

*Chapter 2*

## **ROLE OF THE AMYGDALAR SEROTONERGIC SYSTEM IN EMOTIONAL REGULATION AND DISORDERS**

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### **ABSTRACT**

Many studies indicate a key role of the amygdala in the pathophysiology of psychiatric traits and disorders, such as anxiety disorders, depression and aggression. Serotonin (5-HT) inhibits the output of the amygdala upon exposure to negative emotional stimuli in healthy subjects.

Moreover, serotonin reuptake inhibitors (SSRIs) are the first choice of drug in the treatment of anxiety disorders, and they also may be effective in treating depression and aggression. SSRIs attenuate anxiety-induced c-Fos expression in the basolateral nucleus of the amygdala (BLA), and local injection of SSRIs into this nucleus produces anxiolytic effects. The BLA further receives a strong 5-HTergic innervation from the raphe, and therefore it was regarded as a major target of SSRIs. Based on these observations, the goal of this chapter is to explore 5-HT receptor subtypes and potential mechanisms through which the SSRIs might exert their effects in the amygdala.

The BLA has a cortex-like architecture with glutamatergic, spiny, pyramidal-shaped projection neurons showing a burst or regular firing pattern with adaptation following current injection. BLA interneurons are mostly GABAergic, spine-sparse or aspiny, often coexpress calcium-binding proteins like parvalbumin (PV) or calbindin (CB), and show a

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fast firing pattern with little adaptation. 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors are expressed on both types of BLA neurons, and they are coupled to G<sub>i/o</sub> and G<sub>q/11</sub>, respectively.

Thus, direct stimulation of 5-HT<sub>1A</sub> receptors on projection neurons inhibits them, whereas stimulation of 5-HT<sub>1A</sub> receptors on interneurons reduces the output of interneurons, resulting in disinhibition of projection neurons. In contrast, stimulation of 5-HT<sub>2A</sub> receptors has the opposite effect of exciting interneurons, which leads to a higher inhibition of projection neurons, although projection neurons also may be excited directly. Local injection of 5-HT ligands into the BLA showed that 5-HT<sub>1A</sub> receptors mediate anxiolytic effects, and 5-HT<sub>2C</sub> and 5-HT<sub>3</sub> receptors mediate anxiogenic effects. Systemic administration of 5-HT<sub>4</sub>, 5-HT<sub>6</sub> and 5-HT<sub>7</sub> receptor antagonists also induced anxiolysis, but the role of amygdalar 5-HT<sub>1B</sub>, 5-HT<sub>4</sub>, 5-HT<sub>6</sub> and 5-HT<sub>7</sub> receptors is less clear.

Thus, the amygdalar 5-HTergic system modulates physiological and pathological fear and anxiety through 5-HT<sub>1A</sub>, 5-HT<sub>2C</sub> and 5-HT<sub>3</sub> receptors, and probably also the 5-HT<sub>2A</sub> receptor. We hypothesize that SSRIs increase the 5-HT concentration in the BLA, and the surplus of 5-HT preferentially activates 5-HT<sub>1A</sub> receptors on glutamatergic projection neurons rather than on GABAergic interneurons, resulting in a decreased amygdala output.

Alternatively, increased 5-HT concentrations following SSRI application may preferentially stimulate 5-HT<sub>2A</sub> receptors on GABAergic interneurons in the BLA. These two mechanisms are by no means exclusive, and in fact may act together to reduce the overall excitability and output of the amygdala.

## INTRODUCTION

The amygdala is a crucial brain structure for emotions such as anxiety, fear, depression, aggression, impulsive choice and cognition of subjective value. Selective serotonin reuptake inhibitors (SSRIs) are effective in the treatment of anxiety disorders, depression and aggression, where they are thought to act by increasing the extracellular 5-HT concentration in various brain regions including the amygdala (Bosker et al., 2001; Muraki et al., 2001; Kitaichi et al., 2010). In human functional magnetic resonance imaging (fMRI) studies, fear-related stimuli increased the response of the amygdala, and SSRIs attenuated that increase (Del-Ben et al., 2005). Based on these studies, it has been suggested that SSRIs improve fear or anxiety by increasing the 5-HT concentration in the amygdala.

In animal studies, systemic administration of SSRIs had anxiolytic effects in fear-conditioned rats (Hashimoto et al., 1996). Moreover, conditioned fear-induced c-Fos expression in the amygdala was blocked by SSRIs (Izumi et al., 2006), and local administration of SSRIs into the amygdala reduced conditioned fear-induced freezing behavior (Inoue et al., 2004). These results support the notion that the amygdala is the likely region in which SSRIs exert their anxiolytic effects.

However, there are two considerations that must be taken into account when analyzing the effects of SSRIs. The first is whether there are differences between the physiological- and pharmacologically-enhanced function of the 5-HTergic system in the amygdala. The second is the identification of subtypes of 5-HT receptors, through which SSRIs may exert their anxiolytic effects. As generally known, there are more than 15 subtypes of 5-HT receptors, which differ considerably in their distribution and affinity to 5-HT (Hannon and Hoyer, 2008).

In this chapter, we first review the influence of the amygdalar 5-HTergic system on emotionality in humans and animals. Here it must be emphasized that often the resolution of the human amygdala in imaging studies does not allow the identification of distinct nuclei, whereas most studies on the 5-HTergic system in the amygdala of rodents have focused on the basolateral nucleus of amygdala (BLA). Next, we will describe the basic anatomical and physiological organization of the 5-HTergic neural system in the amygdala. Finally, we will discuss the potential mechanisms underlying 5-HTergic regulation in the amygdala.

## **1. HUMAN AMYGDALA IN PSYCHIATRIC DISORDERS/TRAITS**

### **1.1. Anxiety Disorders**

In fMRI studies, presentation of fear- or negative affect-related stimuli (e.g., photographs of angry or anxious faces, threatening words, etc.) activated limbic brain structures, including the amygdala, in healthy subjects (Morris et al., 1998; Whalen et al., 1998; Dannlowski et al., 2007a). Negative emotional stimuli provoked increased fMRI blood oxygen level-dependent (BOLD) responses in the amygdala, frontal cortex and cingulate cortex in patients with generalized anxiety disorder compared to healthy controls (Stein, 2009). However, there are also controversial findings on the responsivity of the amygdala and related brain structures in fear- and anxiety-related disorders (Table 1). In general, the activity of the frontal cortex, hippocampus and amygdala was enhanced in panic disorder patients, but some studies indicated decreased frontal and amygdalar activity (Engel et al., 2009). In social anxiety disorder, an increased responsivity was observed in the amygdala and/or insula, whereas the response of the frontal cortex was dependent on the experimental condition (Freitas-Ferrari et al., 2010). Post-traumatic stress disorder patients showed a higher amygdala response accompanied by a reduced frontal cortical response (Etkin and Wager, 2007). No doubt, these data obtained in human imaging studies indicate that anxiety-related disorders commonly lead to abnormal responses of the amygdala.

### **1.2. Aggression**

The amygdala is also critical in aggression and violence, but aggressive behaviors show high species specificity (Siegel et al., 2007; Takahashi et al., 2011). In rodents, aggression can be defensive to protect their own territory or pups, although more offensive behaviors can also be induced (escalated aggression).

In cats, there are two distinct types of aggression: defensive aggression, which is triggered by fear or a real threat, and is unplanned, enraged and displays sympathetic signs; and the other is predatory attack, which requires planning and decision-making, and is associated with only a few sympathetic signs. This dichotomy observed in animal behavior was also adapted to describe human aggression (Table 3), although the validity of this approach caused some dispute due to the multifactorial nature of aggression in humans. Defensive aggression in humans is observed in “reactive aggression”, and many elements of predatory attack are found in “instrumental aggression” (Siever, 2008).

**Table 1. fMRI responses to negative emotional stimuli in anxiety disorders**

<i>Disorder</i>	<i>Response</i>	<i>Reference</i>
Generalized anxiety disorder	Amygdala↑ Frontal cortex↑ Cingulate cortex↑	Stein (2009)
Panic disorder	Amygdala↑ or ↓ Frontal cortex↑ or ↓ Hippocampus	Engel et al (2009)
Social anxiety disorder	Amygdala↑ Insula↑	Freitas-Ferrari et al (2010)
Post traumatic stress disorder	Amygdala↑ Frontal cortex↓	Etkin & Wager (2007)

**Table 2. fMRI responses to negative emotional stimuli in depression and schizophrenia**

<i>Disorder</i>	<i>Response</i>	<i>References</i>
Depression	Amygdala ↑ or → Cingulate cortex ↓ Insula ↓ Hippocampus ↓	Pittinger & Duman (2008) Clark et al (2009) Savitz & Devets (2009) Victor et al (2010) Elliot et al (2011)
Depression (remitted)	Amygdala →	Anderson et al (2011)
Schizophrenia	Amygdala ↓ Cingulate cortex ↓ Hippocampus ↓	Benes (2010)

Maladaptive reactive aggression is seen in borderline personality disorder and intermittent explosive disorder patients. In fMRI studies, negative emotional stimuli provoked higher amygdala activation in both borderline personality disorder and intermittent explosive disorder patients (Siever et al., 2008; Blair, 2010). Some studies also indicated a reduced activity in the cingulate cortex of borderline personality disorder patients and in the orbitofrontal cortex of intermittent explosive disorder patients.

In contrast, maladaptive instrumental aggression is more typical for psychopathic traits. In this patient group, emotion-related decision-making and negative emotional stimuli provoked a reduced activity in the amygdala and orbitofrontal cortex (Siever et al., 2008; Blair, 2010).

**Table 3. fMRI responses to negative emotional stimuli in aggression-related disorders/traits**

<i>Disorder/trait</i>	<i>Response</i>	<i>References</i>
Borderline personality disorder	Amygdala↑ Cingulate cortex↓ or →	Siever et al (2008) Blair (2010)
Intermittent explosive disorder	Amygdala↑ Orbitofrontal cortex↓	Blair (2010)
Psychopathic traits	Amygdala↓ Orbitofrontal cortex↓	Blair (2010)

## 2. CLINICAL STUDIES ON AMYGDALAR SEROTONIN SYSTEM

### 2.1. Healthy Subjects

5-HTergic function in the human brain was assessed using fMRI following acute tryptophan depletion or administration of SSRIs (Table 4). Because tryptophan is an essential amino acid and the precursor of 5-HT, intake of tryptophan-free amino acid mixture leads to decreased 5-HT synthesis and attenuated 5-HT neurotransmission, whereas administration of SSRIs increases the 5-HT concentration in the synaptic cleft and enhanced 5-HT neurotransmission. In three studies, a negative emotional stimulus (aversive face) provoked greater fMRI BOLD responses in the right amygdala following acute tryptophan depletion (Cools et al., 2005; Fusar-Poli et al., 2007; van der Veen et al., 2007), but Roiser et al. (2008) reported a higher response of the left amygdala during an affective go/no-go task. Single SSRI administration attenuated BOLD responses provoked by negative emotional stimuli either in the right (Del-Ben et al., 2005; Murphy et al., 2009), left (Takahashi et al., 2005; Arce et al., 2008) or bilateral amygdalae (Anderson et al., 2007). However, Bigos et al. (2008) reported the opposite effect, namely, enhanced bilateral amygdala responses upon single SSRI administration. Likewise single SSRI administration increased the BOLD signal in the amygdala in the resting state (McKie et al., 2005), but in a study using arterial spin labeling, single SSRI administration decreased cerebral blood flow in the amygdala (Chen et al., 2011). Chronic administration of SSRIs (for seven days) also attenuated right amygdala responses provoked by negative emotional stimuli (Harmer et al., 2006).

**Table 4. Effects of tryptophan depletion and SSRIs on fMRI responses of the amygdala to negative emotional stimuli in healthy subjects**

<i>Treatment</i>	<i>Response</i>	<i>References</i>
Acute tryptophan depletion	Right amygdala ↑	Cools et al (2005) Fusar-Poli et al (2007) Van der Veen et al (2007)
	Left amygdala ↑	Roiser et al (2008)
Single SSRI	Right amygdala ↓	Del-Ben et al (2005) Murphy et al (2009)
	Left amygdala ↓	Takahashi et al (2005) Arce et al (2008)
	Bilateral amygdala ↓ or ↑	Anderson et al (2007)
Chronic SSRI (7 days)	Right amygdala ↓	Harmer et al (2006)

## 2.2. Anxiety and Fear

SSRIs are the primary first-line treatment for anxiety disorders. Many studies have indicated abnormal amygdala function in anxiety disorders, but only a few studies have focused directly on abnormalities of the 5-HTergic neural system in the amygdala (Table 5). Using positron emission tomography (PET), Lanzenberger et al. (2007) reported that 5-HT<sub>1A</sub> receptor binding is decreased in several limbic brain regions, including the amygdala, in social anxiety disorder patients. In addition, cerebral blood flow was increased in the amygdala of social anxiety disorder patients when they were subjected to public speaking, and the degree of the increase correlated with the patient's polymorphism of the 5-HT transporter (Furmark et al., 2004).

## 2.3. Depression

In fMRI studies, chronic SSRI treatment attenuated BOLD responses in the amygdala of depressed patients after presentation of negative emotional stimuli (Sheline et al., 2001; Fu et al., 2004; Fales et al., 2009) (Table 5). Moreover, the reduced correlation between the activities of the amygdala and cingulate cortex recovered after chronic SSRI treatment (Chen et al., 2008). Enhanced BOLD responses in the amygdala of depressed patients were

associated with polymorphisms of the 5-HT transporter (Dannowski et al., 2007b; Dannowski et al., 2008) and 5-HT<sub>1A</sub> receptor (Dannowski et al., 2007b). In PET studies, binding to 5-HT<sub>1A</sub> receptors (Bhagwagar et al., 2004; Hirvonen et al., 2008) and the 5-HT transporter (Parsey et al., 2006; Miller et al., 2008; Reimond et al., 2008) was decreased in the amygdala.

**Table 5. Changes of the 5-HTergic neural system in the amygdala detected by functional imaging in psychiatric disorders**

<i>Disorder</i>	<i>Response/ Binding density</i>	<i>References</i>
Social anxiety disorder	5-HT <sub>1A</sub> receptor ↓	Lanzenberger et al (2007)
Depression	fMRI response to emotional stimuli by chronic SSRI ↓	Sheline et al (2001) Fu et al (2004) Fales et al (2009)
	5-HT <sub>1A</sub> receptor ↓	Bhagwagar et al (2004) Hirvonen et al (2008)
	5-HT transporter ↓	Parsey et al (2006) Miller et al (2008) Reimond et al (2008)

## 2.4. Aggression

As mentioned above, imaging studies point to an important role of the amygdala in human aggression. Furthermore, the concentration of the 5-HT metabolite 5-hydroxy-indoleacetic acid (5-HIAA) was reduced in the cerebrospinal fluid of aggressive psychopathic patients, and SSRIs were effective in inhibiting aggression in psychiatric patients (Siever, 2008). Nevertheless, the relationship between aggression and the 5-HTergic system in the human amygdala has not yet been established. In PET studies, aggression levels correlated positively with binding to 5-HT<sub>1A</sub> receptors in the prefrontal and cingulate cortices of healthy subjects (Witte et al., 2010). In addition, 5-HT<sub>2A</sub> receptor binding was increased in the orbitofrontal cortex of psychopathic aggressive patients (Rosell, 2010) and the hippocampus of female borderline personality disorder patients (Soloff, 2007). However, 5-HT transporter binding was reduced in the cingulate and orbitofrontal cortices of psychopathic aggressive patients (Frankle et al., 2005; Siever, 2008). Thus, the importance of the frontal 5-HTergic system in human aggression has been determined in several studies, but the role of the amygdalar 5-HTergic system in aggression remains to be elucidated.

### **3. AMYGDALA'S ROLE IN ANXIETY-AND FEAR-MODELS**

#### **3.1. Animal Models of Anxiety and Fear**

Animal models focusing on anxiety- and fear-related behaviors can be classified as (i) paradigms measuring memory-independent forms of innate anxiety, such as the open field test, social interaction test, elevated plus maze test and ultrasonic vocalization test; and (ii) tests based on memory-dependent fear, such as the conditioned fear paradigm.

Many tests utilize the innate anxiety of rodents from open spaces to measure anxiety-related behavior (Pellow et al., 1985); conflict between punishment and reward is also often used to study anxiolytic effects of drugs (Vogel et al., 1971). In the open field test, animals are placed in a large open square, and the time spent near the walls is used as a measure of anxiety, because anxious animals tend to avoid the center of the field. To test social interaction, naïve rats are placed in pairs in an open space, and the time spent in social interaction (grooming, sniffing, etc.) is measured (File, 1985). In this test, the time spent in social interaction correlates negatively with anxiety. In the elevated plus-maze test, animals are placed in an apparatus consisting of two closed arms (with walls) and two open arms (no walls) connected by a central platform. Because animals prefer the closed arms over the open arms, the number of entries to and time spent on the open arms is a correlate of spontaneous and memory-independent anxiety. In the ultrasonic vocalization test, the distress call of pups separated from their mothers is used as an index of anxiety (Noirot, 1972). In the conflict test, animals must tolerate an aversive stimulus that is simultaneously given with a reward. The animal's behavior directed toward the reward increases upon delivery of anxiolytic drugs (conflict is attenuated), as well as nicotine and caffeine, suggesting that the conflict itself may be a different mental state than anxiety or fear (Vogel et al., 1971).

Conditioned fear is a type of classical conditioning (Fanselow, 1980) composed of acquisition, retrieval (expression), and extinction phases (Myers and Davis, 2002). During acquisition, a simple (e.g., light or tone) or complex (e.g., environmental context) sensory stimulus (CS, conditioned stimulus) is paired with an aversive stimulus (US, unconditioned stimulus), such as footshock. Retrieval occurs when the animal is re-exposed to the CS in the absence of the US, and the responses to the CS are measured. The CS may elicit hormonal, autonomic and behavioral conditioned responses, including elevated cortisol levels, increases in heart rate and freezing behavior or a potentiated startle response (Yilmazer-Hanke, 2008). Extinction occurs when the CS is repeatedly presented in the absence of the US, resulting in a decrease in the magnitude of conditioned responses. The fear-conditioning paradigm is regarded as an animal model of memory-dependent fear (Myers and Davis, 2002).

#### **3.2. Amygdala in Anxiety and Fear**

The amygdala is crucial for innate anxiety, as well as the acquisition and retrieval of conditioned fear (Davis, 1997; LeDoux, 2000). Lesioning and pharmacological studies indicated an activation of the amygdala in various forms of innate anxiety, including the light-dark box, elevated plus maze and conflict tests (for review: Davis, 1997).



Nevertheless, most studies investigating the neural circuitry of the amygdala focused on the conditioned fear paradigm. During conditioned fear training (acquisition) and recall (retrieval), the sensory stimulus (e.g., tone or light cue, exposure to a shock chamber) reaches the lateral nucleus of the amygdala (LA), BLA and hippocampus (Figures 1, 2). In the retrieval phase, the CS induces fear after being checked against memory formed during the acquisition phase. Finally, emotions are expressed as a fear reaction through activation of the central nucleus of the amygdala (CeA) (Ehrlich et al., 2009).

Several studies have suggested that the BLA is involved in fear-related neural plasticity. Maren and Fanselow (1995) reported long-term potentiation in BLA. Herry et al. (2008) found that firing rates of neurons in the BLA were changed by the acquisition or extinction of conditioned fear; however, conditioned fear-induced plastic changes were also reported in the CeA, and it was speculated that neural information on conditioned fear is integrated in the CeA (Ehrlich et al., 2009). While the BLA provides glutamatergic projections to both the CeA and intercalated nuclei of the amygdala (ICN), the ICN send GABAergic projections into the CeA.

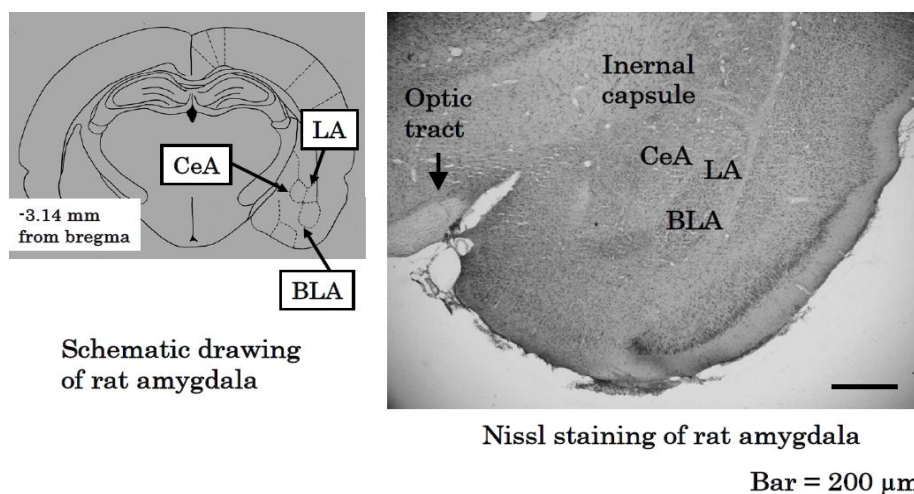


Figure 1. Anatomical location of LA, BLA and CeA. The left panel is a schematic representation of the amygdala (-3.14 mm from bregma, Paxinos and Watson, 1997). The right panel is the Nissl staining section of the same level. BLA, basolateral nucleus of the amygdala; CeA, central nucleus of the amygdala; LA, lateral nucleus of the amygdala.

Therefore, the net output of the CeA mediating conditioned fear is regulated by direct excitatory input from the BLA, which competes with inhibitory input originating from the ICN (Sah and Westbrook, 2008).

### 3.3. Amygdalar Serotonergic System in Anxiety and Fear

#### 3.3.1. Anxiolytic Effect of SSRIs in the Amygdala

In microdialysis studies, extracellular 5-HT release was increased in the BLA during retrieval of conditioned fear (Kawahara et al., 1993; Yokoyama et al., 2005; Zanoveli et al., 2009). Nonetheless, systemic administration of SSRIs also attenuated freezing behavior during conditioned fear retrieval (Hashimoto et al., 1996; Muraki et al., 1999), and local

injection of SSRIs into the amygdala had the same effect (Inoue et al., 2004). In addition, retrieval of conditioned fear increased c-Fos expression in several brain regions including the BLA, and simultaneous systemic administration of SSRIs specifically inhibited the c-Fos expression in the BLA (Izumi et al., 2006). These results are consistent with human clinical studies indicating that the amygdalar 5-HTergic neural system regulates anxiety, and the main target of anxiolytic SSRI effects is the amygdala. Nevertheless, systemic administration of SSRIs did not exert a clear anxiolytic effect in the elevated plus maze test (Pollier et al., 2000; Homberg et al., 2011).

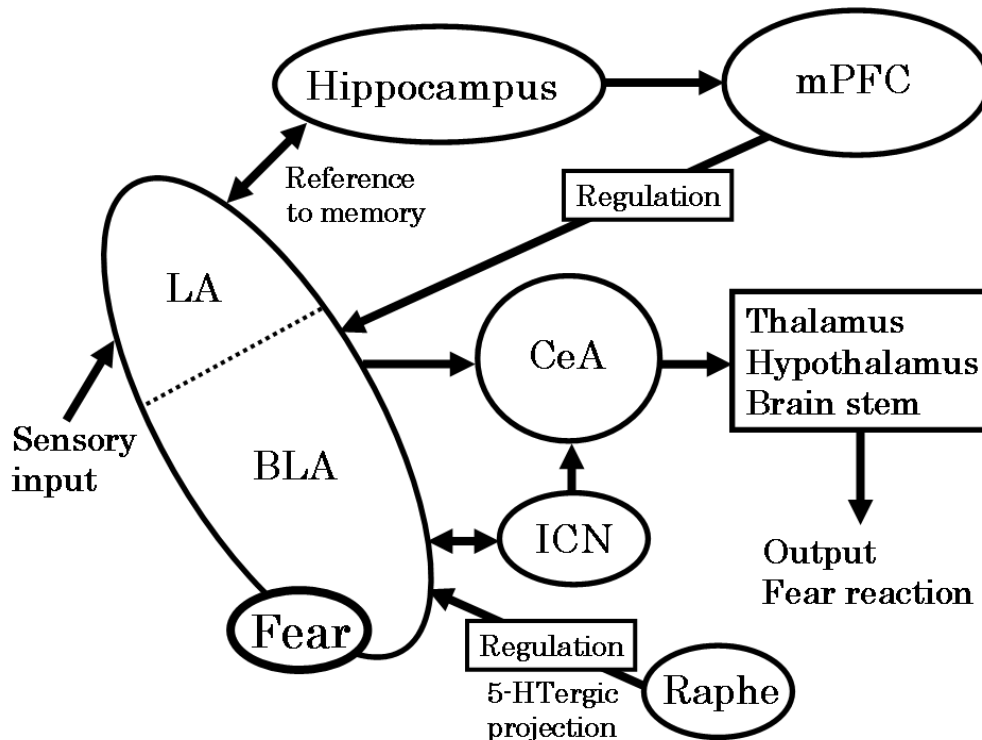


Figure 2. Neural circuitry of conditioned fear. BLA, basolateral nucleus of the amygdala; CeA, central nucleus of the amygdala; ICN, intercalated nuclei of the amygdala; LA, lateral nucleus of the amygdala; mPFC, medial prefrontal cortex.

### 3.3.2. Delivery of Selective 5-HT Ligands into the Amygdala

Selective 5-HT ligands were injected into the amygdala in various models testing anxiety- and fear-related behaviors (Table 6). The local injection of a 5-HT<sub>1A</sub> agonist into the amygdala decreased the retrieval of conditioned fear (Li et al., 2006; Matsuzaki et al., 2011), but had no effect in the elevated plus maze test (Zangrossi and Graeff, 1994; Gonzalez et al., 1996). Systemic administration of 5-HT<sub>2A/2C</sub> agonists induced an anxiolytic effect in the ultrasonic vocalization (Schreiber et al., 1998) and elevated plus maze tests (Nic Dhonnchadha et al., 2003), but their effects in the amygdala were not examined.

**Table 6. Anxiolytic and anxiogenic effects of systemic administration or local injection into the amygdala/BLA of 5-HT-related drugs**

<i>Drug</i>	<i>Anxiety/Fear (test type)</i>	<i>References</i>
SSRI (systemic)	↓ (CF, USV)	Hashimoto et al (1996) Schreiber et al (1998) Muraki et al (1999)
SSRI (local)	↓ (CF)	Inoue et al (2004)
5-HT <sub>1A</sub> agonist (systemic)	↓ (CF)	Matsuzaki et al (2011)
5-HT <sub>1A</sub> agonist (local)	↓ (CF, USV) or → (EPM)	Zangrossi & Graeff (1994) Gonzalez et al (1996) Li et al (2006) Matsuzaki et al (2011)
5-HT <sub>2A/2C</sub> agonist (systemic)	↓ (USV, EPM)	Schreiber et al (1998) Nic Dhonnchadha et al (2003)
5-HT <sub>2A/2C</sub> antagonist (systemic)	↓ (EPM)	Mora et al (1997)
5-HT <sub>2A/2C</sub> antagonist (local)	↑ or → (EPM)	Zangrossi & Graeff (1994) de Mello Cruz et al (2005)
5-HT <sub>2C</sub> agonist (systemic)	↑ (EPM)	Setem et al (1999)
5-HT <sub>2C</sub> agonist (local)	↑ (USV, OF, EWA)	Campbell and Merchant (2003) Overstreet et al (2006)
5-HT <sub>2C</sub> antagonist (systemic)	↓ (SI)	Christianson et al (2010)
5-HT <sub>2C</sub> antagonist (local)	↓ (SI, EWA)	Overstreet et al (2006) Christianson et al (2010)
5-HT <sub>3</sub> agonist (local)	↑ (SI)	Higgins et al (1991)
5-HT <sub>3</sub> antagonist (systemic)	↓ (CF, SI, EPM)	Blackburn et al (1993) Nevins & Anthony (1994)
5-HT <sub>3</sub> antagonist (local)	↓ (SI, EPM)	Higgins et al (1991) Costall et al (1993)
5-HT <sub>4</sub> antagonist (systemic)	↓ (SI)	Kennet et al (1997)
5-HT <sub>6</sub> antagonist (systemic)	↓ (CT)	Wesołowska et al (2010)
5-HT <sub>7</sub> antagonist (systemic)	↓ (CT)	Wesołowska et al (2006)

Legend: BLA, basolateral nucleus of the amygdala; CF, conditioned fear; CT, conflict test; EPM, elevated plus maze test; EWA, ethanol withdrawal anxiety; OF, open field test; SI, social interaction test; USV, ultrasonic vocalization test.

While systemic administration of 5-HT<sub>2A/2C</sub> antagonists also produced anxiolytic effects (Mora et al., 1997), local injection of a 5-HT<sub>2A/2C</sub> antagonist into the BLA produced anxiogenic effects (Zangrossi and Graeff, 1994) or had no effect in the elevated plus maze test (de Mello Cruz et al., 2005). While systemic administration of a 5-HT<sub>2C</sub> agonist exerts anxiogenic effects in the elevated plus maze test (Setem et al., 1999), systemic administration of a 5-HT<sub>2C</sub> antagonist produces anxiolytic effects in the social interaction test (Christianson et al., 2010).

Local injection of a 5-HT<sub>2C</sub> agonist into the BLA has anxiogenic effects in the open field and ultrasonic vocalization tests (Campbell and Merchant, 2003) and ethanol withdrawal anxiety (Overstreet et al., 2006), whereas local injection of 5-HT<sub>2C</sub> antagonists into the BLA is anxiolytic in the social interaction test (Christianson et al., 2010) and ethanol withdrawal anxiety (Overstreet et al., 2006). Likewise local injection of 5-HT<sub>3</sub> agonists into the amygdala were anxiogenic (Higgins et al., 1991), whereas systemic and/or intra-amygdalar 5-HT<sub>3</sub> antagonists were anxiolytic in conditioned fear (Nevins and Anthony, 1994), the social interaction (Higgins et al., 1991; Blackburn et al., 1993) and elevated plus maze tests (Blackburn et al., 1993; Costall et al., 1993). Systemic and/or intra-hippocampal administration of 5-HT<sub>4</sub>, 5-HT<sub>6</sub> and 5-HT<sub>7</sub> antagonist resulted in anxiolysis in the social interaction and conflict tests (Kennett et al., 1997; Wesolowska et al., 2006; Wesolowska, 2010). In summary, these findings suggest that 5-HT exerts its anxiolytic effect in the amygdala via 5-HT<sub>1A</sub> receptors, and its anxiogenic effect via 5-HT<sub>2C</sub> or 5-HT<sub>3</sub> receptors, although the roles of amygdalar 5-HT<sub>2A</sub>, 5-HT<sub>4</sub>, 5-HT<sub>6</sub> and 5-HT<sub>7</sub> receptors need further investigation.

### **3.3.3. Amygdalar 5-HT Receptor Subtypes Mediating SSRI Effects**

Schreiber et al. (1998) reported that systemic administration of SSRIs attenuated anxiety in the ultrasonic vocalization test in rats, and that this effect was blocked by simultaneous systemic delivery of a 5-HT<sub>2A</sub> antagonist, but 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>3</sub> and 5-HT<sub>4</sub> antagonists had no effect. From these results, they concluded that the anxiolytic effect of SSRIs was mediated by the 5-HT<sub>2A</sub> receptor. Other studies indicated that co-administration of 5-HT<sub>1A</sub> antagonists enhance the anxiolytic effects of SSRIs in conditioned fear (Hashimoto et al., 1997; Muraki et al., 2008). They speculated that the blockade of 5-HT<sub>1A</sub> autoreceptors at the somata of 5-HTergic raphe neurons by the antagonist resulted in a disinhibition of these neurons and enhanced presynaptic 5-HT release, and at the same time the SSRIs blocked 5-HT transporters in their presynaptic terminals; as a result, the neurons remained active and the 5-HT concentration in the synaptic cleft increased, potentiating the overall anxiolytic effect. However, this is inconsistent with several studies, which show that local injection of a 5-HT<sub>1A</sub> agonist into the raphe produces anxiolysis (Engin and Treit, 2008). Still, intra-amygdalar or hippocampal administration of a 5-HT<sub>1A</sub> agonist also has anxiolytic effects (Schreiber and De Vry, 1993; Li et al., 2006; Matsuzaki et al., 2011). These seemingly controversial effects of 5-HT<sub>1A</sub> agonists and antagonists in various brain regions might result from differences in their distribution (see Section 6). It is also possible that different synaptic 5-HT concentrations stimulate different subtypes of 5-HT receptors according to their affinities to 5-HT, and that the “appropriate” synaptic 5-HT concentration for anxiolytic effects differs among brain regions.

Therefore, local co-administration of SSRIs and selective 5-HT antagonists into brain regions regulating anxiety-related behavior may be useful.

## **4. AMYGDALA IN ANIMAL MODELS OF STRESS AND DEPRESSION**

### **4.1. Animal Models of Stress and Depression**

Acute or chronic stress generate depression-like behavioral and endocrine changes (immobility, anhedonia and enhanced hypothalamus-pituitary-adrenal (HPA) axis, etc.) in rodents, and antidepressants improve these changes. Common methods used to induce stress in animal models are the forced swim, tail suspension, electric shock, restraint, novel circumstance and social defeat tests.

In these models, immobilization, time threshold to escape from shock, food consumption and sucrose preference are used as measures of stress. Acute stress is often studied using the forced swim and tail suspension paradigms, where the immobilization time during stress is measured.

In the learned helplessness model, animals repeatedly exposed to inescapable electric shock gradually lengthen the time threshold to escape from the shock (Pittenger and Duman, 2008; Nestler and Hyman, 2010; Carr and Lucki, 2011).

The behavioral changes observed in acute stress are improved by a single administration of antidepressants, but these effects might reflect merely the inhibition of monoamine re-uptake, not a “true” antidepressant effect. Therefore, many researchers believe that chronic stress reflects the pathophysiology of depression more accurately than acute stress. Repeated restraint and social defeat are the most commonly used chronic stress models. The enhanced immobility, reduced food consumption and reduced sucrose preference in these tests can also be improved by chronic administration of antidepressants (Carr and Lucki, 2011).

### **4.2. Amygdala in Stress and Depression**

The role of the amygdala in depression was studied in the context of chronic stress in most animal models (Pittenger and Duman, 2008). Acute stress induces neurochemical changes in the amygdala, including monoamine and amino acid transmitters. Inescapable footshock increased the release of 5-HT, dopamine, glutamic acid and GABA in the BLA (Yokoyama et al., 2005; Christianson et al., 2010; Venton et al., 2010). Rezenikov et al. (2009) showed that acute restraint increases GABA release in the BLA, but repeated restraint does not. In addition, acute restraint increased c-Fos expression in the BLA, which was attenuated after repeated restraint (Reznikov et al., 2008). Mozhui et al. (2010) reported that repeated restraint decreases mRNA of glutamate receptor subunits and dampens the electrophysiological activity in the BLA. These studies suggest that the responsivity of the amygdala is lower in chronic than in acute stress, although chronic stress may alter amygdala function and morphology (Figure 3).

For example, repeated restraint stress enhanced acquisition and retrieval of conditioned fear and increased dendritic branching of amygdalar pyramidal neurons (McLaughlin et al., 2009; McEwen, 2010).

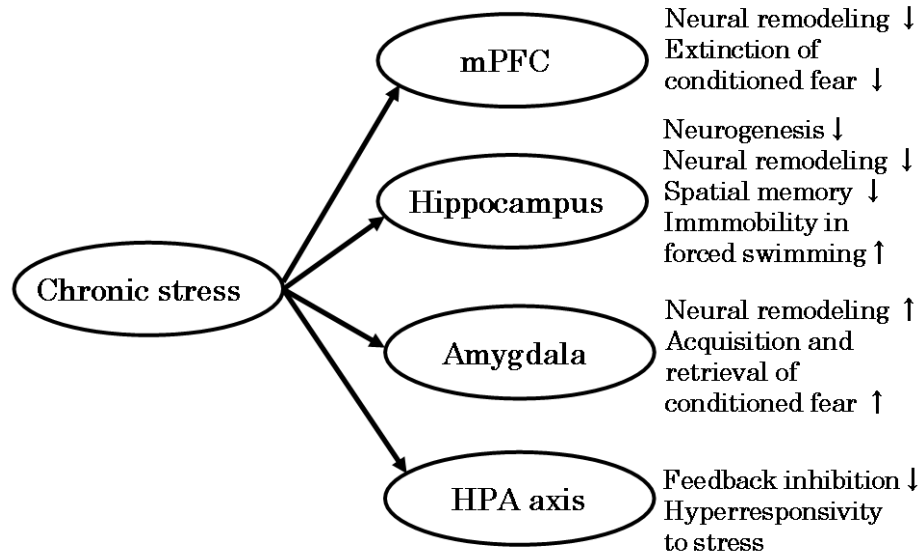


Figure 3. Effects of chronic stress on the brain. HPA axis, hypothalamus-pituitary-adrenal axis; mPFC, medial prefrontal cortex.

Moreover, acute administration of glucocorticoids can induce dendritic hypertrophy in the amygdala (Mitra and Sapolsky, 2008). Although the mechanisms leading to remodeling of neurons in the amygdala by chronic stress are not well understood, investigations in the amygdala, hippocampus and prefrontal cortex support the idea that glucocorticoids are involved in these stress-related structural changes (Hajszan et al., 2009; McLaughlin et al., 2009), and that some of these changes can be reversed by chronic lithium treatment (Johnson et al., 2009). It has been suggested that lithium may exert its effects by inhibiting glutamate release (Johnson et al., 2009) or by increasing extracellular 5-HT levels (Muraki et al., 2001).

### 4.3. Amygdalar 5-HTergic System in Depression

Only few studies have focused on the effect of chronic stress on the amygdalar 5-HTergic system. Repeated forced swimming decreased the 5-HIAA content (Shishkina et al., 2008), and learned helplessness decreased the density of 5-HT<sub>2A</sub> receptors (Wu et al., 1999) in the amygdala.

Moreover, chronic administration of SSRIs together with subchronic lithium increased extracellular 5-HT levels in the medial prefrontal cortex (Muraki et al., 2001), and SSRIs showed neuroprotective effects in the hippocampus (Jin et al., 2009). However, changes in 5-HT release, the 5-HT<sub>1A</sub> receptor, 5-HT transporter or activity of tryptophan hydroxylase after chronic stress were not fully investigated.

## 5. AMYGDALA IN ANIMAL MODELS OF AGGRESSION

### 5.1. Animal Models of Aggression

The most commonly used rodent model of aggression is the resident intruder test. In this test, aggressive behaviors (attacking, biting, aggressive posture) of a male resident rat or mouse in its home cage to a novel intruder are assessed (Takahashi et al., 2011). To enhance the aggression of the resident, he is first isolated or housed with females. Other paradigms are noxious stimulus-induced aggression or maternal aggression (aggression of females during lactation period). For escalating aggression, the repeated victory of a resident over an intruder can also be utilized. In the past, a muricide test (killing of mice by brain-lesioned rat) was also applied (Fujiwara et al., 1980)

### 5.2. Amygdala in Aggression

Defensive aggression can be elicited in cats by electrical stimulation of the periaqueductal gray and medial hypothalamus. Excitation of the medial amygdala (MeA) and medial part of BLA potentiates the aggression elicited by medial hypothalamus stimulation, while excitation of the CeA and lateral part of BLA suppresses it (Siegel et al., 2007). Veening et al. (2005) reported c-Fos expression in the MeA, CeA, BLA, periaqueductal gray and various hypothalamic nuclei of rats induced by aggressive behavior. A lesion in the orbitofrontal cortex also increased aggression in rats (Rudebeck et al., 2007). Thus, amygdalar subregions differentially enhance or inhibit subsystems in the brain (hypothalamus and periaqueductal gray) that control the expression of aggression, whereas the orbitofrontal cortex may regulate the output of the amygdala, although further details of these neural circuits remain to be elucidated (Figure 4).

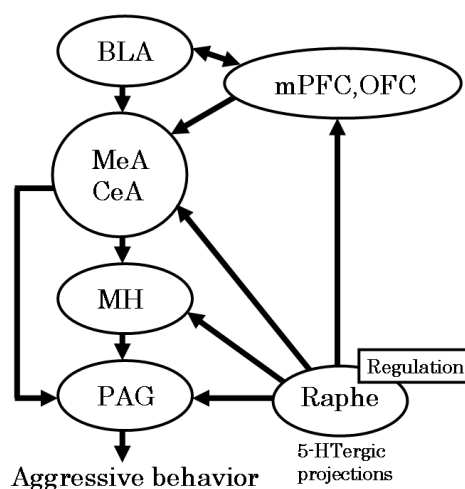


Figure 4. Neural circuitry of aggression. BLA, basolateral nucleus of the amygdala; CeA, central nucleus of the amygdala; MeA, medial nucleus of amygdala; MH, medial hypothalamus; mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; PAG, periaqueductal gray.

### 5.3. Amygdalar 5-HTergic System in Aggression

Although many neurotransmitters modulate aggression (Siegel et al., 2007), 5-HT is among the most well-known because SSRIs have proven to be effective in the treatment of human aggression (Siever, 2008). In a rodent model of aggression, acute systemic administration of a 5-HT<sub>1A</sub> agonist, 5-HT<sub>2A/2C</sub> agonist and antagonist, 5-HT<sub>2C</sub> agonist and antagonist, and SSRIs decreased aggression (Olivier, 2004), but chronic administration of a 5-HT<sub>1A</sub> agonist, 5-HT<sub>2C</sub> agonist and SSRIs paradoxically increased aggression (Mitchell, 2005). In contrast, the delivery of 5-HT into the amygdala (Pucilowski et al., 1985), a 5-HT<sub>1A</sub> agonist into the dorsal and median raphe, dorsal periaqueductal gray, medial hypothalamus, cortical amygdala (CoA) and orbitofrontal cortex, and a 5-HT<sub>1B</sub> agonist into the dorsal and orbitofrontal cortices decreased aggression (Takahashi et al., 2011). Injection of a 5-HT<sub>2A/2C</sub> agonist into the dorsal periaqueductal gray also decreased aggression, but its application into the CeA increased aggression (Ferrari et al., 2005; Takahashi et al., 2011). Likewise, administration of the 5-HT precursor 5-hydroxytryptophan decreased aggression (Olivier, 2004), whereas the inhibition of 5-HT synthesis by para-chlorophenylalanine increased aggression (Valzelli et al., 1981). However, aggression was increased in monoamine oxidase A (MAO<sub>A</sub>) knockout mice, although the brain 5-HT concentration was elevated (Cases et al., 1995), and lesioning of 5-HTergic terminals in the amygdala by the neurotoxin 5,7-dihydroxytryptamine reduced aggression (File et al., 1981). In a brain dialysis study, 5-HT levels in the medial prefrontal cortex (mPFC) decreased during aggressive behavior (Ferrari et al., 2005). Taken together, these findings indicate a close relationship between aggression and the 5-HTergic system, but how 5-HT affects aggression differs between brain regions (including the amygdala), 5-HT receptor subtypes and behavioral models used.

## 6. ANATOMICAL ORGANIZATION OF AMYGDALAR 5-HTERGIC SYSTEM

### 6.1. Neuronal Cell Types in the Amygdala

The amygdala is a heterogenous brain region composed of cortex-like and non-cortex-like nuclei. Here, we will focus on neuronal cell types in amygdalar nuclei with a cortex-like architecture (e.g., LA, BLA), because the function of the 5-HTergic system has been studied best in BLA neurons. These nuclei contain two major cell classes (Figure 5), spiny pyramidal neurons and spiny-sparse non-pyramidal neurons (McDonald, 1982; Millhouse and DeOlmos, 1983; McDonald, 1992a; Washburn and Moises, 1992a; Rainnie et al., 1993; Yajeya et al., 1997). Pyramidal neurons have pyramidal or piriform somata that vary in size, one thick apical dendrite and several thinner basal dendrites, and project to distant brain areas as shown using retrograde tracers (McDonald, 1992b). Some pyramidal neurons have typical pyramidal morphology, but others have atypical morphology, such as neurons that have small, satellite-shaped somata and spiny dendrites (Rainnie et al., 1993).



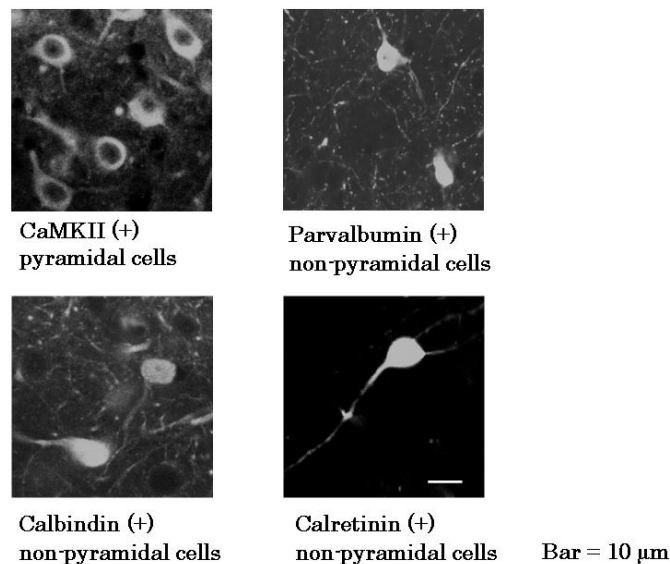


Figure 5. Glutamatergic pyramidal neurons (CaMKII-positive) and examples of GABAergic non-pyramidal neurons in the BLA (parvalbumin-, calbindin- and calretinin-positive). McDonald et al. (2002) indicated that CaMKII is a marker for pyramidal neurons in BLA. This figure is authors' own figure.

Non-pyramidal neurons are lower in number and comprise the GABAergic interneuron population in the BLA; most neurons have small and ovoid somata, but some have bitufted, multipolar or bipolar somata that vary in size (McDonald, 1985; Kempainen and Pitkänen, 2000).

Mascagini and McDonald (2003) classified non-pyramidal neurons in the BLA using markers such as parvalbumin (PV), calbindin (CB), calretinin (CR), cholecystokinin (CCK), somatostatin (SOM), neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP). They identified at least four subpopulations based on coexpression of peptides and calcium-binding proteins. Their type 1) neurons were PV(+)/CB(+), type 2) neurons SOM(+)/CB(+), type 3) neurons with large, multipolar somata CCK(+)/CB(+), and type 4) small bipolar or bitufted neurons CCK(+), VIP(+) or CR(+).

## 6.2. The 5-HTergic Innervation of the Amygdala

The amygdala is rich in monoaminergic and cholinergic innervation. Among cortex-like amygdalar nuclei, the BLA receives the strongest innervation from noradrenergic (Li et al., 2001; Kuramochi et al., 2009), 5-HTergic (Muller et al., 2007; Kuramochi et al., 2009) (Figure 6) and cholinergic neurons (Muller et al., 2011), whereas dopamine neurons provide a stronger innervation to the CeA than to the BLA (Pinard et al., 2008).

The major origin of the 5-HTergic afferents to the amygdala is the dorsal raphe nucleus, although the median raphe nucleus also contributes to the 5-HTergic innervation of the amygdala (Fallon and Ciofi, 1992). Muller et al. (2007) investigated 5-HT-positive terminals

in the BLA in detail, and reported that the main target of 5-HT-positive terminals were spines and distal dendrites of pyramidal cells.

In addition, most if not all of the subpopulations of BLA interneurons were innervated by 5-HT-positive terminals.

### 6.3. Subtypes of 5-HT Receptors in the Amygdala

#### 6.3.1. The 5-HT<sub>1A</sub> Receptor

5-HT<sub>1A</sub> receptors are widely distributed in the brain, with the highest density in the hippocampus, septum and raphe, and moderate densities in the cerebral cortex and amygdala (Chalmers and Watson, 1991; Khawaja, 1995). In the amygdala, 5-HT<sub>1A</sub> receptors show the highest density in the BLA, but these receptors are also expressed in the LA and CeA, although in lower density (Saha et al., 2010).

Based on their distribution, two types of 5-HT<sub>1A</sub> receptors are distinguished in the brain: (i) 5-HT<sub>1A</sub> autoreceptors expressed on the somata of 5-HTergic neurons in raphe nuclei and their stimulation attenuates the firing of 5-HTergic cells and 5-HT release from terminals; and (ii) postsynaptic 5-HT<sub>1A</sub> receptors expressed by neurons in the projection areas of 5-HT neurons. Aznar et al. (2003) demonstrated that 5-HT<sub>1A</sub> receptors exist on both pyramidal neurons, and PV- or CB-positive interneurons in the amygdala. As postsynaptic receptors, they contribute to inhibition of amygdalar neurons, because 5-HT<sub>1A</sub> receptors couple to G<sub>i/o</sub>, and their stimulation inhibits adenylate cyclase, although other signal transduction systems, such as the MAPK and Akt signaling pathways and G protein-coupled K<sup>+</sup> (GIRK) channels are also activated (Polter and Li, 2010).

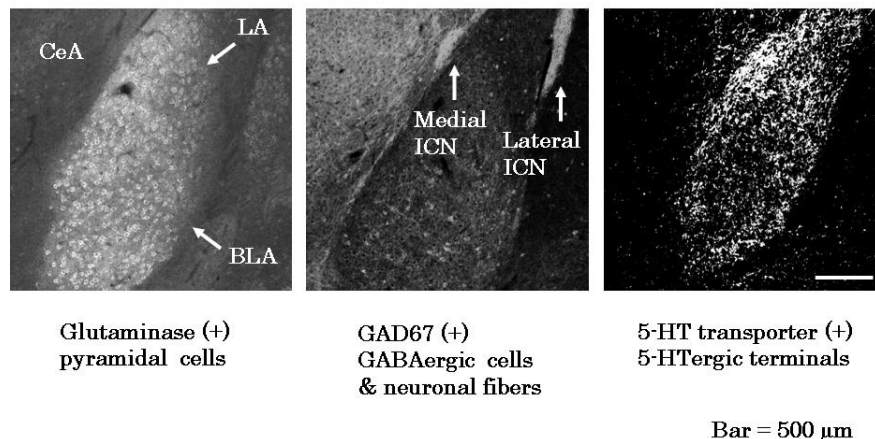


Figure 6. Glutamatergic neurons (glutaminase-positive, left panel), GABAergic neurons and neuronal fibers (GAD67-positive, central panel) and 5-HTergic terminals (5-HT-positive, right panel) in the amygdala, which is stained in the adjacent sections. Izumi et al (2011) indicated that glutaminase is a marker for pyramidal neurons in BLA. The microphotographs are the authors' own images.

### **6.3.2. The 5-HT<sub>1B</sub> Receptor**

5-HT<sub>1B</sub> receptors are present in high densities in the globus pallidus, substantia nigra and accumbens, whereas their density in the cerebral cortex, hippocampus and amygdala is lower (Sari, 2004). In the amygdala, 5-HT<sub>1B</sub> receptors are distributed uniformly in the BLA, LA and CeA (Saha et al., 2010). 5-HT<sub>1B</sub> receptors localized in presynaptic terminals of 5-HTergic neurons act as autoreceptors, and their stimulation attenuates 5-HT release from their terminals. However, 5-HT<sub>1B</sub> receptors are also found in presynaptic terminals of non-5-HTergic neurons as heteroreceptors, or are expressed at postsynaptic sites on pyramidal neurons and granule cells (Sari, 2004; Hannon and Hoyer, 2008). They couple to G<sub>i/o</sub>, and their stimulation inhibits adenylate cyclase.

### **6.3.3. The 5-HT<sub>2A</sub> Receptor**

The density of 5-HT<sub>2A</sub> receptors is high in the cerebral cortex, claustrum, olfactory system and some brainstem nuclei, and moderate in the striatum, accumbens and amygdala. In the amygdala, 5-HT<sub>2A</sub> receptors are expressed at high densities in the BLA and low densities in the CeA; however, the results of immunostaining patterns were inconsistent among the antibodies used (McDonald and Mascagni, 2007; Bombardi, 2011).

5-HT<sub>2A</sub> receptors are localized postsynaptically on the somata and dendrites of pyramidal neurons and interneurons in the BLA, and most 5-HT<sub>2A</sub> receptor-positive GABAergic interneurons in the BLA are also PV or SOM-positive (McDonald and Mascagni, 2007; Hannon and Hoyer, 2008; Bombardi, 2011). Functionally, 5-HT<sub>2A</sub> receptors couple to G<sub>q/11</sub>, and their stimulation enhances the IP3/PKC/Ca<sup>2+</sup> pathway.

### **6.3.4. The 5-HT<sub>2C</sub> Receptor**

The highest density of 5-HT<sub>2C</sub> receptors is seen in the choroid plexus, but moderate densities of this receptor are also found in the olfactory system, cerebral cortex, hippocampus, striatum, thalamus and amygdala (Clemett et al., 2000; Li et al., 2004). In the amygdala, 5-HT<sub>2C</sub> receptors are present in moderate densities in the BLA, but are abundant in the MeA and ICN (Clemett et al., 2000). 5-HT<sub>2C</sub> receptors are exhibited on somata and dendrites of pyramidal neurons and interneurons (Filip and Bader, 2009). They couple to G<sub>q/11</sub>, and their stimulation enhances the IP3/PKC/Ca<sup>2+</sup> pathway. The 5-HT<sub>2C</sub> receptor is the only G-protein-coupled receptor that exhibits RNA editing (Burns et al., 2011).

Adenosine-to-inositol RNA editing modifies the efficacy of the 5-HT<sub>2C</sub> receptor and G-protein coupling. In humans, the RNA editing pattern is thought to be associated with anxiety and depression. In 5-HT transporter knockout mice, RNA editing of the 5-HT<sub>2C</sub> receptor is increased in the amygdala (Moya et al., 2011).

### **6.3.5. The 5-HT<sub>3</sub> Receptor**

5-HT<sub>3</sub> receptors are present in high densities in the dorsal vagal complex, but moderate densities are also found in the cerebral cortex, hippocampus, striatum and amygdala (Barnes et al., 2009). The 5-HT<sub>3</sub> receptor family includes multiple isoforms (5HT<sub>3A</sub>-5-HT<sub>3E</sub>) that are products derived from two different genes (Filip and Bader, 2009). 5-HT<sub>3</sub> receptors consist of five subunits forming ligand-gated non-selective cation channels (Na<sup>+</sup> and Ca<sup>2+</sup> influx, K<sup>+</sup> efflux), and their stimulation induces depolarization of the cell membrane. Heteromeric combination of 5-HT<sub>3A</sub> and 5-HT<sub>3B</sub> subunits is needed to construct a fully functional 5-HT<sub>3</sub>

receptor (Davies et al., 1999; Brady et al., 2001). 5-HT<sub>3</sub> receptors are postsynaptic receptors expressed mainly in CCK-positive interneurons in the cerebral cortex and hippocampus (Morales and Bloom, 1997). In the amygdala, the highest density of 5-HT<sub>3</sub> receptors is seen in the LA and BLA (Mascagni and McDonald, 2007). All 5-HT<sub>3</sub> receptor-positive neurons in the BLA are GABA-positive interneurons, but most are negative for all other GABAergic cell markers, suggesting that they constitute a previously unrecognized subpopulation of interneurons (Mascagni and McDonald, 2007).

#### **6.3.6. The 5-HT<sub>4</sub> Receptor**

The nigrostriatal and mesolimbic systems show the highest density of 5-HT<sub>4</sub> receptors, whereas their density is moderate in the septum, islands of Calleja, hippocampus and amygdala (Hannon and Hoyer, 2008). Nine splice variants of 5-HT<sub>4</sub> receptors were reported (5-HT<sub>4A</sub>-5-HT<sub>4H</sub>, 5-HT<sub>4HB</sub>), but they show similar pharmacological properties (Filip and Bader, 2009). 5-HT<sub>4</sub> receptors are expressed in cell bodies and dendrites of pyramidal neurons and interneurons as postsynaptic receptors (Filip and Bader, 2009), which couple to G<sub>s</sub>. Hence their stimulation enhances adenylate cyclase activity, but other signal transduction systems, such as the ERK and PCL/Ca<sup>2+</sup>/calmodulin pathways, are also activated (Bockaert et al., 2008). 5-HT<sub>4</sub> agonists have antidepressant effects, whereas their antagonists are anxiolytic in animal models (Bockaert et al., 2008; Carr and Lucki, 2011), but it is not currently known whether amygdalar 5-HT<sub>4</sub> receptors contribute to these effects.

#### **6.3.7. The 5-HT<sub>6</sub> Receptor**

The density of 5-HT<sub>6</sub> receptors is high in the olfactory system, cerebral cortex, striatum and accumbens, and moderate in the hippocampus, hypothalamus, thalamus, cerebellum and amygdala (Wesołowska, 2010). 5-HT<sub>6</sub> receptors are mainly localized postsynaptically on dendrites and spines but not on cell bodies of pyramidal neurons and interneurons (Gérard et al., 1997; Hamon et al., 1999). 5-HT<sub>6</sub> receptors couple to G<sub>s</sub>, and their stimulation enhances adenylate cyclase. Local injection of a 5-HT<sub>6</sub> antagonist into the hippocampus produced antidepressant and anxiolytic effects (Wesołowska, 2010), but the role of amygdalar 5-HT<sub>6</sub> receptors in emotion was not examined.

#### **6.3.8. The 5-HT<sub>7</sub> Receptor**

5-HT<sub>7</sub> receptors are present in high densities in blood vessels (Terrón and Martínez-García, 2007) and in the cerebral cortex, hippocampus, thalamus, suprachiasmatic nucleus and amygdala (Thomas and Hagan, 2004). Four splice variants of 5-HT<sub>7</sub> receptors were reported (5-HT<sub>7A</sub>-5-HT<sub>7D</sub>), although they all show similar pharmacological properties (Filip and Bader, 2009). Like most 5-HT receptors, 5-HT<sub>7</sub> receptors are expressed postsynaptically on somata and dendrites of pyramidal neurons and interneurons, but they are also found in the terminals of interneurons as presynaptic receptors (Belenky and Pickard, 2001). 5-HT<sub>7</sub> receptors couple to G<sub>s</sub>, and their stimulation enhances adenylate cyclase, but other signal transduction systems, such as ERK and the G<sub>12</sub>/Rho signaling pathway, can also be activated (Leopoldo et al., 2011). Intra-hippocampal injection of a 5-HT<sub>7</sub> antagonist into the hippocampus induced antidepressant and anxiolytic effects (Wesołowska et al., 2006), but currently there is no study focusing on amygdalar 5-HT<sub>7</sub> receptors in emotional regulation.

## 7. PHYSIOLOGY AND PHARMACOLOGY OF THE 5-HTERGIC SYSTEM IN THE BLA

### 7.1. Electrophysiological Characterization of BLA Neurons

Since the LA and BLA receive the heaviest 5-HTergic innervation within the amygdala, and many pharmacological studies have pointed to the importance of the BLA as a target of anxiogenic and anxiolytic drugs, we will focus mainly on BLA neurons. Recent studies broadly divided BLA neurons into two types based on their electrophysiological characterization, using whole cell recording in brain slices combined with morphological examinations: the pyramidal projection neurons, and non-pyramidal interneurons (Washburn and Moises, 1992b; Rainnie et al., 1993; Yajeya et al., 1997; Sah et al., 2003; Kröner et al., 2005). The projection neurons have broad action potentials, prolonged afterhyperpolarization and show spike frequency adaptation, whereas interneurons have narrow action potentials, fast afterhyperpolarization and show little spike frequency adaptation.

According to the firing patterns in response to current injections, projection neurons can be further divided into several subtypes (Washburn and Moises, 1992a; Washburn and Moises, 1992b; Yajeya et al., 1997; Rainnie, 1999; Kröner et al., 2005; Power and Sah, 2008). In many projection neurons, current injection just above the spike threshold induced one or two spikes immediately after current injection, but in some neurons, there was a delay in the onset of firing after current injection (late-firing). By increasing the current amplitude, the number of spikes in these neurons could be increased, but the spike frequency progressively declined (adaptation or accommodation). When adaptation was strong, neurons fired an initial burst or short train of spikes, and then stopped firing during current injection (burst-firing or strong adapting). If the adaptation was not strong, neurons fired continuously during current injection, but interspike intervals slowed gradually (regular-firing) (Figure 7). Some studies have claimed that there were more regular-firing neurons than burst-firing/strong adapting neurons in the BLA (Washburn and Moises, 1992a; Rainnie et al., 1993), but others claimed the opposite (Yajeya et al., 1997; Kröner et al., 2005; Power and Sah, 2008).

Several authors have consistently reported that BLA interneurons show continuous, modest to high frequency (100-200 Hz) firing in response to current injection without signs of adaptation (fast-firing) (Washburn and Moises, 1992a; Rainnie et al., 1993; Kröner et al., 2005) (Figure 7). Rainnie et al. (2006) still classified BLA interneurons of rats into four types based on their firing pattern, namely, type 1) burst-firing; type 2) regular-firing (a brief burst followed by rhythmic firing); type 3) fast-firing (continuous high frequency firing without adaptation); and type 4) stutter-firing (intermittent fast-firing). Woodruff and Sah (2007) also classified BLA interneurons into four types using transgenic mice expressing enhanced green fluorescent protein (EGFP) coupled to the PV promoter (PV-positive interneurons marked by EGFP): type 1) fast spiking; type 2) delayed firer (a brief burst followed by a silent period and fast firing); type 3) accommodating (continuous firing with adaptation); and type 4) stutterer (intermittent fast-firing). They stated that the fast spiking type was the most frequent (47%). Kaneko et al. (2008) classified BLA interneurons into three types using glutamic acid decarboxylase 67 (GAD67)-GFP mice: type A) low frequency spikes (31-56 Hz); type B) modest frequency spikes (59-112Hz) whose amplitude often progressively attenuated; and type C) high frequency spikes (>109 Hz).

## 7.2. Pharmacological Responses of BLA Neurons to 5-HT Ligands

### 7.2.1. Responses to 5-HT<sub>1A</sub> Receptor Agonists

Stein et al. (2000) investigated the responses of BLA neurons to 5-HT-related drugs using extracellular single cell recording in anesthetized rats. Seven percent and 11% of BLA neurons showed excitatory and inhibitory responses to iontophoretic administration of the 5-HT<sub>1A</sub> agonist 8-OH-DPAT, respectively. The 8-OH-DPAT-induced excitatory effect, derived from the stimulation of 5-HT<sub>1A</sub> receptors on interneurons near the recording projection neuron, was blocked by co-administration of the GABA<sub>A</sub> antagonist bicuculline. The inhibitory effect induced by 8-OH-DPAT could be attributed to the direct stimulation to 5-HT<sub>1A</sub> receptors on the recorded projection neuron. In contrast,

Rainnie (1999) reported that inhibitory postsynaptic currents (IPSCs) (inhibitory input from interneurons) in projection neurons were not changed by bath application of 8-OH-DPAT in whole cell recordings from BLA slices. Based on this observation, he rejected the role of 5-HT<sub>1A</sub> receptors on interneurons in the regulation of the activity of projection neurons. Koyama et al. (1999) investigated the GABAergic inhibitory input to projection neurons using mechanically dissociated BLA neurons by recording spontaneous miniature inhibitory postsynaptic currents (mIPSCs) arising from attached GABAergic nerve terminals to projection neurons. Bath application of 8-OH-DPAT decreased the frequency of mIPSCs but did not change their amplitude.

Therefore, they concluded that 5-HT<sub>1A</sub> receptors exist on presynaptic GABAergic terminals, and their stimulation decreases GABA release from terminals. Cheng et al. (1998) indicated that excitatory postsynaptic potentials (EPSPs) were decreased in BLA slices by bath application of 5-HT. Because this effect was achieved without changing the resting membrane potential, but with an increasing ratio of paired pulse facilitation, this effect was thought to derive from a decreased probability of glutamate release from glutamatergic nerve terminals. Moreover, this 5-HT-induced decrease of EPSPs was blocked by the 5-HT<sub>1A</sub> antagonist NAN-190, and administration of 8-OH-DPAT achieved the same effect as 5-HT. They concluded that 5-HT<sub>1A</sub> receptors are expressed on the presynaptic glutamatergic terminals, and their activation decreases glutamate release from terminals. This is consistent with findings of Aznar et al. (2003), who showed that 5-HT<sub>1A</sub> receptors are expressed by both glutamatergic projection neurons and GABAergic interneurons in the BLA, although currently there is no direct morphological evidence for the presence of 5-HT<sub>1A</sub> receptors in presynaptic terminals of these neurons.

From these results, it may be hypothesized that the activation of 5-HT<sub>1A</sub> receptors on projection neurons inhibits the excitatory output of the BLA, while the activation of 5-HT<sub>1A</sub> receptors on interneurons results in a disinhibition of the BLA (Figure 8). However, it cannot be predicted which effect predominates, the inhibitory effect via 5-HT<sub>1A</sub> receptors on projection neurons or the disinhibition of BLA projection neurons via 5-HT<sub>1A</sub> receptors on interneurons.

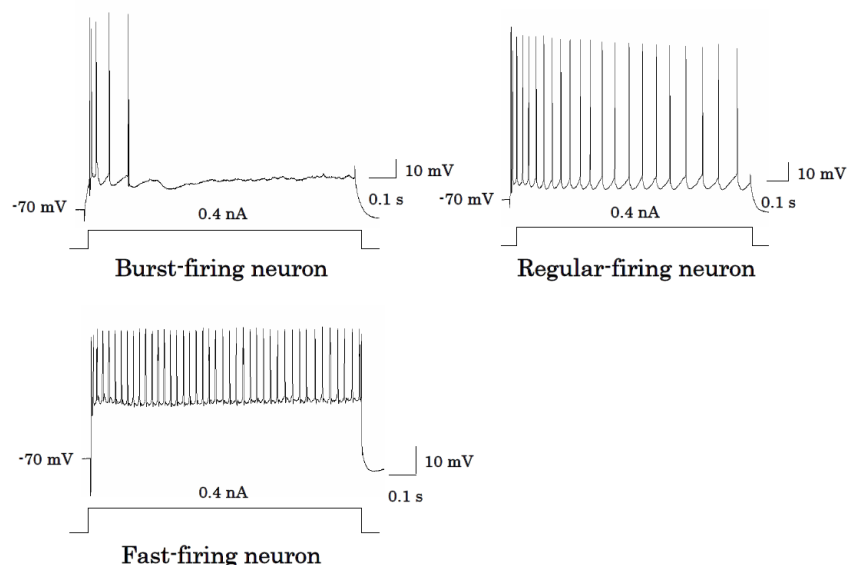


Figure 7. Representative firing patterns of BLA neurons to current injection. This figure is the authors' own figure.

### 7.2.2. Responses to 5-HT<sub>2A/2C</sub> Receptor Agonists

In extracellular single cell recordings, BLA neurons showed an excitatory response to iontophoretic administration of the 5-HT<sub>2A/2C</sub> agonist DOI (Stein et al., 2000). In contrast, most neurons showing inhibitory responses to DOI were found in the CoA and MeA. Because the DOI-induced inhibitory effect was blocked by co-administration of the GABA<sub>A</sub> antagonist bicuculline, this inhibitory effect was derived from stimulation of 5-HT<sub>2A/2C</sub> receptors on GABAergic neurons located next to the projection neurons recorded.

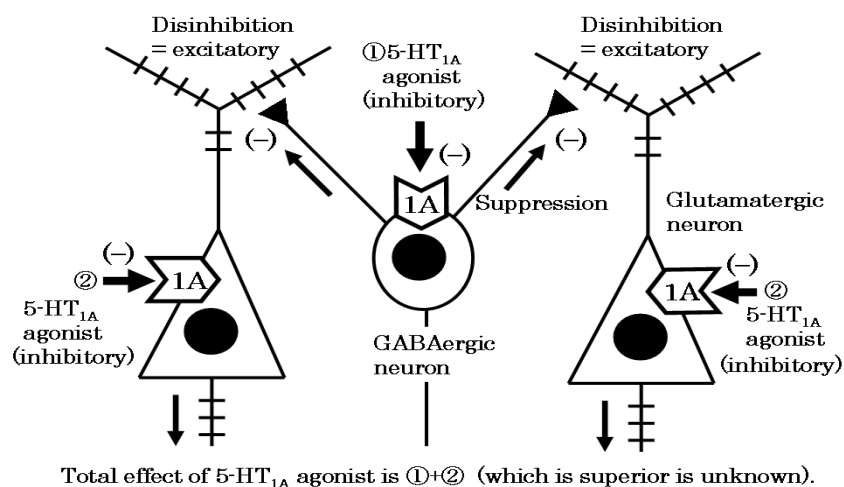


Figure 8. Effect of 5-HT<sub>1A</sub> agonists in the BLA. 5-HT<sub>1A</sub> receptor stimulation reduces cAMP levels and inhibits the target neuron. Inhibition of the GABAergic neuron results in a disinhibition of the projection neuron, whereas direct activation of 5-HT<sub>1A</sub> receptors on projection neurons reduces their activity.

In the BLA, however, DOI may have directly stimulated 5-HT<sub>2A/2C</sub> receptors located on the projection neurons recorded, resulting in excitatory effects. Rainnie (1999) reported that inhibitory post-synaptic potentials (IPSPs) and IPSCs in projection neurons initially increased in frequency, and subsequently decreased in amplitude upon bath application of the non-selective 5-HT<sub>2</sub> agonist  $\alpha$ -methyl-5-HT in whole cell recordings of BLA slices. Jiang et al. (2009) demonstrated that IPSCs were enhanced in frequency and amplitude in projection neurons by  $\alpha$ -methyl-5-HT in whole cell recording of BLA slices, and this effect was blocked by co-administration of a 5-HT<sub>2A</sub> antagonist, but not a 5-HT<sub>2B</sub> or 5-HT<sub>2C</sub> antagonist. Therefore, 5-HT<sub>2A/2C</sub> receptors seem to be expressed by both glutamatergic projection neurons and GABAergic interneurons in the BLA. The stimulation of 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub> receptors on projection neurons probably increases the excitability of the BLA, whereas the stimulation of 5-HT<sub>2A</sub> receptors on interneurons has the opposite effect (Figure 9). In anatomical investigations, 5-HT<sub>2A</sub> receptors are found on both pyramidal neurons and interneurons in the BLA (McDonald and Mascagni, 2007; Bombardi et al., 2011), but the cell type-specific distribution of 5-HT<sub>2C</sub> receptors is unknown. Moreover, as described for 5-HT<sub>1A</sub> receptors, it is unclear whether the activation of 5-HT<sub>2A</sub> and/or 5-HT<sub>2C</sub> receptors facilitates the excitation (via 5-HT<sub>2A/2C</sub> receptors on projection neurons) or inhibition (via 5-HT<sub>2A</sub> receptors on interneurons) of the BLA.

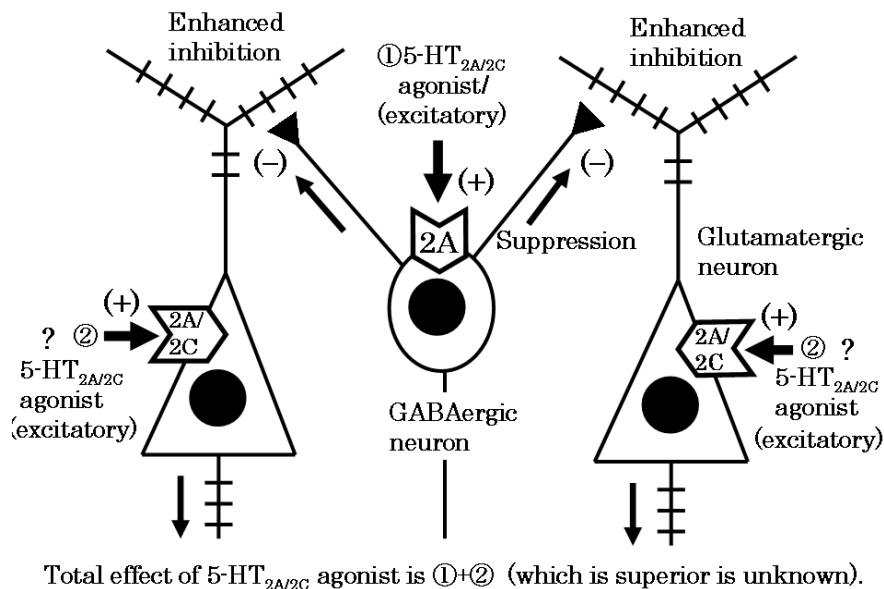


Figure 9. Effect of 5-HT<sub>2A/2C</sub> agonists in the BLA. 5-HT<sub>2A</sub> receptor stimulation increases cAMP levels and excites the target neuron. Excitation of the GABAergic neuron results in inhibition of projection neurons, whereas direct activation of 5-HT<sub>2A</sub> receptors on projection neurons enhances their activity.

### 7.2.3. Responses to 5-HT<sub>3</sub> Receptor Agonists

Like 5-HT<sub>1A</sub> and 5-HT<sub>2A/2C</sub> agonists, iontophoretic application of the 5-HT<sub>3</sub> agonist 2-methyl-5-HT produced both excitatory and inhibitory effects in extracellular single cell recordings in the BLA (Stein et al., 2000). The excitatory effect was attributed to the stimulation of 5-HT<sub>3</sub> receptors on projection neurons, and the inhibitory effect to the stimulation of 5-HT<sub>3</sub> receptors on interneurons. Koyama et al. (2000; 2002) suggested that 5-



HT<sub>3</sub> receptors enhance GABA release from GABAergic nerve terminals. In anatomical investigations, 5-HT<sub>3</sub> receptors were found only in a specific subclass of GABAergic interneurons in the BLA (Mascagni and McDonald, 2007).

### 7.3. Activity of Glutamatergic Projection Neurons in BLA and Anxiety

Both glutamatergic neurons and GABAergic neurons in the BLA (Izumi et al., 2011), and GABAergic neurons in the ICN (Izumi et al., 2011) and CeA (Gozzi et al., 2010) are activated in fear-conditioning paradigms. Tye et al. (2011) indicated that the fear intensity depends on the final output of CeA, which is under the control of inter-CeA inhibitory projections (from centrolateral nucleus to centromedial nucleus) and excitatory projections from the BLA to the CeA. Moreover, the BLA can inhibit the CeA by indirect pathways by activating the GABAergic neurons in the ICN (Figure 10) (Busti et al., 2011).

Thus, glutamatergic neurons in the BLA could have dual roles: they can be “anxiogenic” by directly increasing the CeA output, and “anxiolytic” by decreasing the CeA output through indirect routes. Although the local injection of a 5-HT<sub>1A</sub> agonist into the BLA decreased anxiety (Li et al., 2006; Matsuzaki et al., 2011), there are two possibilities for how this effect may be achieved in the BLA: (i) the 5-HT<sub>1A</sub> agonist may inhibit “anxiogenic” glutamatergic projection neurons through activation of 5-HT<sub>1A</sub> receptors; or (ii) the 5-HT<sub>1A</sub> agonist may stimulate 5-HT<sub>1A</sub> receptors on GABAergic neurons, which causes a disinhibition of “anxiolytic” glutamatergic projection neurons. The dual role of 5-HT on projection neurons and interneurons in the BLA may also apply to the effects mediated by 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub> and 5-HT<sub>3</sub> receptors.

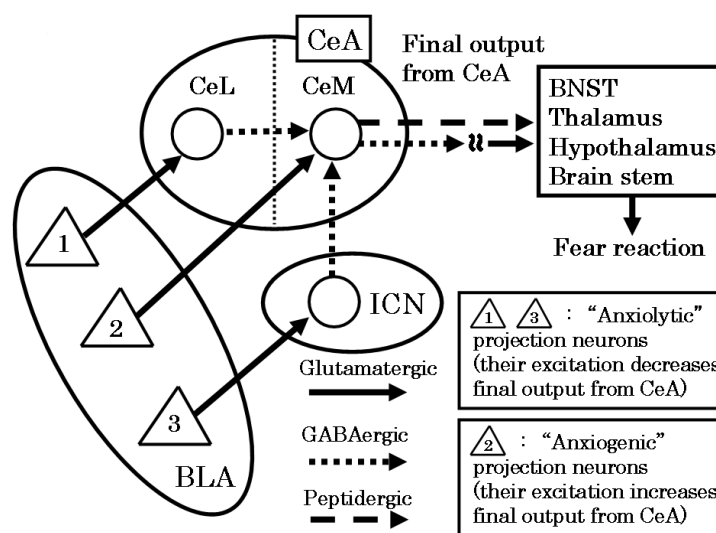


Figure 10. Activities of glutamatergic projection neurons in BLA and anxiety. BLA, basolateral nucleus of the amygdala; BNST, bed nucleus of stria terminalis; CeA, central nucleus of the amygdala; CeL, centrolateral nucleus; CeM, centromedial nucleus; ICN, intercalated nucleus of the amygdala.

## CONCLUSION

The main focus of this chapter is the anxiolytic mechanism of SSRIs. From the descriptions above, it is concluded that the BLA is a major target of SSRIs. Local injection of 5-HT<sub>1A</sub> agonists into the BLA produced anxiolytic effects, whereas local injection of 5-HT<sub>2C</sub> and 5-HT<sub>3</sub> antagonists and systemic administration of 5-HT<sub>4</sub>, 5-HT<sub>6</sub> and 5-HT<sub>7</sub> antagonists produced anxiolytic effects. As SSRIs are known to increase extracellular 5-HT levels, and their injection into the amygdala reduces conditioned fear, it is suggested that the increased 5-HT concentration in the BLA may stimulate 5-HT<sub>1A</sub> receptors on glutamatergic projection neurons or GABAergic interneurons in the BLA, but the overall output of the amygdala decreases. Another possibility is that 5-HT levels increased by SSRIs activate 5-HT<sub>2A</sub> receptors on GABAergic interneurons in the BLA. Moreover, the 5-HTergic neural system in the amygdala may be relevant in depression and aggression, and several subtypes of 5-HT receptors could be targets of new drugs for these disorders/traits.

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## REFERENCES

- Anderson, I. A., Del-Ben, C. M., McKie, S., Richardson, P., Williams, S. R., Elliot, R., Deakin, J. F. W. (2007). Citalopram modulation of neuronal responses to aversive face emotions: a functional MRI study. *Neuroreport*, 18, 1351-1355.
- Anderson, I. A., Juhasz, G., Thomas, E., Downey, D., McKie, S., Deakin, J. F. W., Elliot, R. (2011). The effect of acute citalopram on face emotion processing in remitted depression: a pharmacMRI study. *Eur. Neuropsychopharmacol.*, 21, 140-148.
- Arce, E., Simmons, A. N., Lovero, K. L., Stein, M. B., Paulus, M. P. (2008). Escalation effects on insula and amygdala BOLD activation during emotional processing. *Psychopharmacology*, 196, 661-672.
- Aznar, S., Qian, Z., Shah, R., Rahbek, B., Knudsen, G. M. (2003). The 5-HT<sub>1A</sub> serotonin receptor is located on calbindin- and parvalbumin-containing neurons in the rat brain. *Brain Res.*, 395, 58-67.
- Barnes, N. M., Hales, T. G., Lummis, S. C., Peters, J. A. (2009). The 5-HT<sub>3</sub> receptor--the relationship between structure and function. *Neuropharmacology*, 56, 273-284.
- Belenky, M. A., Pickard, G. E. (2001). Subcellular distribution of 5-HT<sub>1B</sub> and 5-HT<sub>7</sub> receptors in the mouse suprachiasmatic nucleus. *J. Comp. Neurol.*, 432, 371-388.
- Benes, F. (2010). Amygdalocortical circuitry in schizophrenia: from circuits to molecules. *Neuropsychopharmacology REVIEWS*, 35, 239-257.

- Bhagwagar, Z., Rabiner, E. A., Sargent, P. A., Grasby, P. M., Cowen, P. J. (2004). Persistent reduction in brain serotonin<sub>1A</sub> receptor binding in recovered depressed men measured by positron emission tomography with [<sup>11</sup>C]WAY-100635. *Mol. Psychiatry*, 9, 386-392.
- Bigos, K. L., Pollock, B. G., Aizenstein, H. J., Fisher, P. M., Bies, R. R., Hariri, A. R. (2008). Acute 5-HT reuptake blockade potentiates human amygdala reactivity. *Neuropsychopharmacology*, 33, 3221-3225.
- Blackburn, T. P., Baxter, G. S., Kennett, G. A., King, F. D., Piper, D. C., Sanger, G. J., Thomas, D. R., Upton, N., Wood, M. D. (1993). BRL 46470: a highly potent, selective and long acting 5-HT<sub>3</sub> receptor antagonist with anxiolytic-like properties. *Psychopharmacology*, 110, 257-264.
- Blair, R. J. R. (2010). Neuroimaging of psychopathy and antisocial behavior: a targeted review. *Curr. Psychiatry Rep.*, 12, 76-82.
- Bockaert, J., Claeysen, S., Compan, V., Dumuis, A. (2008). 5-HT<sub>4</sub> receptors: history, molecular pharmacology and brain functions. *Neuropharmacology*, 55, 922-931.
- Bombardi, C. (2011). Distribution of 5-HT<sub>2A</sub> receptor immunoreactivity in the rat amygdalar complex and co-localization with  $\gamma$ -amino butyric acid. *Brain Res.*, 1370, 112-128.
- Bosker, F. J., Cremers, T. I. F. H., Jongsma, M. E., Westerink, B. H. C., Wikström, H. V., den Boer, J. A. (2001). Acute and chronic effects of citalopram on postsynaptic 5-hydroxytryptamine<sub>1A</sub> receptor-mediated feedback: a microdialysis study in the amygdala. *J. Neurochem.*, 76, 1645-1653.
- Brady, C. A., Stanford, I. M., Ali, I., Lin, L., Williams, J. M., Dubin, A. E., Hope, A. G., Barnes, N. M. (2001). Pharmacological comparison of human chromomeric 5-HT<sub>3A</sub> receptors versus heterogenic 5-HT<sub>3A/3B</sub> receptors. *Neuropharmacology*, 41, 282-284.
- Burns, C. M., Chu, H., Rueter, S. M., Hutchinson, L. K., Canton, H., Sanders-Bush, E., Emeson, R. B. (2011). Regulation of serotonin-2C receptor G-protein coupling by RNA editing. *Brain Res.*, 1370, 112-128.
- Busti, D., Geracitano, R., Whittle, N., Daezios, Y., Mañko, M., Kaufmann, W., Sätzler, K., Singewald, N., Capogna, M., Ferraguti, F. (2011). Different fear stage engage distinct networks within the intercalated cell clusters of the amygdala. *J. Neurosci.*, 31, 5131-5144.
- Campbell, B. M., Merchant, K. M. (2003). Serotonin 2C receptors within the basolateral amygdala induce acute fear-like responses in an open-field environment. *Brain Res.*, 993, 1-9.
- Carr, G. V., Lucki, I. (2011). The role of serotonin receptor subtypes in treating depression: a review of animal studies. *Psychopharmacology*, 213, 265-287.
- Cases, O., Seif, I., Grimsby, J., Gaspar, P., Chen, K., Pournin, S., Müller, U., Aguet, M., Babinet, C., Shih, J. C., De Mayer, E. (1995). Aggressive behavior and altered amounts of brain serotonin and nor epinephrine in mice lacking MAOA. *Science*, 268, 1763-1766.
- Chalmers, D. T., Watson, S. J. (1991). Comparative anatomical distribution of 5-HT<sub>1A</sub> receptor mRNA and 5-HT<sub>1A</sub> binding in rat brain--a combined in situ hybridization/in vitro receptor auto radiographic study. *Brain Res.*, 561, 51-60.

- Chen, Y., Suckling, J., Obi, C., Fu, C. H., Williams, S. C., Walsh, N. D., Mitterschiffthaler, M. T., Pich, E. M., Bullmore, E. (2008). Functional coupling of the amygdala in depressed patients treated with antidepressant medication. *Neuropsychopharmacology*, 33, 1909-1918.
- Chen, Y., Wan, H. I., O'Reardon, J. P., Wang, D. J. J., Wang, Z., Korczykowski, M., Detre, J. A. (2011). Quantification of cerebral blood flow as biomarker of drug effect: arterial spin labeling pHMRI after a single dose of oral citalopram. *Clin. Pharmacol. Ther.*, 89, 251-258.
- Cheng, L. L., Wang, S. J., Gean, P. W. (1998). Serotonin depresses excitatory synaptic transmission and depolarization-evoked Ca<sup>2+</sup> influx in rat basolateral amygdala via 5-HT<sub>1A</sub> receptors. *Eur. J. Neurosci.*, 10, 2163-2172.
- Christianson, J. P., Ragole, T., Amat, J., Greenwood, B. N., Strong, P. V., Paul, E. D., Fleshner, M., Watkins, L. R., Maier, S. F. (2010). 5-hydroxytryptamine 2C receptors in the basolateral amygdala are involved in the expression of anxiety after uncontrollable traumatic stress. *Biol. Psychiatry*, 67, 339-345.
- Clark, L., Chamberlain, S. R., Sahakian, B. J. (2009). Neurocognitive mechanisms in depression: implications for treatment. *Annu. Rev. Neurosci.*, 32, 57-74.
- Clemett, D. A., Punhani, T., Duxon, M. S., Blackburn, T. P., Fone, K. C. (2000). Immunohistochemical localisation of the 5-HT<sub>2C</sub> receptor protein in the rat CNS. *Neuropharmacology*, 39, 123-132.
- Cools, R., Calder, A. J., Lawrence, A. D., Clarke, L., Bullmore, E., Robbins, T. W. (2005). Individual differences in threat sensitivity predict serotonergic modulation of amygdala response to fearful faces. *Psychopharmacology*, 180, 670-679.
- Costall, B., Domeney, A. M., Kelly, M. E., Tomkins, D. M., Naylor, R. J., Wong, E. H., Smith, W. L., Whiting, R. L., Eglen, R. M. (1993). The effect of the 5-HT<sub>3</sub> receptor antagonist, RS-42358-197, in animal models of anxiety. *Eur. J. Pharmacol.*, 234, 91-99.
- Dannlowski, U., Ohrmann, P., Bauer, J., Kugel, H., Arolt, V., Heindel, W., Suslow, T. (2007a). Amygdala reactivity predicts automatic negative evaluations for facial emotions. *Psychiatry Research: Neuroimaging*, 154, 13-20.
- Dannlowski, U., Ohrmann, P., Bauer, J., Kugel, H., Baune, B. T., Hohoff, C., Kersting, A., Arolt, V., Heindel, W., Deckert, J., Suslow, T. (2007b). Serotonergic genes modulates amygdala activity in major depression. *Genes Brain Behav.*, 6, 672-676.
- Dannlowski, U., Ohrmann, P., Bauer, J., Deckert, J., Hohoff, C., Kugel, H., Arolt, V., Heindel, W., Kersting, A., Baune, B. T., Suslow, T. (2008). 5-HTTLPR biases amygdala activity in response to masked facial expressions in major depression. *Neuropsychopharmacology*, 33, 418-424.
- Davies, P. A., Pistis, M., Hanna, M. C., Peters, J. A., Lambert, J. J., Hales, T. G., Kirkness, E. F. (1999). The 5-HT<sub>3B</sub> subunit is a major determinant of serotonin-receptor function. *Nature*, 397, 359-363.
- Davis, M. (1997) Neurobiology of fear responses: the role of the amygdala. *J. Neuropsychiatry Clin. Neurosci.*, 9, 382-402
- Del-Ben, C. M., Deakin, J. F. W., McKie, S., Delvai, N. A., Williams, S. R., Elliot, R., Dolan, M., Anderson, I. (2005). The effect of citalopram pretreatment on neuronal responses to neuropsychological tasks in normal volunteers: an fMRI study. *Neuropsychopharmacology*, 30, 1724-1734.

- De Mello Cruz, A. P., Pinheiro, G., Alves, S. H., Ferreira, G., Mendes, M., Faria, L., Macedo, C. E., Motta, V., Landeira-Fernande, J. (2005). Behavioral effects of systemically administered MK-212 are prevented by ritanserin microinfusion into the basolateral amygdala of rats exposed to the elevated plus-maze. *Psychopharmacology*, 182, 345-354.
- Ehrlich, I., Humeau, Y., Grenier, F., Ciocchi, S., Herry, C., Lüthi, A. (2009). Amygdala inhibitory circuit and the control of conditioned fear memory. *Neuron*, 62, 757-771.
- Elliot, R., Zahn, R., Deakin, J. F. W., Anderson, I. M. (2011). Affective cognition and its distribution in mood disorders. *Neuropsychopharmacology REVIEWS*, 36, 153-182.
- Engel, K., Bandelow, B., Gruber, O., Wedekind, D. (2009). Neuroimaging in anxiety disorders. *J. Neural. Transm.*, 116, 703-716.
- Engin, E., Treit, D. (2008). The effects of intra-cerebral drug infusions on animals' unconditioned fear reactions: a systematic review. *Prog Neuropsychopharmacol. Biol. Psychiatry*, 32, 1399-1419.
- Etkin, A., Wager, T. D. (2007). Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am. J. Psychiatry*, 164, 1476-1488.
- Fales, C. L., Barch, D. M., Rundle, M. M., Mintun, M. A., Mathews, J., Snyder, A. Z., Sheline, Y. I. (2009). Antidepressant treatment normalizes hypoactivity in dorsolateral prefrontal cortex during emotional interference processing in major depression. *J. Affect. Disord.*, 112, 206-211.
- Fallon, J. H., Ciofi, P. Distribution of Monoamines within the amygdala. In: Aggleton, J. P., editor. *The Amygdala: neurobiological aspects of emotion, memory, and mental dysfunction*. New York: Wiley-Liss; 1992; pp.97-114.
- Fanselow, M. S. (1980). Conditioned and unconditioned components of postshock freezing. *Pavlovian J. Biol. Sc.*, 15, 177-182.
- Ferrari, P. F., Palanza, P., Parmigiani, S., de Almeida, R. M., Miczek, K. A. (2005). Serotonin and aggressive behavior in rodents and nonhuman primates: predispositions and plasticity. *Eur. J. Pharmacol.*, 526, 259-273.
- File, S. E., James, T. A., MacLeod, N. K. (1981). Depletion in amygdalar 5-hydroxytryptamine concentration and changes in social and aggressive behaviour. *J. Neural. Transm.*, 50, 1-12.
- File, S. E. (1985). Animal models for predicting clinical efficacy of anxiolytic drugs: social behaviour. *Neuropsychobiology*, 13, 55-62.
- Filip, M., Bader, M. (2009). Overview on 5-HT receptors and their role in physiology and pathology of the central nervous system. *Pharmacol. Rep.*, 61, 761-777.
- Frankle, W. G., Lombardo, I., New, A. S., Goodman, M., Talbot, P. S., Huang, Y., Hwang, D. R., Slifstein, M., Curry, S., Abi-Dargham, A., Laruelle, M., Siever, L. J. (2005). Brain serotonin transporter distribution in subjects with impulsive aggressivity: a positron emission study with [<sup>11</sup>C]McN 5652. *Am. J. Psychiatry*, 162, 915-923.
- Freitas-Ferrari, M. C., Hallak, J. E. C., Trzesniak, C., Filho, A. S., Machado-de-Sousa, J. P., Chagas, M. H. N., Nardi, A. E., Crippa, J. A. S. (2010). Neuroimaging in social anxiety disorder: a systematic review of the literature. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, 34, 565-580.

- Fu, C. H., Williams, S. C., Cleare, A. J., Brimmer, M. J., Walsh, N. D., Kim, J., Andrew, C. M., Pich, E. M., Williams, P. M., Reed, L. J., Mitterschiffthaler, M. T., Suckling, J., Bullmore, E. T. (2004). Attenuation of the neural response to sad faces in major depression by antidepressant treatment: a prospective, event-related functional magnetic resonance imaging study. *Arch. Gen. Psychiatry*, 61, 877-889.
- Fujiwara, M., Ibi, N., Kataoka, Y., Ueki, S. (1980). Effects of psychotropic drugs on delta 9-tetrahydrocannabinol-induced long muricide. *Psychopharmacology*, 68, 7-13.
- Furmark, T., Tillfors, M., Garpenstrand, H., Marteinsdottir, I., Långström, B., Oreland, L., Fredrikson, M. (2004). Serotonin transporter polymorphism related to amygdala excitability and symptom severity in patients with social phobia. *Neurosci. Lett.*, 27, 189-192.
- Fusar-Poli, P., Allen, P., Lee, F. (2007). Modulation of neural response to happy and sad faces by acute tryptophan depletion. *Psychopharmacology*, 193, 31-44.
- Gérard, C., Martres, M. P., Lefèvre, K., Miquel, M. C., Vergé, D., Lanfumey, L., Doucet, E., Hamon, M., el Mestikawy, S. (1997). Immuno-localization of serotonin 5-HT<sub>6</sub> receptor-like material in the rat central nervous system. *Brain Res.*, 746, 207-219.
- Gonzalez, L. E., Andrews, N., File, S. E. (1996). 5-HT<sub>1A</sub> and benzodiazepine receptors in the basolateral amygdala modulate anxiety in the social interaction test, but not in the elevated plus-maze. *Brain Res.*, 732, 145-153.
- Gozzi, A., Jain, A., Giovanelli, A., Bertollini, C., Crestan, V., Schwarz, A. J., Tsetsenis, T., Ragozzino, D., Gross, C. T., Bifone, A. (2010). A neural switch for active and passive fear. *Neuron*, 67, 656-666.
- Hajszan, T., Dow, A., Warner-Schmidt, J. L., Szigeti-Buck, K., Sallam, N. L., Parducz, A., Leranath, C., Duman, R. S. (2009). Remodeling of hippocampal spine synapses in the rat learned helplessness model of depression. *Biol. Psychiatry*, 65, 392-400.
- Hamon, M., Doucet, E., Lefèvre, K., Miquel, M. C., Lanfumey, L., Insausti, R., Frechilla, D., Del Rio, J., Vergé, D. (1999). Antibodies and antisense oligonucleotide for probing the distribution and putative functions of central 5-HT<sub>6</sub> receptors. *Neuropsychopharmacology*, 21 (2 Suppl.), 68S-76S.
- Hannon, J., Hoyer, D. (2008). Molecular biology of 5-HT receptors. *Behav. Brain Res.*, 195, 198-213.
- Harmer, C. J., Mackay, C. E., Reid, C. B., Cowen, P. J., Goodwin, G. M. (2006). Antidepressant drug treatment modifies the neural processing of nonconscious threat cues. *Biol. Psychiatry*, 59, 816-820.
- Hashimoto, S., Inoue, T., Koyama, T. (1996). Serotonin reuptake inhibitors reduce conditioned fear stress-induced freezing behavior in rats. *Psychopharmacology*, 123, 182-186.
- Hashimoto, S., Inoue, T., Koyama, T. (1997). Effects of the co-administration of 5-HT<sub>1A</sub> receptor antagonists with an SSRI in conditioned fear stress-induced freezing behavior. *Pharmacology Biochem. Behav.*, 58, 471-475.
- Herry, C., Ciocchi, S., Senn, V., Demmou, L., Müller, C., Lüthi, A. (2008). Switching on and off fear by distinct neuronal circuits. *Nature*, 454, 589-590.
- Higgins, G. A., Jones, B. J., Oakley, N. R., Tyers, M. B. (1991). Evidence that the amygdala is involved in the disinhibitory effects of 5-HT<sub>3</sub> receptor antagonists. *Psychopharmacology*, 104, 545-551.

- Hirvonen, J., Karlsson, H., Kajander, J., Lepola, A., Markkula, J., Rasi-Hakala, H., Nagren, K., Salminen, J. K., Hietala, J. (2008). Decreased brain serotonin 5-HT<sub>1A</sub> receptor availability in medication-naïve patients with major depressive disorder: an in-vivo imaging study using PET and [carbonyl-<sup>11</sup>C]WAY-100635. *Int. J. Neuropsychopharmacol.*, 11, 465-476.
- Homberg, J. R., Olivier, J. D., Blom, T., Arentsen, T., van Brunshot, C., Schipper, P., Korte-Bouws, G., van Luitelaar, G., Reneman, L. (2011). Fluoxetine exerts age-dependent effects on behavior and amygdala neuroplasticity in the rat. *PLoS One*, 6, e16646.
- Inoue, T., Li, X. B., Abekawa, T., Kitaichi, Y., Izumi, T., Nakagawa, S., Koyama, T. (2004). Selective serotonin reuptake inhibitor reduces conditioned fear through its effect in the amygdala. *Eur. J. Pharmacol.*, 479, 311-316.
- Izumi, T., Inoue, T., Kitaichi, Y., Nakagawa, S., Koyama, T. (2006). Target brain sites of the anxiolytic effect of citalopram, a selective serotonin reuptake inhibitor. *Eur. J. Pharmacol.*, 534, 129-132.
- Izumi, T., Boku, S., Shinmin, W., Inoue, T., Konno, K., Yamaguchi, T., Yoshida, T., Matsumoto, M., Watanabe, M., Koyama, T., Yoshioka, M. (2011). Retrieval of conditioned fear activates the basolateral and intercalated nucleus of amygdala. *J. Neurosci. Res.*, 89, 773-790.
- Jiang, X., Xing, G., Yang, C., Verma, A., Zhang, L., Li, H. (2009). Stress impairs 5-HT<sub>2A</sub> receptor-mediated serotonergic facilitation of GABA release in juvenile rat basolateral amygdala. *Neuropsychopharmacology*, 34, 410-423.
- Jin, Y., Lim, C. M., Kim, S. W., Park, J. Y., Seo, J. S., Han, P. L., Yoon, S. H., Lee, J. K. (2009). Fluoxetine attenuates kainic acid-induced neuronal cell death in the mouse hippocampus. *Brain Res.*, 1281, 108-116.
- Johnson, S. A., Wang, J. F., Sun, X., McEwen, B. S., Chattarji, S., Young, L. T. (2009). Lithium treatment prevents stress-induced dendritic remodeling in the rodent amygdala. *Neuroscience*, 163, 34-39.
- Kaneko, K., Tamamaki, N., Owada, H., Kakizaki, T., Kume, N., Totsuka, M., Yamamoto, T., Yawo, H., Yagi, T., Obata, K., Yanagawa, Y. (2008). Noradrenergic excitation of a subpopulation of GABAergic cells in the basolateral amygdala via both activation of nonselective cationic conductance and suppression of resting K<sup>+</sup> conductance: a study using glutamate decarboxylase 67-green fluorescent protein knock-in mice. *Neuroscience*, 157, 781-797.
- Kawahara, H., Yoshida, M., Yokoo, H., Nishi, M., Tanaka, M. (1993). Psychological stress increases serotonin release in the rat amygdala and prefrontal cortex assessed by in vivo microdialysis. *Neurosci. Lett.*, 162, 81-84.
- Kempainen, S., Pitkänen, A. (2000). Distribution of parvalbumin, calretinin, and calbindin-D(28k) immunoreactivity in the rat amygdalar complex and colocalization with gamma-aminobutyric acid. *J. Comp. Neurol.*, 426, 441-467.
- Kennett, G. A., Bright, F., Trail, B., Blackburn, T. P., Sanger, G. J. (1997). Anxiolytic-like actions of the selective 5-HT<sub>4</sub> receptor antagonists SB 204070A and SB 207266A in rats. *Neuropharmacology*, 36, 707-712.
- Khawaja, X. (1995). Quantitative autoradiographic characterisation of the binding of [<sup>3</sup>H]WAY-100635, a selective 5-HT<sub>1A</sub> receptor antagonist. *Brain Res.*, 673, 217-225.

- Kitaichi, Y., Inoue, T., Nakagawa, S., Boku, S., Kakuta, A., Izumi, T., Koyama, T. (2010). Sertraline increases extracellular levels not only of serotonin, but also of dopamine in the nucleus accumbens and striatum of rats. *Eur. J. Pharmacol.*, 647, 90-96.
- Koyama, S., Kubo, C., Rhee, J. S., Akaike, N. (1999). Presynaptic serotonergic inhibition of GABAergic synaptic transmission in mechanically dissociated rat basolateral amygdala neurons. *J. Physiol.*, 518 (Pt 2), 525-538.
- Koyama, S., Matsumoto, N., Kubo, C., Akaike, N. (2000). Presynaptic 5-HT<sub>3</sub> receptor-mediated modulation of synaptic GABA release in the mechanically dissociated rat amygdala neurons. *J. Physiol.*, 529 (Pt 2), 373-383.
- Koyama, S., Matsumoto, N., Murakami, N., Kubo, C., Nabekura, J., Akaike, N. (2002). Role of presynaptic 5-HT<sub>1A</sub> and 5-HT<sub>3</sub> receptors in modulation of synaptic GABA transmission in dissociated rat basolateral amygdala neurons. *Life Sci.*, 72, 375-387.
- Kröner, S., Rosenkranz, J. A., Grace, A. A., Barrionuevo, G. (2005). Dopamine modulates excitability of basolateral amygdala neurons in vitro. *J. Neurophysiol.*, 93, 1598-1610.
- Kuramochi, M., Nakamura, S. (2009). Effects of postnatal isolation rearing and antidepressant treatment on the density of serotonergic and noradrenergic axons and depressive behavior in rats. *Neuroscience*, 163, 448-455.
- Lanzenberger, R. R., Mitterhauser, M., Spindelegger, C., Wadsak, W., Klein, N., Mien, L. K., Holik, A., Attarbaschi, T., Mossaheb, N., Sacher, J., Geiss-Granadia, T., Kletter, K., Kasper, S., Tauscher, J. (2007). Reduced serotonin-1A receptor binding in social anxiety disorder. *Biol. Psychiatry*, 61, 1081-1089.
- LeDoux, J. E. The amygdala and emotion: a view through fear. In Aggleton, JP, editor. *The Amygdala: a Functional Analysis* (2<sup>nd</sup> edition). New York: Oxford University Press; 2000; pp.289-310.
- Leopoldo, M., Lacivita, E., Berardi, F., Perrone, R., Hedlund, P. B. (2011). Serotonin 5-HT<sub>7</sub> receptor agents: Structure-activity relationships and potential therapeutic applications in central nervous system disorders. *Pharmacol. Ther.*, 129, 120-148.
- Li, Q. H., Nakadate, K., Tanaka-Nakadate, S., Nakatsuka, D., Cui, Y., Watanabe, Y. (2004). Unique expression patterns of 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors in the rat brain during postnatal development: Western blot and immunohistochemical analyses. *J. Comp. Neurol.*, 469, 128-140.
- Li, R., Nishijo, H., Wang, Q., Uwano, T., Tamura, R., Ohtani, O., Ono, T. (2001). Light and electron microscopic study of cholinergic and noradrenergic elements in the basolateral nucleus of the rat amygdala: evidence for interactions between the two systems. *J. Comp. Neurol.*, 439, 411-425.
- Li, X., Inoue, T., Abekawa, T., Weng, S., Nakagawa, S., Izumi, T., Koyama, T. (2006). 5-HT<sub>1A</sub> receptor agonist affects fear conditioning through stimulations of the postsynaptic 5-HT<sub>1A</sub> receptors in the hippocampus and amygdala. *Eur. J. Pharmacol.*, 532, 74-80.
- Maren, S., Fanselow, M. S. (1995). Synaptic plasticity in the basolateral amygdala induced by hippocampal formation stimulation in vivo. *J. Neurosci.*, 15, 7548-7564.
- Mascagni, F., McDonald, A. J. (2003). Immunohistochemical characterization of cholecystokinin containing neurons in the rat basolateral amygdala. *Brain Res.*, 976, 171-182.



- Mascagni, F., McDonald, A. J. (2007). A novel subpopulation of 5-HT type 3A receptor subunit immunoreactive interneurons in the rat basolateral amygdala. *Neuroscience*, 144, 1015-102.
- Matsuzaki, H., Izumi, T., Horinouchi, T., Boku, S., Inoue, T., Yamaguchi, T., Yoshida, T., Matsumoto, M., Togashi, H., Miwa, S., Koyama, T., Yoshioka, M. (2011). Juvenile stress attenuates the dorsal hippocampal postsynaptic 5-HT<sub>1A</sub> receptor function in adult rats. *Psychopharmacology*, 214, 329-337.
- McDonald, A. J. (1982). Neurons of the lateral and basolateral amygdalar nuclei: a Golgi study in the rat. *J. Comp. Neurol.*, 212, 293-312.
- McDonald, A. J. (1985). Immunohistochemical identification of gamma-aminobutyric acid-containing neurons in the rat basolateral amygdala. *Neurosci. Lett.*, 53, 203-207.
- McDonald, A. J. Cell types and intrinsic connections of the amygdala. In: Aggleton, J. P., editor. *The Amygdala: neurobiological aspects of emotion, memory, and mental dysfunction*. New York: Wiley-Liss; 1992a; pp.67-96.
- McDonald, A. J. (1992b). Projection neurons of the basolateral amygdala: a correlative Golgi and retrograde tract tracing study. *Brain Res. Bull.*, 28, 179-185.
- McDonald, A. J., Muller, J. F., Mascagni, F. (2002). GABAergic innervation of alpha type II calcium/calmodulin-dependent protein kinase immunoreactive pyramidal neurons in the rat basolateral amygdala. *J. Comp. Neurol.*, 446, 199-218.
- McDonald, A. J., Mascagni, F. (2007). Neuronal localization of 5-HT type 2A receptor immunoreactivity in the rat basolateral amygdala. *Neuroscience*, 146, 306-320.
- McEwen, B. S. (2010). Stress, sex, and neural adaptation to a changing environment: mechanisms of neuronal remodeling. *Ann. NY Acad. Sci.*, 1204 (Suppl.), E38-59.
- McKie, S., Del-Ben, C., Elliot, R., Williams, S., del Vai, N., Anderson, I., Deakin, J. F. W. (2005). Neural effects of acute citalopram detected by pharmacMRI. *Psychopharmacology*, 180, 680-686.
- McLaughlin, K. J., Baran, S. E., Conrad, C. D. (2009). Chronic stress- and sex-specific neuromorphological and functional changes in limbic structures. *Mol. Neurobiol.*, 40, 166-182.
- Miller, J. M., Oquendo, M. A., Ogden, R. T., Mann, J. J., Parsey, R. V. (2008). Serotonin transporter binding as a possible predictor of one-year remission in major depressive disorder. *J. Psychiatr. Res.*, 42, 1137-1144.
- Millhouse, O. E., DeOlmos, J. (1983). Neuronal configurations in lateral and basolateral amygdala. *Neuroscience*, 10, 1269-1300.
- Mitchell, P. J. (2005). Antidepressant treatment and rodent aggressive behaviour. *Eur. J. Pharmacol.*, 526, 147-162.
- Mitra, R., Sapolsky, R. M. (2008). Acute corticosterone treatment is sufficient to induce anxiety and amygdalar dendritic hypertrophy. *Proc. Natl. Acad. Sci. US*, 105, 5573-5578.
- Mora, P. O., Netto, C. F., Graeff, F. G. (1997). Role of 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptor subtypes in the two types of fear generated by the elevated T-maze. *Pharmacol. Biochem. Behav.*, 58, 1051-1057.
- Morales, M., Bloom, F. E. (1997). The 5-HT<sub>3</sub> receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon. *J. Neurosci.*, 17, 3157-3167.
- Morris, J. S., Öhman, A., Dolan, R. J. (1998). Conscious and unconscious emotional learning in the human amygdala. *Nature*, 393, 467-470.

- Moya, P. R., Fox, M. A., Jensen, C. L., Laporte, J. L., French, H. T., Wendland, J. R., Murphy, D. L. (2011). Altered 5-HT<sub>2C</sub> receptor agonist-induced responses and 5-HT<sub>2C</sub> receptor RNA editing in the amygdala of serotonin transporter knockout mice. *BMC Pharmacol.*, 11 (Apr. 7), 3.
- Mozhui, K., Karlsson, R. M., Kash, T. L., Ihne, J., Norcross, M., Patel, S., Farrell, M. R., Hill, E. E., Graybeal, C., Martin, K. P., Camp, M., Fitzgerald, P. J., Ciobanu, D. C., Sprengel, R., Mishina, M., Wellman, C. L., Winder, D. G., Williams, R. W., Holmes, A. (2010). Strain differences in stress responsivity are associated with divergent amygdala gene expression and glutamate-mediated neuronal excitability. *J. Neurosci.*, 30, 5357-5367.
- Muller, J. F., Mascagni, F., McDonald, A. J. (2007). Serotonin-immunoreactive axon terminals innervate pyramidal cells and interneurons in the rat basolateral amygdala. *J. Comp. Neurol.*, 505, 314-335.
- Muller, J. F., Mascagni, F., McDonald, A. J. (2011). Cholinergic innervation of pyramidal cells and parvalbumin-immunoreactive interneurons in the rat basolateral amygdala. *J. Comp. Neurol.*, 519, 790-805.
- Muraki, I., Inoue, T., Hashimoto, S., Izumi, T., Ito, K., Ohmori, T., Koyama, T. (1999). Effect of subchronic lithium carbonate treatment on anxiolytic-like effect of citalopram and MKC-242 in conditioned fear stress in the rat. *Eur. J. Pharmacol.*, 383, 223-229.
- Muraki, I., Inoue, T., Hashimoto, S., Izumi, T., Ito, K., Koyama, T. (2001). Effect of subchronic lithium treatment on citalopram-induced increases in extracellular concentrations of serotonin in the medial prefrontal cortex. *J. Neurochem.*, 76, 490-497.
- Muraki, I., Inoue, T., Koyama, T. (2008). Effect of co-administration of the selective 5-HT<sub>1A</sub> receptor antagonist WAY 100,635 and selective 5-HT<sub>1B/1D</sub> receptor antagonist GR 127,935 on anxiolytic effect of citalopram in conditioned fear stress in the rat. *Eur. J. Pharmacology*, 586, 171-178.
- Murphy, S. E., Norbury, R., O'Sullivan, U., Cowen, P. J., Harmer, C. J. (2009). Effect of a single dose of citalopram on amygdala response to emotional faces. *Br. J. Psychiatry*, 194, 535-540.
- Myers, K. M., Davis, M. (2002). Behavioral and neural analysis of extinction. *Neuron*, 36, 567-584.
- Nestler, E. J., Hyman, S. E. (2010). Animal models of neuropsychiatric disorders. *Nat. Neurosci.*, 13, 1161-1169.
- Nevins, M. E., Anthony, E. W. (1994). Antagonists at the serotonin-3 receptor can reduce the fear-potentiated startle response in the rat: evidence for difference types of anxiolytic activity? *J. Pharmacol. Exp. Ther.*, 268, 248-254.
- Nic Dhonnchadha, B. A., Hascoët, M., Jolliet, P., Bourin, M. (2003). Evidence for a 5-HT<sub>2A</sub> receptor mode of action in the anxiolytic-like properties of DOI in mice. *Behav. Brain Res.*, 147, 175-184.
- Noirot, E. (1972). Ultrasounds and maternal behavior in small rodents. *Dev. Psychobiol.*, 5, 371-387.
- Olivier, B. (2004). Serotonin and aggression. *Ann. NY Acad. Sci.*, 1036, 382-392.
- Overstreet, D. H., Knapp, D. J., Angel, R. A., Navarro, M., Breese, G. R. (2006). Reduction in repeated ethanol-withdrawal-induced anxiety-like behavior by site-selective injections of 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> ligands. *Psychopharmacology*, 187, 1-12.

- Parsey, R. V., Hastings, R. S., Oquendo, M. A., Huang, Y. Y., Simpson, N., Arcement, J., Huang, Y., Ogden, R. T., Van Heertum, R. L., Arango, V., Mann, J. J. (2006). Lower serotonin transporter binding potential in the human brain during major depressive episodes. *Am. J. Psychiatry*, 163, 52-58.
- Paxinos, G., Watson, C. *The rat brain in stereotaxic coordinates*. New York: Academic Press; 1997.
- Pellow, S., Chopin, P., File, S. E., Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods*, 14, 149-67.
- Pinard, C. R., Muller, J. F., Mascagni, F., McDonald, A. J. (2008). Dopaminergic innervation of interneurons in the rat basolateral amygdala. *Neuroscience*, 157, 850-863.
- Pittenger, C. and Duman, R. S. (2008) Stress, depression, and neuroplasticity: A convergence of mechanisms. *Neuropsychopharmacology*, 33, 88-109.
- Pollier, F., Sarre, S., Aguerre, S., Ebinger, G., Mormède, P., Michotte, Y., Chaouloff, F. (2000). Serotonin reuptake inhibition by citalopram in rat strains differing for their emotionality. *Neuropsychopharmacology*, 22, 64-76.
- Polter, A. M., Li, X. (2010). 5-HT<sub>1A</sub> receptor-regulated signal transduction pathways in brain. *Cell Signal*, 22, 1406-1412.
- Power, J. M., Sah, P. (2008). Competition between calcium-activated K<sup>+</sup> channels determines cholinergic action on firing properties of basolateral amygdala projection neurons. *J. Neurosci.*, 28, 3209-3220.
- Puciłowski, O., Płaźnik, A., Kostowski, W. (1985). Aggressive behavior inhibition by serotonin and quipazine injected into the amygdala in the rat. *Behav. Neural Biol.*, 43, 58-68.
- Rainnie, D. G., Asprodini, E. K., Shinnick-Gallagher, P. (1993). Intracellular recordings from morphologically identified neurons of the basolateral amygdala. *J. Neurophysiol.*, 69, 1350-1362.
- Rainnie, D. G. (1999). Serotonergic modulation of neurotransmission in the rat basolateral amygdala. *J. Neurophysiol.*, 82, 69-85.
- Rainnie, D. G., Mania, I., Mascagni, F., McDonald, A. J. (2006). Physiological and morphological characterization of parvalbumin-containing interneurons of the rat basolateral amygdala. *J. Comp. Neurol.*, 498, 142-161.
- Reimond, M., Batra, A., Knobel, A., Smolka, M. N., Zimmer, A., Mann, K., Solbach, C., Reischl, G., Schwarzler, F., Grunder, G., Machulla, H. J., Bares, R., Heinz, A. (2008). Anxiety is associated with reduced central serotonin transporter availability in unmedicated patients with unipolar major depression: a [<sup>11</sup>C]DASP PET study. *Mol. Psychiatry*, 13, 606-613.
- Reznikov, L. R., Reagan, L. P., Fadel, J. R. (2008). Activation of phenotypically distinct neuronal subpopulations in the anterior subdivision of the rat basolateral amygdala following acute and repeated stress. *J. Comp. Neurol.*, 508, 458-472.
- Reznikov, L. R., Reagan, L. P., Fadel, J. R. (2009). Effects of acute and repeated restraint stress on GABA efflux in the rat basolateral and central amygdala. *Brain Res.*, 1256, 61-68.
- Roiser, J. P., Levy, J., Fromm, S. J., Wang, H., Hasler, G., Sahakian, B. J., Drevets, W. C. (2008). The effect of acute tryptophan depletion on the neural correlates of emotional processing in healthy volunteers. *Neuropsychopharmacology*, 33, 1992-2006.

- Rosell, D. R., Thompson, J. L., Slifstein, M., Xu, X., Frankle, W. G., New, A. S., Goodman, M., Weinstein, S. R., Laruelle, M., Abi-Dargham, A., Siever, L. J. (2010). Increased serotonin 2A receptor availability in the orbitofrontal cortex of physically aggressive personality disordered patients. *Biol. Psychiatry*, 67, 1154-1162.
- Rudebeck, P. H., Walton, M. E., Millette, B. H., Shirley, E., Rushworth, M. F., Bannerman, D. M. (2007). Distinct contributions of frontal areas to emotion and social behaviour in the rat. *Eur. J. Neurosci.*, 26, 2315-2326.
- Sah, P., Faber, E. S., Lopez De Armentia, M., Power, J. (2003). The amygdalar complex: anatomy and physiology. *Physiol. Rev.*, 83, 803-834.
- Sah, P., Westbrook, R. F. (2008). Behavioural neuroscience: The circuit of fear. *Nature*, 454, 589-590.
- Saha, S., Gamboa-Esteves, F. O., Batten, T. F. (2010). Differential distribution of 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub>-like immunoreactivities in rat central nucleus of the amygdala neurones projecting to the caudal dorsomedial medulla oblongata. *Brain Res.*, 1330, 20-30.
- Sari, Y. (2004). Serotonin<sub>1B</sub> receptors: from protein to physiological function and behavior. *Neurosci. Biobehav. Rev.*, 28, 565-582.
- Savitz, J. B., Devets, W. C. (2009). Imaging phenotypes of major depressive disorder: genetic correlates. *Neuroscience*, 164, 300-330.
- Schreiber, R., De Vry, J. (1993). Neuronal circuits involved in the anxiolytic effects of the 5-HT<sub>1A</sub> receptor agonists 8-OH-DPAT ipsapirone and buspirone in the rat. *Eur. J. Pharmacol.*, 249, 341-351.
- Schreiber, R., Melon, C., De Vry, J. (1998). The role of 5-HT receptor subtypes in the anxiolytic effects of selective serotonin reuptake inhibitors in the rat ultrasonic vocalization test. *Psychopharmacology*, 135, 383-391.
- Setem, J., Pinheiro, A. P., Motta, V. A., Morato, S., Cruz, A. P. (1999). Ethopharmacological analysis of 5-HT ligands on the rat elevated plus-maze. *Pharmacol. Biochem. Behav.*, 62, 515-521.
- Sheline, Y. I., Barch, D. M., Donnelly, J. M., Ollinger, J. M., Snyder, A. Z., Mintun, M. A. (2001). Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. *Biol. Psychiatry*, 50, 651-658.
- Shishkina, G. T., Kalinina, T. S., Dygalo, N. N. (2008). Serotonergic changes produced by repeated exposure to forced swimming: correlation with behavior. *Ann. NY Acad. Sci.*, 1148, 148-153.
- Siegel, A., Bhatt, S., Bhatt, R., Zalzman, S. S. (2007). The neurobiological bases for development of pharmacological treatments of aggressive disorders. *Curr. Neuropharmacol.*, 5, 135-147.
- Siever, L. J. (2008). Neurobiology of aggression and violence. *Am. J. Psychiatry*, 165, 429-442.
- Soloff, P. H., Price, J. C., Meltzer, C. C., Fabio, A., Frank, G. K., Kaye, W. H. (2007). 5HT<sub>2A</sub> receptor binding is increased in borderline personality disorder. *Biol. Psychiatry*, 62, 580-587.
- Stein, C., Davidowa, H., Albrecht, D. (2000). 5-HT<sub>1A</sub> receptor-mediated inhibition and 5-HT<sub>2</sub> as well as 5-HT<sub>3</sub> receptor-mediated excitation in different subdivisions of the rat amygdala. *Synapse*, 38, 328-337.
- Stein, M. B. (2009). Neurobiology of generalized anxiety disorder. *J. Clin. Psychiatry*, 70 (Suppl. 2), 15-19.

- Takahashi, A., Quadros, I. M., de Almeida, R. M. M., Miczek, K. A. (2011). Brain serotonin receptors and transporters: initiation vs. termination of escalated aggression. *Psychopharmacology*, 213, 183-212.
- Takahashi, H., Yahata, N., Koeda, M., Takano, A., Asai, K., Suhara, T., Okubo, Y. (2005). Effects of dopaminergic and serotonergic manipulation on emotional processing: a pharmacological fMRI study. *Neuroimage*, 27, 991-1001.
- Terrón, J. A., Martínez-García, E. (2007). 5-HT7 receptor-mediated dilatation in the middle meningeal artery of anesthetized rats. *Eur. J. Pharmacol.*, 560, 56-60.
- Thomas, D. R., Hagan, J. J. (2004). 5-HT7 receptors. *Curr. Drug Targets CNS Neurol. Disord.*, 3, 81-90.
- Tye, K. M., Prakash, R., Kim, S. Y., Fenno, L. E., Grosenick, L., Zarabi, H., Thompson, K. R., Gradinaru, V., Ramakrishnan, C., Deisseroth, K. (2011). Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature*, 471, 358-362.
- Valzelli, L., Bernasconi, S., Dalessandro, M. (1981). Effect of tryptophan administration on spontaneous and P-CPA-induced muricidal aggression in laboratory rats. *Pharmacol. Res. Commun.*, 13, 891-897.
- van der Veen, F. M., Evers, E. A., Deutz, N. E., Schmitt, J. A. (2007). Effects of acute tryptophan depletion on mood and facial emotion perception related brain activation and performance in healthy women with and without a family history of depression. *Neuropsychopharmacology*, 32, 216-224.
- Veening, J. G., Coolen, L. M., de Jong, T. R., Joosten, H. W., de Boer, S. F., Koolhaas, J. M., Olivier, B. (2005). Do similar neural systems subserve aggressive and sexual behaviour in male rats? Insights from c-Fos and pharmacological studies. *Eur. J. Pharmacol.*, 526, 226-239.
- Venton, B. J., Robinson, T. E., Kennedy, R. T., Maren, S. (2010). Dynamic amino acid increases in the basolateral amygdala during acquisition and expression of conditioned fear. *Eur. J. Neurosci.*, 23, 3391-3398.
- Victor, T. A., Furey, M. L., Fromm, S. J., Öhman, A., Drevets, W. C. (2010). Relationship between amygdala responses to masked face and mood state and treatment in major depressive disorder. *Arch. Gen. Psychiatry*, 67, 1128-1138.
- Vogel, J. R., Beer, B., Clody, D. E. (1971). A simple and reliable conflict procedure for testing anti-anxiety agents. *Psychopharmacologia*, 21, 1-7.
- Washburn, M. S., Moises, H. C. (1992a). Electrophysiological and morphological properties of rat basolateral amygdalar neurons in vitro. *J. Neurosci.*, 12, 4066-4079.
- Washburn, M. S., Moises, H. C. (1992b). Muscarinic responses of rat basolateral amygdalar neurons recorded in vitro. *J. Physiol.*, 449, 121-154.
- Wesołowska, A., Nikiforuk, A., Stachowicz, K., Tatarczyńska, E. (2006). Effect of the selective 5-HT7 receptor antagonist SB 269970 in animal models of anxiety and depression. *Neuropharmacology*, 51, 578-586.
- Wesołowska, A. (2010). Potential role of the 5-HT6 receptor in depression and anxiety: an overview of preclinical data. *Pharmacol. Rep.*, 62, 564-577.
- Whalen, P. J., Rauch, S. L., Etcoff, N. L., McInerney, S. C., Lee, M. B., Jenike, M. A. (1998). Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. *J. Neurosci.*, 18, 411-418.

- Witte, A. V., Flöel, A., Stein, P., Savli, M., Mien, L. K., Wadsak, W., Spindelegger, C., Moser, U., Fink, M., Hahn, A., Mitterhauser, M., Kletter, K., Kasper, S., Lanzenberger, R. (2010). Aggression is related to frontal serotonin-1A receptor distribution as revealed by PET in healthy subjects. *Hum. Brain Mapp.*, 30, 2558-2570.
- Woodruff, A. R., Sah, P. (2007). Networks of parvalbumin-positive interneurons in the basolateral amygdala. *J. Neurosci.*, 27, 553-563.
- Wu, J., Kramer, G. L., Kram, M., Steciuk, M., Crawford, I. L., Petty, F. (1999). Serotonin and learned helplessness: a regional study of 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub> receptors and the serotonin transport site in rat brain. *J. Psychiatr. Res.*, 33, 17-22.
- Yajeya, J., de la Fuente Juan, A., Merchan, M. A., Riobobos, A. S., Heredia, M., Criado, J. M. (1997). Cholinergic responses of morphologically and electrophysiologically characterized neurons of the basolateral complex in rat amygdala slices. *Neuroscience*, 78, 731-743.
- Yilmazer-Hanke, D. M. (2008) Morphological correlates of emotional and cognitive behaviour: Insights from studies on inbred and outbred rodent strains and their crosses. *Behav. Pharmacol.*, 19, 403-434.
- Yokoyama, M., Suzuki, E., Sato, T., Maruta, S., Watanabe, S., Miyaoka, H. (2005). Amygdalic levels of dopamine and serotonin rise upon exposure to conditioned fear stress without elevation of glutamate. *Neurosci. Lett.*, 379, 37-41.
- Zangrossi, H., Graeff, F. G. (1994). Behavioral effects of intraamygdala injection of GABA and 5-HT acting drugs in the elevated plus-maze. *Braz. J. Med. Biol. Res.*, 27, 2453-2456.
- Zanoveli, J. M., Carvalho, M. C., Cunha, J. M., Brandão, M. L. (2009). Extracellular serotonin level in the basolateral nucleus of the amygdala and dorsal periaqueductal gray under unconditioned and conditioned fear states: an in vivo microdialysis study. *Brain Res.*, 1294, 106-115.