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Sex differences in adult Wistar rats in the voluntary consumption of ethanol after pre-exposure to ethanol-induced flavor avoidance learning

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ABSTRACT

Vulnerability to ethanol abuse may be a function of the balance between the opposing (aversive and rewarding) motivational effects of the drug. The study of these effects is particularly important for understanding alcohol addiction. Research in this field seems to point out that ethanol effects are determined by a set of internal factors (sex, ethanol intake history, *etc.*), as well as by environmental conditions surrounding the individual (*i.e.*, stress) and, of course, the interactions between all these factors. This work explores sex differences in sensitivity to aversive effects of ethanol using the procedure of flavor avoidance learning (FAL), as well as the effect of this learning experience on subsequent voluntary ethanol consumption, in adult rats. The results obtained indicated a slight sex based difference in the amount of FAL acquired in that females acquisition was weaker (experiment 1), and a differing influence of previous experience with the aversive effects of ethanol on the voluntary consumption of the drug for each sex (experiment 2). In particular, it was observed that female ethanol-naive rats showed a higher intake level and preference for ethanol than both ethanol-experienced female rats and ethanol-naive male rats. In contrast, the ethanol-experienced male rats showed a greater consumption of and preference for ethanol than ethanol-naive male rats and ethanol-experienced female rats. These data are discussed noting a range of possible explicative factors (sex hormones, hedonic processing, *etc.*), but further studies are warranted to elucidate the mechanisms by which ethanol pre-exposure influences the subsequent intake of ethanol differently by sex.

1. Introduction

Ethanol is probably the most consumed psychoactive drug in the world. Controlled drinking is common, but for many individuals ethanol consumption leads to addictive disorder. The reason why some individuals progress more quickly and easily than others from controlled drinking to the abuse of this substance is an issue that elicits wide and varied research. There are many aspects to consider in relation to repeated addictive ethanol intake, including individual differences (sex, for instance), environmental conditions surrounding the individual, and, of course, the interactions between all these factors.

Positive reinforcement mediated by ethanol has often been considered to be the key mechanism underlying the initiation and maintenance of alcohol consumption (*i.e.*, Pautassi et al., 2009). Ethanol reduces anxiety and dysphoria (Wilson et al., 2004), suggesting that its use could be motivated by its ability to counteract stress, and to reduce anxiety and depression (Kamenetzky et al., 2007; Koob, 2006). Both sources of reinforcement, positive as well as negative, increase the frequency of behaviors aimed at seeking out and consuming ethanol. Procedures such as conditioning of place preference (*i.e.*, Koob and LeMoal, 2008), oral operant and intravenous self-administration (*i.e.*, Green and Grahame, 2008; Sanchis-Segura and Spanagel, 2006), and free ethanol consumption, are generally used to evaluate ethanol-mediated motivational learning (reviewed in De la Torre et al., 2013). The most common way to examine oral ethanol consumption is the two-bottle access procedure, in which animals have continuous free access to two bottles, one containing water and the other a dilute ethanol solution. By measuring the volume of ethanol and water consumed, one can elucidate behavioral patterns among rodents of different genetic backgrounds. On the other hand, negative reinforcing effects can be evaluated *via* approach-avoidance tasks such as the elevated maze, and defensive prod-burying models (Wilson et al., 2004).

It is also likely that variations in ethanol consumption may reflect underlying differences in the innate threshold of the aversive properties of ethanol. The negative post-absorptive effects of ethanol can act as aversive unconditioned stimuli (US), reducing the preference for stimuli associated with the administration of the drug (Cunningham et al., 2002a,b; reviewed in Busse et al., 2005; see also www.CTAlearning.com). In fact, the flavor avoidance learning paradigm (FAL) is largely considered to be one of the most reliable and sensitive paradigms for

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assessing the aversive effects of certain drugs, including alcohol (Awasaki et al., 2011; Escarabajal et al., 2003).

One must keep in mind, however, the derivative effects of repeated exposure to alcohol, in other words, the processes of tolerance and sensitization that reflect an effort on the part of the organism to adapt to the secondary and/or chronic effects of the drug of abuse. Both processes have been implicated in the progressive increase of ethanol ingestion observed in individuals who abuse or are addicted to alcohol. On occasion, the increase has been attributed to an effect of tolerance to the effects of ethanol reinforcement, which assumes that the individuals increase the dose consumed in order to maintain the desired effect of the drug. On the other hand, this greater consumption can also be explained in terms of tolerance to the inhibitory and aversive effects of the drug, which initially limit its consumption but gradually disappear with repeated exposure. Furthermore, this increase in consumption has been explained as sensitization to the reinforcing effects of the drug. That is, progressive increases in drinking may be attributed to an increase in the positive affective consequences of exposure to a given amount of drug (reviewed in Cunningham et al., 2002a,b). Thus, the conditions under which the stimulus properties of ethanol change with repeated exposures are not well defined.

Regarding sex differences, most studies of the effects of repeated alcohol consumption have focused on males, whereas the epidemiological and clinical data have shown substantial differences between the sexes regarding alcohol abuse and dependency. In women, for example, the time lapse between initiation and the onset of alcohol abuse is shorter (Greenfield, 2002), and women also differ from men in their sensitivity to the acute or chronic consequences of ethanol (Fillmore and Weafer, 2004). It is interesting that Dawson and Archer (1993) identified the female gender as a risk factor for developing alcohol dependence despite the fact that the daily average ethanol intake by men is about double that of women after adjusting for body weight and body water. Sexually dimorphic patterns of addictive behavior occur around the time of puberty and continue throughout adulthood suggesting a hormonal basis for this sexual dimorphism (Witt, 2007).

Studies that examine levels of consumption of ethanol in rodents have established that adult females exhibit greater ethanol consumption than do adult males (Caihol and Mormede, 2001; Chester et al., 2006; Le et al., 2001). These differences have been observed in non-consanguine rats (*i.e.*, Lancaster et al., 1996), as well as in rats genetically selected for their high or low ethanol consumption levels (*i.e.*, Le et al., 2001; Chester et al., 2006). Furthermore, these differences between males and females have been observed using various methods, not only 24 h access to ethanol (Cacace et al., 2011, 2012; Doremus et al., 2005; Yoneyama et al., 2008), but also limited access to ethanol with access to two bottles (Le et al., 2001; Vetter-O'Hagen et al., 2009), and with operant self-administration models (Blanchard et al., 1993; Blanchard and Glick, 1995).

Vetter-O'Hagen et al. (2009), using a 2 h limited-access to ethanol model, found that the ethanol consumed varied as a function of sex and age, although the sex differences that emerged at each age were opposites: adolescent males consumed more ethanol than both the adolescent and adult females, while in adulthood, echoing the data in the previous paragraph, the females consumed more ethanol than the males. Nevertheless, it should be noted that not all studies point to these differences in sex, as in the findings obtained by García-Burgos et al. (2009), using a 24 h unlimited-access to ethanol model. While their results do seem to indicate a greater consumption of ethanol in adolescent rats, they suggested no difference by sex.

The above results reveal enough of a discrepancy in the data thus far obtained so as to warrant further study of the relationship between consumption of alcohol and sex. Furthermore, one must keep in mind that the use and abuse of a specific drug may be a function of the balance between its rewarding and aversive effects, warranting inquiry into whether aversive effects of a drug are dependent upon sex. In fact, understanding the relationship between the sex of the subjects and the rewarding/aversive effects of a drug may provide insight into how sex may affect an individual's vulnerability to use and abuse drugs (Rinker et al., 2008).

The present study explores not only the possible sex differences in voluntary ethanol intake, but also the possible differences in sensitivity to the aversive effects of ethanol, using an ethanol-induced FAL procedure.

While robust, FAL appears to be influenced by a variety of factors (see Riley and Freeman, 2004). One factor that has received considerable attention is the sex of the subject used in the FAL procedure. Sex differences in FAL have been examined with a variety of compounds such as lithium chloride (LiCl) (Chambers et al., 1981; Dacanay et al., 1984), cocaine (Busse et al., 2005; Jones et al., 2006) and ethanol (Caihol and Mormede, 2002; Morales et al., 2014; Schramm-Sapyta et al., 2014; Sherrill et al., 2011a). The relative sensitivity of males (compared to females) within this preparation is generally reported, although the strength of the sex difference varies across studies and is dependent upon a number of factors (see Busse et al., 2005). In addition, some studies fail to show any sex differences at all to the aversive effects of drugs of abuse such as nicotine (Rinker et al., 2008) or even ethanol (Morales and Spear, 2013; Vetter-O'Hagen et al., 2009).

Appreciating the diverging documentation to date, we address in this study the sex differences in ethanol-induced FAL, as well as whether these potential differences could have effects, particularly sex-based, on posterior voluntary consumption of ethanol.

Both sexes were included given that, despite evidence for sex differences in ethanol consumption (Blanchard et al., 1993; Doremus et al., 2005; Lancaster et al., 1996), relatively few studies have examined potential sex differences in the emergence of tolerance or sensitization to ethanol, and among those that have, the findings are mixed (Caihol and Mormede, 2002; Linsenbardt et al., 2009; Morales et al., 2011; Sherrill et al., 2011a; Webb et al., 2002). In fact, the conditions under which the stimulus properties of ethanol change with repeated exposure are not well defined, especially with regard to differences in male vs female responses to repeated exposure to ethanol. For this reason we feel the need for deeper study of the question of possible differences due to sex in alcohol consumption in subjects pre-exposed to the drug, a factor which could indicate sex differences in tolerance and/or sensitization to the aversive and/or reinforcing effects of ethanol.

2. Materials and method

2.1. Animals

The subjects were 40 Wistar rats, 20 females and 20 males, provided by Janvier Labs. (Rennes, France). All of them were three months old. The female rats weighed between 230 and 240 g and the male rats between 260 and 300 g at the beginning of the experiment. All animals were individually housed in methacrylate cages, which also served as training chambers during the experiment. A 12:12-hr light/dark cycle was in effect with lights on at 08:00 am. The temperature of the experimental room ranged between 21° and 23 °C. The feeding of the animals throughout the experiment consisted of a daily ration (25 g) of A04 compound feed (Panlab, Barcelona). They were allowed access to water *ad libitum* until the start of the program of deprivation, in the case of experiment 1, at which time the animals had restricted access to water or other fluid. All the behavioral procedures were conducted in agreement with the Bioethics' Committee of the University of Jaén, and followed the Spanish and European Guidelines for the Care and Use of Laboratory Animals (R.D. 53/2013; Directive 2010/63/UE).

2.2. Drugs

As unconditioned stimuli in the FAL task, ethanol was used (96% Aditio; Panreac, España) at 1.5 g/kg intraperitoneally (IP; 12.5% v/v). Saline (0.9%) was used for vehicle injections. The gustatory stimuli