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A combinatorial modulation of synaptic plasticity in the rat medial amygdala by oxytocin, urocortin3 and estrogen

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ABSTRACT

The medial nucleus of the amygdala (MeA) plays a pivotal role in a variety of mammalian social behaviors. Specifically, activity of the hypothalamic pro-social neuropeptide oxytocin in the MeA was shown to be crucial for social recognition memory. The MeA is also a hub of neuroendocrine activity and expresses a large number of receptors of neuropeptides and hormones. These include oxytocin receptor, estrogen receptor alpha and corticotropin-releasing factor (CRF) receptor type 2 (CRFR2). In a previous study we found that intracerebroventricular (ICV) oxytocin application to anesthetized rats promotes long-term depression (LTD) of the MeA response to electrical stimulation of its main sensory input, the accessory olfactory bulb (AOB). We also reported that this type of synaptic plasticity contributes to long-term social recognition memory. Here we used similar methodology to examine the possibility that various neuromodulators pose a combinatorial effect on synaptic plasticity in the MeA. We found that ICV administration of the CRF-related peptide urocortin3 fifteen minutes before oxytocin, caused long-term potentiation (LTP), via CRFR2 activation. Similarly, ICV administration of 17β-estradiol forty-five minutes before oxytocin induced LTP, which was blocked by an antagonist of the estrogen receptors alpha and beta. Notably, none of these two neuromodulators had any effect on its own, suggesting that they both turn the oxytocin-mediated synaptic plasticity from LTD to LTP. Finally, we found that application of 17β-estradiol, forty-five minutes before urocortin3 also caused LTP in the MeA response to AOB stimulation, even without oxytocin application. We suggest that the combinatorial modulation of the bidirectional synaptic plasticity in the AOB-MeA pathway by oxytocin, 17β-estradiol and urocotin-3 serves to modify social information processing according to the animal's internal state.

1. Introduction

Neuromodulators comprise a wide variety of substances, including small molecule transmitters, biogenic amines, neuropeptides, steroid hormones and others. These molecules are released in modes other than classical fast synaptic transmission and act to modify the output of a given neural circuit by changing the properties of its neurons, their synaptic connections or the inputs to the circuit (Bargmann, 2012; Bucher and Marder, 2013). It is widely accepted that neuromodulators act in concert and that any given neural circuit is constantly under the influence of a certain combination of several neuromodulators (Nadim and Bucher, 2014). It should be noted that the combined influence of multiple neuromodulators on the output of a given neural circuit was mainly explored in invertebrates, while neuromodulatory activity in the central nervous system (CNS) of vertebrates was largely studied one neuromodulator at a time (Marder, 2012). Here we aimed to examine the combinatorial influence of several neuromodulators in the medial nucleus of the amygdala (MeA), a mammalian brain area which is thought to be a center of modulation of social information by the neuroendocrine system (Newman, 1999).

The MeA plays a pivotal role in a variety of mammalian social behaviors, including male and female sexual and aggressive behaviors (Noack et al., 2015; Unger et al., 2015), parental behavior (Sheehan et al., 2001) and social fear (Twining et al., 2017). This brain area processes sensory information elicited by the detection of chemosensory social cues in the vomeronasal system, via synaptic inputs arriving from the accessory olfactory bulb (AOB) (Bergan et al., 2014). The MeA expresses multiple receptors of neuromodulators associated with the neuroendocrine system, which are known to regulate various types of social behaviors. These receptors include the oxytocin receptor (Harony-Nicolas et al., 2014), all three estrogen receptors (ERα, ERβ, GPER) (Hazell et al., 2009; Mitra et al., 2003) and the corticotropin-

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releasing factor (CRF) receptor type 2 (CRFR2) (Lewis et al., 2001; Li et al., 2002). The ligands of these receptors are also accessible to MeA neurons, as this area receives oxytocinergic projections from the hypothalamus (Knobloch et al., 2012; Takayanagi et al., 2017) and expresses high levels of the enzyme aromatase which converts androgens to estrogens (Unger et al., 2015) as well as the CRFR2 ligand urocortin3 (Jamieson et al., 2006). Moreover, all of these molecules were reported to modify social behavior by their action in the MeA. Oxytocin activity in the MeA of male mice was found to be crucial for social recognition memory (Choleris et al., 2007; Ferguson et al., 2001) and sex discrimination (Yao et al., 2017). Estrogen activity in the MeA plays a role in social recognition (Lymer et al., 2018) and prosocial behavior (Cushing et al., 2008). Finally, a recent study clearly showed that urocortin3 activity in the MeA regulates social behavior and recognition via CRFR2 activation (Shemesh et al., 2016).

In a previous study, we used evoked field potential (EFP) recordings from anesthetized adult male rats to demonstrate that long-term social recognition memory depends on oxytocin-mediated long-term synaptic depression (LTD) in the AOB-MeA pathway (Gur et al., 2014). Here we used similar methodology to examine possible interactions between oxytocin, estrogen and urocortin3 in controlling plasticity in this synaptic pathway.

2. Materials and methods

2.1. Animals

Adult Sprague-Dawley male rats (8–10 wk, 270–340 g) were born in our facilities and housed in groups of 2–3 animals per cage (60 × 40 × 20 cm) in 12 h light/dark cycle, at 22 ± 2 °C. Food (standard chow diet, Envigo RMS, Israel) and water were available *ad libidum* under veterinary supervision in a semi-pathogen-free (SPF) facility. All experiments were performed according to the National Institutes of Health guide for the care and use of Laboratory animals, and approved by the Institutional Animal Care and Use Committee of the University of Haifa.

2.2. Electrophysiology

All electrophysiological recordings were performed as previously described (Gur et al., 2014). Briefly, the rats were tranquilized with Isoflurane and then anesthetized by injection of Ketamine (10%, 0.09 ml/100 gr) and Medetomidine (0.1%, 0.055 ml/100 gr) subcutaneously. Body temperature was kept constant at approximately 37 °C, using a closed-loop temperature controller (FHC, Bodwin, ME), connected to a rectal temperature probe and a heating-pad placed under the rat. The animals were fixed in a stereotaxic apparatus (Stoelting, Wood Dale, IL) with the head flat. A bipolar 125 µm stimulating electrode (Better Hospital Equipment Corp., Weston, FL) was inserted into the left AOB (A/P = +3.0 mm, L/M = +1.0 mm, D/V = −4.0 mm at 50°) and a recording electrode (0.010" tungsten 1 mm tip exposure, A-M Systems, Sequim, WA) was placed in the left MeA (A/P = −2.4 mm, L/M = +3.18 mm, D/V = −8.5 mm). Additionally, a cannula guide was implanted into the right lateral ventricle (A/P = −1.0 mm, L/M = −1.5 mm, D/V = −3.5 mm) for intracerebroventricular (ICV) injection of the drugs. All experiments started 30 min after the positioning of the electrodes and the cannula guide. Evoked field potential (EFP) responses were amplified (×1000) and filtered (0.1–5 kHz) by AC amplifier (A–M systems), digitized (10 kHz) and analyzed using a self-written MATLAB program. Signal amplitude was measured from the mean of five successive EFP responses to test stimuli (monopolar pulses, 100 µs duration) delivered at 0.1 Hz. Stimulus intensity (0.5–2 mA) eliciting a response that matches 40–50% of the maximal EFP amplitude was used.

Synaptic plasticity was examined by applying theta-burst stimulation (TBS) to the AOB. The TBS comprised 3 sets of 10 trains, each of 10 pulses at 100 Hz (100 µs pulse duration), with 200 ms between trains and 1 min between sets. The TBS was applied following 30 min of stable baseline recordings.

2.3. Administration of substances

2.3.1. Urocortin3

Urocortin3 (American Peptide Company, Sunnyvale, CA) was dissolved in saline (1 mg/ml) to create a stock solution of 240 µM. For ICV injections, the stock solution was diluted 1:5 in saline and 5 µl of the diluted solution (containing 1 µg urocortin3) was administered via the ventricles over 5 min.

2.3.2. Mix of antisuvagine-30 with urocortin3

The CRFR2-specific antagonist, antisauvagine-30 (American Peptide Company) was dissolved in saline (1 mg/ml) to a final concentration of 377 µM. For ICV injections, the injected mix contained 4 µl of the antagonist stock solution (4 µg), mixed with 1 µl of the urocortin3 stock solution (1 µg).

2.3.3. Oxytocin

Oxytocin (American Peptide Company) was dissolved in saline (1 mg/ml) to a concentration of 1 mM. For ICV injections, the stock solution was diluted in saline to a final concentration of 250 nM, and 4 µl (1 ng) was administered over 4 min.

2.3.4. 17β-estradiol

28 mg of 17β-estradiol (TOCRIS) was dissolved in 1 ml DMSO to create a stock solution of 99.506 µM. For ICV injections, the stock solution was diluted 1:56 in 0.9% saline and 5 µl of the diluted solution (2.5 µg) was administered over 5 min.

2.3.5. Mix of ICI 182,780 with 17β-estradiol

1 mg of ICI 182,780 (TOCRIS) was dissolved in 20 µl DMSO to create a stock solution of 50 µg/µl. For ICV injections, 2 µl from stock solution was mixed with 5 µl of 17β-estradiol prepared as described above.

2.4. Statistical analysis

SPSS 19.0 software was used throughout the current study. Parametric paired *t*-test and ANOVA were used if data were found to be normally distributed (Kolmogorov-Smirnov). Statistical analysis of differences between the various groups was done on values representing% change in EFP amplitude from baseline, averaged over the last 60 min of recording.

3. Results

3.1. Oxytocin and urocortin3

To examine the effects of various combinations of the three neuromodulators on plasticity in the AOB-MeA pathway, we recorded EFP responses, induced in the MeA of anesthetized rats by electrical stimulation of the AOB. In these experiments we first repeated the results of our previous publication (Gur et al., 2014) and demonstrated that ICV administration of oxytocin 5 min before theta-burst stimulation (TBS) of the AOB leads to LTD in the MeA response (Fig. 1A, red squares, Oxt), while saline administration did not cause any change (Fig. 1A, green triangles). The difference between the two groups was highly significant (Fig. 1B; student's *t*-test: t = −6.874, df = 10, *** – $p < 0.005$). As previously described (Gur et al., 2014), this depression affected the amplitude of the EFP peak that occurred 5 ms following AOB stimulation, with no change in peak timing (Fig. 1B), suggesting a monosynaptic response. We then tested the effect of urocortin3, ICV-administered 10 min before oxytocin infusion, on the MeA response. Surprisingly, urocortin3 administration 10 min prior to oxytocin