ASAS-SSR Triennial Reproduction Symposium: Looking Back And Moving Forward—How Reproductive Physiology Has Evolved: Male reproductive behavior: sensory signaling in the brain of low-performing domestic rams¹

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ABSTRACT: Rams are selected for genetic traits a producer desires to propagate in his flock. Even though practically all sheep are naturally bred, rams are rarely evaluated for expression of sexual interest or behavior. Research at the U.S. Sheep Experiment Station reported that the proportion of rams with limited interest in ewes was nearly 30% of the total number of breeding rams. Breeding soundness tested rams with low sexual interest sire less than 16% of the lamb crop. Although producers recognize the problem, their usual solution is to increase the number of rams in breeding flocks decreasing the risk of open ewes. Increased costs and a lack of genetic progress are clearly important considerations, but the biological question as to what controls sexual interest remains intriguing. Circulating concentrations of testosterone do not differ by sexual interest among rams. However, following exposure to estrous ewes, sexually active, but not inactive, rams exhibit an increase in LH pulsatile activity, a biological response to sexual stimuli. This begs the question as to whether sexually inactive rams recognize sexual cues. Using *c fos* activity as an indicator of neural activity, differences in the

olfactory pathway were compared among sexually active and inactive rams. Differences in *fos* activity were not detected in the olfactory bulb or medial amygdala. However, sexually inactive rams had lower *fos* activity in the central amygdala, bed nucleus of the stria terminalis, and medial preoptic area of the hypothalamus following exposure to sexual evocative olfactory stimuli. This suggests sexually inactive rams detect olfactory cues but have decreased vigilance to sensory stimuli and a muted response to sexual signals in areas of the brain required for the execution of sexual performance. With the amygdala receiving and integrating sensory signals from many areas of the brain, sexually inactive rams may lack normal reward mechanisms contributing to their lack of sexual interest. Rams with limited sexual interest have decreased dopamine synthesis and a lower expression of dopamine D2 receptors following exposure to sexual stimuli. Thus, a tempered reward pathway in combination with decreased vigilance and sensory processing in the amygdala may reduce stimulation and/or responsiveness in areas of the brain required for expression of sexual behavior.

Key words: ram, reward pathway, sexual behavior

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INTRODUCTION

Variability in the expression of sexual interest in domestic rams was first reported by Hulet et al. (1964). Over an 8-yr period, approximately 30% of 2,175 rams tested were classified as sexually inactive. Thirty years later, Fitzgerald and Perkins (1991) evaluated 374 yearling and 2-yr-old rams

at the same site and classified 50% of them as average or high performing, while 50% of them lacked sexual interest or were classified as low sexual performers. In a competitive breeding environment, high sexually performing rams bred twice as many ewes as low-performing rams (Stellflug et al., 2006). The genetic progress any ram can make within a flock is dependent in-part on his expression of sexual interest. Rams with acceptable breeding soundness and a high serving capacity from three producer flocks comprised 23% to 25% of the ram population but sired between 39% and 70% of the lambs born. Similar numbers of rams tested for breeding soundness with a low serving capacity sired only 3% to 16% of the lamb crop (Alexander et al., 2012). Although producers circumvent the problem of having rams with low sexual interest in the flock by decreasing breeding intensity, the biological question remains of what governs sexual interest. Rams with low sexual interest either do not recognize sexual cues or those cues are processed differently within the central nervous system than in high sexually performing rams.

Recognizing Females in Estrus

For reproduction to occur, females must communicate their ovulatory status and males must accurately detect those cues. Cues of eminent ovulation are received by the male's sensory system where the significance of those cues is extracted and the ram responds with the appropriate behavior. Although sexual behavior seems innate and simple, successful execution of reproductive behavior requires complex processing of sensory cues. Sensory information necessary for the identification of sexually receptive females differ by species. Ewes do not mount other females in estrus or show other overt signs of ovulation. Ewes exhibit proceptive behavior and seek out rams and affiliate with them to garner attention of the ram (Perkins and Fitzgerald, 1994). It is a subtle behavior putting greater importance on other sensory signals to ensure successful reproduction.

Although sheep have excellent vision (Piggins and Phillips, 1996; Jacobs et al., 1998), rams use olfaction to identify ewes in estrus and accurately differentiate the odor of estrus from the other days of the cycle except for the 3 d following estrus (Blissitt et al., 1994). The accessory olfactory system is likely important in confirming what was noted by primary olfaction since flehmen behavior is displayed most frequently on the days leading up to ovulation (Bland and Jubilan, 1987).

Interestingly, expression of the flehmen response requires an intact primary olfaction, but flehmen is displayed even when the vomeronasal organ is occluded (Hart, 1987). Although olfaction plays an important role in the identification of females at ovulation in many species including humans (Miller and Maner, 2010), rodents, goats, and horses, olfaction is surprisingly unimportant to the bull with bulls showing no preference for a heifer in estrus when physical contact is denied (Geary et al., 1991). Bulls express equal numbers of flehmen responses toward heifers in estrus and those in diestrus and show no preference for being near heifers in estrus when physical contact is precluded.

When considering the specific deficit in low sexually performing rams, the question of whether they have the ability to identify ewes in estrus should be considered. It is possible that rams with low sexual interest lack olfactory acuity due to lack of expression of specific chemical receptors. Odorants are detected by G-protein-coupled chemical receptors located on the nasal epithelium (Ache and Young, 2005). Although it is possible that an absence of a single receptor could influence odor detection and the expression of sexual behavior (Keverne, 2004), it seems unlikely because there is a broad variety of chemical components of the pheromones near ovulation (Hart, 1987), and loss of a single chemical receptor would be unlikely to preclude detection of complex odorants. Rams expressing low levels of sexual interest had similar *fos* activity in the olfactory bulb following exposure to isolated olfactory cues of ovulation as sexually active rams (Mirto et al., 2017). *Fos* is a general indicator of neural activity, and although it cannot be determined which olfactory receptors are stimulated, these data indicate the olfactory bulb of low and high sexually performing rams is similarly activated following exposure to urine from ewes in estrus (Mirto et al., 2017). Therefore, it may be concluded that low sexually performing rams detect ewes in estrus but do not act on those cues similar to high-performing rams.

The amygdala is directly innervated by the olfactory bulb (Brennan and Keverne, 1997). Low-performing rams detect olfactory cues; however, it appears that the biological significance of those cues is not properly translated in low sexually performing ram. The amygdala is important for the integration of sensory cues (Davis and Whalen, 2001) with an essential role in fear conditioning but is also important for the interpretation of other sensory stimuli. The amygdala sits in the critical juncture between the stimulus (olfaction) and

the execution (preoptic area of the hypothalamus [POA]) of reproductive behavior and is an area where biological signals could be misinterpreted. The number of receptors for estradiol in the amygdala did not differ among rams with high or low sexual activity (Alexander et al., 1993), but rams with low sexual interest were able to be discriminated from high sexually performing rams based on neuron size in the medial amygdala and POA of the hypothalamus (Alexander et al., 2001b). Although, the significance of neuron size is difficult to determine, a sexually dimorphic nucleus in the POA (SDN-POA) has been identified in sheep. This area is larger in rams than ewes (Roselli et al., 2004) with the organization dependent on prenatal exposure to testosterone (Roselli et al., 2007). Although the area of the SDN-POA has been associated with partner preference (Roselli and Stormshak, 2010), its functional significance remains elusive.

Neural activity in the medial amygdala did not differ among low or high sexually performing rams following fence-line exposure to ewes in estrus (Alexander et al., 2001a) or exposure to isolated olfactory cues of eminent ovulation (Mirto et al., 2017). The main and accessory olfactory bulb has direct connections with the medial and cortical amygdala (Meurisse et al., 2009). The amygdala nuclei are interconnected (Meurisse et al., 2009) allowing for integration of sensory stimuli. Of the amygdala nuclei, the central nuclei are important in fear conditioning (Davis and Whalen, 2001) initiating a state of arousal toward nonspecific stimuli (Gallagher et al., 1990). Fear, however, should be considered a specific state with a heightened sense of awareness which would be advantageous during social settings such as the identification of receptive females. Rams with high sexual interest have increased neural activity in the central amygdala following exposure to olfactory cues associated with estrus than rams with low sexual interest (Mirto et al., 2017). Sexually active rams may be more aware and vigilant than sexually inactive rams and that vigilance facilitates increased sexual activity. In a production setting, increased vigilance may be apparent in the investigation of the ram's environment. For example, sexually active rams track to the feed source more often without consuming feed than do rams with low sexual interest (Uthlaut et al., 2011).

In sheep, the medial and cortical amygdalae have reciprocal connections to the bed nucleus of the stria terminalis (BNST) and medial preoptic area (mPOA) of the hypothalamus (Meurisse et al., 2009). Although differences in *fos* expression in the

medial and cortical amygdalae were not noted by Mirto et al. (2017), sexually inactive rams had fewer numbers of *fos* positive neurons in the BNST and mPOA than sexually active rams exposed to urine from ewes in estrus. Fence-line exposure to ewes in estrus increased *fos* activity in the mPOA and BNST of low sexually performing rams when compared with *fos* activity following exposure to other rams, but no differences were detected among low or high sexually performing rams (Alexander et al., 2001a). These data suggest that low sexually performing rams are detecting ewes in estrus and are able to differentiate among ewes in estrus and other rams, but this detection does not translate to investigatory or mounting activity. In a separate study, low-libido rams with full access to ewes had decreased *fos* expression in the mPOA than rams with high sexual interest. Differences were not noted within the BNST (Borja and Fabre-Nys, 2012). The POA is essential for the expression of male reproductive behaviors—especially mounting and intromission (Hart and Leedy, 1985). Lower *fos* expression in the mPOA may reflect the reduced mounting activity in low-libido rams. Differences in exposure paradigms, and hence sensory stimulus, among these studies would exert different effects on neural activity, and the integration of those sensory signals likely account for the inconsistent results. Efferent projections from the amygdala differentially activate downstream nuclei in low sexually performing rams. Reduced activity in the mPOA could either reflect decreased mounting activity (Fabre-Nys and Martin, 1991) or a lack of input to the mPOA from afferent connections (Mirto et al., 2017).

Role of Testosterone in Male Behavior

When considering male sexual behavior, it is often testosterone that comes immediately to mind. Testosterone is the recognizable face of male sexual behavior. It is easy to equate serum concentrations of testosterone to expression of sexual behavior when animal husbandry practices routinely castrate males to eliminate sexual behavior. However, circulating concentrations of testosterone are a poor predictor of sexual behavior, and sexual interest is maintained in sexually experienced males with 10% of normal concentrations of testosterone (Damassa, 1977). Testosterone is produced well in excess of amounts required to support sexual behavior (Hull, 2002). Numerous studies have confirmed that concentrations of testosterone are similar among rams with low and high sexual interest (Perkins et al., 1992; Alexander

et al., 1993; Pinckard et al., 2000). Clearly, the mechanics of erection and ejaculation are testosterone dependent, and sexual behaviors decline in a predictable sequence following castration in many species (Hull, 2002). Similarly, in rams, mounting activity, but not ejaculation, was relatively retained in sexually experienced rams following castration (Pinckard et al., 2000). Demonstration of sexual interest is likely multifaceted, and while testosterone is clearly required for the initiation of sexual interest, other factors help maintain sexual interest in the absence of testosterone. Reinforcement of sexual behavior occurs through the reward pathway where dopamine and the endogenous opioids likely play an important role. Treatment of male rats with naloxone, an opioid receptor antagonist, decreased the male's preference for the place where he had successfully mated females (Miller and Baum, 1987). Indicating that place preference following sexual conditioning is at least partially dependent on opioid release which triggers ventral tegmental dopamine (Rivier and Rivest, 1991; Spanagel et al., 1992). Interestingly, even though basal and induced concentrations of testosterone are not different among low and high sexually performing rams, high-performing rams exposed to ewes in estrus have an early and immediate rise in testosterone that is not observed in low-performing rams (Alexander et al., 1993). This rise in testosterone may have implications for the reward pathway because nasal application of testosterone caused an immediate rise in dopamine at the nucleus accumbens in male rats (de Souza Silva et al., 2009).

Cortisol and Its Influence in Nonsexually Performing Males

Cortisol and implied stress have been a common undercurrent in the discussion of nonbreeding males in many species. It is accepted that increased cortisol suppresses the reproductive axis (Rivier and Rivest, 1991). Because nondominant males have decreased reproductive success, it seems logical that nonbreeding males would have increased concentrations of cortisol, and this, or the preceding social stress, contributes to a lack of reproductive behavior and success. This may be the case in some species (Creel, 2001). Data supporting a role for cortisol in nonbreeding males, however, remain inconclusive. Human males engaged in hand-to-hand fighting had greater increase in testosterone, as well as cortisol, following a winning match than did losers (Elias, 1981). In many species, the dominant male has greater glucocorticoids than subordinate males (Creel, 2001). It is unclear how

cortisol may influence ram behavior. Samples collected from the spermatic vein in anesthetized rams show sexually inhibited rams have decreased testosterone and increased cortisol (Roselli et al., 2002). This increase was not apparent in conscious rams. Low and high sexually performing rams exhibited similar concentrations of cortisol when exposed to urine from ewes in estrus (Kramer et al., 2017). Although cortisol, in some species, certainly fluctuates with the expression of sexual behavior and would likely be important in the competition for appropriate mates, it is not clear whether increased cortisol is a response to social stress or whether it is a natural response to an increased energy demand (Creel, 2001).

Mating and the Reward Pathway

Midbrain dopamine is important for the reinforcement of motivated behaviors, providing salient reinforcement of that behavior (Bromberg-Martin et al., 2010). A potential role for dopamine in sexual behavior was first noted by increased sexual arousal and activity in Parkinsonian patients treated with L-3,4-dihydroxyphenylalanine (Hyyppä et al., 1970). Since the late 1960s, the role of dopamine in sexual motivation has been further elucidated (van Furth et al., 1995). Dopamine synthesis and secretion is both tonic and induced. Tonic synthesis and release of dopamine from the substantia nigra is necessary for normal motor patterns. Induced dopamine signaling comes primarily from the ventral tegmental area of the midbrain providing rapid input to forebrain structures including the nucleus accumbens (Bromberg-Martin et al., 2010) and mPOA of the hypothalamus (Dominguez and Hull, 2005). Much of that work focused on dopamine connections to the mPOA because in all species studied, the integrity of the mPOA is required for mounting activity, and experimental use of dopamine agonists increased, while antagonists decreased sexual motivation (Hull, 2002). Even though the role of dopamine in the expression of sexual behavior has been questioned (Paredes and Agmo, 2004), it remains accepted that dopamine plays a role in general arousal and motivation, important components of sexual activity.

It is possible that rams with greater sexual interest have increased sensory awareness and arousal thereby increasing the probability of identifying ewes in estrus. This increased arousal may be partially mediated by dopamine (Paredes and Agmo, 2004; Bromberg-Martin et al., 2010). In fact, rams with low sexual interest spend less time at the feed bunk (presumably investigating) without

consuming feed than rams with high sexual interest (Uthlaut et al., 2011). This could be interpreted as monopolizing a feed resource; however, Erhard et al. (1998) demonstrated that rams with low sexual interest had equal numbers of successful agonistic interactions when competing for a limited feed resource or in the presence of a ewe, but rams with low sexual interest spent less time with the ewe and achieved fewer mounts. Rams with high sexual interest had increased neural activity, as measured by *fos*, in the ventral tegmental area when exposed to olfactory cues of ovulation than rams with low sexual interest (Kramer et al., 2017). Interestingly, *fos* activity in the ventral tegmental area was similar among high sexually performing rams exposed to putative sexually evocative or nonevocative olfactory cues. However, neurons staining positive for tyrosine hydroxylase, the first and rate-limiting enzyme for dopamine synthesis, were greater in sexually active rams exposed to estrous ewe urine than those exposed to urine from ovariectomized ewes (Kramer et al., 2017). This indicates that dopamine synthesis is responsive to sensory cues of ovulation. It is possible that decreased dopamine activity in low sexually performing rams decreases general arousal as well as tempering perceived reward following sexual activity. Midbrain dopamine-synthesizing neurons have connections to the mPOA (Dominguez and Hull, 2005). Decreased *fos* activity in the mPOA of low sexually performing rams following exposure to sexual stimuli (Mirto et al., 2017) may be a result of decreased dopamine input in rams with low sexual interest (Dominguez and Hull, 2005).

CONCLUSION

Rams expressing limited sexual interest and behavior are approximately one-third of the ram population. In this highly managed species, mostly dependent on natural breeding, a lack of sexual interest is a potential loss of genetic progress and producer profitability. Rams with low sexual interest have normal concentrations of testosterone and an equal ability to compete for limited feed resource. Without any supporting evidence to suggest sexually disinterested rams have increased social stress, other explanations for their deficit must be considered. Rams expressing low sexual interest seem to equally detect putative sexually evocative signals but have a muted response to these signals at the central amygdala, an area important for alertness and vigilance. The biological significance of these signals may be lost at the level of the amygdala in low sexually performing rams preventing the signal

from moving forward to the POA, an area essential for the expression of male reproductive behavior. Because the amygdala is a highly integrated area, lowered dopamine signaling from the midbrain ventral tegmental area may ultimately influence how sexually evocative signals are interpreted. Rams expressing low sexual interest have a reduced dopamine signaling pathway which may ultimately influence how sexual cues are interpreted.

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