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# The mineralocorticoid receptor antagonist spironolactone reduces alcohol self-administration in female and male rats

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ABSTRACT

Cortisol/corticosterone and the hypothalamic-pituitary-adrenal (HPA) axis serve an important role in modulating alcohol drinking behaviors. To date most alcohol research has focused on the functional involvement of corticosterone and the glucocorticoid receptor (GR), the primary receptor for corticosterone. Recent studies have indicated that the related mineralocorticoid receptor (MR), which binds both corticosterone and aldosterone, may also play a role in alcohol drinking. Therefore, the purpose of the present study was to test the functional role of MR signaling in alcohol self-administration via pharmacological antagonism of the MR with spironolactone. Male and female Long-Evans rats were trained to self-administer a sweetened alcohol solution (15% (v/v) alcohol + 2% (w/v) sucrose). The effects of spironolactone (0, 10, 25, 50 mg/kg; IP) were tested on alcohol self-administration and under "probe extinction" conditions to measure the persistence of responding in the absence of the alcohol reinforcer. Parallel experiments in sucrose self-administration trained rats were used to confirm the specificity of spironolactone effects to an alcohol reinforcer. In female rats spironolactone (50 mg/kg) reduced alcohol self-administration and persistence of alcohol responding. In male rats spironolactone (25 and 50 mg/kg) reduced alcohol self-administration, but not persistence of alcohol responding. Spironolactone reduced sucrose intake in female rats only, and locomotion in male and female rats during sucrose self-administration. There was no effect of spironolactone on persistence of sucrose responding. These studies add to growing evidence that the MR is involved in alcohol drinking, while underscoring the importance of studying both male and female animals.

## 1. Introduction

There is a wealth of literature examining the role of the hypothalamic-pituitary-adrenal (HPA) axis and corticosteroids in alcohol consumption, seeking, and dependence (Koenig and Olive, 2004; Vendruscolo et al., 2012; Vendruscolo et al., 2015). Within the HPA axis, cortisol (corticosterone in rodents) is one of the primary hormonal stress signals, and several studies in male adrenalectomized rats have shown that corticosterone moderates alcohol drinking (Fahlke et al., 1994a; Fahlke et al., 1995). To date, most studies of alcohol and the HPA axis have focused on the role of glucocorticoid receptors (GRs), which primarily bind corticosterone, in alcohol-related behaviors. For example, in preclinical studies of male rats, the GR and progesterone receptor antagonist mifepristone has been shown to block escalation of alcohol drinking following induction of dependence by chronic alcohol vapor exposure (Vendruscolo et al., 2012), and reduce alcohol consumption in a homecage limited-access two-bottle choice study (Koenig and Olive, 2004). Additionally, in alcohol-dependent male rats, GRs are downregulated in the prefrontal cortex (PFC), nucleus accumbens (NAc), and bed nucleus of the stria terminalis (BNST) during acute alcohol withdrawal, and upregulated in the NAc core, ventral BNST, and central amygdala (CeA) 3 weeks into abstinence (Vendruscolo et al., 2012). Overall these studies suggest that glucocorticoid signaling via the GR plays a dynamic role in both acute alcohol consumption and alcohol dependence, though it is important to note the bias towards utilizing male subjects in the literature as the HPA axis is known to be sexually dimorphic, both in normal and diseased states (Bangasser and Valentino, 2014). For example, in female mice with a history of predator stress and alcohol drinking GR is upregulated in the PFC during acute withdrawal, while males show no change (Finn et al., 2018).

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Surprisingly, few alcohol-related studies have focused on the functional role of the mineralocorticoid receptor (MR). The MR has mainly been studied for its peripheral effects such as modulating fluid balance and blood pressure via its endogenous ligand, aldosterone, but is also known to modulate memory formation, fear extinction, and recall in male rats (Zhou et al., 2010; Dorey et al., 2011; Zhou et al., 2011; Ter Horst et al., 2012; Gomez-Sanchez and Gomez-Sanchez, 2014). Furthermore, there are sizeable sex differences in the role of MR in fear extinction, with female mice showing greater extinction deficits following MR deletion in the forebrain than male mice (Ter Horst et al., 2012). While the MR is traditionally thought of as a cytosolic ligand-dependent transcription factor that effects genomic changes on the time-scale of hours, recent studies have identified a membrane bound variant of the MR that can act on the time-scale of minutes (Karst et al., 2005; Khaksari et al., 2007; Dorey et al., 2011; Gomez-Sanchez and Gomez-Sanchez, 2014). In fact, the MR also binds corticosterone, and is expressed in brain regions generally associated with addiction such as the prefrontal cortex, hippocampus, and amygdala (Reul and de Kloet, 1986; Fuller et al., 2000). The MR has also been shown to mediate some responses to corticosterone that GR does not, such as modulating hippocampal glutamate signaling (Karst et al., 2005) and corticosterone-induced impairment of memory retrieval (Khaksari et al., 2007). Earlier studies reported the lack of modulatory effect of MR antagonism on alcohol drinking (Koenig and Olive, 2004; O'Callaghan et al., 2005) (see later discussion), and MR mRNA levels are not changed during acute withdrawal in alcohol dependent male rats (Vendruscolo et al., 2012). However, a recent multi-species study linked lower MR gene expression levels in the central amygdala (CeA) to higher alcohol drinking behavior in male primates with a history of alcohol consumption and more compulsive-like alcohol drinking in alcohol dependent male rats (Aoun et al., 2018). In male and female humans it was confirmed that higher levels of the MR ligand aldosterone correlated with higher alcohol craving in recovering alcohol use disorder (AUD) patients, and that non-abstinent patients had higher levels of aldosterone than abstinent patients (Leggio et al., 2008; Aoun et al., 2018). As such, there is growing evidence that MR signaling may play an important role in alcohol drinking behavior, and that there may be sex differences in this role, but it is unclear if this receptor may prove a potential therapeutic target.

One of the goals of the present study was to assess the role of MR signaling in the maintenance of alcohol self-administration using the MR antagonist spironolactone. Another goal of this study was to examine the effects of spironolactone on behavior under extinction conditions. To do this, probe extinction tests were used in which the cues associated with the reinforcer were presented, but alcohol delivery was withheld. This test allows for the examination of the persistence of responding in the presence of drug-associated cues, but absence of the primary reinforcer, which is an important feature of drug seeking behavior. Based on the literature, we hypothesized that MR antagonism would reduce alcohol self-administration and the persistence of non-reinforced alcohol responding. We also hypothesized that females would be more sensitive to this MR antagonism given greater behavioral response to MR knockout as well as documented HPA axis dimorphism (Ter Horst et al., 2012; Bangasser and Valentino, 2014). To test this hypothesis, male and female Long-Evans rats were trained to self-administer a sweetened alcohol solution (15% (v/v) alcohol + 2% (w/v) sucrose) and administered spironolactone prior to alcohol self-administration and probe extinction sessions. Furthermore, to explore the specificity of this effect to an alcohol reinforcer, spironolactone was tested in a separate group of male and female Long-Evans rats trained on sucrose self-administration. Together with recent studies implicating MR signaling in alcohol drinking behavior, these findings suggest that the MR may be an important avenue for research in the alcohol field.

## 2. Materials and methods

### 2.1. Animals

38 adult Long-Evans rats (19 male/19 female) were single housed under a 12 h light/dark cycle (7am/pm). All experiments were conducted during the light cycle. Animals were continuously monitored and cared for by the veterinary staff of the UNC-Chapel Hill Division of Comparative Medicine. All procedures were carried out in accordance with the NIH Guide for Care and Use of Laboratory Animals and institutional guidelines. All protocols were approved by the UNC Institutional Animal Care and Use Committee (IACUC). UNC-Chapel Hill is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

### 2.2. Apparatus

Self-administration was conducted in operant chambers (Med Associates, Georgia, VT) located within sound-attenuating cabinets equipped with an exhaust fan to provide ventilation and mask outside noise. Chambers were equipped with two retractable levers on opposite sides of the chamber (left and right), and a cue light was located above each lever. When the response requirement was met on the left (active) lever, a cue light (directly above the lever) and a stimulus tone were presented for the duration of the alcohol reinforcer delivery (0.1 mL of solution into a well on the left side of the chamber across 1.66 s). Responding during reinforcer delivery and on the right (inactive) lever was recorded, but had no programmed consequences. Chambers were also equipped with 4 parallel infrared beams across the bar floor to measure general locomotor activity throughout the session. The number of beam breaks for the entire session was collected and this total was divided by the session length (30 min) to represent the locomotor rate (beam breaks/min).

### 2.3. Alcohol self-administration training

Rats were trained to self-administer a 15% (v/v) alcohol + 2% (w/v) sucrose solution (15A/2S) on a fixed ratio 2 (FR2) schedule of reinforcement in 30 min sessions, five days a week (M–F) via sucrose fading as described in (Randall et al., 2017). Sucrose fading began with self-administration of 10% sucrose (10S), then 2A/10S, 5A/10S, 10A/10S, 10A/5S, 15A/5S, 15A/2S on subsequent sessions, 5 sessions of 15A, and then remained at 15A/2S for the duration of training. A sweetened alcohol reinforcer was used as we find this results in stable alcohol self-administration in these long-term studies (Randall et al., 2015; Randall et al., 2017; Jaramillo et al., 2018). Rats that did not consistently self-administer at least 0.4 g/kg alcohol were excluded, and there were two male rats that did not meet this criterion and were not included in this study. Rats had approximately 4 months of self-administration training and were used in an unrelated non-drug study (i.e., involved exposure to a single stressor and self-administration was unaltered (unpublished)) a month prior to the initiation of this study.

### 2.4. Sucrose self-administration training

Rats were trained to self-administer a 0.8% (w/v) sucrose solution (0.8S) on an FR2 schedule over 30 min sessions, five days a week (M–F) via sucrose fading as described above. Sucrose fading began with self-administration of 10S, then 5S, 2S, 1S, then 0.5S for one week before returning to 0.8S for the remainder of the study. This dose of sucrose was selected as it resulted in similar levels of lever responding as the alcohol self-administration group (Jaramillo et al., 2018).

#### 2.4.1. Experiment 1: effect of spironolactone on maintenance of alcohol self-administration

To measure the effect of spironolactone on maintenance of alcohol