5-2018

Chasing the Androgen Receptor: Expression and Localization

Garrett Santini
Lehigh University

Chasing the Androgen Receptor: Expression and Localization

Author: Garrett Santini
Eckardt Scholar Program 2018

Swann Lab
Lehigh University

Abstract:

Testosterone is known to be capable of rescuing of male copulatory behavior in gonadectomized males, but fails to cause this behavior in gonadectomized females. This thesis seeks to analyze the activational effects of testosterone on its receptor, the androgen receptor, in the medial preoptic area (MPOA) of male and female Syrian hamsters. Both the total expression and subcellular localization levels were analyzed to measure for differences in the amount and activity of the AR respectively. While the results have yet to be verified through sufficient replication for statistical analysis, results thus far indicate that there is a sex difference in expression and localization of AR.

Introduction:

Hormones are powerful influencers of behavior (Petrulis et. al). In mammals, sex hormones enact their effects by both influencing development of the organism and acting on sensitized tissues during and after puberty (De Lorme et. al). In order to exert these effects, these hormones bind to receptors, which enact signalling cascades that lead to changes in gene transcription (Cunningham et. al). Despite the fact that males and females share the same hormones and receptors, albeit in varying ratios, downstream effects of hormone replacement persist (Pfaff et. al). In the Syrian hamster (*Mesocricetus auratus*), as well as many other rodent species, testosterone (T) replacement therapy after gonadectomy (GNX) completely restores male-typical sex behavior in males, but fails to incite it in females (Noble et. al, Tiefer & Johnson). This phenomenon demonstrates a sex difference in the activational effect of testosterone, but the exact mechanism through which this occurs is unknown. Rodents have a

sexually dimorphic region of the hypothalamus known as the medial preoptic area (MPOA) that is important in mediating male-typical copulatory behavior in males (Hull & Dominguez). T primarily exerts its effects through signalling in response to binding with its receptor, the androgen receptor (AR) either directly or via its reduced metabolite, DHT. Consequently, AR signalling events in the MPOA in response to T are a potentially important mechanism through which the behavior is promoted.

As noted in a review by Pfaff et. al, several studies have demonstrated that the androgen receptor expression is upregulated in the brain response to T replacement. Unsurprisingly, AR expression in the brain is sexually dimorphic; females express less than males (Wood and Newman). This result is consistent with the hypothesis that androgens upregulate their receptor since males have more circulating androgens, consequently promoting higher expression. It is therefore possible that females simply do not express enough receptor to demonstrate the T-mediated response that leads to sex behavior. However, androgen receptor expression is upregulated in GNX females given T replacement, and its expression is equivalent to that of males given the same dose of T (Pfaff). Importantly, this study was done on the entire brain, not a specific sexually dimorphic nucleus. It is possible that T does not upregulate AR expression in the MPOA enough in females to meet this hypothetical threshold that must be crossed to incite male typical sex behavior.

One of the primary mechanisms through which androgens exert their effects is via signalling that occurs once the steroid has bound to its receptor, the androgen receptor (AR). As the MPOA is densely populated with ARs, that bind testosterone, one possibility may be a difference in the number of receptors. Female hamsters have fewer immunolabeled receptors in

the MPOA than males (Wood and Newman 1999), supporting our theory. However, this result does not address the role of testosterone. Testosterone up regulates its receptor and females have less testosterone than males. The study did explore the role of the non aromatizable androgen DHT. DHT was shown to increase androgen labelling of neurons in both males and females - but the effect was not quantified. Another study examined the effect of short and long days on T-mediated upregulation of AR in the hypothalamic preoptic area (HPOA) of males of Siberian hamsters, *Phodopus sungorus*, (Bittman et. al in 2003).

Given that all of the downstream signalling of T occurs through signalling of the AR, it is a logical molecular brake on this system. While it is certainly possible that feminization of the brain renders the neural circuitry impossible of enabling male copulatory behavior, regardless of cellular signalling, there is evidence that synaptophysin, a cellular marker of presynaptic plasticity, has sex differential expression irrespective of testosterone levels (Smith Dissertation), and indicator that AR expression and/or localization may also be sexually dimorphic irrespective of circulating T. The goal of this thesis was to analyze the expression profile and subcellular localization of AR in order to investigate it as a possible mechanism through which downstream male copulatory behavior is inhibited in females compared to males

Methods:

Adult male and female Syrian hamsters (Mesocricetus auratus), averaging around 60 days old, bred from our breeding colony were used in this experiment. The hamsters were maintained on 14:10 light-dark cycle. All animals were housed in a large cage (45 cm x 24 cm x 20 cm) containing only the animals and sawdust bedding (3 m3), with ad libitum access to food and water located on the wire cage top.

All animals that were gonadectomized were done so at three months of age. All animals were given Metacam orally and then anesthetized with a 3% induction/2% maintenance of isoflurane with 100% O2. All animals were shaved and disinfected at the incision site with 70% ethanol, iodine, and again with 70% ethanol. For castration, an incision was made in the scrotum of adult males. The testis were freed from their musculature and excised through the incision. The major artery was ligated and the testis, fat pads, epididymis and musculature were removed. The wound was then sutured. For ovariectomy, two incisions were made medial to the flank marks, the ovaries and fat pads will be exposed, the uterine horns were ligated and the ovaries and fat pads were removed; then the wounds will be tied shut with suture thread.

Nine weeks after gonadectomy surgery, all gonadectomized males received three weeks of treatment with either subcutaneous injections of mineral oil (0.1mL mineral oil; GNX+MO, OVX+MO) or testosterone (500ug/0.1mL dissolved in mineral oil; GNX+T, OVX+T). Injections were given every 48 hours for a period of three weeks. This is the amount of time supported by the literature that it takes T to fully recover male copulatory behavior (Pfaff).

All groups of animals were age matched and euthanized on the same day (within two hours of the dark cycle) via overdose of somnasol. The brains were extracted, blocked for MPOA and Cortex (control) tissues, and stored at -80 until homogenization. A subcellular fractionation was done to divide the homogenized lysate into a nuclear and a cytosolic fragment using Nuclear Extraction Kit (ab113474). All samples were sampled for total protein using an RCDC protein assay. Two western blots were run for each sample, one for the cytosolic fragment and one on the nuclear fragment. Since the androgen receptor is translocated to the nucleus after activation, Lamin-B (DSHB ADL84.12) was used as a loading control for nuclear

fragments since it is present in significant quantity in the nuclear membrane. Beta Tubulin (DSHB E7) will be used as a loading control for cytosolic fragments since it is present in significant quantity in the cytosol. The secondary used for the loading controls was Jackson ImmunoResearch Peroxidase AffiniPure Goat Anti-Mouse IgG (Lot#116475) Loading controls are required for quantitative comparison since they allow for normalization of how much total protein was loaded into each well. The androgen receptor primary antibody that was used was RbαAR SP107 MAb (Lot# 170622LVG) with secondary antibody Jackson ImmunoResearch Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L, Lot# 115952)

The resulting blots from these experiments will be quantified using image lab software (BIORAD). One way ANOVA will be used to compare AR expression between all six groups, and a posthoc Tuckey's HSD with a bonferroni adjustment. Effect sizes will be calculated using partial eta squared.

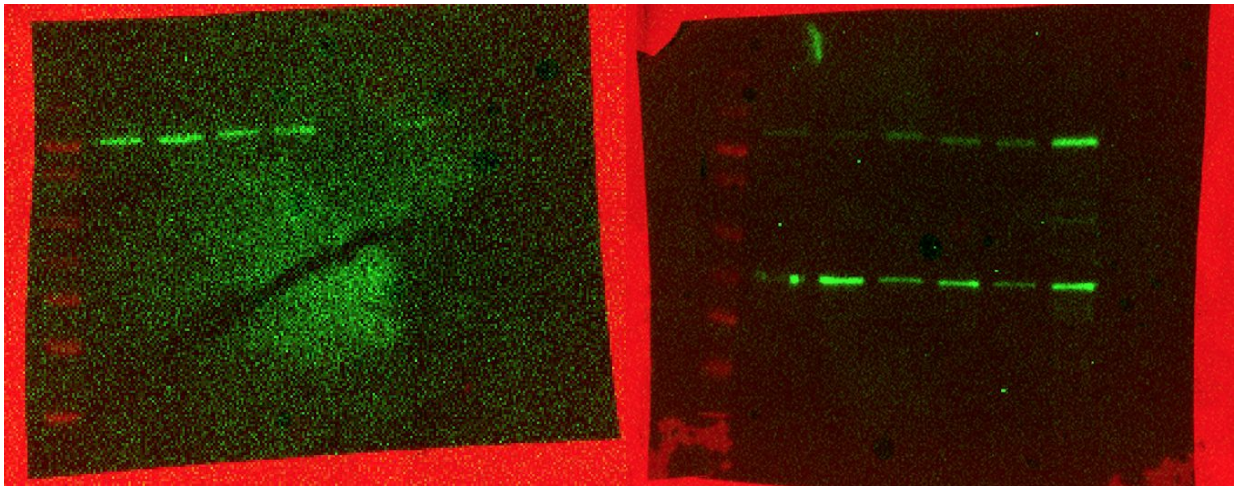


Figure 1: Group 1 Western Blots tagged with RbαAR SP107 MAb (Lot# 170622LVG) with secondary antibody Jackson ImmunoResearch Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L, Lot# 115952). From left to right, the individuals of this group are male, GNX male + MO

(vehicle), GNX Male + T, female, GNX female + MO, GNX female + T. The nuclear blot is on the left and the cytosolic blot is on the right. Background on the nuclear fragment is high and may slightly confound the results.

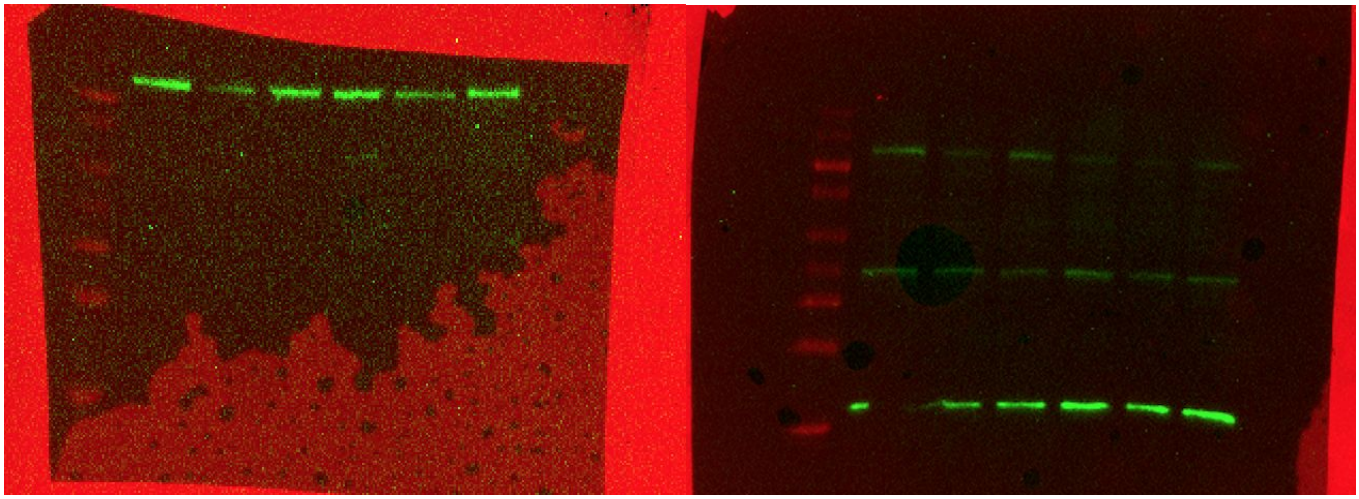
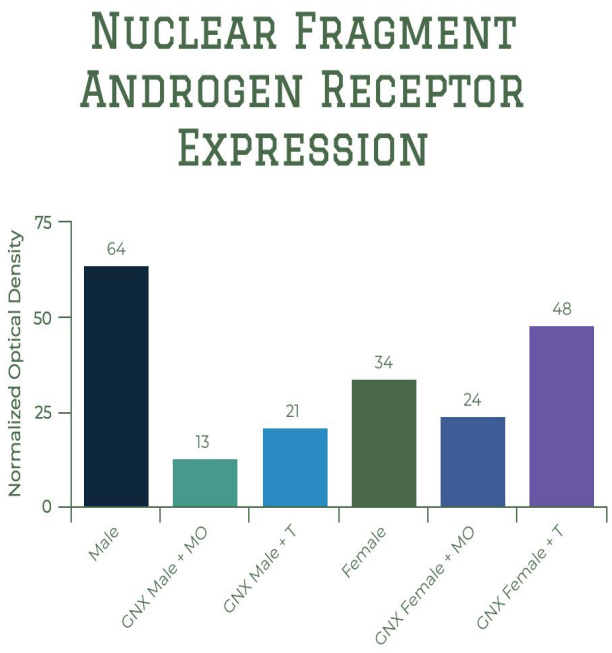


Figure 2: Western Blots for Group 2, in the same order as displayed in figure 1, again with the nuclear fragment on the left and cytosolic on the right.



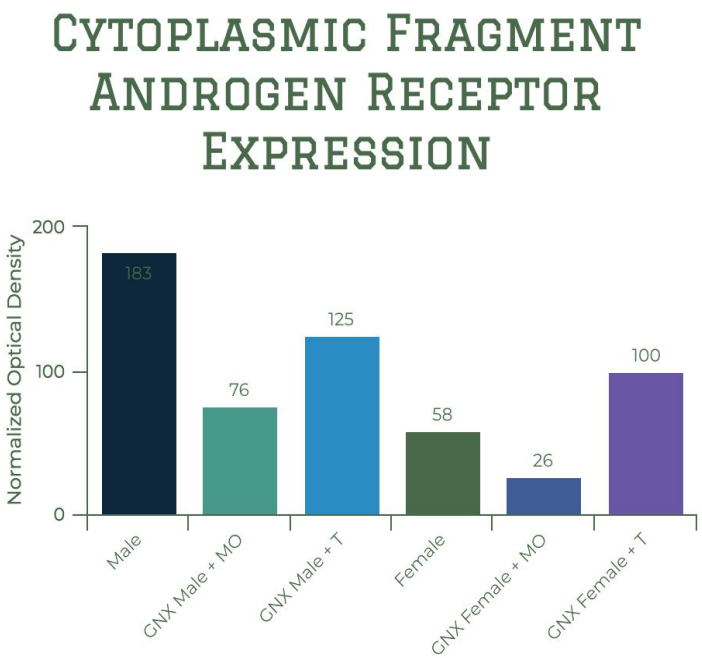


Figure 3: Graphical representation of the normalized optical density of AR expression from the group 2 Western Blots.

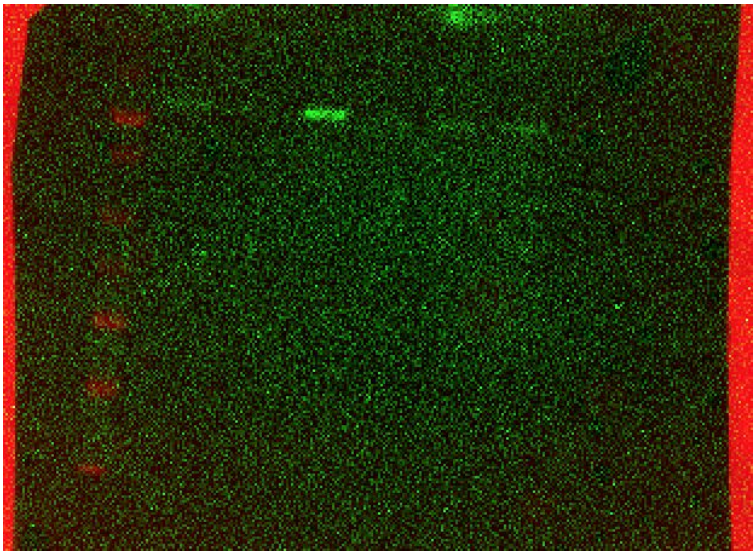


Figure 4: Western Blot for the nuclear fragment of Group 3. This blot is a failed trial due to the low amount of labelling and high intensity of background.

Conclusion:

Although all five groups have been run via Western Blot, only two were completely finished with adequate cytoplasmic and nuclear AR staining without confounding background. While this result is frustrating, in more time the differences will be able to confirmed after more rigorous statistical analysis. Out of Group 1 and Group 2, Group 2 has labelling at the correct molecular weight with minimal background, making it the most indicative of what I believe the answer to the question to be. Overall, the correlating groups are roughly equivalent. The intact

males and females show a difference in expression, but their proportional amount of translocation is about the same. The gonadectomized groups given mineral oil show the lowest expression and the least amount of translocation. This does contradict a previous finding in the lab that gonadectomized males given mineral oil have higher levels of cytosolic AR expression than intact males and males given testosterone, but nonetheless, these are the results. I believe after running more groups, the expression of gonadectomized males given testosterone will increase to be more similar to that of intact males. I predict a marginal sex difference in expression of AR that leads to a sex difference in localization, as well. It appears that translocation is proportionally the same, but differs because males have higher basal expression levels than females. To reemphasize, these results have not yet been statistically validated, but once they are, I hope to submit these data for publication.

Sources:

1. Bittman, E. L., Ehrlich, D. A., Ogdahl, J. L., & Jetton, A. E. (2003). Photoperiod and Testosterone Regulate Androgen Receptor Immunostaining in the Siberian Hamster Brain1. Biology of Reproduction, 69(3), 876-884. doi:10.1095/biolreprod.102.010900
2. Celec P, Ostatníková D, Hodosy J. On the effects of testosterone on brain behavioural functions. Front Neurosci. (2015); 9, pp. 12.
3. Cunningham, R. L., Lumia, A. R., & Mcginnis, M. Y. (2012). Androgen Receptors, Sex Behavior, and Aggression. *Neuroendocrinology, 96*(2), 131-140. doi:10.1159/000337663
4. K. De Lorme, M.R. Bell, C.L. Sisk. The teenage brain: social reorientation and the adolescent brain — the role of gonadal hormones in the male Syrian hamster. Curr Dir Psychol Sci, 22 (2013), pp. 128–133
5. Hull EM, Dominguez JM. Sexual behavior in male rodents. Horm Behav (2007); 52, pp.45–55 R.G.
6. Noble, P.B. Alsum. Hormone dependent sex dimorphisms in the golden hamster (Mesocricetus auratus). Physiol. Behav., 14 (1975), pp. 567–574
7. Pfaff, D. W. (2002). *Hormones, brain and behavior*. pp. 339-393
8. Petrulis, A. (2013). Chemosignals, hormones and mammalian reproduction. *Hormones and Behavior, 63*(5), 723-741. doi:10.1016/j.yhbeh.2013.03.011
9. Tiefer and Johnson, 1975. L. Tiefer, W. Johnson. Neonatal androstenedione and adult sexual behavior in golden hamsters. J. Comp. Physiol. Psychol., 88 (1) (1975), pp. 239–247
10. Wood R, Newman S. Androgen receptor immunoreactivity in the male and female Syrian hamster brain. J Neurobiol., 39 (1999), pp. 359–370.