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Neonatal melanocortin receptor agonist treatment reduces play fighting and promotes adult attachment in prairie voles in a sex-dependent manner

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ABSTRACT

The melanocortin receptor (MCR) system has been studied extensively for its role in feeding and sexual behavior, but effects on social behavior have received little attention. α-MSH interacts with neural systems involved in sociality, including oxytocin, dopamine, and opioid systems. Acute melanotan-II (MTII), an MC3/4R agonist, potentiates brain oxytocin (OT) release and facilitates OT-dependent partner preference formation in socially monogamous prairie voles. Here we examined the long-term impact of early-life MCR stimulation on hypothalamic neuronal activity and social development in prairie voles. Male and female voles were given daily subcutaneous injections of 10 mg/kg MTII or saline between postnatal days (PND) 1-7. Neonatally-treated males displayed a reduction in initiated play fighting bouts as juveniles compared to control males. Neonatal exposure to MTII facilitated partner preference formation in adult females, but not males, after a brief cohabitation with an opposite-sex partner. Acute MTII injection elicited a significant burst of the immediate early gene EGR-1 immunoreactivity in hypothalamic OT, vasopressin, and corticotrophin releasing factor neurons, when tested in PND 6-7 animals. Daily neonatal treatment with 1 mg/kg of a more selective, brain penetrant MC4R agonist, PF44687, promoted adult partner preferences in both females and males compared with vehicle controls. Thus, developmental exposure to MCR agonists lead to a persistent change in social behavior, suggestive of structural or functional changes in the neural circuits involved in the formation of social relationships.

1. Introduction

The melanocortin (MC) system has been studied extensively for its role in coordinating feeding (Poggioli et al., 1986), stress and anxiety (De Barioglio et al., 1991; Lu et al., 2003; Chaki and Okuyama, 2005) and sexual behavior (Argiolas et al., 2000; Rossler et al., 2006), among other physiological and behavioral processes (for review, see Wikberg et al., 2000; Mountjoy, 2010; Tao, 2010). However, the effects of melanocortin receptor activation on social behavior have received little attention. Alpha-melanocyte stimulating hormone (α−MSH) stimulates central OT release from rat hypothalamic slices, an effect that is blocked by an MC4 receptor (MC4R) antagonist (Sabatier et al., 2003). The MC4R also interacts with additional systems involved in the regulation of social behaviors, including dopamine (Lindblom et al., 2001), opioids (Alvaro et al., 1997), and corticotropin-releasing factor (CRF; Lu et al., 2003).

Recently, we have found that melanocortin signaling promotes social attachment in prairie voles (Modi et al., Unpublished results). The socially monogamous, biparental prairie vole (*Microtus ochrogaster*) exhibits a complex repertoire of social behaviors that have been associated with oxytocin, dopamine, opioid, and CRF signaling (Young and Wang, 2004; Aragona et al., 2006; Lim et al., 2007; Bosch et al., 2009; Burkett et al., 2011), and provides an excellent model to assess the neural underpinnings of social behavior (McGraw and Young, 2010). Pair bond formation is assessed using the partner preference test, in which a subject

Abbreviations: oxytocin, OT; oxytocin receptor, OTR; vasopressin, AVP; corticotrophin releasing hormone, CRF; melanocortin, MC; melanocortin receptor, MCR; paraventricular nucleus of the hypothalamus, PVN; brain-derived neurotrophic factor, BDNF; early-growth factor, EGR-1; melanotan-II, MTII; PP, partner preference; IEG, immediate early gene.

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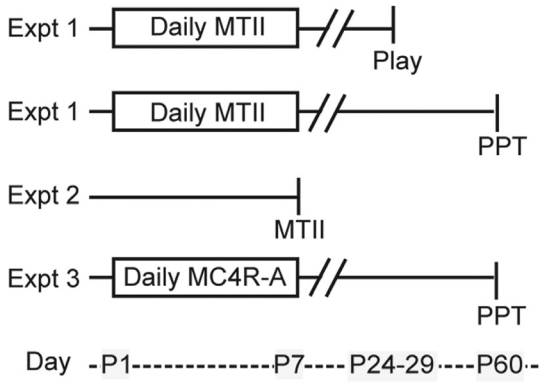

Fig. 1. Experimental design. In Experiment 1, neonates were injected daily from PND1-7 with 10 mg/kg of melanotan-II (MTII) and either tested for juvenile play behavior (Sal, $n = 11$f, 16 m; MTII, $n = 11$f, 15 m) or adult partner preference (Sal, $n = 15$f, 10 m; MTII, $n = 18$f, 12 m). In Experiment 2, PND6-7 neonates were sacrificed 1-hr after an acute injection of 10 mg/kg MTII or saline, or handling only (H, handled). Either immunohistochemistry (IHC) for hypothalamic neuropeptide activation ($n = 6$H, 4 sal, 4MTII) was performed or plasma corticosterone was assayed ($n = 8$H, 8sal, 9MTII). In experiment 3, neonates were injected daily with a melanocortin-4 agonist (MC4R-A, PF-446687, Pfizer) and tested for partner preference in adulthood (Vehicle, $n = 9$f, 9 m; 0.1 mg/kg, $n = 10$f, 15 m; 1 mg/kg, $n = 9$f, 15 m). Animal numbers are outlined in tables on the right. F, female; M, male. PPT, Partner preference test.

animal's preference for a cohabitated partner or novel stranger animal is tested (Williams et al., 1992). In prairie voles, the partially brain penetrant MC3/4 agonist, melanotan-II (MTII), activates oxytocin (OT) neurons in the paraventricular nucleus of the hypothalamus (PVN), potentiates central OT release in response to a physiological stimulus (hypertonic saline), and facilitates partner preference formation (Modi et al., Unpublished results). These effects are thought to be mediated via MC4R as the selective, brain penetrant MC4R agonist, PF446687, also promotes partner preference formation (Modi et al., Unpublished results).

Adult prairie vole sociality is sensitive to early-life manipulations of parental care (Ahern and Young, 2009). One mechanism by which early social experience, in particular parent–infant interactions, may be translated into long-term behavioral alterations is through long-term organizational effects of neuropeptide activation through restructuring neural circuitry (Carter et al., 2009). For example, neonatal treatment with OT at birth impacts later socioemotional behaviors in prairie voles (Bales and Carter, 2003b; Kramer et al., 2003; Cushing et al., 2005; Bales et al., 2007). Interestingly, neonatal administration of α-MSH and ACTH-like peptides to rats leads to enhancements in adult attention to relevant stimuli (Champney et al., 1976), learning (Beckwith et al., 1977b; Acker et al., 1985), social contact in an open field (Beckwith et al., 1977a), and reductions in adult anxiety (Felszeghy et al., 1993). Early α-MSH treatment also impacts hypothalamic cytoskeletal proteins (Wu et al., 2006), hypothalamic dopamine neuron development (Egles et al., 1998), and neurite outgrowth (Joosten et al., 1996), thus leading to functional and structural changes in neural development. As MC agonists impact a variety of neural systems involved in sociality and acute MC4R stimulation promotes adult pair bonding, early MC stimulation may also stimulate encoding of early social information and lead to long-term organizational changes in social behavior.

Here, we investigated the effects of daily MTII and the more selective, small molecule MC4R agonist, PF446687 (Lansdell et al., 2010) injections during the first week of life on juvenile play and adult pair bonding in males and female prairie voles. We also assessed the impact of peripheral MTII on hypothalamic neuropeptide systems using the immediate early gene, early growth response factor-1, as a marker of neuronal activity. Daily neonatal treatment with MTII altered juvenile play behavior and both agonists enhanced adult social bonding, suggesting central MCR stimulation has long-lasting, organizational effects on social neural circuitry.

2. Materials and methods

Animal care and handling. Subjects were laboratory-bred prairie voles, derived from a field-caught Illinois stock. The colony was maintained at 22 °C and on a 14:10 h light:dark cycle with access to food (Purina high-fiber rabbit chow) and water *ad libitum*. Breeder housing consisted of large ventilated cages (34 × 30 × 19 cm) lined with bedding (bed-o-cob, Maumee, OH, USA). Subjects were not exposed to subsequent litters. Pups were weaned into same-sex same-treatment pairs or trios in smaller (30 × 18 × 19 cm) cages at PND21. From each litter, pups were randomly assigned to treatment groups and no more than 2 animals of the same sex and treatment group were used for each experiment. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Emory University Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

Experimental design, The timelines of experiments and animal numbers can be found in Fig. 1 and the legend. Experiments 1 and 3 investigated the behavioral consequences of daily nonselective MC agonist MTII or selective MC4R agonist PF446687 in males and females. Experiment 2 examined the impact of acute MTII injection on hypothalamic systems and plasma corticosterone.

Table 1
Juvenile play ethogram.

Category	Behavior	Definition
Play	Aggressive grooming (AG)	One animal violently grabs at the fur of another and grooms, usually in the back region
	Boxing (B)	Both animals stand on the hind legs and use only their forepaws to attack repeatedly
	Chasing (C)	Active following
	Pinning (Pi)	The action of one animal holding another down overhead and the other is typically on its back or supine
	Pouncing (Po)	Jumping on another animal making contact with forepaws and a common play initiation behavior
	Pulling/biting (PB)	Mouth to body contact
	Supine (Su)	Subject on its back, commonly a result of physical attack or pinning or can be independently induced
	Wrestling/tackling (WT)	Ventrum-to-ventrum embracing with biting without inflicting wounds
Social Affiliation/Exploration	Allogrooming (Allo)	Received or directed grooming to another subject
	Genital investigation (GI)	Sniffing or grooming directed towards other animal's genital region
	Huddling (H)	Immobilized and in close contact
	Sniffing (Sn)	General sniffing the stimulus animal
	Social Contact (SC)	Passive bodily contact

The frequency of juvenile play and social exploration/affiliation with a novel conspecific was coded using the above working definitions of behaviors. Behaviors initiated by the subject animal over a 10 min period for 3 d were coded by a blind observer.