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Review

Serotonergic Neuroplasticity in Alcohol Addiction

Arnauld Belmer[a,b,*], Omkar L. Patkar[a,b], Kim M. Pitman[a,b] and Selena E. Bartlett[a,b,*]
[a]*Translational Research Institute, Queensland University of Technology, Brisbane, Australia*
[b]*Institute of Health and Biomedical Innovation (IHBI), Queensland University of Technology, Brisbane, Australia*

Abstract. Alcohol addiction is a debilitating disorder producing maladaptive changes in the brain, leading drinkers to become more sensitive to stress and anxiety. These changes are key factors contributing to alcohol craving and maintaining a persistent vulnerability to relapse.

Serotonin (5-Hydroxytryptamine, 5-HT) is a monoamine neurotransmitter widely expressed in the central nervous system where it plays an important role in the regulation of mood. The serotonin system has been extensively implicated in the regulation of stress and anxiety, as well as the reinforcing properties of all of the major classes of drugs of abuse, including alcohol. Dysregulation within the 5-HT system has been postulated to underlie the negative mood states associated with alcohol use disorders.

This review will describe the serotonergic (5-HTergic) neuroplastic changes observed in animal models throughout the alcohol addiction cycle, from prenatal to adulthood exposure. The first section will focus on alcohol-induced 5-HTergic neuroadaptations in offspring prenatally exposed to alcohol and the consequences on the regulation of stress/anxiety. The second section will compare alterations in 5-HT signalling induced by acute or chronic alcohol exposure during adulthood and following alcohol withdrawal, highlighting the impact on the regulation of stress/anxiety signalling pathways. The third section will outline 5-HTergic neuroadaptations observed in various genetically-selected ethanol preferring rat lines. Finally, we will discuss the pharmacological manipulation of the 5-HTergic system on ethanol- and anxiety/stress-related behaviours demonstrated by clinical trials, with an emphasis on current and potential treatments.

Keywords: Serotonin, alcohol-related disorders, alcohol addiction, anxiety, stress, depression

Serotonin (5-hydroxytryptamine, 5-HT) is present in almost all organisms from plants to vertebrates. In mammals, 5-HT has been found throughout the body, including the brain, gut, lung, liver, kidney, skin, and platelets. Such a wide distribution indicates that 5-HT is an essential chemical for cell signalling and function in all living animals. In the brain, 5-HT-synthetising neurons are located in the brainstem raphe nuclei, and the distribution of 5-HT projections is widespread, regulating the activity of almost all brain regions. Thus, 5-HT signalling has been implicated in a variety of brain functions, such as sleep-wake cycle, appetite, locomotion, emotion, hormonal regulation, and as a trophic factor. Furthermore, 5-HT is involved in cognitive functions, including attention, control of impulsivity, coping with stress, social behaviour, value-based decision making, learning and memory.

Serotonin exerts its action via 14 classes of receptors (5-HT1-7). With the exception of 5-HT3 receptors, which gate a cation-permeable ion channel, all 5-HT receptors are coupled to G proteins. The core features of transduction via 5-HT receptors are well established: the 5-HT1-5 receptor subtypes

*Correspondence to: Arnauld Belmer and Selena E. Bartlett, Addiction Neuroscience and Obesity Group - Translational Research Institute- Institute of Health and Biomedical Innovation - Queensland University of Technology. 37 Kent St, Woolloongabba, 4102, QLD, Australia. Tel.: +61 7 3443 7291; Fax: +61 7 3443 6966; E-mail: arnauld.belmer@qut.edu.au (A. Belmer), Tel.: +61 7 3443 7238; Fax: +61 7 3443 6966; E-mail: Selena.bartlett@qut.edu.au (S.E. Bartlett).

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are inhibitors while 5-HT2, 4, 6 and 7 receptor subtypes are activators of neuronal activity. Thus, 5-HT can exert a complex effect on the neuronal output of different brain regions, depending on which 5-HT receptors are expressed, and whether they are expressed by glutamatergic (excitatory) or GABAergic (inhibitory) neurons. Additionally, some receptors, such as the 5-HT1A and 1B receptors, have been shown to be also located presynaptically on 5-HT neurons to negatively regulate 5-HTergic neurotransmission [1, 2]. Another main actor in 5-HT signalling is the serotonin transporter (SERT), which is essential to terminate the action of 5-HT in the synapse by reuptaking 5-HT into the terminals.

Hence, serotonin homeostasis is finely regulated and, in humans, alteration in the 5-HT system has been associated with various neuropsychiatric disorders, including stress disorders [3, 4], anxiety [5, 6], depression [7–13], bipolar disorders [14] and substance abuse (cocaine [15, 16]; MDMA [17, 18]). These observations suggest that neurochemical adaptations occur in 5-HT neurons in response to environmental or pharmacological stressors. This is supported by studies in rodents showing that both acute and chronic exposure to stress during early life or adulthood alter the functional responses in serotonergic neurons [19], reduce the density of 5-HT innervation in the central, basolateral amygdala and the hippocampus [20], increase the density of 5-HT1A receptors in the basolateral amygdala [21], reduce the expression of 5-HT1A and 5-HT1B receptors in the prefrontal cortex [22] and the hippocampus [23, 24], increase the expression of the 5-HT transporter, SERT, and the 5-HT synthetizing enzyme, TPH2 in the dorsal raphe nucleus (DRN) [25, 26]. Interestingly, comparable neuroplastic changes in brain 5-HT pathways have been observed in alcohol dependence, suggesting that similar mechanisms are involved. Indeed, a growing body of evidence reveals that alcohol use disorders show a high comorbidity with stress, anxiety and depression, in particular during alcohol abstinence following chronic long term exposure.

In this review, we will describe the changes in 5-HT signalling in limbic brain regions induced by prenatal, acute and chronic alcohol exposure, as well as the changes in 5-HT signalling in stress, anxiety and depression pathways induced by alcohol withdrawal. We will then focus on the 5-HTergic adaptations and changes in stress/anxiety-related behaviours observed in various genetically-selected ethanol preferring rodent lines. Finally, we will discuss the involvement of the 5-HTergic system in ethanol- and anxiety/stress-related behaviours, with an emphasis on current and potential treatments.

ANIMAL MODELS OF ALCOHOL CONSUMPTION

Over several decades, many animal models have been developed to study alcohol dependence. Early studies have employed a "two-bottle choice" procedure in which the animals have continuous access to water and ethanol. Although a slight preference for ethanol develops over drinking sessions, rodents usually limit their consumption to sub-intoxicating levels. Indeed, the taste of ethanol is primarily aversive and rodents do not naturally drink enough ethanol to attain blood ethanol concentration (BEC) equivalent to human alcoholics (0.8 g/L). Therefore, different strategies have been used, such as water deprivation, intragastric administration or systemic injection, to allow for the administration of large doses of ethanol, near lethal, that also produce toxicity and do not reflect the neurochemical process of voluntary drinking. Consequently, studies have tried to enhance the motivation to drink ethanol by adding sweeteners which allows for the addition of gradually increasing concentrations of alcohol in ways that avoid the aversiveness of ethanol. However, studies using this "sucrose-fading" procedure failed to produce stable BECs >0.8 g/L. Later, studies in rats have shown that removal of the ethanol bottle after 24 hours of exposure increases their consumption when ethanol is reintroduced 24 hours afterwards. This "chronic intermittent model" has been shown to produce high drinking patterns of 5-6 g/kg over 24 hours but the BECs were rarely higher than 0.6 g/L. Based on the observation that rodents ingest most of their daily food and water during the dark phase of their circadian cycle, the "Drinking In the Dark" (DID) model was developed. In this model, animals have a limited access to ethanol, 2 hours per day, 3 hours after the onset of the dark period, 4 days per week and on the 5th day, animals are given 4 hour access. This restricted access, alternating between exposure and withdrawal phases, allows for "binge' ethanol intake in mice (3.5–5 g/kg/2 hrs) and BECs over 1 g/L, especially in the C57Bl6 strain, known as alcohol preferrer. Although the mice chronically exposed to the DID for quite a long term (4–6 weeks) reach high BECs, they do not manifest signs of dependence nor ethanol withdrawal symptoms, such as seizures. However, a recent study reported that following 6 weeks of

exposure, mice exhibit increased anxious/depressive behaviours up to 3 weeks after alcohol withdrawal. To induce ethanol dependence in rodents, the "chronic intermittent exposure" (CIE) model has been used for many years. Animals are acutely or chronically (3 to 4 cycles) exposed to ethanol for 14–16 hours using vapour chambers and clearly reach high BECs (1-2 g/L) and show subsequent escalation of ethanol drinking. However, this procedure requires the co-administration of pyrazole, an inhibitor of the alcohol dehydrogenase, to obtain stable blood EtOH concentrations (BECs) during the entire induction course. Because alcohol vapours are passively administered to the animals and ethanol metabolism is inhibited in this procedure, the validity of this model to reproduce brain neuroplastic changes induced by ethanol dependence in human is questionable.

CONSEQUENCES OF PRENATAL ALCOHOL EXPOSURE ON 5-HT SIGNALLING, STRESS AND ANXIETY DURING EARLY LIFE AND ADULTHOOD (TABLE 1)

Foetal alcohol spectrum disorders, caused by maternal alcohol consumption during pregnancy, were first described as foetal alcohol syndrome [27]. These disorders are associated with central nervous system malformations (see [28, 29] for review), mental retardation [30, 31], cognitive impairments, mood disorders and behavioural dysfunctions that can vary in severity, depending on the amount of alcohol consumption, duration, and timing of prenatal alcohol exposure. Because of its important role in brain development, cognition and the regulation of mood, the 5-HT system has received much attention in the neuroplastic adaptations following prenatal alcohol exposure.

5-HT signalling

Incomplete neural tube fusion and missing roof and floor plate tissue in the midline have been observed *in vivo* in foetuses exposed to alcohol, as a result of delayed or prevented formation of the midline and the floor plate tissue [32]. Neurons producing 5-HT are among the earliest to be born in the developing brain and the germinal cells of 5-HT neurons expressed in the raphe adjacent to the midline have been known to rely on trophic factors in midline tissue to differentiate [33]. Thus, alteration in midline formation following prenatal alcohol exposure is likely to alter the development of 5-HT neurons in the offspring's brain. The effect of alcohol on 5-HT neurons begins at neurogenesis (see [29] for review). Animals prenatally exposed to alcohol show reduced density and retarded migration of 5-HT immunoreactive neurons as early as the 13th embryonic day (E13) in the DRN and median raphe (MRN) nuclei in mice [34] and through midgestation (E15) in rats [35] and mice [34, 36, 37]. *In vitro* studies using a 24 hour treatment of foetal rhombencephalic neurons with ethanol have established that the reduction of 5-HT neurons was probably caused by ethanol-associated apoptosis [38–40], a decreased activity of the phosphatidylinositol 3-kinase (PI3K)/pAkt pro-survival pathway [39] and reduced downstream expression of several NF-κB dependent anti-apoptotic genes [40, 41].

The deficit in 5-HT neurons persists into late gestation (E18) [42], in neotates (P5) [43], adolescent (P19-21) [44, 45] and into young adulthood (P35-45) [42, 46] in rats and mice, suggesting a long-lasting neuroplastic effect of ethanol on the 5-HT system [45]. Accordingly, reduced levels of 5-HT, its synthesis enzyme TPH2 (Tryptophan hydroxylase) and its degradation product 5-Hydroxyindoleacetic acid (5-HIAA) have been observed in the brain of embryos, neonates and adult animals exposed to ethanol *in-utero* [45, 47–53]. As a consequence of fewer 5-HT neurons in the raphe, embryos *in-utero* exposed to ethanol show a reduction of 5-HT projections into the medial forebrain bundle (MFB) [36] and fewer 5-HT fibres growing into the ascending pathway in the hypothalamus septal nucleus, frontal and parietal cortices [54]. The forebrain is known to actively develop upon the arrival of 5-HT innervation, this reduction of 5-HT fibre density in ethanol exposed animals likely results in altered growth of brain regions along the ascending 5-HT pathway (hypothalamus, septal nucleus, cortices, and subiculum/hippocampus) [54].

The serotonin transporter (SERT), responsible for the reuptake of 5-HT into presynaptic neurons and nerve terminals, has been shown to be a reliable marker of 5-HT neuron fibres [55]. Short and/or long-lasting alterations in SERT expression and function have been demonstrated in the cortex, hippocampus, medial and lateral amygdala, substantia nigra, DRN, and hypothalamus of offspring of dams that consumed alcohol [56–59]. A study in children with foetal alcohol syndrome (FAS) and foetal alcohol effects (FAE) found a similar reduction of SERT expression in the medial frontal cortex [60].

Along with changes in SERT levels, alterations in 5-HT1A receptor expression have been observed in

Table 1
Changes in 5-HTergic neuroplasticity following prenatal alcohol exposure

Species	Model	Dose of ethanol	Route of administration	BEC	Duration of treatment	Results	Ref #
Sprague Dawley rats	Rhombencephalon neuronal culture	25 to 100 nM	culture media	–	4 days	Increased apoptosis of fetal rhonbencephalic neurons and reduced number of 5-HT neurons. That is prevented by cotreatment with ipsapirone 100nM	38
C57BL6 mice	foetal exposure	20% (EDC)	liquid diet	65 mg/dl on E14	From E8 to E15	20–30% fewer 5-HT-immunoreactive neurons in the Raphe and retard of migration in E15 embryos	37
C57BL6 mice	foetal exposure	20% (EDC)	liquid diet	–	From E8 to E16	Reduced number of 5-HT-immunoreactive neurons inMR and DR. Decreased 5-HT-immunoreactive fibers in the medial forebrain bundle (MFB). Reduced 5-HT fiber diameter	36
C57BL6 mice	foetal exposure	20 or 25% (EDC)	liquid diet	44.3 ± 11.6 (E8), 54.7 ± 14.2 (E11), 142.7 ± 49.5 (E14) and 72.8+19.1 (E17) mg/dl	From E7 or E8.5	60% of embryos at E13 and 20% at E15 showed perforation of the floor plate in the diencephalic vesicle. 70–80% of embryos failed to complete the formation of neural tissue at the roof. 60–80% of embryos showed delayed closure of the ventral canal	32
C57BL6 mice	foetal exposure	25% (EDC)	liquid diet	–	From E7 to E11 or E13	20–30% fewer 5-HT-immunoreactive neurons in MR and DR and retard of migration in E11 and E13 embryos	34
C57BL6 mice	foetal exposure	25% (EDC)	liquid diet	142.7 ± 49.5 (E14) and 72.8+19.1 (E17) mg/dl	From E7 to E18	20% reduction of 5-HT-immunoreactive neurons in the MR and DR at E18 and 30% at P45	42
C57BL6 mice	foetal exposure	25% (EDC)	liquid diet	142.7 ± 49.5 (E14) and 72.8+19.1 (E17) mg/dl	E7 to E15 or E18	Fewer 5-HT-immunoreactive fibers in theMFB and along the projecting pathway through the hypothalamus, septal nucleus, frontal and parietal cortices, and HIP. Underdevelopment of the brain regions along 5-HT fiber projections. Underdevelopment of somatosensory thalamocortical projections, which are known to transiently express 5-HT transporters and to be regulated by 5-HT	54
C57BL6 mice	foetal exposure	25% (EDC)	liquid diet	–	From E7 to E13	Reduced whole brain concentration of 5-HT in E13 animals	48

Wistar rats	foetal exposure	2 × 2.9 g/kg (4 hr interval)	IP injection	446 mg/dl	Only at E8	Increased 5-HT1 function at P45: increased forepaw treading and hind limb abduction induced by 5-MeODMT (2.5 mg/kg, i.p.). Increased 5-HT2A function at P45: increased head twitch response to 5-HTP (150 mg/kg, s.c.)	65
Sprague Dawley rats	foetal exposure	6.6% v/v, *about 35% (EDC)*	liquid diet	–	For 4 to 6 weeks prior to breeding and during gestation (alcohol removed at postnatal day 3)	Brain stem: reduction of 40–60% of 5-HT (E15, E19 and P5) and 24 to 60% of 5-HIAA (E19 and P5). Cortex: reduction of 40% of 5-HT (P5) and 25% of 5-HIAA (E19 and P5). 5-HT1A binding: increased in the brainstem, decreased in the cortex. No difference in 5-HT1B binding	35
Sprague Dawley rats	foetal exposure	6.6% v/v, *about 35% (EDC)*	liquid diet	–	6 weeks prior to breeding and during gestation	28 to 40% reduction of 5-HT-immunoreactive neurons in the MR and DR	43
Sprague Dawley rats	foetal exposure	6.6% v/v, *about 35% (EDC)*	liquid diet	80–120 mg/dl	7 weeks prior to breeding and during gestation	P5: Decreased 5-HT-immunoreactive neurons of 32% in DR and 24% in MR at P5 and 32% in DR and 27% in MR at P19. Maternal treatment with ipsapirone (3 mg/kg/day, from E13 to E20) prevents these deficits	44
Sprague Dawley rats	foetal exposure	6.6% v/v, *about 35% (EDC)*	liquid diet	110 mg/dl	8 weeks prior to breeding and during gestation	Decreased levels of 5-HT and 5-HIAA in the cortex, brainstem and cerebellum of P19 and P35 rats	47
Sprague Dawley rats	foetal exposure	6.6% v/v, *about 35% (EDC)*	liquid diet	110 mg/dl	6 weeks prior to breeding and during gestation	Buspirone 4.5 mg/kg/day subcutaneously from E13 to E20 prevents the 50% reduction of 5-HT content in the cortex at P5 and the 30% reduction at P19.	53
Sprague Dawley rats	foetal exposure	6.6% v/v, *about 35% (EDC)*	liquid diet	120–130 mg/dl	4–6 weeks prior to breeding and during pregnancy	Reduced 5-HT uptake sites in synaptosomes from motor cortex with 30% decrease of Km for 5-HT in P35 animals	58
Sprague Dawley rats	foetal exposure	6.6% v/v, *about 35% (EDC)*	liquid diet	75 to 120 mg/dl	6 weeks prior to breeding and during gestation	Reduced SERT binding in the frontal cortex and parietal cortices, lateral hypothalamus, substantia nigra, medial septum and striatum of P19 and P35 animals. Most of these effects are prevented by maternal treatment with buspirone (4.5 mg/kg/day from E13 to E20, s.c.)	59

(*Continued*)

Table 1
(*Continued*)

Species	Model	Dose of ethanol	Route of administration	BEC	Duration of treatment	Results	Ref #
Sprague Dawley rats	foetal exposure	6.6% v/v, *about 35% (EDC)*	liquid diet	75 to 120 mg/dl	6 weeks prior to breeding and during gestation	Increased 5-HT1A binding in the dentate gyrus but decreased in the parietal cortex and lateral septum in P35 animals. Maternal treatment with buspirone (E13 to E20, 4.5 mg/kg/day, s.c.) prevents most of these alterations. No change in 5-HT2A binding	62
Sprague Dawley rats	foetal exposure	6.6% v/v, *about 35% (EDC)*	liquid diet	130 mg/dl	4–6 weeks prior to breeding and during pregnancy	Decreased of 5-HT1 binding sites: 20% in whole cortex, 38% in motor cortex and 10–30% in somatosensory cortex of both P19 and P37 animals	61
Sprague Dawley rats	foetal exposure	6.6% v/v, *about 35% (EDC)*	liquid diet	–	During whole pregnancy	Increased response to the hypothermic effect of 5-HT1A agonist (8OHDPAT, 0.125 and 0.5 mg/kg, s.c.) in 70–90 day-old animals. Increased head-twitch response to 5-HT2A agonist in 70–90 day-old females	63
Sprague Dawley rats	foetal exposure	6.6% v/v, *about 35% (EDC)*	liquid diet	–	During whole pregnancy	Increased novelty-induced anxiety-like behaviour and anxiolytic effects of 5-HT1A agonist (8OHDPAT, 0.06 mg/kg, s.c.) in females (P22)	64
Sprague Dawley rats	foetal exposure	0.5, 1 and 2 g/kg ethanol once a day	subcutaneously injection	3.32, 40.73 and 106.56 mg/dl in the 0.5, 1 and 2 g/kg groups, respectively	From E15 until birth	5–15% reduction of 5-HT- and TPH-immunoreactive neurons at 3 and 5 weeks after birth, in a dose dependent manner	45
Sprague Dawley rats	foetal exposure	36% (EDC)	liquid diet	–	From E1 to E22	Decreased 5-HT-immunoreactive neurons in the DR (but not MR), rescued by oestrogen treatment (0.05 mg in pellet) in ovariectomized female	46
Sprague Dawley rats	foetal exposure	36% (EDC)	liquid diet	127 mg/100 ml	From E8 to birth	SERT binding in the cortex = decreased (P21, P40 and P60), in HIP = increased (P21, P40 and P60), in the BLA = increased (P21, P40 and P60), LA = increased (P40 and P60)VMH = decreased (P21)	56
Sprague Dawley rats	foetal exposure	36% (EDC)	liquid diet	–	From gestational day 1 to E21 or birth	Reduced SERT mRNA in the brain of E21 foetus. Higher methylation of SERT in the hypothalamus of females at P55	57

Wistar rats	foetal exposure	8–16% w/v as sole drinking fluid	forced drinking	–	3.5 months of 8% and 1 month of 16% before birth	Decreased 5-HTP levels in the whole brain without cerebellum of 5 month-old females	49
Rhesus monkeys	sweetened alcohol	0.6 g/kg/day	liquid diet	20–50 mg/dl	Early gestation (days 0 to 50), middle to late gestation (days 50 to 135) or continuous (days 0 to 135)	Early- and middle-to-late gestation-alcohol exposed monkeys carrying the short allele of the SERT had lower concentrations of 5-HIAA in the CSF	50
Human FAE/FAS children	maternal alcohol consumption	–	voluntary drinking	–	–	Reduced SERT binding in the mPFC of 7–14 year-old FAE/FAS children (SPECT)	60

Abbreviations: NAC, nucleus accumbens; VTA, ventral tegmental area; AMG, amygdala; BLA, basolateral amygdala; LA, lateral amygdala; HIP, hippocampus; PFC, prefrontal cortex; mPFC, medial prefrontal cortex; DR, dorsal raphe; MR, median raphe; TPH, tryptophan hydroxylase; EDC, ethanol derived calories.

offspring prenatally exposed to alcohol, showing a reduction in the density of binding sites in the motor and somatosensory cortices, lateral septum and an increase in the hippocampus and brainstem of young rats (P5-P35) [35, 61, 62]. Additionally, increased hypothermic and anxiolytic responses to the 5-HT1A receptor agonist 8-OHDPAT as well as increased "wet dog shake" response to the 5-HT2A receptor agonist DOI have been observed in young adult female rats prenatally exposed to alcohol [63–65], revealing a female-specific increase in 5-HT1A/2A receptor sensitivity, which is consistent with the ability of alcohol to upregulate oestrogen levels in females (see [66] for review) that in turn, could upregulate 5-HT1A/2A receptor signalling [67, 68].

Since the 5-HT1A receptor is expressed both presynaptically, as an autoreceptor in the dorsal raphe to regulate 5-HT neuronal activity, and postsynaptically in limbic brain regions, alterations in 5-HT1A receptor expression and function could play a pivotal role in the pernicious effects of prenatal alcohol exposure on 5-HT pathway. Indeed, *in vitro* and *in vivo* treatments during pregnancy with the 5-HT1A receptor partial agonist buspirone or ipsapirone prevent the loss of 5-HT or rhombencephalic neurons [38, 43], the reduction in 5-HT and 5-HIAA levels [53], the alteration in 5-HT1A receptor [62] and SERT expression [59] and the decrease of pAkt [38, 39]. Ipsapirone was also able to increase the expression of NF-κB dependent genes in foetal rhombencephalic neurons treated with ethanol [41, 69]. As the 5-HT system has been extensively implicated in the regulation of stress and anxiety, the neuroplastic changes in 5-HT signalling seen with foetal alcohol exposure could alter the regulation of stress- and anxiety-related behaviours, potentially resulting in the development of neuropsychiatric disorders later in life.

Stress and anxiety

Prenatal ethanol exposure has been shown to induce long-term effects on an organism's ability to respond and adapt to stress, as measured by alterations in hypothalamic–pituitary–adrenal (HPA) function [70–76]. In rodents prenatally exposed to ethanol, altered HPA activity can be observed throughout their lifespan. At birth, basal levels of brain, plasma [77–79], and adrenal [79] corticosterone (CORT), as well as stress-induced increased in plasma CORT levels are augmented [79]. From approximately postnatal days 4 to 14, which corresponds to the "stress hyporesponsive period" (reviewed in [80]), prenatally exposed animals displayed an even greater HPA hyporesponsiveness, with reduced adrenocorticotropin (ACTH) and CORT responses following a variety of stressors [77, 79, 81, 82]. In contrast, in adulthood, prenatally exposed animals exhibit HPA hyper-responsiveness, with increased HPA activity following stress [70, 73, 76, 83, 84] and show delayed or deficient recovery to basal levels following chronic or repeated stress [70, 82, 85]. Similarly, HPA hyperresponsiveness is also observed in human infants [15, 86] and in nonhuman primates [87] following prenatal exposure to alcohol.

Although dysfunctions in the HPA axis have been implicated in the pathogenesis of anxiety disorders (reviewed in [88]), studies of basal anxiety in animals prenatally exposed to alcohol have yielded inconsistent results. Some studies have shown an increased basal anxiety in both males and females [64, 89, 90], in other studies only in females [91] or only males [92–94] while others have demonstrated a reduction [95, 96] or no difference [97]. However, increased anxiety in prenatally ethanol-exposed animals has been observed in a sex-independent manner following stress exposure [93, 94].

Serotonin is a key neurotransmitter involved in HPA regulation [98–101], primarily through 5-HT1A/2A receptors [102], and reciprocal interactions between central 5-HT systems and the HPA axis [103, 104]. Additionally, a direct effect of 5-HT on corticotropin releasing hormone (CRH), ACTH, and CORT release [103, 105] have been observed and activation of 5-HT1A/2A receptors activates CRF neurons [106] and increases ACTH and CORT secretion [107]. There is a reciprocal regulatory relationship between 5-HT and the glucocorticoid receptors (GR) [108, 109] and stress induced increases in mineralocorticoid receptor and GR immunoreactivity in the hippocampus are 5-HT dependent [110]. Therefore, changes in 5-HT1A/2A receptor expression and function are likely to be involved, at least in part, in the dysregulation of the stress response [46, 111] and the subsequent predisposition to anxiety-like behaviours following prenatal alcohol exposure.

NEUROADAPTATIONS IN 5-HT SIGNALLING FOLLOWING ALCOHOL EXPOSURE (TABLE 2)

The 5-HT system is not only plastic during embryonic development but also during early life and adulthood (see [112] for review). Therefore,

Table 2
Changes in 5-HTergic neuroplasticity following acute and chronic ethanol exposure

Species	Model	Dose of ethanol	Route of administration	BEC	Duration of treatment	Results	Ref #
Wistar rats	Acute injection or reverse microdialyse	0.5, 1 or 2 g/kg (injection) or 25, 50 or 100 mM (dialyse)	IP or local infusion in the NAC	–	Acute	IP injection: 2.0 g/kg markedly increases 5-HT levels in the NAC within 15 min. Reverse microdialysis: 100 mM ethanol increases 5-HT levels for 1 hr in the NAC	116
Wistar rats	Acute injection or reverse microdialyse	0.5, 1 or 2 g/kg (injection) or 25, 50 or 100 mM (dialyse)	IP or local infusion in the CeA	–	Acute	IP injection: 1.0 and 2.0 g/kg markedly increases 5-HT levels in the AMG within 20 min. Reverse microdialysis: ethanol dose-dependently increases 5-HT levels for 2 hr in the AMG	117
Lewis and Fisher rats	Acute injection	0.5, 1 or 2 g/kg	IP	–	Acute	Ethanol 1 g/kg and 2 g/kg increased 5-HT levels in the NAC (44%) (in Lewis but not Fisher rats)	118
Wistar BgVV and Wistar-Harlan rats	Acute injection	1 g/kg	IP	111–113 mg/dl	Acute	Microdialysis: Ethanol 1 g/kg (ip) or exposure in the elevated-plus maze increases 5-HT release in the mPFC of Wistar-BgVV rats	119
Sprague Dawley rats	Acute injection	0.1, 1 and 10% (v/v)	Local infusion in the VTA	–	Acute	Microdialysis: 10% ethanol increases 5-HT levels in the VTA, which is not blocked by Ca2+ depletion or TTX (1uM)	120
Sprague Dawley rats	Acute injection	16% (w/v)	IP	50–80 mM	Acute	Microdialysis: increased levels of 5-HIAA in the striatum	121
Wistar rats	Acute injection	0.1 and 1 g/kg	IP	–	Acute	Microdialysis: ethanol 0.1 and 1 g/kg (ip) increases 5-HIAA in the NAC	122
Sprague Dawley rats	Acute injection	0.5, 1, and 2 g/kg	IP	–	Acute	Microdialysis: Ethanol 0.5, 1, and 2 g/kg increases 5-HT in the NAC	125
Sprague Dawley, F344 and Lewis rats	Acute injection	20–160 mM	bath (Slices)	–	Acute	Electrophysiology: Ethanol dose-dependly increases VTA neuron firing rate. 5-HT potentiates the increasing effect of ethanol on VTA neuron firing rates, which is replicated by the 5-HT2 agonist DOI (50nM and 2uM)	126
C57Bl6 mice	Acute injection	30 mM	bath (Slices)	–	Acute	Electrophysiology: Ethanol (30 mM) inhibits DR 5-HT neuron excitability via activation of extrasynaptic glycine receptors	128
ICR mice	Acute and repeated	1.0, 2.0, 3.0 or 4.0 g/kg	IP	–	Acute or Repeated (1.0 or 2.0 g/kg once daily for 7 days)	Microdialysis: Acute, 5-HIAA concentrations were increased in the hypothalamus after injection of 3.0 and 4.0 g/kg of ethanol. Repeated, no change observed in 5-HIAA concentration	123

(*Continued*)

Table 2
(*Continued*)

Species	Model	Dose of ethanol	Route of administration	BEC	Duration of treatment	Results	Ref #
Wistar rats	Acute and repeated	2.5 g/kg	IP	–	Acute or 1 repeated injections (24 h after)	Microdialysis: Acute ethanol increases 5-HT levels in the caudate putamen. Repeated: pretreatment with ethanol slightly decreases the elevation in 5-HT induced by ethanol, as compared to a single injection. Electrophysiology in awake animals: Ethanol reduces the firing frequency of 5-HT neurons	124
Sprague Dawley rats	Acute and repeated	acute: 0.25–1.0 g/kg. Chronic: 1–5 g/kg every 6hr for 6 days + challenge (0.25–1 g/kg, i.v.)	acute: intravenous. Chronic: intragastric + intravenous challenge	–	Acute or repeated (for 6 days)	Electrophysiology: Acutely, ethanol decreases the firing rate of 5-HT DR neurons. Reduction of basal electrical activity of 5-HT neurons, 12 h after withdrawal from chronic ethanol. No change in 5-HT1A agonist (8-OHDPAT, 1–16μg/kg, i.v.) sensitivity to reduce the firing rate after withdrawal	127
Wistar rats	Acute and repeated	2 × 2.5 g/kg	IP	237–256 mg/dl	Acute (2 injections in 2 days)	A single ip injection of ethanol 2.5 g/kg increases 5-HT levels in the ventral HIP. A second ip injection of ethanol 2.5 g/kg 24 hrs after does not elevate 5-HT levels	113
Rat (not precised)	Early postnatal gavage	5 g/kg/day	intragastric	325.7 mg/dl	Chronic (P4 to 10)	Early postnatal ethanol exposure increases 5-HT and 5-HIAA concentrations in the HIP of females but not males	129
Rat (not precised)	Early postnatal gavage	6 g/kg/day	intragastric	327.8 to 347.6 mg/dl	Chronic (P4 to 10)	A single ip injection of ethanol 2.5 g/kg increases 5-HT levels in the ventral HIP. A second ip injection of ethanol 2.5 g/kg 24 hrs after does not elevate 5-HT levels	130
Fischer 344 rats	Chronic diet	6.6% v/v	liquid diet	60 to 100 mg/dl	6 weeks	Ethanol reduces 5-HT tissue content in the VTA of 14-month old animal but increases 5-HIAA concentration in the striatum, globus pallidus, NAC, frontal cortex, VTA and ventral pallidum of 24-month old animals	132
Fischer 344 rats	Chronic diet	6.6% v/v	liquid diet	60 to 100 mg/dl	6 weeks	Increased 5-HT2A binding in the NAC of 5-month old ethanol fed rats	146
C57Bl6 mice	SHAC	5% v/v	drinking solution	109 mg/dl	1 or 6 days	Increased extracellular concentration of 5-HT in the NAC of ethanol -inexperienced animals (SHAC1) but the 5-HT levels are no longer elevated in ethanol-experienced animals (SHAC6)	115

Wistar rats	Sucrose fading	gradually from SUC 10%/ ETH 5% to SUC 5%/ETH 10%	drinking solution	15.7 mg/dl	50 days	History of ethanol/sucrose drinking reduces 5-HT content in the medial thalamus and medial hypothalamus and the 5-HIAA/5-HT ratio in the PFC pyriform, motor, auditory, visual and somatosensory cortices and medial thalamus	133
C57Bl6 mice	Chronic free choice drinking (3 water/1 ethanol bottles)	0 to 10% v/v	drinking solution	–	21 days	No change in 5-HTP, 5-HT or 5-HIAA levels in the striatum. Increased 5-HT1A sensitivity to ipsapirone (2 or 3 mg/kg, i.p.)-mediated reduction of 5-HTP accumulation and 5-HT neuron firing rate. Increased 5-HT1A-mediated GTPgammaS coupling in the DR. No difference in 8-OHDPAT-induced hypothermia	142
Wistar rats	Chronic diet + withdrawal + re-exposure	7.2% v/v	liquid diet	288 mg/dl	10 or 21 days	Ethanol reduces 5-HT tissue content in the the cortex and striatum and increases 5-HIAA contents in the HIP after 10 days of exposure	134
Wistar rats	Withdrawal from chronic diet	7.2% v/v	liquid diet	289 mg/dl	21 days + 2, 4 or 6 h of withdrawal	Decreased 5-HT tissue content in the cortex 4 h after withdrawal but Increased levels after audiogenic seizures (>6 hrs). Increased 5-HIAA in the cortex after 2 h of withdrawal. Decreased 5-HT in the striatum after 2, 4 and 6 hrs of withdrawal and after audiogenic seizures. No changes in 5-HT levels in the HIP but decreased 5-HIAA contents after 2 h of withdrawal and after audiogenic seizures	135
Sprague Dawley rats	Withdrawal from chronic diet	9% v/v	liquid diet	255 mg/dl	14 days	Increase in 5-HT1A binding in the DR (+30%) but decrease in the HIP (–20%) and the cortex (–30%). Increase in 5-HT1B in the globus pallidus	143
Sprague Dawley rats	Withdrawal from chronic diet	9% v/v	liquid diet	–	14 days	Effect of 5-HT1A agonist (8-OHDPAT, 2.5 mg/kg ip) is sensitized on lower lip retraction but desensitized on flat body posture after 18 h of withdrawal	144
DBA2 mice	Chronic intermittent vapour	–	Vapour	150–200 mg/dl	5 days, 16 h/day	5-HT2C antagonist (SB242,084, 3 mg/kg, ip) normalizes ethanol-induced anxiety and reduces ethanol-induced fos immunoreactivity in the ventral BNST. Ethanol increases 5-HT2C signalling in the ventral BNST	149

(*Continued*)

Table 2
(*Continued*)

DBA2 mice	Chronic intermittent vapour	–	Vapour	150–200 mg/dl	5 days of 16 h/day followed by 24 h or 7 day withdrawal	Chronic ethanol exposure enhances the net activity of 5-HT neurons by reducing inhibitory transmission during early withdrawal and increasing excitatory transmission during late withdrawal. Chronic ethanol exposure also sensitizes the inhibitory effect of subsequent acute ethanol exposure	162
C57BL/6J, C3H/HeJ and DBA/2J inbred mice	Chronic intermittent vapour followed by 2 bottle choice	22–27 mg/l (vapour) and 10% v/v (drinking)	vapour and drinking solution	50 mg/dl after vapour session	20 days, 3–6 h/d, followed by 5 h withdrawal and 4 h drinking	Alterations in 5-HT2C RNA editing in the NAC and the DR in C57bl6 mice following chronic ethanol exposure	147
C57Bl6 mice				180–200 mg/dl	20 days, 4–8 h/day	Decreased 5-HT and 5-HT/5-HIAA ratio in the DR and HIP. Increased mRNA expression of 5-HT2A, 2C and 7 in the DR, striatum and HIP following 20 days of alcohol vapour exposure. Increased alcohol-induced 5-HT release in the NAC of ethanol vapour-experienced animals.	148
Rhesus monkeys	Chronic 2 bottle choice	4% v/v	drinking solution	–	13 months	Increase 5-HT1A binding (PETSCAN) in cortex, AMG and HIP	145
Macaques	operant self-administration	0.5, 1.0, and 1.5 g/kg	drinking solution	90 mg/dl	12 months	Increased expression and G protein-coupling of 5-HT1A receptors in the HIP	150
Macaques	operant self-administration	0.5, 1.0, and 1.5 g/kg	drinking solution	90 mg/dl	13 months	Decreased SERT binding in the HIP	154
Human	Alcoholics	–	–	–	15 years of drinking	Increased 5-HT1B binding in the pallidum/NAC of alcohol dependent subjects	151
Human	Alcoholics	–	–	–	27 years of drinking	Decreased 5-HT1A-induced prolactin and cortisone release following a challenge of the 5-HT1A agonist Flevinoxan (1 mg/70 kg of body weight, iv)	152

Human	Alcoholics	–	–	–	- (post mortem)	30% reduction of 5-HT1A binding in the anterior cingulate cortex of type 1 alcoholics	153
Human	Alcoholics	–	–	95 g/day (90 kg)	1 to 30 years	The longer duration of excessive alcohol consumption the lower PRL response to D-fenfluramine	155
Human	Alcoholics	–	–	–	3–5 weeks of abstinence	30% reduction of SERT binding in the brainstem	156
Human	Alcoholics	–	–	–	- (post mortem)	30% reduction of SERT binding in the AMG	157
Human	Alcoholics	–	–	–	- (post mortem)	26% reduction of SERT binding in the dorsal striatum	158
Human	Alcoholics	–	–	–	- (post mortem)	35% increase of SERT binding in the NAC	159
Human	Alcoholics	–	–	–	- (post mortem)	25% reduction of SERT binding in the cingulate cortex	161
Human	Alcoholics	–	–	–	- (post mortem)	35% reduction of SERT binding in the cingulate cortex	162
Human	Alcoholics	–	–	128 mg/dl at admission	19 years	Plasma 5-HT concentration decreases during 14 days after withdrawal	160, 163

Abbreviations: NAC, nucleus accumbens; VTA, ventral tegmental area; AMG, amygdala; BLA, basolateral amygdala; LA, lateral amygdala; HIP, hippocampus; PFC, prefrontal cortex; mPFC, medial prefrontal cortex; DR, dorsal raphe; MR, median raphe; TPH, tryptophan hydroxylase; EDC, ethanol derived calories.

acute stressors that impact 5-HT signalling could lead to long lasting neuroplastic adaptations after chronic exposure. Here, we review the involvement of 5-HT signalling in alcohol dependence in the transition from acute to chronic exposure, following alcohol withdrawal and in relation with alcohol withdrawal-induced stress/anxiety.

Acute exposure

Microdialysis experiments in rodents have shown that acute systemic injection of ethanol elevates the extracellular levels of 5-HT and/or its metabolite 5-HIAA in multiple brain regions including the nucleus accumbens (NAc), ventral tegmental area (VTA), prefrontal cortex (PFC) and hippocampus (HIP) [113–125]. Similar increases in 5-HT/5-HIAA levels have been observed in the NAc of mice following acute ethanol drinking under the SHAC paradigm [115].

Since 5-HT potentiates alcohol-induced excitation of the dopamine neurons in reward areas of the brain including the NAc and VTA [126], changes in 5-HT neuron activity might be involved in early neurochemical adaptations that promote the reinforcing effects of alcohol and lead to alcohol addiction [115]. However, electrophysiology experiments have shown that acute systemic injection or bath application of ethanol decreases the firing rate of 5-HT neurons by increasing the inhibitory drive in the DRN [124, 127, 128], suggesting that the stimulatory actions of alcohol on synaptic 5-HT release appear to be mediated by local circuits in the projection areas rather than direct activation of 5-HT neurons.

Chronic alcohol exposure and withdrawal

Short term chronic alcohol exposure (1 week) during the early phase of postnatal development (first 7–10 days in rat, corresponding to the human third trimester) has been shown to increase the hypothalamic and septal concentration of 5-HT, with a greater effect in females [129, 130].

Chronic alcohol exposure leads to adaptive changes within the brain, presumably to re-establish normal cell function, or homeostasis, in response to continuous alcohol-induced alterations in the mesoaccumbens reward pathway. These neuroadaptations are thought to be involved in the development of tolerance and addiction [131]. Chronic studies have shown that 5-HT levels in the NAc, PFC, globus pallidus, VTA and substantia nigra, are no longer elevated after 1 to 7 weeks of alcohol exposure in comparison to acute ethanol exposure [132–134]. Additionally, reduced 5-HT/5-HIAA turnover rate in the NAc suggests 5-HT signalling is decreased [132]. In alcohol dependent rats, 5-HT levels in the NAc, cortex and striatum rapidly decrease during withdrawal [135–137] and are restored by alcohol self-administration [136]. In humans decreased plasma 5-HT levels have been observed in abstinent alcoholics up to 14 days following alcohol withdrawal [138]. Thus, reduced 5-HT neurotransmission after alcohol-withdrawal has been associated with increased stress-induced anxiety, which drives alcohol craving and relapse [139–141].

One study [142] showed the basal activity of 5-HT neurons from the DRN is not altered in mice voluntarily drinking alcohol for 3 weeks, suggesting that alteration in 5-HT signalling is not related to changes in 5-HT neurons activity but could rather involve changes in 5-HT receptor signalling. Indeed, the same study demonstrated that 5-HT1A autoreceptors are hypersensitized and their activation by the partial agonist ipsapirone produced a greater inhibition of 5-HT neuron firing in alcohol exposed animals compared to alcohol naive animals [142]. Similarly, increased 5-HT1A autoreceptor expression and function has been observed in the DRN of rats and primates following chronic ethanol comsumption [143–145]. On the other hand, 5-HT1A postsynaptic binding sites were downregulated in the cortex [143], while 5-HT1B/2A/2C receptors were upregulated in the globus pallidus [143], NAc [146–148], bed nucleus of stria terminalis (BNST) [149] and hippocampus. Similar alterations in postsynaptic 5-HT1A and 1B receptors have been reported the cortex and the hippocampus in monkeys [150] or human alcoholics [151–153].

Consistent with a reduced 5-HT neurotransmission, a decreased expression and function of SERT has also been observed in the hippocampus in monkey [154] and in various brain regions in human alcoholics, including the amygdala, the cortex, the dorsal and the ventral striatum[155–161].

Studies on the consequences of withdrawal from chronic alcohol exposure on 5-HT neuron activity have led to inconsistent results. Pistis and co-workers found that 5-HT neuron basal firing was dose-dependently reduced in rats, 12 h after withdrawal of 6 days of intragastric administration of 1–5 g/kg of ethanol, every 6 hours [127]. On the contrary, by using vapour chambers in DBA2/J mice, Lowery-Gionta and co-workers recently found that 16 hours/day of ethanol vapour exposure for 6 consecutive days

enhances the activity and the excitability of DRN neurons 1 to 7 days after the last exposure [162]. However, the exact nature of the recorded neurons was not demonstrated in this study. Because ethanol is known to increase glycinergic and GABAergic signalling in the DRN [128, 163] the increased neuronal excitability observed by Lowery-Gionta et al. could be attributed to the recording of interneurons in the DRN, which in turn could reduce 5-HT neuron activity. Further work is then needed to understand how 5-HT neuron activity is modulated by withdrawal from chronic alcohol exposure.

5-HT signalling and alcohol withdrawal-induced stress/anxiety

A complex relationship exists between alcohol-drinking behaviour and stress/anxiety. Alcohol has anxiety-reducing properties which can relieve stress, while at the same time acting as a stressor and activating the stress response systems. In particular, chronic alcohol exposure and withdrawal can profoundly disturb the function of the HPA axis, which contributes to the sensitization of anxiety-like behaviour, craving for alcohol, and relapse (see [164] for review).

Compelling evidences reveals that CRF neurons within the HPA axis as well as in extrahypothalamic sites, such as the central nucleus of amygdala and BNST, play a pivotal role in the negative emotional processes associated with alcohol withdrawal/craving (see [164–169] for review). Indeed, extracellular CRF levels are elevated in these regions during ethanol withdrawal [170–172] and restored to basal levels by subsequent ethanol intake [173].

The CRF-immunoreactive fibres arising from the amygdala [174] densely innervate the DRN in a topographically organized manner [175–177] and the behavioural effects induced by CRF are thought to be mediated, in part, by CRF actions on 5-HT systems within the brain [175, 178–181]. Both exposure to a stressor and local infusion of CRF into the DRN have been shown to modulate 5-HT release in forebrain regions, including the PFC, NAc and amygdala [182–185].

Later, studies have shown that both CRF1 and CRF2 receptors are detected in the dorsal raphe nucleus [186–188] and have opposing effects on 5-HT release [175, 189, 190]. Corticotropin-releasing factor has a higher affinity for CRF1 receptors when compared to CRF2 receptors [191, 192], and activation of the former normally inhibits 5-HTergic activity in the dorsal raphé [189, 193] and 5-HT release in the NAc, striatum and lateral septum [194–196]. In contrast, higher levels of CRF are believed to be required for CRF2 receptor activation. Activation of these receptors normally facilitates 5-HTergic activity in the dorsal raphé [175, 189] and the release of 5-HT in the NAc, basolateral amygdala, striatum and lateral septum [194–197]. Combined, these studies suggest that CRF has a dual effect in the dorsal raphé nucleus that depends on both CRF1 or CRF2 receptor activation and the CRF concentration.

Alteration in CRF receptor signalling following chronic exposure to a stressor (or alcohol) can impact the regulation of the 5-HT system. In rats chronically exposed to a stressor, relatively high doses of CRF produce a greater increase in the firing rate of 5-HT neurons [198], suggesting a downregulation of CRF1 and/or upregulation of CRF2 signalling following a sustained CRF release induced by chronic stress exposure. Interestingly, similar downregulation of CRF1 receptor expression in various brain regions and upregulation of CRF2 receptor expression in the DRN have been observed in transgenic mice overexpressing CRF [199]. This CRF-5-HT regulation is likely to play an important role in alcohol addiction, as well as in the negative emotional effects of alcohol withdrawal. Systemic injections of both CRF1 antagonist, CRF2 agonist and the 5-HT1A partial agonist buspirone have been shown to reduce ethanol consumption [200–203], ethanol withdrawal-induced sensitization of anxiety-like behaviours [204–210] and stress induced reinstatement of alcohol seeking [211]. Additionally, the infusion of a CRF antagonist into the DRN reduced ethanol drinking [207] and both infusion of a CRF antagonist into the central amygdala (CeA), DRN, and dorsal-BNST and the 5-HT1A partial agonist buspirone into the raphe reduced ethanol-induced anxiety-like behaviours [212, 213] and stress-induced reinstatement of alcohol seeking [214, 215]. Furthermore, 5-HT2C and 5-HT3 receptors also appear to modulate the mood-altering effects of chronic ethanol intake, as antagonists of these receptors blocked ethanol withdrawal–induced anxiety and stress-induced reinstatement of alcohol seeking [204, 212, 216–218].

NEURONAL ADAPTATIONS IN THE 5-HT SYSTEM IN ALCOHOL PREFERRING RODENT MODELS (TABLE 3)

To further study alcohol drinking behaviours in rodents, high and low alcohol consuming rodent

Table 3
Changes in 5-HTergic neuroplasticity in alcohol-preferring rat lines

Species	Model	Dose of ethanol	Route of administration	BEC	Duration of treatment	Results	Ref #
P vs NP rats	Naive	–	–	–	–	Fewer 5-HT-immunostained (5-HT-IM) neurons and reduced 5-HT content in the DRN of P rats	222
P vs NP rats	Naive	–	–	–	–	Decreased levels of 5-HT and 5-HIAA in the HIP, NAC, and cortex of P rats	224, 228
P vs NP rats	Naive	–	–	–	–	Reduced density of 5-HT fibres in HIP, caudate-putamen, and hypothalamus of the P. Fewer fine 5-HT fibres in PFC and HIP of P rats	225-223
P vs NP rats	Naive	–	–	–	–	Lower 5-HT fibre density is in the cingulate and frontal cortices, HIP and hypothalamus of P rats	226
P vs NP rats	Naive	–	–	–	–	No difference in firing frequencies, the percentages of action potentials in bursts, and the percentages of bursting in P and nP rats, as compared to Wistar rats	229
P vs NP rats	Naive	–	–	–	–	Higher density of 5-HT1A binding sites in mPFC, parietal, cingulate (20–30%), retrosplenial, occipital and temporal (35–40%), entorhinal cortex (15%) cortices, HIP (10–15%), DRN an MRN (15–20%) in periadolescent and adult P rats	232, 233
P vs NP rats	Naive	–	–	–	–	Lower 5-HT1B receptor densities in the cingulate and retrosplenial cortices, septum, and AMG of P rats	235
P vs NP rats	Naive	–	–	–	–	30% decrease of 5-HT3 binding sites in the AMG of P rats	236
P vs NP rats	Naive	–	–	–	–	Reduced 5-HT2A binding sites (50–70%) in mPFC, frontal and parietal cortices of P rats	237

P vs NP rats	Naive	–	–	–	–	5-HT2 binding sites are reduced in mPFC, frontal, cingulate, parietal, and temporal cortices (–15–25%), NAC, olfactory tubercle, and caudate-putamen (40–50%) of P rats	238
P vs NP rats	Naive	–	–	–	–	Higher 5HT2C binding sites in the HIP, AMG and the choroid plexus in P rats. Increased 5HT2C receptor coupling in the choroid plexus of P rats	234
P rats	2 bottle choice	10% v/v	Drinking solution	–	6 or 8 weeks	Microdialysis : Reduced 5-HT extracellular levels (–35%) in the NAC of serotonin following 8 weeks of continuous access to ethanol compared with water controls and animals deprived of ethanol for 2 weeks	230
P vs NP and Wistar rats.	Injection	1.0 g/kg	i.p.	–	5 days.	Decreased basal extracellular 5-HT levels in the NAC of P rats but increased in Wistar and NP rats	231
P vs NP and Wistar rats.	Injection	0.5–1.0 g/kg	i.p.	–	Acute	Basal anxiety is elevated in P rats, which is normalized by ethanol pretreatment	239
sP vs sNP rats	Naive	–	–	–	–	Lower 5-HT and 5-HIAA concentrations in the frontal cortex of sP rats	243
sP vs sNP rats	Naive	–	–	–	–	Reduced 5-HT2-mediated head dog shake response in sP rats	245
sP vs sNP rats	Naive	–	–	–	–	Lower 5HT2A binding sites in mPFC, prefrontal and cingulate cortices of sP rats. No significant difference was found in other areas between groups. Reduced head dog shake response to 5-HT2A receptor agonist microinjected into the mPFC in sP rats	246
sP vs sNP rats	Naive	–	–	–	–	Increased anxiety and higher CRF levels in the AMG in sP rats	247

(*Continued*)

Table 3
(*Continued*)

Species	Model	Dose of ethanol	Route of administration	BEC	Duration of treatment	Results	Ref #
sP rats	2 bottle choice	10% v/v	Drinking solution	–	Acute	Basal anxiety is elevated in P rats, which is normalized by ethanol pretreatment	240
sP vs sNP and Wistar rats	2 bottle choice	10% v/v	Drinking solution	–	14 days	Reduced density of 5-HT fibres in the cingulate cortex, the NAC shell and DR but not in the striatum, NAC core, HIP and MR of sP rats	242
sP vs sNP rats	2 bottle choice	10% v/v	Drinking solution	–	14–15 days	Higher basal anxiety level in ethanol-naïve sP rats, which is normalized by ethanol exposure	248, 249
FH vs Wistar rats	Naive	–	–	–	–	FH rats have more depressive-like behaviours	250
FH vs wistar rats	Naive	–	–	–	–	Decrease 5-HT1A induced hypothermia and 5-HT2-induced hyperthermia. No significant difference in the of 5-HT or 5-HIAA levels in the mPFC, HIP hypothalamus and striatum. Decreases 5-HT and 5-HIAA levels in the brain stem with higher 5-HT turnover rate in the hypothalamus, striatum and HIP	255
FH vs wistar rats	Naive	–	–	–	–	Decreased density of 5-HT1A binding sites in striatum and brainstem and increased density of 5-HT2 binding sites in the striatum and frontal cortex	256
FH vs Sprague-Dawley rats	Naive	–	–	–	–	Chronic fluoxetine treatment causes hypersensitisation of MR 5-HT1A receptors and desensitisation of hypothalamic 5-HT1A receptor in FH rats	258
FH vs Sprague-Dawley rats	Naive	–	–	–	–	Increased 5-HT uptake sites in the HIP, brainstem and striatum and decreased 5-HT levels in the brainstem of FH rats. Higher density of 5-HT2C receptors in the cortex of FH rats	260

FH vs WKY rats	2 bottle choice	5% v/v	Drinking solution	–	28 days of followed by 24 to 48 hrs of withdrawal	Increased SERT expression in the NAC, lateral septum ventral pallidum and VTA of alcohol-naïve FH rats. Increased density of 5-HT1A receptors in the frontal and parietal occipital and temporal cortices and HIP. No change in 5-HT3 receptor binding. Chronic ethanol consumption decreases 5-HT1A binding in the frontal and parietal cortices but increases binding in the entorhinal cortex and HIP. Hippocampal 5-HT1A binding returns to the levels of ethanol-naive rats following withdrawal	257
FH/Wjd vs ACI/N rats	2 bottle choice	10% v/v	Drinking solution	–	6 weeks	FH rats have more depressive-like behaviours	254
FH/Wjd vs ACI/N rats	2 bottle choice	10% v/v	Drinking solution	–	2 weeks	Reduced 5-HT3 in PFC, HIP and AMG of FH/Wjd rats. The anxiolytic effect of 5-HT3 receptor blockade is lost in FH/Wjd rats	259

Abbreviations: NAC, nucleus accumbens; VTA, ventral tegmental area; AMG, amygdala; BLA, basolateral amygdala; LA, lateral amygdala; HIP, hippocampus; PFC, prefrontal cortex; mPFC, medial prefrontal cortex; DR, dorsal raphe; MR, median raphe; TPH, tryptophan hydroxylase; EDC, ethanol derived calories.

lines have been developed through selective breeding. Some of these rat lines include the alcohol-preferring (P) or non-preferring (nP) rats, Sardinian alcohol preferring (sP) or non-preferring (sNP) and alcohol preferring Fawn-Hooded (FW). Here we present the neuroadaptions in the 5-HT system observed in these rat lines following extensive breeding for alcohol preference.

The alcohol-preferring (P) or non-preferring (nP) rats

The P and nP rats have been the most extensively characterised behaviourally and neurochemically (see [219–221] for review). These rats were selectively bred from a colony of Wistar rats selected for preference or non-preference for 10% ethanol over water under a 24 hour free choice drinking protocol. The P rats are capable of consuming 8–10 g/kg of ethanol per day and achieve blood ethanol concentrations (BECs) of 2 g/L.

Interestingly, marked deficiencies in the 5-HT system have been observed in P rats, as compared to nP rats. Decreased 5-HT positive neurons in the DRN and MRN [222] as well as reduced 5-HT positive fibres in the prefrontal cortex, NAc, striatum, hippocampus, and hypothalamus [223–225] were shown in P rats. Hence, ethanol-naïve P rats show lower 5-HT contents in the NAc, frontal cortex, hypothalamus and hippocampus [226, 227].

These alterations in basal 5-HT signalling are likely to be independent of any compensation on the spontaneous activity of 5-HT neurons [228]. Interestingly, 5-HT levels were further decreased in the NAc and 5-HT3 receptor function was downregulated following 12 weeks of alcohol consumption compared to water-exposed animals [229]. The same study showed that, following 2 weeks of withdrawal, 5-HT turnover was increased in deprived animals as compared to water-exposed or non-deprived animals, suggesting an increased 5-HT clearance which may be due to a compensatory response to higher serotonin release during ethanol withdrawal [229]. Similar effects were observed after intraperitoneal (IP) administration of ethanol in chronically exposed animals: 5-HT levels in the NAc were decreased in P rats but increased in nP and wistar rats [226, 230] and higher basal 5-HT levels in the NAc were observed after withdrawal [230]. However, 5-HT levels are elevated in the hippocampus in the P but not the sP rats following an acute IP ethanol challenge and this ethanol-induced increase in 5-HT overflow in the HIP did not show tolerance after a second challenge [231], as was the case in Wistar rats [113].

Such alterations could be associated with changes in 5-HT receptor signalling. Autoradiography studies have demonstrated an increase of 5-HT1A receptor expression in PFC, NAc and HIP [226, 232, 233] and 5-HT2C receptors in the hippocampus, amygdala, and choroid plexus [234]. Whereas expression of 5-HT1A receptors is upregulated in the DRN and MRN [232], 5-HT1B receptors in the cortex, lateral and medial septum and lateral nucleus of the amygdala [235], 5-HT3 receptor in the amygdala [229, 236], 5-HT2A receptors in the PFC, NAc and striatum [237, 238] is downregulated. Interestingly, all these neuroadaptations in 5-HT signalling were associated with a greater degree of anxiety in the P compared to the nP rats [239].

The Sardinian alcohol-preferring (sP) and non-preferring (sNP) rats

Sardinian alcohol-preferring (sP) and alcohol non-preferring (sNP) rats were selected from a large initial population of Wistar rats individually exposed to a two-bottle free-choice regimen, on the basis of ethanol preference or aversion. The sP rats consistently show a high preference for a 10% ethanol solution, with their daily ethanol intake averaging ~6 g/kg but never reaching an intoxicating level [240, 241].

Similar alterations in the 5-HT system have been reported in the Sardinian alcohol-preferring (sP) and non-preferring (sNP) rats. A significant reduction in the number of 5-HT neurons in the DRN was accompanied by a lower density of 5-HTergic fibres in the cortex and NAc shell [242] and reduced 5-HT and 5-HIAA levels in the PFC of sP rats, compared to sNP and Wistar rats [243, 244]. Lower density of 5-HT2A binding sites were also observed in the PFC of sP rats [245, 246]. The sP rats have higher basal levels of CRF in the CeA [247] and a higher innate degree of anxiety than sNP rats, which is reduced to the level of sNP animals after the consumption of alcohol [248, 249].

The Fawn-Hooded (FH) rats

The FH rats are a Wistar-derived inbred strain originally selected for deficiencies in platelet serotonin storage. Later, these rats were reported to drink high amounts of alcohol, 6 g/kg/day of 10% ethanol [250, 251] and exhibit high depression-like behaviour [250, 252, 253], making this strain a good model

to study comorbidity of alcoholism and depression [254].

These peripheral abnormalities in the 5-HT system are accompanied by central alterations, including reduced 5-HT levels in the DRN with higher 5-HT/5-HIAA turnover in the hypothalamus and striatum but lower in the HIP [255]. Also, SERT binding is increased in the NAc, lateral septum, ventral pallidum, VTA, cortex, HIP, brainstem and striatum but decreased in the hypothalamus [256, 257]. 5-HT1A binding is increased in the frontal cortex and HIP but decreased in the striatum [256] and 5-HT1A function is upregulated in the raphe nuclei [258]. Interestingly, following chronic ethanol consumption, 5-HT1A receptor binding is decreased in the frontal cortex but increased the HIP, and, after withdrawal, HIP 5-HT1A receptor binding was restored to the level of alcohol naïve FH rats [257].

Furthermore, reduction in 5-HT3 receptor expression was also observed in the frontal cortex, HIP, and amygdala [259] while 5-HT2 receptors displayed a greater binding in the striatum and the frontal cortex but lower in the HIP [256, 260].

CONCLUSION

It is clear that the neuroplasticity of the 5-HT system is altered in alcohol dependence, which is likely playing a pivotal role in negative emotion-driven craving and relapse. However, alcohol use disorders are complex and multidimensional [261] and the extent of potential abnormalities in 5-HT signalling is likely to vary across patients [262]. A subclassification of alcohol severity has been proposed by Babor and colleagues [263], where type A alcoholism (lower risk/severity) develops during adulthood and is characterized by binge drinking from mild to severe and type B alcoholism (high risk/severity) generally starts during adolescence/early adulthood with severe alcohol abuse remaining stable over time [264].

Study of 5-HT medications for the treatment of alcohol use disorders have led to inconsistent results. Although selective serotonin reuptake inhibitors (SSRIs), antidepressants (Sertraline, Citalopram, Fluvoxamine) have shown promising efficacy for attenuating alcohol consumption [265–270], craving [265, 266] and preventing relapse to alcohol consumption [271], other studies have observed that SSRIs were mostly effective in type A patients [262, 272] or in patients with comorbid depressive disorder and alcohol dependence [273–275], with limited efficacy in type B alcoholics [272].

Clinical trials with buspirone have revealed a promising efficacy of the 5-HT1A partial agonist in reducing alcohol consumption, craving and relapse in alcoholic patients with persistent anxiety [276–280], which could be a useful pharmacological adjunct in the treatment of the psychological symptoms associated with alcohol abstinence. Similarly, the atypical antipsychotic aripiprazole which, aside from its affinity for dopamine receptors, displays a 5-HT1A/2A partial agonist/antagonist activity, was shown to reduce heavy alcohol drinking and craving [281, 282], probably by decreasing visual alcohol-related cue-induced brain activation in alcoholic patients [282, 283]. Additionally, ondansetron, a 5-HT3 receptor antagonist, was shown effective for reducing craving in early onset alcoholics (type B) [284, 285].

Recently, a new class of SSRI antidepressant, namely vortioxetine and vilazodone, has been developed for the treatment of major depressive disorders. This novel class of antidepressant, called serotonin partial agonist-reuptake inhibitor (SPARI) has not only an inhibitory action on 5-HT reuptake (like the classic SSRIs) but also a partial agonist activity at 5-HT1A/1B receptors and an antagonist activity 5-HT2A and 5-HT3 receptors. Accordingly, medications acting concurrently on 5-HT reuptake, 5-HT1A, 5-HT2A and 5-HT3 receptors represent great potential for reducing alcohol consumption, craving and relapse in both type A and type B alcoholic patients. However, further work is still required to determine the efficacy of SPARI medications in the treatment of alcohol use disorders.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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