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Opioid mediation of learned sexual behavior

Kevin S. Holloway, PhD*

Department of Psychology and Neuroscience and Behavior Program, Vassar College, Poughkeepsie, NY, USA

Identifying the role of opioids in the mediation of learned sexual behaviors has been complicated by the use of differing methodologies in the investigations. In this review addressing multiple species, techniques, and pharmaceutical manipulations, several features of opioid mediation become apparent. Opioids are differentially involved in conditioned and unconditioned sexual behaviors. The timing of the delivery of a sexual reinforcer during conditioning trials, especially those using male subjects, acutely influences the role that opioids have in learning. Opioids may be particularly important in the maintenance of conditioned sexual behaviors during periods of non-reinforcement. This appears to be true both for probe trials and procedures designed explicitly to extinguish a sexual conditioned response. These features of opioid mediation of learning do not appear to be restricted to sexual conditioning paradigms. This suggests that, as for other aspects of sexual learning that despite distinctive features conform to underlying behavioral principles, the mediation of conditioned sexual behavior by opioids relies on processes common across reinforcement systems.

Keywords: *opiate*; *conditioning*; *extinction*; *persistence*; *naloxone*; *learning*

Sexual learning has been investigated in a wide variety of animal models including rats, hamsters, mice, blue gouramis and stickleback fish, quail, pigeons, and fruit flies (see Crawford, Holloway, & Domjan, 1993; Domjan & Holloway, 1998; Krause, 2003; and Pfaus, Kippin, & Centeno, 2001 for reviews). Sexual learning can modify responses to a conspecific. Male rats decrease copulatory attempts after exposure to females with surgically closed vaginas (Kagan, 1955; Whalen, 1961). Male rats allowed to intromit but not ejaculate also decreased copulatory attempts (Kagan, 1955); however, if a set number of intromissions (7) were allowed, males achieved ejaculation with fewer intromissions than males without this contingency (Silberberg & Adler, 1974). Female rats will solicit males more frequently and select them for first ejaculation if the males are scented with an odor (Coria-Avila, Ouimet, Pacheco, Manzo, & Pfaus, 2005; Coria-Avila et al., 2008) or marked by a pigmentation (Coria-Avila et al., 2008) previously paired with mating opportunities paced by the female. In male quail, copulatory latencies are shorter after exposure to a sexual conditioned stimulus (CS) (Gutierrez & Domjan, 1996). Female quail will show longer bouts of receptive squatting behavior in the presence of a male after exposure to a sexual CS (Gutierrez & Domjan, 1997).

Similarly, responses to a CS can be altered by sexual learning. Male quail will demonstrate a social proximity response to an arbitrary stimulus previously paired with sexual opportunity (e.g. Domjan, O'Vary, & Greene, 1988; Holloway & Domjan, 1993a, 1993b). Male rats will exhibit increased level changing behavior in a bilevel chamber associated with sexual opportunity (Mendelson & Pfaus, 1989; van Furth & van Ree, 1996; van Furth, Wolterink-Donselaar, & van Ree, 1994). These changes in responding to a CS are evident even if the CS is not arbitrary. For example, experience with a female will result in increased distinctive courtship and mating-related vocalizations in male mice exposed subsequently to just female (but not male) urine (Dizinno, Whitney, & Nyby, 1978). Male quail will learn to approach a small slit window for visual-only access to a hen after copulation with the female (Balthazart, Reid, Absil, Foidart, & Ball, 1995).

The changes due to sexual learning have important functional properties. The sexual performance of male rats exposed to sexually conditioned cues improved in subjects with copulatory difficulties (Cutmore & Zamble,

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1988). In blue gourami fish, exposure to sexual Pavlovian conditioned cues dramatically increased the number of offspring produced (Hollis, Pharr, Duas, Britton, & Field, 1997). Male Japanese quail exposed to a sexual conditioned context ejaculate more semen and more sperm onto a stuffed model (Domjan, Blesbois, & Williams, 1998). Adkins Regan and MacKillop (2003) further demonstrated with male quail that inseminations in a sexual conditioned context will more likely result in fertilized eggs. In a sperm competition situation, exposure to a sexual CS allowed male quail to produce more offspring (Matthews, Domjan, Ramsey, & Crews, 2007).

There is a correspondingly large body of literature exploring the opioid mediation of unlearned sexual behavior in animals (see Argiolas, 1999; Paredes, 2009; Pfaus, 1999; Pfaus & Gorzalka, 1987a; van Furth, Wolterink, & van Ree, 1995 for reviews). In general, opioids and opioid drugs are found to have an inhibitory role in both male and female sexual behavior. Administration of β-endorphin inhibited mounts, intromissions, and ejaculations in male rats (McIntosh, Vallano, & Barfield, 1980) and inhibited lordosis behavior in females (Pfaus & Gorzalka, 1987b; Wiesner & Moss, 1986). Morphine (Pfaus & Gorzalka, 1987b) and methadone (Murphy, 1981) also inhibited male rat sexual responses, and morphine inhibited lordosis in females (Pfaus & Gorzalka, 1987b). Endomorphin-1, an endogenous, μ-opioid receptor specific peptide, injected in the third ventricle, increased ejaculatory latencies and interintromission intervals, and reduced ejaculations (Parra-Gamez, Garcia-Hidalgo, Salazar-Juarez, Anton, & Paredes, 2009). In male quail, the δ-opioid agonist D-Ala^2-Met^5-enkephalinamide injected in the preoptic and anterior hypothalamic areas decreased both aggressive and sexual behaviors (Kotegawa, Abe, & Tsutsui, 1997).

While there is indication that the inhibitory effects of opioids are dose and site of administration specific (e.g. Agmo, Rojas, & Vazquez, 1992; Band & Hull, 1990; Mitchell & Stewart, 1990; van Furth, van Emst, & van Ree, 1995), the general conclusion that opioids inhibit sexual behavior is largely confirmed by studies with opioid antagonists whose administration facilitates sexual responding. Naloxone has been reported to induce copulation in sexually inactive male rats (Gessa, Paglietti, & Pellegrini Quarantotti, 1979). Further, it decreased latency to first mount and decreased the number of intromissions before ejaculation (McIntosh et al., 1980). Naloxone also retarded the onset of sexual exhaustion in male rats (as reported in Pfaus & Gorzalka, 1987a). In male hamsters, naltrexone decreased the latency to first mount and reduced the intromissions before ejaculation (Murphy, 1981). Male Japanese quail exhibited more copulatory behavior when given central injections of naloxone (Kotegawa et al., 1997; Riters, Absil, & Balthazart, 1999). It should be noted that one aspect of male sexual behavior, the postejaculatory refractory period, has been reported in several instances to be increased by naloxone injections (McConnell, Baum, & Badger, 1981; Sachs, Valcourt, & Flagg, 1981). In female rats, lordosis behavior was facilitated by central injections of naloxone (Sirinathsinghji, 1984; Sirinathsinghji, Whittington, Audsley, & Fraser, 1983) although peripheral injections of the opioid antagonists have been reported to be either non- or minimally effective (Wiesner & Moss, 1986).

Given the importance of learning in the sexual behavior system and the seemingly clear mediating role of opioids in non-learned sexual behaviors, the relative paucity of studies specifically designed to explore the role of opioids in sexual learning is surprising. One reason for the seeming lack of contemporary programmatic investigation of the mediation of sexual learning by opioids may be that the wide range of results presented in the existing studies has been difficult to interpret. The goal of this review is to present the available studies of opioid mediation of learned sexual behavior, explore the procedures used, and propose a parsimonious explanation for the disparity of the results. This may in turn stimulate more systematic investigations of the intersection of these two important mediators of sexual behavior.

Experiments addressing the role of opioids in sexual learning

Initial investigations exploring the opioid mediation of learned sexual behavior attempted to evaluate shifting reward values due to manipulation of the opioid system. If opioids mediate sexual reward, then their blockade should affect the acquisition of sexual conditioned responses. Miller and Baum (1987) employed a conditioned place preference (CPP) paradigm. Male rats were allowed to copulate to ejaculation (see Camacho, Portillo, Quintero-Enriquez, & Paredes, 2009, for details of the importance of ejaculation in CPP procedures) with a female 10 times in an initially non-preferred chamber. On alternate days, the males spent the same time alone in the second, preferred chamber. Subsequent to these conditioning trials, males were either castrated or sham-operated. They were then allowed to freely access either chamber after peripheral naloxone (5.0 mg/kg SC) or saline vehicle injections two times, once 7 days following surgery and once 14 days after surgery. In these 15 min test trials, no female was present. Males that were castrated, injected with naloxone, or both showed marked reduction in the amount of time spent in the initially non-preferred chamber (in which they had encountered the female) on day 7. On day 14, this effect was again in evidence with an even more prominent effect in the castrated subjects injected with naloxone. These results were interpreted as indicating a potential reduction in the reward derived from incentive properties of a receptive female.

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In a similar CPP experiment (Agmo & Berenfeld, 1990), male rats were injected peripherally either with distilled water or naloxone (16 mg/kg) prior to one ejaculation and placement in an initially non-preferred chamber for 30 min. Three sexually reinforced trials were conducted, alternated with three trials in which all subjects received distilled water injections and spent 30 min in the initially preferred chamber alone. Again, consistent with the interpretation that opioids mediate sexual reward, naloxone was found to block the acquisition of a CPP.

Unfortunately, another experimental report published in the same year complicated this interpretation of the data. Mehrara and Baum (1990) again used a CPP paradigm. In this case, however, rather than mating with a female prior to placement in the CPP chamber, male rats were allowed to copulate with the female directly in the initially non-preferred chamber. Males were either castrated or intact and received either saline injections or 1 or 5 mg/kg injections of naloxone peripherally prior to sexual reinforced trials. Eight training sessions were conducted, four in which males were exposed to females in the initially non-preferred chambers for up to 1 h to achieve ejaculation and four on alternate days in which the males spent the same amount of time alone in the initially preferred chamber. When compared to saline-injected controls, neither dose of naloxone significantly attenuated the acquisition of a CPP for the initially non-preferred chamber in either the intact or castrated condition (although a trend is reported for naloxone to reduce CPP in castrated subjects). Interestingly, in a second experiment that fundamentally replicated Miller and Baum (1987), naloxone was again found to have an effect similar to this earlier experiment. This time, however, in light of the findings of experiment 1, these new data were interpreted to reflect an impact on the performance of CPP and suggested to the authors that opioids are involved not in the primary reward circuits but in those involved with conditioned incentives.

In these previous CPP studies, males were exposed to an empty chamber on non-reinforced days. An alternative procedure involves exposing male rats to a non-receptive female in the initially preferred chamber on putatively non-reinforced days. Hughes, Everitt, and Herbert (1990) used this arrangement as one of two tests of opioid mediation of sexual learning. Male rats were given eight 15-min reinforced exposures to receptive females in the initially non-preferred chamber alternated with similar exposures to non-receptive females in the initially preferred chamber. β-endorphin or naloxone was infused into the medial preoptic area-anterior hypothalamic area, or naloxone was administered peripherally (5 mg/kg) prior to a 15-min test trial during which the females were removed. Males were allowed to freely choose in which chamber to spend time for the duration of the test. Neither infused β-endorphin nor infused naloxone had an effect on the performance of the CPP. Systemic naloxone reduced the expression of CPP in this condition.

The second test used by Hughes et al. (1990) was a second-order instrumental conditioning procedure. Male rats were first trained to associate a stimulus light (the CS) with copulation to ejaculation. They were then trained to push a lever with presentation of the CS as reinforcement. The female was presented at the end of a session. Initially, this was after one response, but during the course of the experiment, the number of responses necessary to gain access to a female was increased to 100, and then, a fixed interval schedule was introduced and males by the end of the training were making approximately 200 responses (and earning 20 CS exposures) before the female was introduced. β-endorphin infusion into the medial preoptic area of the anterior hypothalamus (mPOA) had no effect on the instrumental behavior. Peripheral administration of naloxone at 5 mg/kg, but not a 1.0 or 2.5 mg/kg, was found to reduce the number of responses made to gain access to the female.

Agmo and Gomez (1993) again used the CPP procedure introduced in Agmo and Berenfeld (1990). However, in this instance, naloxone, in the form of methylnaloxonium, was infused at 5 μg/cannula into the mPOA or nucleus accumbens (NAC) of male rats 1 min before the CPP procedures. As before, males were allowed one ejaculation in a separate holding area and then moved to the initially non-preferred chamber of the CPP apparatus for 30 min. On alternate days, saline was infused, no female was presented, and males were placed for 30 min in the initially preferred chamber. Naloxone infused into the mPOA but not the NAC blocked CPP but did not affect sexual responses directed at the female. This suggested to the authors that the mPOA is the site of sexual reward.

Sexual motivation and sexual learning have also been assessed in male rats through the examination of anticipatory level changing behavior. In a bilevel cage, prior to the introduction of a female, males will exhibit increasing numbers of level changes with repeated exposures to copulation with a female but not following exposures to a non-receptive female. In several nearly identical tests of anticipatory level changing, naloxone was found to prevent the increase of anticipatory level changes. In these procedures, males were placed into a bilevel testing apparatus, and 5 min later, a receptive female was introduced into the chamber (at the level that did not currently contain the male). Peripheral naloxone administration had an effect whether at 1.0 or 10.0 mg/kg doses (van Furth, Wolterink-Donselaar, & van Ree, 1994), in testing during the light phase of the day (van Furth & van Ree, 1994) and if infused directly into the

ventral tegmental area (van Furth & van Ree, 1996). All these data are interpreted as suggesting a role for opioids in sexual motivation. A more recent study, however, calls this and other interpretations of an opioid role in sexual motivation into question.

Agmo (2003b) presented an elegant technique for the assessment of sexual motivation. Male rats were placed into a large test arena. Two chambers were affixed to opposite ends and opposite sides of the long walls of the test arena. Stimuli, such as receptive and non-receptive female conspecifics, could be placed into these chambers, and the resultant male behavior was observed. Using this device, Agmo (2003a) was able to assess sexual incentive motivation while manipulating the opioid system. Subjects were simultaneously presented with a receptive female in one chamber and a sexual-experienced male conspecific in the other. He reports that neither peripherally injected morphine (1, 4, or 8 mg/kg) nor naloxone (1, 4, and 16 mg/kg) had clear effects on incentive sexual motivation. The peripheral opioid agonist loperamide did affect choice in these tests but via non-opioid mechanisms. Therefore, Agmo concluded that opioids are not important for sexual motivation in male rats.

In male Japanese quail, reductions in sexual reward value (Holloway & Domjan, 1993b) or in sexual motivation (Holloway & Domjan, 1993a) resulted in decreased responding in a sexual conditioned approach paradigm. In these procedures, male quail are typically given a brief (30 s–1 min) presentation of a CS followed by access to a quail hen. If it is the case that opioids mediate sexual reward or motivation in quail, then blockade of the opioid receptors by naloxone should result in altered responding to the CS.

One means of testing conditioned sexual approach behavior in quail is to present males with visual access to a female behind a door with a very narrow slit in it as the CS. This is then followed by copulatory access to the hen. Prior to copulation with the hen, the male will spend little time at the slit window. After mating, the male will stand at the window for extensive periods. Riters et al. (1999) used this method to assess the effects of naloxone on the performance of conditioned sexual behavior. Neither peripheral (increasing doses of 1.0, 10.0, and 50.0 mg/kg) nor central injections of naloxone into the third ventricle had an effect on the performance of the conditioned sexual approach behavior. Male quail continued to look at the female through the door slit.

In a follow-up study, Holloway, Cornil, and Balthazart (2004) conducted nine sexual approach conditioning trials with male quail as described above. Then, because visual exposure to a quail hen is known to be rewarding (Holloway & Domjan, 1993b), subjects were tested in non-reinforced extinction trials. That is, the approach to the door slit was assessed across eight trials in which the female was not present behind the door. During these extinction trials, central injections of naloxone were found to markedly reduce sexual conditioned responding in subjects when compared to saline-infused controls.

Because the removal of the female changed the CS between acquisition and extinction trials, it is possible that the reduction in responding was due to naloxone enhancing the males' attention to the CS alteration. To rule out this possibility, male quail were conditioned to approach an arbitrary stimulus object that could be left in place during extinction trials (see Holloway & Domjan, 1993a, 1993b). Even when the CS remained constant across acquisition and extinction trials, central injections of naloxone sharply attenuated sexual conditioned approach responding during the extinction phase (Holloway, Shaw, Cornil, and Balthazart, 2009).

Riters et al. (1999) report different effects of central and peripheral injections of naloxone on unconditioned sexual behavior in male Japanese quail. To test the effects of peripheral injections of naloxone on sexual conditioned behavior, two experiments were conducted with an arbitrary stimulus object as the CS. In the first experiment, male quail were initially conditioned to approach the CS by pairing 30 s exposures to the stimulus with 5 min of copulatory access to a hen. During an extinction phase, males were peripherally injected with naloxone (30 mg/kg) prior to exposure to successive CS alone presentations. Naloxone greatly facilitated the extinction of sexual conditioned approach to the arbitrary CS when compared to saline-injected controls. In the second experiment, male quail were injected with the same 30 mg/kg dose of naloxone prior to each paired CS-quail hen presentation. Contrary to what would be expected if the opioid system is involved in sexual motivation or reward, the naloxone-injected males acquired the sexual conditioned approach response to the CS at the same rate as saline-injected controls (Holloway & Jensen, 1997).

Subsequently, the effects of naloxone (30 mg/kg) administered peripherally during both acquisition and extinction phases of a sexual conditioned approach experiment were explored (Holloway & Meerts, 2003). Once again, in conditioning trials that paired an arbitrary CS with copulation, naloxone had no effect on the development of conditioned approach responding directed to the CS. During successive non-reinforced CS presentations, however, continued naloxone injections significantly and substantially facilitated the extinction of conditioned sexual approach responding.

In all of the above studies, male animals served as subjects. There has been a limited exploration of the role of opioids in mediating learned female sexual behavior. Sexual CPP has been assessed in female rats injected with naloxone (Paredes & Martinez, 2001). Females were allowed to pace mating to ejaculation before being placed in an initially non-preferred chamber for 30 min. On alternate days, they spent the same amount of time in the

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initially preferred chamber. Prior to each reinforced trial, female subjects were injected peripherally with naloxone (4 mg/kg) or distilled water. As was reported for male rats in this CPP paradigm (e.g. Agmo & Berenfeld, 1990), naloxone blocked the acquisition of a sexual CPP.

Subsequently, this sexual CPP experiment with female rat subjects was closely replicated with infusions of naloxone into mPOA, ventromedial nucleus of the hypothalamus (VMH), the amygdala (Me), and the NAC (Garcia-Horsman, Agmo, & Paredes, 2008). Naloxone (5 μg infusions) into the mPOA, VMH, and Me blocked the acquisition of a sexual CPP.

The opioid mediation of learned female sexual behavior has also been investigated in a conditioned partner preference experiment (Coria-Avila et al., 2008). As mentioned previously, initially arbitrary olfactory and visual cues can become associated with paced mating opportunities. Female rats have been reported to prefer males marked with an odor that had previously been present during paced mating (Coria-Avila et al., 2005). In two experiments, Coria-Avila et al. (2008) tested whether the acquisition of this preference could be blocked by peripheral naloxone (4 mg/kg) injected before conditioning trials. In experiment 1, females were presented with almond scented males in paced mating situations and non-scented males in mating situations they could not pace. In experiment 2, albino and pigmented male rats served as paced and unpaced partners in counterbalanced groups. In both experiments, naloxone injected during the acquisition phase was found to disrupt the preference for a male bearing pacing related cues during a non-drug preference test.

It should be noted that conditioned partner preference has also been studied in male rats in the form of conditioned ejaculatory preference. Male rats given peripheral naloxone (5mg/kg) injections prior to 10 conditioning trials with an almond-scented female failed to demonstrate a preference to ejaculate with a similarly scented female in a subsequent open field choice test with scented and unscented females (Ismail, Girard-Beriault, Nakanishi, & Pfaus, 2009).

Sexual pheromones in soiled male bedding can produce a CPP in female mice. Females allowed to explore a large test arena containing on one side a dish of soiled bedding and on the other a dish of clean bedding in 10 min trials on 4 consecutive days were found to prefer spending time on the side containing the soiled bedding in a subsequent non-reinforced test trial. Naloxone (1 and 10 mg/kg) administered peripherally during the conditioning (but not test) phase did not disrupt the acquisition of the sexual CPP (Agustin-Pavon, Martinez-Ricos, Martinez-Garcia, & Lanuza, 2008).

Discussion

As the reviewed experiments make clear, a variety of procedures have been used to assess the role of opioids in learned sexual behavior. Depending on the procedures cited, one could make an argument that opioids either mediate or play no role in the acquisition of sexual conditioned responses and are, therefore, either involved with sexual reward and motivation or not. Even within the most widely used technique, CPP, there are discrepancies that would allow for both sets of conclusions. It seems, however, unlikely that both sets of conclusions are correct.

One way of assessing these procedures is to explore whether commonalities exist in situations where opioids are reported to affect sexual conditioned responding, and of course, in situations where opioids do not seem to be involved in the mediation of learned responses. Mehrara and Baum (1990) presents an interesting start point because they report findings that support both a role for opioids in sexual learning (experiment 2) and suggest that opioids are not involved (experiment 1). The substantive difference between the two experiments is that, in the first, naloxone was administered before the effective CS (the initially non-preferred chamber) and the sexual unconditioned stimulus (US) (copulation to ejaculation) were paired. Thus, CS-US pairings took place under the influence of naloxone administration. In the second experiment, CS-US pairings took place before naloxone administration, and during the testing phase, when naloxone was administered, only the CS was available to the male subject. Indeed, this CS alone test presentation was the case in Miller and Baum (1987) too, and an effect of naloxone was reported. Interestingly, the case can be made that in most of the CPP situations where naloxone was found to attenuate sexual learning, the subject was exposed to the CS alone. In the studies by Agmo and Berenfeld (1990), Agmo and Gomez (1993), Paredes and Martinez (2001), and Garcia-Horsman et al. (2008), naloxone was administered and copulation took place before subjects were placed in the CS chamber. Then, for 30 min, subjects were exposed to the cues of the chamber without further exposure to a sexual partner, but while still under the influence of opioid blockade by naloxone. CS alone presentations in Pavlovian conditioning are, of course, referred to as extinction trials. Of the CPP procedures just mentioned, only in experiment 1 of Mehrara and Baum and the CPP procedures of Agustin-Pavon et al. (2008) was naloxone administered during a typical CS paired US acquisition phase and no significant effect of naloxone was reported in either case. Naloxone's effects in experiment 2 of Mehrara and Baum and Miller and Baum were clearly during extinction trials as arguably were its effects in the other CPP experiments just noted.

Assuming that naloxone has no effect during paired CS-US acquisition trials but does have an effect during extinction, CS-alone, trials similarly allows for all of the quail data to be accounted for. In Riters et al. (1999), naloxone did not affect the performance of sexually conditioned looking behavior. In each of these trials, the male quail's approach to the CS window was reinforced by visual exposure to the quail hen, known to support Pavlovian-conditioned approach behavior (Holloway & Domjan, 1993b). In Holloway and Jensen (1997) and in Holloway and Meerts (2003), direct tests of acquisition following naloxone administration revealed no effects of opioid blockade. In all extinction procedures (Holloway et al., 2004; Holloway et al., 2009; Holloway & Jensen, 1997; Holloway & Meerts, 2003), however, naloxone either significantly reduced sexual conditioned responding or significantly and substantially facilitated extinction.

The assumption that opioid blockade affects learned sexual behavior only by mediating extinction requires some modification to account for the results in other papers. Conditioned sexual behavior is remarkably resistant to extinction in the absence of naloxone administration (see Balthazart et al., 1995). Perhaps, opioids mediate this persistence of conditioned sexual responding. In traditional acquisition trials, during which the CS is rapidly followed by the sexual US, there is no need for persistence. Responding to the CS is quickly followed by sexual opportunity. In extinction trials, the CS is not followed by the US and therefore, if responding is to continue a perseverative mechanism must be activated. Opioids may provide this mechanism, and blocking their activity, therefore, should disrupt not only conditioned sexual responding during extinction but also during long CS exposures followed by a sexual reward and during long, non-primary sexual reinforcer rewarded stretches of instrumental responding. This is what has been reported and reviewed here. The CS in a bilevel chamber procedure is the context of the chamber itself. In the three investigations of anticipatory level changing behavior (van Furth & van Ree, 1994; van Furth & van Ree, 1996; van Furth, Wolterink-Donselaar, & van Ree, 1994), the male rat spent 5 min in the chamber before the presentation of the female. Sexual responding, in the form of what appears to be general search behavior (see Domjan, Mahometa, & Matthews in the current special issue for a discussion of a behavior systems approach to sexual learning), is required to persist over this relatively long interval if evidence of learning is to develop. Naloxone blocked this learning. Similarly, in the instrumental procedure used by Hughes et al. (1990), subjects were evaluated on the number of responses made on a fixed interval schedule to gain access to a receptive female, a clear measure of persistence, and naloxone reduced the number of these responses.

Thus, all but two experiments exploring male sexual behavior and two experiments exploring female sexual behavior conform to the expectations of the interpretation that opioids mediate the persistence of responding in the face of non- or delayed-sexual reinforcement. Interestingly, all of these involve partner preference. It is possible that opioid mediation of unconditioned mechanisms or of non-sexual conditioned mechanisms account for these findings. For example, in the CPP experiment conducted by Hughes et al. (1990), CPP was established by contrasting opportunity to copulate not with an empty chamber but with a non-receptive female. Perhaps, the effects of naloxone on unconditioned behaviors directed to the unreceptive female were sufficient to reduce the relative value of the receptive female. The lack of a CPP here can only be interpreted as suggesting an equal amount of conditioning supported by both the receptive and unreceptive female, not an absence of sexual conditioning. The three remaining instances of results not consistent with the present interpretation all are reported out of the same laboratory and all used a paced mating paradigm. For males, this pacing was required to be quite specific for conditioning to occur. A pacing chamber with one but not four holes was necessary (Ismail, Gelez, Lachapelle, & Pfaus, 2009). In light of this peculiar requirement, it is difficult to interpret the finding of Ismail et al. (2009) that naloxone blocked the acquisition of a conditioned ejaculatory preference for a scented female. It may be that the opportunity for the female to escape periodically during the mating sessions produced a state that required persistence. It may also be that naloxone interfered with the processing of the novel, arbitrary scent information (for a related example see Kelley et al., 2002) in ways not related to sex. Similar issues make interpreting the two experiments with female subjects in Coria-Avila et al. (2008) difficult. Complicating things further, in spite of innovative procedures (e.g. Meerts & Clark, 2009), just precisely what females find rewarding about paced mating is still in question. This includes pacing itself, as Meerts and Clark (2007) were able to condition a CPP in female rats without allowing pacing. In any case, further investigation is warranted.

It is interesting to note that naloxone has been also reported to affect the extinction but not acquisition of conditioned responses in a rodent strain for which alcohol is appetitive (Cunningham, Dickinson, & Okorn, 1995). These effects of naloxone in conditioning procedures using alcohol as a reinforcer may be species and strain related, however (Bormann & Cunningham, 1997; Cunningham, Henderson, & Bormann, 1998). Naloxone also facilitates extinction of lever pressing behavior rewarded with food or sucrose and has minimal impact on responding during rewarded conditioning trials (Norris, Perez-Acosta, Ortega, & Papini, 2009). These

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findings suggest that the mediation of learning in sexual conditioning situations by opioids may be common across other appetitive behavior systems.

The hypothesis presented here, that in sexual learning opioids mediate the persistence of responding in the face of delayed or non-reinforcement, has features in common with the distinction that has been made between 'wanting' and 'liking'. Wanting has been characterized as the value of incentive motivation held by a stimulus absent any hedonic component. Liking on the other hand is the hedonic aspect of a stimulus presentation, the positive sensory component that accompanies reward delivery (Berridge, 2004). Opioids have been implicated in mediating wanting through their activity in the amygdala. Microinjections of [D-Ala2, N-MePhe4, Gly-ol]-enkephalin (DAMGO), a μ receptor agonist, into the central amygdala resulted in vigorous sniffing and nibbling of a CS predicting sucrose pellet delivery. Microinjections of the $GABA_A$ agonist muscimol to inactivate the region resulted in the opposite effect, reduced approach, sniffs, and nibbles of the CS (Mahler & Berridge, 2009). In a closely related experiment, opioid activity in the NAC has been demonstrated to mediate both the wanting and liking components in a sweet reward paradigm (Smith, Berridge, & Aldridge, 2011).

If the administration of opioid antagonists in the sexual conditioning experiments discussed here disrupts the incentive motivation for (wanting) and/or hedonic value (liking) of a CS predicting sexual opportunity or of the sexual stimulus itself, then certainly a decrease in conditioned responding would be expected. This decrease, however, would not be limited to situations where the CS is either not followed by the sexual stimulus or separated in time from the sexual reward. Yet, this is the pattern seen across investigations of the opioid mediation of sexual behavior. Interestingly, Smith, Berridge, and Aldridge (2011) did introduce a timing component to the study of opioid mediation of wanting and liking of a sweet reward. Two CSs were used, one distal in time to the presentation of the sweet stimulus, one proximate. In the sweet-reward system, only responses to the proximate cue were affected by manipulation of opioid activity. This contrasts sharply with the findings from the sexual-reward system presented here. Close temporal pairing of a CS with a sexual reward were unaffected by manipulations of opioid activity (e.g. Holloway & Jensen, 1997; Holloway & Meerts, 2003; Mehrara & Baum, 1990), whereas longer CS-sexual reward intervals resulted in responses sensitive to opioid blockade (e.g. van Furth & van Ree, 1994). As such, while the sparse literature reporting on the opioid mediation of learned sexual behavior can not rule out changes to sexual wanting and liking similar to those detailed by Berridge and his colleagues in sweet-reward system, a third feature, 'persisting"' more fully characterizes the role of opioids in sexual learning.

If the hypothesis that opioids mediate the persistence of conditioned sexual responding in the absence of reward is correct, then several predictions follow. Shorter latencies between the onsets of CS and sexual US presentations should attenuate any effects that opioid antagonists have during acquisition. Similarly, the effects of antagonists should increase as the CS-US interval increases. In instrumental procedures, greater effects of opioid antagonist administration should accompany longer interval and larger ratio contingencies. Across all appetitive sexual conditioning procedures, opioid antagonists should facilitate extinction, as they have been reported to do in the papers reviewed here. More programmatic experimentation is needed to address these predictions.

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***Kevin S. Holloway**
Department of Psychology
Vassar College
124 Raymond Avenue
Poughkeepsie, NY12604, USA
Email: keholloway@vassar.edu