disorder, social phobia, specific phobia, post-traumatic stress disorder, and obsessive-compulsive disorder (DSM-IVR, 1994). Although there are effective medicines for treating some forms of anxiety (e.g. benzodiazepines, selective-5-HT reuptake inhibitors), most anxiety disorders are poorly treated with these agents due to their side effects, dependence liability or slow onset of action.

Our project provided new information to better understand the neurobiological underpinnings of brain dysfunction in anxiety disorders. The research effort of the consortium provided insights on the genetic and environmental determinants of these affective diseases and how these two major risk factors interact to generate the illness.

Scientific Impacts

A) The project enabled a better understanding of circuits and systems involved in anxiety disorders. We identified the plasticity mechanism in hippocampal neural circuits involved in associative learning conducting to fear. The use of pharmacogenetic silencing tools allowed to transiently invalidate circuits that are involved in anxiety responses (contextual fear conditioning). We characterized molecular pathways such as the 5-HT_{1A} and GABA-B receptors that are involved in the functioning of these circuits.

B) The project allowed to produce and to characterize new animal models. This included genetically modified animals but also new experimental paradigms to evaluate the neural circuits underlying anxiety disorders. We obtained new tools for targeting the 5-HT raphe neurons and revealed a genetic heterogeneity in the raphe serotonin neurons that would enable to target specific raphe subtypes. We obtained new genetic tools to question the role of the GABA-B receptors at different times in development. These are new scientific tools that will be useful to the community overall, contributing in short and long term to a better understanding of anxiety disorders.

C) The project allowed to identify new therapeutic targets and compounds for translational research. We discovered new pathways of GABA-B signaling: the KCTD proteins, as modulators of GABA-B receptors. These appear promising paths to drug development. We also characterized the cellular and developmental impacts of GABA-B signaling for adult anxiety behaviors.

D) We identified new interactions between genes and the environment in the control of adult anxiety behaviors that could have high relevance to the pathophysiology of anxiety disorders.

E) We identified the role of microbiota in the control of anxiety disorder. This is a completely new dimension in the field that might also be amenable to therapeutic intervention.

Impacts in the field of treating disorders

As mentioned above several of the scientific discoveries cited above could have an impact on therapeutic or preventive approaches in the field of disorders.

As therapeutic approaches one can envisage that better understanding the neural circuits involved in anxiety will lead to a better behavioural control of the anxiety disorders, by stimulating brain plasticity mechanisms in specific manners. At the other end of the spectrum better and more selective drug intervention could be aimed through the discovery of the molecular pathways identified. Finally indirect therapeutic intervention could be sought for such as acting on the composition of the microbiota.

Prevention: One of the major aims of the proposal was to define a critical period in the early life, during which modification of gene and/or environment has important consequences in anxiety related disorders. Anxiety disorders share an early onset, mostly before age of 16. Large epidemiological studies have identified prospective risk factors in family history, adverse family environment, and personality traits, suggesting the importance of interactions between genes and environment in developing anxiety disorders. Circumstantial evidence points to the role of early stressful experience in causing modified responses to stress in later life and the role of parental care in harmonious brain development. Our research allowed to determine the role of several molecular signaling pathways, namely downstream of serotonin and GABA neurotransmission that could interact with the environment. This obviously calls to question the use of drugs such as antidepressants or GABA modulators at these critical developmental periods.

Impacts for productivity and networking of the European Research effort

The present proposal exemplified well how through pooling expertise and resources, the critical mass necessary for advancing together better on the scientific issues raised in the project. The exchange of novel information, and the integration of methodologies and new tools was real boost to each of the cooperating institutes. Finally, the possibility of working in parallel on a common task allowed us to achieve realistically the objectives within the time frame of the 7th Framework Programme.

Overall more than 70 scientific articles were published by the teams involved in the consortium. These include high impact publications such as Nature, Nature Neuroscience, Neuron, PNAS and Journal of Neuroscience. These publications have been well received and are well cited by the community, and have often lead to press release communications.

The work was also shown at foremost National and International meetings in the field of neuroscience, such as the FENS and the American Society for Neuroscience, to cite only a few.

Overall the consortium participated to more than 90 such international events where parts of the project could be presented.

Impacts for training of young scientists

Overall a large number of young researchers, Master and PhD students and post-doctoral students received excellent training during this period. The different disciplines covered by the associate Partners enabled 18 students to obtain better knowledge of related fields, and to help them put their own research in a larger perspective.

Impact in psychiatry from fundamental research to translational research

Although this project was essentially fundamental research, it gave impetus to several participating teams to foster their interactions with clinical psychiatrists. For Partner 3 is also co-director of the focal area Neurosciences in the translational Department for Clinical and Biological Research. Partner 1a has contributed to organize a translational network, instance the Paris team has engaged into translational networks with clinical psychiatrists in St Anne in a center for Neurobiology and Psychiatry.

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