Psychoneuroendocrinology 92 (2018) 95–102

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

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

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ARTICLE INFO

Keywords:
Social recognition memory
Medial amygdala
Oxytocin
Urocortin3
Estrogen
Synaptic plasticity

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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https://doi.org/10.1016/j.psyneuen.2018.04.006
Received 6 March 2018; Received in revised form 7 April 2018; Accepted 9 April 2018

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

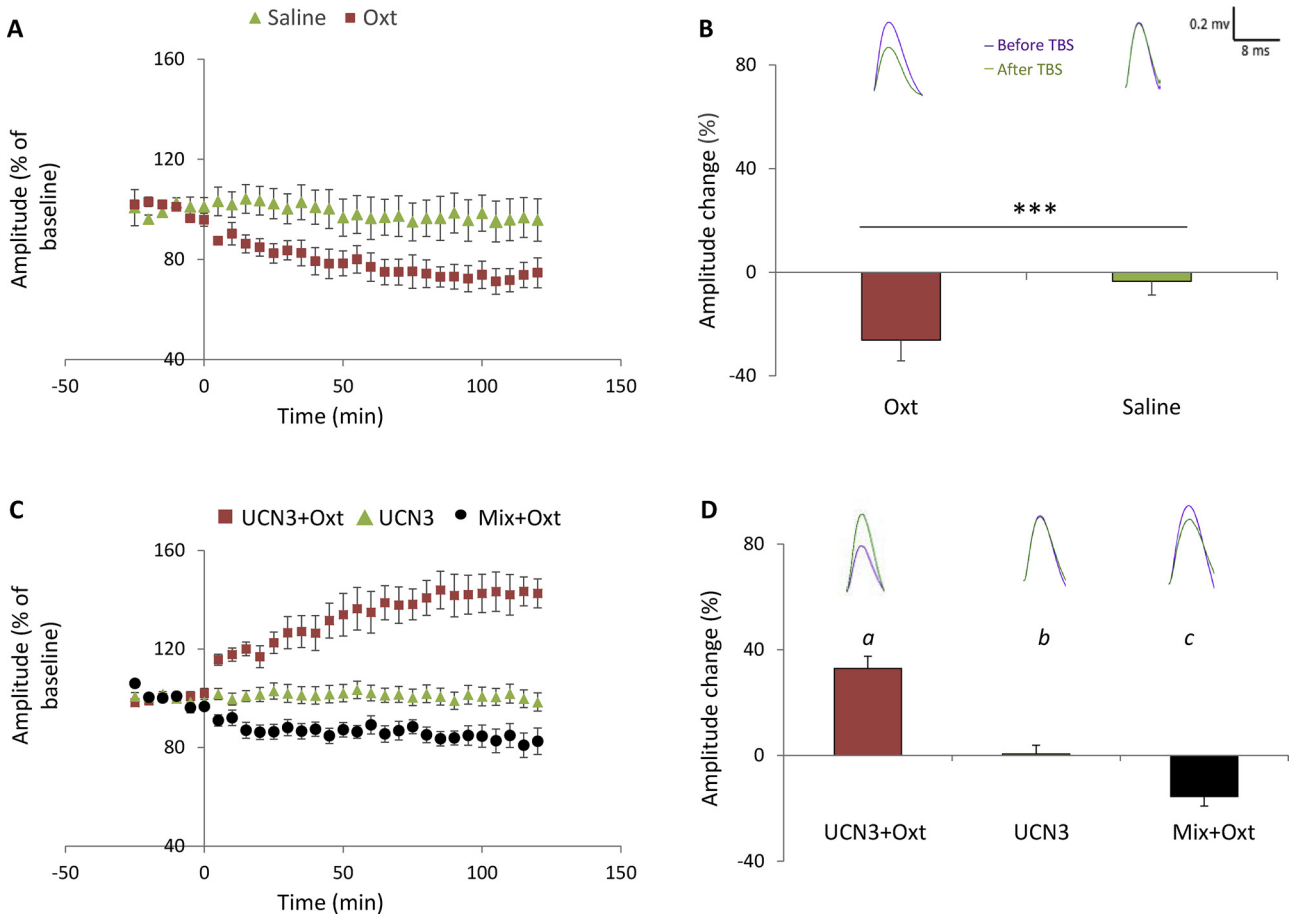

Fig. 1. Urocortin3 turns the oxytocin-dependent LTD in the MeA into LTP in a CRFR2-dependent manner.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A) Mean (± SEM) amplitude (% of baseline) of the MeA evoked field potential (EFP) response to AOB stimulation after theta-burst stimulation (TBS) given at time 0, following administration of oxytocin (Oxt; blue diamonds, n = 12 rats) or saline (green triangle, n = 6).

B) Bars represent mean (± SEM) amplitude change (%) from baseline of the EFP signals recorded in **A**, averaged over the last 60 min of recordings. Representative traces of the mean EFP response before (purple) and 120 min after (green) TBS are shown above the bars, separated from them by letters marking the statistically significant different categories. A statistically significant difference was found between the two groups (student's *t*-test: t = −9.922, df = 16, ***$p < 0.001$).

C) Mean (± SEM) amplitude (% of baseline) of the MeA response to AOB stimulation after TBS given at time 0, following administration of urocortin3 (UCN3; green triangles, n = 11), oxytocin following (10 min) urocortin3 (UCN3 + Oxt, red squares, n = 14) or oxytocin following (10 min) a mixture of urocortin3 and the CRFR2 antagonist antisauvagin-30 (Mix + Oxt; n = 12).

D) Bars represent mean (± SEM) amplitude change (%) from baseline of the EFP signals recorded in **C**. A statistically significant difference was found between all groups, except of the Oxt group and the group exposed to the Oxt and the mix of UCN3 and CRFR2 antagonist. (1-way ANOVA: F = 46.121, df = 2, $p < 0.001$; $a \neq b \neq c - p < 0.05$, Tukey's *post hoc* following main effect)

injection caused a strong long-term potentiation (LTP) of the MeA response that reached a level of ~40% increase 2 h after TBS application (Fig. 1C, red squares, UCN3 + Oxt). Notably, this effect of urocortin3 was oxytocin-dependent, as we observed no change in the response when urocortin3 was applied alone, without oxytocin (Fig. 1C, green triangles, UCN3). Thus, urocortin3 converted the oxytocin-dependent synaptic plasticity in the AOB-MeA pathway from LTD to LTP. This effect seemed mediated via CRFR2, as it was blocked when a CRFR2 antagonist (antisauvagine-30) was applied together with urocortin3. In that case, an LTD was observed again, as in the situation without urocortin3 application (Fig. 1C, black circles, Mix + Oxt). A statistical analysis of the mean effect of each of these treatments revealed a significant difference among all groups (Fig. 1D; 1-way ANOVA: F = 46.121, df = 2, $p < 0.001$; $a \neq b \neq c - p < 0.05$, Tukey's *post-hoc* following main effect), except for the oxytocin group (Oxt), and the group exposed to oxytocin and the mix of urocortin3 and the CRFR2 antagonist (Mix + Oxt). Thus, the CRFR2 antagonist blocked the effect of urocortin3 on the MeA response to oxytocin.

To examine whether the LTP of the MeA response (induced by combining urocortin3 and oxytocin applications) is TBS-dependent, we injected urocortin3 alone (Fig. 2A, green triangles, UCN3) or urocortin3 followed by oxytocin (Fig. 2A, red squares, UCN3 + Oxt) to the ventricles without TBS application. In both of these cases, we observed only a slight increase in the MeA response as compared to saline administration (Fig. 2A, orange diamonds), with no statistically significant difference (Fig. 2B; 1-way ANOVA: $p > 0.05$, F = 1.21, df = 2). We conclude that urocorin-3 modulates the effect of oxytocin on the TBS-induced plasticity in the AOB-MeA pathway, changing it from LTD to LTP.

3.2. Oxytocin and estrogen

We then turned to examine the effects of estrogen alone or in combination with oxytocin. To assess the effect of estrogen on the synaptic plasticity in the AOB-MeA pathway we injected either 17β-estradiol (Fig. 3A, red squares) or its vehicle DMSO (Fig. 3A, blue diamonds) to the ventricles 45 min before TBS application. This time-interval was chosen since 17β-estradiol was previously shown to

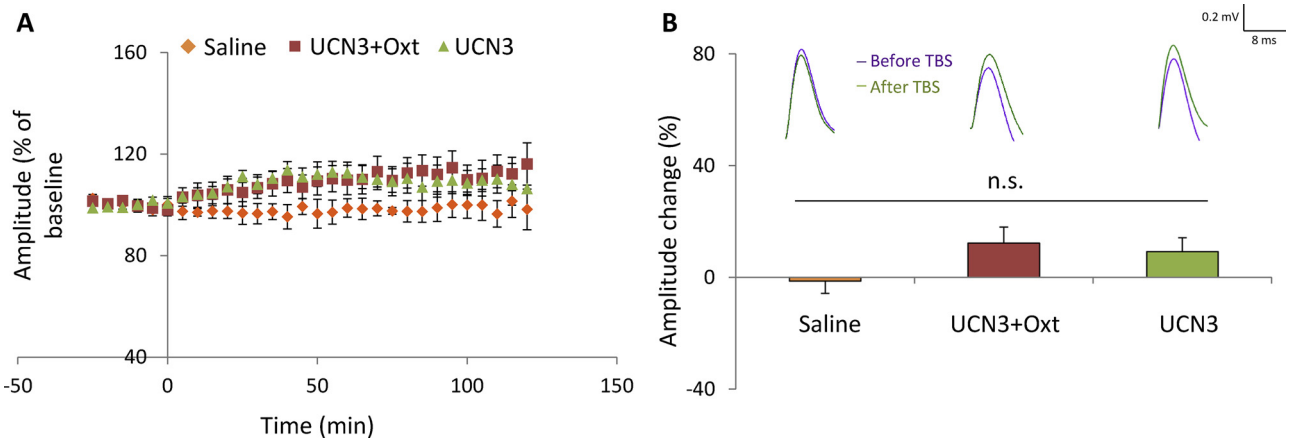

Fig. 2. The effect of Urocortin3 is TBS-dependent.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A) Mean (± SEM) amplitude (% of baseline) of the MeA response to AOB stimulation, following administration of saline (orange diamonds, n = 4) urocortin3 (UCN3, blue triangles, n = 7) or oxytocin followed by urocortin3 (UCN3 + Oxt, black squares, n = 9), without TBS application.

B) Bars represent mean (± SEM) amplitude change (%) from baseline of the EFP signals recorded in **A**, averaged over the last 60 min of recordings. Representative traces of the mean EFP response at the start (purple) and end (green) of recording are shown above the bars.

enhance social memory in awake mice 45–50 min after its application (Ervin et al., 2015b). There was no significant difference between the two treatments (Fig. 3B; student's *t*-test: t = 0.571, df = 15, $p > 0.05$), both of which yielded a small tendency towards LTD, similarly to TBS application alone, as we previously reported (Gur et al., 2014). Thus, in the range of time we examined (45 min before TBS), 17β-estradiol alone does not seem to affect the MeA synaptic plasticity. We then examined the effect of 17β-estradiol or DMSO, when administered 45 min before the oxytocin injection, followed 5 min later by TBS. In contrast to oxytocin administration following DMSO injection, which caused strong LTD (Fig. 3C, red squares, DMSO + Oxt), the combination of oxytocin and 17β-estradiol caused a clear and stable LTP (Fig. 3C blue diamonds, 17β-estradiol + Oxt). To make sure that this effect is due to activation of the classical estrogen receptors ERα or ERβ, we explored the effect of a mix of 17β-estradiol and ICI 182,780, a potent blocker of both ERα and ERβ, and found that application of this mix, followed by oxytocin administration and TBS, did not cause any change in the MeA response (Fig. 3C, green triangles, Mix + Oxt). It should be noted, however, that in this case we did not observe an LTD anymore, suggesting that 17β-estradiol may block the oxytocin-induced LTD by a different pathway, which does not depend on ERα or ERβ. A possible role of the G-protein coupled estrogen receptor GPER in this effect (Lymer et al., 2018) should be clarified in future studies. Overall, the differences among these three treatments were highly significant (Fig. 3D; 1-way ANOVA: F = 21.439, df = 2, $p < 0.01$; $a \neq b \neq c$ – $p < 0.05$ Tukey's *post hoc* following main effect). Thus, we conclude that, similarly to urocortin3, 17β-estradiol modulates the oxytocin-induced synaptic plasticity in the MeA by turning it from LTD to LTP, via a non-genomic activity of either ERα or ERβ.

3.3. Urocortin3 and estrogen

Finally, we examined the effect of a combination of 17β-estradiol and urocortin3, without oxytocin, on the synaptic plasticity in the MeA. We found that this combination also caused an LTP (Fig. 4A) which was significantly different from the effect of each one of these neuromodulators alone (Fig. 4B; 1-way ANOVA: F = 20.237, df = 2, $p < 0.001$; ** – $p < 0.01$,Tukey's *post hoc* following main effect). Thus, while oxytocin alone caused LTD in the AOB-MeA pathway and 17β-estradiol and urocortin3 each did not cause long-term plasticity at all, any combination of two of these three neuromodulators induced LTP in the same synaptic pathway (Fig. 5A). Of these combinations, the one of oxytocin and urocortin3 induced the strongest effect (Fig. 5B; 1-way ANOVA: F = 4.399, df = 2, $p < 0.05$; $a \neq b$ – $p < 0.05$, Tukey's post hoc following main effect).

4. Discussion

4.1. Neuromodulation in the MeA

The MeA plays a pivotal role in many types of social behavior (Li et al., 2017; Newman, 1999; Sheehan et al., 2001; Trezza and Campolongo, 2009; Unger et al., 2015), particularly in social recognition memory (Ferguson et al., 2001; Gur et al., 2014; Lukas et al., 2013; Noack et al., 2015). Moreover, the MeA is a target of many neuromodulators, especially those associated with the neuroendocrine system, as it expresses specific receptors of these ligands and reacts to them. These receptors include (among many others) the melanocortin-4 receptor (Liu et al., 2013), the kisspeptin receptor (Gresham et al., 2016), neuropeptide-S receptor (Grund and Neumann, 2018) and androgen receptor (Blake and Meredith, 2011). Thus, the MeA may be an ideal brain area for exploring the cooperative effects, whether occluding, additive or synergistic, of several neuromodulators on social information processing. Here we concentrated on the combinatorial effects of three neuromodulators that were already shown to affect social recognition by their activity in the MeA: oxytocin, estrogen and urocortin3.

4.2. Urocortin3 in the MeA

Urocortin3 expression in the rat brain is highly selective: cell bodies expressing the neuropeptide were found predominantly in several hypothalamic areas and in the MeA (Li et al., 2002) while immunopositive fibers were observed largely in areas associated with social behavior such as the MeA, lateral septum, bed nucleus of stria terminalis, and ventromedial hypothalamus (Li et al., 2002). Interestingly, these areas are also rich in the CRFR2 receptor, which shows specifically higher affinity to urocorin-3 than to other urocortins (Lewis et al., 2001) as well as much more selective expression in the rat brain, as compared to the widely expressed CRFR1 receptor (Lewis et al., 2001; Li et al., 2002). Thus, urocortin3 and CRFR2 seem to create a ligand-receptor system that appears to be specifically active in social behavior-associated brain regions. Notably, urocortin3 and CRFR2 knockout mice demonstrated specific enhancement of the length of social recognition memory in the social discrimination test (Deussing et al., 2010). In contrast, a recent work by Shemesh et al. (2016) elegantly showed,

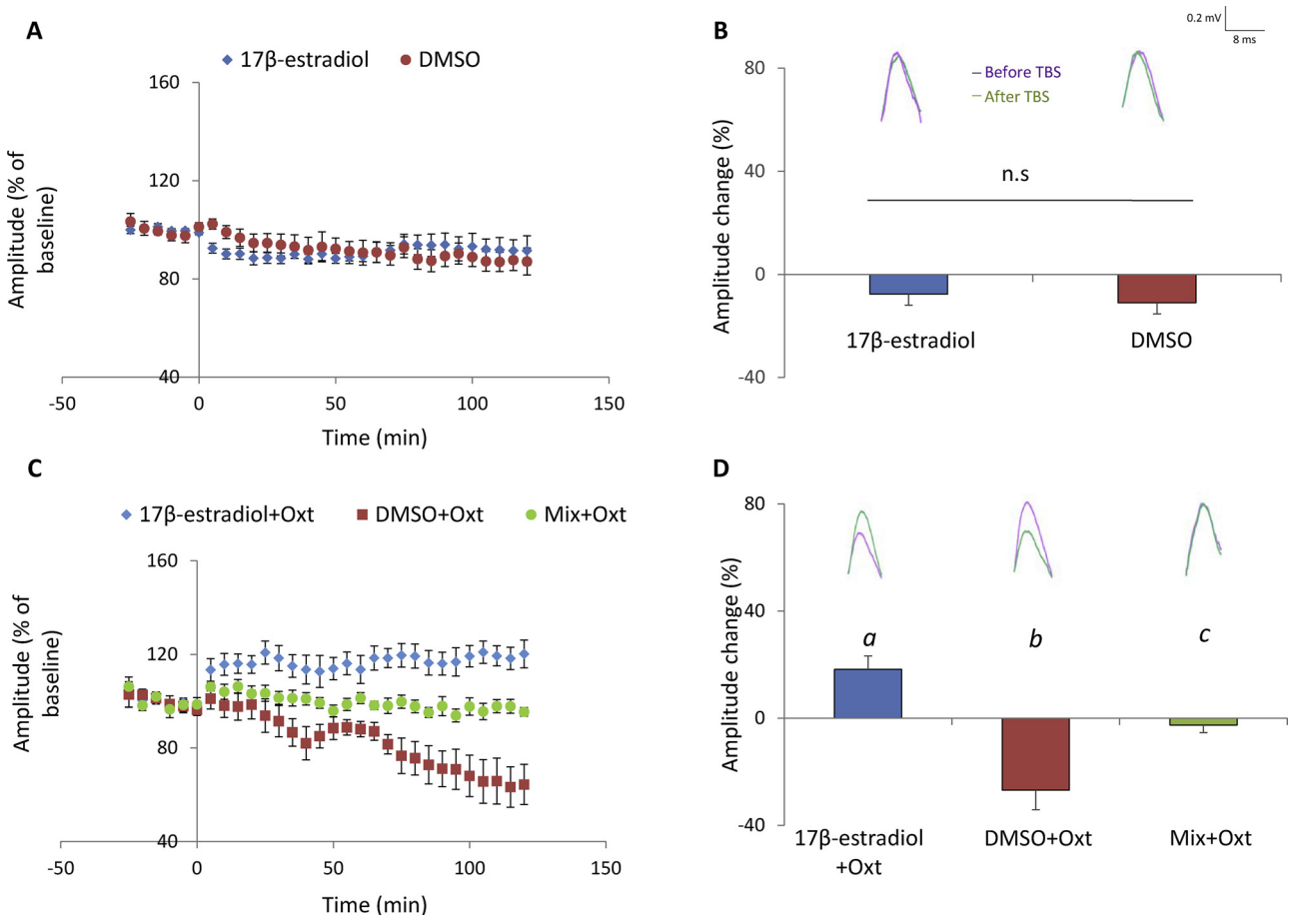

Fig. 3. No effect of 17β-estradiol alone on synaptic plasticity in the AOB-MeA pathway.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A) Figure 3: Mean amplitude (% of baseline) of the MeA response to AOB stimulation following administration of 17β-estradiol (blue diamonds, n = 7) or DMSO (red circles, n = 10), 45 min before TBS application.

B) Bars represent mean (± SEM) amplitude change (%) from baseline of the EFP signals recorded in **A**, averaged over the last 60 min of recordings. Representative traces of the mean EFP response before (purple) and 120 min after (green) TBS are shown above the bars.

C) Mean amplitude (% of baseline) of the MeA response to AOB stimulation after administration of either DMSO (red squares, n = 8), 17β-estradiol (blue diamonds, n = 9), or a mix of 17β-estradiol and the ERα/β blocker ICI 182,780 (green triangles, n = 8), 45 min before Oxt application.

D) Amplitude (± SEM) change (%) from baseline of the EFP signals recorded in **A**, averaged over the last 60 min of recordings. Representative traces of the mean EFP response before (purple) and 120 min after (green) TBS are shown above the bars. (1-way ANOVA: $F = 21.439$, $df = 2$, $p < 0.01$; $a \neq b \neq c$ – $p < 0.05$, Tukey's *post hoc* following main effect).

using a novel paradigm of discrimination between a nest-mate sibling and a novel conspecific in a T-maze system, that the activity of both urocortin3 and CRFR2 in the MeA is crucial for mice to show a preference towards the novel social stimulus; a preference which reflects social memory. The differences in the results of these two studies may reflect differences in their paradigms and the measured parameter (duration of memory vs. social novelty preference). Thus, the role of urocortin3 and CRFR2 in the MeA on social memory seems to be complex.

4.3. Estrogen in the MeA

The MeA expresses high levels of the nuclear estrogen receptors ERα and ERβ in both males and females (Mitra et al., 2003; Stanic et al., 2014). It also expresses moderate levels of GPER (Hazell et al., 2009) as well as the enzyme aromatase which converts testosterone to estradiol (Unger et al., 2015). Multiple studies in female mice showed that estrogen receptors are crucial for social recognition memory (reviewed by Choleris et al., 2012; Choleris et al., 2006; Gabor et al., 2012), at least partially by their activity in the MeA (Lymer et al., 2018). Moreover, these effects on social memory occurred already 40 min following 17β-estradiol administration (Lymer et al., 2018; Phan et al., 2011), suggesting the involvement by rapid, non-genomic actions of ERα and ERβ (reviewed by Ervin et al., 2015a), as well as by GPER activity (Ervin et al., 2015b; Lymer et al., 2018). We therefore used a similar time course in our experiments, injecting 17β-estradiol 40–50 min before TBS.

4.4. Oxytocin in the MeA

Oxytocin role in modulating social behavior in general (reviewed by Caldwell, 2017; Donaldson and Young, 2008) and social memory in particular (reviewed by Maroun and Wagner, 2016) is very well established. Moreover, Oxytocin activity in the MeA was specifically found to be crucial for social recognition memory (Choleris et al., 2007; Ferguson et al., 2001) and sex discrimination (Yao et al., 2017). We previously demonstrated in male rats that long-term social recognition memory relies on oxytocin-mediated LTD of MeA responses to AOB stimulation, in oxytocin-receptor dependent manner (Gur et al., 2014). Here we showed for the first time, that both urocorin-3 and 17β-estradiol convert the oxytocin-mediated LTD into oxytocin-mediated LTP. These results indicate a novel bidirectional synaptic plasticity in the

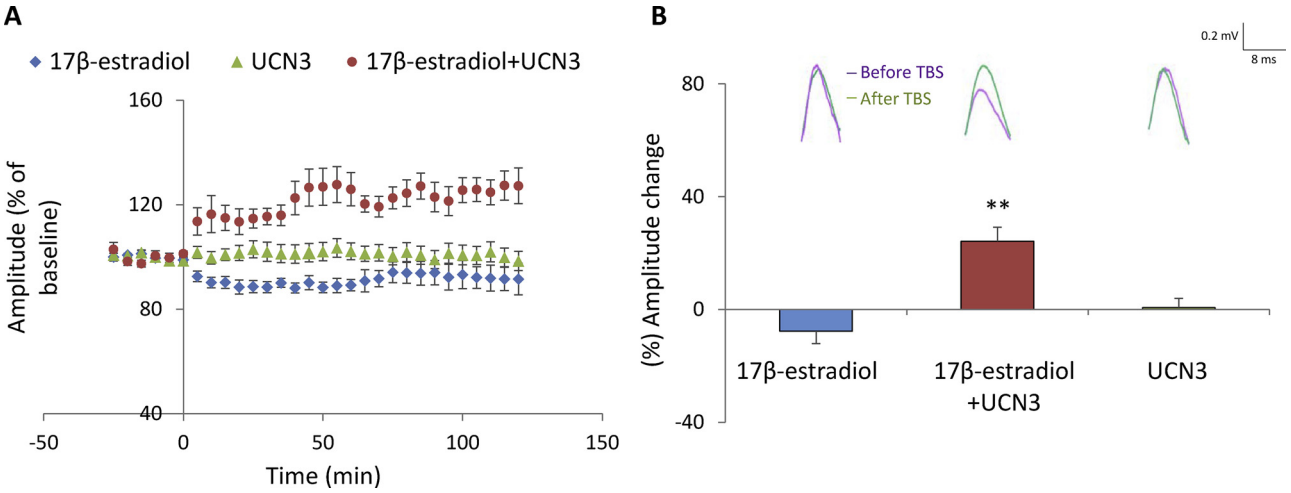

Fig. 4. A combination of urocortin3 and 17β-estradiol, but not each of them alone, induces LTP in the MeA.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A) Mean amplitude (% of baseline) of the MeA response to AOB stimulation after administration of 17β-estradiol followed by urocortin3, (red circles, n = 9, 50 and 5 min before TBS administration, respectively), 17β-estradiol alone (blue diamonds, n = 7, 45 min before TBS) or urocortin3 alone (green triangels, n = 10, 5 min before TBS).

B) Amplitude (± SEM) change (%) from baseline of the EFP signals recorded in **A**, averaged over the last 60 min of recordings. Representative traces of the mean EFP response before (purple) and 120 min after (green) TBS are shown above the bars. A statistically significant difference was observed between the combination of both neuromodulators and the application of each one of them alone (1-way ANOVA: F = 20.237, df = 2, $p < 0.001$; ** –$p < 0.01$, Tukey's *post hoc* following main effect).

AOB-MeA pathway, the direction of which is regulated (whether LTD, LTP or no change) by oxytocin, 17β-estradiol and urocorin-3 in a combinatorial manner.

4.5. Bidirectional synaptic plasticity in the MeA

Long-term synaptic plasticity in the form of either LTP, which enhances synaptic efficacy, or LTD, which attenuates it, is the main known physiological mechanism underlying long-term memory (Nabavi et al., 2014). Bidirectional (LTD/LTP) plasticity in the same synapse was demonstrated in several well- studied synaptic systems of the CNS, including hippocampal CA3 pyramidal neurons (reviewed by Luscher and Malenka, 2012) and cerebellar Purkinje cells (reviewed by Jorntell and Hansel, 2006). In these cases, the transition between LTD and LTP was mainly attributed to different levels of calcium entry, which are dictated by distinct stimulation protocols (Clark and Normann, 2008; Ohtsuki et al., 2009). Recently, bidirectional modulation of synaptic plasticity was shown in several systems to be influenced by various neuromodulators, including BDNF (Montalbano et al., 2013), CRF (Schierloh et al., 2007) and adrenaline (Huang et al., 2013). Moreover, Huang et al. (2012) have demonstrated that in the visual cortex such a bidirectional neuromodulation of synaptic plasticity depends on the type of activated G-protein, with GPCRs coupled to G_s promoting LTP while those coupled to G_q promote LTD. Interestingly, the oxytocin receptor is known for coupling to a large repertoire of G proteins, including G_q, G_i and G_o, as a function of oxytocin concentration or the

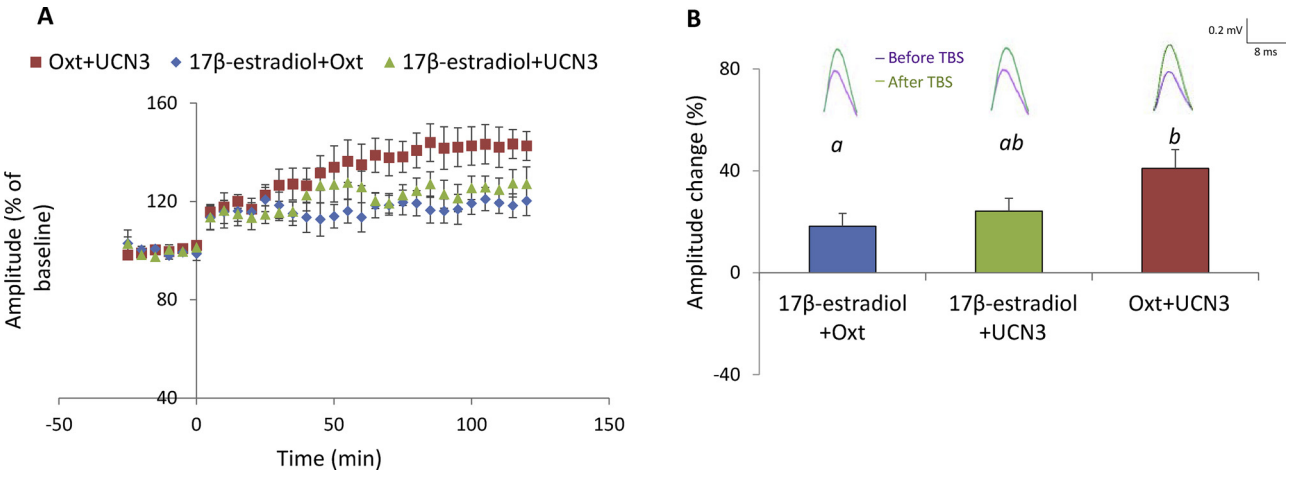

Fig. 5. Any combination of two out of the three neuromodulators induced LTP in the MeA.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A) Mean amplitude (% of baseline) of the MeA response to AOB stimulation after administrating 17β-estradiol with oxytocin (blue diamonds, n = 9), 17β-estradiol with urocortin3 (green triangles, n = 9), or oxytocin with urocortin3 (red squares, n = 14).

B) Amplitude (± SEM) change (%) from baseline of the EFP signals recorded in **A**, averaged over the last 60 min of recordings. Representative traces of the mean EFP response before (purple) and 120 min after (green) TBS are shown above the bars. No significant difference observed between the groups, apart from 17β-estradiol with oxytocin as compared to urocortin3 with axytocin (1-way ANOVA: F = 4.399, df = 2, $p < 0.05$; $a \neq b$ – $p < 0.05$; Tukey's *post hoc* following main effect).

binding of various agonists (Busnelli and Chini, 2017; Busnelli et al., 2012). Thus, one possible explanation for the ability of urocortin3 or 17β-estradiol to convert the effect of oxytocin on the MeA plasticity from LTD to LTP without having any effect by themselves is by shifting, via intracellular pathways, the oxytocin receptor coupling from G_q to other types of G-protein. The combination of the two modulators may even be efficient enough in order to enable the low level of endogenous oxytocin in the MeA to produce LTP.

4.6. The behavioral role of the bidirectional synaptic plasticity in the MeA

In a previous study, we demonstrated the dependence of long-term social recognition memory in adult male rats on oxytocin-induced LTD in the MeA (Gur et al., 2014). We hypothesized that the depression of synaptic inputs arriving to the MeA from the AOB is needed for decreasing an arousal state of social alertness (Tendler and Wagner, 2015), which drives the social investigation behavior of adult male rats. By inducing such synaptic depression, oxytocin allows the relaxation of the social alertness, and reduction in the social investigation behavior during the second encounter with the same social stimulus, which reflects social recognition memory. According to this hypothesis, the results presented here suggest that both urocortin3 and estrogen will impair long-term social recognition memory in adult male rats, by preventing oxytocin from inducing LTD in the AOB-MeA pathway. As regards to urocortin3, this possibility is supported by the fact that, while oxytocin is known for its anxiolytic influence, UCN3 and CRFR2 are linked to the CRF system, mostly known for its involvement in stress responses. Accordingly, UCN3 expression level in the MeA was shown to increase following restraint stress (Jamieson et al., 2006), while social defeat was found to activate CRFR2-expressing cells within the MeA (Fekete et al., 2009). Moreover, CRFR2 activation in the MeA of male rats was found to have clear anxiogenic effects (Alves et al., 2016). Accordingly, *UCN3*-KO and *CRFR2*-KO male mice displayed specific enhancement of social recognition memory (Deussing et al., 2010), and in our hands urocortin3 application to adult male rats impair their social memory in a CRFR2-dependent manner (RG and SW, unpublished data). Altogether, these results support the hypothesis that urocortin3 reverses the enhancing effect, of oxytocin in the MeA, on social recognition memory by preventing it from inducing LTD in the AOB-MeA pathway.

As for estrogen, the picture is more complicated. In their seminal study (Choleris et al., 2003), Choleris et al. demonstrated that female mice that lack each of the *ERα*, *ERβ* and *OTR* (oxytocin receptor) genes show impaired social recognition memory. They further suggested that a four-gene micronet comprising the aforementioned three genes and the *Oxytocin* gene regulates social recognition memory in estrogen-dependent manner. Although these results may involve organizational effects of estrogen on the neuronal network mediating social recognition memory, they were replicated in adult female rats, where *ERα* expression was downregulated using viral-mediated local delivery of shRNA to the MeA (Agmo et al., 2008). Moreover, multiple studies showed that 17β-estradiol application to female mice enhances their social recognition memory within tens of minutes (Ervin et al., 2015b; Lymer et al., 2018; Phan et al., 2011), with both the dorsal hippocampus and MeA implicated in these experiments. It should be noted, however, that all these experiments demonstrated the role of estrogen in social recognition memory of females, while no similar experiments were reported in males. In contrast, here we used male rats for our experiments, following our previous study showing oxytocin-induced LTD in the MeA of adult male rats. Since in males, estrogens in the brain are derived from local conversion of androgens by the intracellular enzyme aromatase, activation of estrogen receptors in the MeA is expected only in aromatase-expressing cells, which are more numerous in males than in females (Stanic et al., 2014; Wu et al., 2009). Therefore, it is likely that the neuronal populations in the MeA that are influenced by estrogen, differ between males and females. Moreover, viral-mediated abolishment of aromatase expressing cells in the MeA achieved markedly different behavioral consequences in male and female mice (Unger et al., 2015). A future direct comparison of the behavioral role of estrogen in the MeA between males and females should clarify this issue.

Finally, as each combination of the different neuromodulators studied here may reflect a distinct internal state of the animal, it is tempting to hypothesize that such states are characterized by different ways of social information processing, which are dictated by the modulation of synaptic activity in the MeA via the presence of various neuromodulators.

Authors contributions

LF contributed to all experiments with estrogen and some experiments with urocortin3. RG contributed to most urocortin3 and oxytocin experiments. Both analyzed their results and contributed to research design and manuscript writing. SW designed the research, assisted the data analysis and wrote the manuscript.

Conflict of interest

The authors have no conflict of interest with this manuscript.

Acknowledgments

We thank Dr. S. Netser for excellent technical support.

This research was supported by The Human Frontier Science Program (HFSP grant RGP0019/2015), the Israel Science Foundation (ISF grant #1350/12) and by the Ministry of Science, Technology and Space of Israel (Grant #3-12068).

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