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The oxytocin system mediates behavioral and neurobiological alterations associated with early adversity

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Early life adversities (ELA) can significantly impact brain development and adult behavior, potentially increasing vulnerability to psychopathologies. Evidence shows that ELA exposure is significantly associated with dysfunctional Oxytocin (OXT), a neuropeptide strongly engaged in social behavior and linked to the processing of rewarding stimuli, such as drugs of abuse. Moreover, it has been recently demonstrated that peripheral OXT may be transported to the brain through several mechanisms, including Receptors for Advanced Glycation End-Products (RAGE), and the RAGE-mediated OXT transport has been shown to play a key critical role in mediating some aspects of social behavior, such as social bonding. However, how OXT system alterations induced by ELA could increase vulnerability to psychopathologies is still under investigation. To investigate this link, we exploit our model of early adversity (Repeated Cross-Fostering, RCF), known to increase the sensitivity to cocaine effects in adult C57BL/6 J (C57) female mice acting on the dopaminergic mesocorticolimbic system. Here, we show that in C57 females, RCF manipulation also impairs social recognition and impacts the OXT system by altering i) OXT levels in the brain and plasma; ii) the expression of RAGE; and iii) the expression of OXT receptor (OxtR). Notably, early restoring brain and plasmatic OXT levels via subcutaneous OXT injection during RCF manipulation counteracts the RCF-induced neurobiological alterations of the OXT system and prevents short and long-lasting behavioral alterations. These findings shed light on the mechanisms by which the oxytocinergic system mediates the long-term effects of early-life adversities on drug addiction vulnerability and social behavior.

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INTRODUCTION

The experience of adversities in early life disrupts normal brain development, possibly inducing detrimental effects on individuals as increasing vulnerability to psychopathology across the lifespan. Clinical and preclinical evidence shows that the experience of early life adversity (ELA) is associated with altered social behavior, increased vulnerability to drug abuse, and impaired emotionality. Specifically, several studies have found a connection between retrospectively documented childhood stress and drug dependence in later life [1–6]. Interestingly, childhood trauma has been reported to increase the likelihood of cocaine relapse and drug use escalation, particularly in women [7], thus suggesting that women might be more vulnerable to ELA effects on cocaine sensitivity [7–9]. According to clinical reports, animal models show a strong association between ELA and vulnerability to addiction-like behaviors in adulthood [10–17], with females showing enhanced susceptibility compared to males, especially to cocaine effects [18–20]. Furthermore, ELA exposure has also been reported to compromise adequate social behavior, impairing social motivation and social recognition [21–24]. In this scenario, early life events involving adverse social experiences with the primary caregivers may alter the brain capacity to correctly process social and rewarding stimuli.

Despite extensive descriptions of the behavioral consequences of ELA (e.g. social impairments and increased substance abuse risk), the underlying neurobiological mechanisms remain largely unclear. The neuropeptide oxytocin (OXT) is a key regulator of all aspects of social behavior, including social bonds [25–28], and clinical and preclinical studies have shown the negative impact of ELA on the OXT system [29]. Moreover, numerous clinical studies reported endogenous OXT levels positively associated with secure attachment and inversely related to insecure attachment in adults [30–32] suggesting the involvement of OXT in attachment bond formation in humans. Interestingly, a dysregulated OXT system has been linked not only to social deficits [33–35] but also to greater susceptibility to developing drug addiction [36, 37]. Accordingly, exogenous OXT in humans has been reported to induce positive

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behavioral effects in some social deficit-related psychiatric disorders, such as autism and schizophrenia [38–41], and to mitigate the adverse effects of drugs of abuse, including cocaine [37, 42–45].

OXT, produced by oxytocinergic neurons in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus, is released both in the brain and periphery [25]. It has been recently demonstrated that peripheral OXT is able to induce central effects acting through multiple, interacting mechanisms, including the receptor for advanced glycation end-products (RAGE) [46, 47]. Notably, the RAGE-mediated OXT transport has been shown to be critical for its behavioral effects related to social bonding [46]. OXT neurons in the PVN project widely to the brain, also sending projections to the main areas of the reward circuit, such as Nucleus Accumbens (NAc), Ventral Tegmental Area (VTA) [48–50] and preFrontal Cortex (pFC) [51], thus indicating OXT as a possible mediator linking ELA effects with response to reinforcing stimuli (e.g., drugs of abuse and social stimuli). However, it is noteworthy that only a few studies have directly demonstrated the relationship between ELA, OXT, and susceptibility to drugs of abuse [37, 52], whilst no studies have examined, to date, if and how altered OXT function within the mesocorticolimbic system induced by ELA can affect response to cocaine and sociability in adulthood. Similarly, to our knowledge, the involvement of the RAGE in ELA-induced alterations of the OXT system and behavioral consequences has never been investigated.

We exploited our model of ELA, the Repeated Cross Fostering (RCF) procedure, to specifically investigate this point. We have previously demonstrated that RCF i) alters the attachment bond between dam and pups [20, 53–55], ii) increases behavioral response to cocaine in adult female C57BL/6 J (C57) mice; iii) affects dopaminergic (DA) mesocorticolimbic release induced by cocaine and stress exposure [56, 57], and iv) alters the cellular neurophysiology of the VTA inducing a long-lasting reduction of the *Ih* current in DA neurons [58].

Here, we extend our findings and disclose the mediating role of OXT in these effects. First, we explore the effect of RCF on the brain and peripheral OXT system. Next, we evaluate the effect of an early OXT treatment on RCF-induced behavioral and neurobiological alterations.

MATERIALS AND METHODS

For detailed methods, see the Supplementary Information (SI).

Animals

C57BL/6 J mice were used for all experiments. All procedures were carried out in accordance with Italian national laws (DL 116/92 and DL 26/2014) regulating the use of animals for scientific purposes, and in compliance with European Communities Council Directives (86/609/EEC and 2010/63/EU). The experimental protocols (numbers 769/2017 and 901/2023) were approved by the Italian Ministry of Health. Adequate measures were taken to minimize animal pain and discomfort.

Repeated Cross-Fostering (RCF) Procedure

RCF was performed as previously described [53–58]. On postnatal day 0 (PND0), C57 pups were housed with their biological mothers. From PND1 to PND4, RCF pups were daily fostered by transferring the entire litter to a different adoptive mother. After PND4, pups remained with the last adoptive mother until weaning.

Early OXT Pharmacological Treatment

During the RCF manipulation (PND1–PND4), RCF pups received subcutaneous injections (20 µL) of either isotonic saline (RCF-Sal) or oxytocin (50 ng dissolved in 20 µL isotonic saline) (RCF-Oxt).

Ultrasonic Vocalization Calls (USVs)

Separation-induced ultrasonic vocalizations (USVs), an indicator of separation anxiety, were measured on PND8. Pups were individually exposed to either home-cage bedding material or clean bedding, and USVs were recorded, as previously described [20, 55].

Adult behavioral tests

Conditioned Place Preference (CPP) experiments were conducted using a place-conditioning apparatus to assess cocaine (2.5 mg/kg)-induced preference, following previously established protocols [57]. For experiments involving *Ih* current inhibition, animals received bilateral intra-VTA injections of the *Ih* channel blocker ZD7288 (ZD) or vehicle and were tested 24 hours post-surgery.

The Three-Chamber Test (TCT) was performed to assess social preference and social recognition. During the first session (10 min; social preference phase), an age- and sex-matched mouse was placed into one cylinder (social stimulus), and a neutral object into the other [20]. In the second session (10 min; social recognition phase), the object was replaced with a novel mouse [59]. Scoring was conducted following established protocols [20, 59]. Open Field Test (OF) and Elevated Plus Maze (EPM) were performed as previously reported [20].

Brain and plasma collection, and Microdissection of brain punches are described in Supplementary Methods.

Enzyme-linked immunosorbent assay (ELISA)

Total protein was extracted from plasma and brain samples using C18 Spin Columns and quantified via ELISA (Enzo Lifesciences kit) according to the manufacturer's instructions. Samples were stored at –20 °C prior to analysis.

Western blot

NAc tissues were homogenized in ice-cold RIPA buffer containing protease and phosphatase inhibitors. After centrifugation, protein concentration was determined using Bradford's assay. Proteins were analyzed by SDS-PAGE and Western blotting using antibodies against oxytocin-neurophysin 1 and beta-tubulin 2, followed by horseradish peroxidase-conjugated secondary antibodies.

Quantitative real-time PCR (qRT-PCR)

OXT receptor (OxtR) mRNA levels were assessed in brain hemispheres from PND5 pups and brain punches from the pFC, NAc, PVN, and VTA from adult mice; vasopressin receptor 1a (V1aR) receptor was also assessed in NAc punches. RNA was extracted using the Total RNA Purification Plus Kit, and cDNA was synthesized using the High-Capacity Reverse Transcription Kit. qRT-PCR was performed using TaqMan assays, with all samples run in triplicate.

Multiplex RNAscope

Coronal brain sections (20 µm) were prepared and processed for RNAscope Multiplex Fluorescent in situ Hybridization using TSAVivid™ fluorophores [60]. Probes targeting OxtR (Mm-Oxtr-C3), D1R (Mm-Drd1-C2), and D2R (Mm-Drd2-C1) were used. Quantification of OxtR-mRNA expression in D1R + and D2R+ neurons of the dorso-medial and dorso-lateral NAc shell was performed.

Immunohistochemistry

Animals were perfused transcardially, and brains were sectioned into 30 µm slices. Sections were incubated with anti-oxytocin-neurophysin 1 antibody and visualized using the avidin-biotin-peroxidase method. OXT-positive neurons in the PVN were counted under a light microscope.

Immunophenotyping and RAGE Expression by High-Dimensional Flow Cytometry

The pFC was isolated post-perfusion and dissociated into single-cell suspensions using a brain dissociation kit and GentleMACS dissociator. High-dimensional flow cytometry was used to assess resident and infiltrating cell types and RAGE expression [61].

Electrophysiology

Horizontal midbrain slices (250 µm thick) containing the VTA were prepared from adult females. After transcardial perfusion with NMDG-based slicing solution, slices were incubated in oxygenated aCSF and allowed to recover. Whole-cell patch-clamp recordings were conducted on

visually identified VTA dopaminergic neurons using K-gluconate-based intracellular solution [58, 62].

Statistics

Statistical analyses were performed using Super ANOVA Statistical Software or Prism 9 (GraphPad). Data normality was assessed using the Shapiro-Wilk or D'Agostino & Pearson omnibus normality tests. Depending on data distribution, Student's t-test (with Welch's correction when needed), one-way or two-way ANOVA, repeated measures ANOVA, Mann–Whitney U test, Friedman test, or Kruskal-Wallis with Dunn's post hoc test were used. Significance was set at $p < 0.05$.

RESULTS

RCF increases separation anxiety in pups and induces opposite OXT alterations in the brain and plasma

First, we evaluated attachment behavior in pups by measuring USVs following separation (Supplementary Fig. 1A). Consistent with previous reports [20, 55], RCF manipulation induced separation anxiety, with RCF pups emitting a similar number of USVs in home-cage versus clean bedding conditions ($t = 0.354$; $p = 0.7306$; Supplementary Fig. 1B). Importantly, no significant differences were observed in maternal behavior or pup retrieval between control (Cont) and RCF dams (Supplementary Table 1; Supplementary Fig. 1C–I). Next, we investigated whether RCF affected central and peripheral OXT levels (Fig. 1A). RCF induced opposite effects: OXT levels were reduced in the brain but increased in plasma compared to Cont pups (Brain OXT: $t = 2.513$; $p = 0.036$; Plasma OXT: $t = 3.394$; $p = 0.0079$; Fig. 1B,C). No significant differences in brain OxtR mRNA expression were detected in pups (Fig. 1D).

To assess whether these alterations persisted into adulthood (PND90), we measured OXT levels in plasma and selected brain regions of the mesocorticolimbic system (NAc, pFC, VTA, and PVN). We also assessed the number of OXT-positive neurons in the PVN. In adult females, elevated plasma OXT levels persisted ($t = 2.186$; $p = 0.0493$; Fig. 1E), and brain OXT levels were selectively reduced in the NAc ($t = 3.006$; $p = 0.022$; Fig. 1F) with no effect in the other brain regions (Fig.1G-I). Western blot analysis confirmed reduced OXT levels in the NAc ($t = 5.71$; $p = 0.0002$; Fig. 1J). To start investigating the OXT system's functionality in RCF mice, we performed a preliminary experiment by measuring cocaine-induced (i.p.; 2.5 mg/kg) OXT release in the NAc using in vivo intracerebral microdialysis coupled with ELISA. Basal OXT levels did not differ between RCF and Cont mice ($t = 0.8276$; $P = 0.4293$); however, cocaine induced a significant OXT increase only in Cont mice (percentage change relative to respective baseline levels ($t = 2.445$; $p > 0.040$); Supplementary Fig. 2B). No significant difference was observed in the number of OXT-positive neurons in the PVN (Fig. 1K).

Altogether, these findings indicate that RCF induces opposite alterations in brain and plasma OXT levels early in life, with persistent effects specifically in the NAc into adulthood.

RCF alters RAGE expression in specific cell types of the pFC

Given the mismatch between unchanged OXT cell numbers and altered OXT levels, we explored whether RCF influenced RAGE expression, a key mediator of peripheral-to-central OXT transport through the BBB [47], in the pFC, as this is one of the best-vascularized areas in the mouse brain [63, 64] and shows bidirectional connections with the NAc [65](Fig. 2A). First, we assessed the expression of RAGE in distinct cellular subtypes of the pFC from Cont mice by means of high- dimensional flow cytometry (see Supplementary Methods for details; Fig. 2B,C). One-way ANOVA showed significantly different expression of RAGE between cell subtypes ($F(7, 40) = 26.88$; $p < 0.0001$) in the Cont group, with non-myeloid leukocytes ($p < 0.0001$) and endothelial cells showing the most robust expression ($p < 0.05$) (Fig. 2C). We then compared RAGE expression in these cell types in RCF *vs*. Cont mice (Supplementary Fig. 3A-E) and found a significant reduction of RAGE in astrocytes ($t = 2.74$; $p = 0.02$; Fig. 2D), non-myeloid leukocytes ($t = 2,23$; $p = 0.049$; Fig. 2E), oligodendrocytes ($t = 2.74$; $p = 0.02$; Fig. 2F).

RCF affects OXT receptor expression in the NAc shell

Given the reduced OXT levels and cocaine-induced release observed in the NAc of adult RCF mice, we investigated whether RCF altered OxtR expression in this brain region (Fig. 3A). The NAc is at the interface between the social and reward networks, integrating multiple signals to regulate social bonding and reward-related behaviors and, interestingly, altered NAc OxtR expression is linked to changes in sociability and drug cue response [66–73].qRT-PCR on NAc punches showed increased OxtR expression in RCF mice ($U = 3$; $p = 0.0082$; Fig. 3B), with no significant differences in the other brain area (Supplementary Fig. 3F-H). Moreover, given the structural and functional similarity between OxtR and V1aR, together with the known cross-reactivity of OXT vasopressin receptor V1aR, we also analyzed V1aR expression in the NAc. No significant difference was observed in V1aR between RCF and Cont group ($t = 0.1214$; $p = 0.906$; Supplementary Fig. 4A).

To further characterize the increased expression of the OxtR, we performed RNAscope Multiplex Fluorescent Assay (RNAscope ISH). Firstly, we observed that OxtRs were mainly expressed on GABAergic neurons of the NAc (Supplementary Fig. 4B). Further, our analysis showed that the expression of OxtR was higher in RCF mice than Cont ($t = 4.079$; $p = 0.0065$) (Fig. 3C, D), thus confirming qRT-PCR results. The increased OxtR expression in RCF mice was evident in both medium spiny neurons (MSNs)-D1+ ($t = 5.297$; $p = 0.0018$) and MSNs-D2+ ($t = 3.215$; $p = 0.018$) subtypes (Fig. 3E-G).

Notably, this upregulation was regionally restricted to the medial shell of the NAc (NAcMed) for both MSN subtypes (MSNs-D1 +: $t = 3.73$; $p = 0.0097$; MSNs-D2 +: $t = 3.315$; $p = 0.016$; Fig. 3H–M).

Together, these findings demonstrate that RCF selectively enhances OxtR expression within MSNs of the NAcMed, suggesting a region- and cell type-specific adaptation of the oxytocinergic system in response to early-life adversity.

OXT treatment during RCF rescues the behavioral and neurobiological deficits in pups

To determine whether OXT system alterations contribute causally to the behavioral phenotype, we administered subcutaneous OXT injections to RCF pups during the RCF period (Fig. 4A). Importantly, early OXT treatment did not alter maternal behavior (Supplementary Fig. 5B–H).

At PND8, separation-induced USVs revealed that RCF-Sal pups showed no significant difference between clean bedding and home-cage bedding exposures ($t = 0.691$; $p = 0.504$), indicative of disrupted attachment behavior. Conversely, RCF-Oxt pups emitted significantly more USVs when exposed to clean bedding compared to home-cage bedding ($t = 3.314$; $p = 0.0158$; Fig. 4B), resembling Cont pups.

Measurement of OXT levels at PND5 revealed that early OXT treatment increased both brain ($t = 6.719$; $p = 0.0001$; Fig. 4C) and plasma ($t = 3.264$; $p = 0.01$; Fig. 4D) OXT levels.

Thus, early OXT treatment restored both OXT levels and separation behavior in RCF pups.

Early OXT treatment during RCF restores OXT levels, OxtR and RAGE expression in adulthood

As we observed that the RCF-dependent alteration of the OXT system persisted from early stages well into adulthood, we asked whether the rescue effects exerted by exogenous OXT during post-natal life persisted along development (Fig. 5A). We thus performed Western Blot (WB) analysis on NAc punches and indeed

found increased OXT levels in the NAc of RCF-Oxt mice compared to RCF-Sal mice (U = 5; p = 0.048; Fig. 5B). Likewise, we found that OXT plasmatic levels were reduced in adult RCF-Oxt compared to RCF-Sal females (t = 2.516; p = 0.03; Fig. 5C).

We then investigated the effect of early OXT treatment on OxtR in the NAc. Both qRT-PCR (t = 2.734; p = 0.011; Fig. 5D) and RNAscope (t = 11.23; p < 0.0001; Fig. 5E,F) techniques showed reduced expression of OxtR mRNA levels in RCF-Oxt mice compared to RCF-Sal mice. Also, focusing on the two MSNs subtypes in the NAc, we found that OXT treatment reduced OxtR expression selectively in MSN-D1R+ neurons (t = 20.35; p < 0.0001; Fig. 5G), located in both the medial and the lateral NAc shell

Fig. 1 RCF affects central and peripheral OXT levels. (**A**) Experimental Timeline: Brain *OxtR* expression and brain and plasma OXT levels were assessed 24 h after the end of the RCF procedure (PND5) and in Cont pups; OXT levels in plasma and across brain areas (NAc, pFC, VTA, and PVN), and the number of OXT-positive cells in PVN were evaluated in adulthood (PND90). (**B**) OXT levels in the brain and (**C**) plasma of RCF and Cont groups (Brain: Cont group = 5; RCF group = 5; Plasma: Cont group = 5; RCF group = 6); (**D**) *OxtR* expression in the whole brain of RCF and Cont pups (PND5) (Cont group = 5; RCF group = 5). (**E**) Plasma OXT levels in adult (PND90) RCF and Cont groups (Cont group = 7; RCF group = 6); (**F**) OXT levels in the NAc of RCF and Cont groups (Cont group = 7; RCF group = 5); (**G**) OXT levels in the pFC of RCF and Cont mice, (**H**) OXT levels in the VTA of RCF and Cont mice (**I**) OXT levels in the PVN of RCF and Cont mice (Cont group = 6; RCF group = 6). (**J**) OXT levels in the NAc evaluated by WB in RCF and Cont groups (Cont group = 11; RCF group = 11); (**K**) *Left panel:* Representative coronal brain sections of the PVN immunostained with OXT primary antibody showing the OXT+ neurons of Cont and RCF mice; *Right panel:* number of OXT+ neurons in the PVN of Cont and RCF groups (N = 5 animals per group, 10 sections/animal). Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

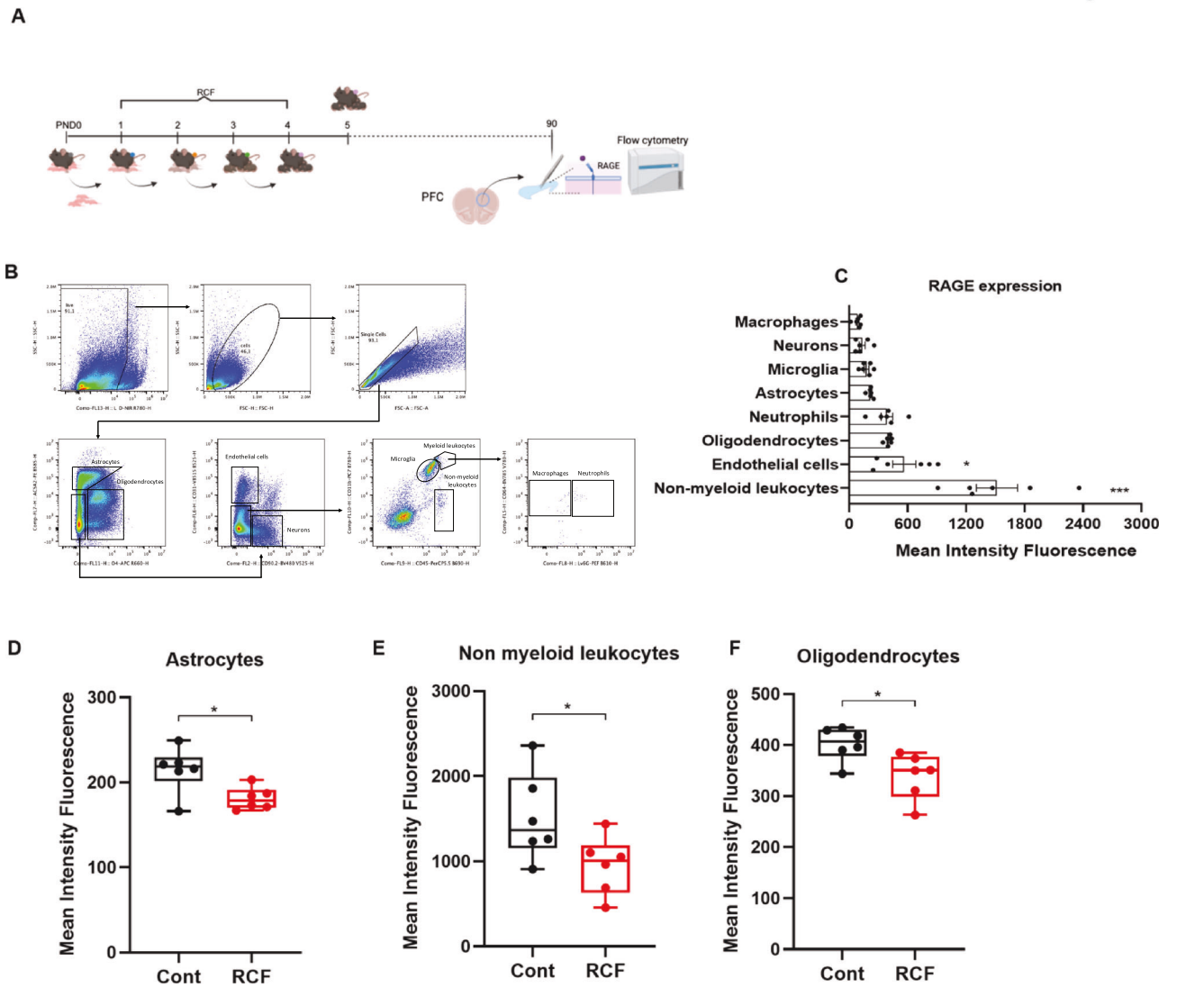
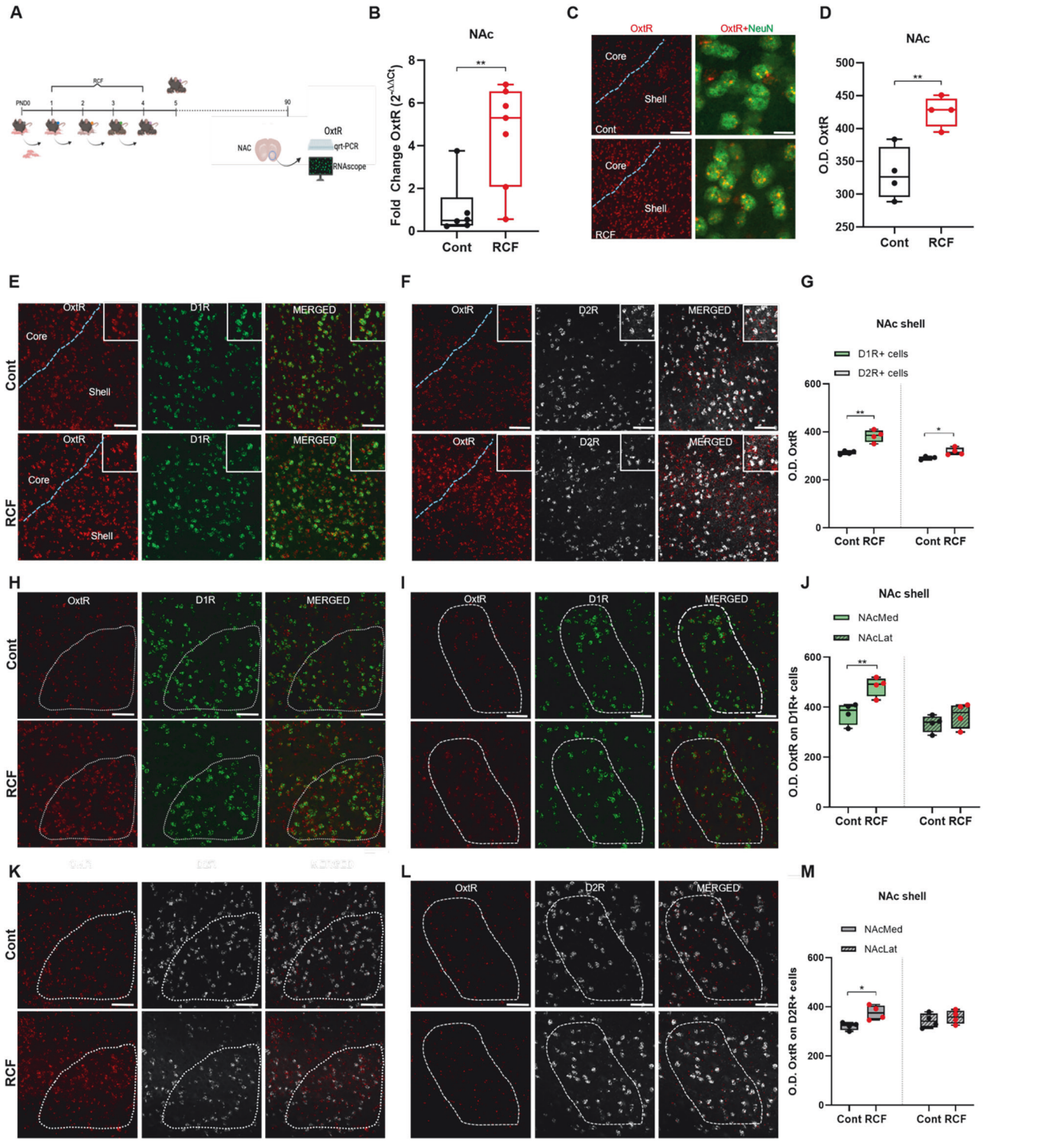


Fig. 2 RCF reduces RAGE expression in pFC specific cellular populations. (**A**) Experimental Timeline: RAGE expression was evaluated in pFC of adult female mice using High-dimensional flow cytometry. (**B**) The gating strategy was used to identify the different resident and infiltrated cell populations within the pFC. (**C**) Distribution of RAGE expression across different cell types in the pFC of Cont mice by High-dimensional Flow Cytometry (n = 6). RAGE expression between Cont and RCF mice in astrocytes (**D**), non mieloyd leukocytes (**E**), and oligodendrocytes (**F**). Data are presented as mean ± SEM. *P < 0.05, ***P < 0.001.

(NacMed: t = 11.91; p < 0.0001; NacLat: t = 19.74; p < 0.0001; Fig. 5H-K), with a non-significant trend on MSN-D2R+ (across the medio-lateral gradient; Supplementary Fig. 4C). Last, to confirm the involvement of RAGE in RCF-induced alterations, we analysed the RAGE expression in the cellular subtypes where alterations were observed in RCF mice (i.e. astrocytes, oligodendrocytes, and non-myeloid leukocytes). Notably, RCF-Oxt mice showed increased RAGE expression in astrocytes (t = 2.37; p = 0.042) (Fig. 5L), non-myeloid leukocytes (t = 4.869; p = 0.0007; Fig. 5M), and oligodendrocytes (t = 2.82; p = 0.018; Fig. 5N), compared to RCF-Sal mice.

Early OXT treatment during RCF restores vulnerability to cocaine effects and social behavior in adulthood

Since exogenous OXT administration effectively reversed neurobiological and behavioral alterations induced by RCF in pups, as



well as neurobiological changes in adulthood, we next assessed whether these rescue effects extended to behavioral outcomes in adult RCF females. We thus tested adult OXT-treated RCF females (PND90) in the Conditioned Place Preference test (CPP) (Fig. 6A) using a subthreshold dose of cocaine (2.5 mg/kg), known to be effective in C57 RCF females [57]. Repeated measure (RM) Two-way ANOVA for CPP data showed an interaction between treatment (RCF-Oxt, RCF-Sal) and pairing (Paired, Unpaired) ($F(1,14) = 5.832$; $p = 0.03$). The following post-hoc analysis showed that whilst RCF-Sal mice showed a significant preference for the chamber associated with cocaine ($p = 0.037$), as previously reported for C57 RCF females [57], RCF-Oxt mice did not show a significant preference for the cocaine-paired chamber (Fig. 6B). Taken together, these results confirmed that early treatment with OXT successfully reversed the typical behavioral alterations observed in female RCF mice [57]. Finally, we tested RCF, Cont, RCF-Oxt, and RCF-Sal mice in the Three Chamber Test (TCT; Fig. 6A) to assess social preference and recognition. As previously

Fig. 3 RCF alters OxtR expression in NAc. (**A**) Experimental Timeline: OxtR expression was evaluated in NAc of Cont and RCF adult mice. (**B**) OxtR expression was evaluated by qRT-PCR in the NAc of Cont and RCF groups (Cont group = 8; RCF group = 7). (**C**) Representative images of OxtR RNAscope ISH performed on coronal brain sections at the level of the NAc; scale bar: 50 μm. High-magnification of representative images of OxtR RNAscope ISH (red) combined with NeuN-immunofluorescence (green) showing the OxtR expression in neurons (right column); scale bar: 15 μm. (**D**) Histograms showing the OxtR-RNA signal measured as optical density (O.D.) in the NAc shell of Cont and RCF groups (N = 4 animals per group, 4 sections/animal). (**E**) Low-magnification of representative images of multiplex RNAscope ISH for OXTR (red) and D1R (green) and merged signal in the NAc shell medium spiny neurons. Scale bar: 25 μm. (**F**) Low-magnification of representative images of multiplex RNAscope ISH for OxtR (red) and D2R (gray) and merged signal in the NAc shell medium spiny neurons. Scale bar: 25 μm. **G** OxtR-RNA signal measured as optical density (O.D.) in the NAc shell D1R+ and D2R+ neurons of Cont and RCF groups (N = 4 animals per group, 4 sections/animal). (**H**) Low-magnification of representative images of multiplex RNAscope ISH for OxtR (red) and D1R (green) and merged signal in the medium spiny neurons of the dorso-medial portion of the NAc shell. Scale bar: 25 μm. (**I**) Low-magnification of representative images of multiplex RNAscope ISH for OxtR (red) and D1R (green) and merged signal in the medium spiny neurons of the dorso-lateral portion of the NAc shell. Scale bar: 25 μm. (**J**) Histograms showing the OxtR-RNA signal measured as optical density (O.D.) in the dorso-medial and dorso-lateral portion of the NAc shell D1R+ neurons of Cont and RCF groups (N = 4 animals per group, 4 sections/animal). (**K**) Low-magnification of representative images of multiplex RNAscope ISH for OxtR (red) and D2R (gray) and merged signal in the medium spiny neurons of the dorso-medial portion of the NAc shell. Scale bar: 25 μm. (**L**) Low-magnification of representative images of multiplex RNAscope ISH for OxtR (red) and D2R (gray) and merged signal in the medium spiny neurons of the dorso-lateral portion of the NAc shell. Scale bar: 25 μm. (**M**) Histograms showing the OxtR-RNA signal measured as optical density (O.D.) in the dorso-medial and dorso-lateral portions of the NAc shell D2R+ neurons of Cont and RCF groups (N = 4 animals per group, 4 sections/animal). Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001. The timeline in Fig. 3a shows two blank box shapes (bottom right, close to the brain, qRT and RNAscope illustrations).

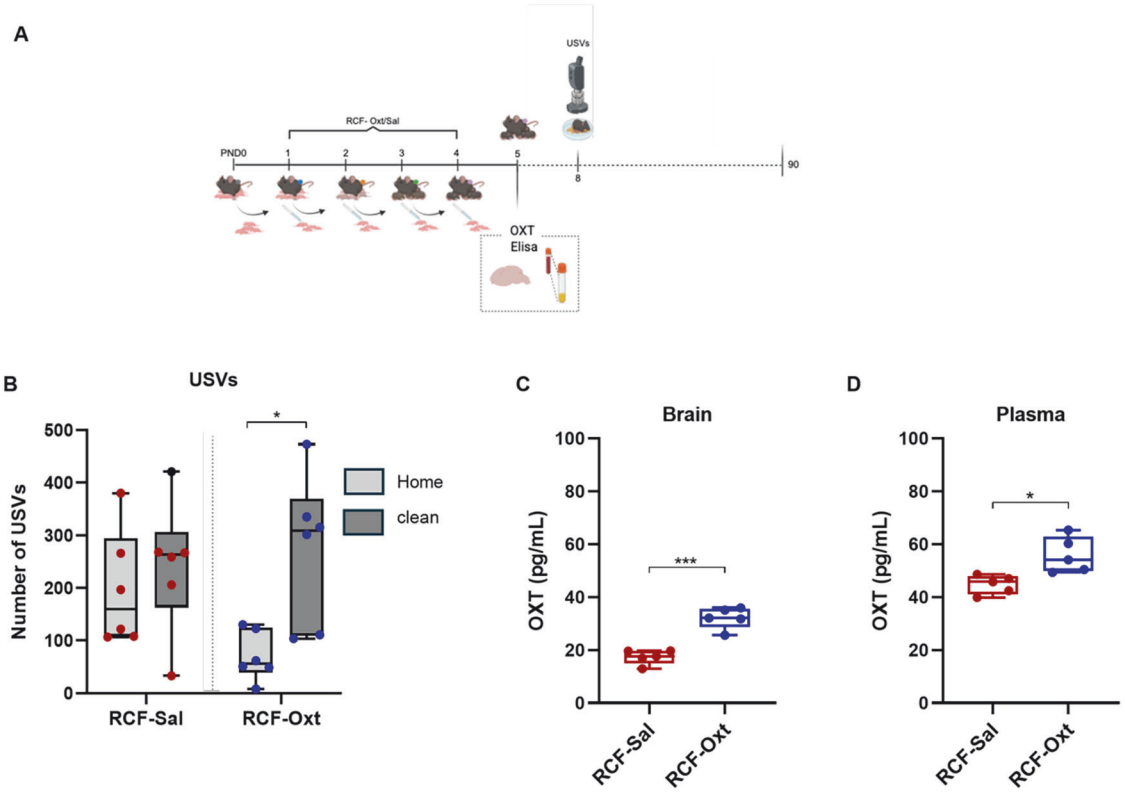
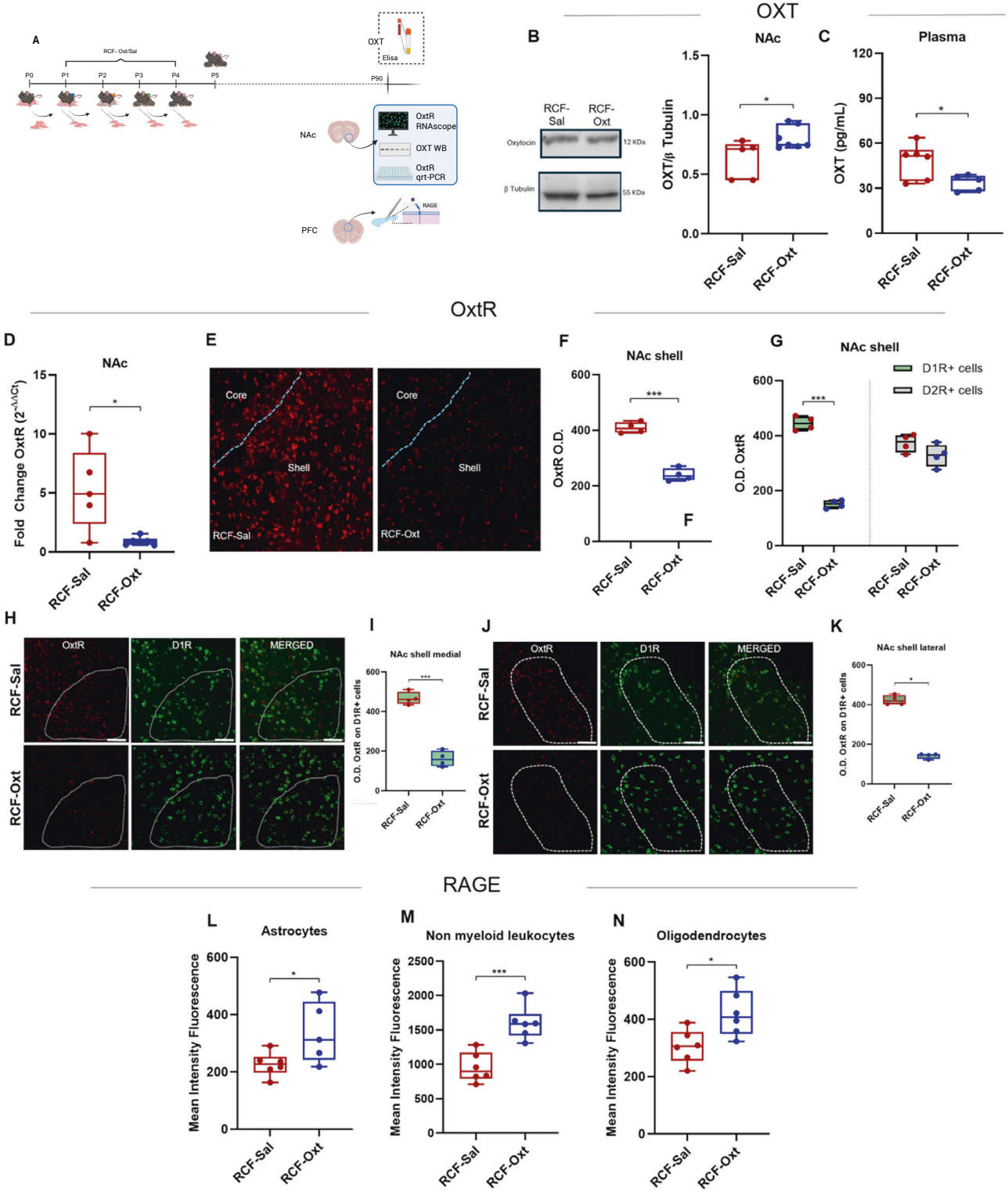


Fig. 4 Impact of OXT treatment during RCF on developmental behavioral deficits and OXT system in pups. (**A**) Experimental Timeline: RCF pups were subcutaneously treated with OXT (RCF-Oxt) or saline (RCF-Sal) during the RCF procedure (PND1-PND4); plasma and brain OXT levels were analysed at PND5, and behavioral tests (USVs) were performed at PND8. (**B**) Number of USVs emitted by RCF-Sal and RCF-Oxt pups when exposed to the home- and clean-cage bedding (RCF-Sal group = 6 per condition; RCF-Oxt = 6 per condition). (**C**) Brain and **D**plasma OXT levels in RCF-Sal and RCF-Oxt mice (RCF-Sal group = 5; RCF-Oxt = 5). *P < 0.05, ***P < 0.001. The timeline in Fig. 4a shows a blank box shape (close to the USVs illustration). Fig. 4b has misaligned lines. The solid and dotted lines in the middle of the plot should be on top of each other.

reported [20], RCF mice showed no social preference deficit (Fig. 6C). However, in the last session of TCT we found a significant difference between RCF and Cont in the time spent interacting with the familiar or the novel subject ($\chi^2(1) = 11.74$, $p = 0.0083$): whilst Cont mice spent more time interacting with the new subject compared to the old one ($p = 0.0056$), RCF mice showed no significant preference, manifesting a deficit in social recognition (Fig. 6D). When analysing results for treated mice, whilst there was no significant difference in social preference between RCF-Oxt and RCF-Sal (Fig. 6E), the RM two way-ANOVA revealed a significant treatment x stimulus (familiar, novel) interaction in the last session of the TCT ($F(1,9) = 9.33$; $p = 0.013$): RCF-Sal mice behaved like RCF (not showing social recognition), and instead RCF-Oxt mice showed social recognition, spending more time interacting with the new subject compared to the old one ($p = 0.0035$) (Fig. 6F), similar to the Cont group. Finally, no significant difference was observed between the RCF-Oxt and RCF-Sal groups in EPM ($t = 0.95$; $p = 0.35$; Supplementary Fig. 5I)



and OF (Time in Periphery: $t = 0.95$; $p = 0.35$; Distance Moved: $t = 0.13$; $p = 0.89$; Supplementary Fig. 5J,K).

Early OXT treatment does not affect *Ih* current in VTA DA neurons of RCF mice

We have previously reported that adult female RCF mice exhibit reduced Ih current and excitability in DA neurons of the intermediate VTA [58]. Given the VTA's central role in reward processing [74, 75], we next assessed whether early OXT treatment modulates *Ih* current in these neurons.

Whole-cell patch-clamp recordings were thus performed from VTA DA neurons on acute ventral slices from adult RCF-Sal or RCF-Oxt females (Supplementary Fig. 6A). Investigation of the intrinsic properties of these neurons showed fully comparable features, indicating that early OXT treatment did not alter macroscopically the basic neurophysiological properties in these neurons

Fig. 5 Early OXT treatment during RCF impacts the adult OXT system. (**A**) Experimental timeline: RCF-Oxt and RCF-Sal were sacrificed around PND90, and the brain and blood were collected for analysis. Different cohorts of mice were used. (**B**) OXT levels evaluated by WB in the NAc of RCF-Sal and RCF-Oxt adult mice (RCF-Sal group = 6; RCF-Oxt group = 7). (**C**) OXT plasma levels in RCF-Oxt mice compared to RCF-Sal mice (RCF-Sal group = 6; RCF-Oxt group = 5). (**D**) *OxtR* mRNA expression was evaluated by qRT-PCR in the NAc of RCF-Sal and RCF-Oxt (RCF-Sal group = 5; RCF-Oxt = 6). (**E**) Representative images of *OxtR* RNAscope ISH performed on coronal brain sections of the NAc regions (core and shell) in RCF-Oxt and RCF-Sal mice; scale bar: 50 μm. (**F**) Histograms showing the *OxtR*-RNA signal measured as optical density (O.D.) in the NAc shell of RCF-Sal and RCF- Oxt groups (N = 4 animals per group, 4 sections/animal). (**G**) Histograms showing the *OxtR*-RNA signal measured as optical density (O.D.) in the NAc shell D1R-and D2R+ neurons of RCF-Sal and RCF-Oxt groups (N = 4 animals per group, 4 sections/animal). (**H**) Low-magnification of representative images of multiplex RNAscope ISH for *OxtR* (red) and D1R (green) and merged signal in the medium spiny neurons of the dorso-medial portion of the NAc shell. Scale bar: 25 μm. (**I**) Histograms showing the *OxtR*-RNA signal measured as optical density (O.D.) in the dorso- medial portion of the NAc shell D1R+ neurons of RCF-Sal and RCF-Oxt groups (N = 4 animals per group, 4 sections/animal). (**J**) Low-magnification of representative images of multiplex RNAscope ISH for D1R (green) and OxtR (red) merged signal in the medium spiny neurons of the dorso-lateral portion of the NAc shell. Scale bar: 25 μm. (**K**) OxtR-RNA signal measured as optical density (O.D.) in the dorso-lateral portion of the NAc shell D1R+ neurons of RCF-Sal and RCF- Oxt groups (N = 4 animals per group, 4 sections/animal). High-dimensional Flow Cytometry analysis of pFC RAGE expression in astrocytes (**L**), non-myeloid leukocytes (**M**) and oligodendrocytes (**N**) of RCF-Oxt and RCF-Sal groups (RCF-Sal group = 6; RCF-Oxt group = 5). Data are presented as mean ± SEM. *P < 0.05, ***P < 0.001.

(Supplementary Table 2). Further, no significant effect of the early OXT treatment on VTA DA neurons was found when investigating spontaneous or evoked excitability (rheobase and 'f-I' relationships), as well as *Ih* current density (Supplementary Fig. 6B-E). As the early OXT treatment was effective in reducing the behavioral effects of cocaine, as assessed by CPP, but not in modulating the *Ih* current in VTA DA neurons, we decided to test further the apparent non-involvement of the *Ih* current in this behavioral phenotype. To this end, pharmacological inhibition of the *Ih* current of VTA DA neurons was performed in Cont mice by intra-VTA injection of a selective *Ih* current inhibitor (ZD) [58] to mimic the effects reported in RCF females (that is, reduced *Ih* current) (Supplementary Fig. 7A). ZD-treated mice (Cont+ZD) and their control group (Cont+Veh) were tested using CPP paradigm. Interestingly, our results showed no significant effect of the treatment (Supplementary Fig. 7B). Overall, our data convincingly demonstrated that ELA exposure significantly and permanently alters the OXT system in female mice, with the NAc appearing to be the mesocorticolimbic area clearly involved in this process.

DISCUSSION

This study extends previous findings on the critical role of the OXT system in short- and long-term behavioral and neurobiological consequences induced by ELA exposure. We report here that RCF disrupts the balance between central and peripheral OXT levels, reducing brain OXT content and increasing plasma OXT levels in pups (PND5). This imbalance was still evident in adulthood, along with altered OxtR expression in NAc, and RAGE expression in the pFC. Most importantly, we demonstrated that early OXT treatment during ELA exposure restored key components of the OXT system function, including brain and plasma OXT levels, OxtR, and RAGE expression. Accordingly, rescuing the OXT function during early life was sufficient to normalize both the pups' behavior and responses to cocaine and sociability in adults.

Early adversity and dysregulation of the OXT System

We observed reduced OXT levels in the brain at PND5 and in the NAc of adult (PND90) mice. This finding is consistent with previous reports showing that ELA can alter neuropeptide signaling in regions involved in emotional regulation, social behavior, and motivation [29, 76]. Here, we also report elevated plasma OXT levels in RCF pups, which persisted into adulthood. Moreover, we did not observe any significant differences in the number of OXT-positive cells within the PVN between Cont and RCF mice, indicating that alterations in OXT dynamics may be due to functional rather than anatomical changes in the OXT-releasing neurons. This interpretation is further supported by in vivo microdialysis results showing impaired OXT release in RCF mice in response to cocaine injection. Although several mechanisms could be proposed to explain the mismatch between increased OXT in plasma and decreased OXT in the brain, one of the most parsimonious involves the reduced expression of RAGE observed in RCF mice.

Impact of RCF on pFC RAGE expression

The pioneering works of Yamamoto and coll. [46, 47] have recently demonstrated, both in vitro and in vivo, that blood OXT can be transported across the BBB by RAGE, also pointing out a role of RAGE-mediated OXT transport in social bonding [46]. However, to our knowledge, the involvement of RAGE in ELA-induced alterations of the OXT system and behavioral consequences has never been investigated. Here, we show a cell-type specific distribution pattern of RAGE in the pFC, a brain region highly involved in motivation and emotional regulation [77] and strongly interconnected with NAc [65]. RAGE was most strongly expressed in non-myeloid leukocytes and endothelial cells, highlighting the vascular interface as a potential site for OXT entry into the CNS. Importantly, RAGE expression was reduced in RCF-exposed mice, particularly in astrocytes, key regulators of BBB permeability [78, 79], as well as in non-myeloid leukocytes and oligodendrocytes, both integral to the neurovascular unit [80]. This down-regulation may hinder OXT transport into the brain, contributing to decreased central and increased peripheral OXT levels observed in RCF animals. Although endothelial RAGE expression was unchanged, the BBB's structural integrity relies on coordinated interactions between endothelial cells, astrocytes, and basement membrane components [78–80], suggesting that astrocytic RAGE downregulation may disrupt OXT trafficking. The high RAGE expression in non-myeloid leukocytes and its reduction in RCF mice further support a multi-cellular mechanism affecting OXT brain entry. Additionally, emerging evidence of communication between oligodendrocytes and endothelial cells [80] raises the possibility of unexplored RAGE-dependent pathways within the neurovascular unit modulating OXT transport. Further studies are warranted to dissect these cell-specific contributions to OXT dysregulation following ELA.

However, although the RAGE's role as a key transporter mediating the entry of peripheral OXT into the brain is well supported by emerging literature, recent studies suggest that this may not be the only mechanism involved in this process. For instance, in an up-to-date paper, Yamamoto and colleagues [81] have identified C4a, a cleaved fragment of the complement component C4, as a new OXT-binding protein able to modulate the bioavailability of circulating OXT, which may affect the availability of OXT to the brain and behavior. Moreover, Rajamannar et al. [82] have recently proposed a "stimulus-secretion-uptake coupling' mechanism to investigate the precise way by which OXT is released from the brain into the general circulation. This mechanism delineates how oxytocin can act as a

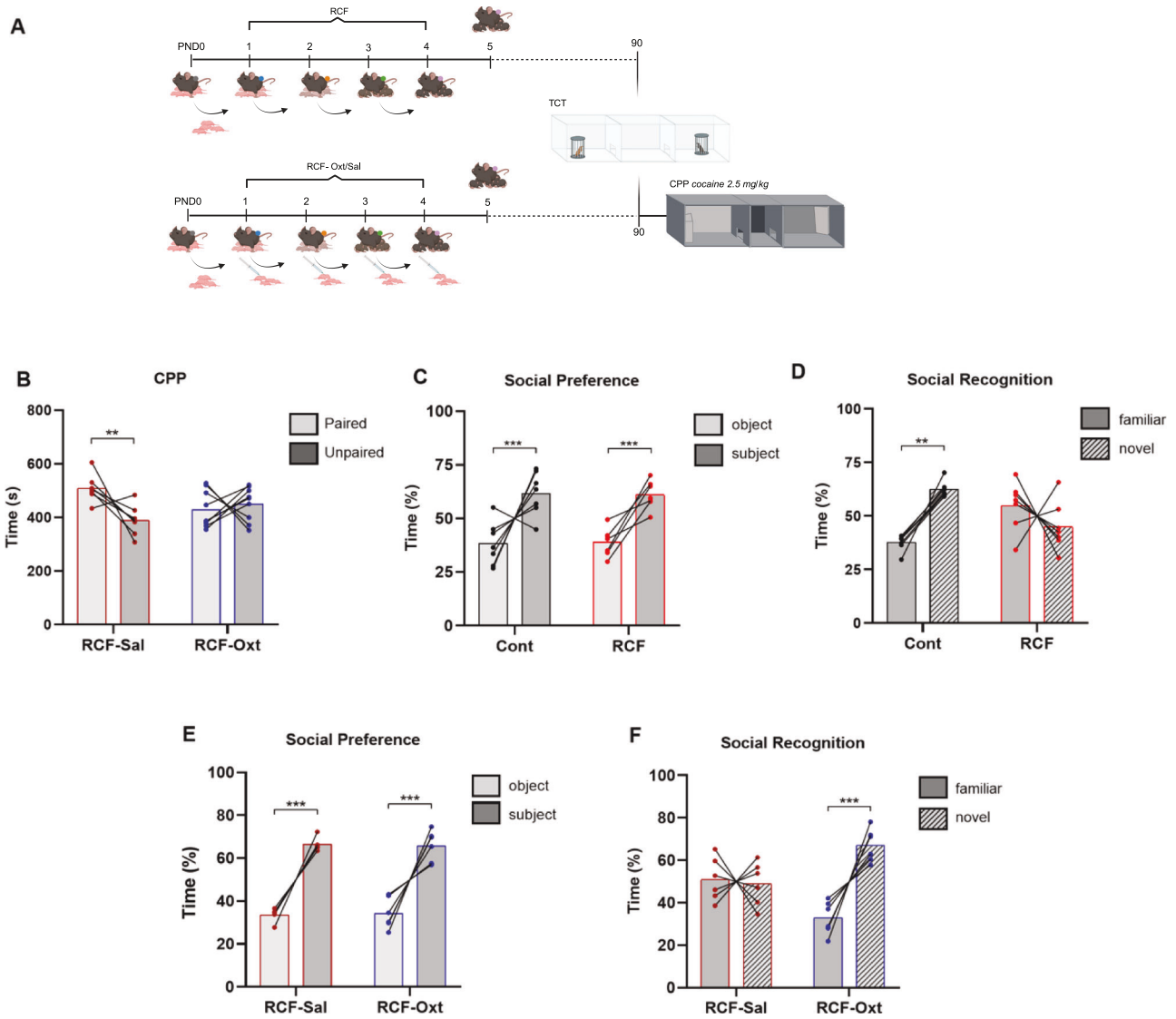


Fig. 6 Early OXT treatment rescues adult behavioral deficits in RCF mice. (**A**) Experimental Timeline: RCF pups were treated with OXT (RCF-Oxt) or saline (RCF-Sal) injections during the RCF procedure (from PND1 to PND4). Another cohort of mice underwent the RCF procedure without treatment. The Conditioned Place Preference (CPP; 2.5 mg/Kg, i.p.) test was performed in adult RCF-Sal and RCF-Oxt mice; Three Chamber Test (TCT) were performed in adult Cont, RCF, RCF-Sal, and RCF-Oxt mice. Different cohorts of RCF-Oxt and RCF-Sal mice were used for CPP and TCT experiments. (**B**) Cocaine-induced CPP in RCF-Oxt and RCF-Sal mice (RCF-Sal group = 7; RCF-Oxt group = 9). (**C**) Social Preference shown by RCF and Cont groups in TCT (Cont group = 7; RCF group = 7). (**D**) Social Recognition shown by RCF and Cont groups in TCT (Cont group = 7; RCF group = 7). (**E**) Social Preference shown by RCF-Oxt and RCF-Sal groups in TCT (RCF-Sal = 5; RCF-Oxt = 6). (**F**) Social Recognition shown RCF-Oxt and RCF-Sal groups in TCT (RCF-Sal = 5; RCF-Oxt = 6). Data are presented as mean ± SEM. **P < 0.01, ***P < 0.00.

self-perpetuating hormone, facilitating its own peripheral uptake through modulation of pituitary hemodynamics and vascular permeability.

Altogether, these findings indicate that the dynamics of peripheral OXT and its central access are likely regulated by multiple, interacting pathways. Further investigation is necessary to understand the mechanisms at play.

OXT Receptor alteration in the nucleus accumbens, social behavior, and cocaine vulnerability

Next, we observed increased OxtR expression in the NAc of RCF mice. Our ex vivo (WB and ELISA) and in vivo (intracerebral microdialysis) experiments on OXT levels and release support the idea that this enhancement may reflect a compensatory response to reduced OXT signaling in RCF mice. Nonetheless, further studies are required to definitively rule out the possibility of an actual enhancement of oxytocinergic signaling.

OXT has been demonstrated to influence social behavior and response to drugs via interactions with brain circuits, particularly the mesocorticolimbic system. Social recognition, the ability to discriminate between familiar from unfamiliar individuals, is critical for establishing social bonds and hierarchies, such as peer and pair bonds [83–86]. Environmental factors and the internal social approach-avoidance balance can influence social recognition, and early adverse experiences, such as a disrupted early attachment bond, can undermine future social discrimination and peer bonding. Notably, impaired social recognition has been linked to impaired OXT system [83]. For instance, OXT knockout mice fail to recognize familiar individuals despite repeated exposure, and this deficit can be rescued by administering low doses of OXT [85]. In this scenario, the impaired social recognition ability shown by adult RCF mice may mirror the altered attachment behavior observed at PND8, which potentially are two different manifestations of a general impairment in the ability to form appropriate social bonds.

The OXT's effects on social bonding are particularly evident in the NAc, where OxtR expression modulates social behavior and resilience to early-life neglect [87]. In our model, increased OxtR expression and reduced OXT levels in the NAc could reasonably underlie the impaired social recognition shown by adult RCF mice. This compensatory response could, in turn, lead to an alteration in the processing of reward-related stimuli, also contributing to increased vulnerability to the effects of cocaine. Current literature in the field reports that reduced OXT release and altered receptor expression contribute to addiction by impairing the OXT system's ability to regulate emotional and reward processes, probably interacting with the dopaminergic system [49]. In line with this, here we report OxtR alterations in both MSNs-D1 and MSNs-D2 neurons in the dorsomedial region of the NAc shell and impaired cocaine-induced OXT release. Several studies have suggested a non-canonical, shared role for both NAc D1R and D2R in encoding positive valence and reward responses to drugs of abuse and food [88], suggesting that they may play synergistically in modulating the response to rewarding stimuli. Moreover, the medial part of the NAc shell sends direct projections to the VTA, forming a feedback loop that enables bidirectional communication, which may regulate DA release in the NAc [89]. Thus, the increased OxtR expression on MSN neurons in the medial part of NAc shell could mediate the increased sensitivity to cocaine exhibited by adult female RCF mice, possibly also modulating the DA response to this drug.

Early OXT treatment rescues OXT system function and behavior

Our most striking finding is the restorative effect of early OXT treatment during RCF exposure on both OXT system function and behavioral outcomes in pups and adult RCF mice. Early OXT treatment normalized brain OXT levels and prevented separation anxiety, an index of the attachment bond, in RCF pups. The restoration of this behavior is consistent with the well-established role of OXT in social bonding [90]. Importantly, OXT administration during the RCF also resulted in normalized adult OXT levels in both brain (NAc) and plasma and restored RAGE expression in the pFC. Moreover, this treatment significantly mitigated long-term vulnerability to cocaine effects and social recognition in adults exposed to RCF, providing strong evidence that early OXT administration is sufficient to restore proper processing of rewarding stimuli. These findings highlight the plasticity of the oxytocinergic system and suggest the potential for early OXT treatments to counteract some of the long-lasting consequences of ELA. Our results are in line with the literature reporting the protective effects of OXT against addiction, including reduction in drug-seeking behaviors, craving, and withdrawal symptoms [51, 52]. In particular, regarding cocaine addiction, OXT mitigates cocaine-seeking behaviors, affecting brain areas such as the NAc and pFC [42, 43]. Interestingly, treatment with OXT during RCF modulates the OxtR expression in MSNs-D1 selectively, and it affects both the medial and lateral portions of the NAc shell, suggesting that OXT may exert a region- and neuron-specific effect in the NAc. The NAc shell, particularly its medial subregion, is implicated in processing more affective or motivational components of reward. In contrast, the lateral NAc shell has been linked to aspects of behavioral control and action initiation. By modulating OxtR expression on MSNs-D1 in both subregions, OXT treatment may restore a more balanced regulation of reward-seeking behavior, dampening the heightened sensitivity to cocaine effects induced by RCF. Moreover, MSNs are also implicated in different aspects of social behavior [91]. Thus, the modulation of OxtR expression in NAc may also regulate the processing of social stimuli in RCF mice, thereby alleviating social recognition deficit. This supports the idea that OXT could modulate addiction-related behaviors and social deficits resulting from early adversity. OXT's ability to restore social recognition has the potential to improve social interactions and emotional regulation in individuals with a history of ELA, which is a known risk factor for developing both addiction and social deficits. Several mechanisms may be responsible for early OXT treatment effects. Firstly, restoring brain OXT levels to a healthy range would likely boost neuropeptide signalling, affecting early social bonding and emotion regulation. Secondly, the restoration of RAGE expression in OXT-treated RCF mice suggests that early treatment may enhance OXT transport across the BBB, thereby ensuring sufficient OXT for optimal brain function. In addition, the normalized OxtR expression into the NAc of OXT-treated RCF mice (together with suitable OXT levels) could restore the proper ability to process natural and pharmacological reinforcing stimuli in adulthood. Finally, given the widespread distribution of OXT receptors in the body, peripherally administered OXT may also influence the brain through peripheral signaling mechanisms. Notably, there is evidence for functional interactions between OXT administration and the vagus nerve (VN) [92, 93]. It has been reported that VN signaling is necessary for the rescue effects of peripheral OXT on methamphetamine self-administration and reinstatement [92], as well as on food intake [93], thus supporting the hypothesis that the vagal pathway mediates the effects of administered OXT on reward processing. In line with this evidence, peripheral OXT administration increases activity of PVN OXT neurons, and this increase is prevented by subdiaphragmatic vagotomy [93]. Altogether, these findings support a model in which the VN functions as a gateway through which peripherally administered OXT signals to the brain to modulate behavior. Further studies are needed to investigate this mechanism in our experimental conditions.

Early OXT treatment does not affect *Ih* current in VTA DA neurons

We have previously reported that early exposure to RCF leads C57 female mice to a long-lasting reduction of the *Ih* current in VTA DA neurons. The *Ih* current helps integrate excitatory synaptic inputs [94] and its modulation has been related to several aspects of addiction, including cocaine- related behaviors. For instance, a reduced *Ih* current in VTA DA neurons has been associated with increased susceptibility to cocaine addiction [95]. Additionally, a modulatory role of OXT on the *Ih* current has also been reported [96]. Here, somewhat at odds with the other rescue effects observed on the RCF phenotype, we found that early OXT treatment does not alter the *Ih* current in VTA DA neurons of adult RCF mice in a long-lasting manner. Our findings suggest that the behavioral rescue effects of early OXT treatment are not related to modulation of the *Ih* current in intermediate VTA DA neurons, but rather most likely result from the specific role played by the NAc in the RCF phenotype, an aspect that warrants further in-depth investigation.

Overall, our results demonstrate that early-life adversity induced by RCF leads to dysregulation of the OXT system, characterized by altered brain and plasma OXT levels, as well as changes in RAGE and OxtR expression in critical nodes of the mesocorticolimbic system. Early OXT treatment prevented these alterations and restored behavioral phenotypes in both pups and adult female mice. These findings shed light on the mechanisms by which the oxytocinergic system mediates the long-term effects of ELA on addiction and social behaviors.

Finally, we have previously shown that RCF increases depression-like behavior in C57 males [20]. Based on known sex-related differences concerning OXT system, we extended our investigation to examine the RCF effects on OXT system in male mice. Interestingly, preliminary data indicate that RCF may influence the oxytocin system in a sex-dependent manner. Specifically, our results suggest that while RCF exerts comparable effects on peripheral OXT levels in both sexes, it differentially affects OxtR expression across brain regions and RAGE expression across cell types, relative to female mice. Ongoing and future studies in male subjects will further explore these sex-specific effects.

DATA AVAILABILITY

All information would be provided upon reasonable request.

REFERENCES

1. Arnow BA. Relationships between childhood maltreatment, adult health and psychiatric outcomes, and medical utilization. J Clin Psychiatry. 2004;65:10–15.
2. De Bellis MD. Developmental traumatology: a contributory mechanism for alcohol and substance use disorders. Psychoneuroendocrinology. 2002;27:155–70.
3. Heffernan K, Cloitre M, Tardiff K, Marzuk PM, Portera L, Leon AC, et al. Childhood trauma as a correlate of lifetime opiate use in psychiatric patients. Addict Behav. 2000;25:797–803.
4. Enoch MA. The role of early life stress as a predictor for alcohol and drug dependence. Psychopharmacology (Berl). 2011;214:17–31.
5. Wu NS, Schairer LC, Dellor E, Grella C. Childhood trauma and health outcomes in adults with comorbid substance abuse and mental health disorders. Addict Behav. 2010;35:68–71.
6. al'Absi M, DeAngelis B, Borodovsky J, Sofis MJ, Fiecas M, Budney A. Early life adversity and substance use: the mediating role of mood and the moderating role of impulsivity. J Psychiatr Res. 2023;168:38–44.
7. Hyman SM, Paliwal P, Chaplin TM, Mazure CM, Rounsaville BJ, Sinha R, et al. Severity of childhood trauma is predictive of cocaine relapse outcomes in women but not men. Drug Alcohol Depend. 2008;92:208–16.
8. McCance-Katz EF, Carroll KM, Rounsaville BJ. Gender differences in treatment-seeking cocaine abusers: implications for treatment and prognosis. Am J Addict. 1999;8:300–11.
9. Sinha R, Rounsaville BJ. Sex differences in depressed substance abusers. J Clin Psychiatry. 2002;63:616–27.
10. Kosten TA, Miserendino MJD, Kehoe P. Enhanced acquisition of cocaine self-administration in adult rats with neonatal isolation stress experience. Brain Res. 2000;875:44–50.
11. Moffett MC, Harley J, Francis D, Moffett MC, Harley J, Francis D, et al. Maternal separation and handling affects cocaine self-administration in both the treated pups as adults and the dams. J Pharmacol Exp Ther. 2006;317:1210–8.
12. Delavari F, Sheibani V, Esmaeili-Mahani S, Nakhaee N. Maternal separation and the risk of drug abuse in later life. Addict Health. 2016;8:107.
13. Alves RL, Oliveira P, Lopes IM, Portugal CC, Alves CJ, Barbosa F, et al. Early-life stress affects drug abuse susceptibility in adolescent rat model independently of depression vulnerability. Sci Rep. 2020;10:13326.
14. Okhuarobo A, Bolton JL, Igbe I, Zorrilla EP, Baram TZ, Contet C. A novel mouse model for vulnerability to alcohol dependence induced by early-life adversity. Neurobiol Stress. 2020;13:100269.
15. Levis SC, Bentzley BS, Molet J, Bolton JL, Perrone CR, Baram TZ, et al. On the early life origins of vulnerability to opioid addiction. Mol Psychiatry. 2021;26:4409–16.
16. Rincón-Cortés M. Sex differences in addiction-relevant behavioral outcomes in rodents following early life stress. Addict Neurosci. 2023;6:100067.
17. Bardo MT, Hammerslag LR, Malone SG. Effect of early life social adversity on drug abuse vulnerability: Focus on corticotropin-releasing factor and oxytocin. Neuropharmacol. 2021;191:108567.
18. Kosten TA, Zhang XY, Kehoe P. Heightened cocaine and food self-administration in female rats with neonatal isolation experience. Neuropsychopharmacol. 2006;31:70–76.
19. Rocks D, Jaric I, Bellia F, Cham H, Greally JM, Suzuki M, et al. Early-life stress and ovarian hormones alter transcriptional regulation in the nucleus accumbens resulting in sex-specific responses to cocaine. Cell Rep. 2023;42:113.
20. Lo Iacono L, Mancini C, Babicola L, Pietrosanto M, Di Segni M, D'Addario SL, et al. Early life adversity affecting the attachment bond alters ventral tegmental area transcriptomic patterning and behavior almost exclusively in female mice. Neurobiol Stress. 2021;15:100406.
21. Kohl C, Wang XD, Grosse J, Fournier C, Harbich D, Westerholz S, et al. Hippocampal neuroligin-2 links early-life stress with impaired social recognition and increased aggression in adult mice. Psychoneuroendocrinology. 2015;55:128–43.
22. Humphreys KL, Kircanski K, Colich NL, Gotlib IH. Attentional avoidance of fearful facial expressions following early life stress is associated with impaired social functioning. J Child Psychol Psychiatry. 2016;57:1174–82.
23. Opendak M, Gould E, Sullivan R. Early life adversity during the infant sensitive period for attachment: programming of behavioral neurobiology of threat processing and social behavior. Dev Cogn Neurosci. 2017;25:145–59.
24. Packard K, Opendak M. Rodent models of early adversity: impacts on developing social behavior circuitry and clinical implications. Front Behav Neurosci. 2022;16:918862.
25. Grinevich V, Desarménien MG, Chini B, Tauber M, Muscatelli F. Ontogenesis of oxytocin pathways in the mammalian brain: late maturation and psychosocial disorders. Front Neuroanat. 2015;8:164.
26. Grinevich V, Knobloch-Bollmann HS, Eliava M, Busnelli M, Chini B. Assembling the puzzle: pathways of oxytocin signaling in the brain. Biol Psychiatry. 2016;79:155–64.
27. Grinevich V, Neumann ID. Brain oxytocin: how puzzle stones from animal studies translate into psychiatry. Mol Psychiatry. 2021;26:265–79.
28. Neumann D. Brain oxytocin: a key regulator of emotional and social behaviours in both females and males. J Neuroendocrinol. 2008;20:858–65.
29. Kompier NF, Keysers C, Gazzola V, Lucassen PJ, Krugers HJ. Early life adversity and adult social behavior: focus on arginine vasopressin and oxytocin as potential mediators. Front Behav Neurosci. 2019;13:143.
30. Eapen V, Dadds M, Barnett B, Kohlhoff J, Khan F, Radom N, et al. Separation anxiety, attachment and inter-personal representations: disentangling the role of oxytocin in the perinatal period. PLoS One. 2014;9:e107745.
31. Pierrehumbert B, Torrisi R, Ansermet F, Borghini A, Halfon O. Adult attachment representations predict cortisol and oxytocin responses to stress. Attach Hum Dev. 2012;14:453–76.
32. Samuel S, Hayton B, Gold I, Feeley N, Carter CS, Zelkowitz P. Attachment security and recent stressful life events predict oxytocin levels: a pilot study of pregnant women with high levels of cumulative psychosocial adversity. Attach Hum Dev. 2015;17:272–87.
33. Takayanagi Y, Yoshida M, Bielsky IF, Ross HE, Kawamata M, Onaka T, et al. Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. Proc Natl Acad Sci USA. 2005;102:16096–101.
34. Parker KJ, Garner JP, Libove RA, Hyde SA, Hornbeak KB, Carson DS, et al. Plasma oxytocin concentrations and OXTR polymorphisms predict social impairments in children with and without autism spectrum disorder. Proc Natl Acad Sci USA. 2014;111:12258–63.
35. Zhang JB, Chen L, Lv ZM, Niu XY, Shao CC, Zhang C, et al. Oxytocin is implicated in social memory deficits induced by early sensory deprivation in mice. Mol Brain. 2016;9:1–13.
36. Buisman-Pijlman FTA, Sumracki NM, Gordon JJ, Hull PR, Carter CS, Tops M. Individual differences underlying susceptibility to addiction: Role for the endogenous oxytocin system. Pharmacol Biochem Behav. 2014;119:22–38.
37. Baracz SJ, Everett NA, Cornish JL. The impact of early life stress on the central oxytocin system and susceptibility for drug addiction: Applicability of oxytocin as a pharmacotherapy. Neurosci Biobehav Rev. 2020;110:114–32.
38. Insel TR. Translating oxytocin neuroscience to the clinic: a national institute of mental health perspective. Biol Psychiatry. 2016;79:153–4.
39. Guastella AJ, Einfeld SL, Gray KM, Rinehart NJ, Tonge BJ, Lambert TJ, et al. Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. Biol Psychiatry. 2010;67:692–4.
40. Gordon I, Jack A, Pretzsch CM, Vander Wyk B, Leckman JF, Feldman R, et al. Intranasal oxytocin enhances connectivity in the neural circuitry supporting social motivation and social perception in children with autism. Sci Rep. 2016;6:35054.
41. Feifel D, Shilling PD, MacDonald K. A review of oxytocin's effects on the positive, negative, and cognitive domains of schizophrenia. Biol Psychiatry. 2015;79:222–33.
42. Leong KC, Freeman LR, Berini CR, Ghee SM, See RE, et al. Oxytocin reduces cocaine cued Fos activation in a regionally specific manner. Int J Neuropsychopharmacol. 2017;20:844–54.
43. Zhou L, Sun WL, Young AB, Lee K, McGinty JF, See RE Oxytocin reduces cocaine seeking and reverses chronic cocaine-induced changes in glutamate receptor function. Int J Neuropsychopharmacol. 2015;18.
44. McGregor IS, Bowen MT. Breaking the loop: Oxytocin as a potential treatment for drug addiction. Horm Behav. 2012;61:331–9.
45. Che X, Cai J, Liu Y, Xu T, Yang J, Wu C. Oxytocin signaling in the treatment of drug addiction: Therapeutic opportunities and challenges. Pharmacol Ther. 2021;223:107820.
46. Yamamoto Y, Liang M, Munesue S, Deguchi K, Harashima A, Furuhara K, et al. Vascular RAGE transports oxytocin into the brain to elicit its maternal bonding behaviour in mice. Commun. Biol. 2019;2:76.
47. Yamamoto Y, Higashida H. RAGE regulates oxytocin transport into the brain. Commun Biol. 2020;3:70.
48. Moaddab M, Hyland BI, Brown CH. Oxytocin excites nucleus accumbens shell neurons in vivo. Mol Cell Neurosci. 2015;68:323–30.
49. Xiao L, Priest MF, Nasenbeny J, Lu T, Kozorovitskiy Y. Biased oxytocinergic modulation of midbrain dopamine systems. Neuron. 2017;95:368–.e5.
50. Liao PY, Chiu YM, Yu JH, Chen SK Mapping central projection of oxytocin neurons in unmated mice using Cre and alkaline phosphatase reporter. Front Neuroanat. 2020;14.
51. Bowen MT, Neumann ID. Rebalancing the addicted brain: Oxytocin interference with the neural substrates of addiction. Trends Neurosci. 2017;40:691–708.
52. Baracz SJ, Robinson KJ, Wright AL, Turner AJ, McGregor IS, Cornish JL, et al. Oxytocin as an adolescent treatment for methamphetamine addiction after early life stress in male and female rats. Neuropsychopharmacology. 2022;47:1561–73.

53. D'Amato FR, Zanettini C, Lampis V, Coccurello R, Pascucci T, Ventura R, et al. Unstable maternal environment, separation anxiety, and heightened CO2 sensitivity induced by gene-by-environment interplay. PLoS One. 2011;6:e18637.
54. Di Segni M, Andolina D, Luchetti A, Babicola L, D'Apolito LI, Pascucci T, et al. Unstable maternal environment affects stress response in adult mice in a genotype-dependent manner. Cereb Cortex. 2016;26:4370–80.
55. Mancini C, Babicola L, Chila G, Di Segni M, Municchi D, D'Addario SL, et al. Secure attachment to caregiver prevents adult depressive symptoms in a sex-dependent manner: A translational study. iScience. 2024;27:111328.
56. Ventura R, Coccurello R, Andolina D, Latagliata EC, Zanettini C, Lampis V, et al. Postnatal aversive experience impairs sensitivity to natural rewards and increases susceptibility to negative events in adult life. Cereb Cortex. 2013;23:1606–17.
57. Di Segni M, Andolina D, Coassin A, Accoto A, Luchetti A, Pascucci T, et al. Sensitivity to cocaine in adult mice is due to interplay between genetic makeup, early environment and later experience. Neuropharmacology. 2017;125:87–98.
58. D'Addario SL, Di Segni M, Ledonne A, Piscitelli R, Babicola L, Martini A, et al. Resilience to anhedonia-passive coping induced by early life experience is linked to a long-lasting reduction of Ih current in VTA dopaminergic neurons: Altered Ih current in the VTA of adult resilient-to-depression mice. Neurobiol Stress. 2021;14:100324.
59. Rein B, Ma K, Yan Z. A standardized social preference protocol for measuring social deficits in mouse models of autism. Nat Protoc. 2020;15:3464–77.
60. Aceto G, Nardella L, Nanni S, Pecci V, Bertozzi A, Nutarelli S, et al. Glycine-induced activation of GPR158 increases the intrinsic excitability of medium spiny neurons in the nucleus accumbens. Cell Mol Life Sci. 2024;81:268.
61. Sciarretta F, Ceci V, Tiberi M, Zaccaria F, Li H, Zhou ZY, et al. Lipocalin-2 promotes adipose–macrophage interactions to shape peripheral and central inflammatory responses in experimental autoimmune. Mol Metab. 2023;76:101783.
62. Ting JT, Lee BR, Chong P, Soler-Llavina G, Cobbs C, Koch C, et al. Preparation of acute brain slices using an optimized N-methyl-D-glucamine protective recovery method. J Vis Exp. 2018;53825.
63. Wilhelm I, Nyúl-Tóth Á, Suciu M, Hermenean A, Krizbai I. Heterogeneity of the blood–brain barrier. Tissue Barriers. 2016;4:e1143544.
64. Kirst C, Skriabine S, Vieites-Prado A, Topilko T, Bertin P, Gerschenfeld G, et al. Mapping the fine-scale organization and plasticity of the brain vasculature. Cell. 2020;180:780–.e25.
65. Khastkhodaei Z, Muthuraman M, Yang JW, Groppa S, Luhmann HJ. Functional and directed connectivity of the cortico-limbic network in mice in vivo. Brain Struct Funct. 2021;226:685–700.
66. Dölen G, Darvishzadeh A, Huang KW, Malenka RC. Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. Nature. 2013;501:179–84.
67. Castro DC, Bruchas MR. A motivational and neuropeptidergic hub: anatomical and functional diversity within the nucleus accumbens shell. Neuron. 2019;102:529–52.
68. Borie AM, Agezo S, Lunsford P, Boender AJ, Guo JD, Zhu H, et al. Social experience alters oxytocinergic modulation in the nucleus accumbens of female prairie voles. Curr Biol. 2022;32:1026–.e4.
69. Penrod RD, Taniguchi M, Kearns AM, Hopkins JL, Reichel CM. Differential roles of oxytocin receptors in the prefrontal cortex and nucleus accumbens on cocaine self-administration and reinstatement of cued cocaine seeking in male rats. Int J Neuropsychopharmacol. 2023;26:817–27.
70. Young LJ, Lim MM, Gingrich B, Insel TR. Cellular mechanisms of social attachment. Horm Behav. 2001;40:133–8.
71. Keebaugh AC, Barrett CE, Laprairie JL, Jenkins JJ, Young LJ. RNAi knockdown of oxytocin receptor in the nucleus accumbens inhibits social attachment and parental care in monogamous female prairie voles. Soc Neurosci. 2015;10:561–70.
72. Williams AV, Duque-Wilckens N, Ramos-Maciel S, Campi KL, Bhela SK, Xu CK, et al. Social approach and social vigilance are differentially regulated by oxytocin receptors in the nucleus accumbens. Neuropsychopharmacology. 2020;45:1423–30.
73. Lobo MK, Nestler EJ. The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons. Front Neuroanat. 2011;5:41.
74. Liu S, Globa AK, Mills F, Naef L, Qiao M, Bamji SX, et al. Consumption of palatable food primes food approach behavior by rapidly increasing synaptic density in the VTA. Proc Natl Acad Sci USA. 2016;113:2520–5.
75. Edwards NJ, Tejeda HA, Pignatelli M, Zhang S, McDevitt RA, Wu J, et al. Circuit specificity in the inhibitory architecture of the VTA regulates cocaine-induced behavior. Nat Neurosci. 2017;20:438–48.
76. Nakama N, Usui N, Doi M, Shimada S. Early life stress impairs brain and mental development during childhood increasing the risk of developing psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2023;126:110783.
77. Cardinal RN, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev. 2002;26:321–52.
78. Galea I. The blood–brain barrier in systemic infection and inflammation. Cell Mol Immunol. 2021;18:2489–501.
79. Barichello T, Collodel A, Hasbun R, Morales R. An overview of the blood–brain barrier. Neuromethods. 2019;146:1–8.
80. Dejana E, Betsholtz C. Oligodendrocytes follow blood vessel trails in the brain. Science. 2016;351:341–2.
81. Yamamoto Y, Zhang A, Yuhi T, Harashima A, Shabalova AA, Nishizawa E, et al. Complement component C4a binds to oxytocin and modulates plasma oxytocin concentrations and social behavior in male mice. Biochem Biophys Res Commun. 2025;771:152004.
82. Rajamannar P, Blechman J, Raz O, Levkowitz G. Neuropeptide oxytocin facilitates its own brain-to-periphery uptake. Cell Rep. 2025;44:115491.
83. Gabor CS, Phan A, Clipperton-Allen AE, Kavaliers M, Choleris E. Interplay of oxytocin, vasopressin, and sex hormones in the regulation of social recognition. Behav Neurosci. 2012;126:97–109.
84. Carter CS, Keverne EB The neurobiology of social affiliation and pair bonding. In: Hormones, Brain and Behavior. Academic Press; 2002. p. 299–337.
85. Ferguson JN, Aldag JM, Insel TR, Young LJ. Oxytocin in the medial amygdala is essential for social recognition in the mouse. J Neurosci. 2001;21:8278–85.
86. Keebaugh AC, Young LJ. Increasing oxytocin receptor expression in the nucleus accumbens of pre-pubertal female prairie voles enhances alloparental responsiveness and partner preference formation as adults. Horm Behav. 2011;60:498–504.
87. Barrett CE, Arambula SE, Young LJ. The oxytocin system promotes resilience to the effects of neonatal isolation on adult social attachment in female prairie voles. Transl Psychiatry. 2015;5:e606.
88. Soares-Cunha C, de Vasconcelos NA, Coimbra B, Domingues AV, Silva JM, Loureiro-Campos E, et al. Nucleus accumbens medium spiny neuron subtypes signal both reward and aversion. Mol Psychiatry. 2020;25:3241–55.
89. Yang H, de Jong JW, Tak Y, Peck J, Bateup HS, Lammel S. Nucleus accumbens subnuclei regulate motivated behavior via direct inhibition and disinhibition of VTA dopamine subpopulations. Neuron. 2018;97:434–.e4.
90. Bosch OJ, Young LJ. Oxytocin and social relationships: from attachment to bond disruption. Curr Top. Behav Neurosci. 2018;35:97–117.
91. Sano H, Nagai Y, Miyakawa T, Shigemoto R, Yokoi M. Increased social interaction in mice deficient of the striatal medium spiny neuron-specific phosphodiesterase 10A2. J Neurochem. 2008;105:546–56.
92. Everett NA, Turner AJ, Costa PA, Baracz SJ, Cornish JL. The vagus nerve mediates the suppressing effects of peripherally administered oxytocin on methamphetamine self-administration and seeking in rats. Neuropsychopharmacology. 2021;46:297–304.
93. Iwasaki Y, Kumari P, Wang L, Hidema S, Nishimori K, Yada T. Relay of peripheral oxytocin to central oxytocin neurons via vagal afferents for regulating feeding. Biochem Biophys Res Commun. 2019;519:553–8.
94. Masi A, Narducci R, Resta F, Carbone C, Kobayashi K, Mannaioni G. Differential contribution of Ih to the integration of excitatory synaptic inputs in substantia nigra pars compacta and ventral tegmental area dopaminergic neurons. Eur J Neurosci. 2015;42:2699–706.
95. Arencibia-Albite F, Vázquez R, Velásquez-Martinez MC, Jiménez-Rivera CA. Cocaine sensitization inhibits the hyperpolarization-activated cation current Ih and reduces cell size in dopamine neurons of the ventral tegmental area. J Neurophysiol. 2012;107:2271–82.
96. Liu JJ, Eyring KW, König GM, Kostenis E, Tsien RW. Oxytocin-modulated ion channel ensemble controls depolarization, integration and burst firing in CA2 pyramidal neurons. J Neurosci. 2022;42:7707–20.

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AUTHOR CONTRIBUTIONS

DM, CM, LLI, SC, CC, MR and RV conceived and designed all the experiments; SLD, AP, MDSLB, performed the in-vivo experiments; SN, MT, GC, GM, SC, VC, MTV performed the ex-vivo experiments; DM, CM, MR MTV and RV wrote the paper in the original draft; all authors reviewed the manuscript; MR and RV funded the study.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

All methods were performed in accordance with Italian national laws (DL 116/92 and DL 26/2014) regulating the use of animals for scientific purposes, and in compliance with European Communities Council Directives (86/609/EEC and 2010/63/EU). The experimental protocols (numbers 769/2017 and 901/2023) were approved by the Italian Ministry of Health.

ADDITIONAL INFORMATION

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