Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres

Research Report

Lack of interaction of endocannabinoids and 5-HT$_3$ neurotransmission in associative fear circuits of the amygdala: Evidence from electrophysiological and behavioural experiments

D. Höfelmann[a,b], B. di Benedetto[c], S.C. Azad[e], V. Micale[d,f], C.T. Wotjak[d], G. Rammes[a,b,*]

[a]Department of Anaesthesiology, Technische Universität, D-81675 Munich, Germany
[b]Neuronal Network Dynamics Max-Planck-Institute of Psychiatry, D-80804 Munich, Germany
[c]Molecular Psychopharmacology, Max-Planck-Institute of Psychiatry, D-80804 Munich, Germany
[d]Neuronal Plasticity, Max-Planck-Institute of Psychiatry, D-80804 Munich, Germany
[e]Department of Anaesthesiology, Ludwig-Maximilians-University, 81377 Munich, Germany
[f]CEITEC-Central European Institute of Technology, Masaryk University, Brno, Czech Republic

ARTICLE INFO

Article history:
Accepted 8 June 2013
Available online 19 June 2013

Keywords:
CB1
5-HT3
Amygdala
Fear conditioning
Slice
IPSC

ABSTRACT

Both the serotonergic and the endocannabinoid system play a major role in mediating fear and anxiety. In the basolateral amygdala (BLA) it has been shown that the cannabinoid receptor 1 (CB1) is highly co-expressed with 5-HT$_3$ receptors on GABAergic interneurons suggesting that 5-HT$_3$ receptor activity modulates CB1-mediated effects on inhibitory synaptic transmission. In the present study, we investigated the possible interactions of CB1 and 5-HT$_3$-mediated neuronal processes in the BLA using electrophysiological and behavioural approaches. Whole-cell patch-clamp recordings were performed in coronal brain slices of mice. Electric stimuli were delivered to the lateral amygdala to evoke GABA$_A$ receptor-mediated inhibitory postsynaptic currents (GABA$_A$-eIPSCs) in the BLA. The induction of LTDi, a CB1-mediated depression of inhibitory synaptic transmission, was neither affected by the 5-HT$_3$ antagonists ondansetron (OND; 20 μM) and tropisetron (Trop; 50 nM) nor by the 5-HT$_3$ agonists SR57227A (10 μM). In auditory fear conditioning tests, mice treated with SR57227A (3.0 mg/kg i.p.) showed sustained freezing, whereas treatment with Trop (1.0 mg/kg i.p.) decreased the expression of conditioned fear. These effects were overruled by the CB1 antagonist rimonabant (RIM; 3.0 mg/kg), which caused increased freezing with or without co-treatment with Trop. In summary, these experiments do not support a functional interaction between CB1 and 5-HT$_3$ receptors at the level of GABA neurotransmission in the BLA nor in terms of fear regulation.

*Corresponding author at: Max-Planck-Institute of Psychiatry, Clinical Neuropharmacology, Kraepelinstraße 2, D-80804 München, Germany. Fax: +49 89 30622402.
E-mail address: rammes@mpipsykl.mpg.de (G. Rammes).

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.06.011

1. Introduction

The endogenous cannabinoid system (ECS) consists of two Gi/o protein coupled receptors, the cannabinoid receptor type 1 (CB1) and the cannabinoid receptor type 2 (CB2), their endogenous ligands as well as the enzymatic machinery involved in endocannabinoid synthesis and degradation. CB1 is widely distributed throughout the central nervous system (CNS) (Lutz, 2007) and CB2 is most strongly expressed in the immune system (Munro et al., 1993). Since the discovery of endocannabinoids (EC), two major members of the EC family have been identified, anandamide and 2-arachydonoyl glycerol (2-AG) (Rodriguez de Fonseca et al., 2005; Luchicchi and Pistis, 2012). Upon synaptic activity, ECs are released postsynaptically as retrograde messengers and bind to presynaptic CB1 which reduces the release of excitatory and inhibitory transmitters (Azad et al., 2004; Chevaleyre et al., 2007; Marsicano et al., 2002; Wilson and Nicoll, 2002). This process mediates short-term and long-term modulation in synaptic transmission (Azad et al., 2004; Chevaleyre et al., 2007; Marsicano and Lutz, 2006), leads to memory modulation (Hampson et al., 2003; Reibaud et al., 1999) and to extinction of aversive memories (Kamprath et al., 2006; Marsicano et al., 2002). ECs are involved in stress-response physiology (Ramikie and Patel, 2012), have analgetic (Martin et al., 1999), antiemetic and antispastic effects (Baker et al., 2000; Breivogel and Childers, 1998; Pertwee, 2001; Porter and Felder, 2001) and they increase appetite, induce sleep and lighten up mood (Maldonado and Valverde, 2003; Porter and Felder, 2001; Robson, 2001). Similar to the ECS, the serotonergic system influences various physiological functions and controls a wide range of behaviours and emotional states (Linthorst, 2005; Lucki, 1998) including analgesia, sleep, aggression, appetite, fear and mood (Southwick et al., 1999). Several studies provided evidence for functional cannabinoid–serotonin (5-HT) interactions (Egertova et al., 1998, 2003; Häring et al., 2007; Hill et al., 2006; Moldrich and Wenger, 2000), suggesting that 5-HT may mediate the antidepressant-like effect of cannabinoids (Bambico et al., 2007; Gobbi et al., 2005; Häring et al., 2007) and indicate that the pharmacological activity of anandamide could be mediated by 5-HT-receptors (Kimura et al., 1998). Interestingly, Barann et al. (2002) showed that ECs inhibit the serotonin type 3 (5-HT_3) receptor. The 5-HT_3 receptor is a ligand-gated ion channel, belonging to the Cys-loop superfamily (Derkach et al., 1989; Maricq et al., 1991). The activation of postsynaptic 5-HT_3 receptors mediate fast excitatory synaptic transmission in the neocortex (Roerig et al., 1997) and in the lateral amygdala (Sugita et al., 1992). Presynaptic 5-HT_3 receptors modulate the release of several neurotransmitters (Koyama et al., 2000, 2002; Oostland et al., 2011). 5-HT_3 receptor antagonists were for long viewed as a promising new target for the treatment of anxiety. However, in animal studies, 5-HT_3 receptor antagonists showed inconsistent effects and ranged from anxiolytic to anxiogenic behaviour (Olivier et al., 2000; Thompson and Lummis, 2006). It has been hypothesised that the putative interaction between 5-HT_3 neurotransmission and cannabinoid signalling may explain such contradictions (Mikics et al., 2009).

The amygdala is an integral element of the limbic circuitry and plays a major role in analgesia (Martin et al., 1999) and in the control of emotional behaviour, including conditioned fear and anxiety (Pape and Pare, 2010). In the basolateral amygdala (BLA), mRNA for CB1 receptors and 5-HT_3 receptors are highly co-localised on GABAergic interneurons (Hermann et al., 2002; Morales et al., 2004). According to the involvement of both cannabinoids and 5-HT_3 receptors in fear and anxiety (Lutz, 2007; Thompson and Lummis, 2007; Witkin et al., 2005) and the fact that ECs directly inhibit 5-HT_3 currents by allosteric mechanisms (Barann et al., 2002) a functional interaction between 5-HT_3 neurotransmission and EC signalling has been hypothesised (Freund et al., 2003). Behavioural relevance for this interaction has been provided recently as 5-HT_3 activation decreases anxiety, and retrograde cannabinoid signalling dampens this effect in normal mice, but not in CB1-KOs (Mikics et al., 2009).

However, on the neuronal level, evidence for a functional interaction of 5-HT_3 neurotransmission and EC signalling is still missing. Therefore, using whole-cell patch-clamp recordings in brain slices and auditory fear-conditioning tests, we examined possible synergistic effects of 5-HT_3 receptor antagonists and the ECS in a pharmacological approach.

2. Results

2.1. *CB1 receptors are co-expressed with 5-HT_3 receptors on BLA interneurons*

In situ hybridisation provides evidence for a co-localisation of CB1 receptor and 5-HT_3 receptor mRNA in GABAergic interneurons in the BLA (Hermann et al., 2002; Morales et al., 2004). First of all, with immunohistochemical methods we investigated the co-localisation of CB1 receptors and 5-HT_3 receptors in BLA neurons at the protein level. Confocal microscopy revealed a co-expression of both receptors on the majority of the presynaptic terminals of BLA interneurons (Fig. 1).

2.2. *Endocannabinoid-dependent synaptic plasticity does not interact with 5-HT_3 receptors*

Stimulation of afferents in the LA with 1 Hz induces an endocannabinoid-dependent long-term depression of inhibitory GABAergic synaptic transmission (LTDi) in neurons of the BLA (Azad et al., 2004). To investigate a possible interaction between the endocannabinoid release and 5-HT_3 receptors, we induced LTDi in the presence of either the 5-HT_3 receptor antagonists ondansetron (OND; 20 μM) and tropisetron (Trop; 50 μM) or the potent 5-HT_3 receptor agonist SR57227A (5 μM) (Bachy et al., 1993). LFS of afferents from the LA induced reliable LTDi in the BLA and was inhibited (96±0.6%; $n=6$; $p>0.05$; Fig. 2C) when the CB1 antagonist rimonabant (RIM; 10 μM) has been applied, demonstrating an EC-dependent mechanism (Azad et al., 2004). Pretreatment of slices with either OND or Trop did not significantly affect the induction of LTDi (OND: 63±5% vs control: 65±4%, $n=7$; $p>0.05$; Fig. 1A; and Trop: 53±9% vs control: 48±6%, $n=7$; $p>0.05$; Fig. 2A,B). One remarkable characteristic of 5-HT_3

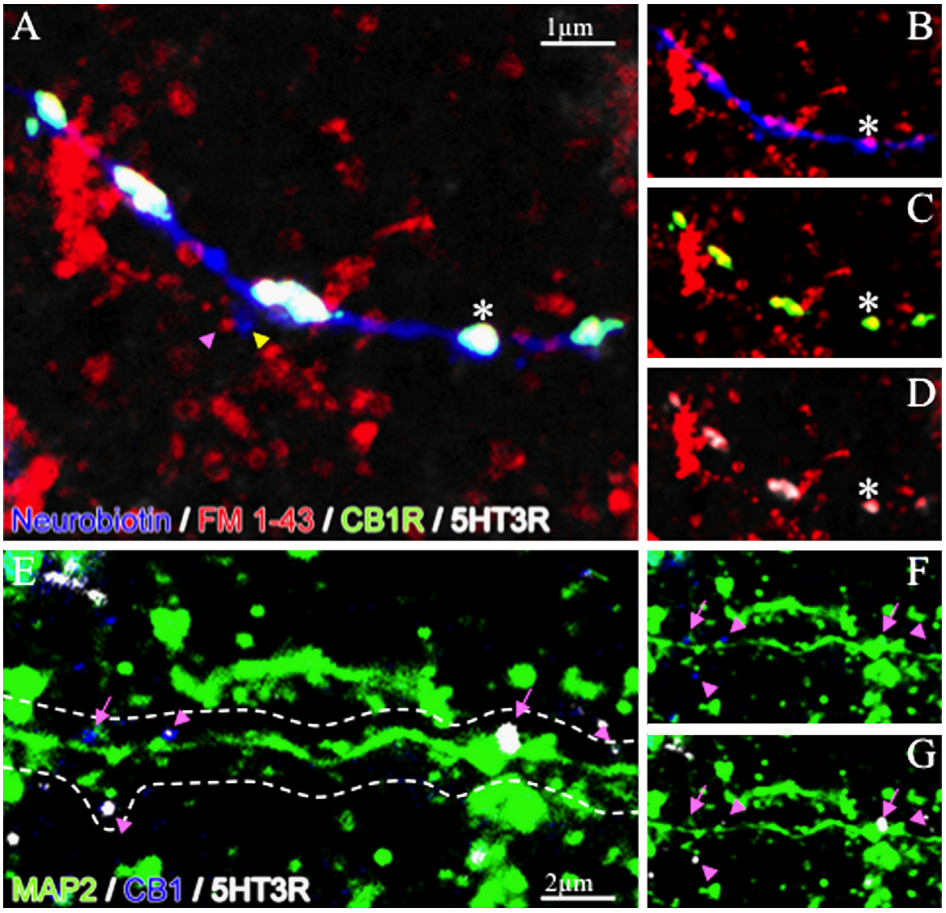

Fig. 1 – Immunohistochemical analysis for the co-localisation of CB1 and $5HT_3$ receptors. (A) Neurobiotin was injected into BLA post-synaptic neurons and revealed with Alexa 405-conjugated streptavidin (blue); FM1-43 (red) was used to label activated presynaptic terminals in the BLA; the presence of CB1R (green) and $5HT_3$ receptors (white) was detected by IF-IHC with the respective antibodies. Arrowheads indicate pre- (pink) or postsynaptic (yellow) terminals. The asterisk shows a presynaptic terminal where FM1-43, CB1 and $5HT_3$ receptors co-localise, as highlighted in the single co-localisation pictures (B,C and D). (E) Dendrites of the BLA were labelled with MAP2 antibody (green), while CB1 and $5HT_3$ receptors were detected by the respective antibodies, labelled in blue and white, respectively. Arrowheads indicate co-localisation of the receptors at presynaptic terminals; arrows point out the existence of also sites of single labelling for one or the other receptor, though they do not represent a majority.

receptors is their pronounced desensitization upon sustained activation by agonists (Koyama et al., 2000; Rammes et al., 2009). Thus, SR57227A has been applied very quickly and only during LFS. When slices were treated with SR57227A during LFS, $eGABA_A$-IPSCs were depressed to 63±11% ($n=6$) and showed no significant difference to control slices (59±5%; $n=5$; $p>0.05$; Fig. 2C). These experiments demonstrate that neither the activation nor the inhibition of $5\text{-}HT_3$ receptors interferes with synaptic processes dependent on the release of ECs.

2.3. *The serotonergic system influences fear extinction*

To study the involvement of the serotonergic neurotransmission in associative fear circuits, C57BL/6N mice were tested in auditory fear conditioning, which is highly dependent on the basolateral amygdala complex (Johansen et al., 2011; Pape and Pare, 2010). In the first experiment, mice underwent auditory fear conditioning (d0), followed by treatment with vehicle or with two different doses of the potent $5\text{-}HT_3$ agonist SR57227A (1 mg/kg or 3 mg/kg) before re-exposure to the tone (d1–d3). There was a significant treatment x day interaction ($F_{4,76}=3.2264$, $p=0.016$), reflecting increased freezing responses upon treatment with 3 mg/kg on d3 (Fig. 3A). Thus, activation of 5-HT3 receptors impaired long-term extinction of conditioned fear.

In the second experiment, we treated new groups of mice with two doses of Trop (0.3, 1 mg/kg) or vehicle before re-exposure to the tone at d1–d3. Two-way ANOVA (treatment, day) for repeated measures (day) revealed a trend for both treatment ($F_{2,27}=3.1022$, $p=0.061$) and treatment x day interaction ($F_{4,54}=2.1631$, $p=0.085$). A separate one-way ANOVA confirmed that 1 mg/kg Trop significantly reduced the expression of conditioned fear at d1 (i.e. in response to the first Trop treatment) ($F_{2,27}=6.6826$, $p=0.004$; Fig. 3B).

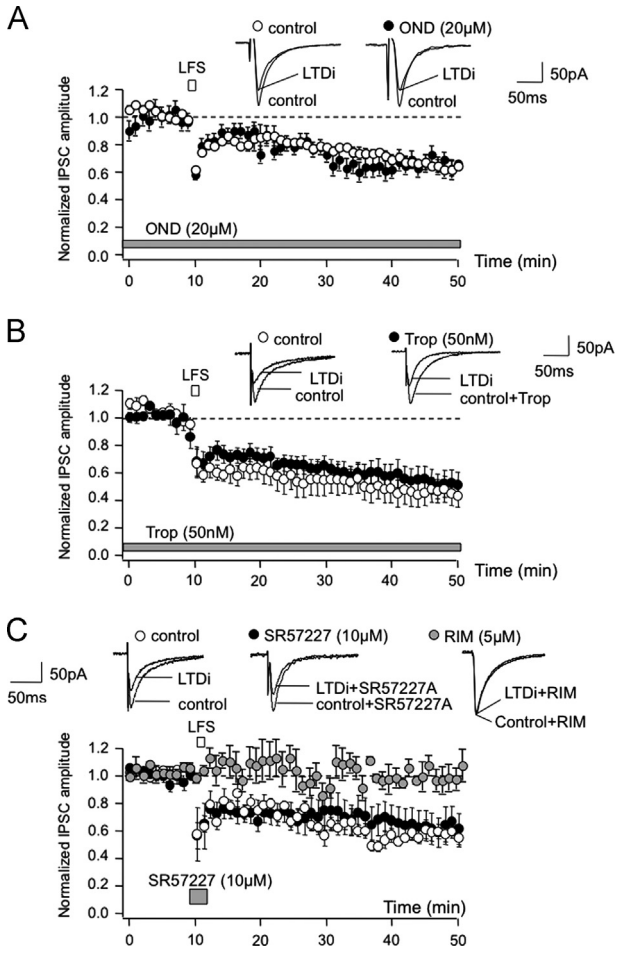

Fig. 2 – The effects of endocannabinoid release on inhibitory synaptic transmission does not interfere with 5-HT$_3$ receptor modulation. Neither 5-HT$_3$ receptor antagonists (ondansetron, OND; tropisetron, Trop) nor a 5-HT$_3$ receptor agonist (SR57227A) interfered with the induction of LTDi. (A–C) LFS of afferents in the LA (100 pulses at 1 Hz) induced LTDi in principal BLA neurons. Pretreatment of slices with either OND (A) or Trop (B), two 5-HT$_3$ receptor antagonists, did not significantly affect the induction of LTDi (OND: 63±5% vs control: 65±4%, $n=7$; $p>0.05$; Trop: 53±9% vs control: 48±6%, $n=7$; $p>0.05$) to 48±0.8% of control ($n=7$; $p<0.05$). (C) LTDi induction was not affected by the application of the 5-HT$_3$ receptor agonist SR57227A. Due to fast 5-HT$_3$ receptor desensitization upon activation, SR57227A was applied rapidly and only present during LFS. eGABA$_A$-IPSCs were depressed to 63±11% ($n=6$) and showed no significant difference to control slices (59±5%; $n=5$; $p>0.05$). LTDi is EC-dependent as the CB1 receptor antagonist RIM (5 μM) inhibits LTDi (grey circles). These experiments demonstrate that neither the activation nor the inhibition of 5-HT$_3$ receptors interferes with synaptic processes dependent on the release of ECs.

In the third experiment we tested whether the anti-fear effects of the effective dose of Trop (1 mg/kg) would counteract the fear promoting consequences of the CB1 receptors antagonist/inverse agonist rimonabant (RIM; 3 mg/kg). In this context we treated new groups of mice with vehicle–vehicle, Trop–vehicle, vehicle–RIM or Trop–RIM 30 min before re-exposure to the tone at d1. A two-way ANOVA (treatment1, treatment2) revealed a significant interaction between the two treatments ($F_{1,48}=10.162$, $p=0.002$). Post-hoc analyses confirmed that Trop reduces the expression of conditioned fear (as seen before) and that the fear promoting consequences of RIM overruled these effects (Fig. 3C).

3. Discussion

The present findings do not support an interaction between EC signalling and 5HT$_3$ neurotransmission in the BLA. LTDi, a process dependent on endocannabinoid release, was not affected by the application of a 5HT$_3$-receptor agonist nor 5HT$_3$-receptor antagonists.

It has been reported that CB1 receptors are also involved in the regulation of serotonergic responses mediated by e.g. 5-HT(2A/C) and 5-HT(1A) (Braida et al., 2007; Marco et al., 2004; Mato et al., 2007). However, due to the pharmacological interaction of ECs with 5-HT$_3$ receptors (Barann et al., 2002) we focused solely on the interaction of CB1 with the ionotropic serotonergic receptor.

Previous studies demonstrate that the ECS influences similar to the serotonergic system physical functions and controls behaviours and emotional states (Linthorst, 2005; Lucki, 1998). Furthermore, several studies supplied evidence for a functional interaction between the EC and the serotonergic system (Egertova et al., 1998, 2003; Häring et al., 2007; Hill et al., 2006; Moldrich and Wenger, 2000) especially with 5-HT$_3$ neurotransmission (Freund et al., 2003; Mikics et al., 2009). In fact, it has been shown that activation of presynaptic 5HT$_3$ receptors enhances GABA release in BLA neurons (Koyama et al., 2000, 2002). ECs control both excitatory and inhibitory neurotransmission (Azad et al., 2003; Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001) and were shown to affect anxiety by modulating GABAergic and glutamatergic functions (Haller et al., 2007; Rubio et al., 2008). CB1 and 5HT$_3$ receptors are co-expressed by GABAergic interneurons in the BLA, hippocampus and cortex (Hermann et al., 2002; Morales and Wang, 2002; Morales et al., 2004), brain structures involved in the control of anxiety (Davidson, 2002; Engin and Treit, 2008). It has been suggested that 5HT$_3$ neurotransmission is one of the important mechanisms through which cannabinoids affect anxiety (Freund and Hajos, 2003): postsynaptic 5HT$_3$ receptors mediate the serotonergic inputs received from the raphe nuclei, whereas presynaptic 5HT$_3$ and CB1 control GABAergic output to postsynaptic principal cells. This raises the possibility of a tight interaction between 5HT$_3$ neurotransmission and cannabinoid signalling in modulating GABAergic transmission and anxiety.

In the present study IF-IHC demonstrates a clear co-localisation of 5HT$_3$ and CB1 at presynaptic terminals and suggests that processes dependent on EC release might be affected by the activity of 5HT$_3$ receptors. Previous studies showed that LTDi in the amygdala involves EC signalling (Azad et al., 2004; Marsicano et al., 2002). Here we demonstrate that neither the application of a 5HT$_3$-receptor agonist nor antagonists interferes with the induction of LTDi, thus

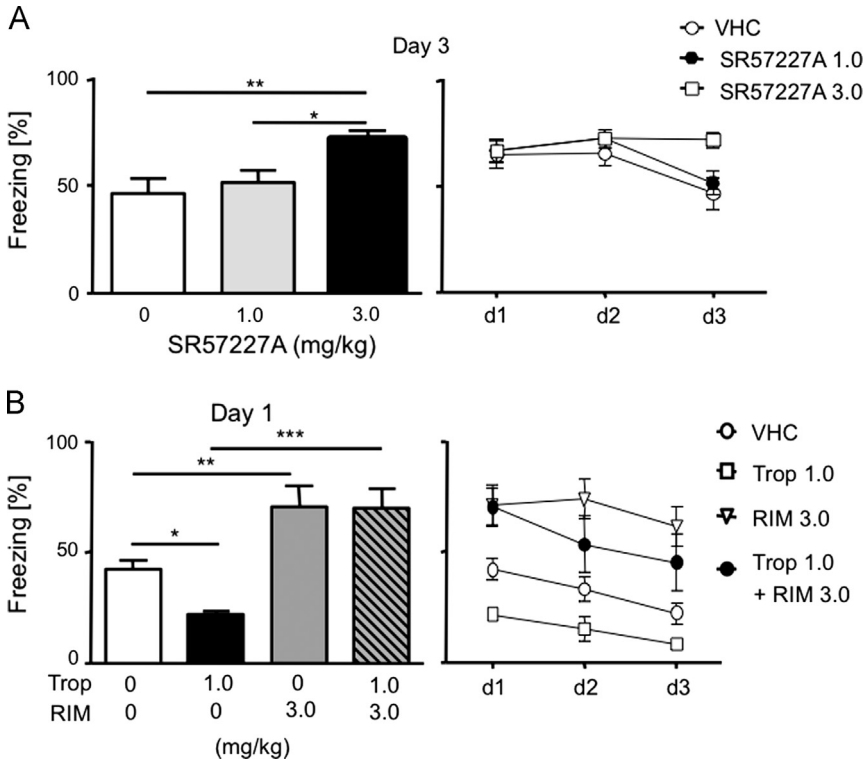

Fig. 3 – 5-HT3 receptors control the expression of conditioned fear. Mice underwent auditory fear conditioning (d0), followed by re-exposure to the tone in a novel environment at the next days (d1–d3). They were treated either with different doses of SR57227A (A) or Trop (B) or vehicle 30 min before tone presentation. (A) SR57227A (3 mg/kg) impaired the extinction of conditioned fear by d3. (B) Trop (1 mg/kg) decreased the level of conditioned fear already during the first re-exposure to the tone. (C) The fear alleviating consequences of Trop (1 mg/kg) failed to ameliorate the fear-promoting effects of the CB1 receptor antagonist/inverse agonist rimonabant (RIM; 3mg/kg). Freezing duration was expressed as a percentage of the 3-min observation period. Data are presented as mean ± SEM. n = 8 - 10 mice per group. $^{*}p<0.05$ $^{**}p<0.01$ (2-way ANOVA followed by Newman–Keuls post-hoc test).

excluding a functional interaction of $5HT_3$ neurotransmission and EC signalling in this form of synaptic plasticity.

At the behavioural level, treatment with the specific $5HT_3$ receptor agonist SR57227A led to sustained freezing, whereas treatment with Trop caused a decrease in the expression of conditioned fear. The results corroborate previous findings, which reported a reduction of fear-potentiated startle responses in rats (Nevins and Anthony, 1994) and humans (Harmer et al., 2006) upon treatment with 5-HT_3 receptor antagonists. Importantly, the fear-promoting effects of the CB1 antagonist RIM (Kamprath et al., 2006; Marsicano et al., 2002; Plendl and Wotjak, 2010) were unaffected by prior treatment with Trop, indicating that the attenuation of CB1 signalling overrules the fear alleviating consequences of inhibited 5-HT_3 signalling. These results are contradictory to the findings that 5-HT_3 activation decreases anxiety in CB1-KOs but not in normal mice, indicating a functional interaction between 5-HT_3 neurotransmission and CB1 signalling (Mikics et al., 2009). One possible explanation for this discrepancy might be the usage of different behavioural paradigms testing anxiety. Mikics et al. (2009) tested mice in the elevated plus maze reflecting anxiety- rather than fear-related behaviour. Furthermore, the anxiolytic effects of the 5-HT3 receptor agonist were observed in CB1-deficient mice, which are prone to compensatory changes.

In the prefrontal cortex, the hippocampus and BLA, co-expression of CB1 and 5-HT_3 receptors are restricted to CCK-containing GABAergic interneurons (Bodor et al., 2005; Katona et al., 1999, 2001; Marsicano and Lutz, 1999; Mascagni and McDonald, 2007; Morales et al., 1996; Morales and Bloom, 1997), suggesting that both receptors reciprocally control the activity of those interneurons and hence GABA release. It has been hypothesised that serotonergic fibres originating from the raphe increase GABA release in the BLA, hippocampus, and the neocortex, via 5-HT_3 receptors and this effect is controlled by retrograde signalling mediated by endocannabinoids (Freund and Hajos, 2003). Convergent evidence has implicated the amygdala, particularly the BLA in the extinction of fear (Barad et al., 2006). In addition, CCK-containing interneurons and the ECS are highly involved in processes of extinction learning (Chhatwal et al., 2005; Chung and Moore, 2007). This combination makes it very likely that in the BLA, CCK-containing interneurons under the control of CB1 and 5-HT_3 receptors may be an important locus of plasticity underlying fear extinction. In the present study, however, pharmacological modulation of 5-HT_3 activity did not interfere with synaptic processes dependent on EC release, neither on neuronal nor behavioural level.

5-HT_3 receptors were repeatedly implicated in the control of anxiety. However, the effects of 5-HT_3 agonists on anxiety

are highly inconsistent and range from anxiolytic to anxiogenic (Andrews and File, 1992; Bourin et al., 2001; Costall et al., 1988; Delagrange et al., 1999; Eguchi et al., 2001; Olivier et al., 1998; Prunier et al., 1997). Even 5-HT_3 antagonists provided inconsistent results (for review see (Olivier et al., 2000; Thompson and Lummis, 2007)). Such inconsistencies were explained by 5-HT_3 effects on serotonin release (Bagdy, 1998; Blier and Bouchard, 1993) and interactions with noradrenaline, glycine, and GABA neurotransmission (Chesnoy-Marchais et al., 2000; Maksay, 1996; Miranda-Morales et al., 2007; Mongeau et al., 1994). The situation might be clearer if fear rather than anxiety is considered. In the present study extinction of aversive memories was inhibited by 5-HT_3 activation, whereas extinction was accelerated when mice were treated with the 5-HT_3 receptor antagonist Trop. It appears to be that activation of 5-HT_3 receptors by endogenous serotonin is maximal at day 1 and day 2, and this activation promotes fear expression. Accordingly, blockade of 5-HT_3 receptors by Trop caused a strong decrease of the freezing response, whereas exogenous agonists of 5-HT3 (e.g., SR57227A) could not further activate the receptor and, thus, had no consequences on fear expression. The situation was different at day 3, when the exogenous agonist promoted fear expression, which reached levels comparable to those shown at day 1 and 2. We can speculate that these effects became evident because of an extinction-related decrease in receptor occupancy by endogenous serotonin. In accordance to our previous findings (Kamprath et al., 2006; Plendl and Wotjak, 2010), treatment with the CB1 receptor antagonist RIM caused an increase in expression of conditioned fear at each experimental day. This increase could not be ameliorated by co-administration of Trop, which speaks against an interdependence of 5-HT_3 and CB1 receptor signalling in expression of conditioned fear. However, we cannot entirely rule out that there are more subtle interactions of the two receptor systems, which might become evident e.g. at lower doses of RIM.

In conclusion, our data do not support a functional interaction between 5-HT_3 receptors and the ECS neither at the level of GABA neurotransmission in the BLA nor in fear conditioning experiments.

4. Experimental procedures

4.1. *Animals*

In the present study, investigations were performed in slices of male C57BL/6N mice (6–8 weeks; Martinsried, Munich, Germany). The mice were housed individually with an inverse 12/12 h light/dark circle for at least 1 week before starting experiments.

All experiments were conducted in agreement with the guidelines of the Ethical Committee on the Use and Care of Animals (Government of Bavaria, Germany). Brain slices were prepared during the light phase. The animals were anaesthetised with isoflurane and decapitated. The brains were fast removed and placed in ice-cold artificial ACSF containing (in mM) 125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, 25 D-glucose, 1,25 NaH_2PO_4, pH 7,4 and bubbled with a 95% O_2/5% CO_2 compound. Using a vibroslicer (FTB, Weinheim, Germany) coronal slices of the amydala (350 μm) were accomplished. The slices were incubated for at least 40 min in a holding chamber with ACSF (22–25°) and planted in the recording chamber being superfused with ACSF at a flow rate of 1.5 ml/min.

4.2. *Immunofluorescent-immunohistochemistry (IF-IHC)*

For immunohistochemical experiments, slices were incubated with 10 μM FM1-43 (Molecular Probes, Eugene, OR) and principal neurons of the basolateral amygdala were injected with neurobiotin tracer by passing 1–5 nA depolarising rectangular pulses of 150 ms duration at 3.3 Hz for 2–10 min. For the specific staining of presynaptic terminals innervating the recorded neuron, afferents were stimulated with 30 Hz for 5 min via the stimulating electrode placed in the lateral amygdala. Slices were then left in the FM1-43 solution for additional 5 min and then washed with ACSF for 30 min. They were then washed in cold PBS buffer (137 mM NaCl, 12 mM Phosphate, 2.7 mM KCl, pH 7.4) and fixed in 4% paraformaldehyde in PBS overnight (O/N) at 4 °C. For IF-IHC, all solution were prepared in PBS. In brief, sections were washed three times in PBS and then permeabilized in 0.2% Triton-X100 for 2 h (hrs) at room temperature (RT). After three washes with PBS, they were then incubated in a blocking solution containing 10% normal goat serum (VectorLabs, Burlingame, CA) for 2 h. Following this pretreatment, sections were incubated with streptavidin Alexa Fluor 405 (Molecular Probes, Life Technologies, NY, US) for 48 h at 4 °C. The day after, they were washed with PBS and then incubated with rabbit anti-CB1 receptor antibody (1:1000, Affinity BioReagents, Golden, CO) O/N at 4 °C. After washing with PBS, sections were incubated for 2 h at RT with a biotinylated anti-rabbit IgG (1:200, Jackson Immunoresearch, Suffolk, UK), washed with PBS and further incubated with avidin and Alexa Fluor 488 conjugate (1:1000, Molecular Probes) for additional 2 h at RT. They were then washed and incubated O/N at 4 °C with rabbit anti-5HT3 receptor antibody (1:200, Calbiochem, San Diego, CA, USA). Afterwards, they were washed and incubated with anti-rabbit Alexa Fluor 647 (1:200, Molecular Probes) for 2 h at RT, washed and mounted onto confocal microscopy chambers (CoverWell imaging chamber gaskets, Molecular Probes) with anti-fading mounting medium (Aqua-Poly/Mount, Polysciences, Eppelheim, Germany).

For complementary control experiments, slices were cut, fixed and pre-treated as described above. Afterwards, they were co-incubated with rabbit anti-CB1 receptor and chicken anti-MAP2 (1:5000, Neuromics, Bloomington, MN, USA) antibodies O/N at 4 °C. The day after, slices were washed and incubated with anti-chicken Alexa Fluor 488 (1:10000, Molecular Probes) for 2 h at RT; for detection of CB1 receptor, the procedure was the same as described before. After the last wash in PBS, slices were further incubated with rabbit anti-5HT3 receptor antibody O/N at 4 °C. The day after they were washed in PBS and processed with the appropriate secondary antibody as described above. Slices were then mounted and analysed with Laser Scanned Confocal Microscopy.

4.3. Confocal microscopy

For the characterisation of CB1 and $5HT_3$ receptor localisation, slices were examined using confocal microscopy (Olympus, Hamburg, Germany). Slices were analysed with a 60× oil-immersion objective, at different magnifications. Pictures are representative of results from three different experiments. The co-localisation was estimated looking at orthogonal projections of the respective signals to ensure pre- or postsynaptic localisation with respect to the dendritic branch analysed.

4.4. Electrophysiology

For whole-cell patch-clamp recordings principal neurons were visualised using infrared video microscopy and the gradient contrast system (Zeiss, Oberkochen, Germany). For technical details, see (Dodt et al., 2002). $GABA_A$ receptor-mediated synaptic transmission ($eGABA_A$-IPSCs) were isolated by application of 50 μM d-(–)-2-amino-5-phosphonopentanoic acid (D-AP-5), 5 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydro-benzo[f]quinoxaline-7-sulphonamide (NBQX) and 200 μM 3-aminopropyl (diethoxymethyl)phosphonic acid (CGP 35348). $eGABA_A$-IPSCs were evoked by square pulse stimuli (0.066 Hz, 5–12 mA, 200 μs) via bipolar tungsten electrodes positioned within the lateral amygdala close to the external capsule and recorded in the BLA. The patch pipettes were pulled from thin-walled borosilicate glass tubes with inner filament (outer diameter 1.5 mm, inner diameter 1.17 mm, GC150TF-10, Clark Electromedical Instruments, Pangbourne Reading, UK) and heat polished using a two-step horizontal puller (DMZ-Universal Puller, Zeitz-Instruments, Munich, Germany). Pipettes had a series resistance of 4–6 MΩ. For recording of $eGABA_A$-IPSCs, glass electrodes contained (in mM): K-D-gluconate 130, KCl 5, Mg-ATP 4, Na_2-phosphocreatine 5, HEPES 10, lidocaine-N-ethyl chloride 5; pH 7.4 adjusted with KOH (osmolarity: 305 mOsm). Patch-clamp recordings were performed at −50 mV holding potential. LTDi were performed at a holding potential −70 mV. LTDi was induced by 100 stimuli at 1 Hz in the current clamp mode (Marsicano et al., 2002). The pipettes were filled with a solution containing (in mM): Mg-ATP 2, $CsCH_3SO_3$ 100, CsCl 60, EGTA 0.2, HEPES 10, $MgCl_2$ 1, QX314 5 and Na_3GTP 0.3 (pH 7.3). Under these conditions, the reversal potential for Cl^- ions was −28 mV. Statistical analysis was performed using the Student's t-test to compare the values of 5 min of a stable baseline (control) to the effect of LTDi or a substance, in each case. The values of 5 min of a stable effect were chosen. $p<0.05$ was considered as a significant difference. Data are expressed as the percentage (means ± SEM) of the average baseline response. Only the last 10 min of the baseline are depicted in all graphs. Currents were recorded using a switched voltage-clamp amplifier (SEC-10L; npi Electronics) with switching frequencies of 75–80 kHz (25% duty cycle). Once electrodes were in ACSF, special care was taken to balance capacity compensation, electrode series resistance and to adjust zero DC so that there were no differences between measurements made in bridge (BC) and switched mode. After impalement of neurons, the capacitance compensation was readjusted to avoid errors in SECC measurements when switching to SEVC (Brennecke and Lindemann, 1974; Finkel and Redman, 1983, 1984; Juusola, 1994). Correct compensation of input capacitance and complete settling of the current injection artefact prior to sampling of the voltage signal in switched mode were controlled by continuously monitoring unsampled electrode potentials on an analogue oscilloscope triggered by the switching frequency. In addition, recording conditions were monitored by injecting hyperpolarizing current pulses (300 ms, −10 mV, 0.066 Hz) through the patch electrode. All recordings were amplified, filtered (3 kHz), and digitised (9 kHz). The digitised data were stored to a Macintosh G3 computer by data acquisition and evaluation program (Pulse v. 8.5; Heka Electronic, Lambrecht, Germany).

4.5. Drugs for electrophysiology

Drugs were applied via superfusion system. The following pharmacological compounds were applied: tropisetron, ondansetron, rimonabant (RIM; SR141716) (NIMH Chemical Synthesis and Drug Supply Program, USA), SR57227A (1-(6-Chloro-2-pyridinyl)-4-piperidinamine hydrochloride; Tocris, Bristol, UK), D-AP-5, CGP35348 (kind gift from Novartis Laboratories, Basel, Switzerland), NBQX. Except otherwise stated, all salts and drugs were purchased from RBI/Sigma (Deisenhofen, Germany).

Stock solutions of RIM (10 mM) were prepared in DMSO and stored at −20 °C. Final DMSO concentrations were ≤0.05%. Before all experiments, fatty acid-free bovine serum albumin (1 mg/ml) was rinsed through the system to avoid binding of RIM to the walls of the tubing.

4.6. Fear conditioning

Male C57BL/6N mice were purchased at an age of 7–9 weeks and housed individually under an inverse 12 h:12 h light/dark cycle (lights off: 09:00 h) for 2 weeks before starting with the experiments. Mice underwent auditory fear conditioning and re-exposure to the tones essentially as described before (Kamprath and Wotjak, 2004; Plendl and Wotjak, 2010). In brief, at the conditioning day (d0), all mice were placed individually into mouse conditioning chambers, where they received a single pairing of a tone (9 kHz, 20 s, 80 dB) with an electric foot shock (0.7 mA, 2 s). At the following days (d1–d3), mice were placed into a novel context, which differed from the conditioning context in texture, bedding, shape and odour of the cleaning solution. After 3 min, the conditioned tone was presented for another 3 min, and mice were returned to their home cages 60 s later. In the first two experiments, mice were treated either with SR57227A (1 or 3 mg/kg) or Trop (0.3 or 1 mg/kg) or vehicle 30 min before exposure to the tone on 3 consecutive days (d1–d3). In the third experiment, new groups of mice received two injections 30 min before re-exposure to the tone 1 day after conditioning. The treatments were as follows: vehicle–vehicle, Trop (1 mg/kg)–vehicle, vehicle–RIM (3 mg/kg) or Trop (1 mg/kg) – RIM (3 mg/kg). The effective dose of RIM was selected on the basis of published data (Kamprath and Wotjak, 2004; Kamprath et al., 2006; Plendl and Wotjak, 2010), that of Trop on the basis of the results of experiment 2. Freezing to the tone was scored off-line by a trained observer. Data (mean ± SEM) were expressed as a percentage of the total observation period (3 min) and analysed by 2-way ANOVA (treatment,

day) for repeated measures (day), one-way ANOVA (treatment) separately per day or 2-way ANOVA (treatment1, treatment 2). Group differences were assessed by Newman–Keuls post-hoc test, if appropriate. The threshold for statistical significance was $p<0.05$. SR57227A and Trop were dissolved in physiological saline and injected intraperitoneally (i.p.) in a volume of 10 ml/kg. RIM (3 mg/kg) was dissolved in vehicle solution (2.5% DMSO and 1 drop of Tween 80 per 3 ml of saline) and injected subcutaneously (s.c.) in a volume of 20 ml/kg. The injections were made 1 h prior each extinction training.

Acknowledgments

V. M. is supported by the project "CEITEC-Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund. S.C.A and G.R. have been supported by a DFG grant (AZ 71/1-1) and (RA 689/7-1), respectively.

REFERENCES

Andrews, N., File, S.E., 1992. Are there changes in sensitivity to 5-HT3 receptor ligands following chronic diazepam treatment?. Psychopharmacology (Berl) 108, 333–337.

Azad, S.C., Eder, M., Marsicano, G., et al., 2003. Activation of the cannabinoid receptor type 1 decreases glutamatergic and GABAergic synaptic transmission in the lateral amygdala of the mouse. Learn. Mem. 10, 116–128.

Azad, S.C., Monory, K., Marsicano, G., et al., 2004. Circuitry for associative plasticity in the amygdala involves endocannabinoid signaling. J. Neurosci. 24, 9953–9961.

Bachy, A., Heaulme, M., Giudice, A., et al., 1993. SR 57227A: a potent and selective agonist at central and peripheral 5-HT3 receptors in vitro and in vivo. Eur. J. Pharmacol. 237, 299–309.

Bagdy, G., 1998. Serotonin, anxiety, and stress hormones. Focus on 5-HT receptor subtypes, species and gender differences. Ann. N. Y. Acad. Sci. 851, 357–363.

Baker, D., Pryce, G., Croxford, J.L., et al., 2000. Cannabinoids control spasticity and tremor in a multiple sclerosis model. Nature 404, 84–87.

Bambico, F.R., Katz, N., Debonnel, G., et al., 2007. Cannabinoids elicit antidepressant-like behaviour and activate serotonergic neurons through the medial prefrontal cortex. J. Neurosci. 27, 11700–11711.

Barad, M., Gean, P.W., Lutz, B., 2006. The role of the amygdala in the extinction of conditioned fear. Biol. Psychiatry 60, 322–328.

Barann, M., Molderings, G., Bruss, M., et al., 2002. Direct inhibition by cannabinoids of human 5-HT3A receptors: probable involvement of an allosteric modulatory site. Br. J. Pharmacol. 137 (5), 589–596.

Blier, P., Bouchard, C., 1993. Functional characterisation of a 5-HT3 receptor which modulates the release of 5-HT in the guinea-pig brain. Br. J. Pharmacol. 108, 13–22.

Bodor, A.L., Katona, I., Nyiri, G., et al., 2005. Endocannabinoid signaling in rat somatosensory cortex: laminar differences and involvement of specific interneuron types. J. Neurosci. 25, 6845–6856.

Bourin, M., Nic Dhonnchadha, B.A., Claude Colombel, M., et al., 2001. Cyamemazine as an anxiolytic drug on the elevated plus maze and light/dark paradigm in mice. Behav. Brain Res. 124, 87–95.

Braida, D., Limonta, V., Malabarba, L., et al., 2007. 5-HT1A receptors are involved in the anxiolytic effect of Delta9-tetrahydrocannabinol and AM 404, the anandamide transport inhibitor, in Sprague-Dawley rats. Eur. J. Pharmacol. 555, 156–163.

Breivogel, C.S., Childers, S.R., 1998. The functional neuroanatomy of brain cannabinoid receptors. Neurobiol. Dis. 5, 417–431.

Brennecke, R., Lindemann, B., 1974. Design of a fast voltage clamp for biological membranes, using discontinuous feedback. Rev. Sci. Instrum. 45, 656–661.

Chesnoy-Marchais, D., Levi, S., Acher, F., 2000. Glycinergic potentiation by some 5-HT(3) receptor antagonists: insight into selectivity. Eur. J. Pharmacol. 402, 205–213.

Chevaleyre, V., Heifets, B.D., Kaeser, P.S., et al., 2007. Endocannabinoid-mediated long-term plasticity requires cAMP/PKA signaling and RIM1alpha. Neuron 54, 801–812.

Chhatwal, J.P., Davis, M., Maguschak, K.A., et al., 2005. Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. Neuropsychopharmacology 30, 516–524.

Chung, L., Moore, S.D., 2007. Cholecystokinin enhances GABAergic inhibitory transmission in basolateral amygdala. Neuropeptides 41, 453–463.

Costall, B., Domeney, A.M., Gerrard, P.A., et al., 1988. Zacopride: anxiolytic profile in rodent and primate models of anxiety. J. Pharm. Pharmacol. 40, 302–305.

Davidson, R.J., 2002. Anxiety and affective style: role of prefrontal cortex and amygdala. Biol. Psychiatry 51, 68–80.

Delagrange, P., Misslin, R., Seale, T.W., et al., 1999. Effects of S-21007, a potent 5-HT3 partial agonist, in mouse anxiety. Chung-Kuo Yao Li Hsueh Pao—Acta Pharmacol. Sin. 20, 805–812.

Derkach, V., Surprenant, A., North, R.A., 1989. 5-HT3 receptors are membrane ion channels. Nature 339, 706–709.

Dodt, H.U., Eder, M., Schierloh, A., et al., 2002. Infrared-guided laser stimulation of neurons in brain slices. Sci. STKE 2002, PL2.

Egertova, M., Giang, D.K., Cravatt, B.F., et al., 1998. A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB1 receptor in rat brain. Proc. Biol. Sci. 265, 2081–2085.

Egertova, M., Cravatt, B.F., Elphick, M.R., 2003. Comparative analysis of fatty acid amide hydrolase and cb(1) cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. Neuroscience 119, 481–496.

Eguchi, J., Inomata, Y., Saito, K., 2001. The anxiolytic-like effect of MCI-225, a selective NA reuptake inhibitor with 5-HT3 receptor antagonism. Pharmacol., Biochem. Behav. 68, 677–683.

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.

Finkel, A.S., Redman, S., 1983. A shielded microelectrode suitable for single-electrode voltage clamping of neurones in the CNS. J. Neurosci. Methods 9, 23–29.

Finkel, A.S., Redman, S., 1984. Theory and operation of a single microelectrode voltage clamp. J. Neurosci. Methods 11, 101–127.

Freund, T.F., Hajos, N., 2003. Excitement reduces inhibition via endocannabinoids. Neuron 38, 362–365.

Freund, T.F., Katona, I., Piomelli, D., 2003. Role of endogenous cannabinoids in synaptic signaling. Physiol. Rev. 83, 1017–1066.

Gobbi, G., Bambico, F.R., Mangieri, R., et al., 2005. Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. Proc. Natl. Acad. Sci. USA 102, 18620–18625.

Haller, J., Matyas, F., Soproni, K., et al., 2007. Correlated species differences in the effects of cannabinoid ligands on anxiety and on GABAergic and glutamatergic synaptic transmission. Eur. J. Neurosci. 25, 2445–2456.
Hampson, R.E., Zhuang, S.Y., Weiner, J.L., et al., 2003. Functional significance of cannabinoid-mediated, depolarization-induced suppression of inhibition (DSI) in the hippocampus. J. Neurophysiol. 90, 55–64.
Häring, M., Marsicano, G., Lutz, B., et al., 2007. Identification of the cannabinoid receptor type 1 in serotonergic cells of raphe nuclei in mice. Neuroscience 146, 1212–1219.
Harmer, C.J., Reid, C.B., Ray, M.K., et al., 2006. 5HT(3) antagonism abolishes the emotion potentiated startle effect in humans. Psychopharmacology (Berl) 186, 18–24.
Hermann, H., Marsicano, G., Lutz, B., 2002. Coexpression of the cannabinoid receptor type 1 with dopamine and serotonin receptors in distinct neuronal subpopulations of the adult mouse forebrain. Neuroscience 109, 451–460.
Hill, M.N., Sun, J.C., Tse, M.T., et al., 2006. Altered responsiveness of serotonin receptor subtypes following long-term cannabinoid treatment. Int. J. Neuropsychopharmacol. 9, 277–286.
Johansen, J.P., Cain, C.K., Ostroff, L.E., et al., 2011. Molecular mechanisms of fear learning and memory. Cell 147, 509–524.
Juusola, M., 1994. Measuring complex admittance and receptor current by single electrode voltage-clamp. J. Neurosci. Methods 53, 1–6.
Kamprath, K., Wotjak, C.T., 2004. Nonassociative learning processes determine expression and extinction of conditioned fear in mice. Learn. Mem. 11, 770–786.
Kamprath, K., Marsicano, G., Tang, J., et al., 2006. Cannabinoid CB1 receptor mediates fear extinction via habituation-like processes. J. Neurosci. 26, 6677–6686.
Katona, I., Sperlagh, B., Sik, A., et al., 1999. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J. Neurosci. 19, 4544–4558.
Katona, I., Rancz, E.A., Acsady, L., et al., 2001. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. J. Neurosci. 21, 9506–9518.
Kimura, T., Ohta, T., Watanabe, K., et al., 1998. Anandamide, an endogenous cannabinoid receptor ligand, also interacts with 5-hydroxytryptamine (5-HT) receptor. Biol. Pharm. Bull. 21, 224–226.
Koyama, S., Matsumoto, N., Kubo, C., et al., 2000. Presynaptic 5-HT3 receptor-mediated modulation of synaptic GABA release in the mechanically dissociated rat amygdala neurons. J. Physiol.-London 529 (2), 373–383.
Koyama, S., Matsumoto, N., Murakami, N., et al., 2002. Role of presynaptic 5-HT1A and 5-HT3 receptors in modulation of synaptic GABA transmission in dissociated rat basolateral amygdala neurons. Life Sciences 72 (4–5), 375–387.
Kreitzer, A.C., Regehr, W.G., 2001. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. Neuron 29, 717–727.
Linthorst, A.C., 2005. Interactions between corticotropin-releasing hormone and serotonin: implications for the aetology and treatment of anxiety disorders. Handb. Exp. Pharmacol. 169, 181–204.
Luchicchi, A., Pistis, M., 2012. Anandamide and 2-arachidonoylglycerol: pharmacological properties, functional features, and emerging specificities of the two major endocannabinoids. Mol. Neurobiol. 46, 374–392.
Lucki, I., 1998. The spectrum of behaviors influenced by serotonin. Biol. Psychiatry 44, 151–162.
Lutz, B., 2007. The endocannabinoid system and extinction learning. Mol. Neurobiol. 36, 92–101.
Maksay, G., 1996. Distinct thermodynamic parameters of serotonin 5-HT3 agonists and antagonists to displace [3H] granisetron binding. J. Neurochem. 67, 407–412.
Maldonado, R., Valverde, O., 2003. Participation of the opioid system in cannabinoid-induced antinociception and emotional-like responses. Eur. Neuropsychopharmacol. 13, 401–410.
Marco, E.M., Perez-Alvarez, L., Borcel, E., et al., 2004. Involvement of 5-HT1A receptors in behavioural effects of the cannabinoid receptor agonist CP 55,940 in male rats. Behav. Pharmacol. 15, 21–27.
Maricq, A.V., Peterson, A.S., Brake, A.J., et al., 1991. Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel. Science 254, 432–437.
Marsicano, G., Lutz, B., 1999. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. Eur. J. Neurosci. 11, 4213–4225.
Marsicano, G., Wotjak, C.T., Azad, S.C., et al., 2002. The endogenous cannabinoid system controls extinction of aversive memories. Nature 418, 530–534.
Marsicano, G., Lutz, B., 2006. Neuromodulatory functions of the endocannabinoid system. J. Endocrinol. Invest. 29, 27–46.
Martin, W.J., Coffin, P.O., Attias, E., et al., 1999. Anatomical basis for cannabinoid-induced antinociception as revealed by intracerebral microinjections. Brain Res. 822, 237–242.
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, 1015–1024.
Mato, S., Aso, E., Castro, E., et al., 2007. CB1 knockout mice display impaired functionality of 5-HT1A and 5-HT2A/C receptors. J. Neurochem. 103, 2111–2120.
Mikics, E., Vas, J., Aliczki, M., et al., 2009. Interactions between the anxiogenic effects of CB1 gene disruption and 5-HT3 neurotransmission. Behav. Pharmacol. 20, 265–272.
Miranda-Morales, M., Garcia-Hernandez, L.M., Ochoa-Cortes, F., et al., 2007. Cross-talking between 5-HT3 and GABAA receptors in cultured myenteric neurons. Synapse 61, 732–740.
Moldrich, G., Wenger, T., 2000. Localization of the CB1 cannabinoid receptor in the rat brain. An immunohistochemical study. Peptides 21, 1735–1742.
Mongeau, R., De Montigny, C., Blier, P., 1994. Effect of long-term administration of antidepressant drugs on the 5-HT3 receptors that enhance the electrically evoked release of [3H] noradrenaline in the rat hippocampus. Eur. J. Pharmacol. 271, 121–129.
Morales, M., Battenberg, E., de Lecea, L., et al., 1996. Cellular and subcellular immunolocalization of the type 3 serotonin receptor in the rat central nervous system. Brain Res. Mol. Brain Res. 36, 251–260.
Morales, M., Bloom, F.E., 1997. The 5-HT3 receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon. J. Neurosci. 17, 3157–3167.
Morales, M., Wang, S.D., 2002. Differential composition of 5-hydroxytryptamine3 receptors synthesized in the rat CNS and peripheral nervous system. J. Neurosci. 22, 6732–6741.
Morales, M., Wang, S.D., Diaz-Ruiz, O., et al., 2004. Cannabinoid CB1 receptor and serotonin 3 receptor subunit A (5-HT3A) are co-expressed in GABA neurons in the rat telencephalon. J. Comp. Neurol. 468, 205–216.
Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterisation of a peripheral receptor for cannabinoids. Nature 365, 61–65.
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 different types of anxiolytic activity?. J. Pharmacol. Exp. Ther. 268, 248–254.
Ohno-Shosaku, T., Maejima, T., Kano, M., 2001. Endogenous cannabinoids mediate retrograde signals from depolarized

postsynaptic neurons to presynaptic terminals. Neuron 29, 729–738.
Olivier, B., Molewijk, H.E., van der Heyden, J.A., et al., 1998. Ultrasonic vocalizations in rat pups: effects of serotonergic ligands. Neurosci. Biobehav. Rev. 23, 215–227.
Olivier, B., van Wijngaarden, I., Soudijn, W., 2000. 5-HT(3) receptor antagonists and anxiety; a preclinical and clinical review. Eur. Neuropsychopharmacol. 10, 77–95.
Oostland, M., Sellmeijer, J., van Hooft, J.A., 2011. Transient expression of functional serotonin 5-HT3 receptors by glutamatergic granule cells in the early postnatal mouse cerebellum. J. Physiol. 589, 4837–4846.
Pape, H.C., Pare, D., 2010. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiol. Rev. 90, 419–463.
Pertwee, R.G., 2001. Cannabinoid receptors and pain. Prog. Neurobiol. 63, 569–611.
Plendl, W., Wotjak, C.T., 2010. Dissociation of within- and between-session extinction of conditioned fear. J. Neurosci. 30, 4990–4998.
Porter, A.C., Felder, C.C., 2001. The endocannabinoid nervous system: unique opportunities for therapeutic intervention. Pharmacol. Ther. 90, 45–60.
Prunier, H., Rault, S., Lancelot, J.C., et al., 1997. Novel and selective partial agonists of 5-HT3 receptors. 2. Synthesis and biological evaluation of piperazinopyridopyrrolopyrazines, piperazinopyrroloquinoxalines, and piperazinopyrido-pyrroloquinoxalines. J. Med. Chem. 40, 1808–1819.
Ramikie, T.S., Patel, S., 2012. Endocannabinoid signaling in the amygdala: anatomy, synaptic signaling, behaviour, and adaptations to stress. Neuroscience 204, 38–52.
Rammes, G., Hosp, C., Eisensamer, B., et al., 2009. Identification of a domain which affects kinetics and antagonistic potency of clozapine at 5-HT3 receptors. PLoS ONE 4, e6715.
Reibaud, M., Obinu, M.C., Ledent, C., et al., 1999. Enhancement of memory in cannabinoid CB1 receptor knock-out mice. Eur. J. Pharmacol. 379, R1–R2.
Robson, P., 2001. Therapeutic aspects of cannabis and cannabinoids. Br. J. Psychiatry 178, 107–115.
Rodriguez de Fonseca, F., Del Arco, I., Bermudez-Silva, F.J., et al., 2005. The endocannabinoid system: physiology and pharmacology. Alcohol Alcohol. 40, 2–14.
Roerig, B., Nelson, D.A., Katz, L.C., 1997. Fast synaptic signaling by nicotinic acetylcholine and serotonin 5-HT3 receptors in developing visual cortex. J. Neurosci. 17, 8353–8362.
Rubio, M., Fernandez-Ruiz, J., de Miguel, R., et al., 2008. CB1 receptor blockade reduces the anxiogenic-like response and ameliorates the neurochemical imbalances associated with alcohol withdrawal in rats. Neuropharmacology 54, 976–988.
Southwick, S.M., Paige, S., Morgan 3rd, C.A., et al., 1999. Neurotransmitter alterations in PTSD: catecholamines and serotonin. Semin. Clin. Neuropsychiatry 4, 242–248.
Sugita, S., Shen, K.Z., North, R.A., 1992. 5-hydroxytryptamine is a fast excitatory transmitter at 5-HT3 receptors in rat amygdala. Neuron 8, 199–203.
Thompson, A.J., Lummis, S.C., 2006. 5-HT3 receptors. Curr. Pharm. Des. 12, 3615–3630.
Thompson, A.J., Lummis, S.C., 2007. The 5-HT3 receptor as a therapeutic target. Expert Opinion Ther. Targets 11, 527–540.
Wilson, R.I., Nicoll, R.A., 2002. Neuroscience—endocannabinoid signaling in the brain. Science 296, 678–682.
Witkin, J.M., Tzavara, E.T., Nomikos, G.G., 2005. A role for cannabinoid CB1 receptors in mood and anxiety disorders. Behav. Pharmacol. 16, 315–331.