CHAPTER 4.6

Genetic factors underlying anxiety-behavior: a meta-analysis of rodent studies involving targeted mutations of neurotransmission genes

Catherine Belzung[1,*], Samuel Leman[1] and Guy Griebel[2]

[1]*EA3248 Psychobiologie des Emotions, Université François Rabelais, UFR Sciences et Techniques, Parc Grandmont, Tours, France*
[2]*Sanofi-Aventis, Psychopharmacology Department, Bagneux, France*

Abstract: This paper aimed at reviewing publications investigating the involvement of genetic factors in anxiety behavior, using a meta-analysis of rodent studies involving targeted mutations of neurotransmission genes. We summarized 311 experiments investigating the involvement of GABAergic, serotoninergic, glutamatergic, and neuropeptidergic targets, and then analyzed these tables according several questions such as: Are some particular behavioral tests used in these studies? Which genetic method has been used? Which phenotypes are observed? Can these results be explained by the species or the strain used? Did this strategy enable to precise the brain area involved in these processes? Is the contribution of the genetic factor limited to the developmental period? Do the effects of the mutation correlate with the results of pharmacological challenge? Does the mutation modify the response to anxiolytic or anxiogenic agents? We propose some conclusions on the genes involved in normal and pathological anxiety behavior.

Keywords: anxiety; genetic; serotonin; glutamate; GABA-neuropeptide

I. Introduction

Anxiety is an all day experience of most people, corresponding to an adaptative reaction to potential threats. The probability of the occurrence of this reaction as well as its intensity may vary among subjects, depending on genetic as well as epigenetic factors. In some subjects, the frequency and intensity of this reaction can become excessive and maladapted: this can correspond to anxiety disorders, such as panic, post-traumatic stress, generalized anxiety, or obsessive-compulsive disorder. In humans, several studies have tried to identify the genetic factors involved either in normal anxiety or in pathological anxiety states. Such a research is mainly based on polymorphism studies. However, polymorphism has not been described for all genes of interest, which limits the progress of human studies. Recently (Belzung and Philippot, 2007), we have shown that rodents are excellent species to study anxiety-related behaviors, as they display most (but not all) of the cognitive processes necessary to human anxiety. Further, targeted genetic modification tools have been particularly well developed in rodents, especially in mice, enabling progress in the study of the genetic factors involved in anxiety using such species.

This paper aimed at reviewing publications that investigated the involvement of genetic factors in anxiety-related behaviors in rodents. Indeed,

*Corresponding author.
E-mail: catherine.belzung@univ-tours.fr

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several arguments indicate that genetic factors may be involved in anxiety behavior. First, the polymorphism of several genes is associated with human anxiety. For example, the Met88Val diazepam binding inhibitor gene polymorphism (Thoeringer et al., 2007), the serotonin transporter promotor region polymorphism (Mazzanti et al., 1999; Hariri et al., 2002; Pezawas et al., 2005; Brocke et al., 2006; Gonda et al., 2007; Hayden et al., 2007; Stein et al., 2007), or the *BDNF* Val66Met polymorphism (Jiang et al., 2005; Lang et al., 2005; Chen et al., 2006b; Hashimoto, 2007; Hunnerkopf et al., 2007) are associated with modifications in anxiety-related phenotypes, suggesting an involvement of genetic factors on these traits. Second, excessive anxiety can be treated by various pharmacological agents targeting specific neurotransmitter systems (see e.g., Millan, 2003 for a review), suggesting that anxiety traits may be related to dysfunctions of these neurotransmitter systems. This indicates that the genes coding for the proteins involved in the secretion, the regulation, or the binding of these molecules may be involved in anxiety processes.

As a huge amount of data is available on genes involved in the anxiety phenotype, we will focus on the results of targeted invalidation or over-expression studies. Other experimental strategies (such as QTL, selected lines, or multiple marker strains) are available, but an exhaustive review of all these studies is not possible in such a limited space. Further, genetic factors can exert their action at two different time points: they can act during the developmental period, modifying the development of the brain (e.g., neurotrophic factors) or act once the animals are adults, interfering either with non-specific processes (such as perception, motricity, pain) or with neurotransmission. We will focus on the second ones, because this may allow comparing the results with the ones obtained using pharmacological activation or inactivation of the proteins that result from the targeted genes (e.g., neurotransmitter receptors). To do that, we will present several tables, which present in an exhaustive way the studies that were undertaken in this field with a focus on the following systems: serotonin (Table 1), glutamate (Table 2), GABA (Table 3), endocannabinoids (Table 4) and a variety of neuroactive peptides (Table 4). In each table, we describe the following parameters: gene involved, genetic method used (knock out, knock in, over-expression), species studied, strain used, experimental device as well as the observed phenotype. For practical reasons, each line of a given table corresponds to an experiment, and not to a published paper. A paper can correspond to several experiments. A total number of 311 experiments have been analyzed in this meta-analysis.

The principal focus of this paper is to analyze these tables, according several questions such as: Are some particular behavioral tests used in these studies? Which genetic method has been used? Was there any particular choice of construction (knock-in, knock-out, and over-expressed models) made for each of the neurotransmission systems? Which phenotypes are observed? Can these results be explained by the species or the strain used? Did this strategy enable to precise the brain area involved in these processes? Is the contribution of the genetic factor limited to the developmental period? Do the effects of the mutation correlate with the results of pharmacological challenge? Does the mutation modify the response to anxiolytic or anxiogenic agents? What do these findings tell us about the link between neurotransmitter systems and anxiety? Do these studies provide useful information about the role played by the various GABAergic, serotoninergic, glutamatergic, and neuropeptidergic targets in anxiety behavior? The response to these questions will enable us to propose some conclusions about the genes involved in normal and pathological anxiety behaviors.

II. Are some particular behavioral tests used in these studies?

Several test situations have been used to assess anxiety behavior, including elevated plus-maze, light/dark test, open-field, free exploration, stress-induced hyperthermia, novelty-induced suppression of feeding, mouse defense test battery, acoustic startle, conditioned fear, Vogel conflict, and cat odor presentation. In these situations, the

Table 1. Effect of targeted mutation of serotonin-related genes on rodents in anxiety tests

Model	Test	Animal	Effects	Reference
5-HT1A KO mice	Open-field	Swiss-Webster/12^{SV} mice	Anxiogenic	Parks et al. (1998)
5-HT1A KO mice	Open-field Elevated plus-maze	n/a	Anxiogenic	Toth and Sibille (1998)
5-HT1A KO mice + diazepam (0.1–1 mg/kg)	Open-field	129/Sv × Swiss-Webster background mice	Diazepam lost its ability to produce anxiolytic-like activity	Sibille et al. (2000)
	Elevated plus-maze		Diazepam lost its ability to produce anxiolytic-like activity	
5-HT1A KO mice	Open-field	Male and female	Anxiogenic	Ramboz et al. (1998)
	Elevated plus-maze	C57BL/6J × 129/Sv mice	Anxiogenic	
5-HT1A KO mice + WAY100635 (0.03–0.3 mg/kg) 5-HT1A KO mice + 8-OH-DPAT (0.1–1 mg/kg) 5-HT1A KO mice + buspirone (0.05–2.5 mg/kg)	Open-field		No effect when compared to vehicle-treated –/–mice	
5-HT1A KO mice	Open-field	129/Sv × C57BL/6J mice	Anxiogenic	Heisler et al. (1998)
	Elevated zero-maze		Anxiogenic	
	Responses to a novel object		Anxiogenic	
5-HT1A KO mice + flesinoxan (0.3–3 mg/kg)	Stress-induced hyperthermia	129/Sv-ter background mice	The anxiolytic-like activity seen in WT mice was lost in KO animals	Pattij et al. (2000)
5-HT1A KO mice	Stress-induced hyperthermia		KO animals were more anxious than WT mice	
5-HT1A KO mice + mCPP (0.3–3 mg/kg)	Stress-induced hyperthermia		WT animals and KO mice displayed similar phenotype	
5-HT1A KO mice + diazepam (1–4 mg/kg)	Stress-induced hyperthermia		The anxiolytic-like activity seen in WT mice was still present in KO animals	
5-HT1A KO mice	Elevated plus-maze	Female and male 129/Sv × C57BL/6J mice (8–10-week-old)	KO animals were more anxious than WT mice	Gross et al. (2002)
5-HT1A KO rescue transgenic mice		Female and male 129/Sv × C57BL/6J mice (18–20-week-old)	Anxious phenotype of 5-HT1A KO mice was no longer seen	
5-HT1A KO mice	Open-field test	Female and male 129/Sv × C57BL/6J mice (8–10-week-old)	KO animals were more anxious than WT mice	
5-HT1A KO rescue transgenic mice		Female and male 129/Sv × C57BL/6J mice (18–20-week-old)	Anxious phenotype of 5-HT1A KO mice was no longer seen	
5-HT1A KO mice	Novelty-induced suppression of feeding	Female and male 129/Sv × C57BL/6J mice (8–10-week-old)	KO animals were more anxious than WT mice	
5-HT1A KO rescue transgenic mice		Female and male 129/Sv × C57BL/6J mice (18–20-week-old)	Anxious phenotype of 5-HT1A KO mice was no longer seen	
5-HT1A KO mice + doxycycline (for 2 months)	Novelty-induced suppression of feeding		KO animals were more anxious than WT mice	

Table 1 (*continued*)

Model	Test	Animal	Effects	Reference
5-HT1A KO rescue transgenic mice + doxycycline (for 2 months)		Female and male 129/Sv × C57BL/6J mice (80–140-day-old)	Anxious phenotype of 5-HT1A KO mice was no longer seen despite 5-HT1A turning off	
5-HT1A KO mice + doxycycline (during development)			KO animals were more anxious than WT mice	
5-HT1A KO rescue transgenic mice + doxycycline (during development)			Reversal of anxious phenotype of 5-HT1A KO mice was no longer seen	
5-HT1A KO mice + doxycycline (for 2 months)	Open-field test	Female and male 129/Sv × C57BL/6J mice (80–140-day-old)	KO animals were more anxious than WT mice	Gross et al. (2002)
5-HT1A KO rescue transgenic mice + doxycycline (for 2 months)			Anxious phenotype of 5-HT1A KO mice was no longer seen despite 5-HT1A turning off	
5-HT1A KO mice + doxycycline (during development)			KO animals were more anxious than WT mice	
5-HT1A KO rescue transgenic mice + doxycycline (during development)			Reversal of anxious phenotype of 5-HT1A KO mice was no longer seen	
5-HT1A KO mice + doxycycline (for 2 months)	Elevated plus-maze		KO animals were more anxious than WT mice	
5-HT1A KO rescue transgenic mice + doxycycline (for 2 months)			Anxious phenotype of 5-HT1A KO mice was no longer seen despite 5-HT1A turning off	
5-HT1A KO mice + doxycycline (during development)			KO animals were more anxious than WT mice	
5-HT1A KO rescue transgenic mice + doxycycline (during development)			Reversal of anxious phenotype of 5-HT1A KO mice was no longer seen	
5-HT1A KO mice	Acoustic startle reflex	Mixed 129/Sv background	(1) No difference between genotypes; (2) 85–120 dB were used	Dirks et al. (2001)
	Footshock-induced sensitization in acoustic startle reflex		No difference between genotypes	
5-HT1B KO mice	Acoustic startle reflex		(1) Reduced reactivity in KO; (2) 85–120 dB were used	
	Footshock-induced sensitization in acoustic startle reflex		Sensitization was reduced in KO	
5-HT1A KO mice	Open-field test	Mixed 129/Sv background	No difference between genotypes	Pattij and Olivier (2001)
	Light/dark test		No difference between genotypes	
	Stress-induced hyperthermia		No difference between genotypes	
	Stress-induced physiological changes		(1) KO mice displayed higher stress response; (2) injection stress was used	
	Stress-induced physiological changes		(1) KO mice displayed higher stress response; (2) novel cage exposure stress was used	

5-HT1A KO mice	Stress-induced hyperthermia Elevated plus-maze	129/Sv background	No difference between genotypes	Pattij et al. (2002b)
5-HT1A KO mice + alprazolam (1–3 mg/kg)	Stress-induced hyperthermia		The anxiolytic-like activity seen in WT mice was still present in KO animals	
5-HT1A KO mice + flumazenil (3–30 mg/kg)			No difference between genotypes	
5-HT1A KO mice + alcohol (2–4 g/kg)			The anxiolytic-like activity seen in WT mice was still present in KO animals	
5-HT1A KO mice + pentylenetetrazole (7.5–30 mg/kg)			No difference between genotypes	
5-HT1A KO mice + diazepam (1 mg/kg)	Elevated plus-maze		The anxiolytic-like activity seen in WT mice was still present in KO animals	
5-HT1A KO mice	Injection stress-induced tachycardia	129/Sv background (12-week-old)	KO mice displayed higher stress response	Pattij et al. (2002a)
5-HT1A KO mice	Rectal temperature procedure-induced hyperthermia		No difference between genotypes	
5-HT1A KO mice + diazepam (4 mg/kg)	Injection stress-induced hyperthermia and tachycardia		Anxiolytic	
5-HT1A KO mice	Novelty stress-induced hyperthermia and tachycardia		KO mice displayed higher stress response	
5-HT1A KO mice	Elevated plus-maze Open-field test Elevated plus-maze Open-field test	Swiss background mice (2–5-month-old) C57BL/6J background mice (2–5-month-old)	KO animals were more anxious than WT mice	Bailey and Toth (2004)
5-HT1A KO mice + diazepam (0.2–1 mg/kg)	Elevated plus-maze	Swiss background mice (2–5-month-old)	The anxiolytic-like activity seen in WT mice was lost in KO animals	
5-HT1A KO mice + diazepam (1 mg/kg)		C57BL/6J background mice (2–5-month-old)	The anxiolytic-like activity seen in WT mice was still present in KO animals	
5-HT1A KO mice + diazepam (0.2–1 mg/kg)		Swiss/B6 background mice (2–5-month-old)	The anxiolytic-like activity seen in WT mice was lost in KO animals	
5-HT1A KO mice	Elevated plus-maze	Female C57BL/6J background mice (5–8-month-old)	KO mice displayed increased anxiety-like behavior	Li et al. (2004)
5-HT1A KO mice + Ad-1AP sense			KO mice displayed increased anxiety-like behavior despite 5-HT1A receptor restauration in the hypothalamus	
5-HT1A KO mice	Light/dark test Vogel conflict test Conditioned fear stress	129/Sv × C57BL/6J mice (8–10-week-old)	KO animals were more anxious than WT mice No difference between genotypes Freezing response of KO mice did not decrease when placed in an ambiguous environment	Klemenhagen et al. (2006)
5-HT1A receptor overexpression transgenic mice	Elevated plus-maze Open-field test Free-exploration test	Male and female NMRI mice (15-week-old)	Mice displayed reduced anxiety-like behavior No phenotypic differences Mice displayed reduced anxiety-like behavior	Kusserow et al. (2004)
5-HT2A KO mice	Open-field test Light/dark test Elevated plus-maze Novelty-induced suppression of feeding	n/a	KO animals appeared less anxious than WT mice	Weisstaub et al. (2006)

Table 1 (*continued*)

Model	Test	Animal	Effects	Reference
5-HT2A KO mice + Emx1-Cre	Open-field test Light/dark test Novelty-induced suppression of feeding		(1) Emx1-Cre restored 5-HT_{2A} function in the cortex; (2) anxiolytic-like phenotype was lost	
5-HT2C KO mice	Open-field Elevated plus-maze Novel object-induced anxiety	n/a	Anxiolytic	Heisler et al. (1998)
5-HT3 KO mice	Elevated plus-maze	C57BL/6J × 129 background (7–20-week-old)	KO animals were less anxious than WT mice	Bhatnagar et al. (2004b)
	Open-field test		No difference between genotypes	
	Light/dark test		No difference between genotypes	
	Conditioned fear stress		KO mice displayed enhancing freezing	
5-HT3 KO mice	Defensive withdrawal	C57BL/6J background (5–7-month-old)	KO animals appeared more anxious than WT mice	Bhatnagar et al. (2004a)
	Defensive withdrawal	Female C57BL/6J background (5–7-month-old)	KO animals appeared less anxious than WT mice	
5-HT3 KO mice	Light/dark test Elevated plus-maze	C57BL/6J × 129 background (90–120-day-old)	KO animals were less anxious than WT mice	Kelley et al. (2003)
5-HT4 KO mice	Restraint stress-induced hypophagia	Mixed 129/Sv × C57BL/6J × B6CBAF1/J background mice	Hypophagia was reduced in KO mice	Compan et al. (2004)
	Open-field test		KO mice were less anxious	
5-HT5A KO mice	Open-field	C57BL/6J mice	Increase in exploratory activity	Grailhe et al. (1999)
	Novel object test		Increase in exploratory activity and curiosity	
	Elevated plus-maze		No effect	
	Shock-probe burying test		Shock of 0.15 mA	
	Acoustic startle test		Bursts of 120 dB	
5-HT1B KO mice	Ultrasonic 'distress' vocalization	Male and female mice from 129/Sv strain	Anxiolytic	Brunner et al. (1999)
	Elevated plus-maze		Weak decrease in anxiety-related behaviors	
5-HT1B KO mice	Elevated plus-maze Object exploration	Male and female mice from 129/Sv strain	No effect Anxiolytic	Malleret et al. (1999)
5-HT1B KO mice	Elevated plus-maze Burying behavior	Mice from 129/Sv strain (20–30 g)	No difference between genotypes KO animals were less anxious than WT mice	Lopez-Rubalcava et al. (2000)
5-HT1B overexpression (in the dorsal raphe nucleus) transgenic rats (3 days)	Open-field test	Sprague–Dawley rats (180–250 g)	Anxiolytic (overexpression was achieved by using herpes simplex virus gene transfer)	Clark et al. (2002)
	Open-field test following restraint stress		Anxiogenic (overexpression was achieved by using herpes simplex virus gene transfer)	
	Elevated plus-maze following restraint stress		Anxiogenic (overexpression was achieved by using herpes simplex virus gene transfer)	

5-HT transporter KO mice	Open-field test	Female and male 129S6/SvEv background mice	No difference between genotypes	Lira et al. (2003)
	Elevated plus-maze		No difference between genotypes	
	Novelty-induced suppression of feeding		KO mice showed an increase in latency to feed	
5-HT transporter KO mice	Elevated plus-maze	Female and male C57BL/6J background mice (3–7-month-old)	5-HTT–/– mice showed increased anxiety-like behavior	Holmes et al. (2003)
	Light/dark test		5-HTT–/– mice showed increased anxiety-like behavior	
	Emergence test		5-HTT–/– mice showed increased anxiety-like behavior	
	Open-field test		5-HTT–/– mice showed increased anxiety-like behavior	
5-HT transporter KO mice + WAY100635 (0.05–0.3 mg/kg)	Elevated plus-maze	Female C57BL/6J background mice (3–7-month-old)	Anxiolytic	
5-HT transporter overexpression transgenic mice	Elevated plus-maze	CBA × C57BL/6J background mice (3–6-month-old)	Transgenic mice displayed reduced anxiety-related behaviors	Jennings et al. (2006)
5-HT transporter overexpression transgenic mice	Hyponeophagia		Transgenic mice displayed reduced anxiety-related behaviors	
5-HT transporter overexpression transgenic mice + paroxetine (10 mg/kg)	Elevated plus-maze		Paroxetine normalized low anxiety in transgenic mice	

Table 2. Effect of targeted mutation of glutamate-related genes on rodents in anxiety tests

Model	Test	Animal strain	Effects	Reference
mGluR5 KO mice	Stress-induced hyperthermia	B6/129 background mice (25–35 g)	(1) Mice displayed an anxiolytic-like phenotype; (2) the stressor was the rectal probing	Brodkin et al. (2002)
			(1) Mice displayed an anxiolytic-like phenotype; (2) the stressor was an intruder	
			(1) Mice displayed an anxiolytic-like phenotype; (2) the stressor was an injection	
mGluR5 KO mice + mGluR5 antagonist (MTEP (16 mg/kg, sc))			(1) The anxiolytic-like activity of MTEP was lost; (2) the stressor was an injection	
mGluR5 KO mice mGluR6 KO mice	Conditioned fear	129Sv/C57BL/6 background mice (8–12-week-old)	No difference between both genotypes KO mice showed reduced fear memory when tested 3, 7, or 14 days after training	Ko et al. (2005)
mGluR7 KO mice	Open-field	Male and female C57BL/6 background mice (8–10-week-old)	No difference between both genotypes	Callaerts-Vegh et al. (2006)
	Elevated plus-maze		KO mice showed decreased anxiety-like behavior	
	Marble burying		KO mice showed decreased anxiety-like behavior	
	Passive-avoidance		(1) KO mice had a reduced latency to enter the unknown dark compartment; (2) electric shocks of 0.2 mA/2 s were applied	
	Conditioned emotional response		(1) KO animals had a higher resistance to extinction of fear-elicited response suppression; (2) A VI-30s schedule was used	
mGluR7 KO mice	Elevated plus-maze Light/dark test Staircase test Stress-induced hyperthermia	129/Ola × C57BL/6 background mice (10–14-week-old)	KO mice showed decreased anxiety-like behavior	Cryan et al. (2003)
mGluR8 KO mice	Open-field Elevated plus-maze	C57BL/6 background mice (6-month-old)	KO mice showed increased anxiety-like behavior	Duvoisin et al. (2005)
mGluR8 KO mice	Elevated plus-maze	ICR background mice (12-week-old)	KO mice showed increased anxiety-like behavior	Linden et al. (2002)
		ICR background mice (24-week-old)		
		ICR background mice (12-week-old)	(1) No difference between both genotypes; (2) animals were tested under fluorescent light conditions	
			(1) No difference between both genotypes; (2) animals were submitted to restraint stress	
NR2A KO mice	Elevated plus-maze Light/dark test Open-field	Male and female C57BL/6 × CBA background mice (at least 10-week-old)	KO mice showed decreased anxiety-like behavior	Boyce-Rustay and Holmes (2006)

Table 3. Effect of targeted mutation of GABA-related genes on rodents in anxiety tests

Model	Test	Animal strain	Effects	Reference
$GABA_A$ alpha1 subunit KO mice	Contextual fear conditioning	129S1/X1 × FVB/N	Anxiogenic	Sonner et al. (2005)
$GABA_A$ alpha1 subunit KO mice + isoflurane			EC50 for isofluorane increased in mutants	
$GABA_A$ alpha1 subunit conditional KO mice in forebrain (amygdala, hippocampus, cortex)			No effect	
$GABA_A$ alpha1 subunit conditional KO mice in forebrain (amygdala, hippocampus, cortex) + isoflurane			EC50 for isofluorane increased in mutants	
$GABA_A$ alpha1 subunit conditional KO mice in forebrain (amygdala, hippocampus, cortex)	Tone fear conditioning		No effect	
$GABA_A$ alpha1 subunit conditional KO mice in forebrain (amygdala, hippocampus, cortex) + isoflurane			EC50 for isofluorane increased in mutants	
$GABA_A$ alpha1 subunit KO mice	Elevated plus-maze	C57BL/6J × 129Sv/SvJ	No effect	Kralic et al. (2003)
$GABA_A$ alpha1 subunit KO mice + ethanol 0.75–1.5 g/kg			No effect (KO more sensitive to the locomotor stimulating effects of ethanol)	
$GABA_A$ alpha1 subunit KO mice	Open-field		No effect	
$GABA_A$ alpha1 subunit KO mice + ethanol 0.75–1.5 g/kg			No effect	
$GABA_A$ alpha1 subunit KO mice	Elevated plus-maze	n/a	No effect	Kralic et al. (2002)
$GABA_A$ alpha1 subunit KO mice + diazepam 0.3–1 mg/kg			Mutants more sensitive to Diazepam: anxiolytic at a 0.6 mg/kg in mutants and at a 1 mg/kg dose in WT	
$GABA_A$ alpha1 H101R point mutation knock-in mice + diazepam 0.5–2 mg/kg	Elevated plus-maze	129/svJ × C57BL/6J	No effect of mutation	Rudolph et al. (1999)
$GABA_A$ alpha1 H101R point mutation knock-in mice + diazepam 1–3 mg/kg	Light/dark		No effect of mutation	

Table 3 (*continued*)

Model	Test	Animal strain	Effects	Reference
$GABA_A$ alpha1 S270H/L27 mutation knock-in mice	Elevated plus-maze	C57BL/6J × 129/SvJ	No effect of mutation	Werner et al. (2006)
$GABA_A$ alpha1 S270H/L277A point mutation knock-in mice + ethanol 0.75 g/kg			Effects of ethanol increased	
$GABA_A$ alpha3 H126R point mutation knock-in mice	Elevated plus-maze	n/a	No effect of mutation	Low et al. (2000)
$GABA_A$ alpha2 H101R point mutation knock-in mice	Light/dark		No effect of diazepam in mutants	
$GABA_A$ alpha3 H126R point mutation knock-in mice	Elevated plus-maze		No effect of mutation	
$GABA_A$ alpha2 H101R point mutation knock-in mice	Light/dark		No effect of diazepam in mutants	
$GABA_A$ alpha5 H105R point mutation knock-in	Light/dark	129/SvJ	No difference in diazepam-induced effects	Crestani et al. (2002)
mice + diazepam 1 mg/kg, p.o.	Elevated plus-maze		No difference in diazepam-induced effects	
$GABA_A$ alpha5 H105R point mutation knock-in mice	Trace fear conditioning		Facilitation in mutant mice	
	Delay fear conditioning		No effect	
	Context fear conditioning		No effect	
$GABA_A$ alpha5 subunit KO mice	Elevated plus-maze	C57BL6 × 129SvEv	No effect	Collinson et al. (2002)
$GABA_A$ alpha5 subunit KO mice + chlordiazepoxide 10 mg/kg				
$GABA_A$ beta3 subunit KO mice	Elevated plus-maze	C57BL6 × 129SvSvJ	Slightly anxiogenic (increased time spent in closed arm)	Liljelund et al. (2005)
$GABA_A$ beta3 subunit KO mice + diazepam 1.5 mg/kg first post-natal week			No effect	
$GABA_A$ beta3 subunit KO mice + diazepam 1.5 mg/kg second post-natal week			Anxiolytic in mutants but not in WT	
$GABA_A$ delta subunit KO mice	Elevated plus-maze	C57BL/6J × 129Sv/SvJ	No effect	Mihalek et al. (1999)
$GABA_A$ delta subunit KO mice + ganaxolone 10 mg/kg i.p.			No effect of ganaxolone in mutants	
$GABA_A$ delta subunit KO mice	Context fear conditioning		No effect	
	Cued fear conditioning		No effect	

$GABA_A$ delta subunit KO mice + THP 10 mg/kg	Elevated plus-maze	Mutants compared to C57BL6	No effect (comparisons probably not made with littermates)	Smith et al. (2006b)
$GABA_A$ delta subunit KO mice + THP 10 mg/kg	Elevated plus-maze after shock		Anxiolyic effect of THP lost in mutants (comparisons probably not made with littermates)	
$GABA_A$ delta subunit KO mice + THP 10 mg/kg + finasteride 50 mg/kg	Elevated plus-maze		Anxiogenic effect lost in mutants (comparisons probably not made with littermates)	
$GABA_A$ delta subunit KO mice + THP 10 mg/kg + finasteride 50 mg/kg	Elevated plus-maze after shock		No effect (comparisons probably not made with littermates)	
$GABA_A$ delta subunit KO mice + lorazepam 0.1 mg/kg			No effect (comparisons probably not made with littermates)	
$GABA_A$ delta subunit KO mice + THP 10 mg/kg + finasteride 50 mg/kg + lorazepam 0.1 mg/kg			No effect (comparisons probably not made with littermates)	
$GABA_A$ delta subunit KO mice + THIP 3 mg/kg	Elevated plus-maze		Anxiolytic effect of THIP lost (comparisons probably not made with littermates)	
$GABA_A$ delta subunit KO mice + THP 10 mg/kg + finasteride 50 mg/kg + THIP 3 mg/kg			Effect lost (comparisons probably not made with littermates)	
$GABA_A$ delta subunit KO mice + flumazenil 2 mg/kg	Elevated plus-maze after shock		No effect (comparisons probably not made with littermates)	
$GABA_A$ delta subunit KO mice + flumazenil 7 mg/kg			No effect (comparisons probably not made with littermates)	
$GABA_A$ delta subunit KO mice + THP 10 mg/kg + finasteride 50 mg/kg + flumazenil 2 mg/kg			Anxiogenic in WT, anxiolytic in mutants (comparisons probably not made with littermates)	
$GABA_A$ delta subunit KO mice + THP 10 mg/kg + finasteride 50 mg/kg + flumazenil 7 mg/kg			Amplitude of anxiolytic effect reduced (comparisons probably not made with littermates)	
$GABA_A$ gamma2L overexpression transgenic mice	Elevated plus-maze Light/dark Mirrored chamber Y maze Open-field	C57BL/6J × DBA/2	No effect of mutation	Wick et al. (2000)
$GABA_A$ gamma2L null allele KO mice	Elevated plus-maze	C57BL/6J × 129/Sv/SvJ	Anxiogenic	Homanics et al. (1999)
$GABA_A$ gamma2L null allele KO mice + ethanol 1.5 g/kg			No difference between genotypes	
$GABA_A$ gamma2 KO mice (heterozygote)	Free exploration Light/dark Elevated plus-maze	129/SvJ × C57BL/6J	Anxiogenic in mutants	Crestani et al. (1999)

Table 3 (*continued*)

Model	Test	Animal strain	Effects	Reference
$GABA_A$ gamma2 KO mice (heterozygote) + diazepam 0.3 mg/kg	Free exploration	129/SvJ × C57BL/6J	Anxiogenic in mutants, reversed by diazepam. Diazepam ineffective in WT	
	Light/dark		Anxiogenic in mutants, reversed by diazepam. Diazepam ineffective in WT	
	Elevated plus-maze		Anxiogenic in mutants, reversed by diazepam. Diazepam ineffective in WT	
$GABA_A$ gamma2 KO mice (heterozygote)	Contextual fear conditioning	129/SvJ × C57BL/6J	No effect	
	Delay fear conditioning		No effect	
	Trace fear conditioning		Freezing increased in mutants	
	Fear conditioning to an ambiguous stimulus		Freezing increased in mutants (interpreted as a cognitive bias for anxiogenic stimuli)	
$GABA_A$ gamma2 knock-down mice	Elevated plus-maze	C57BL/6J	Anxiogenic	Chandra et al. (2005)
	Open-field		Anxiogenic (less distance traveled)	
$GABA_{B1}$ subunit KO mice $GABA_{B2}$ subunit KO mice	Light/dark	BALB/c	Anxiogenic	Mombereau et al. (2005)
$GABA_{B1}$ KO mice + chlordiazepoxide (10 mg/kg, p.o.) $GABA_{B1}$ KO mice + diazepam (7.5 mg/kg, p.o.)	Light/dark	BALB/c	Anxiolytic effect of the benzodiazepine was lost	Mombereau et al. (2004b)
$GABA_{B1}$ subunit KO mice	Light/dark Staircase	BALB/c	Anxiogenic	Mombereau et al. (2004a)

Table 4. Effect of targeted mutation of endocannabinoid- neuropeptide-related genes on rodents in anxiety tests

Model	Test	Animal strain	Effects	Reference
Corticotropin-releasing factor (CRF) and Urocortin				
CRF overproduction transgenic mice	Elevated plus-maze	CRH-Tg^{+}	Animals showed a marked reduction in open arm activity compared with control animals	Stenzel-Poore et al. (1996)
	Open-field		Animals showed a marked reduction in locomotor activity compared with control animals	
CRF overproduction transgenic mice	Exploration tests	n/a	Anxiogenic	Koob and Gold (1997)
CRF overproduction transgenic mice	Acoustic startle reflex	Mice	Not different from wild-type animals	Dirks et al. (1999)
CRF inhibition transgenic mice	n/a	n/a	No behavioral differences were observed between mutant and wild-type mice	Miczek (1997)
CRF-binding protein KO mice	Elevated plus-maze	C57BL/6J × SJL and CD1 mice	Weak anxiolytic effects	Burrows et al. (1998)
CRF-binding protein KO mice	Elevated plus-maze Defensive-withdrawal	C57BL/6J-based mice (2–7 months)	Anxiogenic	Karolyi et al. (1999)
CRF-binding protein KO mice	Elevated plus-maze Open-field	n/a	Anxiogenic	Ramesh et al. (1998)
CRF1 KO mice	Light/dark test	129/Ola or CD1 mice	Anxiolytic	Timpl et al. (1998)
CRF1 KO mice + ethanol withdrawal				
CRF1 KO mice	Elevated plus-maze Light/dark test	C57BL/6J mice	Anxiolytic	Smith et al. (1998)
CRF1 KO mice	Elevated plus-maze Light/dark test	n/a	Anxiolytic	Contarino et al. (1998)
CRF1 KO mice	Exploratory behavior	n/a	Anxiolytic	Kresse et al. (1998)
CRF1 KO mice + ethanol withdrawal			Anxiogenesis-like activity of ethanol withdrawal was lost in KO mice	
CRF1 KO mice	Elevated plus-maze Light/dark test	C57BL/6J × 129 genetic background	Animals showed reduced anxiety-related responses	Contarino et al. (1999)
CRF1 KO mice	Elevated plus-maze	C57BL/6 background mice (about 50 day-old)	KO mice showed reduced anxiety-like behaviors compared to WT animals	Gammie and Stevenson (2006)
CRF1 conditional KO mice restricted to forebrain	Elevated plus-maze Light/dark test	129/Sv × C57BL/6J	Anxiolytic	Muller et al. (2003)
CRF KO mice	Multicompartment chamber Multicompartment chamber + restraint stress Elevated plus-maze Elevated plus-maze + restraint stress Startle reflex after air puff Conditioned fear stress	129SVJ/C57BL6-based mice	Not different from wild-type animals	Weninger et al. (1999)

Table 4 (*continued*)

Model	Test	Animal strain	Effects	Reference
CRF KO mice	Multicompartment chamber Elevated plus-maze	n/a	Not different from wild-type animals	Dunn and Swiergiel (1999)
CRF_2 receptor KO mice	Elevated plus-maze Light/dark emergence task Open-field	Male and female 129SVJ/C57BL6J-based mice	Mutant males (not females) mice showed increased anxiety-like behavior	Kishimoto et al. (2000)
CRF_2 receptor KO mice + α-hel CRF9–41 (1 μg)	Elevated plus-maze	129SVJ/C57BL6J-based mice	No blockade of anxiety-like behavior	
CRF_2 receptor KO mice	Elevated plus-maze Open-field	129SVJ/C57BL6J-based mice	No effect Weak increase in anxiety-related behavior (i.e., time in the center)	Coste et al. (2000)
CRF_2 receptor KO mice	Elevated plus-maze Light/dark test Open-field	Male and female 129SVJ/C57BL6J-based mice	Anxiogenic No effect Anxiogenic	Bale et al. (2000)
Urocortin KO mice	Acoustic startle reflex	Male and female 129S7/C57BL/6 (10–14-week-old)	Startle response was impaired: lower to loud sound and more sensitive to low sound levels	Wang et al. (2002)
	Open-field Elevated plus-maze Light/dark test		No effect	
Nociceptin/orphanin FQ				
Nociceptin-deficient KO mice	Acoustic startle	Male and female 129/Ola × C57BL/6J mice Male and female 129/Ola × C57BL/6J mice	No effect (animals were housed individually) (1) Mutant mice displayed increased anxiety-like behaviors; (2) they were housed in groups and females were submitted to restraint stress	Ouagazzal et al. (2003)
	Light/dark test	Male and female 129/Ola × C57BL/6J mice 129/Ola × C57BL/6J mice Female 129/Ola × C57BL/6 mice	No effect (animals were housed individually) (1) Mutant mice displayed increased anxiety-like behaviors; (2) they were housed in groups No effect (animals were housed in groups and subjected to restraint stress)	
Vasopressin				
V_{1a} reexpression in V_{1a} KO mice	Elevated plus-maze Open-field Light/dark test	C57BL/6J-129/SvJ background mice (2–5-month-old)	Animals treated with LacZ virus were not different from V_{1a} KO mice	Bielsky et al. (2005)
V_{1a} overexpression transgenic mice	Light/dark test	C57BL/6J-129/SvJ background mice (2–5-month-old)	Mice treated with NSE-V_{1a} viral vector showed increased anxiety-related behaviors	
V_{1a} KO mice	Open-field Light/dark test	Female C57BL/6J-129/SvJ background mice (2–5-month-old)	V_{1a} KO mice performed normally	

V_{1b} KO mice	Light/dark test Elevated plus-maze	C57BL/6J-129/SvJ background mice (3–7-month-old)	No phenotypic difference between WT and KO mice	Egashira et al. (2005)
Neurokinin (NK)				
NK_1 receptor disruption transgenic mice	Elevated plus-maze	J129/C57 hybrid mouse pups (20–30 g)	Anxiety-related behavior was decreased in –/–animals	Rupniak et al. (2001)
NK_1 receptor disruption transgenic mice	Elevated plus-maze Novelty-suppressed feeding Maternal separation-induced vocalizations	129/SvEv background mice (12–20-week-old) 129/SvEv background mice (8-day-old)	Anxiety-related behavior was decreased in –/–animals	Santarelli et al. (2001)
Neuropeptide Y (NPY)				
NPY overexpression transgenic rats	Elevated plus-maze Elevated plus-maze Vogel conflict test	Sprague–Dawley background rats (5-month-old; 325–375 g)	No effect Anxiolytic (animals were subjected to restraint stress prior to the test) Anxiolytic	Thorsell et al. (2000)
NPY overexpression transgenic rats	Elevated plus-maze Elevated plus-maze Open-field	Sprague–Dawley background rats (1-year-old)	Anxiolytic (animals were subjected to restraint stress prior to the test) No effect Transgenic rats appeared less anxious than their WT counterparts	Carvajal et al. (2004)
NPY KO mice	Elevated plus-maze Open-field Acoustic startle reflex	129/sv-C57BL6 background mice	KO mice displayed increased anxiety-like behavior	Bannon et al. (2000)
Y_1 KO mice	Open-field Elevated plus-maze Light/dark test	C57BL/6-129svJ background mice	Y_1−/− mice showed reduced anxiety-like behaviors when tested during the light phase or after restraint stress Y_1−/− mice showed reduced anxiety-like behaviors when tested after restraint stress Y_1−/− mice showed increased anxiety-like behaviors when tested during the light phase	Karl et al. (2006)
Y_2 KO mice	Elevated plus-maze Open-field Light/dark test	C57BL/6-129svJ background mice	Y_2−/− mice showed reduced anxiety-like behaviors	Tschenett et al. (2003)
Y_2 KO mice	Elevated plus-maze Open-field	C57BL/6-129svJ background mice (28–30 g)	Y_2−/− mice showed reduced anxiety-like behaviors	Redrobe et al. (2003)
Y_2 KO mice	Elevated plus-maze Open-field	C57BL/6-129svJ background mice (24-month-old)	Y_2−/− mice showed decreased anxiety-like behaviors	Carvajal et al. (2006)
Cannabinoid (CB)				
CB_1 KO mice	Mouse defense test battery	C57BL/6 × 129/Ola background mice (10-week-old)	KO mice displayed reduced defensive aggression responses	Griebel et al. (2005)
CB_1 KO mice	Light/dark test	CD1 background mice	KO mice displayed increased anxiety-like behavior	Martin et al. (2002)

Table 4 (*continued*)

Model	Test	Animal strain	Effects	Reference
CB_1 KO mice	Conditioned fear	C57BL/6NCrl background mice (6–14-week-old)	KO mice were impaired in within-session extinction and adaptation, but not in acquisition of conditioned and sensitized fear	Kamprath et al. (2006)
	Conditioned fear		CB1 deficiency impaired both within-session and long-term adaptation of sensitized fear	
CB_1 KO mice	Open-field Light/dark test	CD1 background mice (4-month-old, 28–30 g)	Mice exhibited a mild anxiety-like behavior	Maccarrone et al. (2002)
Melanin-concentrating hormone (MCH)				
MCH1 KO mice	Elevated plus-maze Stress-induced hyperthermia	129/SvJ × C57BL/6J background mice (25–35 g)	No phenotypic differences KO mice showed attenuated stress response compared to WT animals	Smith et al. (2006a)
MCH1 KO mice	Elevated plus-maze Open-field Stress-induced hyperthermia	129SvJ × C57BL/6J background mice (3–10-month-old)	KO mice showed attenuated anxiety-like behaviors compared to WT animals KO mice are protected against stress-induced hyperthermia	Roy et al. (2006)
	Social interaction		KO mice showed attenuated anxiety-like behaviors compared to WT animals	
Cholecystokinin (CCK)				
CCK1 receptor gene transgenic rats	Open-field	OLETF and LETO rats (4 weeks)	Rats lacking CCK1 receptors displayed reduced locomotor and rearing activities	Kobayashi et al. (1996)
CCK1 mutant rats (OLETF)	Elevated plus-maze Light/dark test	OLETF and LETO rats (7–9-week-old)	OLETF rats were more anxious than LETO rats	Yamamoto et al. (2000)
CCK1 mutant rats (OLETF)	Elevated plus-maze	OLETF and LETO rats (4-week-old)	Motor activity was reduced	Li et al. (2002)
CCK1 KO mice	Elevated plus-maze	C57BL/6J background mice (7-month-old)	No difference in anxiety between WT and KO mice	Miyasaka et al. (2002)
CCK2 KO mice			Anxious phenotype	
CCK1/2 KO mice			No difference in anxiety between WT and KO mice	
CCK2 KO mice	Elevated plus-maze	Female 129sv/C57BL6 background mice	Homozygotes showed increased anxiety	Vasar et al. (2000)
		Male 129sv/C57BL6 background mice	No difference in anxiety between genotypes	
CCK2 KO mice	Elevated plus-maze Open-field Motility conditioned suppression test	Male and female 129sv/C57BL/6 background mice	No difference in anxiety between genotypes	Dauge et al. (2001)
CCK2 KO mice	Elevated plus-maze Light/dark test	Male 129sv/C57BL6 background mice	Homozygotes showed decreased anxiety	Horinouchi et al. (2004)
CCK2 KO mice	Light/dark test	Female 129sv/C57BL6 background mice	Homozygotes showed decreased anxiety	Raud et al. (2005)
	Fear conditioned test		No difference in phenotype	

CCK2 KO mice + DMCM (0.25–1 mg/kg)	Light/dark test		DMCM produced anxiogenic-like effects in –/– but not in + / + mice	
CCK2 KO mice + diazepam (0.5–2 mg/kg)			No influence of mutation on the effects of diazepam	
CCK2 KO mice	Elevated plus-maze	Female 129sv/C57BL6 background mice	Homozygotes showed decreased anxiety	Areda et al. (2006)
	Elevated plus-maze		(1) Mice were exposed to a cat odor prior to testing; (2) no phenotypic differences	
	Cat odor		No phenotypic differences	
CCK2 overexpression (in forebrain) transgenic mice	Open-field Social interaction test Conditioned fear	IF-CCKR-2 mice (2–4-month-old)	Transgenic mice showed increased anxiety-related behaviors (1) Transgenic mice showed increased anxiety-related behaviors; (2) electric shocks of 0.4 mA were applied	Chen et al. (2006a)
CCK2 overexpression (in forebrain) transgenic mice + doxycycline	Open-field		The drug, which inhibits the transgene, abolished the anxious phenotype	
CCK2 overexpression (in forebrain) transgenic mice	Social interaction test			
CCK2 overexpression (in forebrain) transgenic mice + diazepam (0.5 mg/kg)	Open-field		The drug abolished the anxious phenotype	
	Conditioned fear		(1) The drug abolished the anxious phenotype; (2) electric shocks of 0.4 mA were applied	

anxiety-like response is triggered off by different kind of stressors such as suddenness (acoustic startle), forced confrontation with novel space (elevated plus-maze, open-field, light/dark choice test, novelty-induced suppression of feeding), or predator-related stimulus (mouse defense test battery, cat odor), association with a fearful stimulus after conditioning (Vogel test, fear conditioning). The observed response can be a reflex (startle), an avoidance response (cat odor), a freezing response (in the fear conditioning test), a preference for the protected regions of the device (the peripheral part of the open-field, the closed arms of the elevated plus-maze, the dark compartment of the light/dark test), or a decrease of consummatory behavior in the situation associated with stressful events (decrease of drinking when associated with an electric shock in the Vogel test, decrease of feeding in novel space in the novelty-induced suppression of feeding test). These responses are adaptative ones, as they enable the subject to cope with the situation when faced to a danger or a stressful situation. However, in humans suffering from some forms of pathological anxiety such as generalized anxiety, panic, phobia, obsessive-compulsive disorder, or post-traumatic stress disorder, the response toward stimulus from the external world can sometimes be maladapted: these people may display an anxious response in non-stressful situation (excessive trait anxiety in generalized anxiety disorder or excessive autonomous system activation of panic patients in non-stressful environments) or, when faced with some specific threat, they may display an excessive response (e.g., in post-traumatic stress disorder when the patients are faced with stimulus related to the trauma, in obsessive-compulsive disorders when the persons are faced with the stimulus inducing the compulsions, in phobic patients when faced the specific stimulus inducing the phobic response). In rodents, specific experimental situations have been described that may enable to model some of these forms of anxiety: for example, trait anxiety can be assessed using a free exploration situation (Griebel et al., 1993; Belzung and Berton, 1997; Belzung and Griebel, 2001), while the mouse defense test battery has been suggested to model some aspects of panic disorder (Griebel et al., 1995).

A first aspect that can be discussed is the apparent target-related variation in the device used. For example, 26% of the studies assessing the effects of mutations targeting the serotoninergic system used the open-field, while only 5% of the studies assessing GABAergic-related mutations used this situation. Such variation may have occurred by chance, or be related to availability of a given device in the laboratories doing such research. However, it is also possible that this means that some experimental situations are more adapted to reveal anxiolytic or anxiogenic effects of a given neurotransmitter system than others. For example, it can be mentioned that the open-field is more adapted to assess effects of genetic studies targeting the serotoninergic system. In this case, the different tests may not assess equivalent features of anxiety behavior, but measure some particular aspects of this behavior, related to a particular system.

Further, it is obvious that most experiments described in our tables (71%) used forced exploration (38% used elevated plus-maze, 18% used open-field, and 15% used light/dark test) to induce an anxiety-like state and to measure the resulting behavioral phenotype. Forced exploration is known to be stressful in these species (Misslin and Cigrang, 1986) so that these studies investigated an anxious phenotype related to a stressful situation, exacerbating or reducing a normal response. Thus, these studies did not investigate genes involved in the pathological forms of anxiety. Indeed, from our tables, it seems that very few studies used devices related to anxiety disorders: only three used free exploration (less then 1%) and only one used the mouse defense test battery. This is an important point to highlight here. Indeed, one may argue that targeted genetic alteration should induce a pathological phenotype, rather then a normal one. As suggested by Canguilhelm (1943), the factors underlying normal processes may not be the same as the ones involved in pathological processes. Therefore, in order to assess the genetic factors involved in the anxiety disorders, it would be necessary to study the effects of the genetic

alteration using situations modeling normal as well as pathological aspects of anxiety.

III. Which genetic method has been used?

Most studies (85%) involved genetic invalidation of a given gene. This means that the function of a given gene was studied in its absence. This method has been much criticized elsewhere (see e.g., Gerlai, 1996; Wolfer et al., 2002; Crusio, 2004). For example, it is possible that the behavioral alterations that were observed are related to compensation, rather than to the absence of the gene per se. Another point that should be mentioned here is that this strategy has poor significance for clinical research, as very few human behavioral alterations or psychiatric disorders are caused by the absence of a gene. In general, such disease or abnormalities are rather related to modifications of the functioning of a given gene, for example, when a polymorphism has been observed. Such polymorphism can then induce reduction of the expression of a particular gene product. Thus, one may suggest that the development of targeted insertion of a transgene (knock-in strategies) may enable to mimic some features of human polymorphism and would thus have higher isomorphic value and relevance for the study on genetic involvement in a particular anxiety disorder.

Another point to be mentioned here is that most (85%) of the animal models that have been proposed are based on targeting of receptors' genes (serotonin receptors, CRF receptors, $GABA_B$ receptors, cannabinoid receptors) or of subunits (e.g., $GABA_A$ receptors subunits) of receptors of specific neurotransmitters. The remaining studies concerned some transporters (e.g., the serotonin transporter), neuropeptides (e.g., nociceptine), or binding proteins of some neuropeptides (e.g., the CRF binding protein). Very few studies concerned genes involved in the synthesis of a given neurotransmitter. Parallels to the fact that most pharmacological agents used to alter anxiety behavior target specific receptors; which enable to act on specific brain areas (when a given receptor is located in particular brain regions) or on specific intracellular signaling cascades.

IV. Was there any particular choice of construction (knock-in, knock-out, and over-expressed models) made for each neurotransmission system?

It can be observed that particular choices of construction were made, depending on the neurotransmitter system that was targeted. Indeed, for the serotoninergic system, the main strategy used was the genetic invalidation: from Table 1, one can observe that 10 different models were used, 8 of which were knock-outs (of the $5HT_{1A}$, the $5HT_{1B}$, the $5\text{-}HT_{2A}$, the $5HT_{2C}$, the $5HT_3$, the $5HT_4$, and the $5HT_{5A}$ receptors and of the serotonin transporter), the two remaining ones using the over-expression construction (over-expression of the $5HT_{1A}$ and of the $5HT_{1B}$ receptors). On the contrary, in research on the $GABA_A$ receptors, many studies were made using the knock-in strategy. Among the 14 different models, 5 used the knock-in strategy (of various alpha subunits), 7 used the knock-out construction (for various alpha, beta, gamma, and delta subunit). The over-expression and the knock-down strategy were also used in the field of research on involvement of $GABA_A$ receptors in anxiety. In the field of glutamate research, all constructions were based on genetic invalidation. Finally, when targeting neuropeptidergic systems, the knock-out strategy was used in 15 cases, and over-expression in 4 cases. So, one can see that the knock-in and the knock-down strategies were exclusively used in the field of GABA research, while the other strategies (knock-out and over-expression constructions) are found throughout all neurotransmitter system research. This particularity is related to spectacular findings concerning the role of some point mutations in the properties of some specific alpha subunit of the $GABA_A$ pentamer regarding their ability to bind and to react to benzodiazepines. Such precise properties regarding the function of precise amino acids in the binding capacities of the receptors of other neurotransmitter systems have not been described, which may explain the lack of

such studies concerning serotonin, GABA, or neuropeptides.

V. Which phenotypes are observed?

It can be observed that 32% of the studies showed increased anxiety behavior while 25% showed the contrary. This indicates that the procedure used enables to observe both phenotypes, in an equi-probable manner. This applies more or less to each device. For example, in the elevated plus-maze or in the elevated zero-maze, an anxiogenic effect was seen in 35% of the studies, an anxiolytic effect in 35% of the studies while 30% of them did not detect any change. Further, in the light/dark test, 36% of the studies obtained anxiogenesis, 27% anxiolysis, and 36% found no effect; in the novelty-induced suppression of feeding half of the studies observed increased anxiety and the other half showed decreased anxiety. There are some exceptions: in the open-field, an anxiogenesis is observed more frequently (48%) than an anxiolysis (25%) while in the stress-induced hypethermia test, the contrary was observed: anxiolysis in 55% of the studies and anxiogenesis in only 11% of them. However, this does not necessarily mean that the open-field is more pertinent to detect anxiogenesis or that the stress-induced hyperthermia test would be more appropriate to detect anxiolysis; indeed, the number of studies is too small to bring to satisfactory conclusion. Further, a more detailed analysis would be necessary in order to check if, in each particular test, the experimental conditions used (lighting of the device, noise, strains used, etc.) enabled the assessment of anxiolytic as well as anxiogenic effects. Indeed, in some particular cases, this was not fulfilled as ceiling effects were present.

VI. Can these results be explained by the species or the strain used?

Most studies used mice (only 2.9% used rats). Among the mouse strains, very few different genetic backgrounds were used in the different studies. Indeed, 5.1% used outbred strains, 15.4% used C57BL/6 mice, 13.8% used 129 strains, 45.6% used an intercross between C57BL/6 and 129 mice while the remaining ones used an intercross between C57BL/6 or 129 and another strain. So, a large majority of the studies were undertaken in C57BL/6 or 129 genetic backgrounds. It is to be observed that these strains do not exhibit elevated anxiety in inter-strain comparisons, suggesting that they would be more adapted to assess anxiogenic effects. However, this has probably not influenced very much the results of the various studies as anxiogenic phenotypes have been observed as well as anxiolytic ones. One may also observe that most targeted invalidation or over-expression studies were done in only one genetic background. In studies phenotyping other aspects of behavior, such as aggression or learning and memory, it has been shown that some behavioral effects of the mutations were observed in one strain, and not in another strain. It would be useful to undertake also this type of study in research on genetic factors involved in anxiety behavior, as a large inter-strain variation has been documented. This could be very easily done; it may just need several backcrosses.

Another point that should be mentioned is that most studies (65.7%) used an F5-F8 generation intercross between 129 mice and mice from another strain (e.g., C57BL/6). One has to remember that in such a population, recombinant genotypes derived from the two parental mouse strains may be expressed so that that knock-out mice can be genetically different from their control littermates not only at the locus of interest but also at other loci (Gerlai, 1996). Further, in many case, the targeted mutation has been undertaken in embryonic stem (ES) cells from mice belonging to the 129 strain, so that the chromosome with the targeted locus will carry alleles of genes of 129-type. As the probability of genetic recombination is generally inversely related to the distance between the loci of the genes, the 129-type alleles of the genes whose loci are close to the locus of the mutated gene will remain associated with the mutated allele of the gene of interest (Gerlai, 1996). Consequently, the behavioral differences observed between mutants and their wild-type littermates of the hybrid genetic origin might be due to the targeted mutation as well as to the 129 background genes linked to the targeted locus,

inducing false-positive results. This point should be addressed in further studies.

VII. Did this strategy enable to precise the brain area involved in these processes?

Some genes may be pleiotropic, thus exerting multiple effects. This can, for example, be related to the fact that a gene can be expressed in different tissues of the body (e.g., heart, liver, brain) or in different brain areas. Many studies have shown that anxiety-behavior is mostly related to specific brain structures, particularly to limbic ones (amygdala, hippocampus, cortex). These regions are all located in the anterior forebrain. Therefore, when assessing the effect of targeted mutations on anxiety behavior, it would be useful to restrict the targeted mutation to the limbic areas. This can be done using the Cre/LoxP system for generating tissue-specific mutants in which Cre recombinase is expressed under the control of the forebrain specific CaMKII promoter, thus generating mice with forebrain-specific disruption of the gene of interest and Cre-negative littermate controls. This strategy has been used in anxiety research as several mutants of this type have been generated, including knock-out mice for the 5-HT_{2A} receptors, knock-out mice of the $GABA_A$ alpha1 subunit, and knock-out of the CRF_1 receptor. Results confirmed the impact of regions of the anterior forebrain in the effects of these genes and thus the involvement of these brain regions in anxiety-behavior.

VIII. Is the contribution of the genetic factor limited to the developmental period?

Some studies used agents inhibiting expression of the transgene at a given time point (e.g., doxycycline), thus enabling to distinguish the involvement of a gene on anxiety behavior during the developmental period from its impact at a precise time point once adults. This has been done in mice with deletion of the 5-HT_{1A} receptor, showing the crucial importance of this gene during the developmental period. It can be that different patterns of the development of limbic areas may modify in a durable way anxiety-related behaviors. Some genetic factors may thus be involved in the developmental pattern of limbic areas, modifying in an enduring way anxiety behavior and thus affecting trait anxiety. Other genes may be involved in the secretion or in the release of proteins expressed at a particular time point, for example, during stressful situations, thus affecting state anxiety. One may suggest that new pharmacological tools should act rather on proteins related to the second process because its injection in adults cannot modify developmental features.

IX. Do the effects of the mutation correlate with the results of pharmacological challenge?

Do the knock-out mice for a given gene display the same behavior as wild-type mice that have been treated with an antagonist or an inverse agonist of the receptor? Does the over-expression of a gene elicit the same phenotype as the injection of an agonist of the corresponding protein? A correlation is sometimes observed. For example, knock-out of the $\gamma 2$ subunit of the $GABA_A$ receptor elicits a 30% decrease of benzodiazepine receptors and an anxiogenic phenotype in the heterozygotous mice, an effect that is identical to the ones elicited by benzodiazepine receptor inverse agonists. Another example can be found with the CRF_1 receptor, as the CRF_1 receptor knock-out mice display anxiolysis, an effect which is identical to the one induced by CRF_1 receptor antagonists. However, counter-examples can also be found. For example, targeted invalidation of the 5-HT_{1A} receptor induces anxiogenesis while over-expression of the same receptor elicits anxiolysis; conversely, in most pharmacological experiments, 5-HT_{1A} receptor agonists such as buspirone or flesinoxan induce anxiolysis. In most cases, no specific ligands exist for the different target, so that it is not possible to check this point in an exhaustive way. The reasons for discrepancies between genetic and pharmacological data can be related either to the fact that a given target may be important in development (see previous paragraph) or to the fact that some pharmacological agents lack specificity, thus eliciting other effects that the ones related to the target.

X. Does the mutation modify the response to anxiolytic or anxiogenic agents?

In some studies, the effects of pharmacological agents were investigated. Sometimes, the pharmacological agent was a ligand of the protein whose expression was reduced or deleted (e.g., 5-HT_{1A} receptors ligands were studied in 5-HT_{1A} receptor knock-out mice) and sometimes this was not the case (e.g., when benzodiazepines were studied in 5-HT_{1A} receptor knock-out mice). This strategy was not only used in knock-out mice but also in knock-in mice of the GABAergic system (e.g., knock-in of the alpha1, alpha 2, or alpha3 subunits of the $GABA_A$ receptor). In this case, it enabled to assess the effects of single point mutations on the ability of a given compound to interact with the target protein. It is important here to distinguish the effects of a targeted mutation on anxiety from the effects of a given mutation on the effects of anxiolytic compounds. These two processes are not identical. Indeed, our tables show that in some case, a null mutation can alter the response of mice to anxiogenic situations (this is, e.g., the case with the 5-HT_{1A} null mutation) as well as their response to a pharmacological challenge, while in other case, the mutation has no action on anxiety behavior, but modifies the response of ligands of the receptor whose expression has been modified by the mutation.

XI. What do these findings tell us about the link between neurotransmitter systems and anxiety? Do these studies provide useful information about the role played by the various GABAergic, serotoninergic, glutamatergic, and neuropeptidergic targets in the anxiety behavior?

Table 1 summarizes data on serotonin neurotransmission. It is clear that alteration in genes coding for proteins that control serotonin reuptake or modulating 5-HT receptor-mediated signal transduction is involved in anxiety. The role of the serotonin system in establishing normal anxiety levels seems crucial during development, as ablation of the $5HT_{1A}$ gene during the developmental period induces permanent modifications of the anxiety level in the animals once adults, even if the gene has been ''turned on'' when the mice reached adult age. This indicates a role of this protein in developing the brain circuitry that is essential to display a normal reaction to threat. This is coherent with the observation that serotonin plays a role in development before it acts as a neurotransmitter. However, the role of serotonin is not limited to the developmental period. One can also observe that most serotonin receptors control the anxiety level. Indeed, ablation of most serotonin receptors ($5HT_{2A}$, $5HT_{2C}$, $5HT_3$, $5HT_4$, $5HT_{5A}$) induces anxiolysis, with the exception of the $5HT_{1A}$ receptor whose ablation is anxiogenic. The involvement of the $5HT_{2A}$ receptor seems related to a cortical site, as ablation of this receptor in this precise region is sufficient to induce the phenotype. This receptor may thus control the cortical-related process associated with the anxiety response. Further, the fact that ablation of a given receptor induces either anxiolysis or anxiogenesis is not related to the intracellular events that are associated with the receptor: indeed, deletion of the $5HT_{1A}$ receptor, which is negatively coupled to adenylate cyclase, induces anxiogenesis, while ablation of the $5HT_4$ receptor that is positively coupled to adenylate cyclase and of the $5HT_{5A}$ receptor that is negatively coupled to adenytate cyclase both elicit anxiolysis. Unfortunately, few of these studies investigated the level of serotonin in the mutant mice. One can mention the study of Parsons et al. (2001) that showed, using microdialysis, that $5HT_{1A}$ knock-out mice displayed an increased level of serotonin in the hippocampus and the cortex. This suggests that high serotonin level might be associated with elevated anxiety. However, a systematic study of the serotonin level of the different mutant would enable to propose more strong conclusions about the relationship between serotonin and anxiety.

Glutamate is the most widespread excitatory neurotransmitter in brain. It binds to two classes of receptors: the ionotropic and the metabotropic one. Among the ionotropic receptors, three subtypes have been described: the AMPA, the NMDA, and the kainate receptors. Different functional subunits assemble together in heteromultimeric complexes to form these receptors: one

can mention the NR1, NR2A-NR2D, and NR3 for the NMDA receptors. On the other side, the metabotropic glutamate receptors can be subdivided in three groups: group I (mGluR1, mGluR5), group II (mGluR2, mGluR3), and group III (mGluR4, mGluR6, mGluR7, and mGluR8). Table 2 presents the phenotype of different glutamate receptor knock-out mice. The genetic invalidation studies that have been undertaken to alter the glutamatergic neurotransmission targeted the NR2A subunit, the mGluR5, the mGluR6, the mGluR7, and the mGluR8 receptors. These mutations, therefore, altered either the NMDA receptors, or the group I or III metabotropic receptors. All mutations induced anxiolysis, except the mGluR8 invalidation that rather induced anxiogenesis. Again, no relationship can be made with intracellular cascades as the mGluR6, the mGluR7, and the mGluR8 receptors are all negatively coupled with adenylate cyclase. Few data are available on the level of glutamate in these mutants, so that it is not really possible to associate the anxiolytic profile of these mutants with glutamatergic activity.

The data on targeted genetic manipulation on GABAergic targets confirms the involvement of this neurotransmitter in anxiety (Table 3). This is not surprising as GABA is the most abundant inhibitory neurotransmitter in the brain. It binds to two classes of receptors: the $GABA_A$ receptor, which is composed of five subunits, and the $GABA_B$ receptor, that includes two subcategories, the $GABA_{B1}$ and the $GABA_{B2}$ receptors. The subunit composition of $GABA_A$ receptors determines the receptor pharmacology and the density of benzodiazepine receptors. When the number of these benzodiazepine sites is decreased, an anxious phenotype appears. For example, the gamma2 null mutant heterozygotous mice, which display a reduction of approximately 30% of the number of benzodiazepine receptors, display increased anxiety. Generally, the number of benzodiazepine receptors is not modified in the various alpha subunit knock-in mice, so that no anxious phenotype appears in these mice. However, they display modifications in their response to anxiolytic agents. As to the $GABA_B$ receptor, genetic invalidation of the $GABA_{B1}$ as well as of the $GABA_{B2}$ receptor induce anxiogenic effects.

Finally, the effects of mutations involving neuropeptidergic or endocannabinoid targets confirm the implication of these molecules in anxiety behavior (Table 4). Logically, stress peptides such as CRF are involved in this behavior: invalidation of the CRF_2 receptor is anxiogenic while inhibition of the CRF_1 receptor is anxiolytic; this last effect being mediated by the forebrain. Surprisingly, inhibition as well as over-expression of CRF does not elicit main effects on anxiety. Another stress-related peptide, vasopressine, is also involved in anxiety as over-expression of the V_{1B} receptor is anxiogenic. However, a symmetrical effect is not found when invalidating this receptor: no phenotype is seen in the V_{1B} receptor knock-outs. Genetic invalidation studies also focuses on the involvement of cannabinoid targets and showed that invalidation of the CB_1 receptors elicits an anxiogenic effect.

XII. Conclusion and perspectives

In summary, these studies precise the involvement of neurotransmitter genes expressed in normal anxiety. These genes can act either by modifying brain development or by interfering with neurotransmission at a particular moment.

Did this research on targeted genes involved in anxiety really enable to improve treatments of anxiety disorders? To our knowledge, this is unfortunately not really the case. For example, much research focused on the involvement of genes coding for subunits of $GABA_A$ receptors, but no pharmacological treatments binding on specific subunits are available. The same is true for serotoninergic targets. The probability that research on targeted mutations will enable the discovery of new pharmacological tools in the future is also very low because most genes involved in anxiety behavior are in fact non-neurotransmitter genes such as those involved in brain development (e.g., BDNF): such a developmental pattern elicited by particular genes cannot be counteracted by pharmacological treatments applied to the subject once adult. Further, many

epigenetic factors have been involved in anxiety, including maternal care (Calatayud and Belzung, 2001; Meaney, 2001 Calatayud et al., 2004). These factors modify the expression of particular genes such as glucocorticoid receptors via different mechanisms including DNA methylation (Meaney and Szyf, 2005; Weaver et al., 2005, 2006). Therefore, some anxiety-related phenotypes may be related to DNA methylation defects inducing decreased transcription of a given gene, rather than to the absence of a gene. Such mechanisms are completely ignored in targeted gene research and should be considered when aiming at discovering new treatments of anxiety.

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