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Building better strategies to develop new medications in Alcohol Use Disorder: Learning from past success and failure to shape a brighter future

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Abstract

Alcohol Use Disorder (AUD) is a chronic disease that develops over the years. The complexity of the neurobiological processes contributing to the emergence of AUD and the neuroadaptive changes occurring during disease progression make it difficult to improve treatments. On the other hand, this complexity offers researchers the possibility to explore new targets. Over years of intense research several molecules were tested in AUD; in most cases, despite promising preclinical data, the clinical efficacy appeared insufficient to justify futher development. A prototypical example is that of corticotropin releasing factor type 1 receptor (CRF1R) antagonists that showed significant effectiveness in animal models of AUD but were largely ineffective in humans. The present article attempts to analyze the most recent venues in the development of new medications in AUD with a focus on the most promising drug targets under current exploration. Moreover, we delineate the importance of using a more integrated translational framework approach to correlate preclinical findings and early clinical data to enhance the probability to validate biological targets of interest.

Keywords

Alcoholism; Ethanol; Opioids; Brain Imaging; CRF; PET; fMRI

Introduction

Alcohol Use Disorder (AUD) is a major leading cause of death and disability worldwide. AUD account for 44.4% of premature mortality attributable to substance abuse disorders and, within mental and drug use disorders, alcohol dependence is the fourth leading cause of years lost due to disability after depression, anxiety and drug related disorders (Whiteford et al., 2013). According to the WHO alcohol consumption caused 3.3 million deaths

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worldwide in 2012 (WHO, 2014). The Center for Disease Control and Prevention (CDC) estimated that about 88000 people died for alcohol-related causes between 2006 and 2010 thus making alcohol abuse the third leading cause of preventable death after tobacco smoking and eating-related problems in US (Gonzales et al., 2014). In addition, the economic costs of AUD for the US society is estimated at about 250 billion in 2010 (Sacks et al., 2015).

Decades of neuroscience research has dramatically improved our understanding of how alcohol intake drives neuronal circuits and leads to the development of dependence after protracted use. Chronic alcohol drives long-term neuronal adaptations that reshape the cognitive and motivational organization of an individual promoting alcohol dependence. AUD is a chronic relapsing disorder characterized by a progression that starts with the experimentation of the positive reinforcing effects of the substance and ultimately leads to dependence. The latter is characterized by a loss of control over drinking and progressive emergence of compulsive drug-seeking; alcohol is consumed although the individual is aware of the adverse consequences that it causes. Once dependence is established, withdrawal from alcohol causes the emergence of aversive symptoms that promote negative emotional states characterized by dysphoria, anxiety and heightened sensitivity to stressful stimuli. In individuals suffering from this condition resumption of alcohol use leads to a relief from this negative state, thus promoting relapse to alcohol drinking. Relapse is also prompted by environmental cues associated with the positive reinforcing effects of alcohol. Drug seeking and relapse, the main clinical problems with AUD, are under the control of specific brain nuclei including hippocampus, extended amygdala, prefrontal cortex, insula and dorsal striatum (Koob and Volkow, 2010). On the other hand, the pleasurable effects of alcohol are mostly mediated by the mesolimbic dopamine pathway composed of the ventral tegmental area (VTA) and nucleus accumbens (NAc) (Koob, 1998).

Most of approved pharmacotherapies for AUD act centrally by targeting the neurobehavioral substrates that trigger the motivation for alcohol use and mediates drug reward. For example, the panopioid antagonists naltrexone and nalmefene counteract the alcohol reinforcing effects by blocking opioid receptors. Acamprosate, another approved medication, acts at least in part through modulation of the glutamate system, although the exact mechanism of action is this unclear (Spanagel et al., 2014). Differently from these molecules, the old drug disulfiram acts through peripheral mechanisms by inhibiting acetaldehyde dehydrogenase thus causing the accumulation of disliked acetaldehyde.

Unfortunately, the expanding knowledge gained on the neurobiology of AUD does not always translate into the development of new clinically efficacious treatments. Medications for AUD are still inadequate since few are available and they are moderately effective and only in some patients.

The difficulty in finding a treatment for AUD that works for everyone reside in the heterogeneity of the disorder. AUD is the result of an intricate interplay among polygenic, environmental and neurobiological components. This may explain why only a minor proportion of individuals exposed to drugs of abuse become addicted. Research has highlighted that there are specific behavioural and genetic traits that predispose an individual

to drug abuse. For example, individuals with high anxiety or highly impulsive traits are more prone to develop drug dependence (Terracciano et al., 2008; Zuckerman, 1986).

From a clinical perspective an ideal treatment for AUD should drive patients to discontinue alcohol consumption (i.e. abstinence). However, despite complete abstinence is desirable in the presence of severe liver or kidney damage, reduction of alcohol intake is also an important outcome of the therapy since it improves the quality of life and reduces social-related issues caused by alcohol (Miller et al., 1992; Rosenberg, 1993; Walitzer and Connors, 1999). A possible strategy to extend the efficacy of pharmacotherapies for AUD consists in augmenting the range of drugs available, so that clinicians would have more options to adapt the treatment strategy to the individual patient profile. A similar approach is used for depression that, as for AUD, is characterized by a diversity of responses to treatments.

The development of successful treatments requires preclinical models with high predictive validity for treatment outcome. The purpose of animal models is to replicate human conditions and several of these models have allowed, over time, to acquire significant insight into the determinants of psychiatric disorders. The advantage of animal models resides in the opportunity to control important experimental variables including environment and genetics. Moreover, animal research allows manipulations that are not possible in humans such as site-specific brain injection of a pharmacological agent or sophisticated histological and gene expression analyses of the brain that in humans are necessarily limited to post-mortem samples. In the field of alcoholism research, animal models have contributed to define the neuronal substrates involved in alcohol abuse and dependence and have demonstrated some level of predictive and translational validity for AUD.

Animal Models of “drinking”

Excessive alcohol drinking is influenced by numerous factors such as availability of the substance, environment and stressful conditions associated with its use (Ciccocioppo et al., 2001; Katner et al., 1999; Le et al., 1998; Martin-Fardon et al., 2000; Martin-Fardon et al., 2010; Monti et al., 1999; Rohsenow et al., 2000). The development of alcoholism, however, is significantly linked to the presence of specific genetic traits that confer individual vulnerability to escalate alcohol use until dependence is established (Cloninger et al., 1981; Sigvardsson et al., 1996). It is estimated that genetics may account for up to 58% predisposition at the development of AUD (Kendler et al., 1997; Prescott and Kendler, 1999). Decades of alcohol research have led to the awareness that AUD is a polygenic disease, is affected by multiple external causes leading to a very heterogeneous patient populations. As a results, ideal treatments and pharmacotherapies should be tailored to the specific patient subgroups (Goldman et al., 2005; Heilig and Egli, 2006; Heilig et al., 2011). The complexity and heterogeneity of alcoholism make it difficult to find preclinical models and protocols that mimic human conditions. However, each animal models can replicate a specific aspects of human disease and thus they are unparalleled tools for drug development programs.

The preclinical models and procedures currently available have been designed to incorporate the major elements contributing to the development of the disease over time. These include the use of rat lines selectively bred for high ethanol preference and excessive drinking. Furthermore experimental animal models characterized by high alcohol drinking following chronic alcohol intoxication have been developed and validated. The use of these preclinical models, demonstrated that genetic factors are a prerequisite to develop AUD, but repeated consumption of intoxicating quantities of alcohol are fundamental in shaping the transition to AUD. Each one of the available animal models has been shown to possess some level of predictive, face and construct validity (McKinney and Bunney, 1969; Newport et al., 2002; Willner, 1984).

Successful development of naltrexone and acamprosate, two drugs clinically approved for AUD, has offered a robust proof-of-principle of the feasibility of drug development in this field (Sass et al., 1996; Volpicelli et al., 1992a). Moreover, this success has given the possibility to use these compounds to test the predictive validity of animals models. In this respect it is worth mentioning that naltrexone, a drug that reduces alcohol drinking and response to environmental cues in humans also decrease alcohol intake in several lines of rats genetically selected for excessive alcohol drinking and preference as well as in post-dependent models (Koob et al., 2003).

For instance naltrexone is efficacious in the P (Dhaher et al., 2012), the HAD (Krishnan-Sarin et al., 1998), the sP (Sabino et al., 2006), the AA (Koistinen et al., 2001) and the msP lines (Ciccocioppo et al., 2007; Perfumi et al., 2005). Remarkably, naltrexone was shown to reduce alcohol intake also in genetically heterogeneous rats as well as in monkeys (Barr et al., 2010; Kornet et al., 1991; Stromberg et al., 1998; Vallender et al., 2010). Pharmacogenetic studies have clearly demonstrated that the efficacy of naltrexone is largely restricted to those alcoholics carrying a single nucleotide polymorphism (SNP), rs1799971, that encodes for asparagine (N) →aspartate (D) substitution in position 40 of the MOR receptor protein (see for review (Heilig et al., 2011)). Interestingly, in monkeys it was shown that responders to naltrexone carry a MOR C77G polymorphism functionally equivalent to the N40D mutation described in humans and that confer to them the sensitivity to naltrexone (Barr et al., 2010; Vallender et al., 2010).

Further evidence of the validity of preclinical rodent models is offered by acamprosate (Mann et al., 2008) that showed its efficacy on alcohol drinking in a variety of experimental paradigms (Cowen et al., 2005; Olive et al., 2002b). Interestingly, more than a decade ago it was shown that in rats a combination of acamprosate and naltrexone did not offer advantages over administration of single agents (Stromberg et al., 2001). A few years later the US COMBINE clinical trial confirmed the lack of additive effects also in humans (Anton et al., 2006). Altogether these findings provide some level of confidence for the predictive validity of animal models in alcoholism. On the other hand, data from preclinical models should be cautiously evaluated. In fact, oftentimes, contrary to what preclinical models predicted, human treatment has shown a lack of efficacy. Several examples exist, but the classical one is offered by anti-depressants belonging to a class of selective serotonin uptake blockers (SSRIs). These molecules have invariably demonstrated to possess high efficacy in attenuating alcohol drinking in laboratory animals whereas several controlled clinical trials

showed limited effectiveness in humans (Garbutt et al., 1999; Nunes and Levin, 2004). One explanation is that SSRIs elicit an anorectic effect; hence a decrease in alcohol consumption in experimental animals may represent an epiphenomenon secondary to the inhibition of consummatory behavior elicited by these agents rather than an effect on motivation for alcohol.

Voluntary drink of intoxicating doses of alcohol leads to the expression of a withdrawal syndrome once a subject, abruptly, suspend the intake. It is quite difficult to reproduce this condition in animals. However, alcohol preferring rats can spontaneously develop physical dependence if exposed to 10% alcohol over a long period of time (Kampov-Polevoy et al., 2000) and physical signs of withdrawal were induced in alcohol preferring and non-preferring rats after eight weeks of chronic voluntary alcohol consumption (Waller et al., 1982). Moreover it was observed that prolonged abstinence following a long period of ethanol administration increased anxiety levels in rats (Santucci et al., 2008). Anxiety symptoms also occur in abstinent rats after repeated cycles of alcohol intake followed by periods of withdrawal (Holter et al., 1998). As alternatives to the use of alcohol preferring rat line that voluntarily take high alcohol doses preclinical researchers have developed several models of alcohol intoxication using vapor chamber inhalation procedures or forced alcohol administration through diet or intragastric intubation (Becker, 2013; Braconi et al., 2009; Hermann et al.; Majchrowicz, 1975; Penland et al., 2001; Rimondini et al., 2002). These models have been extensively used to study neuroplasticity and maladaptive changes associated to protracted exposure to alcohol with the hope to understand the neurobiological basis of alcohol addiction (Becker, 2013). However, these models lack the voluntary component of alcohol intake which limits their construct validity. Nonetheless these models possess predictive validity as demonstrated by the effectiveness of acamprosate in reducing alcohol intake in rats in which alcohol dependence was induced via vapor inhalation (Le Magnen et al., 1987; Rimondini et al., 2008) or by the efficacy of gabapentin both in animals and humans (Mason et al., 2014; Roberto et al., 2008).

Using these genetic and post-dependent models it has been possible to shed light on negative reinforcement mechanisms responsible for excessive alcohol drinking and high relapse vulnerability in late stages of dependence (Koob, 2014)

Animal Models of “relapse”

Relapse to alcohol use is mainly evoked by exposure to environmental stimuli previously associated with drug consumption. Relapse models based on environmental conditioning rely on cues that become associated with the rewarding effect of ethanol. Once re-exposed to ethanol-associated cues, the subject recalls the pleasurable effect of the drug that consequently trigger the resumption of drug use. Environmental conditioning is a long lasting phenomenon and relapse may occur even after months of abstinence (Monti et al., 2000; Sinha and Li, 2007).

Stress is another key factor in the initiation and maintenance of ethanol abuse in humans, and also plays a crucial role in relapse (Sinha et al., 2009; Sinha and Li, 2007; Sinha and O’Malley, 1999). Stress elicits mood-related symptoms (i.e., anxiety) vegetative (i.e., sleep

disturbances) and several somatic symptoms, that often drive the subject to drinking alcohol to ease these negative affective states (Breese et al., 2011).

Genetically selected alcohol preferring rats and other behavioural models of alcoholism, such as animals subjected to alcohol induced dependence and escalation of drinking, possess the capability to reproduce these complex behavioural traits. MsP rats for example can be trained to self-administer 10% alcohol or water in the presence of different discriminative environmental cues (i., e, lights, sounds our odours). If this self-administration training is followed by an extinction period during which animals are placed in the boxes without receiving the fluids or the respective cues lever pressing progressively decrease. However, seeking behavior is rapidly resumed when alcohol associated cues are presented. On the other hand, lever pressing is not enhanced following presentation of water-paired cues. Using this paradigm it was shown that genetically selected alcohol preferring rats have a more robust and persistent reinstatement of lever pressing compared to their control lines or to non selected animals (Ciccocioppo et al., 2001; Ciccocioppo et al., 2006; Maccioni et al., 2007). Similar to alcohol preferring rats, animals subjected to alcohol drinking escalation or with a history of alcohol dependence show a stronger seeking behaviour following exposure to stress or presentation of cues predictive of drug availability (Liu and Weiss, 2002a; Weiss et al., 2001).

Importantly, high relapse vulnerability in genetically selected alcohol preferring and in post-dependent rats appears to be associated to innate dysregulation of the stress system or to maladaptive reorganization of neuronal transmission. As demonstrated in several studies these changes may result in altered sensitivity to pharmacological manipulation of relapse behaviour. For instance, it has been shown that chronic ethanol exposure produces enhanced sensitivity to the inhibitory effects of D1 and D2 receptor antagonists on cue-induced drug seeking (Liu and Weiss, 2002b). A similar phenomenon was documented also for metabotropic glutamate receptors mGlu2/3 agonist LY379268 that showed higher efficacy in reducing drug-seeking in post-dependent rats compared to nondependent animals (Sidhpura et al., 2010). Conversely, naltrexone was less efficacious in preventing cue-induced drug-seeking in rats with a history of multiple alcohol intoxication and withdrawal (Ciccocioppo et al., 2003).

Genetic background and animal history also have important consequences on the sensitivity to pharmacological manipulations of stress-induced relapse. For instance, CRF1R antagonists are more efficacious in preventing stress-induced alcohol seeking in genetically selected msP rats and in animals with a history of alcohol dependence than in nondependent control animals (Gehlert et al., 2007; Hansson et al., 2006).

These findings have important implication for translational research because they suggest that different animal models and experimental paradigms may reproduce specific aspects of alcoholism or may mimic selected patient subpopulations with distinct sensitivity to pharmacological agents. The correct choice of animal models, the capacity to link them to specific patient sub-phenotypes and the ability to properly direct to them the right pharmacological agents under investigation are critical to increase the success rate of clinical trials in alcoholism.

The data available show that the reinforcing properties of alcohol are very powerful in rats genetically selected for high ethanol preference and that in these rat lines exposure to alcohol-paired cues leads to a compelling motivation for the drug. Furthermore, genetically selected preferring rats show higher sensitivity to stress compared to heterogenous non selected animals. For example, msP rats trained to alcohol self-administration show higher reinstatement of lever pressing after foot-shock stress compared to Wistar controls (Hansson et al., 2006). This innate hypersensitivity to stress can, in part, explain their high predisposition to excessive drinking and relapse vulnerability, as these behaviors may be motivated by the attempt to self-medicate from the negative affective state associated with stress sensitivity (Hansson et al., 2006). Similar results were also obtained with other alcohol preferring rat lines including AA, HAD and P that, compared to control Wistars, show an elevated propensity to relapse following stress exposure (Vengeliene et al., 2003).

Noteworthy, like genetically selected alcohol preferring rats, individuals highly sensitive to stress and/or with impaired stress-coping skills are more likely to abuse alcohol. (Bartlett and Heilig, 2011; Sinha, 2011).

In addition to operant self-administration/reinstatement paradigms, the alcohol deprivation effect (ADE) is also used to model human relapse. In this model animals are allowed to self-administer ethanol for a protracted period of time. Then they are repeatedly deprived of ethanol for a period that can last days or also months. When ethanol become available again a substantial increase in intake over the baseline reached before the deprivation is invariably observed (Heyser et al., 1997; Holter and Spanagel, 1999; McKinzie et al., 1998; Sinclair and Li, 1989; Sinclair and Senter, 1968; Vengeliene et al., 2014). Alcohol preferring rats express alcohol deprivation effect in different conditions (McKinzie et al., 1998; Rodd-Henricks et al., 2000; Sinclair and Li, 1989). It has been suggested that the increase in intake is due to an augmented motivation for alcohol, caused by craving during abstinence. This is demonstrated by the enhanced motivation for alcohol of animals subjected to ADE and tested under progressive ratio schedule of reinforcement (Spanagel and Holter, 2000). The good predictive validity of ADE was demonstrated by the efficacy of acamprosate to reduce the alcohol reinforcing effect following ethanol deprivation (Heyser et al., 1998; Holter et al., 1997). Similar results were also obtained with naltrexone that decreased alcohol intake after abstinence in a rat ADE model (Fredriksson et al., 2015; Holter and Spanagel, 1999). The ADE model embeds also important aspects of construct validity since it mimics the human condition where relapse is triggered by ingestion of alcohol following variables periods of abstinence.

Human Laboratory Paradigm in Alcohol Research.

Human laboratory studies in alcohol addiction can be distinguished into two broad classes; the first one to model drug self-administration the second relapse. The first human laboratory study on alcohol self-administration was published by Mendelson and La Dou (Mendelson and Ladou, 1964) almost 60 years ago. Since then several additional studies have been carried out, especially over the last two decades. In self-administration experiments alcohol can be taken orally or can be delivered through intravenous (IV) infusion; each option offering advantages and disadvantages. The oral route is the more

natural and closely mimics the conditions under which subjects drink alcoholic beverages. On the other hand, following oral consumption experimental parameters can be poorly controlled due to variability across subjects in alcohol absorption, rate of drinking, time of the day, etc. Individual variability in first pass metabolism may also affect alcohol brain concentration. Intravenous infusion helps to control for several of these variables thus allowing a fine regulation of plasma/brain alcohol levels. Another advantage of IV infusion is the possibility to dissect the pharmacological actions of alcohol from the effects due to the subjective taste and/or smell response to alcohol intake. Oral and IV self-administration paradigms if used, for example, in brain imaging studies may lead to substantially different results with the latter allowing a more reliable interpretation of pharmacological effects of alcohol on the brain (Gilman et al., 2012a; Gilman et al., 2012b). Notably, IV alcohol administration has also been used to study brain responses to cues experimentally paired to alcohol, something that would have been difficult following oral administration (Kareken et al., 2012). Alcohol self-administration paradigms have been extensively used to test the effect of naltrexone under experimentally controlled conditions. Many of these studies unambiguously showed the efficacy of naltrexone to reduce alcohol drinking (see for review (Zimmermann et al., 2013). This is one of the strongest evidences in support of the predictive validity of human laboratory paradigms thus further supporting their value in translational medicine.

Stress and environmental conditioning play a key role in shaping addictive behaviors and relapse to drug seeking (Koob and Le Moal, 1997; McLellan et al., 2000; O'Brien et al., 1998). As mentioned above exposure to environmental cues associated with the rewarding effect of ethanol can trigger the resumption of drug use (Koob and Le Moal, 1997; O'Brien et al., 1998). On the other hand stress can trigger negative somatic and affective symptoms that may drive to drug use in order to ease these negative conditions (Brown et al., 1995; Koob and Kreek, 2007; Sinha, 2001).

Over the years a massive effort has been made to model relapse behaviours in humans under experimentally controlled laboratory settings. These models largely rely on provoking relapse after exposure to alcohol associated cues or exposure to stress (Fox et al., 2012; Gilman and Hommer, 2008; Sinha, 2013; Sinha et al., 2011; Zakiniaeiz et al., 2017). Occasionally, studies to evaluate how conditioning factors and stress interact with each other to facilitate relapse have also been carried out (Fox et al., 2013; Sinha et al., 2011; Thomas et al., 2011). The use of these models under laboratory controlled conditions in combination with brain imaging has allowed a tremendous advancement in the understanding of the neurobiological basis of craving and relapse (Gilman and Hommer, 2008; Sinha, 2013). Application of these approaches, together with an increased knowledge of the genetic basis and neurobiology of alcoholism has allowed researchers to distinguish endophenotypes of patients that might respond differently to pharmacological treatments. A prototypical example of this experimental approach has been published by Schacht and co-workers (Schacht et al., 2013). In this work, imaging techniques were used in a cue-reactivity study to distinguish the response to naltrexone in two subgroups of non-treatment-seeking alcohol-dependent individuals, carrying the typical A118G (Asn40Asp) single nucleotide polymorphisms in the μ-opioid receptor (MOP) known to confer different sensitivity to MOP antagonists. Results revealed a complex three-way interaction between ventral striatum and

medial prefrontal cortex activation following exposure to cues, naltrexone treatment, and polymorphism in the MOP opioid and dopamine transporter genes (Schacht et al., 2013). Employing the MOP selective ligand [(11)C] carfentanil a PET displacement study was recently carried out to measure receptor occupancy by naltrexone in carriers of one of the two A118G (Asn40Asp) variants. A trend to a higher receptor occupancy by naltrexone was found in carriers of the G allele which may explain, at least in part, their higher sensitivity to this drug (Weerts et al., 2013). A similar study was carried to investigate how polymorphism in the gene encoding the DA transporter DAT would affect the sensitivity to the effects of the partial agonist aripiprazole. Results revealed that the compound reduced more efficaciously cue-elicited ventral striatal activation and number of drinks in subjects carrying the DAT1 9-repeat allele, that is associated with lower DAT expression, higher synaptic DA tone and greater reward-related brain activation (Schacht et al., 2018). These examples offer a clear demonstration of the importance of combining brain imaging techniques with human behavioural and pharmacological studies to predict the efficacy of medications depending on patient genotypes.

Example of success in AUD

Valuable examples of successful translation from preclinical research to clinical development are represented by acamprosate and naltrexone, two of the three FDA approved drugs to treat AUD. A detailed analysis of the literature about these two compounds is beyond the scope of this article (for review see: (Franck and Jayaram-Lindstrom, 2013). However, it is worth mentioning that research in laboratory animals demonstrated that acamprosate attenuates alcohol consumption in rodents, mitigates some consequences of withdrawal and reduces relapse to alcohol seeking (Heyser et al., 1998). Chronic drug administration, however, has been shown to produce tolerance (Cowen et al., 2005; Lido et al., 2012; Vengeliene et al., 2010). Similarly, naltrexone reduces alcohol consumption and relapse to alcohol seeking in rats, mice and monkeys (Gilpin et al., 2008; Ji et al., 2008).

Acamprosate and naltrexone have shown some level of efficacy in humans and have been approved for clinical use. Nevertheless the Number Needed to Treat (NNT) to prevent one person to return to any drinking was quite high for both drugs (12 for acamprosate and 20 for naltrexone) meaning that only few treated patients respond to drugs (Jonas et al., 2014).

Previous studies in monkeys and in humans showed that naltrexone is not equally efficacious in all individuals treated and a link between the presence of A118G polymorphism at MOP opioid receptor locus (OPRM1) and drug efficacy was proposed (Anton et al., 2008; Barr et al., 2010; Oslin et al., 2003). These results were not confirmed by more recent studies in which the efficacy of naltrexone and GSK1521498, another opioid antagonist, appeared to be unaffected by A118G polymorphism (Oslin et al., 2015; Ziauddeen et al., 2016). Nevertheless, overall preclinical and clinical evidence offers an important demonstration of the feasibility of developing opioid antagonist-based treatments for AUD.

Other promising compounds are under current clinical investigation; one of these is baclofen, a GABA-B agonist registered for muscle spasticity. Initial studies demonstrated the efficacy of this compound in laboratory animals and in alcohol-dependent patients

(Addolorato et al., 2000a). Subsequent clinical studies demonstrated the safety of this compound in alcoholics with liver cirrhosis (Addolorato et al., 2007; Addolorato et al., 2009). The availability of nonhepatotoxic drug it is very important in AUD clinical practice as chronic liver diseases are often present in alcoholics. Acamprosate is also considered a suitable drug as, due to its lack of liver metabolism it can be safely used in patients with mild hepatic insufficiency (Delgrange et al., 1992).

Since the initial studies on baclofen several other observational reports were published to demonstrate its efficacy in AUD with the drug administered in an unusually wide range of doses (from 5 to 630 mg/day) (Thompson et al., 2017). Due to still unclear results and to the difficulty of establishing the dose range at which the drug is effective and has tolerable side effects, in AUD baclofen is currently licensed in France only (Colombo et al., 2002; Soyka and Muller, 2017). An interesting new approach consists of the development of GABA-B positive allosteric modulators (PAM). Preclinical data have shown that these molecules are efficacious to lower doses compared to those needed by orthosteric GABA-B agonists like baclofen. GABA-B PAM showed efficacy in reducing alcohol intake in different animal models (Hwa et al., 2014; Liang et al., 2006; Loi et al., 2013; Maccioni et al., 2010; Maccioni et al., 2012; Orru et al., 2005) as well as reinstatement of alcohol seeking paradigms (Augier et al., 2017; Maccioni et al., 2019; Vengeliene et al., 2018).

In the attempt to identify novel treatments for AUD also various anti-epilectics have been studied. One of this molecule is gabapentin which attenuates alcohol consumption in dependent rats and attenuates anxiety levels induced by ethanol withdrawal in rats (Roberto et al., 2008; Watson et al., 1997). Initial data suggest the efficacy of gabapentin in treating alcohol abstinence in alcoholic patients in which it reduces both anxiety and episodes of heavy drinking (Mason et al., 2014; Myrick et al., 2009). Another interesting pharmacological approach is with topiramate that in mice reduced alcohol intake (Nguyen et al., 2007). Whereas in clinical trials it demonstrated efficacy in reducing drinking and in increasing the days of abstinence (Johnson et al., 2003; Johnson et al., 2007). Moreover, earlier studies demonstrated that zonisamide, another anticonvulsant, also reduce alcohol intake in rodents (Knapp et al., 2007). Later clinical studies confirmed its therapeutic potential also in humans (Arias et al., 2010; Knapp et al., 2015). As shown in Table 2 also gamma-hydroxybutirate (GHB), also known as Sodium Oxybate (SO), displayed important positive effects on craving, attenuates drinking and protects from the expression of alcohol withdrawal (Addolorato et al., 1996; Colombo and Gessa, 2000; Gallimberti et al., 1992). However, GHB is characterised by a substantially high abuse potential that significantly limits its use (Addolorato et al., 2000b). Currently this drug is registered for the treatment of AUD in few European countries. The antiemetic serotonin-3 receptor antagonist ondansetron showed some efficacy in reducing drinking especially in early-onset alcoholics (Johnson et al., 2002; Johnson et al., 2000; Kranzler et al., 2003) and in subgroups of patients with a specific polymorphism of serotonin transporter gene (Johnson et al., 2011).

Finally, encouraging results have been reported for varenicline that shows partial agonist activity to $\alpha 4\beta 2$ nicotinic acetylcholine receptor, approved as a smoking cessation aid. The first evidence of the efficacy of varenicline in reducing alcohol drinking was published in 2007 in the rat (Steensland et al., 2007). Since then several studies confirmed this finding in

both heavy-drinking smokers and primary alcoholics (Falk et al., 2015; McKee et al., 2009; Ray et al., 2014; Schacht et al., 2014). In a lab human study, heavy drinkers subjected to fMRI scan after chronic varenicline compared to placebo reported lower feelings of happiness and excitement following intravenous alcohol. This effect was associated with reduced BOLD signal in the ventral striatum, amygdala, and posterior insula (de Bejczy et al., 2015). In another brain imaging study, varenicline increased control over alcohol-associated thoughts and reduced the activation of the orbito-frontal cortex triggered by environmental cues (Schacht et al., 2014). These mechanisms may explain the efficacy of varenicline on alcohol drinking and support its potential effectiveness as a treatment for AUD.

Learning from failures: the case of CRF1R antagonists

Probably the most notable failure in translational research in AUD is represented by corticotropin releasing factor type 1 receptor (CRF1R) antagonists. The corticotropin releasing factor (CRF) system, has been consolidated by more than 30 years of consistent preclinical data as one of the most promising targets to treat alcoholism. CRF1R, together with CRF2R and CRF-binding protein, are the target of this neuropeptidergic system originally characterised for its role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis system (Bale and Vale, 2004). Subsequently a plethora of preclinical studies linked the CRF system with addiction (see for review (Koob, 2010; Ubaldi et al., 2016a). Briefly, CRF modulates both neuroendocrine stress response by activation of the HPA axis and the affective response to stress by acting on extra-hypothalamic regions (Primus et al., 1997; Sanchez et al., 1999; Van Pett et al., 2000). Through CRF1R located in the extended amygdala (Zorrilla et al., 2014), CRF regulates the withdrawal/negative effect stage of the addiction cycle (Koob and Volkow, 2010), for alcohol (Funk et al., 2006; Hansson et al., 2007; Merlo Pich et al., 1995; Olive et al., 2002a; Roberto et al., 2010; Zorrilla et al., 2001) and other drugs of abuse (Ubaldi et al., 2016a). CRF antagonists reduced anxiety induced by alcohol withdrawal in post-dependent rats (Breese et al., 2005; Gehlert et al., 2007; Knapp et al., 2004; Roberto et al., 2010; Sommer et al., 2008), decreased alcohol self-administration in rodents and prevented stress-induced reinstatement (Chu et al., 2007; Funk et al., 2007; Gehlert et al., 2007; Gilpin et al., 2008; Richardson et al., 2008; Sabino et al., 2006). Data on cocaine, nicotine, cannabinoids, opiates, and benzodiazepines confirmed the potential efficacy of CRF1R antagonism in preventing negative emotions, stress and withdrawal associated with drug abuse (Basso et al., 1999; George et al., 2007; Park et al., 2015; Rodriguez de Fonseca et al., 1997; Skelton et al., 2007; Tucci et al., 2003).

Further evidence linking the CRF1R system to drug addiction comes from genetic studies in alcohol preferring msP rats, an animal line characterized by excessive alcohol drinking, high innate anxiety and inability to engage in active responses to stress (Ayanwuyi et al., 2013; Ciccocioppo et al., 2006; Cippitelli et al., 2015; Hansson et al., 2007; Hansson et al., 2006). In msPs, these maladaptive behaviours are associated with over-expression of the corticotropin-releasing factor (CRF) system possibly triggered by two single nucleotide polymorphisms (SNPs) occurring in the promoter region (position −1836 and −2097) of the CRF1 receptor (CRF1-R) gene. Similar evidences were obtained in human genetic studies showing that CRF1R gene polymorphisms are associated with binge drinking and excessive

drinking in alcohol-dependent patients (Treutlein et al., 2006). Another study also identified gene variations at *Crh1R* locus in a caucasian population with a diagnosis of AUD (Chen et al., 2010).

This wealth of preclinical and human genetic evidences contributed to build substantial interest on CRF1R antagonists for the treatment of alcohol abuse. However, despite these promising findings, two recently developed CRF1R antagonists, pexacerfont and verucerfont, failed to yield expected clinical results (Schwandt et al., 2016). The first compound tested, pexacerfont, did not affect stress-induced alcohol craving and emotional distress, as well as BOLD-fMRI activity induced by both aversive stimuli and alcohol-associated cues (Kwako et al 2014). In addition, activation of HPA-axis induced by dexamethasone/CRF (dex/CRF) was also not affected by pexacerfont (Kwako et al., 2015). It was proposed that binding kinetics could be major determinants of the efficacy of CRF1R antagonists. In the presence of equal binding affinity, compounds with a slow receptor dissociation time constant (off-rate) should have higher efficacy (Fleck et al., 2012). Thus, attention was shifted to verucerfont a drug displaying similar binding affinity with pexacerfont but a slower off-rate constant. Results revealed attenuated brain responses to negative emotional stimuli associated with blunted HPA-axis activation and lower ACTH and cortisol release following CRF stimulation. However, despite these anti-stress like effects, verucerfont did not suppress alcohol craving or negative emotionality (Schwandt et al., 2016).

Noteworthy, lack of clinical efficacy of CRF1R antagonists was recently reported also in trials aimed at investigating the effect of these agents in post-traumatic stress disorder, major depressive disorder, generalized anxiety disorder and social anxiety disorder (Dunlop et al., 2017; Grillon et al., 2015). The reasons why the strong preclinical evidence generated over three decades of research with CRF1R antagonist did not translate into clinical efficacy is unclear.

One study on post-traumatic stress disorder reported that verucerfont is partially efficacious in a subpopulation of patients with a history of childhood abuse carrying a specific single nucleotide polymorphism (GG vs AA) at rs110402 of the CRHR1 gene (Dunlop et al., 2017). Thus, the responsiveness of patients to CRF1R antagonism may depend on previous history of severe stress exposure and/or on their genetic background. This hypothesis is corroborated by preclinical data in msP rats showing higher efficacy of CRF1R antagonists in animals carrying two single nucleotide polymorphisms (AA v GG) in the promoter region of the Crhr1 locus (Ayanwuyi et al., 2013; Ciccocioppo et al., 2006; Cippitelli et al., 2015; Hansson et al., 2007; Hansson et al., 2006). Notably msP rats shows also traits resembling PTSD symptoms which may contribute to their innate propensity to drink high amounts of alcohol and that could explain their high sensitivity to CRF1R blockade (Natividad et al., 2017). Other possible explanations for the failure of CRF1R antagonists in clinical studies can be related to species differences between rodents, monkeys and humans. For instance, it has been shown that brain expression and function of CRF and CRF1R may vary in species differing in parental care, maternal defense or social behavior in general (Hostetler and Ryabinin, 2013). Additionally the differences in preclinical and clinical studies may arise from divergences between human symptomology compared to animal behavior and dosage

and bioavailability of the CRF1 antagonists (Binneman et al., 2008; Dong et al., 2018; Kehne and Cain, 2010; Nielsen, 2006; Zorrilla and Koob, 2004). Despite the inconsistency between preclinical and clinical data the CRF system remains an interesting target for development of AUD medication and evidence of its role in modulating alcohol drinking continue to grow (de Guglielmo et al., 2019).

Although the experience with CRF1R antagonists may appear frustrating at first, the careful analysis of this complex system may be a key to the success of future translational drug development programs.

Examples of emerging targets

Nociceptin:

The neuropeptide nociceptin/orphanin FQ (N/OFQ) and its cognate receptor NOP are widely expressed in the brain. Earlier studies showed that the activation of NOP receptor by its endogenous ligand or by synthetic agonists produces a significant reduction in drinking, reduces alcohol induced conditioned place preference and attenuates reinstatement evoked by stress or cues (Ciccocioppo et al., 2004; Ciccocioppo et al., 1999; Ciccocioppo et al., 2014; Kuzmin et al., 2003; Martin-Fardon et al., 2000; Witkin et al., 2014). Subsequent investigations demonstrated that similar effect was also produced by NOP antagonism. The NOP antagonist LY2940094 decreased voluntary ethanol intake in alcohol preferring and nonpreferring rats (Rorick-Kehn et al., 2016). Moreover, NOP knockout rats self-administer significantly less alcohol that their wild-type counterpart, suggesting a facilitatory role of NOP receptors (Kallupi et al., 2017). Recent clinical data showed the translational potential of NOP modulation as LY2940094 was effective in reducing drinking in humans (Post et al., 2016). The reason why both NOP agonists and antagonists reduce the motivation for alcohol is, now, not completely clear. One recently proposed hypothesis is that NOP agonists may act through mechanisms involving desensitisation of the system (Ciccocioppo et al., 2019).

Ghrelin:

Ghrelin is a 28-aminoacid peptide that binds and activates the growth hormone secretagogue receptor 1a (GHS-R1a) to increase appetite and food intake. Studies on rodent models showed that the blockade of GHS-R1a reduce the consumption and the motivation for alcohol (Bahi et al., 2013; Gomez et al., 2015; Gomez and Ryabinin, 2014; Jerlhag et al., 2009; Kaur and Ryabinin, 2010; Landgren et al., 2012; Suchankova et al., 2013). Accordingly, various studies demonstrated that ghrelin enhance alcohol intake when administered both centrally or systemically (Cepko et al., 2014; Jerlhag et al., 2009; Jerlhag et al., 2011). These results were replicated in humans were ghrelin administration induced alcohol craving (Leggio et al., 2014) providing an initial translational evidence.

Orexins:

Orexin-A/Hypocretin-1 (OxA) and Orexin-B/Hypocretin-2 (OxB), also known as hypocretins, derive from a common precursor peptide and both bind to the orexin receptor 1 (OxR1) and orexin receptor 2 (OxR2), though OxR1 has higher affinity for OxA while OxR2 has equal affinity for both ligands (de Lecea et al., 1998; Sakurai et al., 1998). AUD

patients have increased blood OxA concentration during early withdrawal; peptide levels decrease after prolonged abstinence (Bayerlein et al., 2011; Ziolkowski et al., 2016). Studies in rodents showed increased OxR1 and OxR2 expression in brain areas involved in alcohol abuse (Alcaraz-Iborra et al., 2014; Barson et al., 2015). Together these findings indicate that increased orexin/hypocretin transmission may be associated with enhanced alcohol drinking. Consistently, pre-treatment with OxR1 and, in some cases OxR2, antagonists decreased alcohol and motivation for alcohol under progressive ratio contingencies consumption (Anderson et al., 2014; Brown et al., 2013; Jupp et al., 2011; Lawrence et al., 2006; Lei et al., 2016; Moorman et al., 2017; Olney et al., 2017). In addition, OxR1 antagonism reduced reinstatement of alcohol seeking elicited by alcohol-associated environmental stimuli, stress or by intracranial microinjection of neuropeptide S (Cannella et al., 2009; Jupp et al., 2011; Lawrence et al., 2006; Martin-Fardon and Weiss, 2014; Moorman et al., 2017; Richards et al., 2008; Ubaldi et al., 2016b).

Brain Imaging Technologies, a bridge between preclinal and clinical research.

As already discussed, although no animal model reproduces the complexity of alcohol addiction, they offer the possibility to carry out studies under controlled environmental and genetic conditions, and thus they represent a key tool to analyze genetic and environmental components underlying alcoholism-related traits. Moreover, different animal models enable researchers to dissect out specific alcohol-related endophenotypes, such as alcohol preference, dependence, tolerance etc., and to investigate the different stages of the addiction cycle. One of the challenges in the definition of translational phenotypes is to go beyond predictive and face validity, and to relate neurobiological findings in preclinical species with specific aspects of the human syndrome, also called construct validity (McKinney and Bunney, 1969; Newport et al., 2002; Willner, 1984).

Brain imaging techniques have been widely employed to study the functional and structural organization in the human brain in search of endophenotypes that contribute to the development of alcoholism. Neuroimaging approaches enable measuring objectively the neurobiological bases of the phenotype and can be implemented in animal and human studies, thus representing a translational interface bridging clinical and preclinical research.

Among the various brain imaging techniques, Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) have been broadly used to analyze psychiatric disorders such as ethanol abuse and dependence. Importantly, they can be utilized on both humans and laboratory animals.

MRI is a non-invasive imaging technique characterized by superior soft-tissue contrast, absence of ionizing radiation exposure and spatial resolution in the millimeter range (down to tens of micrometers in ultra-high-field, specialized small-animal scanners). Importantly, MRI is a very versatile technique that enables quantitative assessment of brain morphometry, segmentation of different tissue components (grey and white matter), and assessment of white matter structure and integrity through tractography-based techniques. MRI structural and morphometric investigations make it possible to test the hypothesis that the

pathophysiology of alcohol dependence is associated with quantifiable changes in global or regional neuroanatomy. Moreover, brain MRI images can be sensitized to changes in tissue perfusion and blood oxygenation levels, thus enabling non-invasive mapping of brain function, a technique dubbed functional Magnetic Resonance Imaging (fMRI). Functional MRI approaches offer new and powerful tools to study the association of specific symptoms or aspects of alcohol dependence with specific brain circuits. Through the analysis of correlations between spatially remote fMRI signals, functional and effective connectivity can be explored in the brain at rest, or under a variety of conditions or alcohol-disease-related states. fMRI methods can be also applied to investigate the acute or chronic effects of pharmacological treatment, with the aim of identifying objective markers of response to treatment in animals and patients, thus providing a potentially useful translational tool to help progress novel drugs for medication development for alcoholism from the preclinical to the clinical arena.

Positron emission tomography (PET) is a nuclear medicine imaging technique. Using specific radiopharmaceuticals, with PET is possible to analyze *in vivo* blood flow, metabolism, and receptor binding capacity in specific brain areas. Hence, PET is an imaging method characterized by very high sensitivity (it can detect molecules in picoMolar concentration) and excellent specificity. Despite its limited spatial resolution in humans, PET can also be applied to small animals using new high-resolution scanners (μPET) or *ex vivo* binding techniques. A miniature PE tomography has also been constructed; it is sufficiently small to be worn on the head of a fully conscious and mobile rat, thus allowing animals to be scanned without anesthesia that can be a confounding factor (Schulz et al., 2011; Schulz and Vaska, 2011).

MRI studies showed reduced grey matter volume (GMV) in the cerebral cortex (mostly frontal lobes) (Buhler and Mann, 2011; Demirakca et al., 2011; Fein et al., 2002; Rando et al., 2011), limbic areas, and cerebellum (Fein et al., 2006) of alcoholic patients. Such GMV shrinkage plays a clinically significant role in alcoholism, as it is predictive of relapse risk (Rando et al., 2011). More recently, reduced anterior insula and enlarged amygdala were associated with alcoholism (Senatorov et al., 2015). The effects of alcohol dependence on gray and white matter volume were found to affect both male and female patients (Demirakca et al., 2011). In heavy alcohol drinkers, white matter abnormalities and various functional and metabolic deficits were also reported (reviewed by (Buhler and Mann, 2011) (Gazdzinski et al., 2010; Mechtcheriakov et al., 2007; Pfefferbaum et al., 1995). Interestingly, in MRI experiments similar structural and functional alterations were described in alcohol preferring msP rats (Gozzi et al., 2013).

Functional MRI approaches have also been used to analyze spontaneous fluctuations in the brain resting state, i.e. in the absence of specific tasks or stimulation (Beckmann et al., 2005; Damoiseaux et al., 2006). Correlation patterns are thought to reflect the functional connectivity between remote brain areas, and the large-scale functional organization of the brain. Alterations in functional connectivity have been observed in several neuropsychiatric conditions (Greicius, 2008), including AUDs. Independent Component Analysis of resting state functional connectivity in AUD patients has shown overall integrity of the functional connectivity networks in patients, with reduced connectivity in certain networks, including

the default, salience and executive networks (Muller-Oehring et al., 2015). Other studies (Jansen et al., 2015; Zhu et al., 2017; Zhu et al., 2015) found an increased connectivity within prefrontal and fronto-basal networks in AUD patients or young binge drinkers (Correas et al., 2016). Whether these discrepancies reflect methodological differences in the assessment of functional connectivity, or specific traits or states of the patient subgroups enrolled in different studies is unclear.

[18F]FDG-PET studies reported reduced metabolism in frontal-parietal, orbitofrontal cortex and striatal areas in active and abstinent alcohol-abusers (Gilman et al., 1990; Volkow et al., 1992; Volkow et al., 1994; Volkow et al., 1997; Wang et al., 2000).

Using [11C]raclopride as a radiotracer, blunted amphetamine-induced dopamine release was observed in the nucleus accumbens of alcohol dependent subjects compared with healthy subjects (Martinez et al., 2004). In human alcoholics and post-dependent rats were also reported variations from a hypo- to a hyper-dopaminergic state during three weeks of alcohol abstinence suggesting a composite regulation of brain DA mechanisms that depends by time and state (Hirth et al., 2016). Altogether these results suggest that, following an initially exaggerated dopamine response to ethanol, the dopamine tone in the ventral striatum may fluctuate with time, possibly resulting in a blunted response associated with emergence of craving and relapse. Interestingly, a very similar effect was observed in cocaine addicts (Martinez et al., 2007), thus indicating convergent effects of different drugs of abuse on the brain reward system.

The role of the brain stress systems in sustained alcohol use and relapse has been emphasized (George et al., 2008). The involvement of the neurokinine 1 receptor, a mediator of behavioral stress responses, was shown in an fMRI study in abstinent alcoholics exposed to negative and positive stimuli from the International Affective Picture System (IAPS) and pictures of alcoholic or neutral beverages (George et al., 2008). Alcoholics patients displayed excessive brain responses to images correlated with negative situations while the brain responses were reduced when they were confronted with images of positive values. Treatment with LY686017, a potent and selective Nk1R antagonist, reversed this effect in several brain regions. In particular LY686017 reduced the activation of the insula, an area implicated in craving and in the maintenance of addictive behaviors (George et al., 2008).

Recent neuroimaging studies indicated that individuals with high vulnerability to ethanol abuse had reduced cortical and thalamic GMV (Benegal et al., 2007) and an altered sensitivity of the reward circuitry (Acheson et al., 2009; Andrews et al., 2011; Kareken et al., 2010). Interestingly, these two conditions were observed also in alcoholic patients during withdrawal. These preliminary findings suggest that structural and functional abnormalities could be derived from chronic alcohol use but they may also represent innate feature conferring propensity toward ethanol addiction.

The role played by innate brain abnormalities in alcohol abuse vulnerability have been studied in the msP rat, using multimodal neuroimaging modalities (Gozzi et al., 2013). As previously described the msP rat line closely mimics several fundamental aspects of alcoholism in humans, such binge drinking behavior, negative affective states associated

with alcohol use, hypersensitivity to stress, anxiety and depression (Ciccocioppo et al., 2006; Hansson et al., 2006). Interestingly, these animals appear to have an up-regulated CRF1R system, which may determine the poor ability to cope with stress, like human subjects that drink ethanol as an anxiety-relieving strategy.

In accordance with observations in alcoholics during the withdrawal phase and in individuals at high-risk for ethanol abuse, structural and functional MRI in msP rats showed reduced GMV in the thalamus, ventral tegmental area, insular and cingulate cortex. Also, msP rats highlighted significantly reduced basal Cerebral Blood Volume (bCBV), a marker of resting brain function, in various brain areas. These effects were observed in the cingulate cortex, striatum, thalamus, hypothalamus, ventral hippocampus and corpus callosum (Gozzi et al., 2013). Foci of reduced bCBV were present in the central nucleus of the amygdala, shell of NAc and bed nucleus of the stria terminalis (Gozzi et al., 2013). Interestingly, the striatum, the thalamo-cortical circuits and NAc showed a reduced metabolism. Previous studies on alcohol demonstrated that the functional reactivity of these brain areas is altered in task- or cue-based fMRI activation (Kareken et al., 2004; Vollstadt-Klein et al., 2010). These findings show that the brain of alcohol preferring msP rats exhibit abnormalities similar of those observed in alcoholics. The animals in this study were analyzed in an alcohol-naïve state suggesting that some of the morphological and functional features observed in alcoholics might been present before alcohol exposure and be a factor predisposing to AUD. Whether predisposition to alcohol abuse confers vulnerability to addiction to other substances, as suggested by other forms of drug abuse often observed in alcoholics, remains an open question. To this end, a recent study in msP rats applied a combination of behavioral, neurochemistry and functional imaging techniques to study responses to psychostimulants (Bifone et al., 2018). Pharmacological MRI revealed increased functional reactivity to d-amphetamine in the extended amygdala of msPs, associated with increased extracellular levels of dopamine in the shell of NAc, and with increased propensity to escalate drug use. These results suggest neurobiological and neurochemical substrates involved in the transition from drug use to addiction independently from the drug used. Moreover, it suggests that increased functional responses to stimulants in the extended amygdala may predict individual vulnerability to transitioning to addiction.

With the increasing availability of diagnostic tools and the improvement of their mining capacity, it will be possible to acquire several data modalities for each subject. A multimodal analysis including not only different MRI acquisition paradigms or PET, but also genetic and behavioural data, would allow a comprehensive characterization of the subject, improving the strength of diagnosis and evaluation of treatment outcomes respect to each single modality taken on its own. This approach was recently tested in a longitudinal preclinical MRI study. MsP rats were subjected to voluntary alcohol drinking and then treated with naltrexone during abstinence. Multimodal MRI acquisition were repeated at naïve, intoxicated, and after naltrexone treatment stages. Data showed that it is possible to differentiate multimodal fingerprints of naïve and intoxicated stages and that naltrexone treatment reverted the fingerprint of intoxicated rats back toward the naïve stage (Cosa et al., 2017). This seminal work indicate that a multimodal approach could help detect biomarkers characterizing the transition into an addictive state that could also be integrated in the evaluation of treatment outcome and be helpful in the development of novel drugs.

Conclusions and Remarks

Alcohol abuse and dependence are serious disabling conditions that have a dramatic impact on our society and public health. Neurobiological processes involved in the development of AUD are the result of complex interactions between genetic and environmental determinants. One of the major results in drug development programs for AUD was the approval of naltrexone for clinical use. This opioid antagonist is now considered one of the most important pharmacotherapeutic options in the clinical care of alcoholics. Over the years, in addition to naltrexone, other molecules, such as acamprosate and nalmefene have been approved. Other molecules like gabapentin, baclofen and varenicline, now in the clinic for a different use, are under investigation for future authorization in AUD. Additional compounds targeting a number of other mechanisms (i.e., nociceptin, orexin, ghrelin, dopamine, etc.) are at an earlier stage of development. For some of these compounds preclinical findings are strikingly encouraging. Thus, the hope is to have the possibility to test them in AUD patients soon. Of note the mechanisms used by classical drugs like nalmefene and naltrexone consist of attenuating the rewarding and the reinforcing effect of alcohol. To some extent similar mechanism is used by disulfiram that act as a deterrent by blocking alcohol metabolism. Consistently blockade of orexin, ghrelin or stimulation/ blockade of nociceptin receptor may also lead to reduction of alcohol reward. On the contrary molecules like varenicline and baclofen may partially substitute for alcohol. The successful development of effective medications in AUD provides a proof-of-concept for the feasibility of treating alcoholism with pharmacological agents. On the other hand, the efficacy of currently available medications is not satisfactory and successful development of more efficacious and safer drugs is highly desirable. To enhance the possibility of successfully moving new drugs from bench to bedside it is crucial to establish appropriate strategies of drug development. Learning from negative experiences like the one made with CRF1R antagonists is also of fundamental importance; preclinical data, although promising, does not always predict the clinical efficacy of a new treatment. As highlighted above, several factors may account for this lack of success. Availability of validated preclinical models and the correct use of human laboratory paradigms with appropriate markers able to predict clinical efficacy are two important conditions to reduce the risk of failure. Whereas systematic use of brain imaging methods can help to interpret findings in preclinical models and to translate them to the human level.

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Table 1:

New drug Targets of potential interest in AUD.

Target	Drug	Drug Activity	Key Finding	Reference
PPARγ	• Pioglitazone	Agonism	• Reduce SA • Reduce stress-induced reinstatement	(Stopponi et al., 2011)
PPARα	• Fenofibrate • Tesaglitazar • Bezafibrate	Agonism	• Reduce SA	(Blednov et al., 2015)
PDE4	• Rolipram	Inhibition	• Reduce SA	(Wen et al., 2012)
PDE10	• TP-10	Inhibition	• Reduce SA	(Logrip et al., 2014)
CRF1R	• CRA1000 • MTIP • MPZP • LWH-63 • Antalarmin • KO mice • D-Phe-CRF(12–41)	Antagonism	• Reduce SA • Reduce withdrawal	(Chu et al., 2007; Funk et al., 2007; Gehlert et al., 2007; Gilpin et al., 2008; Knapp et al., 2004; Richardson et al., 2008; Sabino et al., 2006; Sommer et al., 2008)
NOP	• N/OFQ • Ro 64–6198 • LY2940094 • KO rats	Agonism Antagonism	• Reduce CPP • Reduce SA • Reduce withdrawal	(Ciccocioppo et al., 2004; Ciccocioppo et al., 1999; Economidou et al., 2011; Kallupi et al., 2017; Kuzmin et al., 2003; Martin-Fardon et al., 2000; Rorick-Kehn et al., 2016)
OX1	• SB-334867		• Reduce cue- and stress-induced reinstatement	(Lawrence et al., 2006; Richards et al., 2008)
NPSR	• NPS	Agonism	• Increase cue-induced reinstatement • Reduce SA in alcohol-preferring rats	(Cannella et al., 2009; Cannella et al., 2016; Ruggeri et al., 2010)
NK1R	• LY686017 • L822429 • KO mice	Antagonism	• Reduce SA • Reduce CPP • Reduce stress-induced reinstatement	(Ayanwuyi et al., 2015; George et al., 2008; Schank et al., 2011; Thorsell et al., 2010)
mAChR subtype 5	• ML375	NAM	• Reduce SA • Reduce cue-induced reinstatement	(Berizzi et al., 2018)

SA= Self Administration; CPP=Conditioned Place Preference; NAM= Negative Allosteric Modulator.

Table 2:

Medications approved (*) or under clinical investigation for AUD.

Drug	Molecular Target	Efficacy in AUD	Key References	Approved
Topiramate	• Kainate/AMPA receptors • $GABA_A$ • Carbonic anhydrase • Sodium and calcium channels	• Discontinue drinking • Prevent relapse	(Johnson et al., 2003; Johnson et al., 2007)	
Gabapentin	• Calcium channels • $GABA_B$	• Increase abstinence • Reduce drinking	(Mason et al., 2014; Myrick et al., 2009)	
*Nalmefene	• Opioid Receptors	• Reduce drinking	(Gual et al., 2013; Mann et al., 2013; Mason et al., 1994; Mason et al., 1999)	EMA
*Naltrexone	• Opioid Receptors	• Increase abstinence • Reduce drinking	(Bouza et al., 2004; O'Malley et al., 1992; Volpicelli et al., 1992b)	FDA and EMA
GHB (Gamma Hydroxy Butyrate also know as Sodium Oxybate)	• $GABA_a$ • $GABA_B$	• Reduce craving • Increase abstinence	(Addolorato et al., 1996)	Italy and Autria
*Baclofen	• $GABA_B$	• Reduce drinking • Increase abstinence	(Addolorato et al., 2002; Muller et al., 2015)	France
Ondansetron	• $5\text{-}HT_3$	• Early onset AUD	(Johnson et al., 2002; Johnson et al., 2000)	
Varenicline	• Acetylcholine Receptors	• Reduce drinking	(Litten et al., 2013; McKee et al., 2009)	
Zonisamide	• Sodium channels	• Reduce drinking	(Arias et al., 2010; Knapp et al., 2015)	
*Acamprosate	• mGluR5? • Ca^{++}?	• Increase abstinence	(Paille et al., 1995; Sass et al., 1996)	FDA and EMA
*Disulfiram	• Aldehyde dehydrogenase	• Reduce drinking	(Suh et al., 2006)	FDA and EMA