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REPEATED TREATMENT WITH OXYTOCIN PROMOTES HIPPOCAMPAL CELL PROLIFERATION, DENDRITIC MATURATION AND AFFECTS SOCIO-EMOTIONAL BEHAVIOR

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Abstract—Rewarding social behaviors including positive social interactions and sexual behaviors are shown to regulate adult neurogenesis, but the underlying biological mechanisms remain elusive. Oxytocin, a neurohypophysial hormone secreted after exposure to social interaction or sexual behaviors, has a profound role in the formation of social bonding and regulation of emotional distress. While the acute effect of oxytocin was usually studied, relatively scarce evidence showed the behavioral consequence of repeated oxytocin treatment. The purpose of the current study was to investigate the effect of repeated oxytocin treatment on hippocampal cell proliferation, dendritic maturation of new born neurons and social/emotional behaviors. Adult male Sprague–Dawley rats received treatment with either vehicle or oxytocin (1 mg/kg) daily for two weeks. Behavioral tests revealed that oxytocin increased social behaviors and reduced the anxiety- and depression-like behaviors. Cell proliferation, differentiation and the dendritic complexity of new born neurons in the hippocampus were promoted by oxytocin treatment. Depression- and anxiety-like behaviors were induced by repeated treatment of corticosterone (40 mg/kg) for two weeks while oxytocin treatment reversed the behavioral disturbances. Suppression of cell proliferation caused by corticosterone was reverted by oxytocin treatment in which cell proliferation, cell differentiation, and dendritic complexity increased. The present findings reveal that oxytocin not only enhances cell proliferation, but also promotes the development of the new neurons which is associated with the induction of positive emotional and social behaviors. The results also suggest that oxytocin may be a potential therapeutic agent for treatment of emotional and social dysfunction.

Key words: oxytocin, hippocampal cell proliferation, neurogenesis, dendritic complexity, depression-like behaviors, anxiety-like behaviors.

INTRODUCTION

The neurohypophysial hormone oxytocin (OXT) is released both centrally and peripherally under rewarding social stimulation such as social interaction with desirable individuals, sexual activity, and suckling (McNeilly and Ducker, 1972). After being synthesized in magnocellular neurons in the hypothalamic supraoptic and paraventricular nuclei, oxytocin is released into the bloodstream via neuronal connections within hypothalamic and limbic regions (Neumann and Landgraf, 2012). Oxytocin is also released from dendrites and perikarya as a neuromodulator and reaches the oxytocin receptors (OXTR) through diffusion via extracellular fluid and ligand binding (Landgraf and Neumann, 2004). As suggested by the regulators of oxytocin secretion, oxytocin is closely associated with social and emotional behaviors. For instance, administration of oxytocin was shown to improve social attachment, show anxiolytic effect on human subjects and may protect an individual from the negative consequence of stress. which implicates the potential therapeutic value of oxytocin in emotion-related disorders (Landgraf and Neumann, 2004; Leuner et al., 2012). Despite the established roles of oxytocin in regulation of social and emotional behavior, the biological mechanism of its influence remains obscure.

Adult neurogenesis, which describes the production of new functional neurons in the adult central nervous system (CNS), is a complex dynamic process. Internal and external environmental factors can affect the regulation of neurogenesis at different stages including proliferation, migration, differentiation and integration into the existing neural circuitry (Ming and Song, 2005; Song et al., 2012). Neurogenesis can be found predominantly in two distinct regions in the CNS, namely, the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus and the subventricular zone (Song et al., 2012). Cell proliferation, the first step in the neurogenesis process, refers to one complete cell division cycle that can be detected using BrdU which is a marker for DNA synthesis. Once new cells have been born, they differentiate into mature neuronal phenotype and make synaptic connections in the existing circuitry (Malberg, 2004). External factors such as physical exercise and stress

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Abbreviations: CNS, central nervous system; DG, dentate gyrus; FST, Forced Swimming Test; OFT, Open Field Test; SIT, Social Interaction Test; PBS, phosphate-buffered saline.

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have shown to increase and decrease cell proliferation respectively; therefore affecting neurogenesis (Song et al., 2012). Different lines of evidence demonstrate the functional significance of neurogenesis including learning, sexual behaviors and social behaviors (Leuner et al., 2006; Deng et al., 2010). Since neurogenesis has an impact on different emotional and cognitive behaviors, it is expected that the understanding of neurogenesis at different stages of the neurogenesis process would shed light on pathophysiology of emotional and cognitive disorders, and may provide insight on developing novel clinical treatment (Winner et al., 2011).

As both neurogenesis and oxytocin are shown to regulate social and emotional behaviors, it is possible that they may have intricate interaction to regulate the socio-emotional behaviors. One of the evidence to support the relationship between neurogenesis and oxytocin is that postnatal neuronal growth was observed in the vasopressin and oxytocin-containing nucleus of the pig hypothalamus (Rankin et al., 2003). Recently it was revealed that acute and sub chronic (7 day) treatment of oxytocin increases ventral hippocampal cell proliferation, and the neurogenesis-stimulating effect could be found under stressful situation (Leuner et al., 2012). Meanwhile another *in vitro* study showed that oxytocin treatment promotes neuronal differentiation of adipocyte-derived stem cells (Jafarzadeh et al., 2014). To further explore the effect of oxytocin on cell proliferation and the display of emotional and social behavior, the present study tested (1) the effect of repeated exposure to oxytocin on cell proliferation and dendritic maturation of new neurons; (2) the effect of prolonged exposure to oxytocin on social interaction and depression/anxiety-like behaviors and (3) the potential therapeutic effect of oxytocin in a corticosterone-induced depression- and anxiety-like behavior animal model (Gregus et al., 2005; Brummelte et al., 2006). Since oxytocin signaling may play key roles in the underlying mechanisms of pro-neurogenic effect of socially rewarding behaviors, the molecular mechanisms triggered to promote neurogenesis should be further investigated.

EXPERIMENTAL PROCEDURES

Experimental design

Young adult male Sprague–Dawley (SD) rats (31 rats in total, 7–8 weeks of age, 200–220 g) were housed in pairs in polycarbonate cages. They were fed *ad libitum* and the room was maintained on a 12-h alternating light–dark cycle and at 23–25 °C. The housing and behavioral procedures were approved by the Animal Subject Ethics Sub-Committee of the Hong Kong Polytechnic University.

Experiment 1. To study the effect of repeated oxytocin treatment on emotional behaviors and cell proliferation, SD rats ($n = 6$ rats/group) were injected intraperitoneally with oxytocin (1 mg/kg/day; Bachem Americas) or equal volume of vehicle (normal saline). Treatment was given around 17:00 every day for 14 consecutive days. The dose of oxytocin used has shown to stimulate neurogenesis in the DG in previous studies (Leuner et al., 2012). At day 12–14, 50 mg/kg/day of BrdU was intra-peritoneally injected to label proliferative cells. Behavioral tests were performed at days 14 and 15, which was followed by perfusion at day 16.

Experiment 2. Animals were divided into three groups ($n = 6$–7 rats/group): (1) Control group ($n = 6$) with intraperitoneal injection of vehicle (normal saline) and subcutaneous injection of propylene glycol which was the vehicle used to dilute the corticosterone in groups 2 and 3; (2) Corticosterone treatment group ($n = 7$) with subcutaneous injection of corticosterone (40 mg/kg) and (3) Oxt + Cort group ($n = 6$) which received both corticosterone (40 mg/kg) subcutaneously and intraperitoneal injection of oxytocin (1 mg/kg). The co-administration of oxytocin and corticosterone was performed around 17:00 every day for 14 days. In previous studies, repeated administration of corticosterone at 40 mg/kg showed to reliably induce depression-like behavior in rats (Kalynchuk et al., 2004; Gregus et al., 2005; Brummelte et al., 2006). In addition, a dose of 40 mg/kg corticosterone has been reported to diminished cell proliferation in the DG and subventricular zone (Cameron and Gould, 1994; Leuner et al., 2012; So et al., 2012). Behavioral analysis was carried out at days 14 and 15, followed by transcardial perfusion at day 16.

BEHAVIORAL TESTS

Forced Swimming Test (FST)

Swimming sessions were conducted in individual transparent cylinders (40 cm height × 30 cm diameter) filled with water at a depth of 30 cm at room temperature. The cylinders were deep enough to avoid that the rats touch the bottom of the cylinder to support themselves. Two swimming sessions were conducted: an initial 15-min pretest for habituation, followed 24 h later by a 10-min test. Test sessions were video recorded from a front view for scoring later by an observer blinded to the treatment. The behaviors scored in the FST included: (1) time spent immobile – floating in the water without struggling, minimal movement to keep from drowning, with only the necessary movement to keep the head above the water; (2) time spent swimming – making active motions, such as moving around in the cylinder, more than necessary to merely keep the head above water and less movements than those shown when climbing/struggling; and (3) time spent climbing/struggling – showing vigorous active movements with the forelimbs and hind limbs breaking the surface of the water in an clear attempt to get out of the cylinder. The depression-like behavior is exhibited when rats spent more time immobile during the test (Gregus et al., 2005; Lau et al., 2011b). After each session, the rats were removed from the cylinders, dried with cloth towels and returned to their home cages.