ALCOHOL AND WITHDRAWAL: FROM ANIMAL RESEARCH TO CLINICAL ISSUES

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ABSTRACT

The withdrawal syndrome in alcohol-dependent patients appears to be a major stressful event whose intensity increases with repetition of detoxifications according to a kindling process. Disturbances in the balance between excitatory and inhibitory neural processes are reflected in a perturbed physical state while disturbances in the balance between positive and negative reinforcements are reflected in a perturbed mood state. Our purpose is to link the different behavioral outcomes occurring during withdrawal with specific biological brain mechanisms from the animal to the human being. Better understanding of the various biological mechanisms underlying withdrawal from alcohol will be the key to design and to apply appropriate pharmaceutical management, together with appropriate therapy aimed at inducing protracted abstinence.
Dependence and withdrawal

Withdrawal stands at the core of alcohol dependence. A large body of literature has stated that no real alcohol dependence occurs without withdrawal signs.

Withdrawal signs appear within hours of cessation of alcohol intake. They are associated with a negative mood state including anxiety and tension. Alcohol may thus act as a negative reinforcement, as animals and humans beings will tend to avoid environments and behaviors inducing this negative state. At this stage alcohol intake is able to suppress and may even prevent the occurrence of the physical and mood disturbances. Alcohol and related components will therefore be perceived as highly attractive and will represent a potentially powerful reinforcement. Animals and humans will seek environments and develop behaviors aiming at the intake of alcohol.

These synergic negative and positive effects produced by alcohol give rise to withdrawal and protracted withdrawal, a pivotal role in the way out of alcoholism.

Animal studies may provide useful biological insights for a better understanding of human alcohol withdrawal.

During alcohol dependence, neuroadaptations within the central nervous system (CNS) occur allowing the brain to function regularly while being disturbed by alcohol. Ethanol is not a molecule with a single clear effect on a particular neurotransmitter system but it may affect multiple stages of the neurotransmission cascade of the large majority of neurotransmitters.

Our major goal is not to precisely describe the modifications at the different levels of the neurotransmitters, neuromodulators and neurohormones, but rather to define the occurrence of drinking according to the intensity of the physical signs and the mood state induced by withdrawal. When alcohol intake is arrested, CNS changes are by far the main source of signs and symptoms of adjustment of physical and mood state. Physical state is the reflection of a brain homeostatic balance between excitatory and inhibitory mechanisms while mood state depends on a hedonic balance leading to positive and negative reinforcements.

BRAIN HOMEOSTASIS

Cessation of chronic alcoholization leads the brain to an overexcited state observed through signs such as tremor and sometimes seizures.

This overexcitement is due to increase in glutamate transmission (a major neuroexcitatory amino acid) combined with an altered GABA transmission (a major neuroinhibitory pathway). Recent microdialysis studies showed that ethanol withdrawal was associated with increase in glutamate in the striatum [1], the nucleus accumbens (NAC) [2] and hippocampus [3]. Human studies have indicated that excitatory neurotransmitters were elevated in the CSF of alcohol-dependent patients [4]. Changes in GABA$_A$ receptors subunits (particularly a1 – 5 and b2 – 3) reduce the inhibitory activity of GABA contributing to hyperexcitability of neurons [5]. Moreover, intermittent withdrawal is also accompanied by an increase in aspartate (a
neuroexcitatory amino acid) and modification in GABA$_A$ α4 subunit, probably leading to a 'kindled overexcitation' [6,7].

The neural activity modulated by both excitatory and inhibitory influences are dependent on the influx of calcium into the cells through receptor-operated calcium channels and voltage operating channels. A general adaptive increase in these channels is observed during tolerance and withdrawal leading to generalized neuronal excitability induced by calcium entry [8,9].

Adenosine can also function as an inhibitory modulator of seizure activity. Adenosine A1 receptors are upregulated during ethanol withdrawal and repeated ethanol withdrawals [10]. This likely represents an adaptive response to seizure severity induced by repeated episodes of withdrawal.

Adenosine alters the responding levels of second messengers (like cyclic adenosine monophosphate, protein phosphatase, protein kinase A and C) allowing information to be transmitted more or less effectively to the connected neurons. Blockade of adenosine activity can reduce the occurrence of seizures at the cessation of chronic ethanol intake [11] without affecting ethanol consumption [12].

Alcohol intake is always able to rapidly abolish the negative reinforcement induced by its withdrawal and it would thus appear as a powerful medication against physical perturbations. Other classical neurotransmitters also mediate physical signs of alcohol withdrawal but to a lower extent, since agonists and antagonists of monoamines modify in a limited extent to those physical signs [13,14].

**HEDONIC HOMEOSTASIS**

Withdrawal from alcohol leads to a state inducing increase of dysphoria, anxiety, negative emotional state and stress probably because of a decrease in dopamine (DA) release in the NAC. The symptoms can be easily alleviated by alcohol intake. This may be related to an increase of DA activity in the NAC, as observed in rats where self-administered alcohol during withdrawal was able to rapidly reverse the deficit in DA and serotonin in the NAC [15].

DA is the major neurotransmitter released during attracted stimuli (including drugs), particularly in the NAC. Even anticipation of reward (but not punishment) leads to DA release in the NAC [16]. Among various modifications in neurotransmission after alcohol cessation, an increase in acetylcholine (ACHE) was observed in hippocampal microdialysate following the same timing of the deficit in DA accumbal release [17]. In parallel to the putative role of DA in positive reinforcement, ACHe may play a major role in the negative reinforcement [18].

The endogenous opioid system also indirectly activates the mesolimbic dopaminergic system and particularly the opioid receptor may modulate this dopaminergic deficit during alcohol withdrawal [19].

Withdrawal represents a major source of stress and the limbic system will immediately answer by secreting corticotropin-releasing factor (CRF) [20]. The limbic system is a large complex of brain structures regulating emotional processes. It plays a role in the negative reinforcement induced by withdrawal. Direct CRF antagonist injection into limbic areas were able to block anxiogenic-like responses induced by withdrawal [21]. CRF R1 or R2 receptors deficient
knockout mice present opposite impairments in anxiety-like (estimated by open-field test and light-dark test) and memory disorders. They also show opposite activity profiles during withdrawal signs [22]. CRF R1 receptors are directly activated in alcohol withdrawal and lead to anxiogenic-like responses. The time schedule of the CRF release in the amygdala observed during alcohol withdrawal is long lasting (peak value around 12 h after onset of withdrawal) as compared to conventional restraint stress (return to baseline CRF after 1 h) [21]. CRF in cerebrospinal fluid is also elevated during withdrawal in alcohol patients [23] and protracted abstinence enhances neuronal sensitivity to CRF in rats (estimated by EEG and event-related potentials) 15 weeks after complete withdrawal [24]. This long lasting increase in sensitivity to CRF may induce increased sensitivity to daily environmental stresses and thus may lead to secondary conditioned reinforcement.

During such conditioning, environmental stimuli or internal stimuli associated with withdrawal can act as cues for the appearance or exacerbation of the neurochemical modifications leading to a kindling process.

A common trait emerging when analyzing the neurochemical changes appearing during physical and mood state withdrawal modifications, is that alcohol intake leads to the immediate abolishment of undesirable symptoms. With conditioned secondary reinforcement and the kindling process associated with repeated withdrawals, self-administration of alcohol will progressively increase and will stabilize at a higher level [25].

**Alcohol withdrawal syndrome**

The alcohol withdrawal syndrome is a complex set of symptoms occurring in alcohol dependent patients after alcohol cessation. It involves a wide range of brain neurotransmitters implicated in the development of alcohol tolerance and reflects a homeostatic readjustment of the CNS [26]. The signs and symptoms of alcohol withdrawal syndrome have been well described in humans. They can be divided into three sets although many classifications have been made for various purposes [27]. In the first hours following the last alcohol intake, sympathetic hyperactivity is responsible for tachycardia, sweating, tremor, hypertension, anxiety and agitation that usually peak within 24 h. Later on (24 – 48 h after alcohol cessation), epileptic seizures may occur, whereas delirium tremens can be observed from 3 to 7 days after acute abstinence. This third set of symptoms is characterized by auditory and visual hallucinations, confusion and disorientation, clouding of consciousness and pronounced autonomic hyperactivity. It can lead to death from respiratory and cardiovascular collapse [28]. All these symptoms are disabling enough to lead many patients to resume alcohol consumption at the early stages of withdrawal. The severity of alcohol withdrawal syndrome is therefore a major risk factor of early relapse [29]. The understanding of its complex pathophysiology may help finding effective tools in preventing such an event.
DOPAMINERGIC SYSTEM

As mentioned above, among the many neurotransmitters involved in alcohol withdrawal, DA seems to play a central role both in tolerance to and withdrawal from alcohol. Alcohol activates the mesolimbic dopamine system by releasing DA in the NAC and it has been hypothesized that subjects with impaired dopaminergic activity may compensate for this deficiency by the use of alcohol and other substances that increase brain DA levels [30,31]. Both neuroendocrine [32] and genetic studies [33] have highlighted the implication of a reduced DAD2 receptor (DRD2) functioning in severe alcohol dependence. Whether this deficiency plays a role in some of the symptoms observed during withdrawal is still controversial, but the hypothesis of a reduced dopaminergic activity in the mesocorticolimbic reward pathway, responsible for negative mood states after alcohol cessation remains attractive [34]. Indeed, it has been shown that alcoholic patients carrying the A1 allele of the DRD2 gene exhibit lower densities of postsynaptic D2-receptors [35,36], which may result in low DA neurotransmission. These patients displayed increased depressive symptoms during acute alcohol withdrawal [37]. Poor dopaminergic functioning during the early stages of withdrawal and consecutive depressive symptoms could also be linked to higher recoveries in dopamine transporter (DAT) density in some alcoholic patients shortly after alcohol cessation [38]. It has been shown that this protein, which seems to be decreased during prolonged heavy drinking [39], reuptakes DA into presynaptic terminals and thereby terminates dopaminergic activity in synaptic neurotransmission [40]. This is in accordance with a reported increase of DAT levels in depressed patients [41]. However, opposite to concerning DA availability and its clinical consequences have been reported. Many reports suggest a DA overactivity during alcohol withdrawal, which may be linked to decreased GABAergic neurotransmission. It has been shown that GABAergic neurons directly inhibit dopaminergic neurons in the substantia nigra [42]. Therefore reduced GABAergic activity, as observed during alcohol withdrawal, leads to enhanced dopaminergic transmission, which could be responsible for more severe withdrawal symptoms [43]. This is in accordance with many studies showing higher concentrations of the DA metabolite homovanillic acid in alcoholics with delirium compared to patients who did not suffer from clouding of consciousness [44]. Furthermore, insufficient clearance of DA in the synaptic cleft leading to hyperdopaminergic states may be related to severe withdrawal symptoms [45], as suggested by the higher risk of seizures or delirium during withdrawal in patients carrying the A9 allele of the DAT-gene [40][46]. The functional consequence of the presence of this allele could indeed induce an alteration in DAT-gene expression [47] resulting in lower DA reuptake.

In summary, dopaminergic neurotransmission may be responsible for various symptoms of the alcohol withdrawal syndrome. Dopaminergic hypoactivity may cause dysphoric features while its overactivity could induce hallucinations. This can be paralleled to the putative pathophysiological mechanisms underlying psychotic symptomatology in which subcortical mesolimbic rewarding DA projections and mesocortical cognitive projections seem to interact in opposite ways, roughly underlying negative symptoms on the one hand, and positive on the other hand [48].
SEROTONERGIC SYSTEM

As for the serotonergic system, it has been demonstrated that serotonin (5-HT) could play a facilitator role on DA release in the NAC [49,50] while ethanol seems to directly stimulate the release of DA and 5-HT [51]. This could lead to a deficiency in the release of these neurotransmitters during ethanol withdrawal. Furthermore, interactions between impaired serotonergic neurotransmission and anxiety and depression have been postulated. As a 5-HT deficiency seems to be involved in alcohol dependence as well as in the maintenance of excessive alcohol consumption [52 – 54], it could be expected to influence the occurrence of mood symptoms in alcoholics, especially during withdrawal. This hypothesis was tested in alcohol-dependent rats, suggesting that deficits in accumbal 5-HT release may contribute to the negative affective consequences of alcohol withdrawal and motivate ethanol-seeking behavior [15].

In human, a significant reduction in the availability of 5-HT transporters was found in the raphe nuclei area of recently detoxified alcoholics, which was strongly correlated with increased levels of anxiety and depression during early abstinence [55]. Furthermore, reduced transcriptional efficiency of the serotonin transporter (5HTT) may underlie another set of symptoms during alcohol withdrawal. The frequency of the short allele of the polymorphism coding for 5HTT, responsible for modifications in 5-HT activity [56] was indeed strongly increased in alcoholics who reported seizures or delirium including hallucinations [47].

Finally, it has been postulated that deficiency of brain 5-HT activity in alcoholics may result in a decreased impulse-control over drug taking [57], particularly during alcohol withdrawal. According to Cloninger’s typology [58], this may lead to increased craving and anxiety during withdrawal, particularly in type II alcoholics, who are supposed to have low cerebrospinal fluid levels of 5-HT and its metabolite 5-HIAA, as opposed to late-alcoholism-onset-type I subjects [59]. Behavioral differences between type I and type II alcoholics were also observed by George et al. [60] who, after a m-chlorophenylpipерazine (m-CPP) challenge test, linked this finding to a hypo-5-HT2C receptor function.

NORADRENERGIC SYSTEM

Finally, attention should also be focused on another neurotransmitter involved in the pathophysiology of alcohol withdrawal, namely noradrenaline. An important aspect of withdrawal is increased central excitation, which leads to a sympathetic overactivity. Plasma and cerebrospinal fluid levels of noradrenaline are increased because of the overstimulation of noradrenergic neurons by the increased glutamate transmission and the loss of noradrenergic autoinhibition by reduced postsynaptic α2-adrenoceptor function [61]. Many studies have reported a persistent hypo-α2-adrenoceptor functioning during and after withdrawal as assessed by a blunted growth hormone (GH) response to clonidine [62 – 64]. GH response to clonidine is also known to be impaired in endogenous depression [65,66] as well as in panic disorder [67,68]. Impaired α2-adrenoceptor function might therefore be hypothesized to be responsible for anxiety and depressive symptoms occurring during alcohol withdrawal. However, no evidence for a relationship between dysphoric features and α2-adrenoceptor subsensitivity has been found to date [69]. Alternatively, the noradrenergic overdrive observed in alcohol withdrawal may solely be implicated in autonomic symptoms.
such as tachycardia, hypertension, tremor and sweating. As mentioned above, alcohol withdrawal reflects altered function of the brain processes involved in the development of tolerance to alcohol. In particular, impaired balance between inhibitory functions and excitatory systems seems to be implicated in the occurrence of some of the symptoms observed during acute withdrawal. On the one hand, chronic alcohol consumption enhances $g$-aminobutyric acid (GABA) inhibitory action, while on the other hand, it blocks some of the brain excitatory systems such as the glutamate $N$-methyl-$d$-aspartate (NMDA) receptor. The increase in the number of these receptors observed during chronic alcohol intoxication, has been interpreted as an attempt by the brain to compensate for diminished glutamate transmission [26]. Thus, when alcohol is acutely removed, GABA receptors are no longer stimulated while NMDA function is excessive. The latter is also due to the magnesium depletion observed in chronic alcoholism [70]. NMDA overactivity seems, among other pathways, to induce noradrenaline release, and may therefore contribute to the increased sympathetic activity observed during withdrawal. (cf. Table 1)

Table 1. Putative relationships between neurotransmitters disturbances and alcohol withdrawal symptoms

<table>
<thead>
<tr>
<th>Neurotransmitters</th>
<th>Chronic alcohol</th>
<th>Withdrawal</th>
<th>Withdrawal symptoms</th>
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<tbody>
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<td>Glutamate</td>
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<td>Seizures</td>
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<td>Aspartate</td>
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<td>Sympathetic overdrive</td>
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<tr>
<td>GABA</td>
<td>$\searrow$</td>
<td>$\searrow$</td>
<td>Anxiety</td>
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<tr>
<td>Noradrenaline</td>
<td>$\searrow$</td>
<td>$\nearrow$</td>
<td>Sympathetic overdrive</td>
</tr>
<tr>
<td>Dopamine</td>
<td>$\searrow$</td>
<td>$\searrow$ or $\nearrow$</td>
<td>Depression or hallucinations/ delirium tremens</td>
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<tr>
<td>Serotonin</td>
<td>$\nearrow$ or $\searrow$</td>
<td>$\searrow$</td>
<td>Anxiety</td>
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<td></td>
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<td>Depression</td>
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Alcohol withdrawal syndrome as a stressor

When managed in a medical setting, the severity of the symptoms and signs of withdrawal in alcohol-dependent patients is usually maximal on the first day of abstinence, and then decreases over a period of 5 – 7 days.

In the search for neurobiological markers of the syndrome, it has been shown that acute alcohol withdrawal is associated with hypercortisolaemia [71 – 75] and increase of salivary cortisol levels [76]. The magnitude of cortisol increase was related to the severity of withdrawal symptoms. The mechanism of hypercortisolaemia during alcohol withdrawal remains unclear. It does not appear to be due to hypothalamo-pituitary-adrenal axis hyperactivity since plasma ACTH concentration is decreased at baseline [72] and after injection of corticotrophin-releasing hormone [77,78].

Biochemical studies indicate that increased plasma levels of norepinephrine are associated with certain symptoms of alcohol withdrawal like blood pressure and heart rate response to standing [79] and that the severity of the withdrawal syndrome correlates positively with the amount of released norepinephrine [80,81].

In addition to medullo-adrenal and sympathetic hyperactivity, there are also indications of central adrenergic hypersecretion during alcohol withdrawal. High concentrations of norepinephrine and its major metabolite 3-methoxy-4-hydroxyphenylglycol (MOPEG) have been reported in cerebrospinal fluid in alcohol withdrawn patients [82,83]. In the beginning of the withdrawal manifestations, there is a positive correlation between MOPEG levels and sleeping problems, tremors, restlessness, visual hallucinations and elevated muscle tension [84].

These converging observations that alcohol withdrawal syndrome is a powerful stressor could be of major importance for the understanding of the development of addiction. It has become clear that administration of addictive drugs to laboratory rats can cause time-dependent sensitization that is probably a function of their nonspecific, stressful nature [85]. This is underlined by the fact that there is cross-sensitization between a stress challenge after previous drug administration [86] and a drug challenge after a previous stressful experience [87]. Repeated intracerebroventricular administration of CRF has similar sensitizing effects on later responsivity to amphetamine [88]. Stressful experiences in humans can result in a spectrum of long-term changes in behavioral, autonomic and hormonal responsivity. An extreme form of such alterations is found in patients with post-traumatic stress disorder. A number of animal models has been developed in which intense stressful experiences (such as shocks or social confrontations) result in long-term altered responsivity of behavioral, autonomic and hormonal responses to aversive challenges [89].

Daily exposure to emotional stress enhances the initiation of self-administration of morphine and cocaine, and ventral tegmental intracranial electrical self-stimulation in rats [90,91]. Moreover, social defeat directly followed by threat for four consecutive days increases the amount of cocaine self-administration and defeated rats acquire cocaine self-administration in approximately half the time of non-defeated rats [92,93]. These studies suggest that certain types of stress can enhance the addictive properties of drugs, possibly by enhancing the
sensitivity of the mesocorticolimbic dopaminergic system to these substances. Conversely, drug-induced sensitization of the mesocorticolimbic DA system may underlie increased responsiveness to stress.

Therefore, we hypothesize that drug withdrawal syndrome and especially alcohol withdrawal syndrome could be one important factor contributing to the progressive reinforcement of drinking through the progressive sensitization of the brain reward system by stress hormones. This could also be responsible for the higher risk to develop dependence to other drugs in alcoholics.

Such a sensitization to alcohol withdrawal is in accordance with the kindling hypothesis of alcohol withdrawal stated by Ballenger and Post [94]. This hypothesis has recently gained considerable support. Indeed, clinical studies have demonstrated a positive relationship between the number of previous withdrawal reactions and the risk of developing seizures during oncoming withdrawal reactions [95,96, Verbanck et al., unpublished].

These findings are in agreement with animal studies showing increased withdrawal behavior during repeated episodes of alcohol intoxication and withdrawal [97 – 99]. This process can be prevented by blocking the early withdrawal reactions with phenobarbital or diazepam [100,101], even if diazepam was reported to be unable to prevent the occurrence of seizures [102].

**Alcohol withdrawal as a neurotoxic event**

Acute effects of ethanol disrupt glutamatergic neurotransmission by reducing the sensitivity of the NMDA receptor [103]. Prolonged inhibition of NMDA receptors by ethanol induces their upregulation. When chronic alcohol consumption ceased, the upregulation combined with an increased release of excitatory amino acids can result in acute excitotoxicity [4]. Neurobiological effects of alcohol on the brain, such as intoxication, withdrawal symptoms and Wernicke–Korsakoff syndrome can be understood as consequences of effects of ethanol on the glutamatergic system.

Specifically, it was demonstrated that ethanol withdrawal is associated with increased extracellular glutamate [1]. This increase in release of glutamate could have major long-term consequences for the alcoholic patient. There is evidence of specific alcohol-associated frontal lobe injury [104]. While alcohol-induced oxidative stress may cause global brain impairment, the frontal lobes can be specifically injured by glutamate-mediated excitotoxicity because frontal lobes are particularly rich in excitatory amino acid pathways [105]. Using neuropsychological and radioisotopical methods, we demonstrated recently that frontal lobe hypometabolism in detoxified alcoholics induces specifically a major impairment of executive functions [106,107]. We also demonstrated that this impairment of executive functions (decision-making, flexibility, double-task) is a major clinical issue because it contributes dramatically to the risk of short-term relapse after detoxification [108].

The withdrawal syndrome in alcohol-dependent patients appears to be a major stressful event whose intensity increases with repetition of detoxifications according to a kindling process. These phenomena could be at least partly responsible for a progressive increase of the
reinforcing power of addictive drugs including alcohol, and for brain damage through an excitotoxic mechanism due to an increasing release of excitatory amino acids during withdrawal. Progressive neurotoxic lesions due to excitotoxicity in the frontal lobe could be a major consequence of repeated alcohol withdrawal, with major neuropsychological deficits negatively influencing the outcome after detoxification.

Hippocampus, another neural structure rich in excitatory amino acids projections, also appears to be particularly sensitive to the neurotoxic effects ethanol administration [109,110], especially after intermittent exposure [111,112]. This was demonstrated to result in memory impairments in rats [113]. Hippocampus lesions seem to be due to both excitotoxicity [114,115] and glucocorticoids [116 – 120]. A more recent drug, acamprosate, appears to have promising neuroprotective properties against the consequences of multiple withdrawals in animal models [121].

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