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What's in a brain: neuroanatomy and neurochemistry of anxiety disorders in dogs

Angststoornissen bij honden: neuroanatomische en neurochemische circuits

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ABSTRACT

This review deals with the neurocircuitry of fear and anxiety disorders, with the focus on neuroanatomy and neurochemistry. This knowledge is required to correctly diagnose and treat dogs with anxiety-related behavioral disorders.

Research to date has shown the involvement of the frontal cortex, the amygdala, the thalamus and the hippocampus as core regions in regulating fear. Imbalances (hyper- or hypoactivation) in this fear circuitry can trigger inappropriate fear responses, i.e. anxiety disorders.

Serotonin, dopamine and norepinephrine are the main neurotransmitters of emotion in the brain, but gamma-aminobutyric acid (GABA), glutamate, and the hypothalamic-pituitary-adrenal (HPA) axis producing glucocorticoids are also important in the neurochemistry of anxiety.

SAMENVATTING

De diagnose en behandeling van honden met angststoornissen zijn slechts mogelijk indien het onderliggende en gesofisticeerde angstmechanisme ter hoogte van de hersenen in acht wordt genomen.

Neuroanatomische bevindingen tonen de betrokkenheid van de frontale cortex, de amygdala, de thalamus en de hippocampus aan in de regulatie van angst. Aberraties ter hoogte van dit angstnetwerk kunnen angststoornissen veroorzaken.

Naast neuroanatomische bevindingen spelen ook verschillende neurotransmittersystemen een belangrijke rol. Hierbij staan vooral serotonine, dopamine en norepinefrine centraal, naast gamma-aminobutyraat, glutamaat en de glucocorticoïd producerende hypothalamische-hypofysaire-bijnieras.

INTRODUCTION

In the past decades, dogs have been developing an increasingly unique relationship with humans by living more closely to them than ever before (Houpt *et al.*, 1996). This unique human-canine relationship, however, can be endangered by canine behavioral problems. Dogs showing aggressive behavior (towards humans or other dogs), anxiety (social, non-social, or separation), house soiling and destructive behavior have a high risk of being abandoned, surrendered to animal shelters, or even euthanized by their owners (Patronek *et al.*, 1996 ; Houpt *et al.*, 1996 ; Salman *et al.*, 1998 ; Salman *et al.*, 2000). Anxious behavior leads to low quality of life, and anxiety-based aggressive behavior can force this welfare issue to expand and involve a safety issue as well. In view of these facts and the unique position of dogs in our households, the importance of increasing research in canine behavioral problems cannot be emphasized enough.

Fear can be defined as a normal protective response to a threatening situation. According to Ennaceur, fear "*is an emotional reaction which can be induced by exposure to novelty and can be expressed through escape, avoidance or anxiety responses*" (Ennaceur *et al.*, 2006). In contrast, anxiety is an organism's preparatory response to contexts in which a threat may occur (Cisler, 2009). Hence, the two terms are highly interrelated and anxiety should be interpreted as a fearful state accompanied by a certain amount of worry or uncertainty.

From the moment that fearful behaviors (i) are triggered by harmless stimuli or (ii) arise at a certain intensity or frequency that is greater than what would be expected in a specific situation or (iii) affect the dog's safety, quality of life, or relationship with his or her ow-

ners, it can be said that an anxiety disorder is occurring (LeDoux, 1998; Case, 2005).

To investigate anxiety disorders, it is imperative to fully understand the neurocircuitry of fear and anxiety. Accordingly, the aims of this article are to describe the neuro-anatomical pathways underlying fear, and to discuss the available information linking anxiety and the neuro-chemical systems in the brain.

Neuroanatomy of anxious responses

When sensory stimuli are registered in the brain, they first enter the sensory thalamus, which acts as a central relay station before transferring the peripheral stimuli to the amygdala and the prefrontal cortex (Ettinger *et al.*, 2007). Two pathways, the so-called low road and high road, leave the thalamus. The low road goes directly to the lateral nucleus of the amygdala, without passing by the prefrontal cortex, whereas the high road goes through the prefrontal cortex before reaching the lateral nucleus of the amygdala. Projections go further to the central nucleus of the amygdala, from which different target regions are reached, such as the periquaductal gray, the locus coeruleus, the hypothalamus, the parabrachial nucleus and multiple cortical areas (Armony and LeDoux, 1997; Walker *et al.*, 2003). Finally, each target region mediates specific signs of fear or anxiety, as depicted in Figures 1 and 2.

Differences between the low and the high road are found on multiple levels. The low road reacts more rapidly and will reach the amygdala first, resulting in a primitive and unthinking fear response. By contrast, the high road passes through the prefrontal cortex, which is the most evolved and most recently developed area of the brain. The prefrontal cortex is capable of performing a detailed analysis of the stimuli, thus enabling the individual human or animal to restrain the primitive amygdala and reach a more appropriate (i.e. in proportion to the stimuli) and thinking response to the sensory stimuli. It is important to understand that the low road response will have an inhibitory effect on the prefrontal cortex, thus preventing an initial thinking response, whereas the high road passing through the prefrontal cortex will afterwards inhibit the amygdala, thus allowing the high road response to mediate the limbic response. Both responses are critical for survival in the context of a threat. The unthinking response happens instantly and prepares the organism to act without thinking. In other words, it quickly inhibits thinking in favor of instant reaction. The thinking response, on the other hand, can analyze a situation and its variables, compare it to previous situations, and then take appropriate action. This thinking response is slower than the unthinking response; its advantage, however, is that it is subject to learning processes by means of which it can inhibit unthinking responses originating from the amygdala, thus leading to appropriate socialization (Armony and LeDoux, 1997).

In support of this view, the results from many human and animal model studies have associated the amygdala with emotional memory, appetitive or attentional processes, and fear and fear-learning, as well as identifying it as a key brain region in anxiety disorders (LeDoux, 1998; Davis, 1998; Uys *et al.*, 2003; Walker *et al.*, 2003; Kent and Rauch, 2003; Keele, 2005; Bartz and Hollander, 2006; Etkin and Wager, 2007). For instance, amygdala lesions have been associated with the impairment of emotional processing, including dysfunctional fear learning in which stimulation of the amygdala has resulted in subjective feelings of fear not

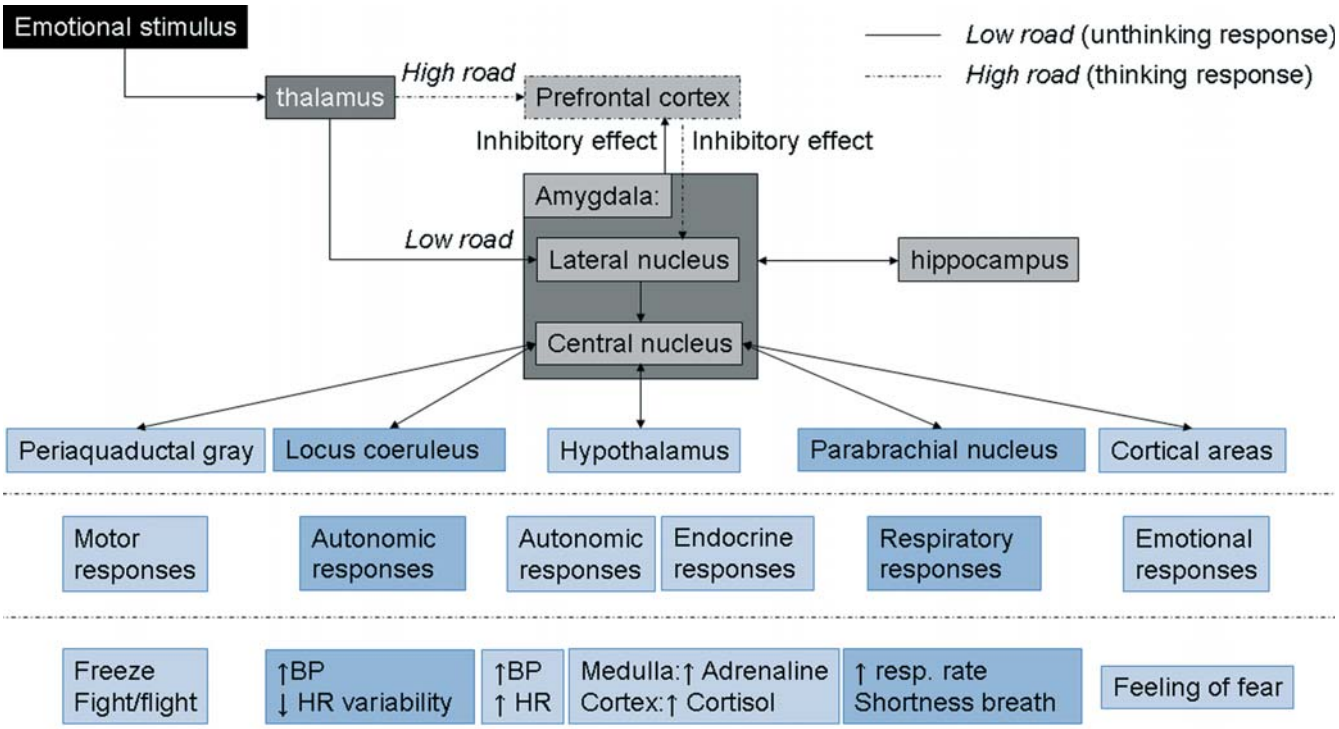

Figure 1. Neuro-anatomical path from the incoming emotional stimulus to the behavioral signs expressed.

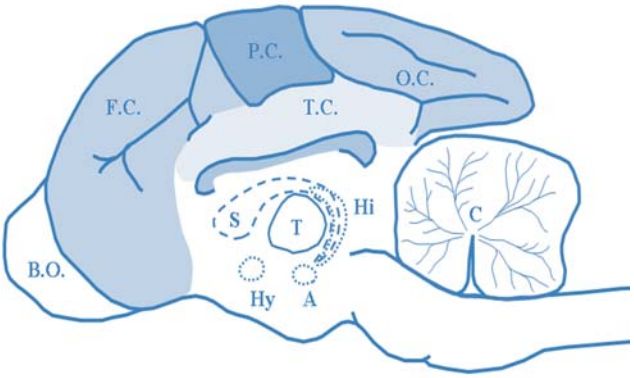

Figure 2. The canine brain with the main regions involved in anxiety. (B.O.: bulbus olfactorius; F.C.: frontal cortex; P.C.: parietal cortex; T.C.: temporal cortex; O.C.: occipital cortex; C: cerebellum; S: striatum; T: thalamus; Hi: hippocampus; A: amygdala; Hy: hypothalamus)

based in objective reality (LeDoux, 2000; Keele, 2005). Objective and subjective are used to emphasize.

Amygdala hypersensitivity is not the only factor that can cause anxiety. The lack of ventral medial prefrontal cortex (vmPFC) control via projections to the amygdala can also be responsible for anxiety disorders (Kent and Rauch, 2003). Moreover, lesions of the dorsal medial prefrontal cortex in rats are correlated with an exaggerated freezing response to a conditioned stimulus, a fact which underscores the role of the medial prefrontal cortex in fear reduction (Morgan *et al.*, 1993). The normal prefrontal cortex will inhibit impulsive and primary emotions triggered by the amygdala, but the presence of lesions on the prefrontal cortex will lead to the absence of inhibition (Bufkin and Luttrell, 2005).

Finally, the hippocampus, which is the centerpiece of the limbic system and plays a critical role in learning and memory, is also involved in this "fear network" of the brain and plays an important role in contextual fear conditioning (Gorman *et al.*, 2000; Bremner, 2004). Especially the ventral hippocampus plays a role in anxiety-related behaviors, providing information about the context of potentially threatening stimuli, whereas the dorsal hippocampus may play a role in different forms of memory and spatial learning (Bannerman *et al.*, 2003). Rats with lesions on the ventral half of the hippocampus show reduced levels of anxiety (reduced levels of freezing) in different studies (Kjelstrup *et al.*, 2002; Bannerman *et al.*, 2003; Bannerman *et al.*, 2004).

As Walker, Toufexis and Davis (2003) explain, the central nucleus of the amygdala is the main output system of the amygdala. It projects to the different anatomical targets (e.g. hypothalamus, brainstem nuclei), which mediate the different (sympathetic) signs and symptoms of fear, anxiety or stress responses. Therefore, lesions at the level of this central nucleus will stop the expression of all responses (LeDoux, 1995). Lesions at a lower level, for instance at the parabrachial nucleus, will only cause disruption of the respiratory responses.

Neurochemistry of anxious responses

Neurotransmitters are endogenous chemical messengers that orchestrate neuronal signal transduction. This communication between two neurons occurs at the level of the connection space between the two, i.e. at the synapse or synaptic cleft. The synapse represents a gap between a pre-synaptic and a post-synaptic neuron. When a neuron is stimulated, an electrical impulse is sent from the top of the neuron to the pre-synaptic axon terminal. Subsequently, the electrical impulse, being unable to cross the gap to the post-synaptic neuron, releases (by opening calcium channels) the chemical messenger, i.e. the neurotransmitter, in the synapse. The neurotransmitter binds to specific receptors of the post-synaptic neuron and allows the message to continue to the next neuron. Through the action of the neurotransmitter, which can be relatively straightforward (e.g. opening an ion channel) or more sophisticated (e.g. activation of a gene), behavior can be influenced (Figures 3 and 4). Disturbed actions will result in aberrant behavioral patterns. The neurotransmission is ended by the reuptake of the neurotransmitter into the pre-synaptic neuron, an action which is performed by specific transporters (e.g. serotonin transporters).

Based on their molecular structure, three major categories of neurotransmitters exist: (1) **monoamines** (serotonin, dopamine, norepinephrine, adrenaline, acetylcholine, histamine, …), (2) **amino acids** (primarily glutamate, gamma-aminobutyric acid (GABA), aspartate and glycine) and (3) **peptides** (vasopressin, somatostatin, neurotensin, ...). But also hormones, puri-

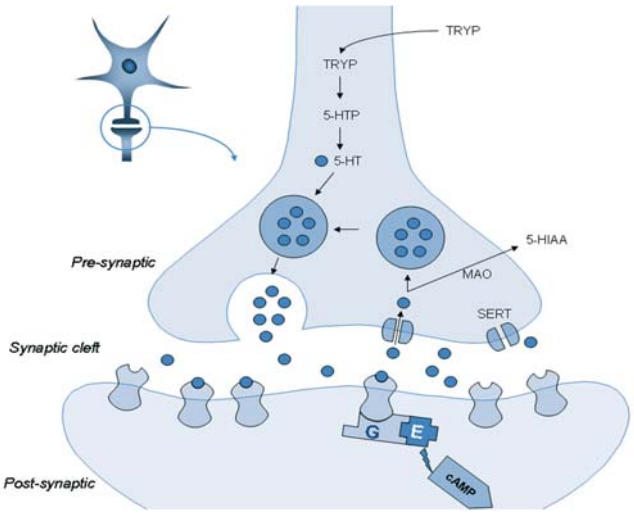

Figure 3. Schematic representation of the serotonergic neurotransmission. Upon the occurrence of electrical impulses and the influx of Ca^{++}, storage vesicles filled with 5-HT release their content into the synapse, thus allowing the neurotransmitter to bind to specific receptors. This initiates a cascade of chemical messengers depicted in Figure 4. Serotonergic neurotransmission is ended by re-uptake of 5-HT by serotonin transporters (SERTs) into the pre-synaptic neuron, where 5-HT is either degraded to 5-HIAA by monoamine oxidase (MAO) or else is re-stored in storage vesicles. Tryptophan (TRYP) and 5-hydroxytryptophan (5-HTP) are precursors of 5-HT.

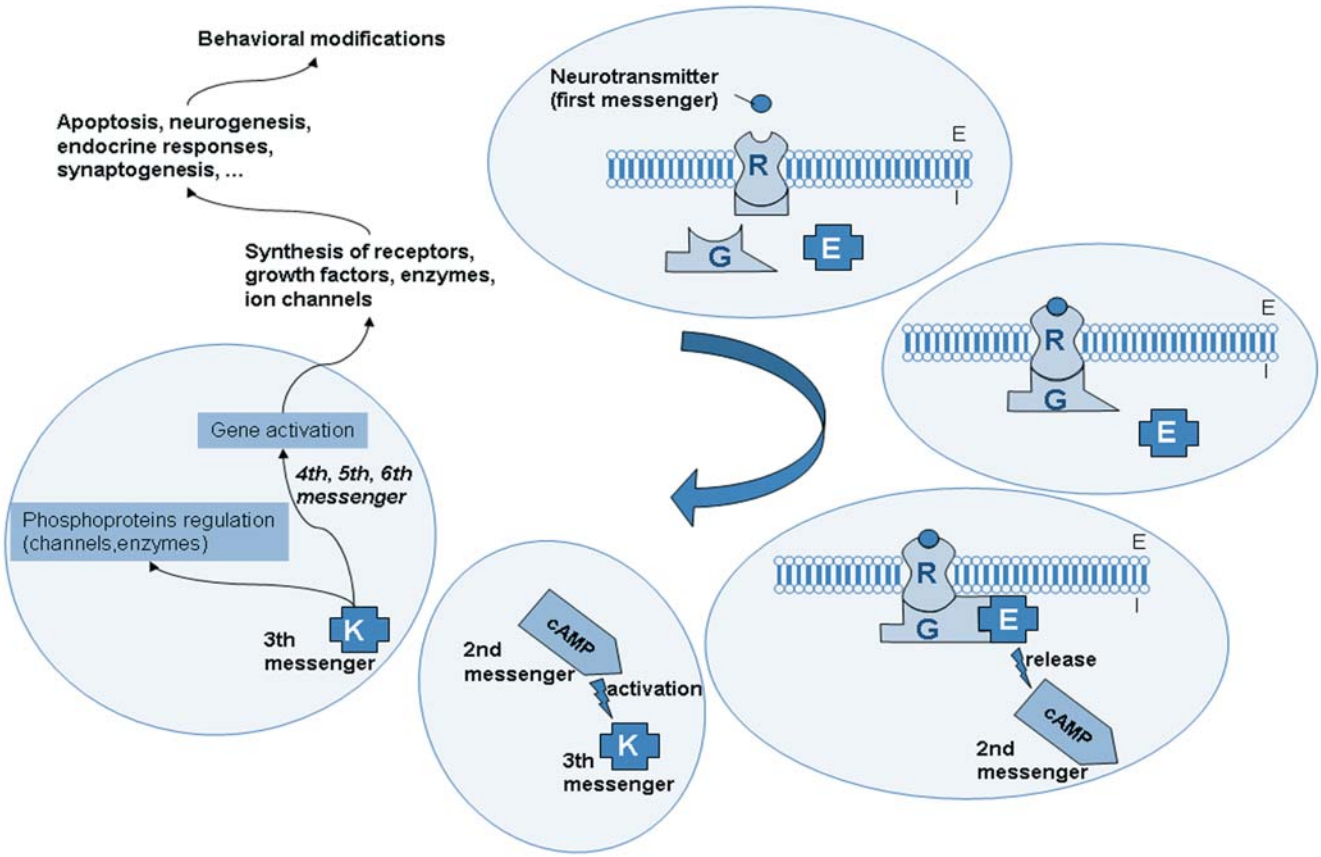

Figure 4. Signal transduction cascade. Example of the signal transduction cascade for G-protein linked neurotransmitters such as serotonin, dopamine, norepinephrine and glutamate. R: receptor; G: G-protein; E: enzyme; cAMP: cyclic adenosine monophosphate; K: protein kinase.
The cascade is activated by the binding of a neurotransmitter, the first messenger, to a unique Receptor. Once bound, the Receptor transforms its conformation so that the receptor can bind to the G-protein. Upon binding of the Receptor and the 'G-protein', the conformation of the G-protein changes, thus allowing the Enzyme to bind. It is the Enzyme that synthesizes and releases the second messenger. Subsequent messengers target two major groups: phospoproteins and genes. Modification of these two groups leads to biological responses such as synaptogenenis, neurogenesis, and apoptosis, but can also possibly lead to learning, memory, endocrine or antidepressant responses, and even to the production of chronic pain and anxiety disorders.

nes and even gases such as nitric oxide (NO) and carbon monoxide (CO) can function as brain neurotransmitters.

Serotonin, dopamine, norepinephrine, GABA, the hypothalamic-pituitary-adrenal axis producing glucocorticoids, and glutamate will be examined successively within the framework of anxiety.

Serotonin and Anxiety

Serotonin (5-hydroxytryptamine; 5-HT) is a key neurotransmitter in the brain with multiple behavioral implications. Serotonin has been linked with psychiatric symptoms such as obsessionality (Murphy and Pigott, 1990), depression (Gorman and Kent, 1999 ; Lira *et al.*, 2003), eating disorders (Audenaert *et al.*, 2003; Jahng *et al.*, 2007) and impulsivity behaviors (Fairbanks *et al.*, 2001; Bjork *et al.*, 2002; Pattij and Vanderschuren, 2008), but on the basis of early research it is also assumed that serotonin plays a central role in fear and anxiety (Murphy and Pigott, 1990; Handley and McBlane, 1993; Stein and Stahl, 2000). This central role in behavior is not surprising since the serotonergic neurons, which mostly originate in the raphe nuclei (RN), extensively innervate the (prefrontal) cortex as well as the hippocampus, thalamus and amygdala, limbic regions long associated with emotional behavior (Hensler, 2006).

A first line of evidence indicating a serotonergic involvement in anxiety is based on peripheral measurements of the main serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), in which increased cerebrospinal fluid or plasma levels of 5-HIAA were observed in patients with panic disorder (Sullivan *et al.*, 2006; Esler *et al.*, 2007).

Other evidence is provided by experimental studies focusing on the serotonin transporter (SERT). For instance, a recent study by Lee et al. describes depression- and/or anxiety-like behaviors in rats with decreased SERT expression after neonatal maternal separation (Lee *et al.*, 2007). Moreover, the complete lack of SERT (in serotonin transporter knock-out mice) led to increased anxiety- and depression-like behaviors (Lira *et al.*, 2003; Wellman *et al.*, 2007) in several behavioral tests, whereas over-expression of the SERT decreased these behaviors (Hariri and Holmes, 2006;

Jennings *et al.*, 2006), thus suggesting a negative correlation between anxiety- and depression-like behavior and the expression of serotonin reuptake transporters. It is important to note that in addition to the absence of SERT in SERT knock-out mice, abnormal neuronal morphology was noted both in the amygdala and in the prefrontal cortex (Wellman *et al.*, 2007) regions of the fear neurocircuitry.

Beside SERT influences on fear and anxiety, evidence for the involvement of serotonin receptors in anxiety is abundant. Direct evidence is provided by the anxiolytic effect of 5-HT2A receptor agonists and the anxiogenic effect of 5-HT2A receptor antagonists (Nic Dhonnchadha *et al.*, 2003). Moreover, genetic studies observed single nucleotide polymorphisms (SNPs) of the 5-HT2A receptor gene in human patients with panic disorder (Inada *et al.*, 2003; Unschuld *et al.*, 2007), and neuroimaging studies on human and canine anxiety disorders revealed disturbed 5-HT2A receptor densities (Frokjaer *et al.*, 2008; Perani *et al.*, 2008; Vermeire *et al.*, 2009). Other receptors, such as the 5-HT1A receptor, also play a role in anxiety as the co-administration of a selective serotonin reuptake inhibitor (SSRI) and a 5-HT1A receptor antagonist reduces the delay of therapeutic onset known in sole SSRI treatment (Watson and Dawson, 2007).

Selective serotonin reuptake inhibitors have proved active in anxiety disorders and are the recommended first-line medication for these disorders (Stein *et al.*, 2000; Fernandez *et al.*, 2001; Bandelow *et al.*, 2002; Davidson *et al.*, 2004; Westenberg and Liebowitz, 2004; Lee *et al.*, 2005). SSRIs enhance the serotonergic transmission by a pharmacological inhibition of the serotonin reuptake (via SERTs) back into the presynaptic neuron, causing an increased amount of serotonin to be available in the synaptic cleft to bind to pre- and postsynaptic receptors (Gorman *et al.*, 2000). It must be noted, however, that this SERT blocking action to relieve anxiety is in contradiction with the previously described negative correlation between SERT expression and anxiety. Other drugs that increase the synaptic availability of serotonin, such as monoamine oxidase (MAO) inhibitors, are also effective in anxiety disorders (Bandelow *et al.*, 2002; Lee *et al.*, 2005; Maron and Shlik, 2006). It is important to keep in mind that because all monoamine neurotransmitters (5-HT, DA and NE) are destroyed by MAO, MAO inhibitors are also effective in increasing the synaptic availability of dopamine and norepinephrine.

Anxiety and the role of other neurotransmitters

The classic hypothesis of the pivotal role of serotonin in the modulation of anxiety has long been formulated with the idea that serotonin promotes anxiety, while the suppression of serotonin decreases anxiety (Iversen, 1984). However, the conflicting findings from different studies have resulted in the questioning of this hypothesis and the suggestion that multiple neurotransmitter systems and more complex anxiety mechanisms are involved (Handley and McBlane, 1993).

Preclinical and clinical studies support the hypothesis that, besides serotonin, also dopamine plays an important role in obsessive-compulsive disorder (OCD). OCD is a chronic human illness characterized by the presence of recurrent, persistent and unwanted thoughts (obsessions) resulting in distress and anxiety. In order to reduce this anxiety, patients are driven to perform repetitive, ritualistic acts (compulsions). Due to the component of anxiety, OCD is classically categorized as an anxiety disorder (Goodman *et al.*, 1990; Harvey *et al.*, 2002; Bartz and Hollander, 2006; Korff *et al.*, 2007). Patients refractory to the first-line agent SSRI are often treated with additional dopamine blockers (Zohar and Westenberg, 2000; Uys *et al.*, 2003). Furthermore, different pharmacological studies using rat models have demonstrated that the use of dopamine agonists such as apomorphine or (meth)amphetamine is anxiogenic and induces stereotypic behaviors that reflect some OCD symptoms (Szechtman *et al.*, 1998; Vasilev *et al.*, 2003; Hall *et al.*, 2008). Conversely, dopamine D_1 and D_2 antagonists were able to reduce stereotyping behavior in deer mice and bank voles (Kennes *et al.*, 1988; Presti *et al.*, 2003). Recent studies have also provided proof of the direct involvement of the dopaminergic system in the pathophysiology of OCD, showing that OCD patients have lower striatal dopamine D_1 and D_2 binding ratios compared to control subjects (Denys *et al.*, 2004; Olver *et al.*, 2008). Other OCD neuroimaging studies focusing on the dopamine transporter (DAT) have provided evidence for altered DAT densities in OCD, but have failed to show conclusive results with both higher and lower DAT ratios being reported (van der Wee *et al.*, 2004; Hesse *et al.*, 2005).

The dopaminergic system is not only involved in obsessive-compulsive behavior. Some studies also sug-

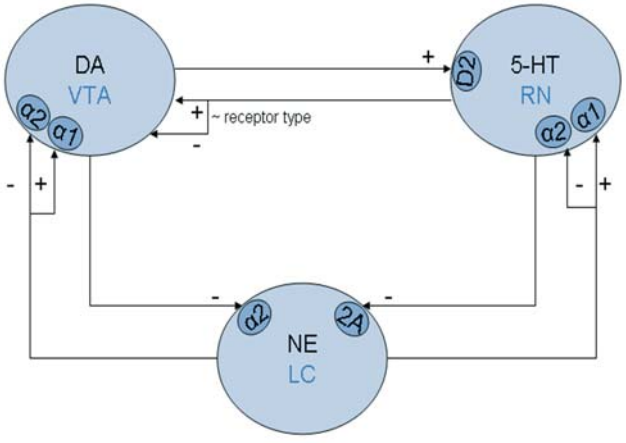

Figure 5. Interplay between the monoamine neurotransmitters Dopamine (DA), Serotonin (5-HT) and Norepinephrine (NE). VTA: ventral tegmental area; RN: raphe nuclei; LC: locus coeruleus. Note that this is a simplified representation and that other interactions (e.g. with glutamate and GABA) and autoregulation via autoreceptors are not shown. The adrenergic receptors α_1 and α_2, the dopamine receptor D_2 and the serotonin A_2 receptor are included in the scheme.

gest the involvement of the dopaminergic system in patients with social anxiety disorder (SAD), in whom lower dopaminergic and D_2 receptor binding potentials (Schneier *et al.*, 2000) and decreased DAT densities (Tiihonen *et al.*, 1997) have been reported. Another piece of evidence for the involvement of the dopaminergic system in SAD is the association between SAD and Parkinson's disease (PD). Studies have shown that patients with PD, a disease which involves disturbed dopamine function, are more likely to develop SAD, thus suggesting that hypodopaminergic function is a central factor not only in PD, but also in SAD (Richard *et al.*, 1996; Muller *et al.*, 2005). Further evidence for the involvement of dopamine in anxiety is provided by the fact that D_1 and D_2 receptor antagonists were able to reduce anxiety-like behavior induced by nicotine injection in rats and that the administration of D1/D2 receptor agonist apomorphine resulted in anxiogenic behavior (Zarrindast *et al.*, 2010). Five major dopaminergic pathways exist, originating from the ventral tegmental area (VTA) and the substantia nigra (SN), and terminating in the striatum, the limbic areas and the cortical areas.

Another neurotransmitter system that has been a focus of interest in anxiety disorders is the norepinephrine (NE) system. The noradrenergic neurons ascend from the locus coeruleus (LC) and innervate the cerebral cortex and limbic regions such as hippocampus, thalamus, hypothalamus and amygdala (Neumeister *et al.*, 2005), thus suggesting the involvement of NE in the regulation of mood and emotion. Moreover, abnormal norepinephrine metabolite levels in plasma and urine, as well as elevated plasma and CSF norepinephrine levels have been noted in human anxiety disorders (Wyatt *et al.*, 1971; Sevy *et al.*, 1989). Increased expression of α_2- and β-adrenergic receptors has been reported in depressive patients and suicide victims, which are disorders with high anxiety comorbidity (Mann *et al.*, 1986; Meana *et al.*, 1992), and α_2-antagonists such as yohimbine have been found to potentiate the action of the SSRI fluoxetine (Sanacora *et al.*, 2004). Furthermore, a number of pharmacological studies have demonstrated an improvement in anxiety symptoms after central NE depletion or lesions in rats (Lapiz *et al.*, 2001; Sziray *et al.*, 2010). Overall, increased norepinephrine activity is suspected in anxiety disorders.

Gamma-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the brain, and there is increasing evidence suggesting that a dysfunctional GABAergic system is involved in anxiety disorders (Domschke and Zwanzger, 2008). Blocking GABA receptors with antagonist leads to severe anxiety in humans and in animals, whereas increasing the GABA activity with agonist reduces fear and anxiety (Malizia *et al.*, 1998; Cameron *et al.*, 2007; Zarrindast *et al.*, 2008). Clinical results suggest a relative deficiency in GABA neurotransmission in anxiety disorders (Nemeroff, 2003). Agents like the benzodiazepines increase the affinity of the $GABA_A$ receptor for GABA by inducing allosteric changes in the GABA binding site; another agent, tiagabine, influences the GABA system by increasing the synaptic GABA availability. Both agents modulate GABA transmission and are used in the management of anxiety disorders like general anxiety disorder, panic disorder and posttraumatic stress disorder, often in combination with a SSRI (Uhlenhuth *et al.*, 1999; Schwartz, 2002; Crane, 2003).

The main excitatory neurotransmitter found in the brain is the glutamate system. It extensively innervates anxiety-related areas of the brain such as the frontal cortex (anterior cingulated cortex, orbitofrontal cortex, medial prefrontal cortex, insular cortex), the amygdala and the hippocampus (Cortese and Phan, 2005). Glutamate has recently been linked to stress response and anxiety, in addition to its previously known involvement in learning, neuro-development and neuro-degeneration. This system acts through glutamate and its different receptor types (ionotropic and metabotropic), but also by affecting the release of dopamine, serotonin and GABA. The administration of NMDA was followed by anxiogenic-like behavior, whereas antagonists appear to have anxiolytic effects in rats tested using the elevated plus maze evaluation method (Zarrindast *et al.*, 2008; Rezvanfard *et al.*, 2009). Glutamate excitotoxicity has been observed after exposure to severe or chronic stress leading to potential neuronal damage and/or death. Over time, preclinical animal studies and human drug trials have provided clear evidence of the efficacy of drugs altering glutamate transmission in the treatment of anxiety (Cortese and Phan, 2005; Platt, 2007; Mathew *et al.*, 2008).

A final aspect of anxiety neurochemistry can be found in the stress response by the hypothalamic-pituitary-adrenal (HPA) axis. Upon stress or threat, the hypothalamus releases corticotropin releasing factor (CRF), which prompts the pituitary gland to produce and disperse adrenocorticotropin-releasing hormone (ACTH) into the bloodstream. The ACTH subsequently activates the release of glucocorticoids (mainly cortisol) from the adrenal cortex. Stress also triggers the sympathetic nervous system, which, together with cortisol, stimulates the release of adrenaline and norepinephrine from the adrenal medulla. Under normal physiological conditions, glucocorticoids will over time exert a negative feedback on CRF release through the hypothalamus, the hippocampus and the pituitary gland, thus terminating the stress response. The hippocampus is important because of its inhibitory control over the ACTH release. However, in the event of persistently elevated glucocorticoids, hippocampal damage and atrophy will occur, resulting in a disinhibition of the HPA axis and chronic elevated levels of stress hormones (Swaab *et al.*, 2005; Stahl, 2008b).

Multiple studies have shown altered HPA axis function in human anxiety disorders with elevated CRF levels in cerebrospinal fluid (posttraumatic stress disorder) and SNP's in the CRF gene (panic disorder). Furthermore, whereas reductions in anxiety symptoms have been noted after the administration of CRF-1 re-

ceptor antagonist in depressive patients, intracerebroventricular CRF injection has been reported to induce anxiety-like behaviors (Swaab *et al.*, 2005; Mathew *et al.*, 2008). Taken together, the present data suggest that the HPA axis plays a role in stress-related behaviors. It is worthwhile noting that the neuroendocrine response to stress is further complicated by interactions between the HPA axis and the neurotransmitters serotonin, dopamine and norepinephrine (Tsigos and Chrousos, 2002; Heisler *et al.*, 2007).

Despite the separate descriptions above of each of the different systems involved in anxiety, significant interplay exists between them, and especially between the different systems in the monoaminergic network (Figure 5). The exact mechanisms of these interactions are far from being understoodalthough it is clear that the neurotransmitters are sensitive to and influenced by changes in the other neurotransmitters.

For instance, various studies indicate a strong serotonin-dopamine interaction, with the dopamine neurons innervating the serotonergic cells of the raphe nuclei (RN) and, vice versa, the serotonin neurons innervating the dopaminergic ventral tegmental area (VTA) and the substantia nigra (SN). Depending on the 5-HT receptor type involved, 5-HT either stimulates or inhibits the dopamine release. However, the fact that no consensus exists on which receptor type exerts which effect (Bortolozzi *et al.*, 2005; Perani *et al.*, 2008; Esposito *et al.*, 2008; Di Matteo *et al.*, 2008; Stahl, 2008a) suggests the functioning of a complex 5-HT/DA equilibrium. A more straightforward control is noticeable in the excitatory effect of dopamine on the serotonergic neurons located at the raphe nuclei (Monti and Jantos, 2008; Di Giovanni *et al.*, 2008).

The extensive interconnectivity between the serotonergic and the norepinephrine systems, with their main projections from the raphe nuclei and the locus coeruleus, respectively, has been known for many years (Mongeau *et al.*, 1997). In the case of the NE control over 5-HT release, both stimulation (through the somatodendritic alpha 1 receptors on 5-HT neurons) and inhibition (through the postsynaptic alpha 2 receptors on 5-HT neurons) have been noted. Inversely, only a unidirectional inhibitory effect has been observed from 5-HT on the NE release. Similarly, an inhibitory effect of NE over the VTA dopamine neurons has been observed for alpha 2 receptors, and a stimulatory effect for alpha 1 receptors (Guiard *et al.*, 2008). In turn, dopamine exerts an inhibitory effect over LC norepinephrine.

Finally, the GABA and glutamate systems, which are ubiquitous in the brain, have interactions with nearly all neurons and act autonomously or as intermediate steps in other neurotransmitter systems, exerting their principal excitatory effect by glutamate and their principal inhibitory effect by GABA (Di Giovanni *et al.*, 2008). These interactions are also bidirectional, with GABA and glutamate influencing the monoamine systems, and vice versa. One example of this is the inhibition or stimulation of glutamate release when 5-HT binds to the 5-HT1A receptors or 5-HT2A receptors, respectively.

CONCLUSION

Anxiety appears to be a complex mechanism involving numerous brain regions and brain neurotransmitters. This focus on neuroanatomy necessarily involves the cortical and subcortical brain regions, including the prefrontal cortex, the amygdala, the hippocampus and the thalamus. Serotonin, dopamine and norepinephrine are the main neurotransmitters involved, but other neurotransmitters and hormones such as GABA, glutamate and the glucocorticoids must also be taken into account. An awareness of this complex interplay and equilibrium in the brain is essential for correctly approaching the problem of behavioral disorders in general, and anxiety in particular.

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