HHS Public Access

Author manuscript

Neuropharmacology. Author manuscript; available in PMC 2022 July 07.

Published in final edited form as:

Neuropharmacology. 2021 March 15; 186: 108446. doi:10.1016/j.neuropharm.2020.108446.

Genetically selected alcohol-preferring msP rats to study alcohol use disorder: Anything lost in translation?

Anna Maria Borruto[a], **Serena Stopponi**[a], **Hongwu Li**[b], **Friedbert Weiss**[c], **Marisa Roberto**[d], **Roberto Ciccocioppo**[a,*]

[a]School of Pharmacy, Pharmacology Unit, University of Camerino, Camerino, Italy

[b]College of Chemical Engineering, Changchun University of Technology, Changchun, China

[c]Department of Neuroscience, The Scripps Research Institute, La Jolla, CA, USA

[d]Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA, 92037, USA

Abstract

For several decades, genetically selected alcohol-preferring rats have been successfully used to mimic and study alcohol use disorders (AUD). These rat lines have been instrumental in advancing our understanding of the neurobiology of alcoholism and enabling pharmacological studies to evaluate drug efficacy on alcohol drinking and relapse. Moreover, the results of these studies have identified genetic variables that are linked to AUD vulnerability. This is an up-to-date review that focuses on genetically selected Marchigian Sardinian alcohol-preferring (msP) rats. To support the translational relevance of the findings that are obtained from msP rats and highlight important similarities to AUD patients, we also discuss the results of recent brain imaging studies. Finally, to demonstrate the importance of studying sex differences in animal models of AUD, we present original data that highlight behavioral differences in the response to alcohol in male and female rats. Female msP rats exhibited higher alcohol consumption compared with males. Furthermore, msP rats of both sexes exhibit higher anxiety- and depressive-like behaviors in the elevated plus maze and forced swim test, respectively, compared with unselected Wistar controls. Notably, voluntary alcohol drinking decreases foot-shock stress and depressive-like behavior in both sexes, whereas anxiety-like behavior in the elevated plus maze is attenuated only in males. These findings suggest that male and female msP rats both drink high amounts of alcohol to self-medicate negative affective symptoms. For females, this behavior may be driven by an attempt to treat stress and depressive-like conditions. For males, generalized anxiety appears to be an important additional factor in the motivation to drink alcohol.

*Corresponding author. School of Pharmacy, Pharmacology Unit, University of Camerino, Via Madonna delle Carceri, 9 62032, Camerino, MC), Italy. roberto.ciccocioppo@unicam.it (R. Ciccocioppo).

CRediT authorship contribution statement

Anna Maria Borruto: Writing: Original draft preparation, Writing: Reviewing and editing, Investigation, Formal Analysis. Serena Stopponi: Writing: Reviewing and editing. Hongwu Li, investigation, Friedbert Weiss, Marisa Roberto, Roberto Ciccocioppo: Conceptualization, Funding acquisition, Supervision, Writing: Reviewing and editing.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2020.108446.

Keywords

Alcoholism; Sex differences; Genetic selection; Animal models; Relapse; Self-administration; fMRI; Brain imaging

1. Introduction

According to a recent report by the World Health Organization (WHO), alcohol is one of the most commonly abused psychotropic drugs in the world, second only to tobacco, leading to more than 3 million deaths as a result of its harmful use. At the global level, an estimated 237 million men and 46 million women suffer from alcohol use disorder (AUD), with the highest prevalence among men and women in Europe (14.8% and 3.5%, respectively) and the United States (11.5% and 5.1%, respectively). The *Diagnostic and Statistical Manual of Mental Disorders*, 5th edition (DSM-5), describes AUD as a chronic relapsing disorder with substantial heritability (American Psychiatric Association, 2013). It is the most prevalent mental disorder at the global level (Disney et al., 1999; Slutske et al., 1999). The chronicity and relapsing nature of AUD is caused by maladaptive changes in the brain that occur in response to prolonged exposure to alcohol (Costin and Miles, 2014). Furthermore, AUD is associated with many physical (e.g., liver and heart disease) and psychiatric (e.g., anxiety and depression) comorbidities, the loss of productivity, and impairments in interpersonal functioning (Grant et al., 2015; Li et al., 2004).

Alcohol use disorder is also characterized by individual variability. Not all people are equally vulnerable to the disease. Various factors, including the age at which people start drinking, comorbid psychiatric conditions, and mental, social, and cultural status, can contribute to the development and progression of AUD. Nevertheless, family history studies have shown that genetic factors are key elements in shaping the vulnerability to AUD.

Well-designed human and animal studies have clearly shown that individuals may be genetically predisposed to AUD, although this does not exclude the importance of environmental factors (Bierut et al., 2002; Edenberg, 2002; Gianoulakis and de Waele, 1994). Genetic predisposition is estimated to contribute to approximately 50–60% of the vulnerability to AUD (Costin and Miles, 2014; Goodwin et al., 1974; Prescott and Kendler, 1999), as shown by adoption studies (Bakhireva et al., 2018; Bohman et al., 1981; Cloninger et al., 1981; Goodwin et al., 1977; Waaktaar et al., 2018) and twin-pair studies (Heath et al., 1997; Kendler et al., 1992, 1995). The multigenic nature of AUD hampers the identification of specific genes that confer either vulnerability or resilience to the disease, thus limiting successes in this field of study (Costin and Miles, 2014). Because of the complexity of AUD, preclinical research has encountered significant difficulties in successfully mimicking all characteristics of the disease in laboratory animals. However, the general consensus is that there are minimal criteria, such as predictive, face, and construct validity, that must be met to consider an animal model valid (for details, see Ciccocioppo (2013)).

Despite these difficulties, extensive work over the past few decades has been performed to develop new preclinical procedures and animal models that are able to mimic the human condition. One of the major contributions in this field has come from studies of

genetically selected alcohol-preferring rats. These models allow studies of genetic factors that are associated with excessive alcohol drinking, links to specific phenotypes, and the impact of the environment on disease progression. Furthermore, the establishment of *in vivo* brain imaging techniques and new experimental paradigms that promote excessive alcohol drinking and intoxication in rodents have further helped bridge the gap between preclinical and clinical research. Unfortunately, despite these advances, female rodents have been relatively understudied. Sex differences in the response to alcohol are a neglected area of research that deserve particular attention.

The present review provides an update on different rat lines that have been genetically selected for high alcohol preference, with a particular focus on Marchigian Sardinian alcohol-preferring (msP) rats. We describe major advances in the field that have been achieved through the use of this rat line. We also present original data that underscore the importance of studying sex differences in AUD. Furthermore, we provide a summary of *in vivo* brain imaging data that demonstrate the importance of experimental approaches to improve the translational impact of preclinical research. Lastly, we critically discuss the translational validity of genetically selected alcohol-preferring rat lines as model to fully mimic the human AUD conditions. In particular we analyze the case of corticotropin releasing factor one (CRF1) receptor antagonists that in several animal models, including the msP rat, demonstrated significant efficacy, but then failed in the clinic.

2. Historical overview of genetically selected alcohol-preferring rats

Genetically selected alcohol-preferring rat lines were originally developed to gain insights into genetic factors that affect voluntary alcohol intake and preference. Through genetic selection and selective breeding, animals with high alcohol preference and animals with low alcohol preference have been generated. As a result of this work, at least six different rat lines have been generated around the world.

In 1951, the first selective breeding program was initiated at Universidad de Chile (UCh), where high alcohol-drinking (UChB) and low alcohol-drinking (UChA) lines were developed (Mardones et al., 1953; Mardones and Segovia-Riquelme, 1984; Quintanilla et al., 2006). Eriksson and colleagues later initiated another selective breeding program at Alko Research Laboratories in Finland, where the Alko Alcohol high alcohol-preferring (AA) and Alko Non-Alcohol low alcohol-preferring (ANA) rat lines were generated (Eriksson, 1968). After the success of these two initiatives, additional selective breeding programs were initiated in several laboratories. These programs resulted in the development of alcohol-preferring (P) and non-preferring (NP) rat lines at Indiana University/Purdue University Indianapolis (Indianapolis, IN, USA; Li et al., 1979) and Sardinian alcohol-preferring (sP) and non-preferring (sNP) rats in Cagliari, Italy (Ciccocioppo et al., 2006; Colombo et al., 2006; Li et al., 1979). Years later, starting from selectively bred rats from the N/NIH founder stock (a cross of eight inbred rat strains with varying levels of alcohol intake) in Indianapolis, the same research team launched a new bidirectional breeding program that resulted in the generation of High Alcohol Drinking (HAD) and Low Alcohol Drinking (LAD) rat lines (Hansen and Spuhler, 1984; Murphy et al., 2002).

Over the years, some of these rat lines have been transferred to other laboratories, from which additional genetically selected alcohol-preferring rat lines were derived. For example, using P rats as founders, an inbred alcohol-preferring (iP) rat line was developed in Indianapolis. This new iP line was made available to colleagues at the Howard Florey Institute (University of Melbourne, Australia) that are now maintaining it. The iP line conserved the high alcohol-drinking phenotype of the founder stock. Over the years, it has been used in several genetic and pharmacological studies (Carr et al., 2007; Ciccocioppo et al., 2006; Cowen et al., 2005; Kimpel et al., 2007; Rodd et al., 2007).

In Italy, a few pairs of sP rats have been transferred from the University of Cagliari to the University of Camerino. Starting from this founder stock, a new line of alcohol-preferring rats was generated, namely Marchigian Sardinian alcohol-preferring (msP) rats (for details, see (Ciccocioppo et al., 2006; Ciccocioppo et al., 1999a,b,c). We have used this rat line in an extensive program to characterize these rats genetically, behaviorally, and pharmacologically. Moreover, in an attempt to obtain translationally meaningful information, msP rats have been used in various functional magnetic resonance imaging (fMRI) studies, some of which also involve cohorts of AUD patients (De Santis et al., 2019, 2020). Unfortunately, as for the other genetically selected rat lines, females msP rats have been rarely used in experiments. Thus, what we know about this rat line comes mostly from males. To start filling this gap, this review reports original data that show different behavioral responses to alcohol in male and female msP rats.

3. Genetically selected Marchigian Sardinian alcohol-preferring rats: a rodent model to study the neurobiology of alcohol use disorder

MsP rats were originally derived from Wistar rats. They have been selectively bred for high alcohol preference and consumption at the University of Camerino (Marche, Italy) for more than 80 generations, beginning from the 13th generation of sP rats that were originally developed at the University of Cagliari (Sardinia, Italy; (Colombo et al., 2006). The first publication on msP rats appeared in 1991 (Ciccocioppo et al., 2006). Since then, more than 80 studies have explored behavior, genetics, neurobiology, and responses to pharmacological manipulations in this rat line (see the key studies in Table 1).

3.1. Genetics of msP rats

Over the years, several studies have investigated genetic factors that are responsible for the high alcohol drinking phenotype in msP rats. For example, an extensive genetic mapping of the msP line was performed using microarrays and gene sequencing (Ciccocioppo, 2013; Ciccocioppo et al., 2006; Hansson et al., 2006, 2007). The results of these studies showed that several genes that encode aldehyde dehydrogenase (ADH) isoforms are altered in msP rats compared with unselected Wistar controls (Ciccocioppo et al., 2006). The *Aldh2* gene was downregulated, and *Aldh1a1*, *Aldh1a4*, *Aldh3a2*, and *Aldh5a1* were upregulated. Furthermore, the msP line exhibited overactivity of the corticotropin-releasing factor (CRF) system in different brain regions, driven by two single-nucleotide polymorphisms (SNPs) at CRF_1 receptor locus (Ayanwuyi et al., 2013; Cippitelli et al., 2015; Hansson et al., 2006; Logrip et al., 2018). As a consequence of this overexpression, msP rats exhibited a

high anxious-like phenotype, were sensitive to stress, and had depression-like symptoms that were all improved by alcohol drinking (Ciccocioppo, 2013; Ciccocioppo et al., 2006). In msP rats, a few days of voluntary alcohol drinking attenuated the overexpression of CRF_1 receptors in various brain areas, suggesting the possibility that these animals drink to alleviate negative symptoms that are associated with an overactive stress system (Hansson et al., 2007). Pharmacological studies provided direct support for this hypothesis. In fact, the blockade of CRF_1 receptors by antalarmin reduced alcohol self-administration in msP rats but not in unselected Wistar controls (Hansson et al., 2006). The same phenomenon was reported following treatment with nociceptin receptor (NOP) agonists, which acted as functional CRF_1 receptor antagonists in reduced drinking in msP rats but not in Wistar controls (Economidou et al., 2008; Ubaldi et al., 2016; Witkin et al., 2014). Interestingly, Wistar rats that were chronically exposed to intoxicating doses of alcohol through intermittent vapor exposure exhibited neuroadaptive changes in the CRF system that resembled its innate dysregulation in msP rats. At the behavioral level, this genetic trait was associated with higher anxiety-like behavior, greater sensitivity to stress, and greater sensitivity to CRF_1 receptor antagonists (Ciccocioppo et al., 2009; Gehlert et al., 2007; Herman et al., 2016; Kirson et al., 2018; Natividad et al., 2017; Sommer et al., 2008). At the cellular level, the dysregulation of γ-aminobutyric acid (GABA)ergic and glutamatergic synapses in the central nucleus of the amygdala (CeA) and greater sensitivity to CRF/CRF_1 and cannabinoid CB_1 receptor compounds was reported in msP rats compared with Wistar controls, supporting the role of these systems in the anxiety-like and excessive drinking phenotypes (Herman et al., 2016; Kirson et al., 2018; Natividad et al., 2017). Notably, CRF_1 receptor gene polymorphisms have also been identified in humans. Importantly, these polymorphisms appear to be linked to AUD (Quadros et al., 2016). Indeed, they correlate with the lifetime prevalence of drunkenness, high levels of alcohol drinking, and binge drinking episodes (Chen et al., 2010; Treutlein et al., 2006). Altogether, these findings indicate that humans and msP rats, at least to some extent, share common genetic factors that predispose them to AUD.

Differences between msP and Wistar rats have been detected at the level of expression of genes that are linked to glutamatergic and GABAergic transmission (Ciccocioppo et al., 2006). For example, alterations of the gene that encodes the metabotropic glutamate receptor (*Grm3*) and the solute carrier family 6 (*Slc6a1*) gene that encodes the GBA transporter have been linked to alcohol sensitivity in mice (Hu et al., 2004).

Other genes of interest that were found to be differentially expressed in msP rats encode the opioid receptor μ1 (*Oprm1*) gene, opioid receptor-like (*Oprl-1*; referred to as the NOP receptor) gene, and neuropeptide Y receptor 5 gene, which have all been linked to alcohol abuse (Ciccocioppo et al., 2000, 2006; Heilig and Thorsell, 2002; Schroeder et al., 2005; Thorsell et al., 1999). Specifically, msP rats exhibited higher expression of nociceptin/orphanin FQ (N/OFQ) and NOP receptor mRNA in numerous brain regions compared with their Wistar counterparts, accompanied by a significant increase in NOP receptor binding in the CeA, the bed nucleus of the stria terminalis (BNST), the ventral tegmental area (VTA), and several cortical structures (Economidou et al., 2008). Notably, Wistar rats that were exposed to chronic alcohol exhibited neuroadaptive changes in the N/OFQ-NOP system that resembled innate dysregulation that is detected in msP rats. These observations indicate

an association between innate upregulation of the N/OFQ-NOP system and high alcohol preference. Supporting this possibility, NOP receptor blockade with selective antagonists markedly reduced alcohol intake in msP rats (Borruto et al., 2020). This effect, however, also extended to unselected lines of rats and mice (Brunori et al., 2019; Rorick-Kehn et al., 2016). In summary, consistent with the complex polygenic nature of AUD, genetic data indicate that the selection of msP rats has been accompanied by alterations of several genes that affect the pharmacokinetic and pharmacodynamic properties of alcohol.

3.2. Behavioral characterization of msP rats

If exposed to a standard home-cage two-bottle choice (2BC; 10%, v/v, alcohol *vs.* water) regimen with continuous access for 24 h/day, msP rats drink approximately 7–8 g/kg of alcohol daily (Ciccocioppo et al., 1999, 2006). Drinking occurs mostly during the dark phase of the light/dark cycle, during which they consume around 80% of their daily alcohol. Drinking is organized into bouts. The largest bout occurs within the first hour of the dark phase. The second large bout occurs in the middle of the dark phase, and a third bout usually occurs immediately before the new light phase begins. These drinking bouts produce blood alcohol levels (BALs) around 70–80 mg/dl but can peak over 100 mg/dl to produce pharmacologically meaningful effects (Ciccocioppo et al., 2006). If subjected to operant alcohol self-administration, msP rats exhibit robust lever-responding for alcohol that is acquired spontaneously and much faster than unselected Wistar controls (Ayanwuyi et al., 2013; Cannella et al., 2016; Domi et al., 2019). Moreover, compared with Wistar rats, if subjected to a progressive-ratio (PR) schedule of reinforcement, msP rats reach a significantly higher breakpoint for alcohol, suggesting stronger motivation for alcohol (Ciccocioppo et al., 2006; Domi et al., 2019). In msP rats, the intragastric (IG) administration of 0.7–1.5 g/kg alcohol produces the expression of significant conditioned place preference (CPP; (Ciccocioppo et al., 1999a,b,c). In Wistar rats, the administration of these doses of alcohol leads to conditioned aversive responses (Fidler et al., 2004).

In addition to a high alcohol drinking phenotype compared with Wistar controls, msP rats have a higher propensity to relapse when exposed to stimuli that predict alcohol availability or in response to stress (Ayanwuyi et al., 2013; Cannella et al., 2016; Ciccocioppo, 2013; Ciccocioppo et al., 2004, 2014; Cippitelli et al., 2008; Fotio et al., 2020; Stopponi et al., 2013).

Environmental contexts, such as alcohol-paired cues, are well known to be determining factors that can trigger relapse in alcoholic patients (Cooney et al., 1997; Martin-Fardon and Weiss, 2013). Likewise, msP rats that are trained to self-administer 10% alcohol in the presence of discriminative cues, following a withdrawal period during which lever pressing is extinguished, resume a marked level of responding when re-exposed to alcohol cues in the absence of the primary reinforcer (Ciccocioppo, 2013; Ciccocioppo et al., 2006). Similar results have also been reported in heterogeneous Wistar rats (Augier et al., 2016; Bachteler et al., 2005). However, the magnitude and persistence of the reinstating effect of alcohol-associated cues is much higher in msP rats than in heterogeneous Wistar rats (Ciccocioppo et al., 2006). A similar phenomenon has also been shown in other lines of

genetically selected alcohol-preferring rats, such as P and sP rats (Ayanwuyi et al., 2013; Ciccocioppo, 2013; Koob et al., 2005; Vengeliene et al., 2003).

The msP rat line is also characterized by high sensitivity to stress, which may contribute to their excessive alcohol drinking phenotype (Hansson et al., 2006). For example, in a self-administration study with an implemented extinction-reinstatement paradigm, stress that was induced by intermittent footshock reinstated the alcohol-paired response in both msP and Wistar rats (Hansson et al., 2006). However, msP rats had the highest level of reinstatement after a 0.3 mA electric shock, whereas the same response in Wistar rats was achieved at a current intensity between 0.6 and 1.0 mA. At this current intensity, msP rats exhibited marked freezing behavior, thus demonstrating higher sensitivity to footshock stress compared with their Wistar counterparts. The high stress sensitivity in msP rats was also revealed in other behavioral paradigms, including the forced swimming test, elevated plus maze, open field test under unfamiliar conditions, and the defensive and marble burying tests (Ayanwuyi et al., 2013; Cippitelli et al., 2015; Natividad et al., 2017; Stopponi et al., 2018). Biochemical and electro-physiological data indicate that the hyper-anxious, high stress-sensitivity phenotype of msP rats depends on the dysregulation of endocannabinoid signaling in the CeA that is triggered by their innate hyperactivity of the CRF/CRF_1 receptor system (Natividad et al., 2017; Stopponi et al., 2018). Notably, anxiety- and depressive-like symptoms and relatively poor stress coping ability in msP rats are attenuated by voluntary alcohol consumption and repeated intragastric alcohol administration (Ciccocioppo et al., 1999a,b,c; Domi et al., 2019). Altogether, these findings reflect the results of several clinical studies that showed that a large subpopulation of alcoholic patients is characterized by a low ability to engage in adequate stress-coping responses, and alcohol is consumed to ameliorate negative affective symptoms that are associated with anxiety and depression (Koob and Le Moal, 2005).

Following abstinence episodes, AUD patients usually report a greater urge to drink that normally terminates in relapse, followed by severe alcohol intoxication episodes (Boening et al., 2001; McBride et al., 2002; Vengeliene et al., 2005). Resembling humans, rodents that are trained to chronically drink alcohol and subjected to periods of forced abstinence consume higher amounts of alcohol when re-exposed to it. This phenomenon is known as the alcohol deprivation effect (ADE), which has been described in both genetically selected alcohol-preferring rats and in unselected heterogeneous rats. Specifically, msP rats that were exposed to chronic alcohol exhibited a robust ADE when they were returned to alcohol after a forced abstinent period of 10 days (Perfumi et al., 2005). However, similar to unselected rodent lines, the increase in drinking in msP rats is transient and usually returns to baseline levels after a couple of days (Holter and Spanagel, 1999; Vengeliene et al., 2014). Genetically selected alcohol-preferring rats do not appear to differ from unselected heterogeneous stock rats in this regard.

One of the major criticisms of using alcohol-preferring rats, and animal models of AUD in general, is that they only minimally incorporate the diagnostic criteria of the DSM-IV and DSM-5, which are mostly based on interviews and self-report questionnaires that assess the quantity and frequency of drinking and perceived consequences. Another common critique of these models is that only a small proportion of human alcohol users develop

AUD. Laboratory animal experiments, including those that utilize genetically selected rats, are usually performed on the entire subject population without considering individual variability. In an attempt to address these important critiques, we recently developed a model to explore interindividual differences in the propensity to shift from controlled to compulsive alcohol intake between msP rats and unselected Wistar controls. This model was originally developed by Deroche-Gamonet and Piazza for cocaine and has been used for psychostimulants in different laboratories (Belin et al., 2011; Deroche-Gamonet and Piazza, 2004; Spanagel, 2017). This model was named the "0/3crit model of addiction," based on the DSM-IV diagnostic criteria for addiction (American Psychiatric Association, 2000). It consists of a multidimensional experimental approach that seeks to identify subpopulations of rats that possess vulnerability (3crit) and resilience (0crit) to drug addiction-like behaviors by measuring three traits: (1) inability to refrain from drug seeking, (2) high motivation for the drug, and (3) maintenance of drug use despite negative consequences. By comparing msP and unselected Wistar rats, we adapted this experimental model to characterize their alcohol-addiction phenotype based on the 0/3 crit model (Domi et al., 2019). The results showed significant interindividual variability among both msP and Wistar rats. Only a subset of subjects (~13%) were positive for all three AUD criteria that were tested. Interestingly, the number of msP rats that could be classified as 3crit was three-times higher than Wistar rats (9.5% *vs.* 3.17%). Conversely, the 0crit group was enriched of Wistar rats. These findings are consistent with human data that show that only a proportion of subjects with a chronic alcohol drinking habit actually develop AUD, with genetic factors accounting for approximately 50% of this progression (Wagner and Anthony, 2002). A secondary finding of the study was that the amount of alcohol that was consumed positively correlated with the expression of anxiety-like behavior in msP rats but not in Wistar rats. This observation further supports the hypothesis that this alcohol-preferring rat line resembles a specific subgroup of AUD patients, in which drinking is motivated by tension-relief purposes. Future studies should assess the predictive validity of this model in pharmacological studies. The development of this model may represent an important advancement in the field because one of the most frequently debated issues in the alcohol research community is whether existing preclinical models effectively mimic AUD patients.

3.3. Sex-related behavioral differences in msP rats

Studies of sex differences in substance use disorders are a largely neglected area of research that is receiving growing attention (Becker and Chartoff, 2019; Becker et al., 2017; Perry et al., 2013). Historical data indicate that the rate of AUD is greater in men than in women. However, this gap is progressively reducing (White et al., 2015). In recent years, the rate of AUD in women has increased by 84%, relative to a 35% increase in men (Grant et al., 2017). Much evidence indicates significant sex differences in the reasons for initiating alcohol use and for the trajectory of AUD (Peltier et al., 2019; Schulte et al., 2009). More frequently than men, women initiate alcohol consumption as a coping strategy to attenuate negative affective states (e.g., anxiety, depression, stress, and feelings of isolation). In men, drinking is often initiated for social reasons, especially in young people who are trying to be accepted by groups (Buchmann et al., 2010; Buckner et al., 2006; Crutzen et al., 2013; Oscar-Berman et al., 2014; Peltier et al., 2019).

Sex differences in the trajectory of AUD have also been documented in humans and to some extent have been replicated in laboratory animals. For example, women have a common tendency to experience a shift from recreational alcohol use to compulsive drinking more rapidly than men. Female rats are more prone to escalate alcohol consumption than males (Becker et al., 2012, 2017; Perry et al., 2013). Moreover, stress plays an important role in all phases of AUD, but its consequences are more pronounced in females than in males. Women are more likely to relapse in response to stressful events (Greenfield et al., 2007; Hudson and Stamp, 2011; Hyman et al., 2008; Sinha et al., 2006; Walitzer and Dearing, 2006). Moreover, females escalate their drug use more rapidly than males (Anglin et al., 1987; Becker et al., 2017; Bobzean et al., 2014). After a prolonged period of alcohol consumption, women have a higher risk of developing physical pathologies, such as breast cancer, cardiovascular problems, and liver inflammation, than men (Ashley et al., 1977; Smith-Warner et al., 1998; Urbano-Marquez et al., 1995).

Clinical research on AUD currently lacks a sufficient number of gender-related studies, but even more problematic is the situation in preclinical science. In fact, especially in rodents, most studies have been performed only in males. This has resulted in the generation of incomplete data to guide clinical trials (Landis et al., 2012; Zucker and Beery, 2010).

A few years ago, NIH issued a new series of guidelines to emphasize the need to study both males and females also at the preclinical level (Clayton and Collins, 2014; Collins and Tabak, 2014; Fattore and Melis, 2016b). In response to these recommendations, preclinical researchers in the addiction field made substantial efforts to include both sexes in their investigations (for review, see (Becker and Koob, 2016; Fattore and Melis, 2016a). However, the gap of knowledge in studies of sex differences remains large, and the research community should further commit to fill it (Lee, 2018; Sanchis-Segura and Becker, 2016). For this reason, in our laboratory, we recently began a research program to investigate sex differences in response to alcohol in msP rats and heterogeneous Wistar controls and evaluate alcohol-related behaviors in both males and females. Below we report the results of a series of experiments in which adult males and females of both strains were compared for voluntary alcohol drinking and the effects of alcohol on anxiety- and depressive-like responses (for details see Supplementary Materials).

The first series of experiments used the 2BC procedure (choice between water and 10% alcohol). Singly housed male and female msP and Wistar rats were given continuous (24 h/day) access to 10% alcohol and water under free-choice condition. The first phase of the experiment consisted of an acclimation period that continued until baseline drinking was stable. Parallel groups with access to water only were used as controls. At this point, we monitored voluntary fluid consumption at 2, 8, and 24 h by recording the volume of intake from graduated cylinders as previously described (Borruto et al., 2020). Under baseline condition, the level of drinking in Wistar rats was very low, and we could not distinguish any difference between males and females. Conversely, msP rats consumed a high volume of alcohol, and female msP rats took significantly higher amounts of alcohol compared with male msP rats (Fig. 1A–C), whereas preference for alcohol was very high and did not differ between sexes (Fig. 1D). Overall, these findings are consistent with the results of studies in other rat strains, in which higher alcohol consumption was reported in females than in males

(Cailhol and Mormede, 2001; Li et al., 2019); for review, see (Becker and Koob, 2016; Hilderbrand and Lasek, 2018).

In earlier experiments, compared with Wistar rats, we repeatedly observed that msP rats exhibited higher levels of anxiety- and depressive-like symptoms that were attenuated by alcohol drinking. These behaviors have never been systematically explored in female msP rats. Here, we report data from the elevated plus maze, forced swim test and footshock stress response in male and female msP and Wistar rats following exposure to 2BC 10% alcohol drinking or water only. The results showed that both naive male and female msP rats exhibited higher anxiety-like behavior compared with Wistar rats (Fig. 2A and B). However, alcohol drinking reduced anxiety-like behavior in males (Fig. 2A) but not in females (Fig 2B) in the elevated plus maze test. When the rats were tested in the forced swim test, we found that both male and female msP rats exhibited significantly longer immobility time (a measure of depression) compared with their Wistar counterparts. Alcohol drinking reduced immobility time in msP rats of both sexes (Fig. 3A and B). Lastly, msP rats of both sexes showed higher freezing in response to foot-shock stress compared to unselected Wistar rats. Alcohol consumption attenuated the freezing time both in male and female msP rats (Fig. 4A and B). These latter results expand our earlier findings in male msP rats showing their higher sensitivity to foot-shock stress induced freezing compared to Wistar controls (Cippitelli et al., 2015; Hansson et al., 2006). Most important, as previously demonstrated, these differences are not due to a different pain/sensitivity threshold of these two rat lines (Cippitelli et al., 2015; Hansson et al., 2006). Based on these findings, we hypothesize that male and female msP rats are both characterized by traits that confer negative mood conditions that co-segregated with alcohol drinking during genetic selection. However, the motivation for alcohol in males is probably linked to its ability to attenuate anxiety. In female rats, alcohol drinking appears to be linked to its antidepressant (and possibly anti-stress) properties. Although speculative, this hypothesis is consistent with human data that show that psychiatric comorbidity is different between male and female AUD patients. For example, women alcohol abusers are more likely than men who abuse substances to be diagnosed with post-traumatic stress disorder (Cottier et al., 1992; Kessler et al., 1995). Numerous studies have shown an association between AUD in women and a history of child maltreatment (Anda et al., 2002; Dinwiddie et al., 2000; Fergusson et al., 1996; Wilsnack et al., 1997) and physical and sexual assault in adulthood (Kilpatrick et al., 1997, 2003). Further studies are needed to better characterize the impact of sex differences on alcohol abuse-related behaviors of msP rats. On the other hand, these preliminary findings corroborate the observation that alcohol-related sex differences present in AUD patient can be detected also in msP rats, which support the translational value of this type of investigation.

3.4. Limits and caveats in msP rats

Genetically selected alcohol-preferring rats have been successfully used to explore various aspects of AUD that are impossible to study in humans or unselected rodent lines. On the other hand, alcoholism is a heterogeneous disorder to which several genetic, environmental and personality factors can contribute (Cloninger, 1987; Cloninger et al., 1981; Heilig et al., 2011). Therefore, the complexity of this disease would be hardly represented by

a single animal model. For instance, the msP line, in addition to showing an innate preference for alcohol, is characterized by high-stress sensitivity and proclivity to negative affect, resembling a specific subpopulation of AUD patients that drink to self-medicate from negative mood and stress-relieving purposes. Hence, it is conceivable that msP rats more closely mick this cohort of individuals rather than other AUD subpopulations. If so, the generalization of the findings obtained in msP rats to AUD patients other than those that drink for self-medication and tension relieving purposes may be a mistake. Often, pharmacological experiments demonstrated that the use of this rat line is associated with good predictive validity. For example, acamprosate and naltrexone, two medications approved for AUD, are also effective in reducing the motivation for alcohol in msP rats (Bachteler et al., 2005; Perfumi et al., 2003). Whereas the 5-HT2 receptor antagonist ritanserin that is not efficacious in humans failed to attenuate drinking also in msP rats (Johnson et al., 1996; Panocka et al., 1993). However, in other circumstances, the validity of the msP model appeared questionable. The most striking example is that of CRF1 receptor antagonists. In fact, genetic, pharmacological and, molecular data in msP rats converged to suggest the therapeutic potential of this class of molecules (Bachteler et al., 2005; Panocka et al., 1993; Perfumi et al., 2005). However, when tested in AUD patients they systematically failed (Kwako et al., 2015; Schwandt et al., 2016).

In msP rats the neurochemical effects of alcohol have never been investigated and the only paper published so far demonstrated that following an amphetamine challenge, msP rats revealed a higher peak in extracellular dopamine levels in the NAc shell compared to the Wistar counterpart (Bifone et al., 2019). In the absence of these data, it is hard to fully clarify the neurochemical mechanisms subserving the high motivation for alcohol of msP rats. In the past, few neurochemical studies have been carried out in the sP line from which the msPs have been derived (Fadda et al., 1999; Richter et al., 2000). Starting three decades ago, msP rats have been re-derived from sP progenitors and we are now at the 91st generation of separate breeding. It is conceivable that during such a long period of separate selection significant *genetic drift* might have occurred making msP and sP rats different at both genotypic and phenotypic levels (Crabbe et al., 2010). Hence, any generalization between msP and sP rats should be taken cautiously.

Clinical studies demonstrated that alcohol abuse during adolescence represents a critical risk factor in the development of alcoholism in adulthood (Amodeo et al., 2017; Bates and Labouvie, 1997; Gilpin et al., 2012; Spear, 2018). In msP rats, spontaneous preference for alcohol appears very early in life making this animal line ideal to investigate the consequences of adolescent drinking. Systematic studies to investigate this phenomenon have not been conducted yet, but considering its importance future efforts should be dedicated to the exploration of this research area.

4. Neuroimaging studies: structural and functional MRI in msP rats

In clinical research, fMRI techniques are widely used to investigate structural and functional brain properties in healthy and pathological states (i.e., AUD). Several developments in the field have allowed the possibility of performing fMRI also in rodents (Bifone and Gozzi, 2012; Gozzi et al., 2011; Zahr and Pfefferbaum, 2017). This technological advancement

allows preclinical researchers to generate data that can be directly translated to humans, thus providing a unique possibility to explore the face and construct validity of an animal model. Over the years, we have conducted a few fMRI experiments in msP rats, some of them directly comparing this rat line with AUD patients (De Santis et al., 2019, 2020).

The first fMRI study in alcohol-naïve msP rats performed basal cerebral blood volume (bCBV) mapping followed by voxel-based morphometry (VBM). We observed a reduction of gray matter (GM) volume in different thalamic and cortical regions in these animals compared with Wistar controls. Particularly striking was the reduction of bCBV, a marker of resting brain function, in cortical regions and the striatum (Gozzi et al., 2013). Consistent with these data, people with a high risk for alcohol abuse and AUD patients during the withdrawal phase exhibited lower metabolism in striatal regions and thalamo-cortical circuits (Kareken et al., 2004; Vollstadt-Klein et al., 2010). Moreover, loss of the GM signal in cortical regions in msP rats was compatible with their innate tendency to exhibit depressive- and anxiety-like symptoms. In fact, lower GM volume in the rostro-dorsal cingulate, which was reminiscent of msP rats, was also observed in patients who suffered from depression and anxiety (Spampinato et al., 2009; van Tol et al., 2010). Overall, these data suggest that some of the morphological and functional features that are observed in msP rats resemble characteristics of AUD patients. A tempting hypothesis is that these characteristics that are present before alcohol exposure may predispose individuals to AUD.

In addition to this innate condition, chronic alcohol consumption is well known to lead to numerous functional, neuro-morphological, and neuro-metabolic deficits in alcoholic patients (Buhler and Mann, 2011). For example, several studies reported lower GM and white matter volumes in heavy drinkers (Buhler and Mann, 2011; Demirakca et al., 2011; Fein et al., 2002; Mechtcheriakov et al., 2007; Pfefferbaum et al., 1995; Rando et al., 2011). These abnormalities are particularly pronounced in the frontal lobes, but conspicuous brain atrophy has also been reported in limbic areas and the cerebellum (Fein et al., 2006; Makris et al., 2008). In a recent translational study that used a diffusion tensor imaging (DTI) approach, we observed similar microstructural changes in white matter in AUD patients and msP rats following a period of voluntary alcohol drinking (De Santis et al., 2019). These changes continued to progress over the first 2–6 weeks of abstinence in both humans and msP rats, suggesting an underlying process that continues to evolve, even after alcohol cessation. In a subsequent imaging study, we found that chronic alcohol consumption produced a widespread increase in mean diffusivity (MD) in GM in both alcohol-preferring rats and alcoholic patients, which persisted into early abstinence (De Santis et al., 2020). We found that these alterations were associated with a marked decrease in extracellular space tortuosity that was linked to an increase in microglial reactivity in msP rats. A mathematical elaboration that was applied to the results of these imaging experiments indicated that such changes in MD can facilitate the extra synaptic release and extracellular propagation of neurotransmitters like dopamine. This may contribute to an increase in the rewarding effects of alcohol and the motivation for alcohol that is a common feature in chronic drinkers and genetically selected alcohol-preferring rats.

One socially and scientifically debated question is whether the predisposition to alcohol dependence confers vulnerability to addiction to other substances of abuse. To test

this hypothesis, we combined functional brain imaging, neurochemistry, and behavioral techniques in a group of msP rats and evaluated their response to psychostimulants (Bifone et al., 2019). The results revealed that msP rats that were challenged with an intravenous injection of D-amphetamine exhibited a higher level of activation of regions of the extended amygdala, detected by fMRI, compared with Wistar controls. This higher activation was associated with an increase in the extracellular release of dopamine in the nucleus accumbens shell and a higher propensity to escalate operant cocaine self-administration (Bifone et al., 2019). Altogether, these results support the hypothesis that there are some common genetic traits that predispose individuals to the development of substance use disorders, independent of the drug used. Considering the importance of this observation, it will be important to conduct further studies to confirm this initial observation.

5. Concluding remarks

Alcohol use disorder is one of the major burdens of disease at global level. Despite its impact on society, however, few Food and Drug Administration- or European Medicines Agency-approved pharmacotherapeutic options are available for its treatment. These include disulfiram, naltrexone, and acamprosate and in Europe also nalmefene. Moreover, clinical experience has demonstrated that their efficacy is limited to only certain subgroups of patients (Heilig and Egli, 2006). The successful development of these medications provides a proof-of-concept that supports the feasibility of drug development programs for AUD. This optimism, however, is tempered by numerous failures with several drugs that showed efficacy in laboratory animals but not in humans. The most striking negative experience involves CRF_1 receptor antagonists. In fact, a wealth of preclinical data that were generated over 30 years demonstrated remarkable efficacy of this class of molecules in reducing excessive drinking and relapse to alcohol seeking and preventing negative symptoms that are associated with alcohol withdrawal (Zorrilla et al., 2013).

Genetic and behavioral studies in msP rats have consistently supported the potential of CRF_1 receptors as a promising treatment target for AUD. For instance, msP rats overexpress CRF_1 receptors in the brain, triggered by two SNPs in the promoter region of the respective gene (Ayanwuyi et al., 2013; Cippitelli et al., 2015; Hansson et al., 2006; Logrip et al., 2018). Similar evidence was obtained in human genetic studies that showed that CRF_1 receptor gene polymorphisms are associated with binge drinking in AUD patients (Chen et al., 2010; Treutlein et al., 2006). Gene variations at the *Crh1R* locus was also reported in a Caucasian population with a diagnosis of AUD (Chen et al., 2010; Treutlein et al., 2006). Data suggest that these mutations conferred to msP rats higher sensitivity to the pharmacological blockade of CRF_1 receptors. For example, CRF_1 receptor antagonists are more effective in attenuating alcohol drinking, stress-induced relapse, and anxiety that is associated with alcohol abstinence in this rat line than in Wistar controls (Ciccocioppo, 2013; Ciccocioppo et al., 2006). This negative experience raises the general question of whether animal models of AUD, more specifically genetically selected alcohol-preferring rats, have adequate predictive validity. Importantly, AUD is a heterogeneous disorder to which multiple genetic factors, environmental conditions, and cultural experiences can contribute. It is unlikely, therefore, that a single animal model (e.g., genetically selected alcohol-preferring rats) can mimic all these complexities. More reasonably, animal models are useful for studying specific aspects

of AUD. For instance, msP rats may mimic conditions under which drinking is triggered by the necessity to self-medicate a negative affective state. To overcome these limitations, we believe that preclinical research should refine its approach to study AUD and other complex psychiatric pathologies. Preclinical experiments should incorporate key elements that have emerged from human studies, including individual vulnerability. In fact, among alcohol users, only a small proportion of individuals develop AUD over time. It is unlikely that this condition can be captured in preclinical studies in which experimental subjects are tested together as a homogeneous group without distinguishing them based on their individual response to alcohol. To overcome this limitation, we recently proposed the use of the 0/3crit model to study AUD based on DMS-5 criteria, including individual animals' propensity to exhibit (1) an inability to refrain from drug seeking, (2) high motivation for alcohol, and (3) the maintenance of drug use despite negative consequences.

Moreover, it is imperative to integrate preclinical research with experiments that explore sex differences in the response to alcohol and related pharmacological treatments. In fact, human studies have very clearly documented that men and women may initiate drinking for completely different reasons and may respond differently to alcohol and AUD medications (Buchmann et al., 2010; Buckner et al., 2006; Crutzen et al., 2013; Oscar-Berman et al., 2014; Peltier et al., 2019). Furthermore, not all individuals with AUD are the same. This disorder is a very heterogeneous condition and associated with multiple endophenotypes. A valid animal model should not be proposed as a phenocopy of AUD in general. Rather, it should simulate specific endophenotypic characteristics that resemble distinct subpopulations of patients. If such distinctions are not considered, then such preclinical models would not be endowed with sufficient predictive, face, or construct validity. Finally, future studies should expand the use of *in vivo* brain imaging in laboratory animals because these techniques allow direct comparisons between the results from animal models and humans.

Over the years, preclinical research on AUD has advanced substantially, and the use of alcohol-preferring rats has made important contributions to this effort. However, there is still much room for improvement to reduce the risks of failure when translating from preclinical studies to the human condition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding and acknowledgements

This work was supported by National Institute on Alcohol Abuse and Alcoholism grants AA014351 (to FW and RC) and AA017447 (to MR and RC), ERA-NET neuron Psy-Alc (to RC), the Eva-Maria and Rutger Hetzler Foundation (to RC) and PRIN 2017SXEXT5(to RC). We thank Michael Arends for editing the manuscript.

Abbreviations

2BC	two-bottle choice
5-HT$_{2A}$	serotonin 5-hydroxytryptamine-2A

AA	Alko Alcohol-preferring rat line
ADE	alcohol deprivation effect
ADH	aldehyde dehydrogenase
AUD	alcohol use disorder
ANA	Alko Nonalcohol-preferring rat line
BAL:	blood alcohol level
bCBV	basal cerebral blood volume
BNST	bed nucleus of the stria terminalis
CeA	central nucleus of the amygdala
CPP	conditioned place preference
CRF	corticotropin-releasing factor
CRF_1	corticotropin-releasing factor 1 receptor
DTI	diffusion tensor imaging
DSM	Diagnostic and Statistical Manual of Mental Disorders
GABA	γ-aminobutyric acid
GM	gray matter
HAD	High Alcohol Drinking rat line
IG	intragastric
MD	mean diffusivity
LAD	Low Alcohol Drinking rat line
MRI	magnetic resonance imaging
msP	Marchigian Sardinian alcohol-preferring rat line
NIH	National institutes of Health
N/OFQ	nociceptin/orphanin FQ
NOP	nociception/orphanin FQ receptor
NP	Alcohol Non-preferring rat line
OPRM-1	opioid receptor mu 1
OPRL-1	opioid-related nociceptin receptor 1
P	alcohol-preferring rat line

PR	progressive ratio
sNP	Sardinian alcohol non-preferring rat line
SNP	single nucleotide polymorphism
sP	Sardinian alcohol-preferring rat line
UChA	Universidad de Chile low alcohol-drinking rat line
UChB	Universidad de Chile “Bebidores” high alcohol-drinking rat line
VTA	ventral tegmental area
WHO	World Health Organization

References

American Psychiatric Association, 2000. In: Diagnostic and Statistical Manual of Mental Disorders: DSM-IV-TR. American Psychiatric Association, Washington, DC.

American Psychiatric Association, 2013. In: Diagnostic and Statistical Manual of Mental Disorders, fifth ed. American Psychiatric Association, Arlington, VA.

Amodeo LR, Kneiber D, Wills DN, Ehlers CL, 2017. Alcohol drinking during adolescence increases consumptive responses to alcohol in adulthood in Wistar rats. Alcohol 59, 43–51. 10.1016/j.alcohol.2016.12.002. [PubMed: 28187948]

Anda RF, Whitfield CL, Felitti VJ, Chapman D, Edwards VJ, Dube SR, Williamson DF, 2002. Adverse childhood experiences, alcoholic parents, and later risk of alcoholism and depression. Psychiatr. Serv 53 (8), 1001–1009. 10.1176/appi.ps.53.8.1001. [PubMed: 12161676]

Anglin MD, Hser YI, McGlothlin WH, 1987. Sex differences in addict careers. 2. Becoming addicted. Am. J. Drug Alcohol Abuse 13 (1–2), 59–71. 10.3109/00952998709001500. [PubMed: 3687885]

Ashley MJ, Olin JS, le Riche WH, Kornaczewski A, Schmidt W, Rankin JG, 1977. Morbidity in alcoholics. Evidence for accelerated development of physical disease in women. Arch. Intern. Med. 137 (7), 883–887. 10.1001/archinte.137.7.883. [PubMed: 879927]

Augier E, Dulman RS, Rauffenbart C, Augier G, Cross AJ, Heilig M, 2016. The mGluR2 positive allosteric modulator, AZD8529, and cue-induced relapse to alcohol seeking in rats. Neuropsychopharmacology 41 (12), 2932–2940. 10.1038/npp.2016.107. [PubMed: 27339394]

Ayanwuyi LO, Carvajal F, Lerma-Cabrera JM, Domi E, Bjork K, Ubaldi M, Cippitelli A, 2013. Role of a genetic polymorphism in the corticotropin-releasing factor receptor 1 gene in alcohol drinking and seeking behaviors of marchigian Sardinian alcohol-preferring rats. Front. Psychiatr 4, 23. 10.3389/fpsyt.2013.00023.

Bachteler D, Economidou D, Danysz W, Ciccocioppo R, Spanagel R, 2005. The effects of acamprosate and neramexane on cue-induced reinstatement of ethanol-seeking behavior in rat. Neuropsychopharmacology 30 (6), 1104–1110. 10.1038/sj.npp.1300657. [PubMed: 15668725]

Bakhireva LN, Garrison L, Shrestha S, Sharkis J, Miranda R, Rogers K, 2018. Challenges of diagnosing fetal alcohol spectrum disorders in foster and adopted children. Alcohol 67, 37–43. 10.1016/j.alcohol.2017.05.004. [PubMed: 29316477]

Bates ME, Labouvie EW, 1997. Adolescent risk factors and the prediction of persistent alcohol and drug use into adulthood. Alcohol Clin. Exp. Res 21 (5), 944–950. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/9267549. [PubMed: 9267549]

Becker JB, Chartoff E, 2019. Sex differences in neural mechanisms mediating reward and addiction. Neuropsychopharmacology 44 (1), 166–183. 10.1038/S41386-018-0125-6. [PubMed: 29946108]

Becker JB, Koob GF, 2016. Sex differences in animal models: focus on addiction. Pharmacol. Rev 68 (2), 242–263. 10.1124/pr.115.011163. [PubMed: 26772794]

Becker JB, McClellan ML, Reed BG, 2017. Sex differences, gender and addiction. J. Neurosci. Res 95 (1–2), 136–147. 10.1002/jnr.23963. [PubMed: 27870394]

Becker JB, Perry AN, Westenbroek C, 2012. Sex differences in the neural mechanisms mediating addiction: a new synthesis and hypothesis. Biol. Sex Differ 3 (1), 14. 10.1186/2042-6410-3-14. [PubMed: 22676718]

Belin D, Berson N, Balado E, Piazza PV, Deroche-Gamonet V, 2011. High-novelty-preference rats are predisposed to compulsive cocaine self-administration. Neuropsychopharmacology 36 (3), 569–579. 10.1038/npp.2010.188. [PubMed: 20980989]

Bierut LJ, Saccone NL, Rice JP, Goate A, Foroud T, Edenberg H, Reich T, 2002. Defining alcohol-related phenotypes in humans. The collaborative study on the genetics of alcoholism. Alcohol Res. Health 26 (3), 208–213. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/12875049. [PubMed: 12875049]

Bifone A, Gozzi A, 2012. Neuromapping techniques in drug discovery: pharmacological MRI for the assessment of novel antipsychotics. Expet Opin. Drug Discov 7 (11), 1071–1082. 10.1517/17460441.2012.724057.

Bifone A, Gozzi A, Cippitelli A, Matzeu A, Domi E, Li H, Ciccocioppo R, 2019. phMRI, neurochemical and behavioral responses to psychostimulants distinguishing genetically selected alcohol-preferring from genetically heterogenous rats. Addiction Biol. 24 (5), 981–993. 10.llll/adb.12671.

Bobzean SA, DeNobrega AK, Perrotti LI, 2014. Sex differences in the neurobiology of drug addiction. Exp. Neurol 259, 64–74. 10.1016/j.expneurol.2014.01.022. [PubMed: 24508560]

Boening JA, Lesch OM, Spanagel R, Wolffgramm J, Narita M, Sinclair D, Wiesbeck GA, 2001. Pharmacological relapse prevention in alcohol dependence: from animal models to clinical trials. Alcohol Clin. Exp. Res 25 (5 Suppl. ISBRA), 127S–131S. 10.1097/00000374-200105051-00022. [PubMed: 11391061]

Bohman M, Sigvardsson S, Cloninger CR, 1981. Maternal inheritance of alcohol abuse. Cross-fostering analysis of adopted women. Arch. Gen. Psychiatr 38 (9), 965–969. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/7283667. [PubMed: 7283667]

Borruto AM, Fotio Y, Stopponi S, Brunori G, Petrella M, Caputi FF, Ciccocioppo R, 2020. NOP receptor antagonism reduces alcohol drinking in male and female rats through mechanisms involving the central amygdala and ventral tegmental area. Br. J. Pharmacol 177 (7), 1525–1537. 10.1111/bph.14915. [PubMed: 31713848]

Brunori G, Weger M, Schoch J, Targowska-Duda K, Barnes M, Borruto AM, Cippitelli A, 2019. NOP receptor antagonists decrease alcohol drinking in the dark in C57BL/6J mice. Alcohol Clin. Exp. Res 43 (10), 2167–2178. 10.1111/acer.14165. [PubMed: 31386211]

Buchmann AF, Schmid B, Blomeyer D, Zimmermann US, Jennen-Steinmetz C, Schmidt MH, Laucht M, 2010. Drinking against unpleasant emotions: possible outcome of early onset of alcohol use? Alcohol Clin. Exp. Res 34 (6), 1052–1057. 10.1111/j.1530-0277.2010.01180.x. [PubMed: 20374211]

Buckner JD, Eggleston AM, Schmidt NB, 2006. Social anxiety and problematic alcohol consumption: the mediating role of drinking motives and situations. Behav. Ther 37 (4), 381–391. 10.1016/j.beth.2006.02.007. [PubMed: 17071215]

Buhler M, Mann K, 2011. Alcohol and the human brain: a systematic review of different neuroimaging methods. Alcohol Clin. Exp. Res 35 (10), 1771–1793. 10.1111/j.1530-0277.2011.01540.x. [PubMed: 21777260]

Cailhol S, Mormede P, 2001. Sex and strain differences in ethanol drinking: effects of gonadectomy. Alcohol Clin. Exp. Res 25 (4), 594–599. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/11329501. [PubMed: 11329501]

Cannella N, Kallupi M, Li HW, Stopponi S, Cifani C, Ciccocioppo R, Ubaldi M, 2016. Neuropeptide S differently modulates alcohol-related behaviors in alcohol-preferring and non-preferring rats. Psychopharmacology (Berlin) 233 (15–16), 2915–2924. 10.1007/s00213-016-4333-7. [PubMed: 27235017]

Carr LG, Kimpel MW, Liang T, McClintick JN, McCall K, Morse M, Edenberg HJ, 2007. Identification of candidate genes for alcohol preference by expression profiling of congenic rat

strains. Alcohol Clin. Exp. Res 31 (7), 1089–1098. 10.1111/j.1530-0277.2007.00397.x. [PubMed: 17451403]

Chen AC, Manz N, Tang Y, Rangaswamy M, Almasy L, Kuperman S, Porjesz B, 2010. Single-nucleotide polymorphisms in corticotropin releasing hormone receptor 1 gene (CRHR1) are associated with quantitative trait of event-related potential and alcohol dependence. Alcohol Clin. Exp. Res 34 (6), 988–996. 10.1111/j.l530-0277.2010.01173.x. [PubMed: 20374216]

Ciccocioppo R, 2013. Genetically selected alcohol preferring rats to model human alcoholism. Curr Top Behav Neurosci 13, 251–269. 10.1007/7854_2012_199. [PubMed: 22328453]

Ciccocioppo R, Angeletti S, Panocka I, Massi M, 2000. Nociceptin/orphanin FQ and drugs of abuse. Peptides 21 (7), 1071–1080. 10.1016/s0196-9781(00)00245-x. [PubMed: 10998542]

Ciccocioppo R, Economidou D, Cippitelli A, Cucculelli M, Ubaldi M, Soverchia L, Massi M, 2006. Genetically selected Marchigian Sardinian alcohol-preferring (msP) rats: an animal model to study the neurobiology of alcoholism. Addiction Biol. 11 (3–4), 339–355. 10.1111/j.1369-1600.2006.00032.x.

Ciccocioppo R, Economidou D, Fedeli A, Angeletti S, Weiss F, Heilig M, Massi M, 2004. Attenuation of ethanol self-administration and of conditioned reinstatement of alcohol-seeking behaviour by the antiopioid peptide nociceptin/orphanin FQ in alcohol-preferring rats. Psychopharmacology (Berlin) 172 (2), 170–178. 10.1007/s00213-003-1645-1. [PubMed: 14624331]

Ciccocioppo R, Gehlert DR, Ryabinin A, Kaur S, Cippitelli A, Thorsell A, Heilig M, 2009. Stress-related neuropeptides and alcoholism: CRH, NPY, and beyond. Alcohol 43 (7), 491–498. 10.1016/j.alcohol.2009.08.003. [PubMed: 19913192]

Ciccocioppo R, Panocka I, Froldi R, Colombo G, Gessa GL, Massi M, 1999a. Antidepressant-like effect of ethanol revealed in the forced swimming test in Sardinian alcohol-preferring rats. Psychopharmacology (Berlin) 144 (2), 151–157. 10.1007/s002130050988. [PubMed: 10394996]

Ciccocioppo R, Panocka I, Froldi R, Quitadamo E, Massi M, 1999b. Ethanol induces conditioned place preference in genetically selected alcohol-preferring rats. Psychopharmacology (Berlin) 141 (3), 235–241. 10.1007/S002130050830. [PubMed: 10027504]

Ciccocioppo R, Panocka I, Polidori C, Regoli D, Massi M, 1999c. Effect of nociceptin on alcohol intake in alcohol-preferring rats. Psychopharmacology (Berlin) 141 (2), 220–224. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/9952048. [PubMed: 9952048]

Ciccocioppo R, Stopponi S, Economidou D, Kuriyama M, Kinoshita H, Heilig M, Teshima K, 2014. Chronic treatment with novel brain-penetrating selective NOP receptor agonist MT-7716 reduces alcohol drinking and seeking in the rat. Neuropsychopharmacology 39 (11), 2601–2610. 10.1038/npp.2014.113. [PubMed: 24863033]

Cippitelli A, Ayanwuyi LO, Barbier E, Domi E, Lerma-Cabrera JM, Carvajal F, Ciccocioppo R, 2015. Polymorphism in the corticotropin-releasing factor receptor 1 (CRF1-R) gene plays a role in shaping the high anxious phenotype of Marchigian Sardinian alcohol-preferring (msP) rats. Psychopharmacology (Berlin) 232 (6), 1083–1093. 10.1007/s00213-014-3743-7. [PubMed: 25260340]

Cippitelli A, Cannella N, Braconi S, Duranti A, Tontini A, Bilbao A, Ciccocioppo R, 2008. Increase of brain endocannabinoid anandamide levels by FAAH inhibition and alcohol abuse behaviours in the rat. Psychopharmacology (Berlin) 198 (4), 449–460. 10.1007/s00213-008-1104-0. [PubMed: 18446329]

Clayton JA, Collins FS, 2014. Policy: NIH to balance sex in cell and animal studies. Nature 509 (7500), 282–283. 10.1038/509282a. [PubMed: 24834516]

Cloninger CR, 1987. Neurogenetic adaptive mechanisms in alcoholism. Science 236 (4800), 410–416. 10.1126/science.2882604. [PubMed: 2882604]

Cloninger CR, Bohman M, Sigvardsson S, 1981. Inheritance of alcohol abuse. Cross-fostering analysis of adopted men. Arch. Gen. Psychiatr 38 (8), 861–868. 10.1001/archpsyc.1981.01780330019001. [PubMed: 7259422]

Collins FS, Tabak LA, 2014. Policy: NIH plans to enhance reproducibility. Nature 505 (7485), 612–613. 10.1038/505612a. [PubMed: 24482835]

Colombo G, Lobina C, Carai MA, Gessa GL, 2006. Phenotypic characterization of genetically selected Sardinian alcohol-preferring (sP) and -non-preferring (sNP) rats. Addiction Biol. 11 (3–4), 324–338. 10.1111/j.1369-1600.2006.00031.x.

Cooney NL, Litt MD, Morse PA, Bauer LO, Gaupp L, 1997. Alcohol cue reactivity, negative-mood reactivity, and relapse in treated alcoholic men. J. Abnorm. Psychol 106 (2), 243–250. 10.1037//0021-843X.106.2.243. [PubMed: 9131844]

Costin BN, Miles MF, 2014. Molecular and neurologic responses to chronic alcohol use. Handb. Clin. Neurol 125, 157–171. 10.1016/B978-0-444-62619-6.00010-0. [PubMed: 25307574]

Cotder LB, Compton WM 3rd, Mager D, Spitznagel EL, Janca A, 1992. Posttraumatic stress disorder among substance users from the general population. Am. J. Psychiatr 149 (5), 664–670. 10.1176/ajp.149.5.664. [PubMed: 1575258]

Cowen MS, Adams C, Kraehenbuehl T, Vengeliene V, Lawrence AJ, 2005. The acute anti-craving effect of acamprosate in alcohol-preferring rats is associated with modulation of the mesolimbic dopamine system. Addiction Biol. 10 (3), 233–242. 10.1080/13556210500223132.

Crabbe JC, Phillips TJ, Belknap JK, 2010. The complexity of alcohol drinking: studies in rodent genetic models. Behav. Genet 40 (6), 737–750. 10.1007/s10519-010-9371-z. [PubMed: 20552264]

Crutzen R, Kuntsche E, Schelleman-Offermans K, 2013. Drinking motives and drinking behavior over time: a full cross-lagged panel study among adults. Psychol. Addict. Behav 27 (1), 197–201. 10.1037/a0029824. [PubMed: 22925011]

De Santis S, Bach P, Perez-Cervera L, Cosa-Linan A, Weil G, Vollstadt-Klein S, Canals S, 2019. Microstructural white matter alterations in men with alcohol use disorder and rats with excessive alcohol consumption during early abstinence. JAMA Psychiatry 76 (7), 749–758. 10.1001/jamapsychiatry.2019.0318. [PubMed: 30942831]

De Santis S, Cosa-Linan A, Garcia-Hernandez R, Dmytrenko L, Vargova L, Vorisek I, Canals S, 2020. Chronic alcohol consumption alters extracellular space geometry and transmitter diffusion in the brain. Sci Adv 6 (26). 10.1126/sciadv.aba0154 eaba0154.

Demirakca T, Ende G, Kammerer N, Welzel-Marquez H, Hermann D, Heinz A, Mann K, 2011. Effects of alcoholism and continued abstinence on brain volumes in both genders. Alcohol Clin. Exp. Res 35 (9), 1678–1685. 10.1111/j.1530-0277.2011.01514.x. [PubMed: 21599718]

Deroche-Gamonet V, Belin D, Piazza PV, 2004. Evidence for addiction-like behavior in the rat. Science 305 (5686), 1014–1017. 10.1126/science.1099020. [PubMed: 15310906]

Dinwiddie S, Heath AC, Dunne MP, Bucholz KK, Madden PA, Slutske WS, Martin NG, 2000. Early sexual abuse and lifetime psychopathology: a co-twin-control study. Psychol. Med 30 (1), 41–52. 10.1017/S0033291799001373. [PubMed: 10722174]

Disney ER, Elkins IJ, McGue M, Iacono WG, 1999. Effects of ADHD, conduct disorder, and gender on substance use and abuse in adolescence. Am. J. Psychiatr 156 (10), 1515–1521. 10.1176/ajp.156.10.1515. [PubMed: 10518160]

Domi A, Stopponi S, Domi E, Ciccocioppo R, Cannella N, 2019. Sub-dimensions of alcohol use disorder in alcohol preferring and non-preferring rats, a comparative study. Front. Behav. Neurosci 13, 3. 10.3389/fnbeh.2019.00003. [PubMed: 30760988]

Economidou D, Hansson AC, Weiss F, Terasmaa A, Sommer WH, Cippitelli A, Heilig M, 2008. Dysregulation of nociceptin/orphanin FQ activity in the amygdala is linked to excessive alcohol drinking in the rat. Biol. Psychiatr 64 (3), 211–218. 10.1016/j.biopsych.2008.02.004.

Edenberg HJ, 2002. The collaborative study on the genetics of alcoholism: an update. Alcohol Res. Health 26 (3), 214–218. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/12875050. [PubMed: 12875050]

Eriksson K, 1968. Genetic selection for voluntary alcohol consumption in the albino rat. Science 159 (3816), 739–741. 10.1126/science.159.3816.739. [PubMed: 17795073]

Fadda P, Tronci S, Colombo G, Fratta W, 1999. Differences in the opioid system in selected brain regions of alcohol-preferring and alcohol-nonpreferring rats. Alcohol Clin. Exp. Res 23 (8), 1296–1305. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/10470971. [PubMed: 10470971]

Fattore L, Melis M, 2016a. Editorial: exploring gender and sex differences in behavioral dyscontrol: from drug addiction to impulse control disorders. Front. Psychiatr 7, 19. 10.3389/fpsyt.2016.00019.

Fattore L, Melis M, 2016b. Sex differences in impulsive and compulsive behaviors: a focus on drug addiction. Addiction Biol. 21 (5), 1043–1051. 10.1111/adb.12381.

Fein G, Di Sclafani V, Cardenas VA, Goldmann H, Tolou-Shams M, Meyerhoff DJ, 2002. Cortical gray matter loss in treatment-naive alcohol dependent individuals. Alcohol Clin. Exp. Res 26 (4), 558–564. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/11981133. [PubMed: 11981133]

Fein G, Landman B, Tran H, McGillivray S, Finn P, Barakos J, Moon K, 2006. Brain atrophy in long-term abstinent alcoholics who demonstrate impairment on a simulated gambling task. Neuroimage 32 (3), 1465–1471. 10.1016/j.neuroimage.2006.06.013. [PubMed: 16872844]

Fergusson DM, Horwood LJ, Lynskey MT, 1996. Childhood sexual abuse and psychiatric disorder in young adulthood: II. Psychiatric outcomes of childhood sexual abuse. J. Am. Acad. Child Adolesc. Psychiatry 35 (10), 1365–1374. 10.1097/00004583-199610000-00024. [PubMed: 8885591]

Fidler TL, Bakner L, Cunningham CL, 2004. Conditioned place aversion induced by intragastric administration of ethanol in rats. Pharmacol. Biochem. Behav 77 (4), 731–743. 10.1016/j.pbb.2004.01.010. [PubMed: 15099918]

Fotio Y, Borruto AM, Benvenuti F, Demopulos G, Gaitanaris G, Roberto M, Ciccocioppo R, 2020. Activation of peroxisome proliferator-activated receptor gamma reduces alcohol drinking and seeking by modulating multiple mesocorticolimbic regions in rats. Neuropsychopharmacology, 10.1038/s41386-020-0754-4.

Gehlert DR, Cippitelli A, Thorsell A, Le AD, Hipskind PA, Hamdouchi C, Heilig M, 2007. 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-b]pyridazine: a novel brain-penetrant, orally available corticotropin-releasing factor receptor 1 antagonist with efficacy in animal models of alcoholism. J. Neurosci 27 (10), 2718–2726. 10.1523/JNEUROSCI.4985-06.2007, 27/10/2718 [pii]. [PubMed: 17344409]

Gianoulakis C, de Waele JP, 1994. Genetics of alcoholism: role of the endogenous opioid system. Metab. Brain Dis 9 (2), 105–131. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/8072460. [PubMed: 8072460]

Gilpin NW, Karanikas CA, Richardson HN, 2012. Adolescent binge drinking leads to changes in alcohol drinking, anxiety, and amygdalar corticotropin releasing factor cells in adulthood in male rats. PLoS One 7 (2), e31466. 10.1371/journal.pone.0031466. [PubMed: 22347484]

Goodwin DW, Schulsinger F, Knop J, Mednick S, Guze SB, 1977. Psychopathology in adopted and nonadopted daughters of alcoholics. Arch. Gen. Psychiatr 34 (9), 1005–1009. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/901132. [PubMed: 901132]

Goodwin DW, Schulsinger F, Moller N, Hermansen L, Winokur G, Guze SB, 1974. Drinking problems in adopted and nonadopted sons of alcoholics. Arch. Gen. Psychiatr 31 (2), 164–169. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/4851437. [PubMed: 4851437]

Gozzi A, Agosta F, Massi M, Ciccocioppo R, Bifone A, 2013. Reduced limbic metabolism and fronto-cortical volume in rats vulnerable to alcohol addiction. Neuroimage 69, 112–119. 10.1016/j.neuroimage.2012.12.015. [PubMed: 23261637]

Gozzi A, Tessari M, Dacome L, Agosta F, Lepore S, Lanzoni A, Bifone A, 2011. Neuroimaging evidence of altered fronto-cortical and striatal function after prolonged cocaine self-administration in the rat. Neuropsychopharmacology 36 (12), 2431–2440. 10.1038/npp.2011.129. [PubMed: 21775976]

Grant BF, Chou SP, Saha TD, Pickering RP, Kerridge BT, Ruan WJ, Hasin DS, 2017. Prevalence of 12-month alcohol use, high-risk drinking, and DSM-IV alcohol use disorder in the United States, 2001-2002 to 2012-2013: results from the national epidemiologic survey on alcohol and related conditions. JAMA Psychiatry 74 (9), 911–923. 10.1001/jamapsychiatry.2017.2161. [PubMed: 28793133]

Grant BF, Goldstein RB, Saha TD, Chou SP, Jung J, Zhang H, Hasin DS, 2015. Epidemiology of DSM-5 alcohol use disorder: results from the national epidemiologic survey on alcohol and related conditions III. JAMA Psychiatry 72 (8), 757–766. 10.1001/jamapsychiatry.2015.0584. [PubMed: 26039070]

Greenfield SF, Brooks AJ, Gordon SM, Green CA, Kropp F, McHugh RK, Miele GM, 2007. Substance abuse treatment entry, retention, and outcome in women: a review of the literature. Drug Alcohol Depend. 86 (1), 1–21. 10.1016/j.drugalcdep.2006.05.012. [PubMed: 16759822]

Hansen C, Spuhler K, 1984. Development of the National Institutes of Health genetically heterogeneous rat stock. Alcohol Clin. Exp. Res 8 (5), 477–479. 10.1111/j.1530-0277.1984.tb05706.x. [PubMed: 6391259]

Hansson AC, Cippitelli A, Sommer WH, Ciccocioppo R, Heilig M, 2007. Region-specific down-regulation of Crhr1 gene expression in alcohol-preferring msP rats following ad lib access to alcohol. Addiction Biol. 12 (1), 30–34. 10.1111/j.1369-1600.2007.00050.x.

Hansson AC, Cippitelli A, Sommer WH, Fedeli A, Bjork K, Soverchia L, Ciccocioppo R, 2006. Variation at the rat Crhr1 locus and sensitivity to relapse into alcohol seeking induced by environmental stress. Proc. Natl. Acad. Sci. U. S. A 103 (41), 15236–15241. 10.1073/pnas.0604419103. [PubMed: 17015825]

Heath AC, Bucholz KK, Madden PA, Dinwiddie SH, Slutske WS, Bierat LJ, Martin NG, 1997. Genetic and environmental contributions to alcohol dependence risk in a national twin sample: consistency of findings in women and men. Psychol. Med 27 (6), 1381–1396. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/9403910. [PubMed: 9403910]

Heilig M, Egli M, 2006. Pharmacological treatment of alcohol dependence: target symptoms and target mechanisms. Pharmacol. Ther 111 (3), 855–876. 10.1016/j.pharmthera.2006.02.001. [PubMed: 16545872]

Heilig M, Goldman D, Berrettini W, O'Brien CP, 2011. Pharmacogenetic approaches to the treatment of alcohol addiction. Nat. Rev. Neurosci 12 (11), 670–684. 10.1038/nrn3110. [PubMed: 22011682]

Heilig M, Thorsell A, 2002. Brain neuropeptide Y (NPY) in stress and alcohol dependence. Rev. Neurosci 13 (1), 85–94. 10.1515/revneuro.2002.13.1.85. [PubMed: 12013027]

Herman MA, Varodayan FP, Oleata CS, Luu G, Kirson D, Heilig M, Roberto M, 2016. Glutamatergic transmission in the central nucleus of the amygdala is selectively altered in Marchigian Sardinian alcohol-preferring rats: alcohol and CRF effects. Neuropharmacology 102, 21–31. 10.1016/j.neuropharm.2015.10.027. [PubMed: 26519902]

Hilderbrand ER, Lasek AW, 2018. Studying sex differences in animal models of addiction: an emphasis on alcohol-related behaviors. ACS Chem. Neurosci 9 (8), 1907–1916. 10.1021/acschemneuro.7b00449. [PubMed: 29227676]

Holter SM, Spanagel R, 1999. Effects of opiate antagonist treatment on the alcohol deprivation effect in long-term ethanol-experienced rats. Psychopharmacology (Berlin) 145 (4), 360–369. 10.1007/s002130051069. [PubMed: 10460312]

Hu JH, Ma YH, Yang N, Mei ZT, Zhang MH, Fei J, Guo LH, 2004. Up-regulation of gamma-aminobutyric acid transporter I mediates ethanol sensitivity in mice. Neuroscience 123 (4), 807–812. 10.1016/j.euroscience.2003.11.018. [PubMed: 14751274]

Hudson A, Stamp JA, 2011. Ovarian hormones and propensity to drug relapse: a review. Neurosci. Biobehav. Rev 35 (3), 427–436. 10.1016/j.neubiorev.2010.05.001. [PubMed: 20488201]

Hyman SM, Paliwal P, Chaplin TM, Mazure CM, Rounsaville BJ, Sinha R, 2008. Severity of childhood trauma is predictive of cocaine relapse outcomes in women but not men. Drug Alcohol Depend. 92 (1–3), 208–216. 10.10l6/j.drugalcdep.2007.08.006. [PubMed: 17900822]

Johnson BA, Jasinski DR, Galloway GP, Kranzler H, Weinreib R, Anton RF, Clyde C, 1996. Ritanserin in the treatment of alcohol dependence–a multi-center clinical trial. Ritanserin Study Group. Psychopharmacology (Berlin) 128 (2), 206–215. 10.1007/s002130050126. [PubMed: 8956382]

Kareken DA, Claus ED, Sabri M, Dzemidzic M, Kosobud AE, Radnovich AJ, Li TK, 2004. Alcohol-related olfactory cues activate the nucleus accumbens and ventral tegmental area in high-risk drinkers: preliminary findings. Alcohol Clin. Exp. Res 28 (4), 550–557. 10.1097/01.alc.0000122764.60626.af. [PubMed: 15100605]

Kendler KS, Heath AC, Neale MC, Kessler RC, Eaves LJ, 1992. A population-based twin study of alcoholism in women. J. Am. Med. Assoc 268 (14), 1877–1882. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/1404711.

Kendler KS, Walters EE, Neale MC, Kessler RC, Heath AC, Eaves LJ, 1995. The structure of the genetic and environmental risk factors for six major psychiatric disorders in women. Phobia, generalized anxiety disorder, panic disorder, bulimia, major depression, and alcoholism. Arch.

Gen. Psychiatr 52 (5), 374–383. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/7726718. [PubMed: 7726718]

Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson CB, 1995. Posttraumatic stress disorder in the national comorbidity survey. Arch. Gen. Psychiatr 52 (12), 1048–1060. 10.1001/archpsyc.1995.03950240066012. [PubMed: 7492257]

Kilpatrick DG, Acierno R, Resnick HS, Saunders BE, Best CL, 1997. A 2-year longitudinal analysis of the relationships between violent assault and substance use in women. J. Consult. Clin. Psychol 65 (5), 834–847. 10.1037//0022-006X.65.5.834. [PubMed: 9337502]

Kilpatrick DG, Ruggiero KJ, Acierno R, Saunders BE, Resnick HS, Best CL, 2003. Violence and risk of PTSD, major depression, substance abuse/dependence, and comorbidity: results from the National Survey of Adolescents. J. Consult. Clin. Psychol 71 (4), 692–700. 10.1037/0022-006x.71.4.692. [PubMed: 12924674]

Kimpel MW, Strother WN, McClintick JN, Carr LG, Liang T, Edenberg HJ, McBride WJ, 2007. Functional gene expression differences between inbred alcohol-preferring and -non-preferring rats in five brain regions. Alcohol 41 (2), 95–132. 10.1016/j.alcohol.2007.03.003. [PubMed: 17517326]

Kirson D, Oleata CS, Parsons LH, Ciccocioppo R, Roberto M, 2018. CB1 and ethanol effects on glutamatergic transmission in the central amygdala of male and female msP and Wistar rats. Addiction Biol. 23 (2), 676–688. 10.1111/adb.12525.

Koob GF, Le Moal M, 2005. Plasticity of reward neurocircuitry and the 'dark side' of drug addiction. Nat. Neurosci 8(11), 1442–1444. 10.1038/nn1105-1442. [PubMed: 16251985]

Kwako LE, Spagnolo PA, Schwandt ML, Thorsell A, George DT, Momenan R, Heilig M, 2015. The corticotropin releasing hormone-1 (CRH1) receptor antagonist pexacerfont in alcohol dependence: a randomized controlled experimental medicine study. Neuropsychopharmacology 40 (5), 1053–1063. 10.1038/npp.2014.306. [PubMed: 25409596]

Landis SC, Amara SG, Asadullah K, Austin CP, Blumenstein R, Bradley EW, Silberberg SD, 2012. A call for transparent reporting to optimize the predictive value of preclinical research. Nature 490 (7419), 187–191. 10.1038/naturell556. [PubMed: 23060188]

Lee SK, 2018. Sex as an important biological variable in biomedical research. BMB Rep 51 (4), 167–173. 10.5483/bmbrep.2018.51.4.034. [PubMed: 29429452]

Li J, Chen P, Han X, Zuo W, Mei Q, Bian EY, Ye J, 2019. Differences between male and female rats in alcohol drinking, negative affects and neuronal activity after acute and prolonged abstinence. Int. J. Physiol. Pathophysiol. Pharmacol 11 (4), 163–176. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/31523363. [PubMed: 31523363]

Li TK, Hewitt BG, Grant BF, 2004. Alcohol use disorders and mood disorders: a national institute on alcohol abuse and alcoholism perspective. Biol. Psychiatr 56 (10), 718–720. 10.1016/j.biopsych.2004.03.006.

Li TK, Lumeng L, McBride WJ, Waller MB, 1979. Progress toward a voluntary oral consumption model of alcoholism. Drug Alcohol Depend. 4 (1–2), 45–60. 10.1016/0376-8716(79)90040-1. [PubMed: 41697]

Logrip ML, Walker JR, Ayanwuyi LO, Sabino V, Ciccocioppo R, Koob GF, Zorrilla EP, 2018. Evaluation of alcohol preference and drinking in msP rats bearing a Crhr1 promoter polymorphism. Front. Psychiatr 9, 28. 10.3389/fpsyt.2018.00028.

Makris N, Oscar-Berman M, Jaffin SK, Hodge SM, Kennedy DN, Caviness VS, Harris GJ, 2008. Decreased volume of the brain reward system in alcoholism. Biol. Psychiatr 64 (3), 192–202. 10.1016/j.biopsych.2008.01.018.

Mardones J, Segovia N, Hederra A, 1953. Heredity of experimental alcohol preference in rats. II. Coefficient of heredity. Q. J. Stud. Alcohol 14 (1), 1–2. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/13037991. [PubMed: 13037991]

Mardones J, Segovia-Riquelme N, 1984. Effects of different diets on voluntary consumption of ethanol in UChA and UChB rats. Acta Physiol. Pharmacol. Latinoam 34 (1), 25–30. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/6236670. [PubMed: 6236670]

Martin-Fardon R, Weiss F, 2013. Modeling relapse in animals. Curr Top Behav Neurosci 13, 403–432. 10.1007/7854_2012_202. [PubMed: 22389178]

McBride WJ, Le AD, Noronha A, 2002. Central nervous system mechanisms in alcohol relapse. Alcohol Clin. Exp. Res 26 (2), 280–286. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/11964569. [PubMed: 11964569]

Mechtcheriakov S, Brenneis C, Egger K, Koppelstaetter F, Schocke M, Marksteiner J, 2007. A widespread distinct pattern of cerebral atrophy in patients with alcohol addiction revealed by voxel-based morphometry. J. Neurol. Neurosurg. Psychiatry 78 (6), 610–614. 10.1136/jnnp.2006.095869. [PubMed: 17088334]

Murphy JM, Stewart RB, Bell RL, Badia-Elder NE, Carr LG, McBride WJ, Li TK, 2002. Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. Behav. Genet 32 (5), 363–388. 10.1023/a:1020266306135. [PubMed: 12405517]

Natividad LA, Buczynski MW, Herman MA, Kirson D, Oleata CS, Irimia C, Parsons LH, 2017. Constitutive increases in amygdalar corticotropin-releasing factor and fatty acid amide hydrolase drive an anxious phenotype. Biol. Psychiatr 82 (7), 500–510. 10.1016/j.biopsych.2017.01.005.

Oscar-Berman M, Valmas MM, Sawyer KS, Ruiz SM, Luhar RB, Gravitz ZR, 2014. Profiles of impaired, spared, and recovered neuropsychologic processes in alcoholism. Handb. Clin. Neurol 125, 183–210. 10.1016/B978-0-444-62619-6.00012-4. [PubMed: 25307576]

Panocka I, Ciccocioppo R, Pompei P, Massi M, 1993. 5-HT2 receptor antagonists do not reduce ethanol preference in Sardinian alcohol-preferring (sP) rats. Pharmacol. Biochem. Behav 46 (4), 853–856. 10.1016/0091-3057(93)90212-c. [PubMed: 7508629]

Peltier MR, Verplaetse TL, Mineur YS, Petrakis IL, Cosgrove KP, Picciotto MR, McKee SA, 2019. Sex differences in stress-related alcohol use. Neurobiol. Stress 10, 100149. 10.1016/j.ynstr.2019.100149. [PubMed: 30949562]

Perfumi M, Mattioli L, Forti L, Massi M, Ciccocioppo R, 2005. Effect of Hypericum perforatum CO2 extract on the motivational properties of ethanol in alcohol-preferring rats. Alcohol Alcohol 40 (4), 291–296. 10.1093/alcalc/agh133. [PubMed: 15870093]

Perfumi M, Santoni M, Cippitelli A, Ciccocioppo R, Froldi R, Massi M, 2003. Hypericum perforatum CO2 extract and opioid receptor antagonists act synergistically to reduce ethanol intake in alcohol-preferring rats. Alcohol Clin. Exp. Res 27 (10), 1554–1562. 10.1097/01.ALC.0000092062.60924.56. [PubMed: 14574225]

Perry AN, Westenbroek C, Becker JB, 2013. Impact of pubertal and adult estradiol treatments on cocaine self-administration. Horm. Behav 64 (4), 573–578. 10.1016/j.yhbeh.2013.08.007. [PubMed: 24013034]

Pfefferbaum A, Sullivan EV, Mathalon DH, Shear PK, Rosenbloom MJ, Lim KO, 1995. Longitudinal changes in magnetic resonance imaging brain volumes in abstinent and relapsed alcoholics. Alcohol Clin. Exp. Res 19 (5), 1177–1191. 10.1111/j.1530-0277.1995.tb01598.x. [PubMed: 8561288]

Prescott CA, Kendler KS, 1999. Genetic and environmental contributions to alcohol abuse and dependence in a population-based sample of male twins. Am. J. Psychiatr 156 (1), 34–40. 10.1176/ajp.156.l.34. [PubMed: 9892295]

Quadros IM, Macedo GC, Domingues LP, Favoretto CA, 2016. An update on CRF mechanisms underlying alcohol use disorders and dependence. Front. Endocrinol 7, 134. 10.3389/fendo.2016.00134.

Quintanilla ME, Israel Y, Sapag A, Tampier L, 2006. The UChA and UChB rat lines: metabolic and genetic differences influencing ethanol intake. Addiction Biol. 11 (3–4), 310–323. 10.1111/j.1369-1600.2006.00030.x.

Rando K, Hong KI, Bhagwagar Z, Li CS, Bergquist K, Guarnaccia J, Sinha R, 2011. Association of frontal and posterior cortical gray matter volume with time to alcohol relapse: a prospective study. Am. J. Psychiatr 168 (2), 183–192. 10.1176/appi.ajp.2010.10020233. [PubMed: 21078704]

Richter RM, Zorrilla EP, Basso AM, Koob GF, Weiss F, 2000. Altered amygdalar CRF release and increased anxiety-like behavior in Sardinian alcohol-preferring rats: a microdialysis and behavioral study. Alcohol Clin. Exp. Res 24 (12), 1765–1772. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/11141034. [PubMed: 11141034]

Rodd ZA, Anstrom KK, Knapp DJ, Racz I, Zimmer A, Serra S, Colombo G, 2004. Factors mediating alcohol craving and relapse: stress, compulsivity, and genetics. Alcohol Clin. Exp. Res 29 (7), 1325–1333. 10.1097/01.alc.0000171487.62079.a3.

Rodd ZA, Bertsch BA, Strother WN, Le-Niculescu H, Balaraman Y, Hayden E, Niculescu AB, 2007. Candidate genes, pathways and mechanisms for alcoholism: an expanded convergent functional genomics approach. Pharmacogenomics J. 7 (4), 222–256. 10.1038/sj.tpj.6500420. [PubMed: 17033615]

Rorick-Kehn LM, Ciccocioppo R, Wong CJ, Witkin JM, Martinez-Grau MA, Stopponi S, Statnick MA, 2016. A novel, orally bioavailable nociceptin receptor antagonist, LY2940094, reduces ethanol self-administration and ethanol seeking in animal models. Alcohol Clin. Exp. Res 40 (5), 945–954. 10.1111/acer.13052. [PubMed: 27084498]

Sanchis-Segura C, Becker JB, 2016. Why we should consider sex (and study sex differences) in addiction research. Addiction Biol. 21 (5), 995–1006. 10.1111/adb.12382.

Schroeder JP, Overstreet DH, Hodge CW, 2005. The neuropeptide-Y Y5 receptor antagonist L-152,804 decreases alcohol self-administration in inbred alcohol-preferring (iP) rats. Alcohol 36 (3), 179–186. 10.1016/j.alcohol.2005.10.001. [PubMed: 16377459]

Schulte MT, Ramo D, Brown SA, 2009. Gender differences in factors influencing alcohol use and drinking progression among adolescents. Clin. Psychol. Rev 29 (6), 535–547. 10.1016/j.cpr.2009.06.003. [PubMed: 19592147]

Schwandt ML, Cortes CR, Kwako LE, George DT, Momenan R, Sinha R, Heilig M, 2016. The CRF1 antagonist verucerfont in anxious alcohol-dependent women: translation of neuroendocrine, but not of anti-craving effects. Neuropsychopharmacology 41 (12), 2818–2829. 10.1038/npp.2016.61. [PubMed: 27109623]

Sinha R, Garcia M, Paliwal P, Kreek MJ, Rounsaville BJ, 2006. Stress-induced cocaine craving and hypothalamic-pituitary-adrenal responses are predictive of cocaine relapse outcomes. Arch. Gen. Psychiatr 63 (3), 324–331. 10.1001/archpsyc.63.3.324. [PubMed: 16520439]

Slutske WS, True WR, Scherrer JF, Heath AC, Bucholz KK, Eisen SA, Tsuang MT, 1999. The heritability of alcoholism symptoms: “indicators of genetic and environmental influence in alcohol-dependent individuals” revisited. Alcohol Clin. Exp. Res 23 (5), 759–769. 10.1111/j.1530-0277.1999.tb04181.x. [PubMed: 10371393]

Smith-Warner SA, Spiegelman D, Yaun SS, van den Brandt PA, Folsom AR, Goldbohm RA, Hunter DJ, 1998. Alcohol and breast cancer in women: a pooled analysis of cohort studies. J. Am. Med. Assoc 279 (7), 535–540. 10.1001/jama.279.7.535.

Sommer WH, Rimondini R, Hansson AC, Hipskind PA, Gehlert DR, Barr CS, Heilig MA, 2008. Upregulation of voluntary alcohol intake, behavioral sensitivity to stress, and amygdala Crhr1 expression following a history of dependence. Biol. Psychiatr 63 (2), 139–145. 10.1016/j.biopsych.2007.01.010. S0006-3223(07)00070-4 [pii].

Spampinato MV, Wood JN, De Simone V, Grafman J, 2009. Neural correlates of anxiety in healthy volunteers: a voxel-based morphometry study. J. Neuropsychiatry Clin. Neurosci 21 (2), 199–205. 10.1176/appi.neuropsych.21.2.199. [PubMed: 19622691]

Spanagel R, 2017. Animal models of addiction. Dialogues Clin. Neurosci. 19 (3), 247–258. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/29302222. [PubMed: 29302222]

Spear LP, 2018. Effects of adolescent alcohol consumption on the brain and behaviour. Nat. Rev. Neurosci 19(4), 197–214. 10.1038/nrn.2018.10. [PubMed: 29467469]

Stopponi S, de Guglielmo G, Somaini L, Cippitelli A, Cannella N, Kallupi M, Ciccocioppo R, 2013. Activation of PPARgamma by pioglitazone potentiates the effects of naltrexone on alcohol drinking and relapse in msP rats. Alcohol Clin. Exp. Res 37 (8), 1351–1360. 10.1111/acer.12091. [PubMed: 23550625]

Stopponi S, Fotio Y, Domi A, Borruto AM, Natividad L, Roberto M, Cannella N, 2018. Inhibition of fatty acid amide hydrolase in the central amygdala alleviates comorbid expression of innate anxiety and excessive alcohol intake. Addiction Biol. 23 (6), 1223–1232. 10.1111/adb.12573.

Thorsell A, Carlsson K, Ekman R, Heilig M, 1999. Behavioral and endocrine adaptation, and up-regulation of NPY expression in rat amygdala following repeated restraint stress. Neuroreport 10 (14), 3003–3007. 10.1097/00001756-199909290-00024. [PubMed: 10549813]

Treutlein J, Kissling C, Frank J, Wiemann S, Dong L, Depner M, Schumann G, 2006. Genetic association of the human corticotropin releasing hormone receptor 1 (CRHR1) with binge drinking and alcohol intake patterns in two independent samples. Mol. Psychiatr 11 (6), 594–602. 10.1038/sj.mp.4001813.

Ubaldi M, Cannella N, Ciccocioppo R, 2016. Emerging targets for addiction neuropharmacology: from mechanisms to therapeutics. Prog. Brain Res 224, 251–284. 10.1016/bs.pbr.2015.07.018. [PubMed: 26822362]

Urbano-Marquez A, Estruch R, Fernandez-Sola J, Nicolas JM, Pare JC, Rubin E, 1995. The greater risk of alcoholic cardiomyopathy and myopathy in women compared with men. J. Am. Med. Assoc 274 (2), 149–154. 10.1001/jama.1995.03530020067034.

van Tol MJ, van der Wee NJ, van den Heuvel OA, Nielen MM, Demenescu LR, Aleman A, Veltman DJ, 2010. Regional brain volume in depression and anxiety disorders. Arch. Gen. Psychiatr 67 (10), 1002–1011. 10.1001/archgenpsychiatry.2010.121. [PubMed: 20921116]

Vengeliene V, Bachteler D, Danysz W, Spanagel R, 2005. The role of the NMDA receptor in alcohol relapse: a pharmacological mapping study using the alcohol deprivation effect. Neuropharmacology 48 (6), 822–829. 10.1016/j.neuropharm.2005.01.002. [PubMed: 15829254]

Vengeliene V, Bilbao A, Spanagel R, 2014. The alcohol deprivation effect model for studying relapse behavior: a comparison between rats and mice. Alcohol 48 (3), 313–320. 10.1016/j.alcohol.2014.03.002. [PubMed: 24811155]

Vengeliene V, Siegmund S, Singer MV, Sinclair JD, Li TK, Spanagel R, 2003. A comparative study on alcohol-preferring rat lines: effects of deprivation and stress phases on voluntary alcohol intake. Alcohol Clin. Exp. Res 27 (7), 1048–1054. 10.1097/01.ALC.0000075829.81211.0C. [PubMed: 12878910]

Vollstadt-Klein S, Wichert S, Rabinstein J, Buhler M, Klein O, Ende G, Mann K, 2010. Initial, habitual and compulsive alcohol use is characterized by a shift of cue processing from ventral to dorsal striatum. Addiction 105 (10), 1741–1749. 10.1111/j.1360-0443.2010.03022.x. [PubMed: 20670348]

Waaktaar T, Kan KJ, Torgersen S, 2018. The genetic and environmental architecture of substance use development from early adolescence into young adulthood: a longitudinal twin study of comorbidity of alcohol, tobacco and illicit drug use. Addiction 113 (4), 740–748. 10.1111/add.14076. [PubMed: 29057620]

Wagner FA, Anthony JC, 2002. From first drug use to drug dependence; developmental periods of risk for dependence upon marijuana, cocaine, and alcohol. Neuropsychopharmacology 26 (4), 479–488. 10.1016/S0893-133X(01)00367-0. [PubMed: 11927172]

Walitzer KS, Dearing RL, 2006. Gender differences in alcohol and substance use relapse. Clin. Psychol. Rev 26 (2), 128–148. 10.1016/j.cpr.2005.11.003. [PubMed: 16412541]

White A, Castle IJ, Chen CM, Shirley M, Roach D, Hingson R, 2015. Converging patterns of alcohol use and related outcomes among females and males in the United States, 2002 to 2012. Alcohol Clin. Exp. Res 39 (9), 1712–1726. 10.1111/acer.12815. [PubMed: 26331879]

Wilsnack SC, Vogeltanz ND, Klassen AD, Harris TR, 1997. Childhood sexual abuse and women's substance abuse: national survey findings. J. Stud. Alcohol 58 (3), 264–271. 10.15288/jsa.1997.58.264. [PubMed: 9130218]

Witkin JM, Statnick MA, Rorick-Kehn LM, Pintar JE, Ansonoff M, Chen Y, Ciccocioppo R, 2014. The biology of Nociceptin/Orphanin FQ (N/OFQ) related to obesity, stress, anxiety, mood, and drug dependence. Pharmacol. Ther 141 (3), 283–299. 10.1016/j.pharmthera.2013.10.011. [PubMed: 24189487]

Zahr NM, Pfefferbaum A, 2017. Alcohol's effects on the brain: neuroimaging results in humans and animal models. Alcohol Res. 38 (2), 183–206. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/28988573. [PubMed: 28988573]

Zorrilla EP, Heilig M, de Wit H, Shaham Y, 2013. Behavioral, biological, and chemical perspectives on targeting CRF(1) receptor antagonists to treat alcoholism. Drug Alcohol Depend. 128 (3), 175–186. 10.1016/j.drugalcdep.2012.12.017. [PubMed: 23294766]

Zucker I, Beery AK, 2010. Males still dominate animal studies. Nature 465 (7299), 690. 10.1038/465690a. [PubMed: 20535186]

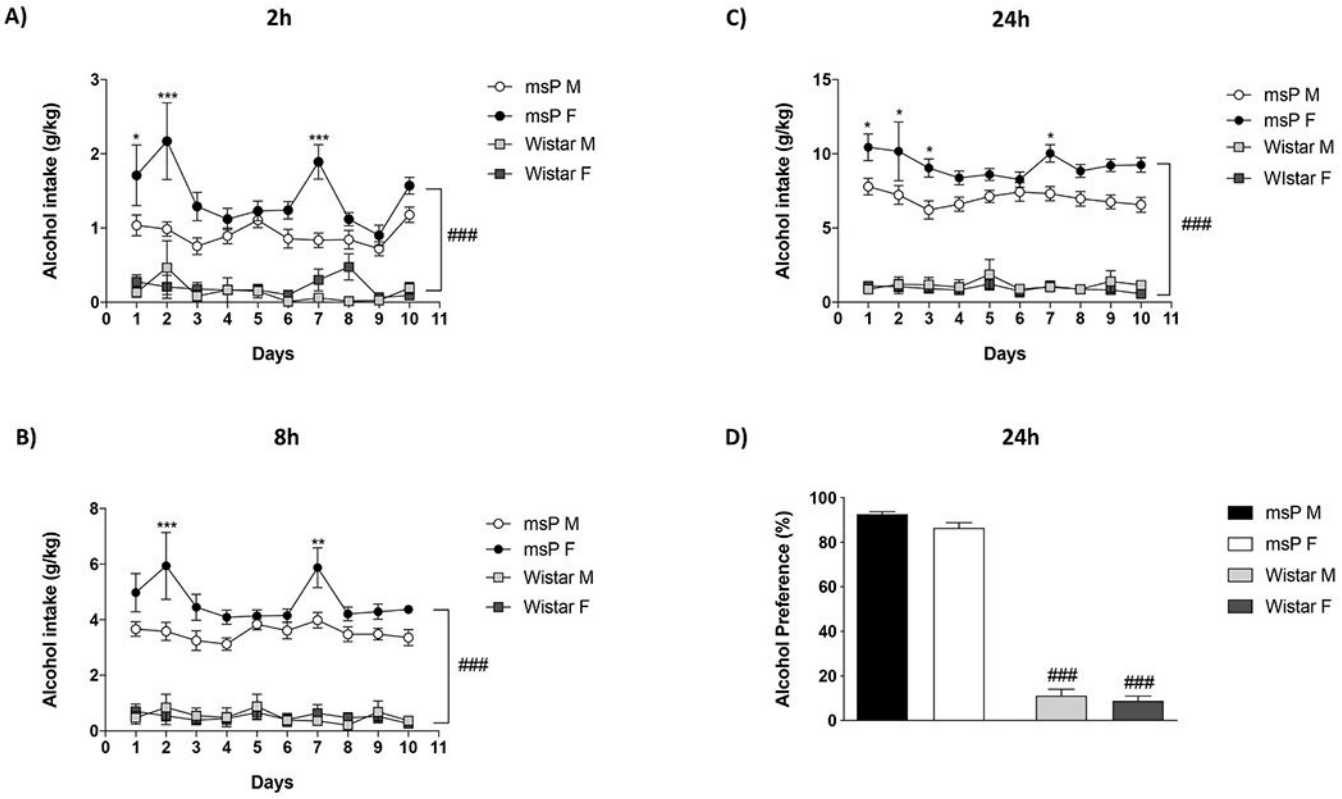

Fig. 1.

Voluntary 10% alcohol intake in male msP (n = 14/group) and Wistar (n = 12/group) rats and female msP (n = 10/group) and Wistar (n = 12/group) rats at (A) 2 h (B) 8 h, and (C) 24 h. Alcohol intake is expressed as g/kg to reduce the influence of differences in body weight. Alcohol preference is expressed as the mean percentage ± SEM of the last 3 days/24 h of alcohol intake over water (D). The data are expressed as mean ± SEM. $^{###}p < 0.001$, $^{##}p < 0.01$, $^{#}p < 0.05$, difference between msP and Wistar; $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, difference between males and females (three-way ANOVA followed by Newman-Keuls *post hoc* test).

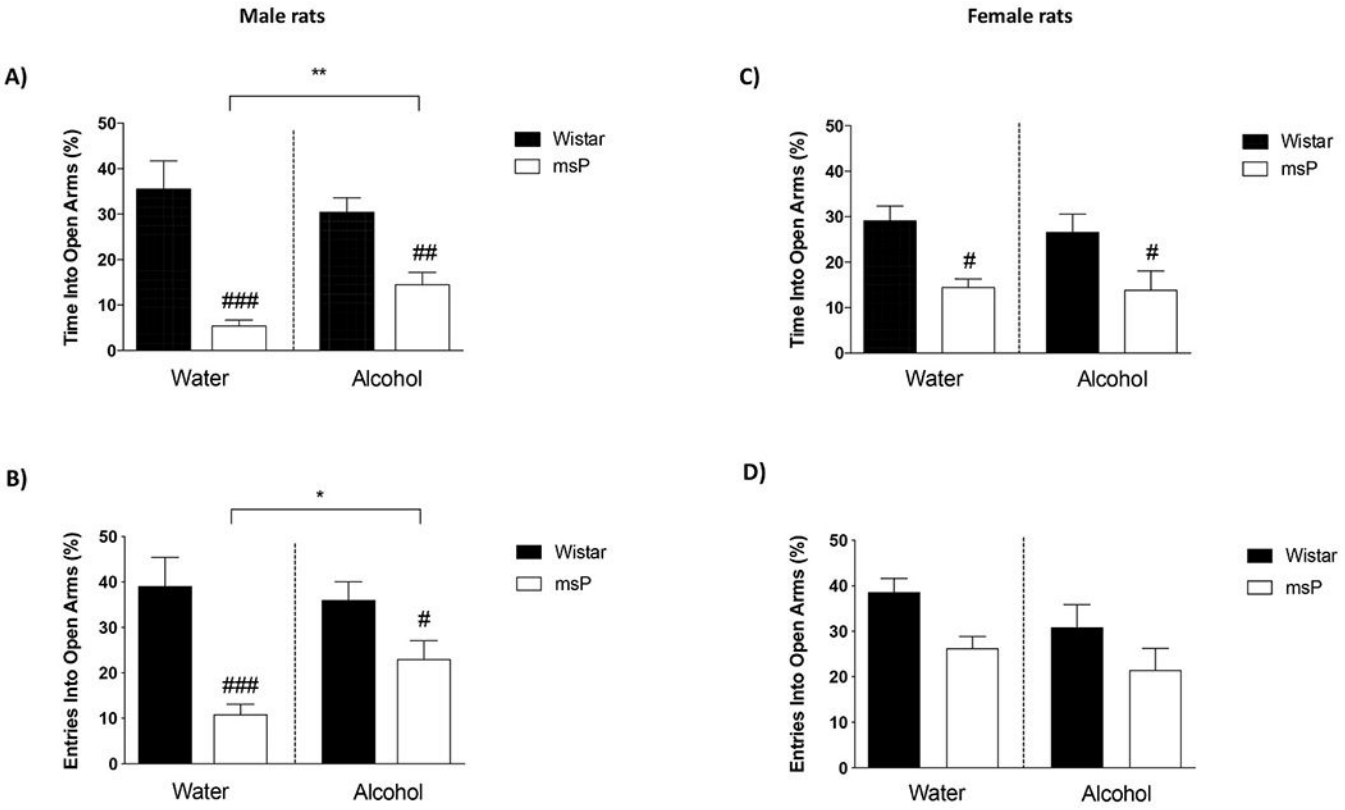

Fig. 2.

Anxiety-like behavior in (A–B) male msP (n = 14/group) and Wistar (n = 12/group) rats and (C–D) female msP (n = 10/group) and Wistar (n = 12/group) rats in the elevated plus maze. The data are expressed as the mean percentage ±SEM of open arm time and open arm entries. $^{###}p < 0.001$, $^{##}p < 0.01$, difference between msP and Wistar; $^{**}p < 0.01$, $^{*}p < 0.05$, difference between alcohol and water (two-way ANOVA followed by Newman-Keuls *post hoc* test).

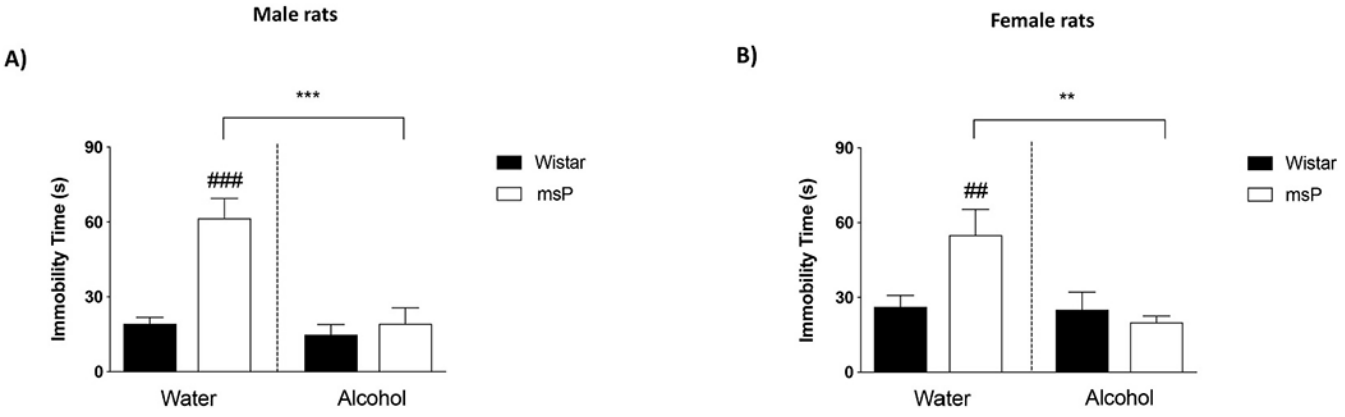

Fig. 3.

Depressive-like behavior in (A) male msP (n = 14/group) and Wistar (n = 12/group) rats and (B) female msP (n = 10/group) and Wistar (n = 12/group) rats in the forced swim test. The data are expressed as mean ± SEM. $^{###}p < 0.001$, $^{##}p < 0.01$, difference between msP and Wistar; $^{***}p < 0.001$, $^{**}p < 0.01$, difference between alcohol and water (two-way ANOVA followed by Newman-Keuls *post hoc* test).

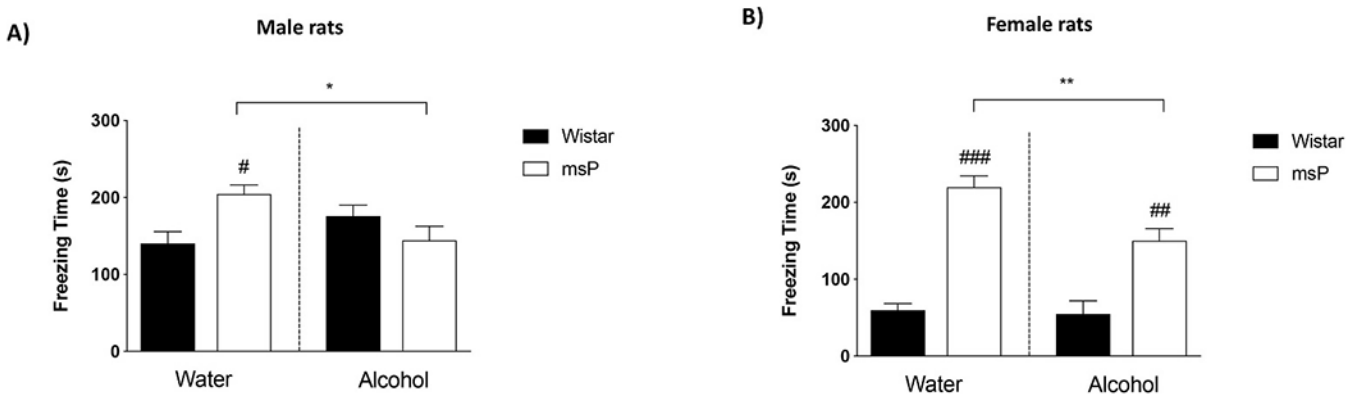

Fig. 4.

Foot-shock induced freezing in (A) male msP (n = 10/group) and Wistar (n = 10/group) rats and (B) female msP (n = 10/group) and Wistar (n = 10/group) rats. The data are expressed as mean ± SEM of the total time spent in freezing during 7 consecutive 1-min blocks. $^{###}p < 0.001$, $^{#}p < 0.05$, difference between msP and Wistar; $^{**}p < 0.01$, $^{*}p < 0.05$, difference between alcohol and water (two-way ANOVA followed by Newman-Keuls *post hoc* test).

Table 1

Summary of the key genetic, behavioral and neuroimaging studies on msP rats.

Type of study	Result	Reference
Genetic	msP rats show innate upregulation of CRF1-R expression and density in multiple corticolimbic regions.	Hansson et al. (2006)
Genetic	Voluntary alcohol consumption produces down-regulation of Crhr1 transcript levels in multiple corticolimbic regions.	Hansson et al., 2007
Genetic	Genetic polymorphism in the CRF1-R promoter region is associated to an increased sensitivity to the effects of the pharmacological blockade of CRFI-R.	Ayanwuyi et al., 2013
Genetic	1 Polymorphism in the CRF1-R gene plays a role in shaping the high anxious phenotype of msP rats.	Cippitelli et al. (2015)
Genetic	msP rats show innate upregulation of N/OFQ and NOP mRNA expression in multiple corticolimbic regions.	Economidou et al., 2008
Behavior	Access to alcohol reduce the anxious and depressive phenotype of msP rats.	Ciccocioppo et al., 1999
Behavior	Alcohol induces conditioned place preference in msP rats.	Ciccocioppo et al., 1999
Behavior	msP rats are highly motivated to lever press for alcohol and show high level of seeking behavior in response to stress and environmental cues.	Ciccocioppo et al., 2004; Cippitelli et al., 2008
Behavior	msP rats exhibit a robust ADE when they are returned to alcohol after a forced abstinent period.	Perfumi et al., 2005
Behavior	Characterization of alcohol-addiction phenotype in msP rats based on the 0/3 crit model.	Domi et al. (2019)
Neuroimaging	Innate reduction of gray matter volume in different thalamic and cortical regions in msP rats.	Gozzi et al. (2013)
Neuroimaging	Microstructural changes in white matter after voluntary alcohol drinking associated to a decreased extracellular space tortuosity.	De Santis et al., 2019; De Santis et al., 2020
Neuroimaging	Amphetamine challenge induces strong functional responses in the extended amygdala of msP rats.	Bifone et al. (2019)