

Inhibition of Fatty Acid Binding Protein 5 and 7 Blunts Locomotor Response to Cocaine in Female Mice

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Introduction

1.1 Endocannabinoids

- The medicinal properties of cannabinoids have been well demonstrated but are overshadowed by such adverse effects of cognitive and motor dysfunction.
- The endocannabinoid system (ECS) is host to many important physiological functions including depression (Rutkowska and Jachimczuk, 2004, Bambico et al., 2010, Umathe et al., 2011, Kruk-Slomka et al., 2015), reward behavior (Maldonado et al., 2006), anxiety (Batista et al., 2014), stress (Hill and McEwen, 2010, Hillard, 2014, Gray and Vecchiarelli, 2015), and pain-sensation (Hohmann and Suplita, 2006).
- The ability of the ECS to impact the DA system has become of recent interest in the treatment for addiction.
- CB1 receptors are ubiquitous throughout the brain and are widely distributed in the mesolimbic dopamine circuit, which act as a regulatory mechanism on motivational and emotional processing (Laviolette and Grace, 2006; Melis et al. 2014; Wang and Lupica, 2014).

1.2 Fatty Acid-binding Proteins

- FABPs are a family of proteins characterized by their ability to bind fatty acids.
- FABPs act as intracellular transport of endocannabinoids, such as anandamide (AEA), and shuttle them to their point of catabolism (e.g. Fatty acid amide hydrolase (FAAH) for AEA)(Kaczocha et al. 2009).
- Deletion of FABP genes have been shown to effectively raise levels of AEA and other related N-Acylethanolamine's like palmitoylethanolamide (PEA) and oleoylethanolamide (OEA). The same effect has been achieved using the FABP 5/7 inhibitor SBF126 (Kaczocha et al., 2015).
- However, the behavioral effects from targeting FABPs and modifying the ECS have not been well characterized in the literature.

Hypothesis

Genetic deletion and/or pharmacological inhibition of FABP 5/7 will alter reward behavior

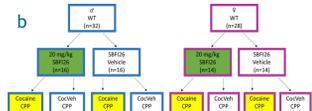
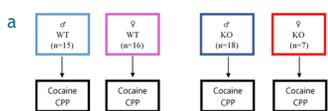
Methods

2.1 Animals

- All animals used in the following experiments were of a C57BL/6 background.
- Male and female wild-type (WT) and FABP 5/7 double knockouts (KOs) were generated as previously described (Matsumata et al., 2012).
- Mice were assigned to groups that were tested for cocaine CPP but were also co-treated with either vehicle or SBF126.
- For locomotor sensitization, male and female WT mice were used
- Animals were single housed in temperature controlled conditions (22° C) on a reverse light cycle (9:00-21:00). Food and water were provided *ad libitum*.

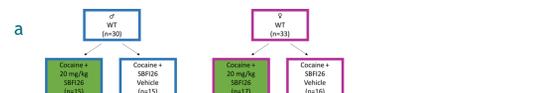
2.2 Cocaine Conditioned Place Preference (CPP)

- CPP is a paradigm that tests reward behavior by comparing time spent in an environment which has been paired with a rewarding stimulus versus a control environment.
- We carried out two separate Cocaine CPP experiments, one testing genetic deletion of FABP 5/7 (CPP-1) (Fig 2.2a) and the other to test pharmacological inhibition of FABPs 5 and 7 (CPP-2) (Fig 2.2b).
- After a single day, 15 minute preconditioning session to test for baseline preference, animals underwent 8 consecutive days of conditioning which alternated between vehicle and treatment days (10 mg/kg cocaine and/or 20 mg/kg SBF126).
- Following conditioning, all animals underwent an extinction period which lasted until no preference was observed, which was immediately followed by cocaine-induced reinstatement.



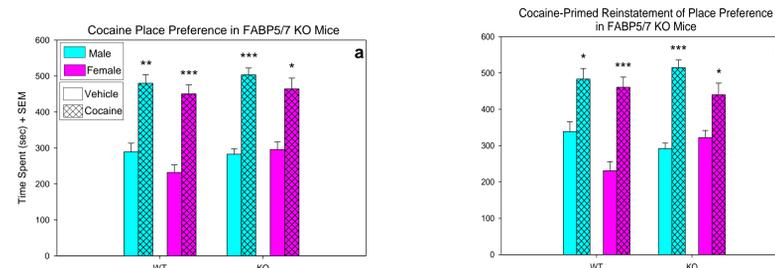
2.3 Locomotor Sensitization

- After analysis, no significant difference was found in locomotor activity between vehicle and cocaine days in the female Coc/SBF126 group, which suggests that SBF126 may blunt the locomotor response to cocaine
- To investigate this finding further, a 12 day cocaine locomotor sensitization test was conducted.
- All subjects received cocaine, but only half received SBF126 (Fig 2.3a). Two days of vehicle injections were followed by 5 days of cocaine and SBF126 or vehicle injections, and one last day of vehicle injections.

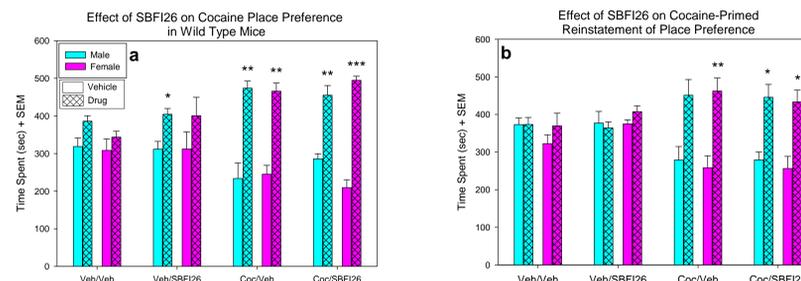


Figures/Results

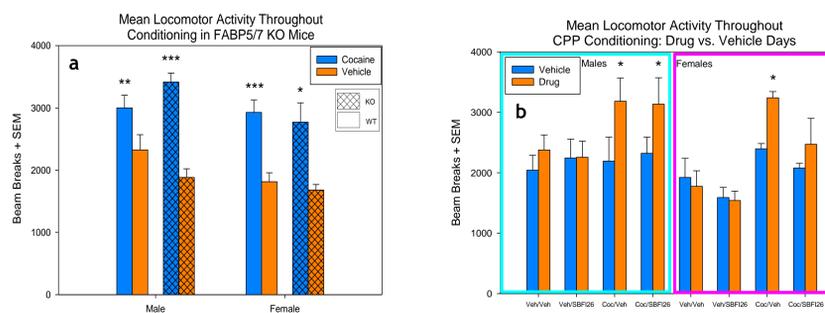
3.1 Cocaine CPP and Genetic Deletion of FABP 5/7 (CPP-1)



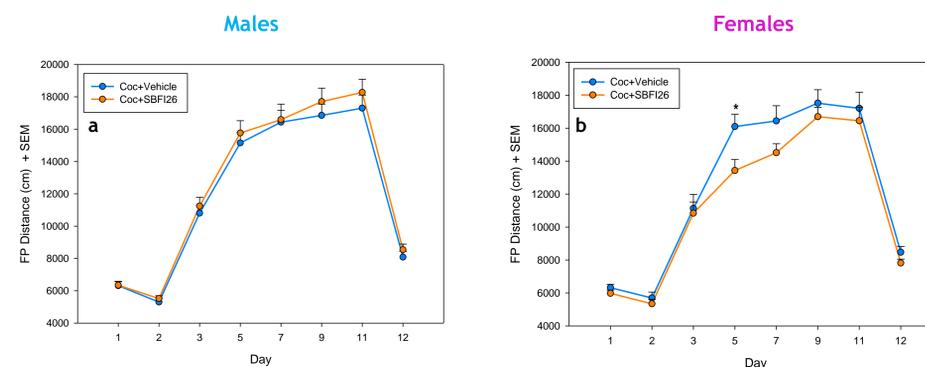
3.2 Cocaine CPP and Pharmacological Inhibition of FABPs 5 and 7 (CPP-2)



3.3 Locomotor Activity Across CPP Conditioning (CPP-1 and CPP-2)



3.4 Locomotor Sensitization to Cocaine



Results Continued

3.1 CPP-1

- All groups showed normal acquisition of cocaine CPP ([t(14) = 4.07, p = 0.001], FABP5/7 +/+ male; [t(15) = 5.00, p < .001], FABP5/7 +/+ female; [t(17) = 7.04, p < .001], FABP5/7 -/- males; [t(6) = 3.61, p < 0.05], FABP5/7 -/- female, Fig 3.1a).
- All groups also displayed cocaine-primed reinstatement ([t(14) = 2.59, p < 0.05], FABP5/7 +/+ male; [t(15) = 4.93, p < .001], FABP5/7 +/+ female; [t(17) = 6.47, p < .001], FABP5/7 -/- male; [t(6) = 2.71, p < 0.05], FABP5/7 -/- female, Fig 3.1b).

3.2 CPP-2

- Cocaine place preference was observed in all mice that received cocaine during conditioning ([t(6) = 5.73, p < .01], coc/veh male; [t(7) = 4.83, p < .01], coc/SBF126 male, [t(7) = 5.14, p < .01], coc/veh female, [t(5) = 11.13, p < .0013], coc/SBF126 female). Additionally, male veh/SBF126 animals showed preference for the drug-paired chamber [t(7) = 2.77, p < .05] (Fig 3.2a).
- Cocaine-primed reinstatement of place preference was observed in all groups that received cocaine during conditioning with the exception of the male coc/veh: ([t(6) = 2.41, p = .052], coc/veh male; [t(7) = 3.18, p < .05], coc/SBF126 male, [t(7) = 5.19, p = .001], coc/veh female, [t(5) = 3.22, p < .05], coc/SBF126 female, Fig 3.2b)

3.3 CPP Locomotor Activity

- For CPP-1, paired-samples t-tests comparing mean locomotor activity on drug conditioning days to saline conditioning days showed that all four groups displayed cocaine-induced hyperlocomotion ([t(14) = 3.10, p < 0.01], FABP5/7 +/+ male; [t(15) = 4.90, p < .001], FABP5/7 +/+ female; [t(15) = 8.17, p < .001], FABP5/7 -/- male; [t(6) = 3.34, p < 0.05], FABP5/7 -/- female, Fig 3.3a).
- For CPP-2, Males showed significantly higher mean locomotor activity on drug days in both the Coc/Veh [t(15) = 3.206, p < .01] and Coc/SBF1 groups [t(15) = 3.693, p < .01] compared to vehicle days. In the females however, only the Coc/Veh group showed significantly higher locomotor activity on drug days [t(7) = 5.105, p < .01]. A two-way ANOVA using sex and group as factors to compare locomotor activity on drug days also found a main effect of both group [F(3,84) = 10.126, p < .001] and sex [F(1,84) = 7.570, p < .01]. Holm-Sidak comparisons showed a significant difference between sexes within the Veh/SBF1 group as well, with the females showing lower activity (Fig 3.3b).

3.4 Locomotor Sensitization

- Two-way RM ANOVAs using group and day as factors showed that SBF126 had no significant effect on cocaine locomotor sensitization in male (F(1, 110) = .90, p = NS; Fig 3.4b) or female (F(1, 110) = 1.85, p = NS; Fig 3.4b) wild type mice. However, while the male data are nearly identical during sensitization, there was an apparent delay in the increase of locomotor activity seen in the female SBF126 group. Holm-Sidak post-hoc test showed this was only significant on day 5, and seemed to normalize towards the end of the experiment.

Summary

4.1 Genetic Deletion of FABP 5/7

- This work expands on previously published work on the role of FABP5 and FABP7 inhibition on reward and locomotor behavior (Thanos et al., 2016).
- Based on our results, FABP 5/7 deletion appears to have no effect on acquisition of CPP or cocaine-induced hyperlocomotorization.

4.2 Pharmacological Inhibition of FABP 5 and FABP 7

- In contrast to work previously done by our lab with SBF126 which was administered 50 minutes prior to CPP conditioning, the results of this study suggest that SBF126 may have rewarding properties depending on time of administration (90 minutes prior) (Thanos et al. 2016). SBF126 otherwise does not appear to have an effect on CPP acquisition or cocaine-primed reinstatement of preference.
- Furthermore, the results of our locomotor sensitization experiment suggest that SBF126 may dampen the locomotor response of cocaine administration, a finding that warrants further investigation.

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