Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Jennifer MJ McGee Date

Food Reward in Dopamine β-Hydroxylase Knockout Mice

By

Jennifer MJ McGee
Master of Arts

Psychology

_____

Dr. Darryl Neill, PhD
Advisor

_____

Dr. Patricia Brennan, PhD
Committee Member

_____

Dr. Hillary Rodman, PhD
Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____ Date

Food Reward in Dopamine β-Hydroxylase Knockout Mice

By

Jennifer MJ McGee
B.A., George Washington University, 2006

Advisor: Dr. Darryl Neill, PhD

An abstract of
a thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Master of Arts
in Psychology
2010

Abstract

Food Reward in Dopamine β-Hydroxylase Knockout Mice

By: Jennifer Marie-Juergensen McGee

While dopamine (DA) has been implicated as an essential neurotransmitter for reward, norepinephrine (NE) has largely been ignored. To examine the role of NE in food reward, we tested mice that have been genetically altered to lack an enzyme necessary for NE synthesis (dopamine β-hydroxylase; DBH-/-) using fixed ratio (FR) responding for 14 mg food pellets. The results suggest that genetic depletion of DBH produces effects on responding for food that, in males, are highly dependent on the ratio requirement of the schedule. When the number of responses required were low, FR1-FR14, male DBH-/- mice responded significantly more than DBH+/- controls. However, as the ratio requirement increased to FR16 through FR30, there were no differences between the two genotypes. There were no differences in low or high ratio requirements between genotypes in females and a post hoc analysis demonstrated the only significant difference between female genotypes was at FR1. 24-hour food intake was also measured and it was determined that male DBH-/- consumed more food per body weight than male controls, but that female DBH-/- mice consumed less than controls. At the behavioral level, these results were interpreted as reflecting two factors: the physiological need for food intake, and the incentive value of the food. At the neuroscience level, it is proposed that the chronic lack of NE causing an upregulation of DA receptors, resulting in at low FR values.

Food Reward in Dopamine β-Hydroxylase Knockout Mice

By

Jennifer MJ McGee
B.A., George Washington University, 2006

Advisor: Dr. Darryl Neill, PhD

A thesis submitted to the Faculty of the
James T.Laney School of Graduate Studies of Emory University
In partial fulfillment of the requirements for the degree of
Master of Arts
In Psychology
2010

Table of Contents

Food Reward in Dopamine β-Hydroxylase Knockout Mice

Norepinephrine (NE) is primarily distributed through the brain via two pathways. Ascending noradrenergic projections from the locus coeruleus form the dorsal adrenergic bundle, which innervates the hippocampus, cerebellum, cerebral cortex and the ventral tegmental area (VTA) (Jones & Moore, 1977; Moore & Bloom, 1979; Oades & Halliday, 1987). NE is also produced in nuclei located within the pons and medulla, forming the ventral adrenergic bundle, which primarily innervates the hypothalamus and amygdala. Owing to these widespread projections, NE is understood to regulate many behaviors including memory (Khakpour-Taleghani et al., 2008; Murchison et al., 2004), attention (Arnsten, 2009), and has recently been suggested as a critical neurotransmitter for reward (Weinshenker & Schroeder, 2006). While decades of research have shown that dopaminergic transmission in the nucleus accumbens (NAc) is clearly important for both the rewarding/reinforcing and motor-activating effects of psychostimulants and other pleasurable stimuli, there is growing evidence that NE is also important in these behavioral responses, particularly considering the evidence that NE may function as a neuromodulator of DA.

Early research showed that selective lesions of DA releasing terminals in the NAc, through injections of 6-hydroxydopamine, attenuate the amphetamine-induced increase in locomotion (Kelly, Seviour, & Iversen, 1975) and reduce the amount of cocaine rats will self-administer, with the more extensive lesions resulting in extinction (D.C.S. Roberts, G.F. Koob, Klonoff, & H.C. Fibiger, 1980). This decrease in responding is not found when similar lesions are produced in the caudate (Taylor & Robbins, 1986) or in the frontal cortex (Martin-Iverson, Szostak, & H.C. Fibiger, 1986).

The main dopaminergic input to the NAc comes from the VTA, and selective destruction of the VTA also results in a reduction of cocaine intake, implying that this VTA to NAc pathway is important, if not critical, for the rewarding properties of psychostimulants (Roberts & Koob, 1982). However, Roberts and Koob note that the extent of the lesion did not significantly correlate with cocaine intake, allowing for the possibility that other modulating neurotransmitters may be involved.

Because DA transmission from the VTA to the NAc appears to be critical for drug reward, the ways in which NE may contribute to the function of this dopaminergic pathway is essential for this discussion. The LC projects to many different areas of the brain, but considering the evidence presented above, the connections to the ventral tegmental area (VTA) are arguably the most important for a discussion about reward. Application of selective NE reuptake blockers in the VTA results in an increase of released DA in the VTA (Chen & Reith, 1994), possibly by altering the firing pattern of DA neurons (Linnér et al., 2001). In addition, systemic administration of the antidepressant desipramine, which inhibits the norepinephrine transporter, results in an increase of extracellular DA in the NAc (Li, Yan, & Coffey, 1996) and the prefrontal cortex (Carboni, Tanda, Frau, & Di Chiara, 1990). While these results may be explained by the ability of NE transporters to take DA out of the cleft and into NE terminals (Yamamoto & Novotney, 1998), selectively destroying locus coerulus neurons results in a reduction of basal DA (Lategan, Marien, & Colpaert, 1990; Lategan, Marien, & Colpaert, 1992). This suggests that NE may be necessary to modulate released DA.

Further, there is evidence that DA release in the NAc is regulated by the prefrontal cortex using both DA and NE, but through opposing mechanisms. Pascucci

and colleagues (2007) demonstrated that DA release in the medial prefrontal cortex (mPFC) resulted in an inhibition of accumbal DA release whereas NE release in this same cortical area resulted in a temporary increase of accumbal DA release. The paradigm employed by Pascucci et al (2007) utilized a stressful situation to demonstrate the different roles of the neurotransmitters, but it is possible that this cortical modulation of accumbal DA release occurs with other stimuli and situations. In addition, stress is a common precondition for drug relapse, suggesting that the prefrontal modulation could be important in many drug and behaviors resulting from conditioned stimuli. These data show that NE is an important modulator of DA, and may have some implication for reward.

NE has long been implicated in opioid withdrawal (Maldonado, 1997) but there is recent evidence of its involvement in the rewarding properties of opioids as well. Morphine enhances NE release in the mPFC (Ventura, Alcaro, & Puglisi-Allegra, 2005) which, as referenced earlier, causes an increase of DA in the NAc. Further, destruction of NE afferents in this area abolishes the morphine-induced increase of DA in the NAc (Ventura et al., 2005). Both the specific depletion of NE afferents and general lesions of the mPFC eliminate morphine-induced conditioned place preference (Hao et al., 2008; Ventura et al., 2005), suggesting a critical role of NE in morphine reward. Morphine self-administration also dose-dependently increases the level of extracellular NE in the anterior cingulate cortex of the frontal lobe (Sudakov, Rusakova, Trigub, Kudrin, & Klodt, 2007). Finally, NE is important for the behavioral responses to morphine, as shown by a reduction in morphine induced locomotor hyperactivity after inhibiting α1-

adrenergic receptors by prazosin in rats (Drouin, Blanc, Villgier, Glowinski, & Tassin, 2002).

NE may also be critical for psychostimulant reward and dependence. Cocaine (Sofuoglu, Nelson, Babb, & Hatsukami, 2001), amphetamine (Ventura, Cabib, Alcaro, Orsini, & Puglisi-Allegra, 2003), and nicotine (Westfall, 1974) all increase extracellular levels of NE throughout the brain. Since forebrain DA release by the VTA is essential for motivated behaviors and addiction, the modulatory role of NE could be a critical component to reward. In addition, prefrontal NE depletion blocks amphetamine CPP (Ventura et al., 2003) and administration of the α1-adrenergic receptor inhibitor prazosin reduces amphetamine induced locomotor hyperactivity in mice (Snoddy & Tessel, 1985)

Aside from chemical disruptions and lesions, genetically altering the function of the noradrenergic pathway has elucidated more ways in which NE may contribute to the rewarding properties of drugs and other stimuli. Mice that have been genetically altered to lack the gene that codes for DBH (*DBH -/-)* are unable to produce NE and are good candidates for examining the effect of the chronic loss of NE on reward. A number of behavioral paradigms and biological assays have led to the hypothesis that the chronic lack of NE leads to the up-regulation of DA receptors, rendering the mouse hypersensitive to psychostimulants, such as cocaine (Schank et al., 2005). Typically, when NE is unavailable, DA release is attenuated reducing the effect of psychostimulants (Ventura, Morrone, & Puglisi-Allegra, 2007). In *DBH -/-* mice, however, this attenuated DA is a chronic condition that leads the DA receptors to upregulate in response (Shank, et al. 2006). This up-regulation of high affinity DA receptors results in hypersensitivity to released DA (Schank et al., 2005; Weinshenker & Schroeder, 2006). Interestingly,

although there is a an attenuation of subcortical DA these mice have normal levels of extracellular DA in the prefrontal cortex (Schank et al., 2005), an area, as discussed above, which may be critical for the rewarding and motor activating effects of many drugs of abuse.

In one experiment, Schank et al. (2005) demonstrated that the dose response curve for cocaine shifted to the left in DBH-/- mice. The lowest dose of cocaine (5mg/kg) resulted in a significant place preference for DBH-/- mice, but not for DBH+/- and the highest dose (20 mg/kg) produced a strong place aversion in DBH-/- mice but a place preference for the controls (Schank et al., 2005). Similarly, DBH-/- mice also showed an aversion to 25 mg/kg of morphine, an amount that produced place preference in littermate controls (Olson et al., 2006). *DBH -/-* mice also demonstrated hypersensitivity to amphetamine, as measured through locomotor activity, and exhibited stereotypy after a much lower dose of amphetamine than controls (Weinshenker, Miller, Blizinsky, Laughlin, & Palmiter, 2002). In another genetic knockout, mice lacking α1b-adrenergic receptors (α1b-AR) do not exhibit place preference for morphine and do not show the normal increase in motor behavior associated with d-amphetamine, cocaine, or morphine (Drouin et al., 2002) These mice also exhibit a strong aversion to both cocaine and morphine when given an oral preference test (Drouin et al., 2002)

As the evidence for a role of NE in drug reward becomes clearer, we wanted to determine how NE might contribute to the mechanisms behind food reward. Understanding how NE is involved in food reward may be essential when considering the larger problems of weight maintenance and obesity in humans. Although there is substantial evidence that DA plays a crucial role in food reward, independent of its

effects on arousal and movement (for review see Wise, 2006), little evidence exists on how NE may affect operant responding for food. It has been shown that after food deprivation levels of NE increase in the prefrontal cortex in response to food, which may not be due to the rewarding properties of the food, but due to the motivation and attention acquired when deprived (Fallon, Shearman, Sershen, & Lajtha, 2007). In the absence of freely available food, wild animals would require an increase in attention to properly forage for food. However, in one experiment, the novel exposure to a food reward (chocolate) in non-deprived animals also increased extracellular NE levels in the mPFC and DA in the NAc (Ventura, Morrone, & Puglisi-Allegra, 2007). In animals where the NE cortical afferents were chemically depleted, the food-induced increase of DA in the NAc was abolished, suggesting a critical modulatory role of NE in not only morphine reward as stated previously, but also in food reward (Ventura et al., 2007). Selectively destroying mPFC NE afferents also abolishes conditioned place preference for food (as well as cocaine) in non food-restricted animals (Ventura et al., 2007), and prevents maladaptive food seeking behavior like choosing to endure foot shocks for the sake of food (Latagliata, Patrono, Puglisi-Allegra, & Ventura, 2010). This type of lesion also significantly increases the rate of responding for food pellets on a fixed ratio 30 schedule in food deprived rats (Price, Murray, & H.C. Fibiger, 1977), but only after significant training. Complete bilateral lesions of the locus coerulus, however, impair the increase in the rate of running for a food reward in an L-shaped runway task without disrupting motor or exploratory behavior (Anlezark, Crow, & Greenway, 1973). This disparity might be explained by the release of DA that occurs when a reward is periodically delivered. In many operant schedules, the food reward is only delivered after multiple

responses which causes DA to be released (Richardson & Gratton, 1998); however, in the runway task the food is always available and always in the same location. Measuring the length of time it takes an animal to find food may be assessing a different component of drug reward, namely seeking, and not reward per se.

In contrast to work on drug reward, there has not been any research to date on genetic NE alterations and food reward. There is evidence that these mice have an increased metabolism and therefore show increased food consumption (Thomas & Palmiter, 1997), but this is evidence for the role of NE in feeding and not necessarily in reward. The present study used non food-deprived DBH-/- mice to examine how the chronic lack of NE affects operant responding for a food reward.

Methods

Animals

20 adult *DBH-/-* mice and 20 adult *DBH+/-* control mice were used, 10 females and 10 males of each genotype (all aged 5-9 months). *DBH -/-* mice were developed and generated by Dr. David Weinshenker at Emory University, as previously described (Thomas et al., 1995). The absence of NE during gestation has fatal consequences. For this reason, L-threo-3, 4-dihydroxyphenylserine (DOPS) is added to the maternal drinking water, providing sufficient NE to the gestating pups. After birth, the pups are no longer exposed to DOPS and cannot produce NE on their own, but survive. Heterozygotic littermates (*DBH+/-)* have normal levels of NE, exhibit no known behavioral differences from wild type strains, and therefore were used as control animals. All animals were group housed in a temperature and humidity-controlled environment on

a 12-h lighting cycle (lights on at 0700) and had unrestricted access to food and water. Behavioral testing was conducted between 1100 and 1500h. All procedures were approved by the Emory University Animal Care and Use Committee.

Apparatus

The mice were trained and tested in operant conditioning chambers measuring 15.9 cm x 14.0 cm x 12.7 cm (MED Associates, Georgia, VT). On one wall of the chamber, a food receptacle was flanked by two nose poke holes measuring 1.3 cm in diameter by 1.0 cm deep. Each hole was equipped with a light and an infrared sensor. Only the hole on the left of the receptacle was active and lit; the hole on the right was inactive and unlit. Entry of the animal's nose 0.64 cm into the active hole triggered an infrared sensor and defined the response. A single nutritionally balanced 14mg food pellet (BioServe, Inc., Frenchtown, NJ) was delivered after the required number of nose pokes. Experimental contingencies were computer-controlled using MED-PC software (MED Associates, Georgia, VT).

Training

All mice were trained in the operant testing chambers during a 12-h overnight session. Water was available ad libitum but food could only be obtained by nose-poking. Food pellets were available on a fixed ratio (FR) 1 schedule; after one nose poke in the correct hole they received the reward. After the overnight session, training continued for 5 days. For each day of training, the mice were placed in the testing chamber and allowed to nose poke on an FR1 schedule for 20 minutes. To be included in the experiment, the mice had to reach a criterion of 15 nose pokes during the overnight session and have an average of 10 responses per day over the five training sessions. Two

male mice, one DBH-/- and one DBH+/-, did not reach both criterion and therefore their data were not included in the analysis.

Testing

On test days, home cages were moved into the testing room allowing the animals to have free access to their regular rodent chow immediately before and after the testing session. Mice were then placed in the operant chamber for 20 minutes. The number of nose pokes into both the active and inactive holes was recorded. Testing occurred every day between 1100 and 1500h for 15 days. Each testing day the response requirement increased by two (FR2, FR4, FR6…FR30).

Results

Operant Responding

During the overnight training sessions, an independent samples t-test showed that DBH-/- mice responded more than controls $t(36) = 3.50$, $p = .001$ (Figure 1). Four days of FR1 maintenance training also resulted in higher responding for DBH-/- mice $F(1,36) = 48.62$, $p < 0.001$ (Figure 2).

Figure 3a-c displays the responses made across the different fixed ratio schedules. Although there were no discernable differences between the genotypes when analyzed across all testing sessions, $F(1, 35) = 1.12$, $p > 0.05$, when the sessions were divided into 'low' and 'high' ratio requirements differences emerged. Because norepinephrine plays a critical role in attention and memory, we divided the sessions in half to determine if those sessions with low attentional requirements (FR1-FR14) resulted in different behaviors than those sessions with high attentional requirements (FR15-FR30). During the testing

sessions that had a low ratio requirement DBH-/- mice responded more than controls $F(1, 36) = 6.01$, $p = 0.019$. Increasing FR values increased responding for both genotypes $F(2.38, 85.68) = 17.20$, $p < 0.001$. The interaction between genotype and FR value failed to reach statistical significance, $F(2.83, 85.68) = .562$, $p = 0.60$. (Mauchly's test indicated that the assumption of sphericity had been violated (chi-square = 189.24, $p < .05$), therefore degrees of freedom for the two preceding statistics were corrected using Greenhouse-Geisser estimates of sphericity (epsilon = 0.34)).

During the testing sessions that had a high ratio requirement (FR16-FR30), DBH-/- mice were indistinguishable from DBH+/- controls $F(1, 35) = 0.12$, $p = 0.734$. Again, Mauchly's test indicated that the assumption of sphericity had been violated (chi-square = 90.82, $p < .05$), therefore degrees of freedom for the following two statistics were corrected using Greenhouse-Geisser estimates of sphericity (epsilon = 0.53). Increasing FR values failed to change responding for both genotypes $F(3.74, 130.73) = 1.44$, $p = 0.226$, and the interaction between genotype and FR value also failed to reach statistical significance, $F(3.74, 130.73) = 1.12$, $p = 0.35$.

Food Intake

DBH-/- mice have an elevated metabolism, and although the mechanism is unclear, this could be related to an increase in food intake relative to heterozygous controls (Thomas & Palmiter, 1997). Although Thomas and Palmiter demonstrated that DBH-/- males do consume significantly more food then controls, female mice were not evaluated. Since the present experiment utilized food as a motivator, we measured food intake for both male and female DBH-/- and DBH+/- mice.

The mice utilized for this experiment were experimentally naïve adults, aged 3-6 months. Each mouse was individually housed in a controlled environment, similar to the FR experiment described above, and had continuous access to water and standard rodent laboratory chow. Body weight and food intake in grams was recorded every 24 hours for 5 days.

As previously reported (Thomas & Palmiter, 1997), male DBH-/- ate significantly more when adjusted for body weight, $t(19) = 4.25$, $p < 0.001$. However, female DBH-/- ate significantly less when adjusted for body weight, $t(6) = 2.75$, $p = .033$ (Figures 4a-b).

Sex Differences

Because there was a significant sex difference in food intake, we re-analyzed our data post hoc to check for sex differences in responding.

During the overnight training sessions, a repeated measures ANOVA showed that male DBH-/- mice responded more than controls $F(1,17) = 10.01$, $p = .006$, but female mice, while showing a strong trend in the same direction, did not differ by genotype $F(1,19) = 3.79$, $p = 0.065$ (Figure 5). Five days of FR1 maintenance training revealed higher responding for both DBH-/- males, $F(1,16) = 72.29$, $p < 0.001$, and females, $F(1,18) = 14.30$, $p = 0.001$ (Figures 6a-b)

During the testing sessions that had a low ratio requirement (FR1-FR14), male DBH-/- responded more than male DBH+/- controls $F(1, 16) = 5.76$, $p = 0.029$, (Figure 7). Mauchly's test indicated that the assumption of sphericity had been violated (chi-square = 64.69, $p < .05$), therefore degrees of freedom for the following two statistics were corrected using Greenhouse-Geisser estimates of sphericity (epsilon = 0.44).

Increasing FR values increased responding for both male genotypes $F(3.11, 49.72) = 6.37$, $p = 0.001$. The interaction between genotype and FR value failed to reach statistical significance, $F(3.11, 49.72) = 1.019$, $p = 0.394$.

During the testing sessions that had a high ratio requirement (FR16-FR30), male DBH-/- mice were indistinguishable from male DBH+/- controls $F(1, 16) = 0.23$, $p = 0.638$, (Figure 8). Again, Mauchly's test indicated that the assumption of sphericity had been violated (chi-square = 43.22, $p < .05$), therefore degrees of freedom for the following two statistics were corrected using Greenhouse-Geisser estimates of sphericity (epsilon = 0.53). Increasing FR values failed to change responding for both genotypes $F(3.68, 58.92) = 0.80$, $p = 0.522$, and the interaction between genotype and FR value also failed to reach statistical significance, $F(3.68, 58.92) = 1.56$, $p = 0.201$.

Responding by female DBH-/- mice did not vary from DBH+/- controls when response requirement was low $F(1, 18) = 1.92$, $p = .183$ (Figure 9) or high $F(1, 17) = .030$, $p = .865$ (Figure 10).

Discussion

It has previously been shown that *DBH-/-* mice are hypersensitive to amphetamine (Weinshenker et al., 2002) and cocaine (Schank et al., 2005). To determine if this hypersensitivity extends to food reward, we measured operant responses for food pellets. The present results suggest that the genetic depletion of DBH produces effects on responding for food that, in males, are highly dependent on the ratio requirement of the schedule. When the number of responses required was low, i.e. FR1-FR14, male DBH-/- mice responded significantly more than DBH+/- controls. However, as the ratio

requirement increased, FR16-FR30, there were no differences between the two genotypes. There were no significant differences in responding during low or high ratio requirements between genotypes in females. A post hoc analysis demonstrated the only significant difference between female genotypes was during sessions of FR1.

Although evidence demonstrates that male DBH-/- mice have a faster metabolism and therefore have a higher 24 hour food intake than DBH-/+ controls (Thomas & Palmiter, 1997), the design of the current experiment discourages this interpretation of our results. Our mice were not food deprived, the testing sessions were only 20 minutes long, and the tests occurred in the middle of the day. Therefore, the increase in responding when response requirements are low may be due to an increase of reward salience/incentive and not due to hunger. In addition, female DBH-/- mice eat significantly less than littermate controls yet respond for food pellets at a rate similar to controls. If the increase in responding were solely due to differences in appetite, we would expect the female mice to respond in the FR sessions significantly less than the controls. The results of the 12-hour overnight session are even more telling. If the mice were solely motivated by the unconditioned quest for food, then the female mice should have eaten less, as measured by the amount of responses generated, since their 24-hour food intake is significantly less then female controls. However, the DBH-/- females tended to eat more during the overnight session, suggesting that the conditioned motivation of nose-poking for pellets was stronger than the unconditioned motivation for food.

The difference in responding between low and high ratio requirements could be a result of altered DA signaling (Figure 4). In rodents, noradrenergic projections to

midbrain DA neurons modulate DA release (Grenhoff, Nisell, Ferré, Aston-Jones, & Svensson, 1993; Grenhoff & Svensson, 1993a; Jones & Moore, 1977; Schank et al., 2005; Ventura, Alcaro, & Puglisi-Allegra, 2005; Ventura et al., 2003). Due to the absence of NE in DBH-/- mice, DA release is attenuated and, consequently, both D1 and D2 dopamine receptors are upregulated in the nucleus accumbens and caudate putamen (Schank et al., 2005). This increase in DA receptors could be what produces the hypersensitivity to psychostimulants, since both cocaine and amphetamine increase synaptic levels of DA (Schank et al., 2005; Weinshenker et al., 2002). Enhanced DA release is also an effect of the periodic delivery of food (McCullough & Salamone, 1992), and evidence suggests it is the anticipation of food delivery, brought about by a conditioned stimulus or trained behavior, that facilitates the enhanced DA release. For example, increases in DA were found in the NAc when rats were exposed to a stimulus that had been previously presented before food delivery, but no increase was reported when presented with and allowed to consume an un-signaled meal (Blackburn, Phillips, Jakubovic, & Fibiger, 1989). Similarly, rats responding on a fixed ratio schedule (FR5) showed increases in extracellular DA whereas those rats who received pellets en masse with no behavioral requirement did not (Salamone, Cousins, McCullough, Carriero, & Berkowitz, 1994). Therefore, it is possible that low FR values triggered an increase in dopamine release and, due to the upregulated DA receptors, enhanced the incentive motivation of DBH-/- mice. The greater incentive resulted in increased responding during low FR values.

However, response maintenance in the absence of reward presentation requires a certain level of arousal and attention to remain on task. Since both NE and DA have

been implicated as critical neurotransmitters in attention (Arnsten, 2009) higher ratios could tax the attentional system of the DBH-/- mice rendering them unable to complete the task. At some point, the length of time between the first nose poke and the delivery of the food reward becomes too long and the altered arousal system, in a sense, outweighs the enhancement caused by increased DA transmission. The longer the interval between the behavior and the reward, the more difficult it is for the DBH-/- mice to sustain responding. Currently, this lab is using the three choice serial reaction time test to determine the proficiency of DBH-/- mice to maintain attention for a food reward. Preliminary results suggest that these mice perform much more poorly than DBH+/-, as we would expect with the hypothesis outlined here (unpublished data).

One question that remains is why female DBH-/- do not statistically differ from their littermate controls. Female DBH-/- and DBH+/- mice have not thus far been shown to have genotypic differences in their responses to cocaine, amphetamine, and ethanol (Schank et al., 2005; Weinshenker et al., 2002; Weinshenker, Rust, Miller, & Palmiter, 2000). In these same studies, the female mice were statistically indistinguishable from males of the same genotypes and the sexes were combined in the analyses. However, in our test there was a clear sex difference in both food intake and responding for food pellets, and no genotypic difference between the females. If we consider the food intake data and the responding on FR schedules data together, the fact that there was no statistical difference between female genotypes in all the FR values above FR1 is actually quite interesting. This suggests that although they may be less intrinsically motivated to consume food, the act of performing for a food pellet is enticing enough to warrant increased responding. As mentioned earlier, we would expect the females to respond

less, thereby consuming less food, during the session if the main motivation was hunger or a drive to eat. Since this was not the case, we may speculate that the female DBH-/- mice, similar to the males, are driven by the DA that is released by the periodic food delivery. It is unclear why a sex difference might have emerged for the 24-hour food intake, but considering the cause of the increased metabolism of male DBH-/- has yet to be determined, more research is required.

References

Anlezark, G. M., Crow, T. J., & Greenway, A. P. (1973). Impaired learning and decreased cortical norepinephrine after bilateral locus coeruleus lesions. *Science (New York, N.Y.), 181*(100), 682-684.

Arnsten, A. F. T. (2009). Toward a new understanding of attention-deficit hyperactivity disorder pathophysiology: an important role for prefrontal cortex dysfunction. *CNS Drugs, 23 Suppl 1*, 33-41. doi:10.2165/00023210-200923000-00005

Blackburn, J. R., Phillips, A. G., Jakubovic, A., & Fibiger, H. C. (1989). Dopamine and preparatory behavior: II. A neurochemical analysis. *Behavioral Neuroscience*, *103*(1), 15-23.

Carboni, E., Tanda, G. L., Frau, R., & Di Chiara, G. (1990). Blockade of the noradrenaline carrier increases extracellular dopamine concentrations in the prefrontal cortex: evidence that dopamine is taken up in vivo by noradrenergic terminals. *Journal of Neurochemistry*, *55*(3), 1067-1070.

Chen, N. H., & Reith, M. E. (1994). Effects of locally applied cocaine, lidocaine, and various uptake blockers on monoamine transmission in the ventral tegmental area of freely moving rats: a microdialysis study on monoamine interrelationships. *Journal of Neurochemistry*, *63*(5), 1701-1713.

Drouin, C., Blanc, G., Villigier, A., Glowinski, J., & Tassin, J. (2002). Critical role of alpha1-adrenergic receptors in acute and sensitized locomotor effects of D-amphetamine, cocaine, and GBR 12783: Influence of preexposure conditions and pharmacological characteristics. *Synapse*, *43*(1), 51-61.

Fallon, S., Shearman, E., Sershen, H., & Lajtha, A. (2007). Food Reward-Induced Neurotransmitter Changes in Cognitive Brain Regions. *Neurochemical Research*,

32(10), 1772-1782. doi:10.1007/s11064-007-9343-8

Grenhoff, J., Nisell, M., Ferré, S., Aston-Jones, G., & Svensson, T. H. (1993). Noradrenergic modulation of midbrain dopamine cell firing elicited by stimulation of the locus coeruleus in the rat. *Journal of Neural Transmission. General Section*, *93*(1), 11-25.

Grenhoff, J., & Svensson, T. H. (1993). Prazosin modulates the firing pattern of dopamine neurons in rat ventral tegmental area. *European Journal of Pharmacology*, *233*(1), 79-84.

Hao, Y., Yang, J., Sun, J., Qi, J., Dong, Y., & Wu, C. F. (2008). Lesions of the medial prefrontal cortex prevent the acquisition but not reinstatement of morphine-induced conditioned place preference in mice. *Neuroscience Letters*, *433*(1), 48-53. doi:10.1016/j.neulet.2007.12.040

Jones, B. E., & Moore, R. Y. (1977). Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study. *Brain Research*, *127*(1), 25-53.

Kelly, P. H., Seviour, P. W., & Iversen, S. D. (1975). Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Research*, *94*(3), 507-522. doi:doi: DOI: 10.1016/0006-8993(75)90233-4

Khakpour-Taleghani, B., Lashgari, R., Aavani, T., Haghparast, A., Naderi, N., & Motamedi, F. (2008). The locus coeruleus involves in consolidation and memory retrieval, but not in acquisition of inhibitory avoidance learning task. *Behavioural Brain Research*, *189*(2), 257-262. doi:doi: DOI: 10.1016/j.bbr.2008.01.004

Latagliata, E. C., Patrono, E., Puglisi-Allegra, S., & Ventura, R. (2010). Food seeking in

spite of harmful consequences is under prefrontal cortical noradrenergic control. *BMC Neuroscience, 11*(1), 15.

Lategan, A. J., Marien, M. R., & Colpaert, F. C. (1990). Effects of locus coeruleus lesions on the release of endogenous dopamine in the rat nucleus accumbens and caudate nucleus as determined by intracerebral microdialysis. *Brain Research, 523*(1), 134-138.

Lategan, A. J., Marien, M. R., & Colpaert, F. C. (1992). Suppression of nigrostriatal and mesolimbic dopamine release following noradrenaline depletion by DSP-4: A microdialysis study. *Life Sciences, 50*(14), 995-999. doi:doi: DOI: 10.1016/0024-3205(92)90093-5

Li, M., Yan, Q., & Coffey, L. L. (1996). Extracellular Dopamine, Norepinephrine, and Serotonin in the Nucleus Accumbens of Freely Moving Rats During Intracerebral Dialysis with Cocaine and Other Monoamine Uptake Blockers. *Journal of Neurochemistry, 66*(2), 559-568.

Linnér, L., Endersz, H., Ohman, D., Bengtsson, F., Schalling, M., & Svensson, T. H. (2001). Reboxetine modulates the firing pattern of dopamine cells in the ventral tegmental area and selectively increases dopamine availability in the prefrontal cortex. *The Journal of Pharmacology and Experimental Therapeutics, 297*(2), 540-546.

Maldonado, R. (1997). Participation of noradrenergic pathways in the expression of opiate withdrawal: biochemical and pharmacological evidence. *Neuroscience and Biobehavioral Reviews, 21*(1), 91-104.

Martin-Iverson, M., Szostak, C., & Fibiger, H. (1986). 6-Hydroxydopamine lesions of the

medial prefrontal cortex fail to influence intravenous self-administration of cocaine. *Psychopharmacology, 88*(3). doi:10.1007/BF00180830

McCullough, L. D., & Salamone, J. D. (1992). Involvement of nucleus accumbens dopamine in the motor activity induced by periodic food presentation: a microdialysis and behavioral study. *Brain Research*, *592*(1-2), 29-36. doi:doi: DOI: 10.1016/0006-8993(92)91654-W

Moore, R. Y., & Bloom, F. E. (1979). Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. *Annual Review of Neuroscience*, *2*, 113-168. doi:10.1146/annurev.ne.02.030179.000553

Murchison, C. F., Zhang, X., Zhang, W., Ouyang, M., Lee, A., & Thomas, S. A. (2004). A Distinct Role for Norepinephrine in Memory Retrieval. *Cell*, *117*(1), 131-143. doi:doi: DOI: 10.1016/S0092-8674(04)00259-4

Oades, R., & Halliday, G. (1987). Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. *Brain Research Reviews, 12*(2), 117-165. doi:doi: DOI: 10.1016/0165-0173(87)90011-7

Olson, V. G., Heusner, C. L., Bland, R. J., During, M. J., Weinshenker, D., & Palmiter, R. D. (2006). Role of Noradrenergic Signaling by the Nucleus Tractus Solitarius in Mediating Opiate Reward. *Science, 311*(5763), 1017-1020. doi:10.1126/science.1119311

Pascucci, T., Ventura, R., Latagliata, E. C., Cabib, S., & Puglisi-Allegra, S. (2007). The Medial Prefrontal Cortex Determines the Accumbens Dopamine Response to Stress through the Opposing Influences of Norepinephrine and Dopamine. *Cereb. Cortex, 17*(12), 2796-2804.

Price, M., Murray, G., & Fibiger, H. (1977). Schedule dependent changes in operant responding after lesions of the dorsal tegmental noradrenergic projection. *Pharmacology Biochemistry and Behavior*, *6*(1), 11-15. doi:doi: DOI: 10.1016/0091-3057(77)90153-8

Richardson, N. R., & Gratton, A. (1998). Changes in Medial Prefrontal Cortical Dopamine Levels Associated with Response-Contingent Food Reward: An Electrochemical Study in Rat. *Journal of Neuroscience*, *18*(21), 9130-9138.

Roberts, D., Koob, G., Klonoff, P., & Fibiger, H. (1980). Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. *Pharmacology Biochemistry and Behavior*, *12*(5), 781-787. doi:doi: DOI: 10.1016/0091-3057(80)90166-5

Roberts, D. C., & Koob, G. F. (1982). Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacology Biochemistry and Behavior*, *17*(5), 901-904. doi:doi: DOI: 10.1016/0091-3057(82)90469-5

Salamone, J. D., Cousins, M. S., McCullough, L. D., Carriero, D. L., & Berkowitz, R. J. (1994). Nucleus accumbens dopamine release increases during instrumental lever pressing for food but not free food consumption. *Pharmacology Biochemistry and Behavior*, *49*(1), 25-31. doi:doi: DOI: 10.1016/0091-3057(94)90452-9

Schank, J. R., Ventura, R., Puglisi-Allegra, S., Alcaro, A., Cole, C. D., Liles, L. C., Seeman, P., et al. (2005a). Dopamine [beta]-Hydroxylase Knockout Mice have Alterations in Dopamine Signaling and are Hypersensitive to Cocaine. *Neuropsychopharmacology*, *31*(10), 2221-2230.

Schank, J. R., Ventura, R., Puglisi-Allegra, S., Alcaro, A., Cole, C. D., Liles, L. C., Seeman, P., et al. (2005b). Dopamine [beta]-Hydroxylase Knockout Mice have Alterations in Dopamine Signaling and are Hypersensitive to Cocaine. *Neuropsychopharmacology, 31*(10), 2221-2230.

Snoddy, A. M., & Tessel, R. E. (1985). Prazosin: Effect on psychomotor-stimulant cues and locomotor activity in mice. *European Journal of Pharmacology, 116*(3), 221-228. doi:doi: DOI: 10.1016/0014-2999(85)90156-6

Sofuoglu, M., Nelson, D., Babb, D. A., & Hatsukami, D. K. (2001). Intravenous cocaine increases plasma epinephrine and norepinephrine in humans. *Pharmacology, Biochemistry, and Behavior, 68*(3), 455-459.

Sudakov, S. K., Rusakova, I. V., Trigub, M. M., Kudrin, V. S., & Klodt, P. M. (2007). Self-administration of morphine by rats causes monoamine release in the anterior cingulate cortex. *Bulletin of Experimental Biology and Medicine, 144*(2), 210-213. doi:10.1007/s10517-007-0291-8

Taylor, J., & Robbins, T. (1986). 6-Hydroxydopamine lesions of the nucleus accumbens, but not of the caudate nucleus, attenuate enhanced responding with reward-related stimuli produced by intra-accumbens d-amphetamine. *Psychopharmacology, 90*(3). doi:10.1007/BF00179197

Thomas, S. A., & Palmiter, R. D. (1997). Thermoregulatory and metabolic phenotypes of mice lacking noradrenaline and adrenaline. *Nature, 387*(6628), 94-97. doi:10.1038/387094a0

Ventura, R., Morrone, C., & Puglisi-Allegra, S. (2007). Prefrontal/accumbal catecholamine system determines motivational salience attribution to both

reward- and aversion-related stimuli. *Proceedings of the National Academy of Sciences, 104*(12), 5181-5186. doi:10.1073/pnas.0610178104

Ventura, R., Alcaro, A., & Puglisi-Allegra, S. (2005). Prefrontal Cortical Norepinephrine Release Is Critical for Morphine-induced Reward, Reinstatement and Dopamine Release in the Nucleus Accumbens. *Cereb. Cortex*, *15*(12), 1877-1886.

Ventura, R., Alcaro, A., & Puglisi-Allegra, S. (2005). Prefrontal cortical norepinephrine release is critical for morphine-induced reward, reinstatement and dopamine release in the nucleus accumbens. *Cereb Cortex*, *15*, 1877 - 1886.

Ventura, R., Cabib, S., Alcaro, A., Orsini, C., & Puglisi-Allegra, S. (2003). Norepinephrine in the prefrontal cortex is critical for amphetamine-induced reward and mesoaccumbens dopamine release. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *23*(5), 1879-1885.

Weinshenker, D., Miller, N. S., Blizinsky, K., Laughlin, M. L., & Palmiter, R. D. (2002). Mice with chronic norepinephrine deficiency resemble amphetamine-sensitized animals. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(21), 13873-13877. doi:10.1073/pnas.212519999

Weinshenker, D., Rust, N. C., Miller, N. S., & Palmiter, R. D. (2000). Ethanol-Associated Behaviors of Mice Lacking Norepinephrine. *J. Neurosci.*, *20*(9), 3157-3164.

Weinshenker, D., & Schroeder, J. P. (2006). There and Back Again: A Tale of Norepinephrine and Drug Addiction. *Neuropsychopharmacology*, *32*(7), 1433-1451.

Westfall, T. C. (1974). Effect of nicotine and other drugs on the release of 3H-

norepinephrine and 3H-dopamine from rat brain slices. *Neuropharmacology*, *13*(8), 693-700.

Wise, R. A. (2006). Role of brain dopamine in food reward and reinforcement. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1471), 1149-1158. doi:10.1098/rstb.2006.1854

Yamamoto, B. K., & Novotney, S. (1998). Regulation of extracellular dopamine by the norepinephrine transporter. *Journal of Neurochemistry, 71*(1), 274-280.

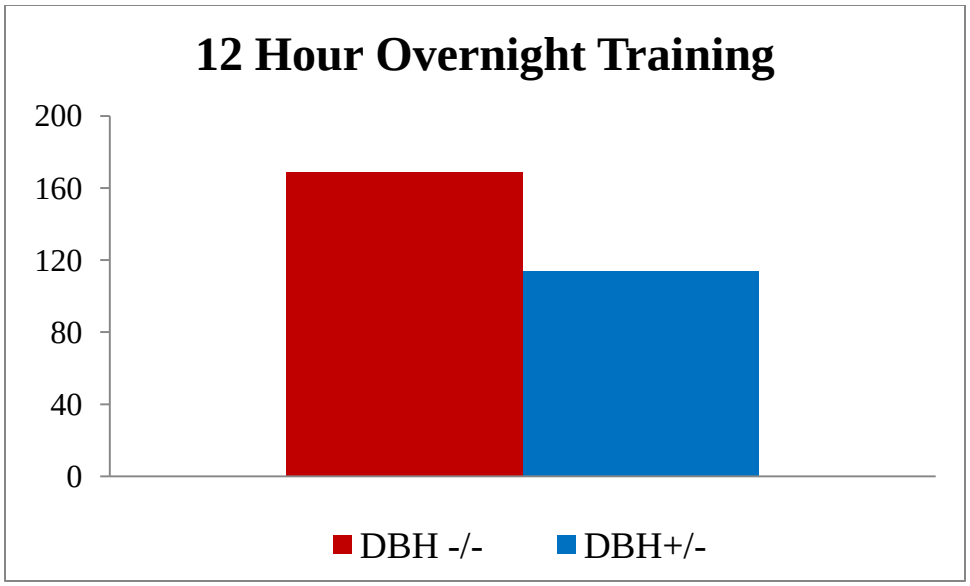

Figure 1 : Responses during the 12 hour overnight training session. DBH-/- mice responded significantly more than DBH+/- controls. t(36) = 3.50, p = .001

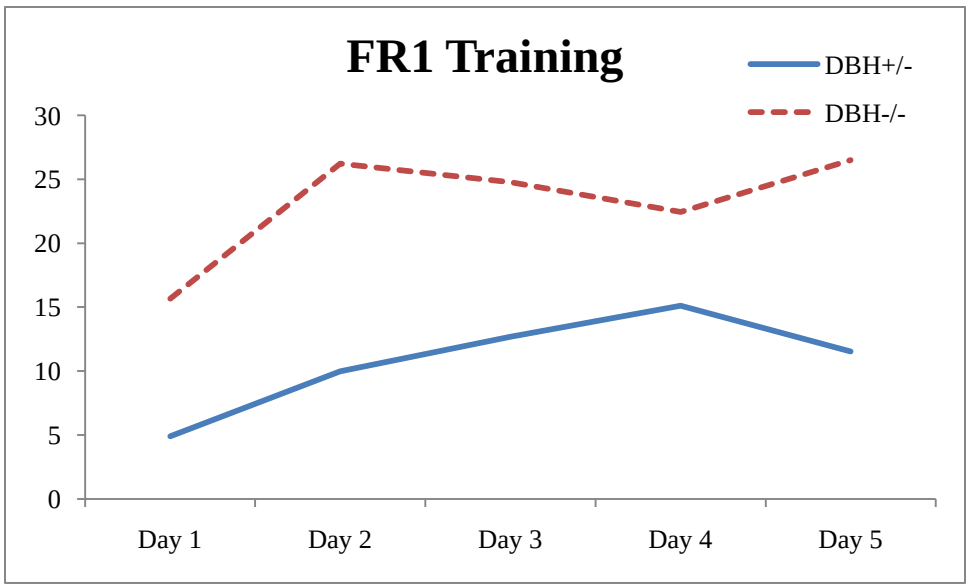

Figure 2: Responses on FR1 across training sessions. DBH-/- responded significantly more than DBH+/- , $F(1,36) = 48.62$, $p < 0.001$

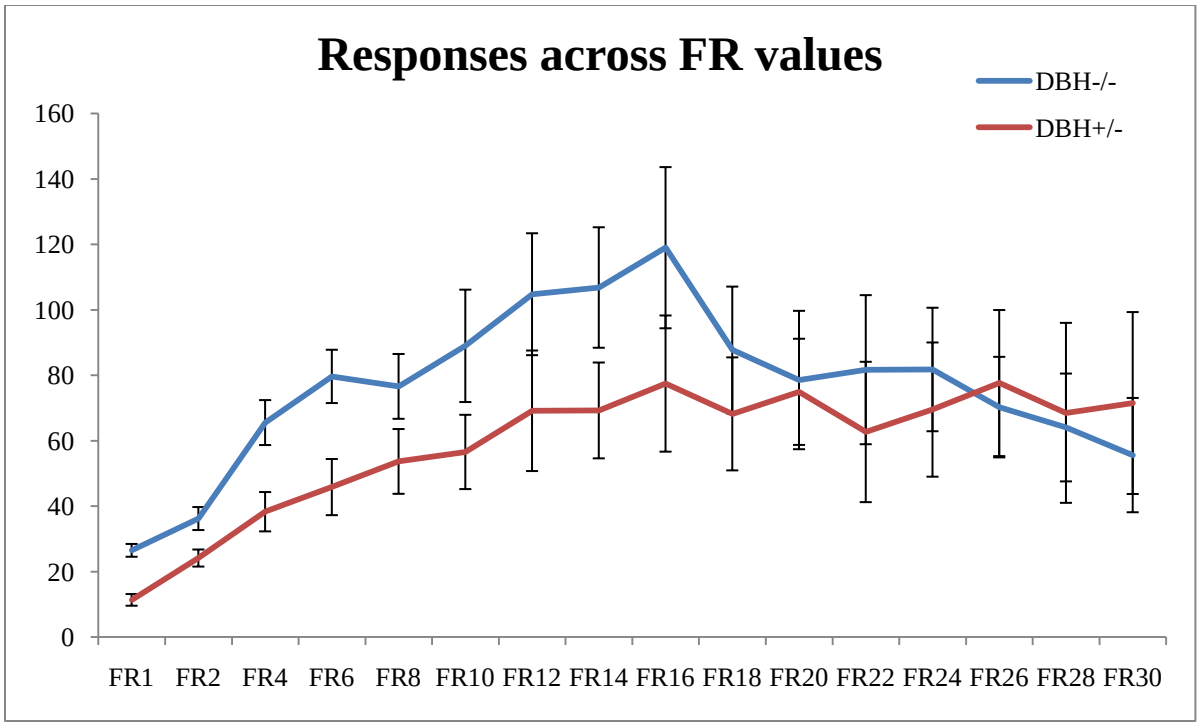

Figure 3a: There is no statistical difference between genotypes when compared across all testing sessions, $F(1, 35) = 1.12$, $p > 0.05$.

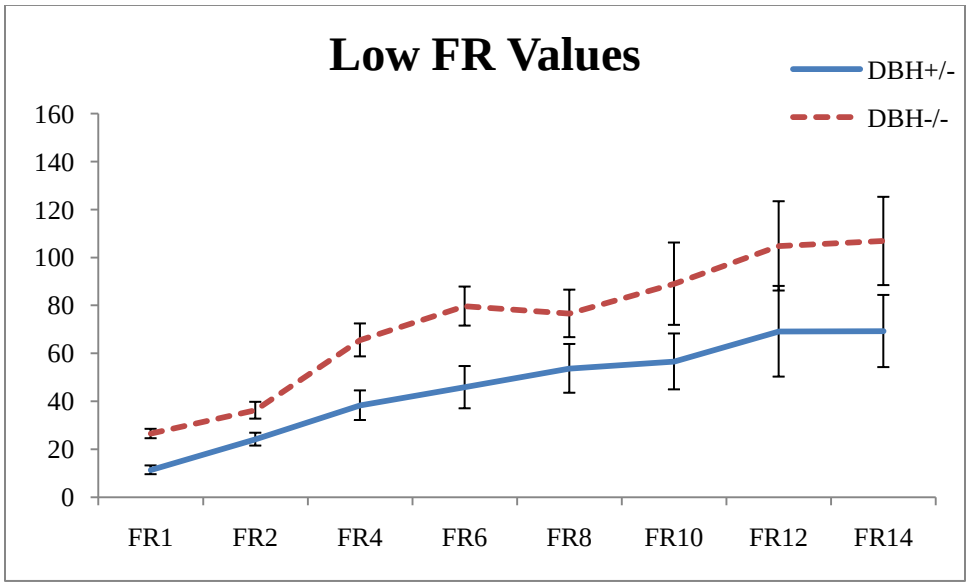

Figure 3b: When the response requirement was low (FR1-FR14), the DBH-/- mice responded more than DBH+/- controls. $F(1, 36) = 6.01$, $p = 0.019$

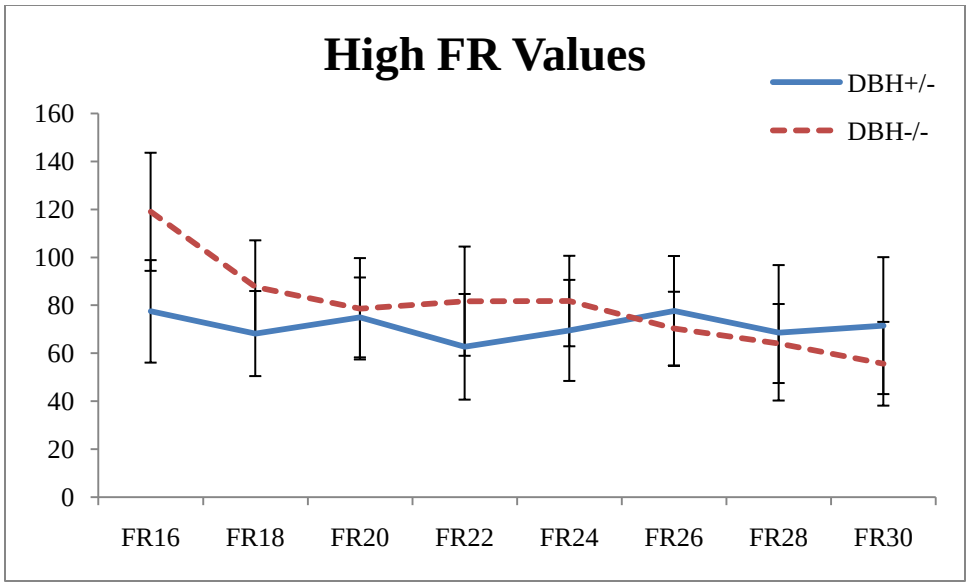

Figure 3c: When the response requirement was high (FR16-FR30), there was no statistical difference between genotypes. $F(1, 35) = 0.12$, $p = 0.734$

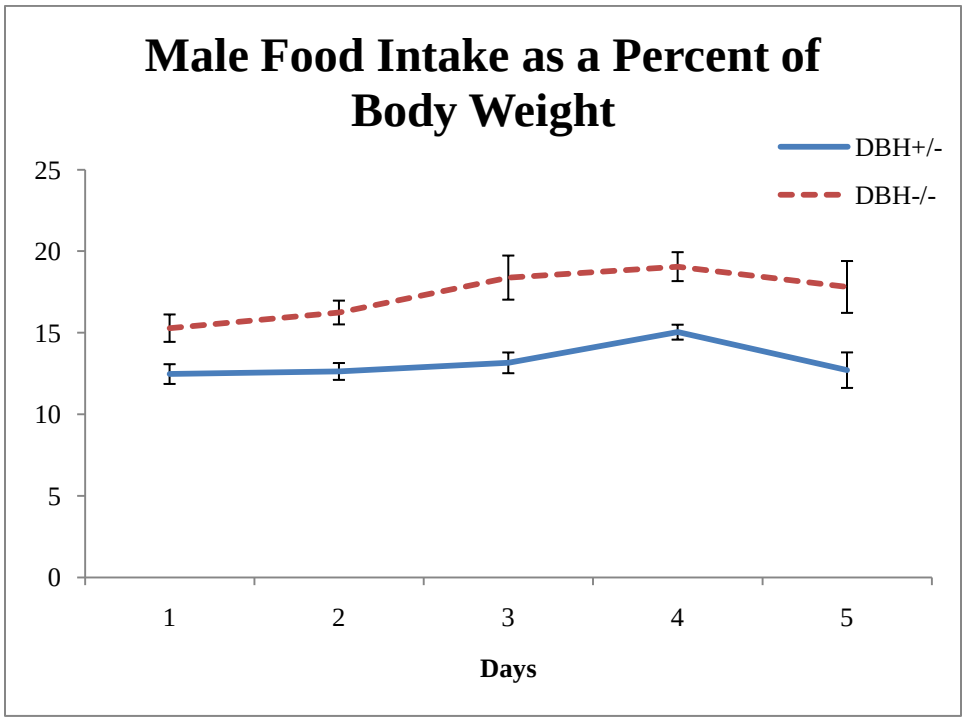

Figure 4a: Male DBH-/- mice ate significantly more food per body weight than male DBH+/- mice, $t(19) = 4.25$, $p < 0.001$.

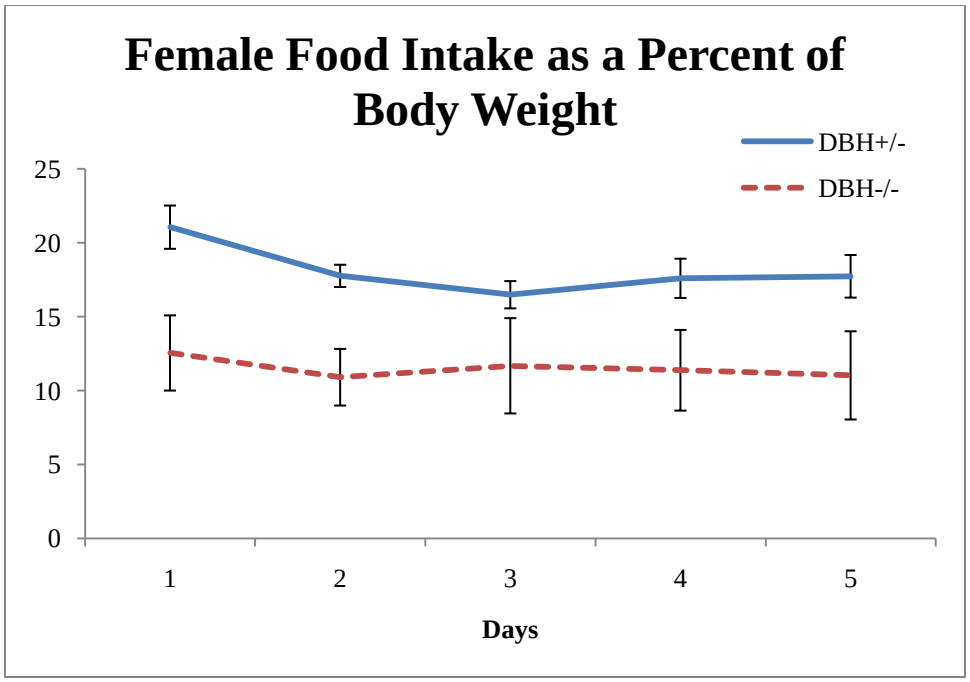

Figure 4b: Female DBH-/- mice ate significantly less food per body weight than female DBH+/- mice, $t(6) = 2.75$, $p = .033$

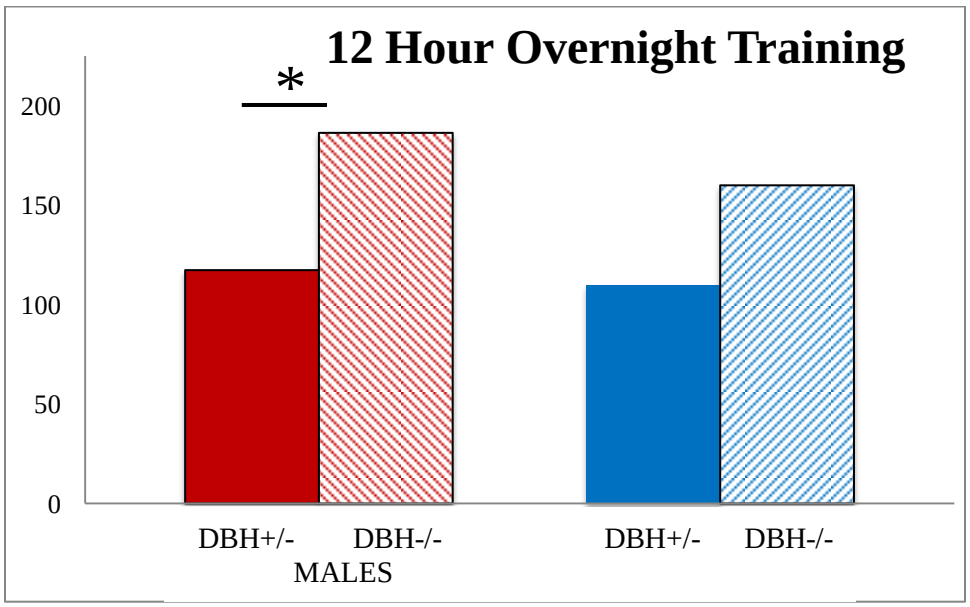

Figure 5: Responses during the 12 hour overnight training session. Male DBH-/- responded more than DBH+/- controls $F(1,17) = 10.01$, $p = .006$, but female mice did not differ by genotype $F(1,19) = 3.79$, $p = 0.065$.

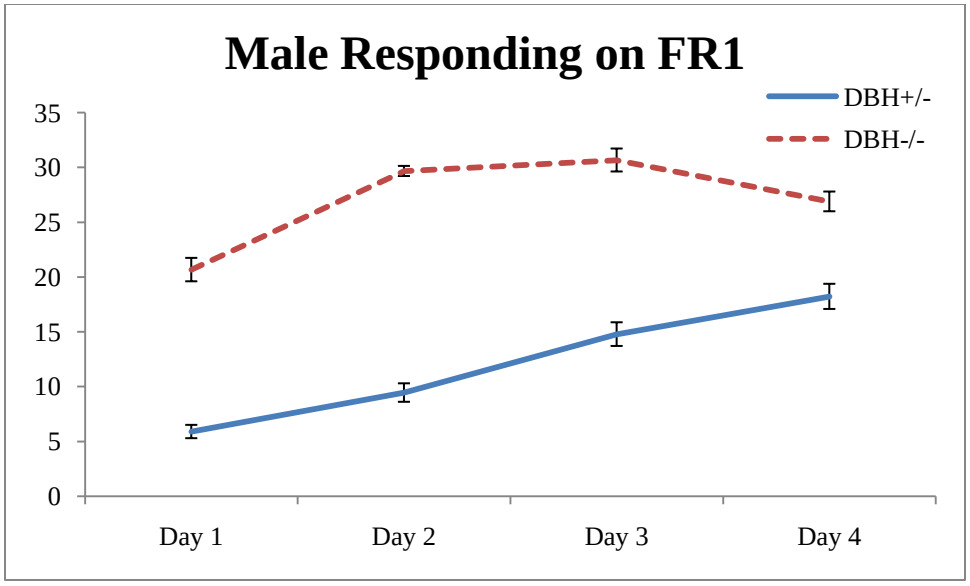

Figure 6a: Male responses on FR1 across four days of training, $F(1,16) = 72.29$, $p < 0.001$

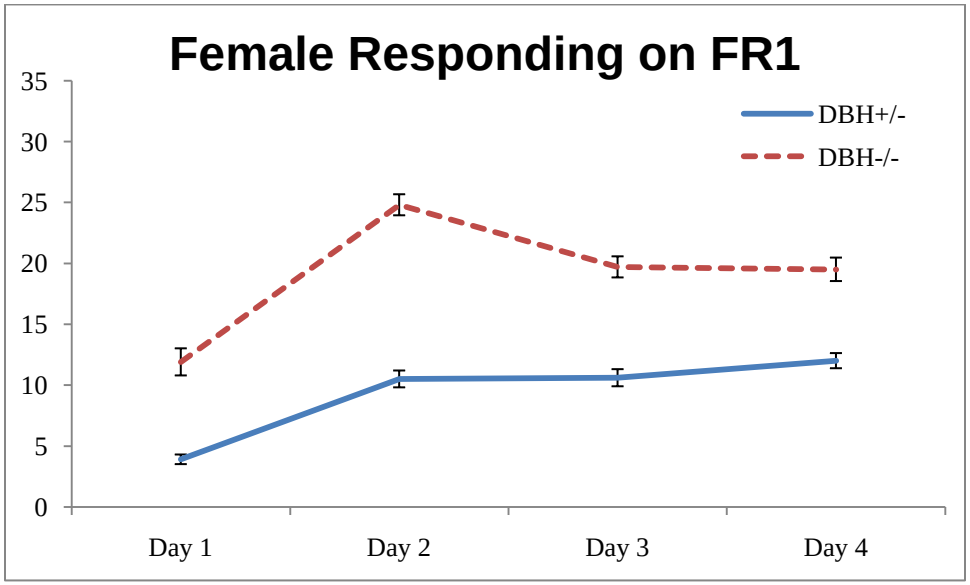

Figure 6b: Female responses on FR1 across four days of training, $F(1,18) = 14.30$, $p = 0.001$

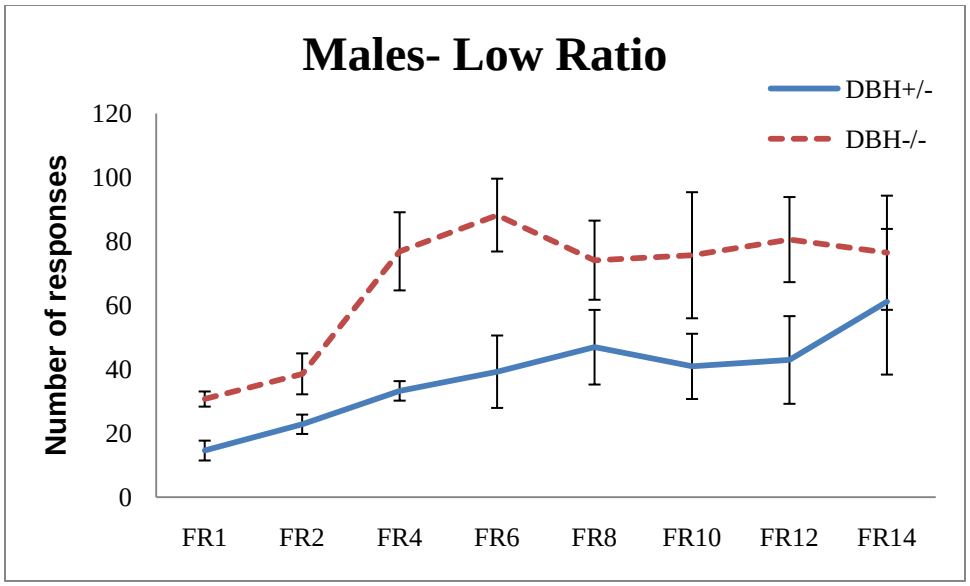

Figure 7: Male responding during low FR values, $F(1, 16) = 5.76$, $p = 0.029$

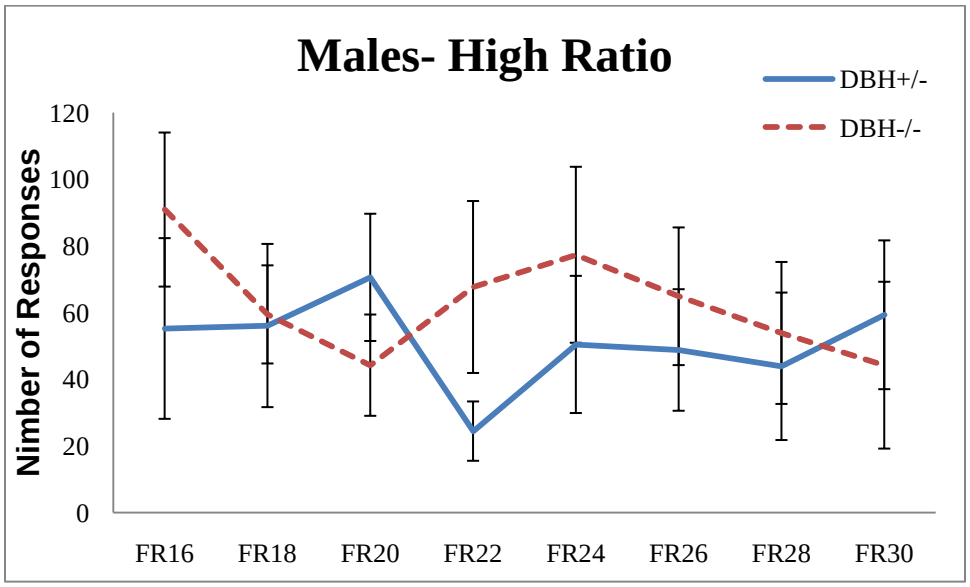

Figure 8: Male responding during high FR values $F(1, 16) = .231$, $p = 0.638$

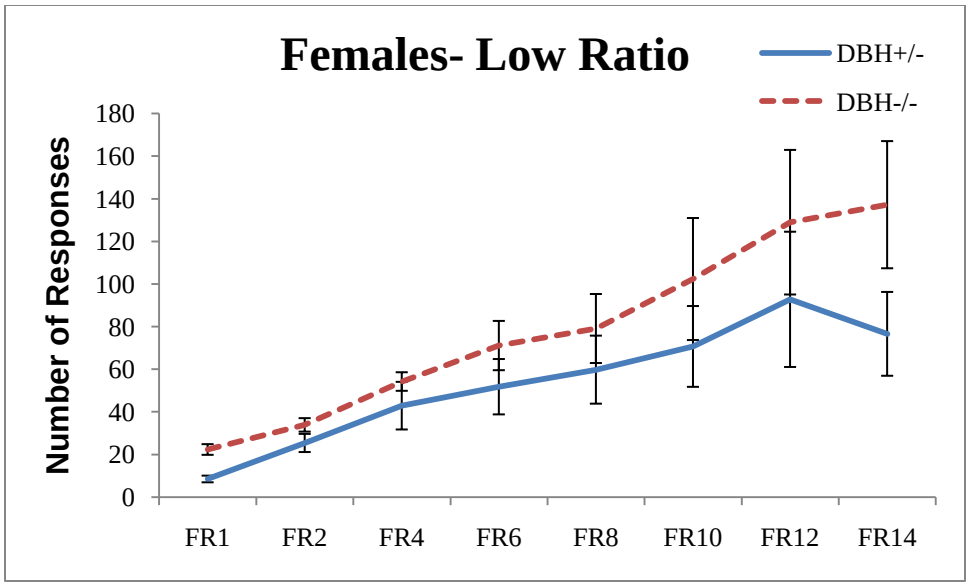

Figure 9: Female responding during low FR values, $F(1, 18) = 1.92$, $p = .183$

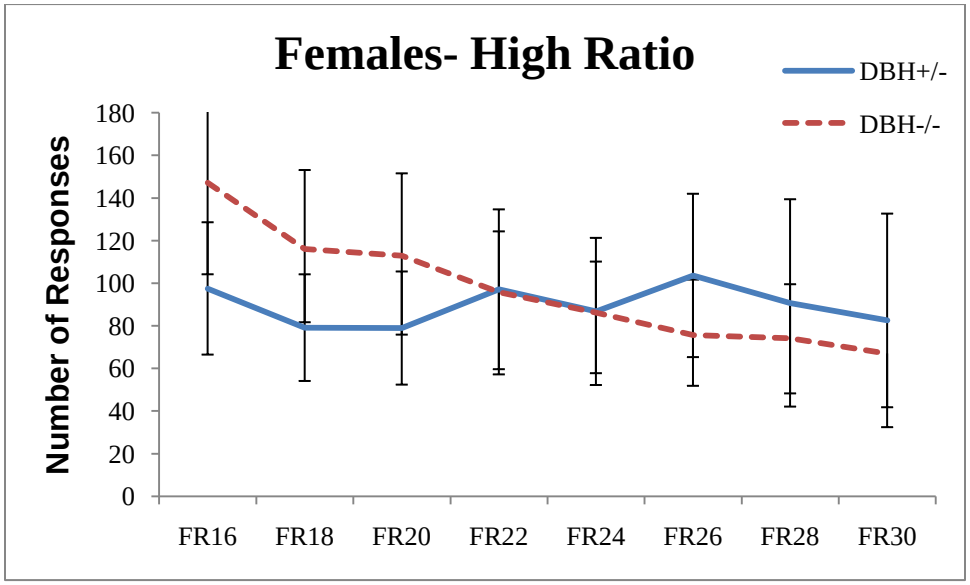

Figure 10: Female responding during high FR values, $F(1,17) = .030$, $p = .865$

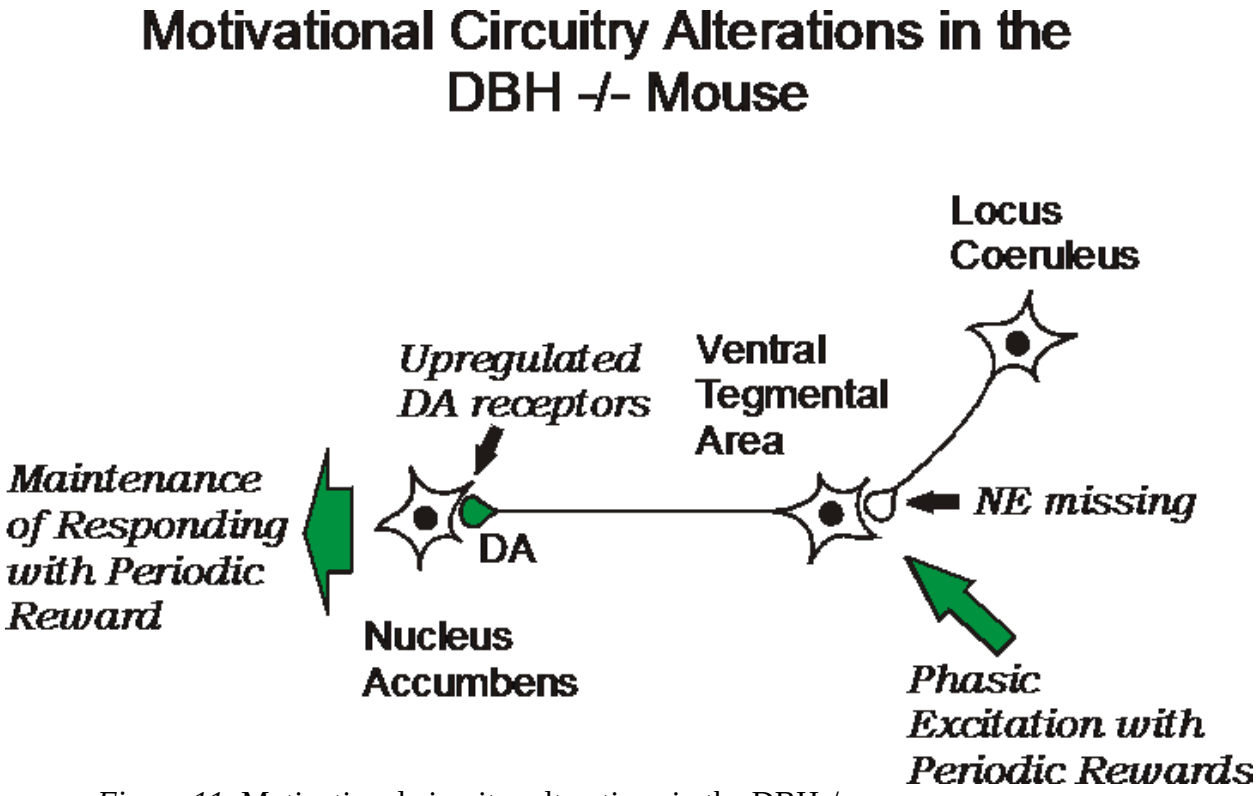

Figure 11: Motivational circuitry alterations in the DBH-/- mouse.