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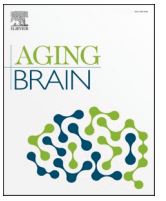

Age-related decline in social interaction is associated with decreased *c-Fos* induction in select brain regions independent of oxytocin receptor expression profiles

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ABSTRACT

Social behavior decreases with aging, and we have previously found a substantial decline in social investigative behavior of old female rats. In this study we examined the neural activation pattern (*c-Fos* mRNA) of young (3 month) and old (18 month) female rats after brief 10 min exposure to a novel female rat in order to identify forebrain regions that show selective age-related alterations in their neural response to social investigation. We also measured relative oxytocin receptor expression (*Oxtr* mRNA) as a possible factor in age-related declines in *c-Fos* induction after social interaction. Young rats exposed to a social partner had a greater *c-Fos* mRNA response than those exposed to novel context alone in the lateral septum and septohypothalamic area, with blunted increases evident in old rats. In addition, *c-Fos* mRNA levels in the lateral septum were positively correlated with social investigative behavior. Interestingly, age-related differences in *c-Fos* gene induction were unrelated to the local amount of *Oxtr* expression within specific brain regions, although we found an age-related decline in *Oxtr* expression in the ventromedial hypothalamus. This functional neuroanatomical characterization may point to certain brain regions that are especially sensitive to age-related declines associated with social interaction behavior.

Introduction

Cognitive decline is inevitable with advanced aging of the brain. However, cognition encompasses a complex set of abilities and each of these abilities likely has its own vulnerability and rate of progression with advanced aging [15]. One important aspect of age-

Abbreviations: AC, anterior cingulate cortex; AOP, anterior olfactory nucleus, posterior; BF, barrel field cortex; BST, bed nucleus of the stria terminalis; BSTlp, bed nucleus of the stria terminalis, lateral posterior part; BSTma, bed nucleus of the stria terminalis, medial anterior part; CeA, central nucleus of the amygdala; DG, dentate gyrus; DEn, dorsal endopiriform nucleus; DTT, dorsal tenia tecta; IL, infralimbic cortex; LSd, lateral septum, dorsal; LSv, lateral septum, ventral; MeA, medial amygdala; *Oxtr*, oxytocin receptor; PL, prelimbic cortex; SHy, septo-hypothalamic area; VCl, ventral claustrum; VO, ventral orbital cortex; VMH, ventromedial amygdala.

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related cognitive decline relates to social cognition and social behavior more generally. A decline in social interactions is observed in the elderly as a result of both a reduction in interpersonal social network size and decreased preference for interacting with new people [6]. Importantly, decreased levels of social contact are associated with greater cognitive decline and poorer health outcomes in old adults [6,47,31]. Identifying specific age-related changes in neural processes associated with age-related decreases in social interaction may provide valuable insights into social behavior-relevant neurocircuit function that is vulnerable to the aging process [24].

Previous research has noted that there is a highly conserved set of brain structures that play an active role in multiple types of social behaviors [14,18,31,47,33,48]. Key nodes within this circuit include regions of the hypothalamus (e.g., anterior and ventromedial hypothalamus), lateral septum, brainstem regions (e.g., periaqueductal gray), preoptic area, and the extended amygdala, including both the medial amygdala and medial bed nucleus of the stria terminalis [18,34]. In rodents, social interaction behaviors rely heavily on olfactory signals and as such, social interaction also activates brain regions associated with olfactory processing (e.g., olfactory nuclei and piriform cortex) [10].

Regulation of appropriate situation specific social interaction behaviors depends on a wide range of neurochemical and neuropeptide components including dopamine, oxytocin, and vasopressin [21,34]. These intercellular mediators and their cognate receptors are expressed in key nodes of the mammalian social behavior circuit (described above). Consistent with this, pharmacological manipulation of these signaling systems can influence partner preference in prairie voles [57] as well as social recognition memory in rodents [9]. In particular, the oxytocinergic neuropeptide signaling system has been implicated in regulation of various aspects of social behavior, including affiliative, parental, and territorial behaviors [4,12,21,26]. There is also some evidence for aging-related declines in oxytocin signaling [2,8,17,20], and this decrease may contribute to age-related declines in social behavior.

We have previously reported a decline in social exploration of novel same sex rats in late-aged rats (18+ months), with this decline being more pronounced in females than males [41]. We have also found several brain regions of young female rats that exhibit greater neuronal activation as indicted by induction of the immediate early gene *c-Fos* when exposed to a novel rat than when exposed to the novel context alone [42]. The primary goal of this study was to determine if a decline in social interaction observed in 18-month-old female rats is also associated with attenuated neuronal activation of select forebrain regions during the social interaction experience. For this assessment, we measured expression of the immediate early gene *c-Fos*. *c-Fos* gene expression is widely used as a marker of recent increased neuronal activity in the rodent brain [1,19]. In addition, the expression of the *c-Fos* gene does not simply reflect relative firing rate of neurons, but instead, *c-Fos* gene expression is rapidly induced by experiential events that drive changes in neuroplasticity [16,23]. The use of *in situ* hybridization to measure relative *c-Fos* mRNA levels provides not only for high spatial resolution, but also better temporal resolution than is provided by measurement of FOS protein [1]. We compared the *c-Fos* mRNA induction in young (3 month) and old (18 month) female Fischer 344 (F344) rats that were placed in a test environment and then allowed to interact for 10 min with a novel young adult female rat. In addition, we also examined whether there were age-related differences in oxytocin receptor expression. We selected a number of forebrain regions of interest for evaluation. These brain regions include cortical and subcortical structures that previously showed increased neural responses in young female rats to these test conditions [42]. Importantly, several of our regions of interest are found in the highly conserved social behavior network described above [14,31,47,34,33,48]. In addition, some of these regions have enriched oxytocin receptor expression levels and may therefore be especially important for age-related declines in social behavior. We hypothesized that within social interaction responsive brain regions, aged rats would show blunted *c-Fos* gene expression following social interaction, relative to young adult rats.

Material and methods

Subjects

Female F344 rats were obtained from the National Institute of Aging colony maintained by Charles River Laboratories at 3 month (young adults) and 18 month (old) of age. Rats were given at least 1 week to acclimate to the Binghamton University vivarium before the onset of experimentation. Social partners for the social interaction condition were 3 month old F344 female rats. Vivarium conditions were maintained at 22 ± 1 °C with 12:12 light:dark cycle (lights on 0700 h). Rats were pair-housed in standard polycarbonate cages with pine shavings for bedding material. Rats were provided *ad libitum* access to food and water. Rats were handled for 3 min per day for two days prior to the onset of behavioral testing. At all times, rats were maintained and treated in accordance with the guidelines set forth by the Office of Laboratory Animal Welfare and in accordance with the protocol approved by the IACUC at Binghamton University.

Experimental procedure

Testing was conducted in rooms with dim lighting (10–20 lx) between 0800 h and 1200 h. Experimental subjects were placed in Plexiglas social interaction chambers (Binghamton Plate Glass, Binghamton, NY) 45 × 30 × 30 cm. Clean pine shavings lined the bottoms of the chambers. Each test chamber was divided into two compartments that were equal in size by a clear Plexiglas partition containing a rectangular aperture (9 × 7 cm) that allowed for movement of rats between the compartments. After testing of each subject, the soiled wood shavings were removed, chambers were cleaned with water, and clean shavings were added for the next subject. Behavior of the rats was recorded by a camcorder (Panasonic model AF-X8, Secaucus, NJ). At the end of each day, chambers were wiped down with a 3 % hydrogen peroxide solution.

Rats of both age conditions were randomly assigned to one of three experimental behavioral conditions: home cage controls (HCC; 10 young and 10 old rats), context exposure (CXT; 10 young and 10 old rats), and social interaction exposure (SI; 10 young and 10 old

rats). HCC rats were handled prior to the test day, but did not undergo any behavioral testing. Both CXT and SI rats were habituated to the testing apparatus for 30 min on the day prior to testing and tissue collection. On the test day, CXT rats were placed into the testing apparatus for 30 min. SI rats were placed into the apparatus for 20 min, after which a novel 3 month old female social partner was placed in a chamber for a 10-min social interaction test (Fig. 1). Rats were returned to their home cage after testing and brains were collected 30 min after the end of testing, a time interval that we have previously found results in peak *c-Fos* mRNA expression in most brain regions after exposure to a variety of novel experiences [37,36].

Brain tissue collection and sectioning

Rats were rapidly decapitated (unanesthetized) 30 mins after the end of testing (CXT and SI). Home cage control rats (HCC) were decapitated at the same time. Brains were removed and flash-frozen in methylbutane (EMD Millipore, Darmstadt, Germany; cat. no. MX0760-1) chilled to −20° to −30 °C with dry ice and stored at −80 °C until processing. Coronal brain sections (12 µm) were cut on a cryostat (Leica model 1850), thaw-mounted on Colorfrost® plus microscope slides and stored at −80 °C. A rostral, intermediate and caudal series of sections (6 sections per slide, 6 slides per series, 2 series per target region) were collected centered around three forebrain regions that included our regions of interest (ROI) as demarcated in the Paxinos and Watson rat brain atlas [38] (Fig. 2). The rostral series of sections (centered around + 3.24 mm relative to bregma) contained the following ROIs: anterior cingulate cortex (AC), prelimbic cortex (PL), infralimbic cortex (IL), insula, ventral orbital cortex (VO), anterior olfactory nucleus/posterior (AOP), dorsal tenia tecta (DTT) layers 2 (DTT II) and layers 3/4 (DTT III/IV). The mid-level series of sections (centered around -0.24 mm relative to bregma) contained the dorsal lateral septum (LSd), ventral lateral septum (LSv), bed nucleus of the stria terminalis (BST) medial anterior portion (BSTma), BST lateral posterior portion (BSTlp), septo-hypothalamic area (SHy), ventral claustrum (VCl), and dorsal endopiriform nucleus (DEn). Concerning the septo-hypothalamic area, some rat brain atlases [39,52] include this region as part of the ventral lateral septum. However, we observed greater *c-Fos* expression and lower *Oxtr* expression in this brain region compared to the nearby lateral septum (see Results). Consequently, using key anatomical landmarks [3,38] that included the relative position to the anterior commissure, lateral ventricle and fornix (Fig. 4), we analyzed the septo-hypothalamic area as a distinct ROI separate from the ventral lateral septum. The caudal series of sections (centered around −2.76 mm relative to bregma) contained the barrel field cortex (BF) layers 2/3 (BF 2/3) and layers 5/6 (BF 5/6), hippocampal CA1 region, hippocampal CA3 region, dentate gyrus (DG), medial amygdala (MeA), ventromedial amygdala (VMH) and central nucleus of the amygdala (CeA).

Behavioral measures

The total number of crossovers (movement through the aperture) demonstrated by the CXT and SI groups was recorded for each 10-min time bin of testing and was used as an index of locomotor activity. For rats in the SI group, social investigation was defined as nose contact with any part of the body of the partner, whereas frequency of contact behavior was scored as the sum of crawling over and under the partner and social grooming.

Radiolabelled in situ hybridization and densitometry analysis

The radiolabelled *in situ* hybridization procedure used to assess *c-Fos* mRNA expression has been described previously [32]. Briefly,

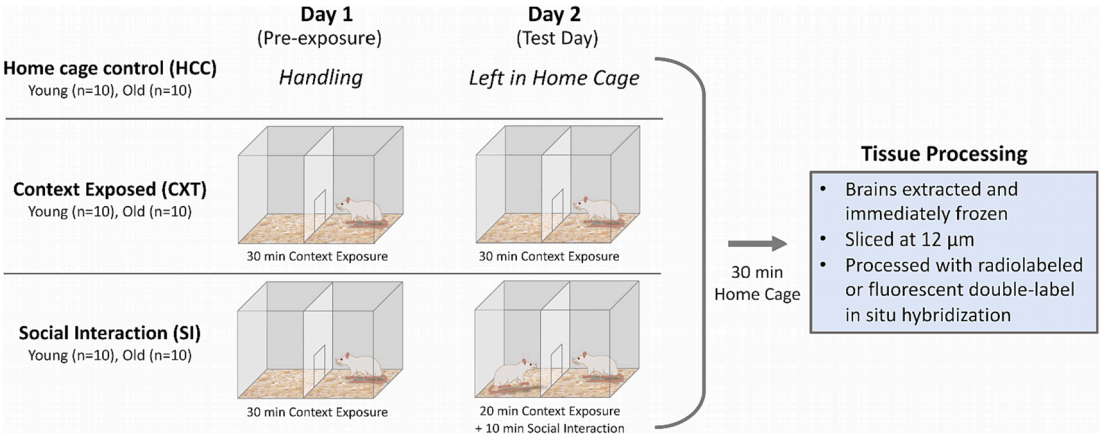

Fig. 1. Experimental Time-line. On the Test Day, young (3 months) and old (18 months) female rats were exposed to one of three experimental behavioral conditions: 1) home cage controls (HCC), 2) 30 min of context exposure (CXT) or 3) 20 min of context exposure followed by 10 min of social interaction exposure within the same context (SI). Rats in CXT and SI conditions were returned to their home cage for 30 min before sacrifice for brain extraction. The day before testing (Pre-exposure), CXT and SI rats were exposed to the context for 30 min. HCC rats were briefly handled the day before testing.

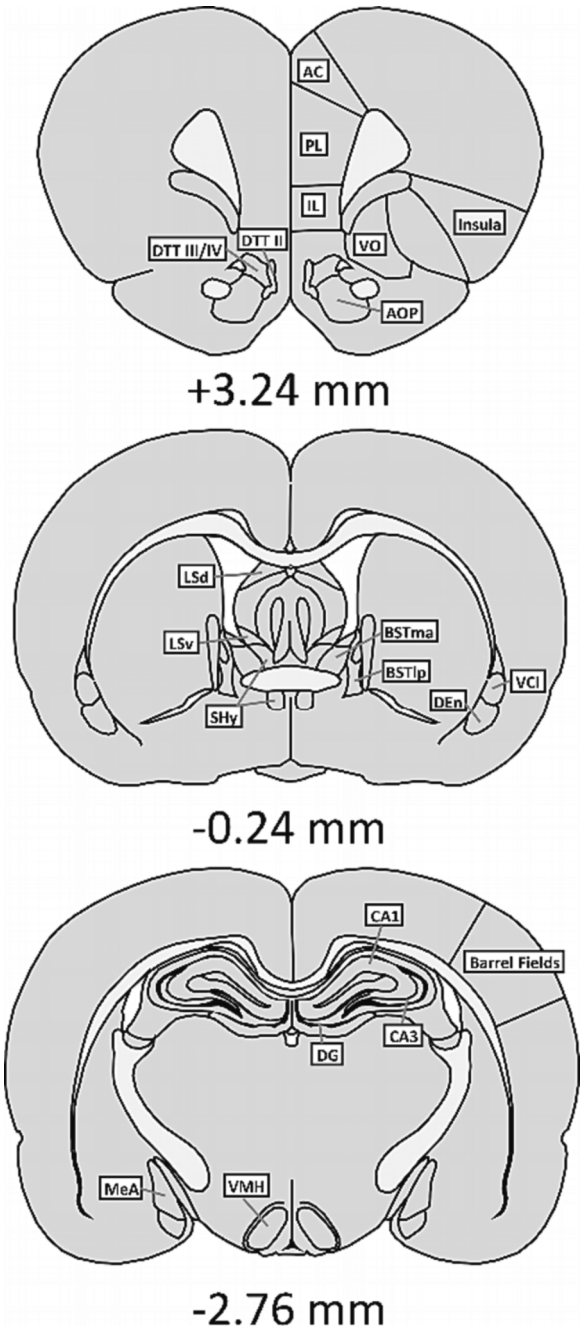

Fig. 2. Forebrain Regions of Interest. Coronal brain sections were collected at a rostral (~+3.24 mm), mid-level (~−0.24 mm) and caudal (~−2.76 mm) location relative to bregma. Regions of interest at each rostral/caudal level are denoted on rat brain atlas diagrams adapted from Paxinos and Watson [38]. Regions of Interest: AC (anterior cingulate cortex), PL (prelimbic cortex), IL (infralimbic cortex), VO (ventral orbital cortex), DTT II (dorsal tenia tecta layer 2), DTT III/IV (DTT layer 3/4), AOP (anterior olfactory nucleus, posterior), dLS (dorsal lateral septum), vLS (ventral LS), BSTma (bed nucleus of stria terminalis, medial anterior), BSTlp (BST, lateral posterior), SHy (septo-hypothalamic area), DEn (dorsal endopiriform nucleus), VCl (ventral claustrum), CA1 (hippocampal CA1 region), CA3 (hippocampal CA3 regions), DG (dentate gyrus), BF 2/3 (barrel field cortex layers 2/3), BF 5/6 (BF layers 5/6), CeA (central nucleus of the amygdala), MeA (medial amygdala), VMH (ventromedial hypothalamus).

sections were fixed in 4 % paraformaldehyde for 1 h, followed by a 10 min acetylation step in 0.1 M triethanolamine with 0.25 % acetic anhydride. Sections were then dehydrated using graded alcohols. Sections were hybridized overnight at 55 °C in hybridization buffer containing a [35S]-UTP-labeled riboprobe generated from a 680 base pair cDNA template that corresponds to nucleotides 596–1171 of *c-Fos* mRNA (Accession # X06769.1; courtesy of Dr. T. Curran, St Jude Children's Hospital, Memphis, TN, USA). The next day, sections were incubated with RNase A (200 μg/ml) at 37 °C for 1 h. Sections were then given a high stringency incubation of 0.1 × saline

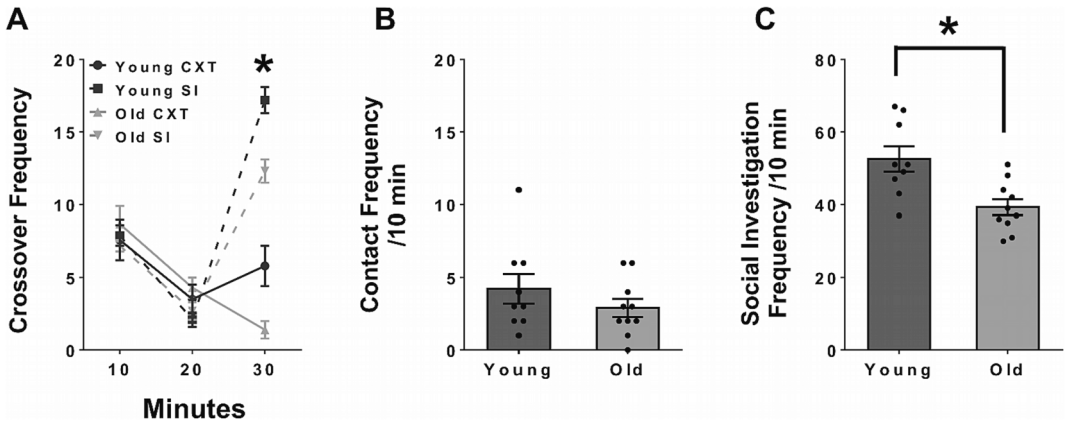

Fig. 3. Young rats exhibited greater activity and social behavior in the presence of a conspecific than old rats. (A) There was no difference in activity levels (crossover frequency) between young (3 months) and old (18 months) rats when placed for 30 min in a context alone (CXT). In the social interaction condition (SI), rats significantly increased their activity levels during the third 10-min bin; *p <.001, age effect during the third 10-min bin driven by the greater increased activity of young rats compared to old rats during social interaction. (B) For the SI condition there was no age difference in contact frequency, but, (C) young rats engaged in significantly greater social investigation; *p <.05 (Student's *t*-test).

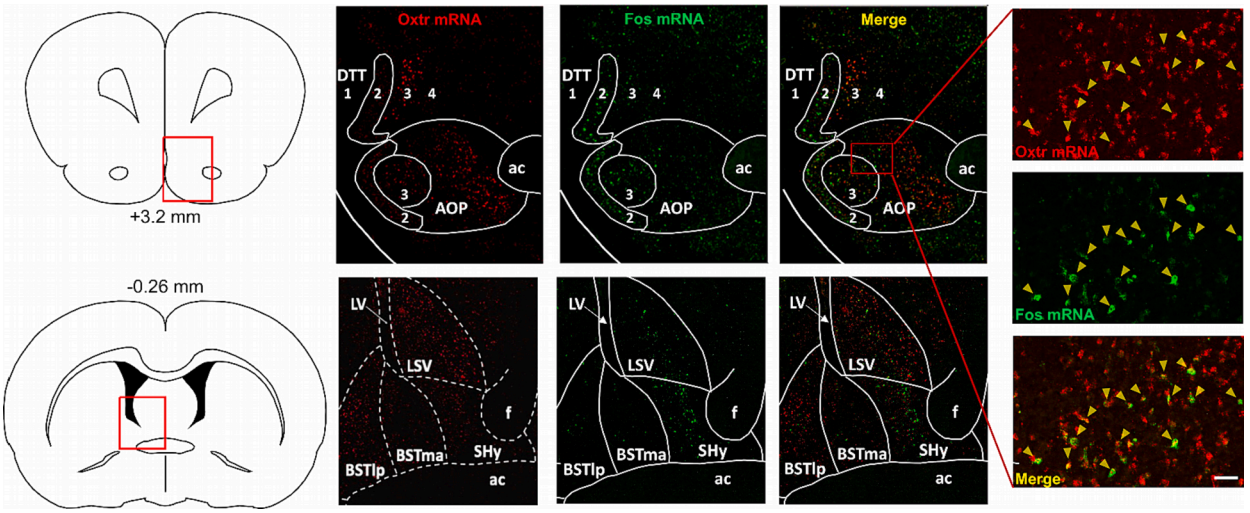

Fig. 4. Sample photomicrographs showing double-label fluorescent *in situ* hybridization for *c-Fos* and oxytocin receptor (*Oxtr*) mRNA of a young female rat after social interaction. Top row shows an example photomicrograph taken from a rostral brain section (approximate location denoted by red dashed line on corresponding rat brain atlas diagram) featuring the anterior olfactory nucleus (AOP) and dorsal tenia tecta (DTT). Note enriched *Oxtr* expression (red fluorescence) in AOP and layer 3, but not layer 2 of the DTT, with *c-Fos* expression (green fluorescence) in each of these brain regions. Bottom row shows an example photomicrograph taken from a mid-level brain section (approximate location denoted by red dashed line on corresponding rat brain atlas diagram) featuring the ventral lateral septum (LSV), lateral bed nucleus of the stria terminalis (BST) lateral posterior (BSTlp) and medial anterior (BSTma) subregions, and the septohypothalamic nucleus (SHy). Note enriched *Oxtr* expression in LSV and BSTlp compared to BSTma and SHy. The three panels on the far right show an enlarged portion of the AOP (red dashed rectangle indicated on the merged top row panel), illustrating the extensive colocalization of *c-Fos* mRNA positive cells with *Oxtr* mRNA positive cells (arrow heads). Scale bar = 50 μm. ac = anterior commissure, LV = lateral ventricle, f = fornix.

sodium citrate (65 °C) for 1 h. Finally, sections were dehydrated and placed into light-tight cassettes with an overlying sheet of highly sensitive X-ray film (BioMax MR; Eastman Kodak, Rochester, NY) for structure-appropriate times (1–3 weeks). Tissue sections from each of the 60 brains for a given rostral-caudal brain level were included in the same *c-Fos* mRNA assay. In order to assess oxytocin receptor (*Oxtr*) mRNA expression, the same procedure was followed with use of a [35S]-UTP-labeled riboprobe generated from a 924 base pair cDNA template that corresponds to nucleotides 132–1055 of *Oxtr* mRNA (Accession # 012871.4; courtesy of Dr. Serge Campeau and Dr. Heidi Day, University of Colorado Boulder, CO, USA). Tissue sections from each of the 60 brains for a given rostral-caudal brain level were included in a single assay. Uncalibrated optical density measurements from left and right hemispheres on 4–6 sections per brain for each ROI was performed by an individual blind to the treatment condition. Optical density values were averaged across each of the tissue sections/hemispheres for each brain to yield a single value for that brain.

Double-label fluorescent in situ hybridization

The first stages of the *in situ* hybridization procedure was the same as described above for the radiolabelled procedure, after which additional steps were performed in order to fluorescently tag specific hybridization duplexes. Sections were hybridized overnight at 55 °C in hybridization buffer containing both a Digoxigenin-11-UTP (Roche #11 209 256 910) labeled riboprobe against *Oxtr* mRNA and a Flourescein-12-UTP (Roche #11 427 857 910) labeled riboprobe against *c-Fos* mRNA (same cDNA templates as used for radiolabeled *in situ* hybridization). Fluorescent labeling of the hybridized *Oxtr* and *c-Fos* probes was carried out using the TSA-Plus Cyanine 3/Fluorescein System (Perkin-Elmer #NEL753). Sections were incubated in freshly made 2 % H_2O_2 in 0.05 M PBS for 30 min with gentle agitation to quench endogenous peroxidases. Sections were then blocked for 1 h in 0.5 % blocking reagent (Perkin-Elmer #FP1012) in tris buffered saline (TBS). Next, sections were incubated in anti-digoxigenin-horseradish peroxidase (HRP; 1:1000 in blocking buffer, Roche #11 207 733 910) for 30 min. Sections were then incubated with Cy3 fluorophore and tyramide amplification reagent (1:100 in amplification diluent, Perkin Elmer #NEL753) for 45 min. Sections were then washed in TBS and stored overnight in 0.05 M PBS at 4 °C. The next day, sections underwent a second peroxidase quenching step, again for 30 min, followed by a 2 h incubation in anti-fluorescein-HRP (1:250 in blocking buffer, Perkin-Elmer #NEL753). Sections were then incubated with the fluorescein fluorophore and tyramide amplification reagent (1:100 in amplification diluent) for 1 h. Finally, sections were washed in TBS before being coverslipped with Prolong Gold Antifade Mountant with DAPI (Invitrogen #P36931). Tissue sections from each of the 60 brains for a given rostral-caudal brain level were either included in a single assay or were evenly split in a counterbalanced fashion across treatment groups into two assays. For *Oxtr* mRNA expression measures, in which the only factor of interest was Age, tissue sections from all 30 rats (AOP, DTT III/IV) or 15 rats of each age counterbalanced across the three test day treatment conditions (LSv, BSTlp, VMH) were assayed.

Fluorescent imaging and cell counts

Tiled three-channel images of regions of tissue sections were collected with 10x objective using high efficiency red, green and blue fluorescent filter sets to image *Oxtr* mRNA, *c-Fos* mRNA and DAPI, respectively (Zeiss Axio Imager M1 epifluorescent microscope, Axiocam 305 monochrome camera, and ZEN software, Carl Zeiss Microscopy, Thornwood NY). Cell counting was performed using ImageJ by an individual blind to the treatment group assignments. Images were opened and channels were split to isolate the green (*c-Fos*) channel. The background was subtracted using a 'rolling ball radius' of 50 pixels. Tissue sections for analysis were chosen that closely matched the proximal tissue landmarks for the ROI according to the Paxinos and Watson 7th Edition Atlas [38]. A hand drawn ROI was then affixed in the appropriate location based solely on tissue landmarks, such as nearby white matter and ventricular profiles. Fluorescent intensity thresholding was applied to acquired images using consistent settings across images. Cells were counted using the 'Analyze Particles' feature, selecting for contiguous particles $\geq$ 25 pixels (~15 μm in diameter). For each ROI, three images from three nearby sections were analyzed per animal and counts were averaged.

Statistical analyses

Test day behavioral activity (crossover frequency) was evaluated using a 3-way mixed-factor ANOVA, with Age (young, old) and Test Condition (context, social interaction) as between-group factors and 10-min bins as a within-subjects factor. Post hoc tests of within and between group differences in crossover behavior across bins was assessed using Fisher Least Significance Difference Test (FLSD). Age differences in social investigation and contact behavior were analyzed using a *t*-test. *c-Fos* mRNA expression in each ROI was assessed by separate analyses for each region using 2 (Age: young, old) × 3 (Test Condition: homecage, context, social interaction) between groups ANOVAs. Significant main effects of Age were followed up with pairwise post hoc tests of Age within each Test Condition (Holm-Sidak's multiple comparison post hoc test). Significant main effects of Test Condition were followed up with pairwise post hoc tests collapsing across Age (Holm-Sidak's multiple comparison post hoc test). Exploratory pairwise post hoc tests of Test Condition within each age group were also performed and are indicated on the data figures and Tables (Holm-Sidak's multiple comparison post hoc test or FLSD). Only brain regions that had a significant Age by Test Condition interaction (ANOVA) are featured in the main conclusions of the study. Correlation analysis of crossover behavior or social investigation behavior with *c-Fos* mRNA expression (double-label fluorescent *in situ* hybridization) used Pearson correlation coefficient. Age differences in *Oxtr* mRNA were analyzed by Student's *t*-test (two-tailed test, unless indicated otherwise). For all statistical tests, alpha level was a priori set at $p \leq 0.05$. Data presented in figures show group mean ± SEM.

Results

Context and social interaction behavior

A 2 (Age) × 3 (Time) ANOVA of locomotor activity (crossover frequency) revealed significant Age by Time, [$F(2,72) = 11.42$, $p < 0.001$], as well as Test Condition by Time, [$F(2,72) = 72.26$, $p < 0.001$] interactions (Fig. 3). Post hoc tests indicate that similar levels of activity were exhibited in young and old rats in the first 10 min ($p = 0.75$) and second 10 min ($p = 0.39$) in the chamber (Fig. 3A). Young and old rats that remained in the chamber alone for an additional 10-min period (CXT condition) did not show any changes in locomotor activity during the third 10-min bin relative to the first 20-min ($p = 0.73$). In contrast, there was a substantial increase in locomotor activity levels for young and old rats that had a conspecific placed into the chamber (SI condition) for the final 10-min

period of testing ($p < 0.00001$). Regardless of condition, young females demonstrated more crossovers during the third 10-min bin than their old counterparts ($p < 0.0001$), although this effect appears to be driven by the SI group (Fig. 3A). Social contact behavior did not differ as a function of age [$t(17) = 1.13$, $p = 0.28$; Fig. 3B], but the young females exhibited more social investigation than the old rats [$t(17) = 3.31$, $p = 0.004$; Fig. 3C].

c-Fos mRNA (radiolabeled in situ hybridization)

We first used radiolabeled *in situ* hybridization to examine *c-Fos* mRNA expression in a number of cortical and subcortical forebrain regions of interest. This is a high throughput method that is effective for mapping relative mRNA expression patterns across a wide range of brain regions. This method also allows for direct comparison to our findings in a previous study in which we used this method to examine relative *c-Fos* mRNA in young female and young male rats (4 month old) undergoing the same test day conditions as used in this study [42].

Separate 2 (Age) × 3 (Test Condition) ANOVAs of each ROI revealed main effects of Test Condition for all ROIs [$F(2,54) > 9.0$, and $p < .001$ for all ROIs] (Supplemental Table 1). As expected, placement of rats for 30 min in a chamber (with or without social interaction for the final 10 min) produced a large increase in *c-Fos* mRNA in all brain regions examined compared to the home cage control condition (Supplemental Table 1, Supplemental Fig. 1). In a number of brain regions there was a trend for overall greater *c-Fos* mRNA in the social interaction condition compared to the context only condition. However, post hoc tests of test condition (collapsing across age) indicate that this difference was only statistically significant in layer 2/3 of the Barrel Fields ($p = .006$). Exploratory post hoc tests within each age group support a greater *c-Fos* mRNA response in the social interaction condition compared to context that was restricted to young rats in layers 2/3 of the Barrel Fields ($p = .002$), layer 5/6 of the Barrel Fields ($p = .045$) and the dorsal lateral septum ($p = .031$) (Supplemental Table 1, Supplemental Fig. 1A). This differential age effect, however, was not large enough to produce a significant Age by Test Condition interaction. In addition, for several brain regions there was an overall main effect of Age, with less *c-Fos* mRNA levels in old rats compared to young rats in the CA3 region of the hippocampus [$F(1,54) = 9.49$, $p = .003$], dentate gyrus [$F(1,54) = 12.10$, $p = .001$] and medial amygdala [$F(1,54) = 5.24$, $p = .03$] (Supplemental Table 1, Supplemental Fig. 1B).

c-Fos mRNA in oxytocin receptor enriched brain regions and other nearby regions of interest (fluorescent double-labeled in situ hybridization)

In follow-up to our radiolabelled *in situ* hybridization analyses, we used a *c-Fos/Oxtr* double-labeled fluorescent *in situ* hybridization procedure to assess whether group differences in *c-Fos* mRNA expression profiles were greater in brain regions with relatively high oxytocin receptor expression (Fig. 4). Visual inspection of *Oxtr* mRNA expression in the forebrain revealed especially dense *Oxtr* mRNA levels in the ventromedial hypothalamus, central nucleus of the amygdala, anterior olfactory nucleus and layers III/IV of the tenia tecta, consistent with previous reports [12]. We observed relatively lower *Oxtr* mRNA levels within the septal and bed nucleus of the stria terminalis subregions, but distinct enrichment within the ventral lateral septum and lateral posterior bed nucleus of the stria terminalis compared to the nearby medial anterior bed nucleus of the stria terminalis and septohypothalamic nucleus (Fig. 4). We

Table 1
Relative *c-Fos* mRNA – Fluorescent *in situ* Hybridization: Cell Counts (SEM) and 2-Way ANOVA Probabilities.

Brain Region	Young (3 Months)			Old (18 Months)			ANOVA Probabilities		
	HC	CXT	SI	HC	CXT	SI	Age	Test Condition	Interaction
With Enriched Oxtr Expression									
AOP	0.7(0.3)	28.3(4.4)*	51.7(8.7)*†	0.4(0.3)	26.4(3.7)*	36.8(9.2)*	0.24	**<0.001**	0.40
DTT III/IV	0.0(0.0)	10.2(1.1)*	11.6(1.5)*	0.1(0.1)	6.8(0.8)*	11.0(2.8)*	0.28	**<0.001**	0.44
BSTlp	1.5(0.4)	7.7(1.3)*	9.5(1.2)*	2.5(0.7)	7.0(1.8)*	9.0(1.3)*	0.95	**<0.001**	0.76
LSv	0.2(0.1)	8.4(1.4)*	12.7(2.0)*	0.3(0.1)	3.2(1.0)*$	5.8(1.0)*$	**<0.001**	**<0.001**	**0.01**
VMH	1.3(0.6)	5.8(1.2)*	6.8(1.3)*	1.1(0.8)	4.2(0.9)*	6.0(0.6)*	0.31	**<0.001**	0.79
Without Enriched Oxtr Expression									
DTT II	0.9(0.4)	23.3(3.0)*	29.3(3.9)*	0.1(0.1)	22.8(3.6)*	25.1(5.6)*	0.51	**<0.001**	0.83
BSTma	0.3(0.1)	4.8(0.8)*	9.0(1.6)*†	0.5(0.3)	3.3(0.7)*	6.6(1.3)*	0.14	**<0.001**	0.39
SHy rostral	0.8(0.4)	31.6(9.0)*	72.2(14.8)*†	3.0(1.1)	40.4(13.2)*	32.0(8.6)*	0.22	**<0.001**	**0.03**
SHy medial	1.2(0.5)	58.9(7.1)*	81.9(15.2)*	2.4(0.8)	32.0(9.1)*	36.7(7.4)*$	**0.001**	**<0.001**	**0.025**
SHy caudal	0.8(0.4)	48.0(5.1)*	68.8(13.7)*	1.0(0.4)	26.4(5.9)*	27.6(2.9)*$	**<0.001**	**<0.001**	**0.01**
VCl	0.2(0.1)	11.4(2.0)*	23.9(2.1)*†	0.4(0.1)	11.6(2.3)*	17.3(3.6)*	0.24	**<0.001**	0.21
DEn	0.1(0.1)	9.6(1.0)*	18.4(2.8)*†	0.4(0.1)	8.6(2.1)*	11.3(2.1)*	0.07	**<0.001**	0.10

Significant ANOVA probabilities ($p < .05$) are highlighted in bold text. Post hoc tests:*$p < .05$, significantly different from HC of same age group; †$p < .05$, significantly different from CXT of same age group; $$p < .05$, significantly different from Young of same test condition (Holm-Sidak's test). Abbreviations: HC (home cage), CXT (context only), SI (social interaction), *Oxtr* (oxytocin receptor), AOP (anterior olfactory nucleus, posterior), DTT II (dorsal tenia tecta layer 2), DTT III/IV (DTT layers 3 and 4), BSTlp (bed nucleus of stria terminalis, lateral posterior), BSTma (BST, medial anterior), LSv (lateral septum, ventral), VMH (ventromedial hypothalamus), SHy (septo-hypothalamic area), VCl (ventral claustrum), DEn (dorsal endopiriform nucleus).

found enriched *Oxtr* mRNA signal present within most identifiable cells within the anterior olfactory nucleus, tenia tecta layers III/IV, lateral septum, lateral posterior bed nucleus of the stria terminalis and the ventromedial hypothalamus (see anterior olfactory nucleus as an example, Fig. 4). Thus, it was not practical to perform separate counts of *c-Fos* mRNA positive cells within *Oxtr* mRNA positive and negative cells. Instead, we grouped the *c-Fos* mRNA cell counts for these regions of interest according to regions with enriched or not enriched *Oxtr* mRNA expression. We also observed on mid rostral-caudal tissue sections relatively high *c-Fos* mRNA expression in the dorsal endopiriform nucleus and ventral claustrum, two cortical brain regions reported to receive oxytocin neuron innervation [50]. We therefore included these two regions within our analysis. Despite the reported oxytocin neuron innervation of these two closely related brain regions [49], neither had enriched *Oxtr* mRNA expression, as previously reported [50].

Consistent with the results of relative *c-Fos* mRNA expression assessed by radiolabeled *in situ* hybridization, there was a large main effect of test condition for all brain regions examined with fluorescent *in situ* hybridization [$F(2,54) > 14.0$, and $p < .001$ for all ROIs] (Table 1). As expected, post hoc tests of test condition that collapsed across age found that for all brain regions there was increased *c-*

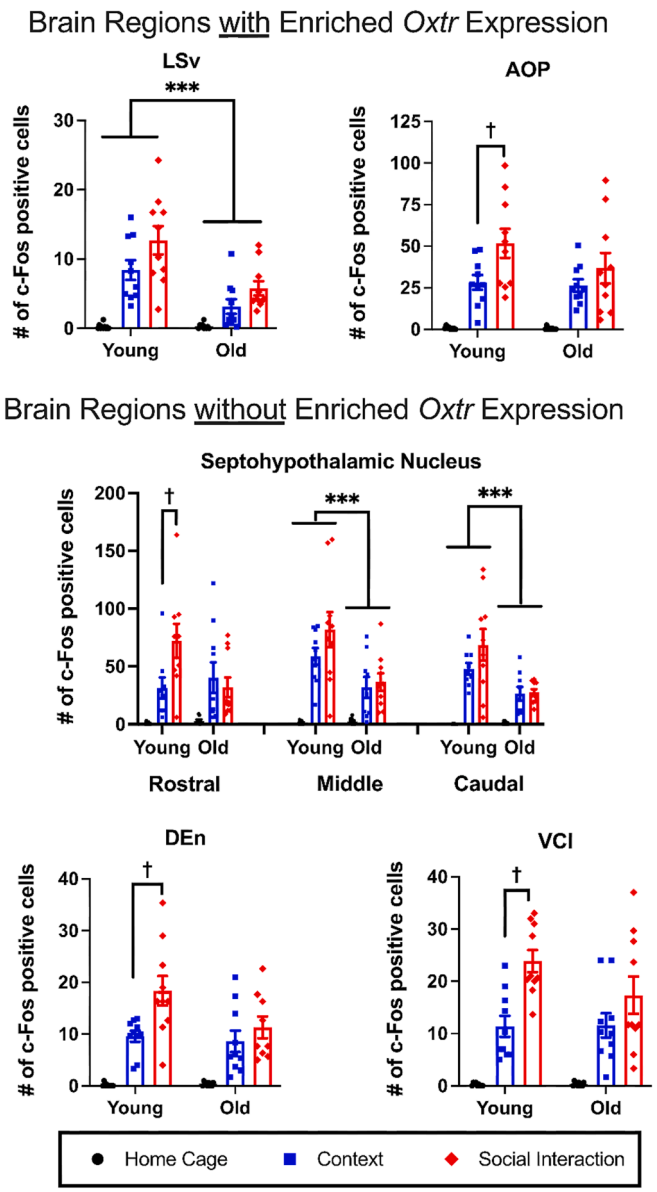

Fig. 5. Effect of age and social interaction on c-*Fos* expression patterns in brain regions of interest with or without enriched oxytocin receptor (*Oxtr*) expression (double-label fluorescent *in situ* hybridization). Bar graphs of brain regions of interest in which there were age-related differences in *c-Fos* mRNA (# of *c-Fos* mRNA positive cells). †$p < .05$, significantly different from context test condition of same age group (exploratory Holm-Sidak's test); ***$p < .001$, overall Age Effect (ANOVA). Abbreviations: LSv (lateral septum, ventral), AOP (anterior olfactory nucleus, posterior), DEn (dorsal endopiriform nucleus), VCl (ventral claustrum).

Fos mRNA for the context and social interaction conditions compared to the home cage condition (Holm-Sidak test). In addition, these post hoc tests also found increased *c-Fos* mRNA for the social interaction condition compared to the context alone condition in the anterior olfactory nucleus ($p = .016$), ventral lateral septum ($p = .015$), medial anterior bed nucleus of the stria terminalis ($p < .001$), ventral claustrum ($p < .001$) and dorsal endopiriform nucleus ($p = .004$).

Because we observed especially high levels of *c-Fos* mRNA within the septohypothalamic nucleus after social interaction, we further examined the extent to which treatment effects on *c-Fos* mRNA were similar across the rostral, middle and caudal portions of the septohypothalamic nucleus (Table 1, Fig. 5). For each rostral-caudal level of the septohypothalamic nucleus there was a significant Age by Test Condition interaction [rostral: $F(2,51) = 3.88$, $p = .03$; middle: $F(2,53) = 3.95$, $p = .025$; caudal: $F(2,51) = 4.83$, $p = .01$]. Post hoc tests indicated that there was a significantly greater amount of *c-Fos* mRNA in the social interaction condition compared to the context condition for young rats ($p = .021$), but not old rats within the rostral portion of the septohypothalamic nucleus (Table 1, Fig. 5). There was also a main effect of Age within the middle [$F(1,53) = 11.91$, $p = .001$] and caudal [$F(1,51) = 14.31$, $p < .001$] portion of the septohypothalamic nucleus, with overall lower levels of *c-Fos* mRNA in the old rats (Table 1, Fig. 5). Similar results were found for the ventral lateral septum with a significant Age by Test Condition interaction [$F(2,54) = 4.73$, $p = .01$] and a main effect of Age [$F(1,54) = 17.29$, $p < .001$] (Table 1, Fig. 5). In addition, exploratory post hoc tests for Test Condition suggest that there was a greater amount of *c-Fos* mRNA in the social interaction condition compared to the context condition for young rats, but not old rats, within the anterior olfactory nucleus ($p = .023$), endopiriform nucleus ($p = .005$), and ventral claustrum ($p < .001$) (Fig. 5; Table 1). This age difference, however, was not large enough to produce a significant Age × Test Condition interaction in these brain regions.

No age-related effects were found in the dorsal tenia tecta, bed nucleus of the stria terminalis or the ventromedial hypothalamus. It should also be noted that there appeared to be no simple relationship between the presence or absence of age-related *c-Fos* mRNA differences with relative *Oxtr* mRNA expression levels within the brain regions of interest (Fig. 5; Table 1).

Correlation between test day behavior and c-Fos mRNA

We used simple linear correlational analysis (Pearson r) to explore the relationship between relative *c-Fos* mRNA expression in our brain regions of interest (double-labelled fluorescent *in situ* hybridization) and overall activity levels or social investigation behavior during the third 10 min period on the behavioral test day. We found a positive correlation between *c-Fos* mRNA levels and overall activity (crossover frequency) when including all rats (CXT and SI, young and old) in the anterior olfactory nucleus ($r = 0.47$, $p = .002$), medial anterior bed nucleus of the stria terminalis ($r = 0.46$, $p = .003$), ventral lateral septum ($r = 0.36$, $p = .02$), ventral claustrum ($r = 0.48$, $p = .002$) and dorsal endopiriform nucleus ($r = 0.44$, $p = .006$) (Supplemental Table 2). These positive correlations were observed in most of these same brain when including only young rats, but not when including only old rats (Supplemental Table 2). Interestingly, we also found a positive correlation between social investigation behavior and *c-Fos* mRNA levels of young and old rats in the ventral lateral septum ($r = 0.50$, $p = .03$) (Fig. 6), lateral posterior bed nucleus of the stria terminalis ($r = 0.49$, $p = .03$), and the dorsal endopiriform nucleus ($r = 0.47$, $p = .05$) (Supplemental Table 2).

Age comparison of relative Oxtr mRNA expression level

Despite the high level of *Oxtr* mRNA expression in the ventromedial hypothalamus, we observed low levels of *c-Fos* mRNA positive cells (≤7 cells per tissue section) under all conditions (Table 1). However, we noted that the total number of *Oxtr* mRNA expressing

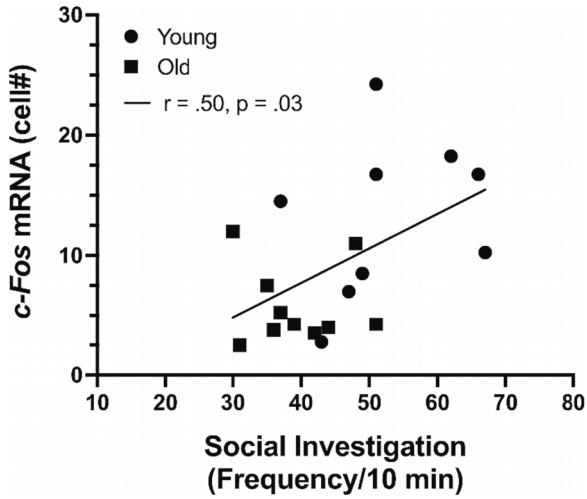

Fig. 6. Correlation between social investigation behavior and *c-Fos* mRNA in the ventral lateral septum. Scatter plot showing social investigation behavior (nose contacts with conspecific) and *c-Fos* mRNA cell counts in the lateral septum of young and old rats. Line of best linear fit illustrates a positive correlation, $r = 0.50$, $p = .03$ (Pearson correlation coefficient).

cells in the ventromedial hypothalamus was significantly less in old rats than young rats (fluorescent *in situ* hybridization; t(26) = 3.63, p = .001) (Fig. 7A). To further confirm this age difference in *Oxtr* mRNA expression in the ventromedial hypothalamus, we also used radiolabeled *in situ* hybridization to measure *Oxtr* mRNA in the ventromedial hypothalamus (Fig. 7B and C). This analysis confirmed a significant reduction of Oxtr mRNA levels in the ventromedial hypothalamus of old rats compared to young rats (t(54) = 1.90, p = .03, one-tailed Student's *t*-test). On the other hand, we did not see an age difference in *Oxtr* mRNA in the central nucleus of the amygdala, which was present on the same coronal sections (Young = 0.087 ± 0.01 and Old = 0.078 ± 0.010; mean ± sem OD, t(54) = 0.63, p = .53) (Fig. 7C). We then examined whether there was an age effect on the number of *Oxtr* mRNA positive cells (fluorescent *in situ* hybridization) in some of the other brain regions that had relatively strong *Oxtr* expression (anterior olfactory nucleus, dorsal tenia tecta layer 3/4, ventral lateral septum, and lateral posterior bed nucleus of the stria terminalis). There was not an age-related difference in *Oxtr* expression in each of these brain regions (Fig. 7D).

Discussion

Consistent with our previous report [41], we found in this study that young adult female rats (3 months old) engaged in greater exploratory social behavior than old female rats (18 months old) when placed for 10 min with a novel female rat. Interestingly, we also observed some differences in acute neural activity patterns supportive of neuroplasticity (*c-Fos* mRNA expression) across forebrain regions that differed with age and social condition. Notably, we observed in young rats an increased *c-Fos* mRNA response to the social interaction condition that was selective for a subset of brain regions.

Our results may point to some brain regions that are especially responsive to the social interaction experience. *c-Fos* gene expression is rapidly and transiently induced in response to increased neuronal activity associated with discrete experiential events that drive

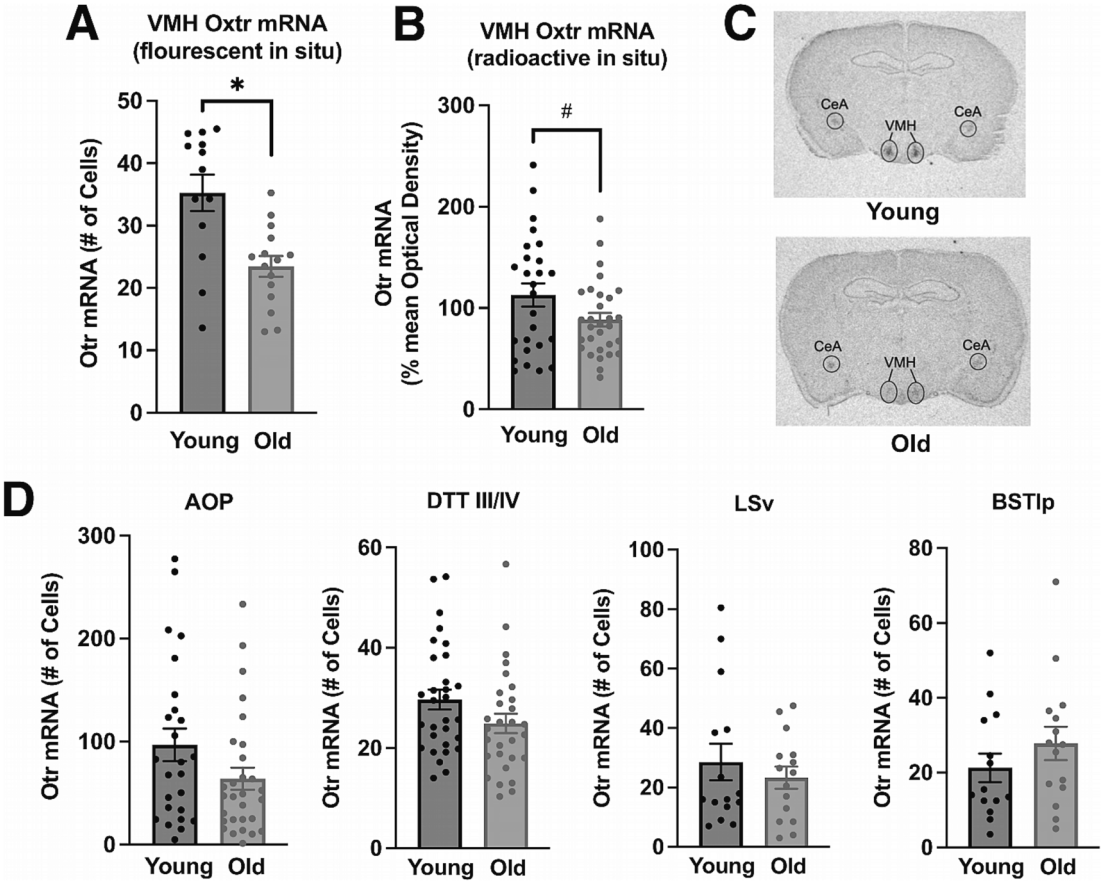

Fig. 7. Old rats have decreased oxytocin receptor (*Oxtr*) mRNA levels in the ventromedial hypothalamus. Decreased *Oxtr* mRNA levels in the ventromedial hypothalamus (VMH) of old rats as determined by (A) fluorescent *in situ* hybridization, or (B) radioactive *in situ* hybridization, *p = .001 (Students two-tailed *t*-test); #p = .03 (confirmatory Students one-tailed *t*-test). (C) Representative autoradiograms of *Oxtr* mRNA (radiolabelled *in situ* hybridization) in young and old rat brain. Note age difference in optical density in the VMH, but not central nucleus of the amygdala (CeA). (D) Bar graphs of *Oxtr* mRNA levels (fluorescent *in situ* hybridization) in other brain regions with enriched *Oxtr* expression. Abbreviations: AOP (anterior olfactory nucleus, posterior), DTT-III/IV (dorsal tenia tecta layers 3 and 4), LSv (lateral septum, ventral), BSTlp (bed nucleus of stria terminalis, lateral posterior).

neuroplasticity [1,19,23,30]. Using the *in situ* hybridization procedure, we can detect increased levels of *c-Fos* mRNA within 10 min after event onset that peaks around 30 min after a brief discrete event [37,36]. It is likely that the 10 min social interaction condition in this study triggered an additional wave of *c-Fos* gene induction in some brain regions that was superimposed on the *c-Fos* gene induction produced by the first 20 min of the test context exposure. We observed increased *c-Fos* mRNA with the social interaction condition in some neocortical (barrel fields), allocortical (anterior olfactory nucleus, ventral claustrum, and dorsal endopiriform nucleus) and subcortical (lateral septum, medial anterior bed nucleus of the stria terminalis and septohypothalamic nucleus) brain regions. Two of these brain regions (lateral septum and bed nucleus of the stria terminalis) are key nodal points within a conserved vertebrate social behavior neural network [14,31,47,33,34,48].

In a previous study [42], that used this same social interaction test procedure to examine possible sex differences in young rat *c-Fos* mRNA response profiles, we also found that young female rats had increased neural responses in the barrel fields, lateral septum, and several subregions of the bed nucleus of the stria terminalis. However, the specific subregions of the bed nucleus of the stria terminalis that exhibited increased *c-Fos* mRNA expression in response to social interaction were not consistent between this study and our previous study. This subregion inconsistency could possibly be due to the lower spatial resolution of the radiolabelled *in situ* hybridization autoradiograms used in our previous study compared to the photomicrographs of the fluorescent *in situ* hybridization measurements of this study.

Importantly, we found in this study that in some brain regions there was an attenuation of the *c-Fos* mRNA response to the social interaction condition in old rats. This was most reliable in the lateral septum and septohypothalamic area as indicated by a significant age by treatment condition interaction. In addition, there was a positive correlation between *c-Fos* expression levels in the lateral septum and social investigation behavior. The pattern of an attenuated *c-Fos* mRNA response to the social interaction condition in old rats was also apparent in a number of other brain regions examined, suggesting that this experiment was underpowered to detect this interaction. Exploratory post hoc analyses also support a blunted *c-Fos* mRNA response of old rats to the social interaction condition in the barrel fields, anterior olfactory nucleus, ventral claustrum and dorsal endopiriform nucleus. It should be noted that this blunted *c-Fos* mRNA response to the social interaction condition was not necessarily due to an overall blunted *c-Fos* mRNA response of old rats to all aspects of the test condition. In most of the brain regions in which there was a decreased response to the social interaction condition, there was not a corresponding decreased response to the context alone. In a previous behavioral experiment using the same general experimental design [41], we also saw decreased exploratory behavior of old rats when exposed for 10 min to a novel conspecific rat. But we did not see decreased exploratory behavior of old rats exposed for 10 min to a novel inanimate object. Similarly, in this experiment there was not age-related differences in exploratory behavior to the context alone condition.

On the other hand, in some other brain regions that were not specifically responsive to the social interaction condition in young or old rats, old rats displayed an overall blunted *c-Fos* mRNA response to the test conditions. This blunted response pattern was observed in the medial amygdala and in the CA3 and dentate gyrus of the hippocampal formation. Thus, there is some evidence for more widespread dampened neural processing in old rats that extends beyond the social interaction condition. In contrast, the prefrontal cortex subregions were spared from an age-related decline in *c-Fos* mRNA levels during either the context or social interaction conditions. This is reminiscent of the increased fMRI activity seen in the prefrontal cortex of older adults when performing various cognitive tasks compared to younger adults. This increased activity has been proposed to reflect age-related compensation in prefrontal cortex-dependent cognitive processing [7]. A limitation of our study is that we did not perform neuronal cell counts in each of our brain regions of interest. It is possible, therefore, that the decreased *c-Fos* mRNA expression observed in old rats was due to lower neuronal density within some brain regions of interest. This does not seem likely to be the case in brain regions in which old rats had similar levels of *c-Fos* mRNA expression to young rats in response to context alone, unless the decreased *c-Fos* mRNA response of old rats was due to a cell loss of a subpopulation of neurons that respond selectively to social interaction. It should be noted that a number of studies have examined neuronal cell density in the rat brain over the life span, and most studies fail to observe significant neuronal decline in cortical and subcortical brain regions, even in advanced aging (>24 months) [25,27,40,44].

An additional limitation of our study is that we did not monitor estrus cycle phase in our female rats at the time of behavioral testing and brain collection. In rodents, previous studies have demonstrated sex differences in the time spent investigating a novel same-sex partner [5,42,51]. Furthermore, there is some evidence that estradiol administration is anxiogenic, reducing time spent engaging in social interaction behaviors [22]. Since ovarian hormones differ across the estrous cycle, with estrogen levels highest in proestrous, it is possible that social interactions will vary across the cycle as well. However, Stack et al. [51] found no difference in time spent engaged in social interaction between female rats in diestrous and proestrous [51]. Similarly, Scholl et al. [46] did not observe differences in social interaction time between female rats in all phases of the estrous cycle [46].

A key hypothesis of our study was that age-related differences in *c-Fos* mRNA expression profiles during the social interaction condition may be especially pronounced in oxytocin target brain regions. The oxytocinergic system is an important neuromodulator that promotes a wide range of adaptive social behaviors [4,11,12]. There is also some evidence for decreases in oxytocinergic signaling with aging [2,8,17,20]. We were particularly interested in performing a more detailed examination of *c-Fos* mRNA expression within subregions of the septum and bed nucleus. These brain regions are not only two components of the social behavior neural network, but also targets of oxytocin input from hypothalamic neurons [12]. The septum and bed nucleus of the stria terminalis, however, are heterogenous brain regions with multiple subnuclei, some of which are enriched for oxytocin receptor expression and others that are not. Moreover, the relationship between these various subregions and social behavior has been largely unexplored. We therefore used *c-Fos* and *Oxtr* mRNA double-labeled fluorescent *in situ* hybridization to examine the *c-Fos* mRNA response of young and old rats to social interaction within septal and bed nucleus subregions as well as other nearby brain regions of interest. Somewhat contrary to our hypothesis, we saw in old rats a blunting of *c-Fos* mRNA with the social interaction condition not only in a brain region that was enriched in oxytocin receptors (ventral lateral septum), but also one that was not (septohypothalamic nucleus). The

septohypothalamic nucleus as defined by Paxinos and Watson [38] overlaps with a ventral extension of the lateral septum, as outlined in some other rat brain atlases [39,52]. We found that both the septohypothalamic nucleus and the ventral lateral septum were functionally similar in terms of a blunted *c-Fos* mRNA response of old rats to social interaction. However, the septohypothalamic nucleus was phenotypically distinct from the nearby ventral lateral septum in terms of its limited oxytocin receptor expression. This phenotypic feature is shared with the adjacent medial anterior bed nucleus of the stria terminalis. Interestingly, neural activity of the septohypothalamic region in humans is strongly associated with social affiliative emotion [29].

Our results do not necessarily rule out a role of diminished oxytocinergic signaling to contribute to the decreased social interaction behavior of old rats. For example, blunted neural activity in oxytocinergic target brain regions, as we saw in the lateral septum, may have network wide effects in other brain regions that are either not directly regulated by oxytocin, or do not display high levels of oxytocin receptor mRNA expression. One brain region in which our exploratory analysis supported a blunted *c-Fos* mRNA response to the social interaction condition in old rats was the barrel fields portion of neocortex. The barrel fields consist of the portion of the primary somatosensory cortex that has receptive fields for each of the separate facial whiskers. Whisking is a principal form of somatosensation for rats and is vital to exploratory behavior [43]. Barrel fields *c-Fos* mRNA expression levels seem to be very sensitive to top-down modulation of brain activity. For example, barrel fields *c-Fos* mRNA levels reflect both habituation and sensitization of neurobehavioral responses to various stressor exposure regimens [13,55]. Interestingly, there is evidence for oxytocin to enhance signal-to-noise processing in a socially specific manner within primary sensory processing brain regions [35,45].

Although we did not see a clear relationship between a brain region's oxytocin receptor enrichment level and age-related deficits in *c-Fos* expression, we still considered the possibility that there were age-related differences in oxytocin receptor expression in our brain regions of interest. There was overall pronounced *Oxtr* mRNA expression in the ventromedial hypothalamus, anterior olfactory nucleus, lateral posterior bed nucleus of the stria terminalis, ventral lateral septum, dorsal tenia tecta layer 3 and central nucleus of the amygdala. These observations are in good agreement with other studies of oxytocin receptor binding, immunoreactivity or mRNA distribution in mouse and rat brain [12,28,54,56]. Quantification of oxytocin receptor expression in each of these brain regions indicated a selective age-related decline in *Oxtr* mRNA that was restricted to the ventromedial hypothalamus. One other study examined age-related differences in oxytocin receptor levels based on radioligand binding [2]. That study compared oxytocin receptor binding levels in young (3 mos) and old (20 mos) male Sprague-Dawley rats, and found a similar decline in the ventromedial hypothalamus, without a decline in the bed nucleus of the stria terminalis, central nucleus of the amygdala or anterior olfactory nucleus. The results of our study suggest that the previously observed age-related decline in ventromedial hypothalamic oxytocin radioligand receptor binding occurs at the transcriptional level. Our results also extend this age-related decrease in oxytocin receptor expression to female rats. Close correspondence between relative OXTR protein and *Oxtr* mRNA levels across a range of brain regions and ages has previously been reported [28]. The age-related decline in ventromedial hypothalamic *Oxtr* expression is not likely due to neuronal loss as a previous study found no age-related decreases in ventromedial hypothalamic neuronal density of 24 month old female rats [25]. Testosterone and estradiol have been shown to have strong positive regulatory effects on oxytocin receptor expression in the ventromedial hypothalamus [53]. Thus, the decline in ventromedial hypothalamic oxytocin receptor expression in old age may be related to age-related declines in gonadal steroid hormone production and/or action. Nevertheless, it seems unlikely that this age-related decline in ventromedial hypothalamic oxytocin receptor expression can account for the age-related decline in social behavior. We observed very limited neural activation of the ventromedial hypothalamus with the social interaction condition compared to our other brain regions of interest, suggesting that this brain region is not strongly engaged by the social interaction condition.

Conclusions

In summary, we found that decreased social exploration in old female rats was associated with decreased *c-Fos* gene expression in select forebrain regions, some of which are known elements of a conserved vertebrate social behavior neural network. Age-related declines in the generally prosocial oxytocinergic signaling system [11] were considered as a possible mediator of decreased social exploration in old rats. However, only some of the affected brain regions were ones that had high oxytocin receptor expression. It is also noteworthy that we found a significant reduction in oxytocin receptor mRNA in the ventromedial hypothalamus of old female rats. Taken together, our results support age-related neuroprocessing changes in select brain regions involved in social exploration, with support for age-related decline in oxytocin receptor expression in a specific brain region (ventromedial hypothalamus) that may or may not contribute to the altered social behavior and neural activity patterns. Whether these age-related reductions in *c-Fos* expression reflect neurocircuit specific attenuation of social stimuli processing and/or declines in social exploration motivation is an important question for future studies. Attaining this information may be valuable in developing neurobehavioral strategies to protect against tendencies for reduced social interactions that emerge with advanced age.

CRediT authorship contribution statement

J. Russell Ravenel: Investigation, Formal analysis, Writing – original draft. **Amy E. Perkins:** Conceptualization, Formal analysis, Investigation, Writing – review & editing. **Angela Tomczik:** Investigation, Writing – review & editing. **Ana Defendini:** Investigation, Writing – review & editing. **Helen K. Strnad:** Investigation, Writing – review & editing. **Elena Varlinskaya:** Conceptualization, Writing – review & editing. **Terrence Deak:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Robert L. Spencer:** Formal analysis, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbas.2024.100107.

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