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Presence of a pair-mate regulates the behavioral and physiological effects of opioid manipulation in the monogamous titi monkey (*Callicebus cupreus*)

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Summary The role of opioid receptors in infant—mother attachment has been well established. Morphine, a preferential μ opioid receptor (MOR) agonist, attenuates separation distress vocalizations and decreases physical contact between infant and mother. However, there is little research on how opioid receptors are involved in adult attachment. The present study used the monogamous titi monkey (*Callicebus cupreus*) to explore the role of opioid receptors in the behavioral and physiological components of pair-bonding. In Experiment 1, paired male titi monkeys ($N = 8$) received morphine (0.1, 0.5, or 1.0 mg/kg), the opioid antagonist naloxone (1.0 mg/kg), vehicle, or a disturbance control and were filmed with their pair-mate for 1 h. In Experiment 2, the same eight males received morphine (0.25 mg/kg), naloxone (1.0 mg/kg), vehicle, or a disturbance control and were filmed for an hour without their pair-mates. All video sessions were scored for social and non-social behaviors. Blood was sampled immediately prior to drug administration and at the end of the hour session. Plasma was assayed for cortisol, oxytocin, and vasopressin. In Experiment 1, opioid manipulation had no effect on affiliative behaviors; however, morphine dose-dependently decreased locomotor behavior and increased scratching. In Experiment 2 in which males were separated from their pair-mates, naloxone increased locomotion. Morphine dose-dependently attenuated the rise in cortisol, while naloxone potentiated the increase of cortisol. The cortisol increase following naloxone administration was greater when a male was alone compared to when the male was with his pair-mate. Naloxone increased vasopressin but only when the male was tested without his pair-mate. The present study found that the absence of a pair-mate magnified naloxone's effects on stress-related hormones and behaviors, suggesting that the presence of a pair-mate can act as a social buffer against the stress-inducing effects of naloxone.

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1. Introduction

The role of the opioid system in infant–mother bonds is well established (Herman and Panksepp, 1978; Panksepp et al., 1980; Kalin et al., 1988, 1995; Nelson and Panksepp, 1998). Infants separated from their mother or primary attachment figure emit stereotyped separation vocalizations (Panksepp et al., 1980; Hoffman et al., 1995; Mason and Mendoza, 1998). Preferential MOR agonists (such as morphine and oxymorphone) attenuate these distress vocalizations in young that develop selective attachments to their mothers, such as guinea pigs (Herman and Panksepp, 1978), dogs (Panksepp et al., 1978a), monkeys (Kalin et al., 1988), and chickens (Panksepp et al., 1978b, 1980). In contrast, non-specific opioid antagonists such as naltrexone or naloxone, either have no effect or intensify these vocalizations (Herman and Panksepp, 1978; Panksepp et al., 1980; Kalin et al., 1988; Nelson and Panksepp, 1998). Polymorphisms of the *OPRM1* gene coding for the MOR in monkeys have also been found to affect separation vocalizations (Barr et al., 2008).

Nelson and Panksepp (1998) have argued that individuals seek to obtain an optimal opioidergic tone, which can be modulated by an attachment figure. Disruptions to opioidergic activity through separation from an attachment figure result in behavioral and physiological changes such as separation vocalizations and activation of the hypothalamic–pituitary–adrenal (HPA) axis. In addition to its effects on separation vocalizations, exogenous opioid manipulation has also been shown to affect the HPA axis (Wand et al., 1998). Acute administration of MOR agonists decreases cortisol concentrations in humans (Zis et al., 1984), macaque monkeys (Broadbear et al., 2004), and sheep (Parrott and Thornton, 1989), while naloxone and naltrexone increase cortisol concentrations in humans (Wand et al., 1998), nonhuman primates (Fabre-Nys et al., 1982), and sheep (Parrott and Thornton, 1989). It is hypothesized that distress vocalizations emitted during separation act to attract the mother, thereby reinstating an infant's ideal opioidergic tone and HPA homeostasis (Panksepp et al., 1980; Nelson and Panksepp, 1998).

In addition to opioids regulating infant–mother attachments, opioids also appear to have a role in affiliative behavior in adult, nonhuman, Old World primates living in large social groups. In talapoin monkeys and macaques, morphine administration decreases the number of grooming solicitations and the amount of grooming received, while naloxone administration increases grooming solicitations and the receipt of grooming (Fabre-Nys et al., 1982; Keverne et al., 1989; Martel et al., 1995). Receipt of grooming results in a release of β-endorphins (Keverne et al., 1989), which primarily bind to MORs (Goodman et al., 1983). It has been proposed that morphine administration results in overactivation of MORs, which ends in an adjustment in an animal's behavior to maintain a homeostatic level of MOR activation by decreasing the amount of grooming received (Nelson and Panksepp, 1998). In contrast, administration of naloxone or naltrexone is predicted to increase the amount of grooming received, which would compensate for the decrease in MOR activation.

The role of opioids in affiliative behavior among adult animals has also been explored in the formation and maintenance of monogamy. The filial-like attachment bond formed between adults in monogamous species enables the possibility of exploring the potential contribution of opioids in this unique social system (Mason and Mendoza, 1998). The neurobiology of adult attachment has focused primarily on neuropeptides such as oxytocin (OT) (Williams et al., 1994; Carter et al., 1995; Smith et al., 2010; Young et al., 2011a; Schneiderman et al., 2012) and vasopressin (AVP) (Winslow et al., 1993; Jarcho et al., 2011; Young et al., 2011a; Gouin et al., 2012) as well as the neurotransmitter dopamine (Aragona et al., 2003; Curtis et al., 2006; Young et al., 2011a). In the monogamous prairie vole, systemic administration of the opioid antagonist naltrexone, or local administration of the specific MOR antagonist CTAP into the dorsal striatum, prevents pair-bond formation (Burkett et al., 2011). Shapiro et al. (1989) found that morphine can reduce side-by-side contact in prairie voles at high doses that also affect locomotor behavior. However, opioid blockade with naloxone or naltrexone has repeatedly failed to increase physical contact in prairie voles whereas it does in nonmonogamous primates (Shapiro et al., 1989; Burkett et al., 2011; Resendez et al., 2012). One of the questions we are concerned with is whether the differences between voles and macaques are due to their social structure or phylogenetic status. The manner in which opioid receptors influence affiliative behaviors may vary depending on the neurophysiological differences found in monogamous versus nonmonogamous species or those found in rodent versus primate species. The present study attempts to answer this question through the use of a monogamous primate species.

The titi monkey (*Callicebus cupreus*) is a socially monogamous nonhuman primate that forms strong, heterosexual pair-bonds (Mason, 1966), and therefore acts as an excellent nonhuman primate model to examine whether the opioid system is involved in adult attachment. Titi monkeys spend a significant amount of time in physical contact and proximity

Table 1 Social composition information.

♂ Subject	♀ Pair-mate	# of offspring	Offspring sex	Offspring age (years)
29775	37133	0	–	–
30410	36993	0	–	–
31716	29852	1	♂	1.5
32878	34866	0	–	–
34438	37623	0	–	–
34531	36163	1	♂	0.6
35383	30557	0	–	–
36187	35292	1	♀	0.1

(Mason, 1966). Furthermore, involuntary separation of pair-mates results in a separation distress response similar to that seen in infants in that there is an increase in separation distress vocalizations and HPA activity (Cubicciotti and Mason, 1975; Mendoza and Mason, 1986a; Mason and Mendoza, 1998; Ragen et al., 2012). By examining the effects of opioid manipulation in a primate species that also exhibits a monogamous social structure, we will be able to answer the question whether the contrasting findings between voles and macaques are due to a monogamous social structure or phylogenetic differences. We will also be able to determine whether opioids regulate the separation distress response in an adult attachment compared to an infant–mother attachment.

If opioid manipulation in titi monkeys is more akin to those in other nonhuman primate species, opioid blockade with naloxone would increase affiliative behavior while activation would decrease it. Additionally, opioid activation with morphine would be able to suppress the separation distress response experienced upon brief social separation. We also sought to determine whether opioid manipulation could affect hormones involved in attachment, including cortisol, OT, and AVP. Based on previous studies we hypothesized that naloxone would increase cortisol and OT while morphine would decrease cortisol and OT; however we made no specific hypothesis in relation to AVP since previous findings have been inconsistent (Fabre-Nys et al., 1982; Zis et al., 1984; Parrott and Thornton, 1989; Van Wimersma Greidanus and Van De Heijning, 1993; Wand et al., 1998; Vuong et al., 2010).

2. General methods

2.1. Subjects

Subjects were eight titi monkey (*Callicebus cupreus*) males, housed at the California National Primate Research Center. Subjects were a mean age of 9.3 years (range = 5.4–14.1) and had been housed together with their female pair-mates for a mean (±SEM) of 33.3 ± 10.9 months. Subjects' mean (±SEM) weight was 1.41 ± 0.06 kg. Female pair-mates were a mean age of 7.3 years (range = 4.1–13.8) and a mean (±SEM) weight of 1.16 ± 0.06 kg. All females had previously given birth with either the current or previous pair-mate. All females were socially housed in family groups prior to pairing. Animals were housed indoors in cages sized 1.2 m × 1.2 m × 1.8 m. Social group composition consisted of the male-female pair and any offspring. Table 1 provides details of the social group composition for each pair. Rooms were on a 12:12 light:dark cycle with lights on at 0600 and lights off at 1800. Housing treatments were similar to those found in Mendoza (1999). Subjects were fed daily at 0800 and 1300 on a diet of New World monkey chow, apples, carrots, rice cereal, and bananas. Water was available ad libitum. All procedures were approved by the University of California, Davis Institutional Animal Care and Use Committee.

2.2. Drugs

Morphine sulfate dissolved in saline was obtained at a concentration of 10 mg/ml (Cardinal Health, Dublin, OH), and further dilutions were made using physiological saline. Naloxone hydrochloride (Sigma–Aldrich, St. Louis, MO) was dissolved in physiological saline and filtered through a 20 μm filter. Naloxone solution was mixed fresh before each test session. Drugs and vehicle were injected intramuscularly (IM) in a volume of 0.1 ml/kg.

2.3. Blood sampling and hormone assessment

As a component of general laboratory practice, all animals were trained to enter a transport box (0.3 m × 0.3 m × 0.3 m) when prompted. Once captured in the transport box, the subject was manually restrained and a blood sample

Table 2 Ethogram.

Behavior[a]	Description
Proximity (s)	Subject is within arm's reach (~6 in.) of female
Contact (s)	Passive physical contact between subjects that does not involve tail-twining
Tail-twine (s)	Sitting side-by-side with tails wrapped around each other for at least one rotation
Total passive contact (s)	Combined time spent in contact and tail-twining
Receive grooming (s)	The focal animal is being groomed by the attachment figure
Grooming solicitations	Male presents body part in front of the female to be groomed
Grooming (s)	Male combs through the fur of the female with his hands and/or mouth
Autogroom (s)	Male combs through own fur with his hands and/or mouth
Male approach	Male moves within six or less inches of the female
Male leave	Male moves away from the female to a distance >6 in.
Female approach	Female moves within <6 in. of the male
Female leave	Female moves away from the male to a distance >6 in.
Follow (s)	Male locomotes at the same time as the female while staying within 6 in. of female
Locomotion (s)	Male displaces his body by at least one body length
Limb movement (s)	Male moves arm or leg >1 in. while body is stationary. Does not include behaviors such as grooming or scratching
Scratch (s)	Rapid and repeated raking of the fur or skin with own hand or foot
Isolation peeps	Short, high pitched vocalizations which usually occur in rapid succession
Long-calls (s)	Loud, sustained vocalizations

[a] Behaviors followed by (s) are durations measured in seconds. All other behaviors are frequencies.

(1 ml) was collected with a heparinized needle from the femoral vein. Mean (±SEM) time from cage entry to blood collection was 2.88 ± 0.08 min. Collected blood was immediately placed on wet ice and later centrifuged at 4 °C for 15 min; plasma was extracted and stored at −70 °C until assay. Plasma AVP and OT concentrations were estimated in duplicate using commercial enzyme immunoassay kits (Enzo Life Sciences, Farmingdale, NY) that were validated for titi monkeys (Bales et al., 2005). Intra- and inter-assay coefficients of variation (CV) were 9.91% and 14.85%, respectively for AVP, and 9.22% and 15.90%, respectively for OT. Plasma cortisol concentrations were estimated in duplicate using commercial radioimmunoassay kits (Siemens Healthcare, Malvern, PA). Prior to assay, samples were diluted 1:4 in PBS gel buffer. Assay procedures were modified with the addition of 0.5 and 2.5 μg/dl concentrations of standards along with the provided range of 1.0—50 μg/dl. Assay sensitivity has been determined to be 0.26069 μg/dl. This assay procedure has been validated for titi monkeys (Mendoza, unpublished data) and has been used repeatedly to analyze levels of plasma cortisol in titi monkeys (Hoffman et al., 1995; Bales et al., 2007; Jarcho et al., 2011; Laugero et al., 2011). Intra- and inter-assay CVs were 5.62% and 7.71%, respectively.

2.4. Experiment 1

Opioid manipulation affects affiliative behavior in macaque infant—mother dyads as well as filial relationships between adult Old World monkeys (Fabre-Nys et al., 1982; Keverne et al., 1989; Kalin et al., 1995); however, this effect appears to be absent in the monogamous prairie vole (Burkett et al., 2011; Resendez et al., 2012). The first goal of Experiment 1 was to determine whether the effects of MOR manipulation on affiliative behavior were more similar to that of a non-monogamous primate species or a monogamous rodent species. The second goal was to determine the dose of morphine to be used in Experiment 2 that would not affect locomotor behavior but influence separation distress behavior.

2.4.1. Design and procedures

All animals in the home cage, except for the female pair-mate, were captured in transport boxes. The subject was restrained to obtain a blood sample followed by an IM injection of morphine (0.1, 0.5, or 1.0 mg/kg), naloxone (1.0 mg/kg), or vehicle. For the disturbance control, animals were captured in the transport box and immediately returned to their home-cage. Each subject received all six treatments, and treatments were counterbalanced. Seven days separated testing sessions, which occurred between 1000 and 1200. The dose of naloxone was chosen due to its ability to potentiate separation behaviors in infant macaques (Kalin et al., 1988) as well as increase affiliative behavior in juvenile macaques (Schino and Troisi, 1992). All offspring remained in transport cages in a room outside of visual and auditory range. The separation of offspring from the male likely had no effect on distress behavior or HPA activity since it has been previously demonstrated that separation from offspring does not result in an increase in separation distress behavior or HPA activity in fathers (Mendoza and Mason, 1986b). One subject had a one-month infant that remained with the pair during testing. The test session was filmed for 60 min with the male as the focal animal. Various social and non-social behaviors (Table 2) were scored at a later point by an experimenter blind to the drug condition. After the 60-min session, the male was recaptured to obtain a second blood sample. All animals were then returned to their home cage. One subject did not receive the full amount of the high dose of morphine; data for this animal in the high dose of morphine were excluded from the analysis.

2.5. Experiment 2

The goal of Experiment 2 was to explore the effects of opioid manipulation on the behavioral and physiological separation distress response in male titi monkeys. Previous studies have found that morphine decreases the separation distress response in offspring separated from their mothers (Herman and Panksepp, 1978; Panksepp et al., 1978a,b, 1980; Kalin et al., 1988), therefore we hypothesized that morphine would decrease locomotion and isolation peeps in male titi monkeys; behaviors that reliably increase upon separation from a pair-mate (Mendoza and Mason, 1986a; Ragen et al., 2012). Since one of the behaviors being measured was locomotion as an effect of separation we chose a dose that we hoped would be able to reduce locomotion but not as a result of sedation. Based on the results of Experiment 1, we chose a 0.25 mg/kg dose of morphine for Experiment 2 since the medium dose of 0.5 mg/kg produced mild sedation and the low dose of 0.1 mg/kg resulted in no physiological changes. The beginning of Experiment 2 occurred three weeks after the end of Experiment 1 to avoid any possible carry over effects of the drugs (Keith et al., 1996; Sim et al., 1996; Williams et al., 2013).

2.5.1. Design and procedures

All animals in the home cage were captured in transport boxes, and the subject was restrained to obtain a blood sample followed by an IM injection of morphine (0.25 mg/kg), naloxone (1.0 mg/kg), or vehicle. The male was then returned to his home cage alone. All offspring and the female pair-mate remained in the transport cages in a room outside of visual and auditory contact. As stated before, separation of the offspring likely had no effect on behavior or HPA activity (Mendoza and Mason, 1986b). For disturbance control sessions, males were captured and immediately returned to the cage without injection. Each subject received all treatments, which were counterbalanced. As in Experiment 1, seven days separated testing sessions, which occurred between 1000 and 1200. Males were filmed for 60 min, and a variety of behaviors including those related to the social separation response (Table 2) were scored at a later point by an experimenter blind to the drug condition. Immediately after the 60-min session, the male was recaptured to obtain a second blood sample. All animals were then returned to the home cage. We were unable to obtain one baseline sample in the vehicle treatment and one baseline sample in the morphine treatment.

2.6. Statistics

Data were analyzed by generalized linear mixed model (GLMM) (Littell et al., 1996) in SAS 9.2 (SAS Institute, Cary,

Table 3 Behavior with pair-mate.

Behavior	Condition						*F*-Statistic[#]
	Control	Vehicle	1.0 mg/kg naloxone	0.1 mg/kg morphine	0.5 mg/kg morphine	1.0 mg/kg morphine	
Proximity[†]	242.3 ± 35.5	153.6 ± 36.7	130.6 ± 30.5	124.6 ± 45.5	239.6 ± 111.0	483.0 ± 181.0	2.43
Contact	812.5 ± 169.9	1009.5 ± 228.3	527.6 ± 116.3	1119.6 ± 324.3	844.1 ± 208.1	1007.4 ± 355.7	1.14
Tail-twine	859.4 ± 249.5	1342.6 ± 288.8	1660.0 ± 367.9	1001.3 ± 356.4	1372.6 ± 240.2	881.9 ± 381.8	0.88
Total passive contact	1671.9 ± 318.2	2352.1 ± 248.3	2187.63 ± 378.7	2120.9 ± 434.4	2216.8 ± 205.8	1889.3 ± 326.9	0.24
Receive grooming	218.0 ± 101.8	77.8 ± 42.5	21.0 ± 10.2	40.1 ± 34.6	49.2 ± 16.8	62 ± 14.3	0.91
Grooming solicitations	2.5 ± 1.1	1.3 ± 0.86	0.0 ± 0.0	0.3 ± 0.2	0.4 ± 0.4	0.4 ± 0.3	1.30
Grooming[†]	57.3 ± 22.8	93.3 ± 50.2	11.6 ± 9.4[*]	22.0 ± 22.0[*]	1.6 ± 1.6[**]	0.1 ± 0.1[**]	3.72[b]
Autogroom[†]	16.0 ± 8.3	11.4 ± 6.0	14.0 ± 15.8	13.6 ± 6.7	32.5 ± 21.2	4.4 ± 4.1	1.24
Male approach[†]	11.4 ± 4.3	11.0 ± 3.3	10.5 ± 2.5	7.8 ± 1.9	4.0 ± 1.3[**]	1.4 ± 0.4[***]	6.35[c]
Male leave[†]	8.6 ± 2.6	6.0 ± 1.2	9.9 ± 5.1	6.4 ± 1.7	1.6 ± 0.5[*]	1.7 ± 0.9[*]	3.43[b]
Female approach[†]	13.1 ± 3.3	7.0 ± 1.9	12.0 ± 6.7	6.5 ± 2.4	6.6 ± 2.3	8.0 ± 3.9	0.28
Female leave[†]	15.5 ± 5.0	11.4 ± 3.1	11.4 ± 3.6	7.6 ± 2.6	8.4 ± 2.4	7.3 ± 3.6	0.49
Follow[†]	1.3 ± 0.7	1.1 ± 0.6	1.4 ± 1.2	8.8 ± 0.6	0.4 ± 0.3	0.0 ± 0.0	1.12
Locomotion	112.87 ± 35.4	77.6 ± 25.7	113.3 ± 73.9	53.8 ± 14.8	26.8 ± 8.9	13.9 ± 4.5[*]	2.59[a]
Limb movement	139.5 ± 29.2	155.2 ± 49.3	157.8 ± 57.7	73.6 ± 9.8	54.1 ± 7.7[*]	22.9 ± 6.0[**]	3.80[b]
Scratch[†]	153.0 ± 35.0[*]	108.4 ± 35.1	51.4 ± 24.9	158.1 ± 67.6	236.3 ± 44.7[*]	89.4 ± 16.7	3.91[b]

[a] $p = 0.054$.
[b] $p < 0.05$.
[c] $p < 0.01$.
[#] *F*-Statistic comparing all conditions except control.
[†] Adjusted *p*-value for unplanned comparison.
[*] <0.05 compared vehicle.
[**] <0.01 compared vehicle.
[***] <0.001 compared vehicle.

NC). For behavioral variables, the model included Treatment, with animal ID (identification) as a random variable. For hormone variables, the model included Treatment, Time, Time × Treatment interaction with ID as a random variable. The Time variable refers to the baseline sample (pre) and the blood sample taken 60 min after drug administration (post). If ID was not significant then it was removed from the model. If the data were not normally distributed, a square root or quad root transformation was performed. If data were still not normal after transformation, the GLMM was still performed. An F-test is recommended for non-normally distributed data (Feir-Walsh and Toothaker, 1974). For all variables, if the omnibus statistical test was significant, post hoc comparisons were done using least-squared means tests. Due to the multiple behavioral statistical tests performed for Experiment 1, p-values were adjusted for all unplanned comparisons using the Hochberg–Benjamini false discovery rate (Benjamini and Hochberg, 1995). To reduce the number of post hoc behavioral comparisons in Experiment 1, and thereby reducing the chance of a Type I error, we only compared the drug values to the vehicle. Statistical significance was set at $\alpha = 0.05$, and all tests were two-tailed.

For behavioral variables, t-tests were performed to compare the vehicle treatment and the disturbance control treatment to determine if handling, the blood draw and vehicle had a significant effect. Except for scratching, there were no significant differences in behavior between these treatments ($p > 0.05$); therefore, the disturbance control treatment was excluded from the GLMM analyses.

3. Results

3.1. Experiment 1

3.1.1. Behavior

There was a significant effect of Treatment on Grooming, Male Approach, and Male Leave but not for other affiliative/social behaviors (for all comparisons, see Table 3). There was a significant effect of Treatment on Grooming [$F(4, 27) = 3.72$, $p < 0.05$]. Compared to vehicle, there was less Grooming in response to naloxone [$F(1, 27) = 6.55$, $p < 0.05$], the low [$F(1, 27) = 6.45$, $p < 0.05$], medium [$F(1, 27) = 10.05$, $p < 0.01$], and high [$F(1, 27) = 11.42$, $p < 0.01$] doses of morphine. There was a significant effect of Treatment on Male Approach [$F(4, 27) = 6.35$, $p < 0.01$]. Compared to vehicle, the high [$F(1, 27) = 15.44$, $p < 0.001$] and medium [$F(1, 27) = 9.00$, $p < 0.01$] doses of morphine resulted in significantly fewer Male Approaches. There was a significant effect of Treatment on Male Leave [$F(4, 34) = 3.43$, $p < 0.05$]. Compared to vehicle, the high [$F(1, 34) = 6.50$, $p < 0.05$] and medium [$F(1, 34) = 5.86$, $p < 0.05$] doses of morphine resulted in significantly fewer Male Leaves.

Treatment had a significant effect on movement related behaviors. There was an effect of Treatment on Locomotion, although this only approached significance [$F(4, 34) = 2.59$, $p = 0.054$] (Fig. 1A). The high dose of morphine resulted in significantly less Locomotion compared to vehicle [$F(1, 34) = 7.24$, $p < 0.05$]. There was a significant effect of Treatment on Limb Movement [$F(4, 27) = 3.80$, $p < 0.05$]. Compared to vehicle, the high [$F(1, 27) = 8.70$, $p < 0.01$] and medium [$F(1, 27) = 5.48$, $p < 0.05$] doses of morphine resulted in a significant decrease in Limb Movement. Treatment had a significant effect on Scratch [$F(4, 27) = 3.91$, $p < 0.05$]. The medium dose of morphine resulted in a significant increase in Scratch compared to vehicle [$F(1, 27) = 6.30$, $p < 0.05$].

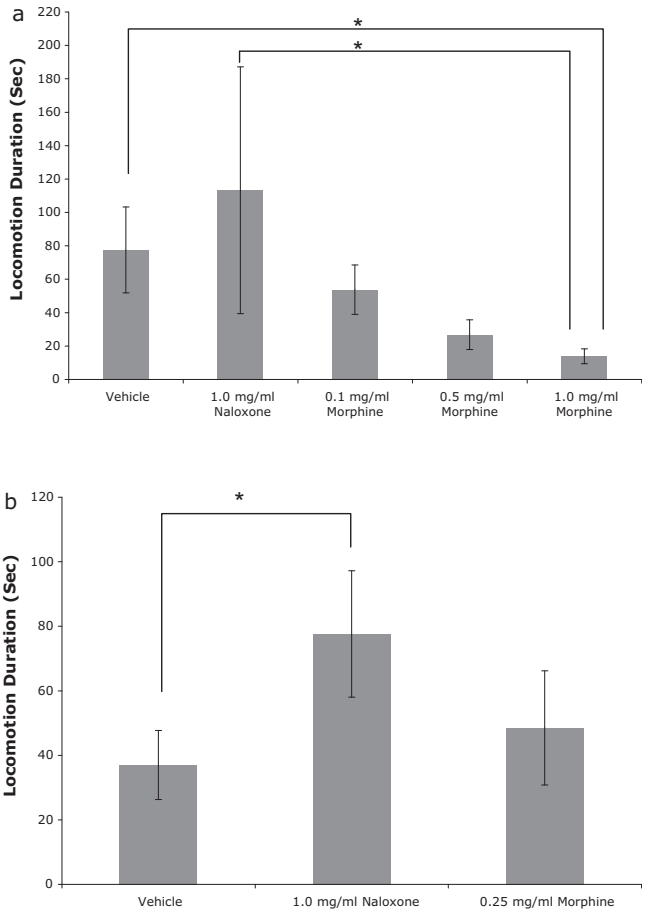

Figure 1 (A) Mean (±SEM) duration of locomotion in seconds comparing vehicle, naloxone, and the three doses of morphine when the male was with his pair-mate. (B) Mean (±SEM) duration of locomotion in seconds comparing vehicle, naloxone, and morphine when the male was without his pair-mate. *$p < 0.05$.

3.1.2. Cortisol

There was a significant effect of Time [$F(1, 61) = 34.59$, $p < 0.0001$], Treatment [$F(4, 61) = 10.05$, $p < 0.0001$], and Time × Treatment interaction [$F(4, 61) = 5.35$, $p < 0.001$] on cortisol (Fig. 2A). Overall, mean plasma cortisol concentrations of the post samples were greater than the pre (baseline) samples [main effect of Time, $F(1, 61) = 34.59$, $p < 0.0001$]. Mean plasma cortisol in the naloxone treatment was significantly greater than the other five treatments: vehicle [$F(1, 61) = 5.62$, $p < 0.05$], low [$F(1, 61) = 27.98$, $p < 0.0001$], medium [$F(1, 61) = 28.52$, $p < 0.0001$], and high [$F(1, 61) = 13.18$, $p < 0.001$] doses of morphine. Cortisol concentrations in the low and medium doses of morphine were significantly lower compared to vehicle [$F(1, 61) = 8.58$, $p < 0.01$; $F(1, 61) = 8.82$, $p < 0.01$; respectively]. Cortisol was greater in the post sample compared to the pre sample in response to vehicle [$F(1, 7) = 11.36$, $p < 0.01$], naloxone

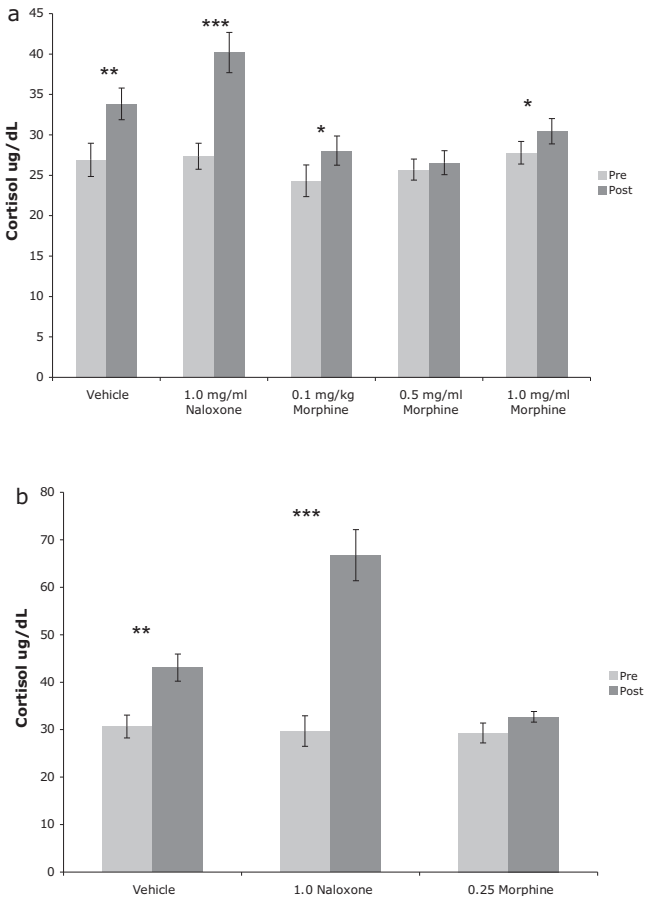

Figure 2 (A) Mean (±SEM) plasma cortisol concentrations pre and post injection of vehicle, naloxone, and all three doses of morphine when the male was with his pair-mate. (B) Mean (±SEM) plasma cortisol concentrations pre and post injection of vehicle, naloxone, and morphine when the male was without his pair-mate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

[$F(1,14) = 18.81$, $p < 0.001$], low [$F(1,7) = 10.92$, $p < 0.05$], and high [$F(1, 6) = 7.32$, $p < 0.05$] doses of morphine. However, there was no increase in cortisol from the pre to post sample in the medium dose of morphine [$F(1, 7) = 0.91$, $p > 0.05$].

We examined whether the change in cortisol from pre to post was different between treatment conditions. Treatment had a significant effect on cortisol change [$F(4, 34) = 10.22$, $p < 0.0001$; see Fig. 3A]. The change in cortisol in response to naloxone was greater compared to vehicle [$F(1, 34) = 8.12$, $p < 0.01$], the low [$F(1, 34) = 19.40$, $p < 0.001$], medium [$F(1, 34) = 33{,}41$, $p < 0.0001$], and high [$F(1, 34) = 22.56$, $p < 0.0001$] doses of morphine. The increase in cortisol in the vehicle treatment was greater than the medium [$F(1, 34) = 8.58$, $p < 0.01$] and high [$F(1, 34) = 3.96$, $p = 0.054$] doses of morphine, although this only approached significance.

3.1.3. Vasopressin

When a male was tested with his pair-mate there was no effect of Time [$F(1, 61) = 0.00$, $p > 0.05$], Treatment [$F(1, 4) = 1.06$, $p > 0.05$], or Time × Treatment interaction [$F(1, 4) = 0.67$, $p > 0.05$] on AVP concentrations (Fig. 4A).

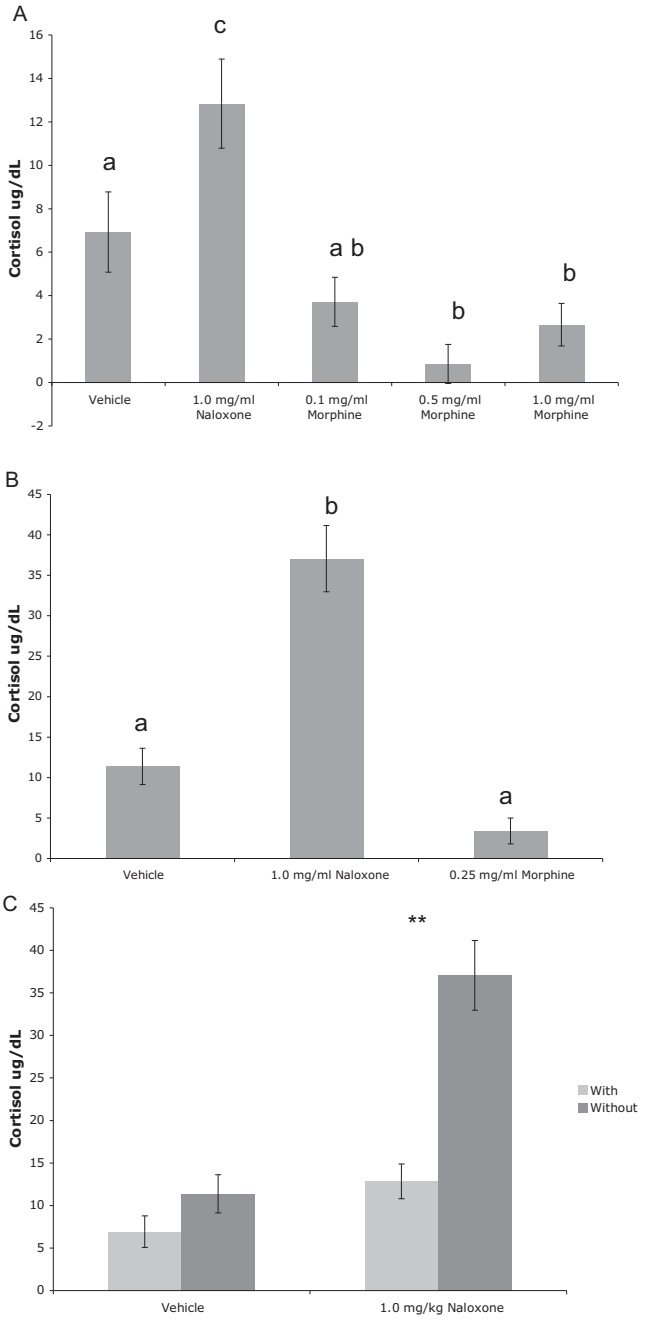

Figure 3 (A) Mean (±SEM) change in cortisol from pre to post samples comparing vehicle, naloxone, and all three doses of morphine when the male was with his pair-mate. (B) Mean (±SEM) change in cortisol from pre to post samples comparing vehicle, naloxone, and morphine when the male was without his pair-mate. (C) Mean (±SEM) change in cortisol from pre to post samples comparing males with and without their pair-mate in response to vehicle and naloxone. Different letters indicate a significant difference at *$p < 0.05$. **$p < 0.01$.

3.1.4. Oxytocin

When a male was tested with his pair-mate there was no significant effect of Time [$F(1, 61) = 0.14$, $p > 0.05$], Treatment [$F(4, 61) = 0.49$, $p > 0.05$], or Time × Treatment interaction [$F(4, 61) = 0.70$, $p > 0.05$] on plasma OT concentrations (Fig. 4C).

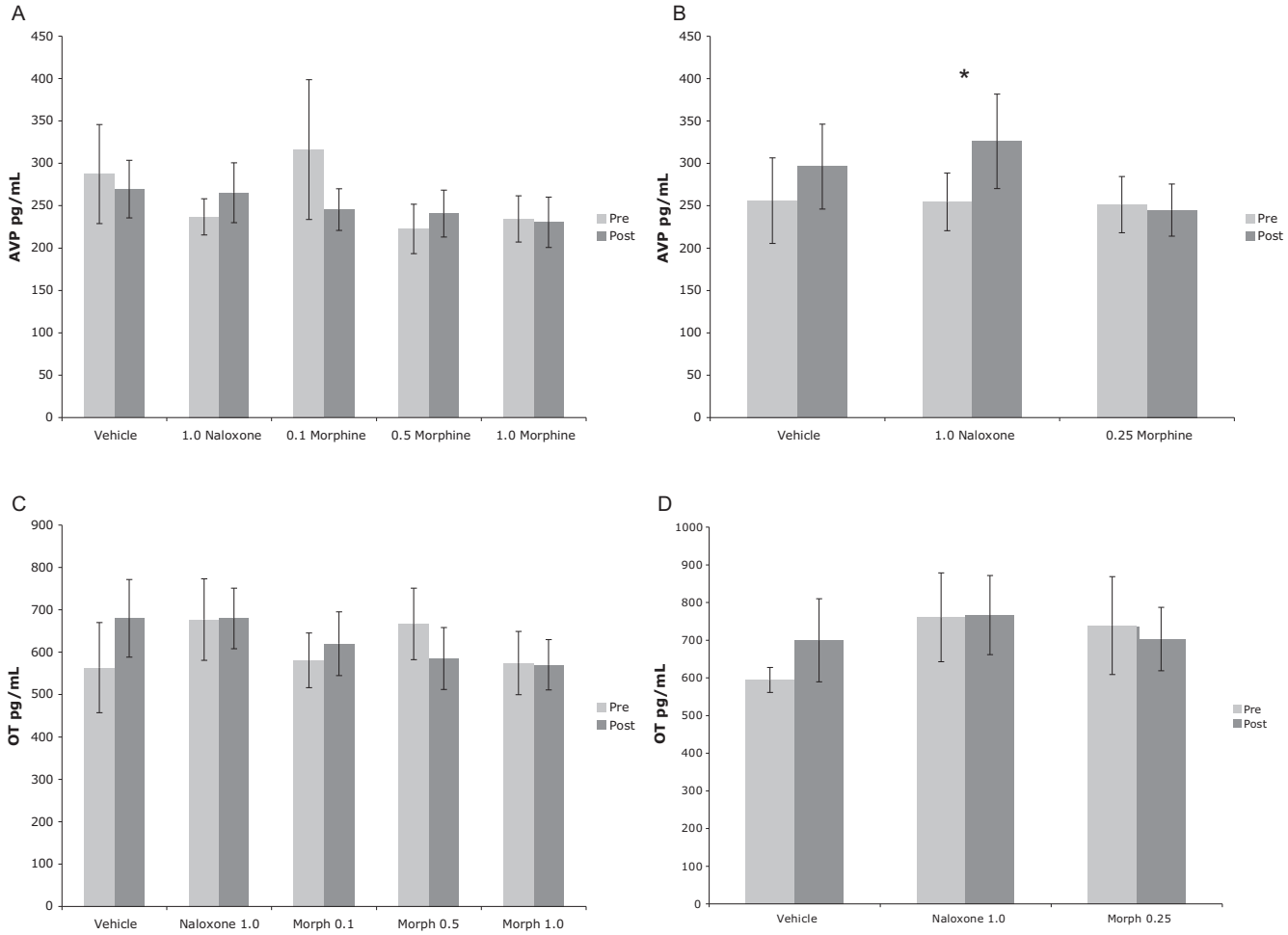

Figure 4 (A) Mean (±SEM) AVP pre and post injection of vehicle, naloxone, and all three doses of morphine when the male was with his pair-mate. (B) Mean (±SEM) AVP pre and post injection of vehicle, naloxone, and morphine when the male was without his pair-mate. (C) Mean (±SEM) OT pre and post injection of vehicle, naloxone, and all three doses of morphine when the male was with his pair-mate. (D) Mean (±SEM) OT pre and post injection of vehicle, naloxone, and morphine when the male was without his pair-mate. *$p < 0.05$.

3.1.5. Summary

Opioid manipulation had no effect on affiliative behaviors with the exception of grooming, in which both naloxone and all three doses of morphine decreased grooming behavior. We also found that the medium and high doses of morphine reduced movement related behaviors including locomotion and limb movement. The high dose of morphine decreased the number of male approaches and leaves, and morphine dose-dependently increased scratching behavior.

The hormonal effects of opioid manipulation were greatest on HPA activity. All treatments except for the medium dose of morphine resulted in a significant increase in cortisol from pre to post. Furthermore, we found that the rise in cortisol levels was greatest in response to naloxone treatment, while the rise in plasma cortisol in response to the medium and high dose of morphine was significantly less than that of vehicle. Neither morphine nor naloxone had any effect on either AVP or OT.

Table 4 Behavior without pair-mate.

Behavior	Condition				*F*-Statistic[#]
	Control	Vehicle	1.0 mg/kg naloxone	0.25 mg/kg morphine	
Isolation peeps	483.6 ± 123.3	480.5 ± 124.7	416.4 ± 96.8	426.6 ± 164.2	0.20
Long calls	46.0 ± 26.0	22.8 ± 17.8	19.4 ± 8.2	34.4 ± 22.0	1.12
Locomotion	61.0 ± 21.0	37.0 ± 10.7	77.6 ± 19.6[*]	48.5 ± 17.7	3.71[a]
Scratch	15.1 ± 3.9	13.8 ± 5.0	36.9 ± 26.0	59.5 ± 29.3	1.72

[a] $p = 0.051$.
[#] *F*-Statistic comparing all conditions except control.
[*] <0.05 compared vehicle.

3.2. Experiment 2

3.2.1. Behavior

Treatment had no significant effect on Isolation Peeps, Long Calls, or Scratch (for all comparisons, see Table 4). There was an effect of Treatment on Locomotion, which approached significance [$F(2, 14) = 3.71$, $p = 0.051$] (Fig. 1B). Naloxone resulted in significantly more Locomotion compared to vehicle [$F(2, 14) = 6.97$, $p < 0.05$].

3.2.2. Cortisol

When a male was tested alone there was a significant effect of Time [$F(1, 33) = 68.61$, $p < 0.0001$], Treatment [$F(2, 33) = 24.69$, $p < 0.0001$] and Time × Treatment interaction [$F(2, 33) = 21.71$, $p < 0.0001$] on cortisol (Fig. 2B). Overall, mean plasma cortisol was significantly greater in the post samples compared to the pre samples [$F(1, 33) = 68.61$, $p < 0.0001$]. Cortisol levels in the naloxone treatment were greater than vehicle [$F(1, 33) = 18.66$, $p < 0.001$] and morphine [$F(1, 33) = 44.89$, $p < 0.0001$]. Cortisol levels in the morphine treatment were significantly less compared to vehicle [$F(1, 33) = 5.38$, $p < 0.05$]. Cortisol was greater in the post sample compared to pre in the vehicle [$F(1, 6) = 25.70$, $p < 0.01$] and naloxone [$F(1, 7) = 81.54$, $p < 0.0001$] treatment. However, cortisol in the post sample was not greater than the pre sample in the morphine treatment [$F(1, 13) = 4.88$, $p > 0.05$].

Treatment had a significant effect on the change in cortisol from pre to post [$F(2, 19) = 39.36$, $p < 0.0001$, see Fig. 3B]. The increase in cortisol in response to naloxone was significantly greater than that of vehicle [$F(1, 19) = 36.84$, $p < 0.0001$] and morphine [$F(1, 19) = 63.31$, $p < 0.0001$], but there was no significant difference between vehicle and morphine [$F(1, 19) = 3.31$, $p > 0.05$].

Finally we tested whether the change in cortisol in response to vehicle and naloxone were different when a male was tested alone compared to when he was tested with his pair-mate (Fig. 3C). The increase in cortisol in response to vehicle was not significantly greater when the male was tested alone compared to being tested with his pair-mate [$F(1, 13) = 2.38$, $p > 0.05$]. However, the increase in cortisol in response to naloxone was significantly greater when a male was tested alone compared to being tested with his pair-mate [$F(1, 14) = 27.89$, $p < 0.001$].

The lack of effect in the vehicle condition is interesting since previous studies have found that cortisol levels after separation from a pair-mate are significantly greater compared to when they are with their pair-mate (Mendoza and Mason, 1986a; Hoffman et al., 1995). Because of this lack of effect, we wanted to make sure that subjects were actually experiencing separation distress by analyzing isolation peeps between Experiment 1 and Experiment 2. Males did emit more isolation peeps when separated from their pair-mate (Experiment 2) compared to when they were with their pair-mate (Experiment 1) [$F(1, 14) = 20.1$, $p < 0.001$].

Cortisol levels in the pre sample were statistically the same [$F(1, 13) = 0.75$, $p > 0.05$] in Experiment 1 and 2. We compared cortisol levels in the post samples between Experiments 1 and 2 to determine whether there was actually an increase in cortisol when males were separated from their pair-mate. Cortisol levels were, in fact, greater when the male was separated from his pair-mate (Experiment 2) compared to when he was with his pair-mate (Experiment 1) [$F(1, 14) = 7.14$, $p < 0.05$].

3.2.3. Vasopressin

When a male was tested alone there was an effect of Time on AVP [$F(1, 33) = 4.70$, $p < 0.05$] but no effect of Treatment [$F(2, 33) = 2.38$, $p > 0.05$] or Time x Treatment interaction [$F(2, 33) = 1.33$, $p > 0.05$] (Fig. 4B). AVP was significantly greater in the post sample compared to the pre sample [$F(1, 33) = 4.70$, $p < 0.05$].

Since naloxone had differing effects on stress related behavior (i.e. locomotion) and hormones (i.e. cortisol) depending on the presence of a pair-mate, we decided to analyze whether the presence of a pair-mate also affected naloxone's effect on AVP, another hormone released in response to a stressor (Ring, 2005). When a male was administered naloxone while with his pair-mate there was no significant increase in AVP [$F(1, 7) = 5.48$, $p > 0.05$]; however, there was a significant increase in AVP in response to naloxone when a male was tested alone [$F(1, 7) = 5.68$, $p < 0.05$].

3.2.4. Oxytocin

When a male was tested alone there was no significant effect of Time [$F(1, 33) = 0.07$, $p > 0.05$], Treatment [$F(2, 33) = 1.13$, $p > 0.05$], or Time × Treatment interaction [$F(2, 33) = 0.39$, $p > 0.05$] on plasma OT (Fig. 4D).

3.2.5. Summary

In Experiment 2 we failed to decrease behaviors associated with the separation distress response, specifically isolation peeps and locomotion, when we administered morphine. However, we did find that naloxone was able to increase locomotion when the male was separated from his pair-mate.

Neuroendocrine responses to morphine and naloxone were similar to those in Experiment 1. Administration of vehicle and naloxone increased plasma cortisol concentrations. There was no significant change in plasma cortisol following morphine administration. Like Experiment 1, naloxone treatment resulted in the greatest rise in cortisol. Interestingly, the rise in cortisol when a male was administered naloxone was significantly greater when he was alone compared to when he was with his pair-mate. In Experiment 2 morphine and naloxone again failed to affect plasma OT, but AVP was greater in the post sample compared to baseline upon administration of naloxone when a male was tested without his pair-mate.

4. Discussion

The present study examined whether the opioid system was involved in affiliative behavior and the separation distress response in a monogamous primate and whether manipulation of this system is more similar to nonmonogamous primates or a monogamous rodent. We also investigated the effects of opioid manipulation on plasma OT, AVP, and cortisol concentrations. We did not find any changes in the majority of social behaviors in response to morphine or naloxone in titi monkeys. However, we did find that the presence of a pair-mate was able to ameliorate the negative effects of naloxone as seen through naloxone's effects on locomotion, cortisol, and AVP.

Our results differ from studies exploring the effects of opioid manipulation in nonmonogamous, nonhuman primates in which the majority have found that morphine decreases affiliative behavior, particularly grooming solicitations and receiving grooming, while opioid blockade increases these behaviors (Keverne et al., 1989; Schino and Troisi, 1992; Martel et al., 1995). The current study replicates findings in prairie voles that did not find an effect of opioid blockade on affiliative behaviors (Shapiro et al., 1989; Burkett et al., 2011; Resendez et al., 2012). These results suggest opioid receptors in titi monkeys function more similarly to that of a monogamous species rather than that of a nonmonogamous, nonhuman primate. We should mention, however, that only one dose of naloxone was used, but this dose has been found to be effective in increasing affiliative behavior in juvenile macaques (Schino and Troisi, 1992).

The one affiliative behavior that was altered by opioid manipulation was grooming. Interestingly, both naloxone and all three doses of morphine resulted in a decrease in grooming behavior compared to vehicle. The middle and high doses of morphine in the present study resulted in moderate to large decreases in motor behaviors, which in turn could impede grooming. Naloxone's impairment on grooming behavior contrasts with findings in other studies in adult nonhuman primate relationships where opiate blockade only affects grooming solicitations and receiving grooming (Fabre-Nys et al., 1982; Keverne et al., 1989; Martel et al., 1995). Acute naltrexone administration increases clinging behavior between mother and infant macaques, however grooming behavior was not measured in that study (Kalin et al., 1995). Martel and colleagues (1993) found that naloxone administration to rhesus macaque mothers resulted in those mothers grooming their infants less frequently; however, it is important to note that the Martel et al. (1993) study used a lower dose and administration was chronic rather than acute as in the present study. Naloxone may be producing negative affect as seen in other species (Mucha and Walker, 1987; Martin del Campo et al., 1994), and this may reduce motivation to engage in grooming behavior. The decrease in grooming behavior at the 0.1 mg/kg dose of the present study mirrors findings from Kalin et al. (1995), which found that administration of 0.1 mg/kg dose of morphine to mother macaques decreases clinging with their infants, however grooming behavior was not measured in that study either. It is possible that an appropriate level of opioid activation (neither too high nor too low) is needed to support normal grooming behavior. This has been found with other neurotransmitter systems in that both dopamine agonists and antagonists can impair social behavior (Young et al., 2011b).

In Experiment 2 neither morphine nor naloxone had any effect on isolation vocalizations when males were separated from their pair-mate. Opioid manipulation has previously been demonstrated to affect separation distress behaviors, particularly isolation vocalizations in infants. Morphine and other MOR agonists reliably decrease isolation vocalizations in a variety of species (Herman and Panksepp, 1978; Panksepp et al., 1978a,b, 1980; Kalin et al., 1988; Nelson and Panksepp, 1998), with opioid blockade sometimes resulting in an increase in isolation vocalizations (Panksepp et al., 1980; Kalin et al., 1988). It appears that unlike infant—mother bonds, opioids are not involved in regulating isolation vocalizations emitted by male titi monkeys when separated from their attachment figure.

While opioid manipulation did not affect most social behaviors, it affected a variety of non-social behaviors. Morphine dose-dependently increased scratching behavior, with the medium dose having the greatest efficacy. The highest dose of morphine most likely did not increase scratching behavior due to its sedative effects. This bell shaped curve of scratching behavior in response to morphine has been observed in other studies (Ko et al., 2004). MOR activation consistently results in an increase in the itch/scratch response (Twycross et al., 2003) in humans (Smith and Beecher, 1962), monkeys (Ko et al., 2004) and rodents (Kuraishi et al., 2000), and appears to be mediated primarily through central mechanisms when administered systemically or spinally (Twycross et al., 2003; Ko et al., 2004).

Morphine also had a sedative effect. Sedation was dose-dependent and was reflected in a reduction of locomotor activity and limb movement, with the highest dose having the greatest impact. At the middle and high doses of morphine, there was also a decrease in subjects approaching and leaving their pair-mate. Although this might reflect alterations in social attraction, it is more likely due to a sedative effect since approaches and leaves were affected and were accompanied by a reduction in limb movement. Locomotor effects of opioids on nonhuman primates have not been well studied. Chronic administration of the preferential MOR agonist, methadone, to bonnet macaques results in periods of extreme sedation as well as periods of increased activity (Crowley et al., 1975). Morphine doses up to 2.0 mg/kg have failed to reduce locomotor behavior in juvenile marmosets and adult talapoin monkeys (Keverne et al., 1989; Guard et al., 2002). It is possible there are species differences in opioid receptors or opioid receptor distribution resulting in titi monkeys being more sensitive to the sedative effects of morphine. Naloxone affected locomotor behavior, but was dependent upon whether the male was with or without his pair-mate. Naloxone administration to a male when he was with his pair-mate did not result in a change in locomotion, but there was a significant increase in locomotor behavior compared to vehicle when the male received naloxone without his pair-mate. Infant guinea pigs also experience an increase in locomotion when administered naloxone in isolation (Herman and Panksepp, 1978).

The presence of a pair-mate also affected how naloxone altered endocrine function. Drug treatments had no effect on AVP when the male was tested with his pair-mate, but administration of naloxone in the absence of a pair-mate resulted in a significant increase in plasma AVP compared to baseline levels. Some studies have found that MOR antagonists can increase AVP while MOR activation decreases AVP. However, these findings are not consistent and are dependent on the hydration state of the organism and differ depending on in vitro versus in vivo methodologies (Van Wimersma Greidanus and Van De Heijning, 1993; Vuong et al., 2010). Since subjects were provided ad libitum water, the increase in AVP in response to naloxone was not influenced by a state of dehydration but rather whether the male was in the presence of his pair-mate. This effect may be related to AVP's role in the stress response (Ring, 2005) instead of its antidiuretic effects.

The stress related hormone, cortisol, was altered by opioid manipulation. When the male was tested with his pair-mate, administration of vehicle resulted in an increase in plasma cortisol compared to baseline. This was likely due to the stress of capture and blood draw. An increase in cortisol was also seen in response to naloxone and the low and high doses of morphine. The middle dose of morphine was able to suppress the increase in cortisol in response to the capture and blood draw. The rise in cortisol from baseline also differed depending on treatment. When administered naloxone, the rise in cortisol was greater compared to all other treatments. In contrast, the rise in cortisol in response to the middle and high doses of morphine was less than vehicle.

A similar effect of opioid manipulation on plasma cortisol occurred when males were tested alone. There was an increase in cortisol in response to vehicle and naloxone, and morphine was able to prevent this increase. The rise in cortisol from baseline after naloxone administration was greater compared to the rise in cortisol levels following vehicle and morphine administration. Interestingly, the rise in cortisol in response to vehicle was not different when comparing males with and without their pair-mate. Despite this lack of effect, subjects did emit significantly more isolation peeps when separated compared to when he was with his pair-mate indicating that a separation response did occur. We also found that post samples of cortisol were greater when males were tested alone compared to when they were tested with their pair-mate while baseline levels of cortisol were same between the two experiments. This is consistent with previous research (Mendoza and Mason, 1986a; Hoffman et al., 1995). Previous studies analyzing HPA activity in response to social separation in titi monkeys have not measured increases in the hormone from a baseline sample, as was done in the present study, and have only looked at cortisol levels after the social separation, and baseline cortisol samples were taken on different days from separation (Mendoza and Mason, 1986a; Hoffman et al., 1995). The fact that we applied both a physical (capture and bleeding) and social stressor (temporary separation from pair-mate) is a possible explanation for why the increase in cortisol from baseline in response to vehicle was the same when a male was with and without his pair-mate for the hour following the first bleed.

One of the most interesting and unexpected findings of this study was the relationship of social context to the response to naloxone. Naloxone can be considered a chemical stressor in that it induces a phenotype similar to environmental stressors. Systemic administration of naloxone to drug naïve subjects results in dysphoria in humans (Martin del Campo et al., 1994), conditioned place aversions in rodents (Mucha and Walker, 1987), as well as increases in HPA activity in a variety of species (Fabre-Nys et al., 1982; Parrott and Thornton, 1989; Wand et al., 2011). When a male was administered naloxone with his pair-mate, the behavioral and physiological response was different compared to when he received the drug without his pair-mate. When administered naloxone alone, the rise in cortisol was significantly greater compared to when he was tested with his pair-mate. Furthermore there was a significant increase in plasma AVP and locomotion, which was not observed when the male received naloxone with his pair-mate. Cortisol and AVP are released in response to a stressor (Mendoza et al., 2000; Sapolsky et al., 2000; Ring, 2005), and locomotion in titi monkeys is an indicator of arousal and occurs when exposed to certain stressors including social separation (Mendoza and Mason, 1986a; Ragen et al., 2012).

These results suggest that the presence of a pair-mate provides a source of social buffering to dampen the aversive components of naloxone. Social buffering has been observed in titi monkeys previously. Titi monkeys exposed to a novel environment experience an increase in cortisol and locomotion; however, these increases are attenuated when an animal is with its pair-mate. Social buffering effects to naloxone are also observed through sheep HPA activity (Parrott and Thornton, 1989) and guinea pigs' distress vocalizations (Herman and Panksepp, 1978). The social buffering effects seen in the present study lends support to the hypothesis that removal of an affiliative partner results in a decrease in endogenous opioids and opioid activation (Panksepp et al., 1980; Nelson and Panksepp, 1998). In titi monkeys, this is indicated by an enhancement of naloxone's ability to act as a chemical stressor.

There are potentially multiple neuroanatomical sites where naloxone is acting to regulate affect and stress physiology. There is evidence that MORs located in the nucleus accumbens (Wand et al., 2011), ventral pallidum (Skoubis and Maidment, 2003), caudate/putamen (Wand et al., 2011), amygdala (Ribeiro et al., 2005), and hypothalamus (Wand et al., 2011) are mediating reward and stress related systems. MORs in the striatum are associated with positive affect (Olds, 1982), and blockade of these receptors could contribute to naloxone inducing negative affect and thereby increasing locomotion. MOR activation is believed to provide an inhibitory tone on the HPA axis (Wand et al., 1998, 2011). Blockade of these MORs by naloxone may result in an increase in cortisol and AVP, while activation by morphine could result in inhibiting HPA activation when a titi monkey experiences a stressor (Wand et al., 1998, 2011). This is supported by the evidence that the medium dose (0.5 mg/kg) of morphine suppressed the increase in cortisol when a male was tested with his pair-mate, and the 0.25 mg/kg dose of morphine suppressed the increase in cortisol when a male was tested alone. Furthermore, the change in cortisol from baseline in the medium and high morphine doses was less compared to vehicle.

The dose-dependent effects of morphine on cortisol were not linear unlike what has been found in humans (Zis et al., 1984). It is possible that the high dose of morphine produced a negative affective state that could have prevented a further decrease in cortisol via a top-down mechanism. In humans there is variability in the pleasurable effects of opiates, which are affected by dose and whether the subject is an addict (since high doses of opiates can be aversive in non-addicts) (Fraser and Isbell, 1952; Smith and Beecher, 1962). Methodological differences may also explain this difference; studies in humans look at baseline cortisol levels instead of cortisol levels after a stressor as in the present study (Zis et al., 1984).

There are also limitations of this study that should be addressed. The most important is that of making comparisons between Experiments 1 and 2, specifically the differing effects of naloxone on locomotion, vasopressin and cortisol. All of the sessions without the pair-mate occurred after the

sessions were with their pair-mate which could have introduced a confound of order, potentially resulting in sensitization to the drugs or paradigm. This unfortunately was inevitable due to the need to choose the appropriate dose of morphine for Experiment 2. However, it is unlikely that these sensitizations occurred due to the three weeks that separated Experiment 1 from Experiment 2 (Keith et al., 1996; Sim et al., 1996; Williams et al., 2013). It is also important to note that the baseline levels of cortisol from Experiment 1 were the same as those find in Experiment 2, suggesting that there is some hormonal consistency between Experiments 1 and 2. Finally, we feel confident that the differing effects of naloxone are real in that they replicate other experiments that have shown that being in the presence of an attachment or affiliative figure can buffer the behavioral and/or physiological responses to naloxone (Herman and Panksepp, 1978; Parrott and Thornton, 1989). Another limitation of the study is that we only used males and not females, and effects could be sex-specific. Finally, during Experiment 1, two of the subjects had offspring that were removed during the session. It is known that neither father nor mother form an attachment toward the infant and do not undergo separation distress upon removal of the infant (Mendoza and Mason, 1986b; Hoffman et al., 1995). However, it is possible that removal of the infant could have affected social behavior between the male and female.

In future studies we may want to alter the experimental paradigm of opioid manipulation in order to attempt to get an effect of opioid manipulation on social behavior. In the present study we put the subject back with his pair-mate immediately following drug administration. This paradigm did not result in changes in social behavior. It is possible that if we used a different paradigm, such as giving the drug prior to a reunion after a separation, we would be able to find an effect of the drug on social behavior. A study by Kalin et al. (1995) utilized this kind of paradigm in that they separated an infant and mother macaque for 30 min, administered naltrexone or morphine, and then reunited the animals. Using this design, they found that naltrexone increased clinging and morphine decreased clinging. This type of paradigm could increase the chances of finding an effect of opioid manipulation in the titi monkeys. Another possible study would be to manipulate the opioid system in infant titi monkeys. This would give us more information on whether lack of separation distress behavior found in adult titi monkeys is due to their adult attachment rather than a species differences. Opioid manipulation in infant titi monkeys would be especially interesting since they form a selective attachment toward their father rather than their mother (Mendoza and Mason, 1986b) which would provide us with information on whether the opioid system acts similarly with paternal attachment as it does with maternal attachment.

By examining how opioid manipulation affects behavior and hormones in titi monkeys we found that opioid receptors play a unique function in a socially monogamous species. Opioid functioning in titi monkeys is different than its role in affiliative and separation distress behaviors in nonmonogamous species. Naloxone in titi monkeys had minimal effects on affiliative behaviors, which are closer to findings in the monogamous prairie vole than findings in non-monogamous primates. Furthermore, opioid manipulation had no effect on the behavioral components of the separation distress response, which is in contrast to infant—mother attachments. Even though we did not observe major changes in social behaviors, we did find that the presence of pair-mate does influence opioid functioning. This was observed by the presence of a pair-mate ameliorating naloxone's effect on hormonal and affective systems related to stress. The present study does support the hypothesis of Panksepp and colleagues (1980), in that an attachment figure provides a homeostatic level of opioid activation and removal of an attachment figure disrupts that homeostasis. However, unlike infant—mother attachments where the disruption in opioid activation can be observed through separation vocalizations, in titi monkey adult attachments we observed this effect in the differing behavioral and hormonal outcomes of opioid blockade when a male is with or without his pair-mate.

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Conflict of interest

None of the authors had any conflicts of interest.

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