The Effect of Chronic Cocaine on Behavioral Sensitization and Allopregnanolone Levels in Rat Serum and Brain Samples

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Introduction

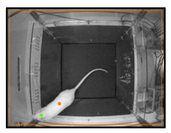
- Neurosteroids are steroids produced de novo in the brain that affect neuronal excitability. Chronic substance use is often associated with decreased levels of neurosteroids[1,2].
- Allopregnanolone, (3α,5α)-3-hydroxy-5-pregnan-20-one, is a progesterone-derived neurosteroid that is a positive allosteric modulator of $GABA_A$ receptors with potential to affect dopaminergic transmission[1].
- Human studies have demonstrated the possibility of allopregnanolone as a treatment for cocaine use disorder. Specifically, elevating circulating allopregnanolone via progesterone administration mediated a reduction in drug craving and stress responsivity[3].
- Chronic cocaine confers sensitization in rats[4]. In humans, it is associated with decreased levels of pregnenolone, a precursor to allopregnanolone[2].
- Basal plasma and cerebral concentrations of allopregnanolone are elevated in female rats compared to males[5].
- This study aims to elucidate the mechanism of an allopregnanolone-mediated inhibition of drug reinstatement observed in humans[3] by testing whether chronic cocaine reduces allopregnanolone in an animal model.
- We hypothesize that chronic cocaine administration will sensitize subject's locomotion and stereotyped behavior while attenuating allopregnanolone levels in serum and specific brain regions involved in the mesolimbic dopamine pathway and neurosteroidogenesis.

Materials and Methodology

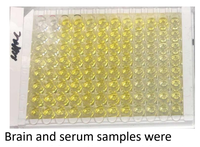
Experimental design:

- 32 Sprague-Dawley rats: 16 male, 16 female
- Drug regiment: 2 days of saline IP injections, followed by 14 days of 15 mg/kg cocaine HCl or saline IP injections (once/day)
- Immediately after injections, animals were placed in operant boxes for 2 hours to measure locomotor activity. Distance travelled was measured via Anymaze software and stereotyped behavior was scored by the experimenter[6].
- Animals were euthanized 24 hours after the final injection. Allopregnanolone was extracted from serum and brain samples and ELISAs were used to quantify allopregnanolone.

Animals were housed in our facility at the UNC School of Medicine. Animals were weighed before every injection and HCl cocaine or saline was administered intraperitoneal.



Anymaze software tracked body movement over time to quantify locomotion. Stereotyped behavior was scored in accordance with Ellinwood EH Jr, Balster RL[6].



Brain and serum samples were collected from the animal following rapid decapitation. Allopregnanolone was isolated via a solid-phase extraction and was quantified using Arbor Assays enzyme immunoassay.

Results

Figure 1. Locomotor activity

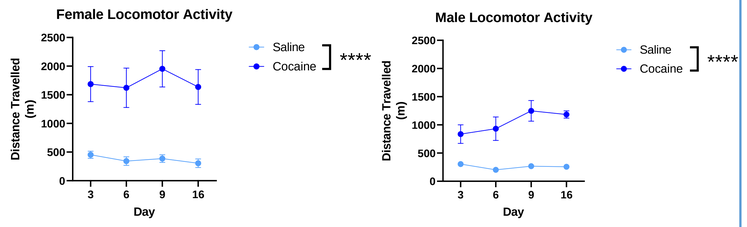


Figure 1. Locomotor activity was potentiated in females and males receiving chronic cocaine

There was a main effect of treatment on locomotion as cocaine-treated females travelled significantly further distances than saline-treated females ($p<0.001$). This was also replicated within the males ($p<0.001$). There was no treatment x time interaction.

Figure 2. Stereotyped behavior

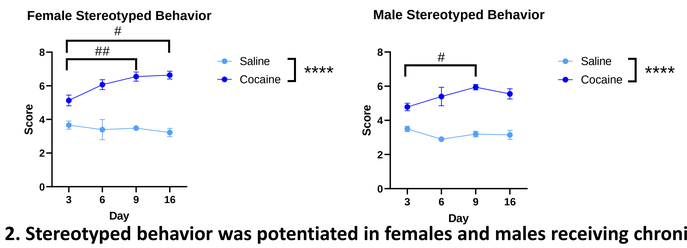


Figure 2. Stereotyped behavior was potentiated in females and males receiving chronic cocaine

There was a main effect of treatment on stereotyped behavior as cocaine-treated females received higher stereotypy scores than saline-treated females ($p<0.001$). This was also replicated within the males ($p<0.001$). Within the females, there was a significant treatment-by-time interaction between days 3 and 9 ($p<0.01$) and between days 3 and 16 ($p<0.05$). Within the males, there was a significant treatment x time interaction between days 3 and 9 ($p<0.05$).

Figure 3. Allopregnanolone Levels in Serum and Brain Regions

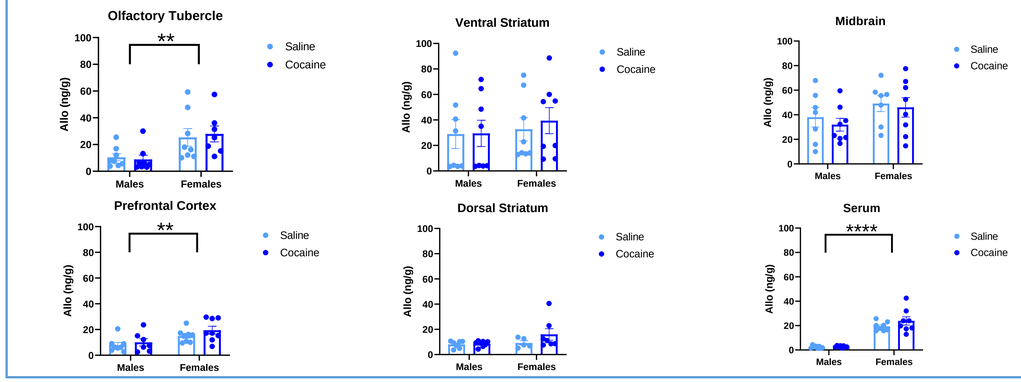


Figure 3. Allopregnanolone levels were not mediated by chronic cocaine administration

Within all brain regions and in serum there was no main effect of treatment on allopregnanolone levels in either sex. There was a main effect of sex, grouped by treatment, in both the olfactory tubercle and in the prefrontal cortex ($p<0.01$), but in no other brain regions. Additionally, there was a main effect of sex, grouped by treatment, in serum ($p<0.001$). Importantly, brain regions not experiencing a sex difference are involved in dopaminergic transmission.

Conclusions

- Chronic cocaine increased locomotor and stereotyped behavior compared to saline, but sensitization was only observed in stereotyped behavior.
- Chronic cocaine did not significantly affect allopregnanolone levels in serum nor in any brain region.
- We found potent sex differences in allopregnanolone levels in serum, olfactory tubercle, and the prefrontal cortex, consistent with literature.
- We found a lack of a sex difference in the dorsal striatum, ventral striatum, and midbrain, potentially elucidating sex differences in addiction and reinstatement.

Future Directions

- Does allopregnanolone decrease spontaneous dopamine release within the mesolimbic dopamingeric pathway? Is this effect sex-dependent?
- Can allopregnanolone restore chronic cocaine-mediated increases in dopamine release?

References

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Acknowledgments

UNC Department of Biology, Biology 395: Undergraduate Research in Biology
This research was funded by NC TraCS UL1TR001111 project 2KR1011813 and a Foundation of Hope research award.