Altered Expression of Sexual Behavior in Young Adult Male Sprague-Dawley Rats Maintained on a Ketogenic Diet

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INTRODUCTION

BACKGROUND:

- The high fat, low carb ketogenic diet (KD) has shown therapeutic potential for neuropsychiatric disorders, including drug addiction.
  - Previously our lab found that rats on the KD showed less cocaine-induced stereotyped locomotor responses than standard diet rats (Martinez, Lees, Ruskin, & Masino, 2019).
  - Meg Huston in our lab has found that rats on the KD have shown reduced motivation for cocaine, as evidenced by the inability of these rats to form a conditioned place preference (CPP) for this drug.
- What is not known is whether the KD more broadly affects the expression of behaviors associated with natural rewards such as sex.
  - One previous study looked into the KD's impact on sexual activity in obese people, but this study was very limited (Castro et al., 2018).

HYPOTHESIS: The KD more broadly disrupts how the brain processes rewarding stimuli, resulting in disrupted expression of behaviors associated with natural rewards such as sex

PREDICTIONS: If this hypothesis is true, then we would expect the KD animals to fail to show the same sex behavior patterns as standard diet animals.

METHODS

ANIMALS: Young adult male and female Sprague Dawley rats were pair housed in polycarbonate cages with wire tops and maintained on a 12:12 L:D cycle (lights off 12 pm). All testing occurring during the dark phase of the light cycle. Food and water were available *ad libitum.*

DIET: Rat were put on assigned diets at the age of five weeks. Standard diet (SD; LabDiet 5001): 13.5% calories from fat, 58.0% calories from carbohydrates, and 28.5% from protein. KD (Bio-Serv F3666): 93.4%93.4% calories from fat, 1.8% calories from carbs and 4.7% calories from protein.

OVARIECTOMY (OVX) SURGURIES: Prior to surgery, females were anesthetized with isoflurane gas (induction 4-5%; maintenance during surgery 2-3%). Females were then injected subcutaneously with the analgesic carprofen (2.5 mg/kg) and the antibiotic enrofloxacin (10 mg/kg). The flanks were shaved and prepped with alternating wipes of 70% ethanol and betadine. Incisions were made through skin and underlying muscle wall. Ovaries were externalized and then removed via cauterization. Muscle incisions were closed via absorbable sutures. The skin incisions were closed using wound clips. The females were given a second injection of carprofen within 24 hours of the surgery. Wound clips were removed 7-10 days post surgery.

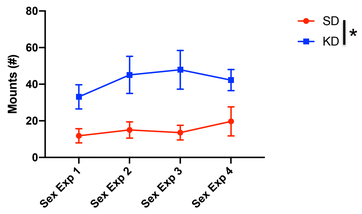
HORMONE INJECTIONS: OVX females were injected subcutaneously with 0.1 mL estradiol benzoate (15.90 mM; E8515, Sigma-Aldrich) and progesterone (132.81 µM; PO130, Sigma-Aldrich) both diluted in cottonseed oil. Estradiol benzoate was given 48 hours prior to sex experience and progesterone given 4-6 hours prior to sex experience.

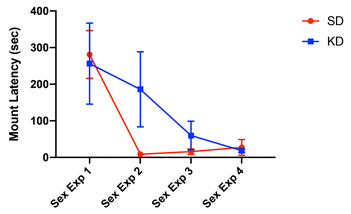
SEXUAL EXPERIENCE SESSIONS: Male rats (8-9 weeks of age) had a total of four sex experiences with OVX, hormone primed females. The sessions took place in glass aquariums. Males were habituated to the aquariums 30 minutes before testing. Each session was video recorded. An OVX, hormone primed female was placed in the cage after the 30 minute habituation. The number of mounts and intromissions were scored live by a researcher. The session was concluded by the researcher after thirty minutes or once the male had ejaculated. A mount is defined as the male mounting the female from the rear and occasionally putting his forearms around her. Sex behaviors were operationalized as previously described in Ågmo (1997). Intromissions start with a mount, that leads to a deep thrust from the male to have vaginal penetration. Ejaculation starts with an intromission and ends with the male holding onto the female for 1-3 seconds after.

RESULTS

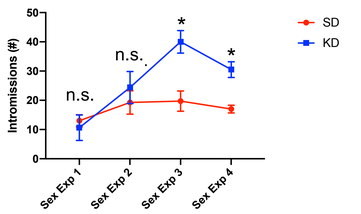
Behavioral Measures

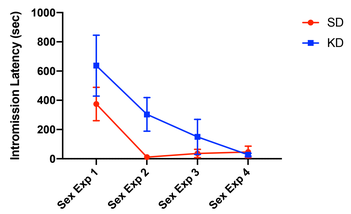
Mounts



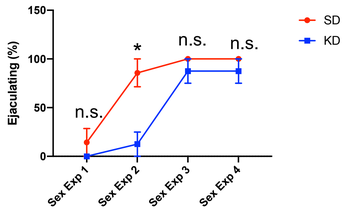


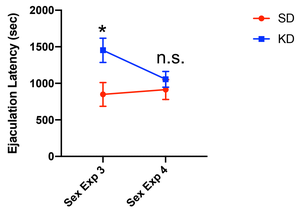
Intromissions





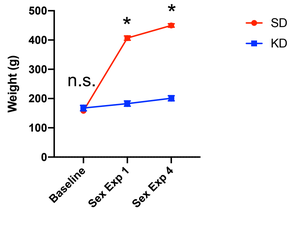
Ejaculation

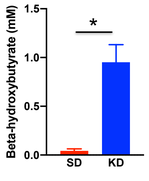


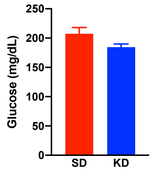


Mean +/- SEM # of mounts, # of intromissions, % ejaculating, as well as the latency to perform each of these behaviors, across consecutive sex experience sessions. There was a significant main effect of diet for # of mounts (KD>SD). There was a significant interaction of diet x time for # intromissions. Specifically, KD males had greater # of intromissions than SD males on sex experience 3 and 4. Finally, there were significant interactions of diet x time for ejaculation % and ejaculation latency. Specifically, KD males were less likely to ejaculate than SD males on sex experience 2 and took longer to ejaculate on sex experience 3. p<0.05.*

Physiological Measures







Mean +/- SEM weights (g), beta-hydroxybutyrate (mM), glucose (mg/dL). There was a significant interaction of diet x time for weight. Specifically, KD males had lower weights than SD males on sex experience 1 and 4. However, it is important to note that weights were equivalent at baseline (prior to KD assignment). There is significant effect of diet on blood ketone levels (KD>SD). Finally, there is no significant effect of diet on blood glucose levels. . p<0.05.*

DISCUSSION

BEHAVIORAL MEASURES:

- KD males had more mounts, more intromissions (sex experience 3 and 4), were less likely to ejaculate (sex experience 2) and took longer to ejaculate (sex experience 3).
  - This suggests that male rats on KD may require more sexual stimulation to reach ejaculation than males on the SD, thereby reducing their mating efficiency.
  - However, for the most part the sex behavior of KD males normalizes to SD male levels with repeated experience.

PHYSIOLOGICAL MEASURES:

- The KD males had reduced weight gain over time and had higher levels of blood ketones than SD males.
  - These effects match results from our previous studies with KD males and confirm that these animals were successfully maintaining ketosis while on the KD.

FUTURE DIRECTIONS

FEMALES: See if female sex behaviors are similarly impacted by the KD.

REINFORCING PROPERTIES OF SEX: See if KD animals are able to form a conditioned place preference for sex.

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