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Research report Dr Georgina M. Renard

Contact details of the applicant:

Name: Georgina Maria Renard
Title: PhD in Biological Science
Address: Av. Gran Bretaña 1111 – Playa Ancha – Valparaiso - Chile
Tel No: +56 32 2508038
Email address: georgina.renard@uv.cl
Current Position: Assistant Professor

Role of the limbic vasopressinergic system in sex differences of addictive-like behaviors

Drug addiction is a chronic brain disease characterized by compulsive drug use, loss of control over its consumption, and emergence of a negative emotional state (dysphoria, anxiety, irritability) that exacerbates during withdrawal (Koob & Le Moal 1997, Koob & Le Moal 2008). Importantly, in the last years more women become to have problems with drugs of abuse. Furthermore, women become dependent more quickly than men after a first casual drug use and also experience a withdrawal syndrome with high levels of desire and relapse (Bobzean *et al.* 2014, Brady & Randall 1999). Russo and colleagues (2003) demonstrated that the acquisition of cocaine conditioned place preference (CPP; an animal model for the study of the rewarding effects of drugs of abuse) by cocaine requires less time (conditioning sessions) and smaller doses of the drug in female rats compared to male rats. In addition, it has been shown that female rats have stronger amphetamine (AMPH) preference in CPP than male rats (Brown *et al.* 2011). Thus, one important question in addiction research is determining sex differences in vulnerability to drug addiction. In this project, I propose that the vasopressinergic projections from the medial amygdala (MeA) to the lateral septum (LS) are implicated in sex differences of addictive behaviors. In this sense, the vasopressinergic system features marked sex differences in the brain. In the 80s, studies by De Vries and colleagues (1983) showed that the density of vasopressinergic fibers in the LS is larger in male rats compared to female rats. Besides, one of the most problematic consequences of drug abuse are the alteration of different social behaviors (Young *et al.* 2011). Remarkable, brain arginine-vasopressin (AVP) has a major role in regulating these kind of behaviors (Caldwell *et al.* 2008).

My hypothesis is:

"MeA-LS vasopressinergic system is affected by amphetamine in a sex dependent manner"

This hypothesis is based mainly on the following observations:

First, both the LS and the Amygdala are implicated in addictive process (Sartor & Aston-Jones 2012a, Sartor & Aston-Jones 2012b) and their connections were scarcely studied.

Second, vasopressinergic fibers that project from the amygdala to the LS are denser in male than in female rats and the LS expressed high levels of V_{1A} receptors (de Vries 2008, Ostrowski et al. 1992). So, this system could be modulating addictive-like behaviors in a sex dependent manner.

Third, it has been shown that drugs of abuse produce alterations in AVP protein expression and AVP mRNA levels in some nuclei in the brain (Rodriguez-Borrero *et al.* 2010, Zhou *et al.* 2005, Zhou *et al.* 2011).

Specific aim 1: To study the effect of amphetamine (AMPH) on conditioned place preference (CPP) and locomotor sensitization in male and female rats.

Specific aim 2: To determine changes in protein AVP levels in LS and AVP release in the LS of male and female rats exposed to AMPH CPP.

Results

Conditioned Place Preference

Our results showed that the 50% of females and 78% of males had significant preference for the AMPH-associated compartment.

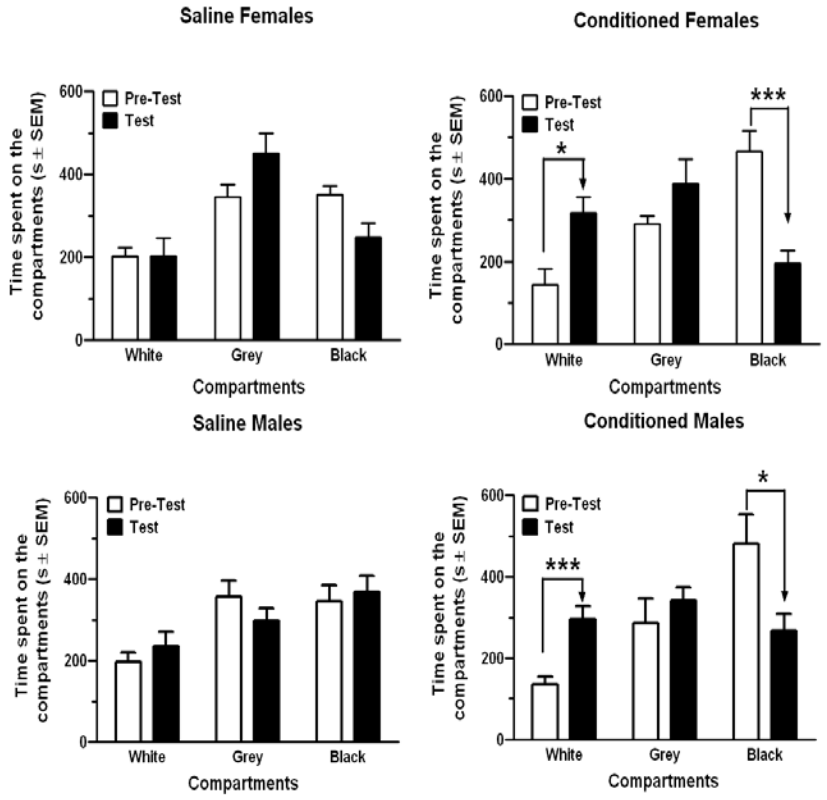

Figure 1. Time spent on different compartments of the conditioned place preference apparatus before (pre-test) and after (test) the conditioning to AMPH of (A) control (n=7) and AMPH-treated females (n=6) and (B) control (n=6) and AMPH-treated males (n=7).*p< 0,01 (A) y **p< 0,001 (B) .

AVP content in LS is higher in saline males than females. Amphetamine conditioned males showed reduced AVP content in LS than saline males. Currently we are performing additional experiments to study if this difference is significative.

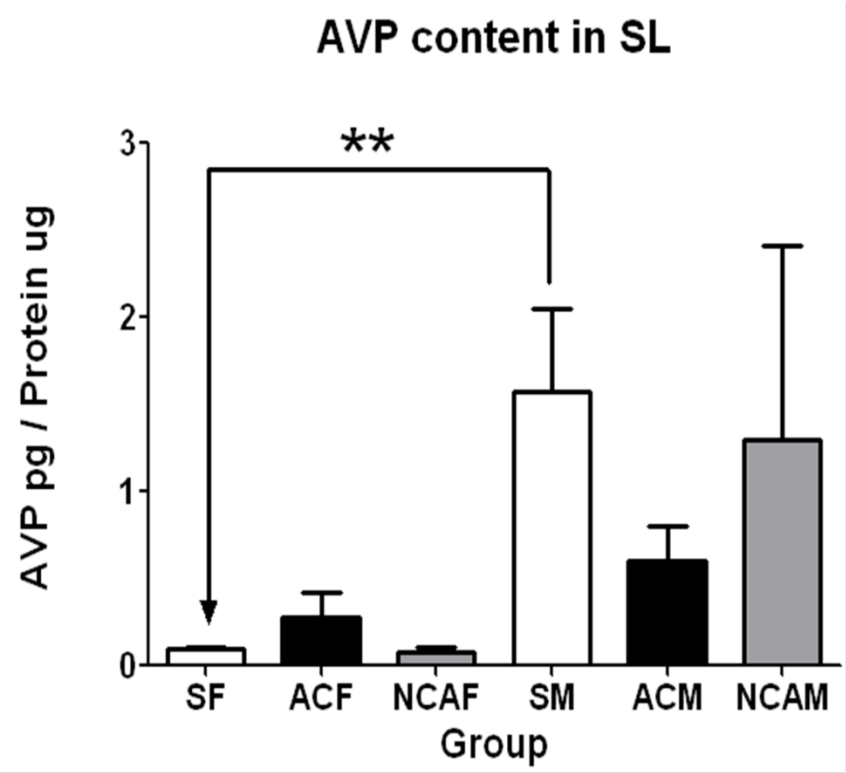

Figure 4. AVP content expressed as pg/ug of total protein in lateral septum of saline females (SF; n=4), amphetamine conditioned females (ACF; n=4), non-conditioned amphetamine females (NCAF; n=3), saline males (SM; n=4), amphetamine conditioned males (ACM; n=4) and non-conditioned amphetamine males (NCAM; n=2)

Locomotor sensitization

Animals that do not show sensitization to AMPH show higher locomotors activity at first AMPH injection than sensitized animals.

Both males and females obtained the same rate of locomotor sensitization to AMPH and small differences between male and female rats were found in this addictive like behavior.

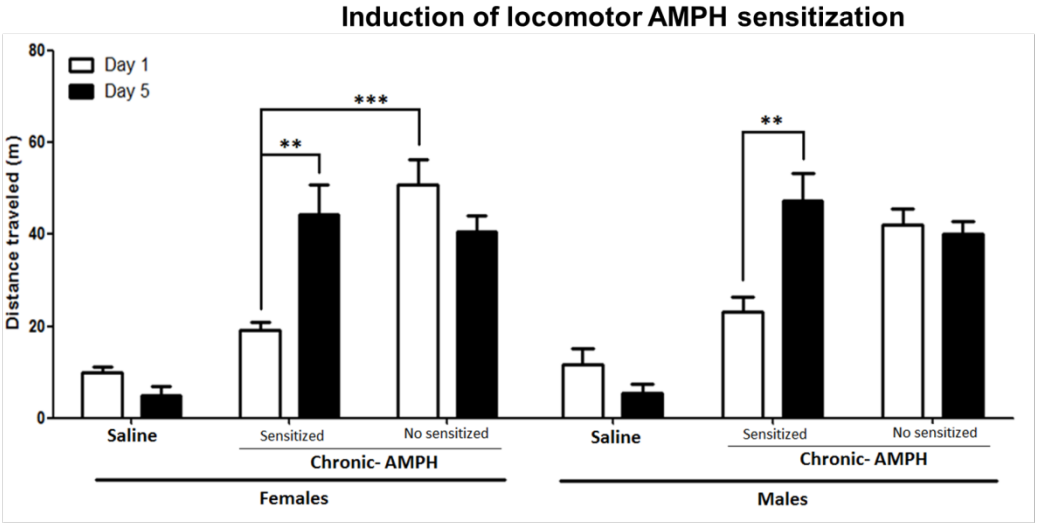

Fig. 1. Locomotor sensitization to AMPH expressed in distance traveled (m) at day 1 and 5 of the induction process in female and male rats (n = 11 saline females and 7 saline males, 11 sensitized females and 7 sensitized males, 3 not sensitized females and 2 not sensitized males). ** $p < 0.01$; *** $p < 0.001$

AMPH produce a decrease in AVP content in LS.

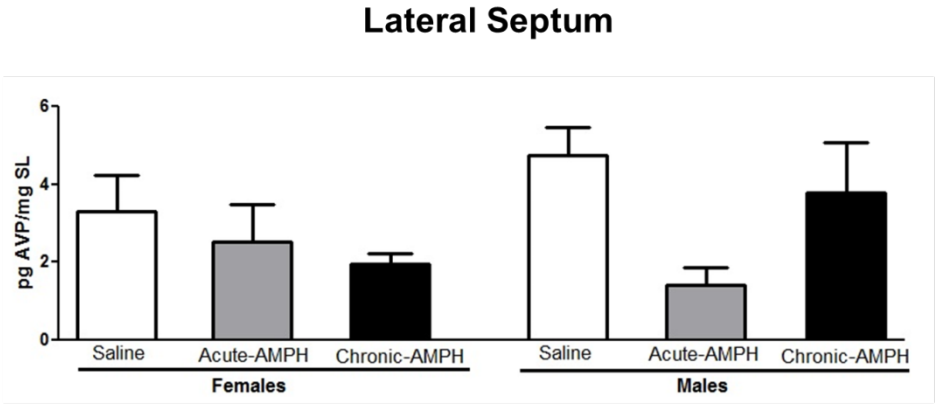

Fig. 4. AVP content in the lateral septum (LS) of control and AMPH-treated male and female rats.

Results communications

The results summarized above were presented at the III Symposium Miguel Ozorio de Almeida (SMOA), Rio de Janeiro, Brazil (May 2015) and in the Join meeting between the Chilean Societies of Pharmacology, Physiological Sciences and Neurosciences, Coquimbo, Chile (September 2105).

The fund was spent on the obtaining the following chemicals and materials:
* Chemicals for the histochemical: Primary antibody (The experiments are underway)

- Two conditioned place preference apparatus.
- Two open field apparatus.
- Comercial ELISA Kit for vasopressin quantification.
- Micro-pipettes.
- Rats and rat's food.
- Part of the financial support was used to pay travel expenses for 2 undergraduate students in Brazil.