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Brain regional differences in social encounter-induced Fos expression in male and female rats after post-weaning social isolation

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1. Introduction

Early life adversity, including during adolescence, is a strong predictor of aggression and antisocial behavior in adults (Haller et al., 2014). Post-weaning social isolation (PSI) of rats or other social species, also known as isolation rearing, is a well-established model of early life adversity that produces inappropriate social behavior and heightened emotional reactivity to social experience. Although long-term social isolation that occurs during different periods of development (either neonatally or in adulthood) may also impact behavior, the alterations in social behavior that are produced by PSI are particularly pronounced (Hall, 1998). These alterations are persistent and in some cases irreversible (Tulogdi et al., 2014), and are thought to be largely due to a lack of opportunities for social play during the sensitive period of adolescence. Play is adaptive in the early life of social species including humans and rodents (Panksepp, 1981), allowing individuals to practice the skills needed for proper social conduct and developmentally appropriate behaviors in adulthood.

PSI consists of depriving adolescent rats of social experience by housing them individually (as compared to housing in same-sex groups, typically 3 or 4 per cage) for a period of 4 to 8 weeks after weaning at postnatal day 21 (P21). This period encompasses the entire periadolescent period, which in the rat has been described as lasting roughly from P28 to P60 (Spear, 2000). Adolescence in the rat has also been defined as beginning as early as P21 and consisting of early (P21-P30), middle (P30-P45), and late (P45-P60) periods (Tirelli et al., 2003). By isolating rats immediately after weaning it is ensured that the entire periadolescent period is accounted for. Social isolation during this period produces

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persistent behavioral changes known as the isolation syndrome (Hatch et al., 1965) that includes outcomes such as increased anxiety-like behavior (Einon and Morgan, 1977), overreaction to novelty (Hall, 1998), and altered social behavior (Ferdman et al., 2007; Wongwitdecha and Marsden, 1996). Among the alterations in social behavior produced by postnatal social isolation is increased aggression; PSI of male rats produced increases in aggression (Zhao et al., 2009) and isolation rearing-induced aggression was exacerbated when testing occurred in an unfamiliar environment (Wongwitdecha and Marsden, 1996). Although most studies of PSI have been performed in male rats, exposure to PSI can also result in abnormal patterns of social behavior, including increased aggression, in females as well as males (Wall et al., 2012).

A number of previous studies have reported that social interaction, including play behavior, produces alterations in the immediate early gene *c-fos* (Gordon et al., 2002; Northcutt and Nguyen, 2014), or in its protein product, Fos (Weatherington et al., 2012; Paredes-Ramos et al., 2014), in a number of brain regions, in particular within a network involving corticostriatal and corticolimbic connections (van Kerkhof et al., 2014). Exposure to a novel conspecific can produce differential alterations in mPFC function (Hermes et al., 2011; Levine et al., 2007), including alterations in Fos (Wall et al., 2012) in rats after PSI as compared to normally housed controls. In our previous report, a single, brief social encounter with a novel same-sex rat induced large increases in the expression of Fos in the mPFC of group-reared rats (GRP). The social encounter-induced increase in mPFC Fos expression was profoundly blunted in rats that had experienced PSI. Interestingly, there were subregion differences in the magnitude of the Fos response between GRP males and females; the response was greater in males in the infralimbic region and greater in females in the anterior cingulate (Wall, 2012). Because the mPFC is still maturing during the adolescent period, it was of particular interest in studies of adolescent adversity including PSI. However, alterations in other brain regions, including the amygdala (Lukkes et al., 2012) and the hippocampus (Bonab et al., 2012) can also result from PSI.

The goal of the present study was to determine the effects of a brief social encounter on Fos protein expression in multiple cortical and subcortical brain regions to assess whether PSI-induced neuronal hypofunction during social interaction is specific to the mPFC. Fos is a transcription factor that is induced rapidly and transiently in many types of neurons, and is sensitive to neuronal activation in much of the brain (Curran and J. I. Morgan, 1995). We were particularly interested in brain regions important for the regulation of emotion and those that may have connections with the mPFC. Thus, we used tissue from the same rats used in Wall et al. (2012) to assess Fos expression in regions within the cortex, thalamus and epithalamus, hippocampus, amygdala, and basal ganglia of male and female rats exposed to either PSI or group housing. An additional goal of the study was to determine relationships between social behavior and Fos activation in the forebrain.

2. Results

PSI blunted the social encounter-induced increase in Fos expression that was observed in GRP rats in most of the brain regions assessed. In several regions the difference was more

pronounced in males, in that a social encounter produced greater increases in Fos in GRP males than in GRP females as well as PSI males and females.

2.1. Cortex and thalamus

In these regions, a social encounter produced robust increases in Fos expression that only reached significance in GRP males (Fig. 1).

2.1.1. Piriform cortex—In the PIR (Fig. 1A) there was a significant Rearing $\times$ Social Encounter $\times$ Sex interaction, $F(1,55) = 7.56$, $p < .01$. Post-hoc tests indicated that Fos was only induced by a social encounter in GRP males, thus GRP males exposed to a social encounter had significantly more Fos in the PIR than GRP male home cage controls $p < .05$; no other Social Encounter groups were significantly different than their respective home cage controls. In addition, a significant Rearing $\times$ Social Encounter interaction was observed, $F(1,55) = 12.64$, $p < .01$. Post hoc tests collapsed across Sex indicated that Fos expression produced by a social encounter was greater in GRP than in PSI rats, $p < .05$. Main effects of Rearing, $F(1,55) = 6.58$, $p < .05$, and Social Encounter, $F(1,55) = 20.83$, $p < .001$, were observed in the PIR, indicating greater Fos expression in GRP rats and in rats that had experienced a social encounter, respectively.

2.1.2. Cingulate cortex—In the CgC (Fig. 1B) there was a significant Rearing $\times$ Social Encounter $\times$ Sex interaction, $F(1,55) = 5.69$, $p < .05$. Post-hoc tests indicated that Fos was only induced by a social encounter in GRP males, thus GRP males exposed to a social encounter had significantly more Fos in the CgC than GRP male home cage controls as well as PSI males exposed to a social encounter, $p < .05$. In addition, a significant Rearing $\times$ Social Encounter interaction was observed, $F(1,55) = 13.83$, $p < .001$. Post hoc tests collapsed across Sex indicated that Fos expression produced by a social encounter was greater in GRP than in PSI rats, $p < .05$. Main effects of Rearing, $F(1,55) = 8.37$, $p < .01$, and Social Encounter, $F(1,55) = 21.98$, $p < .001$, were observed in the CgC, indicating greater Fos expression in GRP rats and in rats that had experienced a social encounter, respectively.

2.1.3. Anterior insular cortex—In the AIC (Fig. 1C) there was a significant Rearing $\times$ Social Encounter $\times$ Sex interaction, $F(1,55) = 4.881$, $p < .05$. In addition, there was a significant Rearing $\times$ Social Encounter interaction, $F(1,55) = 13.18$, $p < .01$. Post hoc tests collapsed across Sex indicated that Fos expression was induced by a social encounter only in GRP rats, $p < .05$.

2.1.4. Somatosensory cortex—In the SSC (Fig. 1D), there was a significant main effect of Social Encounter, $F(1,55) = 65.35$, $p < .001$, but no other main effects or interactions.

2.2. Limbic regions

A social encounter increased Fos expression in each of these regions, with regional differences in the effects of housing and sex (Fig. 2).

2.2.1. Paraventricular nucleus of the thalamus—In the PVT (Fig. 2A) there was a significant Rearing $\times$ Social Encounter $\times$ Sex interaction, $F(1,55) = 5.43$, $p < .05$. Post-hoc tests indicated that Fos was only induced by a social encounter in GRP males, thus GRP males exposed to a social encounter had significantly more Fos in the PVT than GRP male home cage controls $p < .05$. In addition, a significant Rearing $\times$ Social Encounter interaction was observed, $F(1,55) = 9.82$, $p < .01$. Post hoc tests collapsed across Sex indicated that Fos expression produced by a social encounter was greater in GRP than in PSI rats, $p < .05$. Main effects of Rearing, $F(1,55) = 18.72$, $p < .01$, and Social Encounter, $F(1,55) = 61.16$, $p < .001$, were observed in the PVT, indicating greater Fos expression in GRP rats and in rats that had experienced a social encounter, respectively. Interestingly, in the PVT there was also a significant main effect of Sex, $F(1,55) = 9.57$, $p < .01$, indicating that Fos expression was greater in males than females.

2.2.2. Habenula—In the HAB (Fig. 2B), a significant Rearing $\times$ Social Encounter interaction was observed, $F(1,55) = 28.30$, $p < .001$. Post hoc tests collapsed across Sex indicated that Fos expression produced by a social encounter was greater in GRP than in PSI rats, $p < .05$. Main effects of Rearing, $F(1,55) = 21.38$, $p < .001$, and Social Encounter, $F(1,55) = 102.10$, $p < .001$, were observed in the HAB, indicating greater Fos expression in GRP rats and in rats that had experienced a social encounter, respectively.

2.2.3. Basolateral amygdala—In the BLA (Fig. 2C), a significant Rearing $\times$ Social Encounter interaction was observed, $F(1,55) = 7.89$, $p < .01$. Post hoc tests collapsed across Sex indicated that Fos expression produced by a social encounter was greater in GRP than in PSI rats, $p < .05$. A significant main effect of Social Encounter, $F(1,55) = 26.27$, $p < .001$, was observed in the BLA, indicating greater Fos expression in rats that had experienced a social encounter.

2.2.4. Central nucleus of the amygdala—A main effects of Social Encounter, $F(1,55) = 60.39$, $p < .001$, was observed in the CEA (Fig. 2D), indicating greater Fos expression in rats that had experienced a social encounter. A strong trend for a Rearing $\times$ Social Encounter interaction did not reach significance, $F(1,55) = 3.98$, $p = .05$.

2.3. Hippocampal formation and lateral septum

There was very little Fos expression in the hippocampus, and in some groups there was no Fos expression in No Social controls (Fig 3).

2.3.1. CA1 and CA3 subfields—A significant main effect of Social Encounter, $F(1,55) = 6.38$, $p < .05$, was observed in CA1 (Fig. 3A), indicating greater Fos expression in rats that had experienced a social encounter. No other main effects or interactions were observed. A significant main effect of Social Encounter, $F(1,55) = 8.67$, $p < .01$, was also observed in CA3 (Fig. 3B), indicating greater Fos expression in rats that had experienced a social encounter. There was a strong trend for a Rearing $\times$ Social Encounter interaction, $F(1,55) = 3.69$, $p = .06$.

2.3.2. Dentate Gyrus—In the DG (Fig. 3C), a significant Rearing $\times$ Social Encounter interaction was observed, $F(1,55) = 9.26$, $p < .01$. Post hoc tests collapsed across Sex indicated that Fos expression produced by a social encounter was greater in GRP than in PSI rats, $p < .05$. Main effects of Rearing, $F(1,55) = 7.01$, $p < .05$, and Social Encounter, $F(1,55) = 15.06$, $p < .001$, were observed in the DG, indicating greater Fos expression in GRP rats and in rats that had experienced a social encounter, respectively.

2.3.3. Lateral septum—In the LS (Fig. 3D), a significant Rearing $\times$ Social Encounter interaction was observed, $F(1,55) = 14.25$, $p < .001$. Post hoc tests collapsed across Sex indicated that Fos expression produced by a social encounter was greater in GRP than in PSI rats, $p < .05$. Main effects of Rearing, $F(1,55) = 12.29$, $p < .01$, and Social Encounter, $F(1,55) = 13.03$, $p < .01$, were observed in the LS, indicating greater Fos expression in GRP rats and in rats that had experienced a social encounter, respectively.

2.4. Basal Ganglia

A social encounter increased Fos expression in all regions, and no interactions of Rearing or Sex were observed (Fig 4).

2.4.1. Nucleus Accumbens Shell and Core—In the NAcS (Fig. 4A) and NAcC (Fig. 4B), significant main effects of Social Encounter were observed, $F(1,55) = 15.67$, $p < .001$, and $F(1,55) = 17.43$, $p < .001$ respectively, indicating greater Fos expression in rats that had experienced a social encounter. No other main effects or interactions were observed in the NAc.

2.4.2. Dorsomedial and Dorsolateral Striatum—A significant main effect of Social Encounter, $F(1,55) = 19.38$, $p < .001$, was observed in DMS (Fig. 4C), indicating greater Fos expression in rats that had experienced a social encounter. A significant though less robust main effect of Social Encounter, $F(1,55) = 6.16$, $p < .05$, was also observed in DLS (Fig. 4D), indicating greater Fos expression in rats that had experienced a social encounter. No other main effects or interactions were observed in the striatum.

2.5. Relationships between Fos expression and social behavior

Time spent actively engaged in social behavior in these rats has previously been reported (Wall) and thus are not presented here. However, it was of interest to determine whether relationships between those behaviors with the brain regions assessed here would be observed. In that study, isolation rearing increased social and aggressive behaviors in both male and female rats, and males engaged in more social and aggressive behaviors than females (Wall et al., 2012). Using brain tissue from the same animals, we performed correlations between Fos and social interaction, aggressive grooming of the stimulus rat, and chasing of the stimulus rat, and these correlations were performed separately for males and females. We observed significant negative correlations between these behaviors and several of the brain regions assessed here, particularly in males. R values for these correlations are shown in Table 1. Only HAB (in females) and PVT (in males) were significantly negatively correlated with total Social Interaction. No regions were significantly correlated with Aggressive Grooming in females, but AIC, CgC, LS, PIR, and PVT were significantly

negatively correlated with Aggressive Grooming in males. Several regions were negatively correlated with Chasing in both females (AIC, BLA, and CEA) and males (AIC, BLA, CgC, LA, PIR, and PVT). Overall, Chasing was the social behavior most correlated with Fos expression. AIC and PVT were the regions with the most correlations with social behavior, and BLA, CEA, LS, and PIR also had more than one correlation with social behavior. In all cases, significant correlations were negative. There were no correlations between the hippocampal formation or the basal ganglia and social behavior.

3. Discussion

Increases in Fos expression were produced by a single, brief social encounter in male and female rats that were exposed to either normal social housing or social isolation during the postweaning period encompassing adolescence; however, these alterations were dependent upon housing condition, brain region, and sex. We assessed a number of forebrain regions involved in emotion regulation, social behavior, and cognition. Our primary finding was a widespread blunting of social encounter-induced Fos induction in PSI rats, which was observed in many, but not all, of the brain regions assessed. In addition, in GRP rats, the social encounter-induced increase in Fos was greater in males than in females in several of the brain regions assessed.

There were 3 distinct patterns of activation as reflected in social encounter-induced Fos expression in the forebrain. In one pattern, GRP males were the only group with a statistically significant increase in Fos produced by a social encounter (PIR, CgC, AIC, PVT). In the second pattern, GRP rats only, both males and females, had significant increases in Fos produced by a social encounter (HAB, BLA, DG, and LS). In the third pattern, all groups had significant increases in Fos produced by a social encounter (SSC, CEA, CA1, CA3, NACC, NACS, DLS, and DMS). Thus, in half of the regions sampled, a difference was observed in social encounter-induced Fos expression between GRP and PSI, and in half of those cases the difference was due to Fos increases in GRP males only. In no region was Fos expression produced by a social encounter significantly greater in PSI than GRP, or greater in females than males. Moreover, there were no differences between groups in Fos expression in the absence of a social encounter. These results suggest that specific neural networks are involved in social experience and that these networks may be differentially influenced by social history and sex. The neural consequences of PSI, at least in response to a social challenge, appear to be the most pronounced in the limbic regions of the cortex as well as thalamus, epithalamus, dentate gyrus, and lateral septum, and are the least pronounced in the basal ganglia as well as the hippocampal CA subfields and central nucleus of the amygdala. However, it is clear based on these findings that the neural consequences of PSI are neither specific to the mPFC nor manifest throughout the brain.

3.1. Social encounter-induced Fos activation was only observed in GRP males in some regions

The present results demonstrate a robust increase in Fos expression in several regions of GRP males after a single social encounter that was larger than that of GRP females and of PSI males and females. Regions in which robust increases in Fos expression were specific to

male GRP rats were confined to limbic regions of the cortex (PIR, AIC, and CgC) and to the PVT, which interacts closely and reciprocally with them. Thus, these findings demonstrate effects of housing as well as sex differences within GRP rats, and is consistent with our earlier finding of greater social encounter-induced Fos expression in the infralimbic region of the mPFC of male GRP rats (Wall et al., 2012). Increases in Fos expression have been reported in female rats in the PFC and orbital frontal cortex, but not piriform cortex, after social play (Paredes-Ramos et al., 2014). The piriform cortex is particularly important for processing olfactory information, and activation of Fos in this region has been observed after exposure to a novel odor in rats (Lehallier et al., 2012). In line with this, large increases in Fos expression in the PIR were observed when an odor was paired with social play in sexually experienced female rats (Paredes-Ramos et al., 2014). Male rats were not tested in that study, and we know of no previous studies that examine sex differences in activation of PIR during social interaction. Activation of the piriform cortex has also been shown to be produced by exposure to the elevated plus maze, suggesting sensitivity to anxiogenic stimuli (Galvis-Alonso et al., 2010), and social interaction is often used as a test of anxiety (Seth and File, 2003).

Agranular insular cortex has been known for some time to be important for the integration of visceral and limbic responding (Krushel and van der Kooy, 1988). Recent research has uncovered a role for the agranular insular cortex in social play and appropriate social behavior as well. Prenatal ethanol exposure, a treatment that alters social behavior, blunted social experience-induced Fos expression in the AIC and PL when animals were tested as adults (Hamilton et al., 2010). Our results for the AIC are in contrast to those of Hamilton et al who did not observe a sex difference in fos activation in the AIC after social experience after either prenatal ethanol or controls treatment; in that study increases in fos mRNA were observed in the AIC of both males and females. However, several factors may account for this difference. In the present study animals experienced an encounter with a novel rat, while in the Hamilton et al. study animals were exposed to their familiar cage-mates after a 24 hr separation. Furthermore, that study was done in adult rats (P90 to P130), while the present study was performed during late adolescence (P49 to P52). The PVT receives input from the mPFC as well as the AIC (Allen et al., 1991) and it is notable that the pattern of Fos activation was the same in the PVT as in the AIC (present results) and in the mPFC (Wall et al., 2012). Paredes-Ramos et al. (2014) have speculated that the cortex is important for the fine-tuning of play behavior; thus, because play behavior is more prominent in males than in females (Argue and McCarthy, 2015; Meaney et al., 1981), the role of the cortex may be more important in males.

3.2. Social encounter-induced Fos activation was only observed in GRP rats (both males and females) in some regions

Social encounter-induced Fos activation was observed in male and female GRP rats, both male and female, in LS, HAB, BLA, and DG. The lateral septum has been reported to be important for the regulation of social recognition (Bredewold et al., 2014), and social play behavior (Veenema et al., 2013) has been proposed by a mechanism involving vasopressin. In the studies by Veenema (2013, 2014), sex-dependent effects on social outcomes were obtained by manipulations of the vasopressin system in the lateral septum. While we did not

observe sex differences in our results, it is possible that vasopressin signaling in the lateral septum is dysregulated in PSI rats.

The habenula complex has direct efferent and afferent connections with regions that have previously been reported to be involved in social play behaviour, such as the thalamus and septum (Lecourtier and Kelly, 2007; Siviý and Panksepp, 2011). To the best of our knowledge, no previous studies have investigated whether the habenula is altered after PSI. However, inactivation of the habenula reduced responsiveness to play solicitation in a recent study in male rats, consistent with a role for appropriate social behavior (van Kerkhof et al., 2013). Thus it is curious that habenula activation was reduced in PSI rats that engaged in more social interaction. One interpretation is that this reflects a mismatch between the perception of the social behaviors engaged in as playful versus aggressive in the PSI rats.

The BLA has been implicated by a number of researchers in normal social play behavior. For example, social play was decreased, and expression of Fos in the BLA was increased, after MDMA administration (Navarro et al., 2004). Fos expression increased in the BLA after a 10 min social interaction in young adult male rats (von Heimendahl et al., 2012) and in the BLA of adolescent males after 15 min of play (van Kerkhof et al., 2014). In contrast to our results and those of von Heimendahl et al., (2012), decreases in BLA Fos were observed in the BLA of males after non-threatening social interaction in male juveniles, but no change was observed in female juveniles (Weathington et al., 2012). The present finding of blunting of social encounter-induced Fos in the dentate gyrus of PSI rats is consistent, at least in part, with a study by Bonab et al. (2012), in which reductions in glucose metabolism throughout the hippocampus (subregions were not individually assessed) were observed in male isolates. In that study, there was no acute challenge, social or otherwise, although it is possible that the PET procedure was stressful in itself. Reductions in glucose metabolism were also found in thalamic regions and the frontal cortex in isolates (Bonab et al., 2012), in line with our findings in the cortex and PVT.

3.3. Social encounter-induced Fos activation was observed in all rats in some regions

This pattern of Fos activation was observed in SSC, CEA, CA1, CA3, NACC, NACS, DLS, and DMS. In contrast to the results obtained in other cortical regions, there were no group differences in social encounter-induced Fos expression in the SSC. This may reflect a resistance to alteration in brain regions involved in motor function, as a similar pattern was observed throughout the basal ganglia, consistent with our earlier microdialysis study that showed no differences between rearing groups in social interaction-induced dopamine and serotonin release in the nucleus accumbens shell (Grotewold et al., 2014). van Kerkhof et al. (2014) also observed increased *c-fos* expression in the dorsal and ventral striatum of male rats after social play. However, our results are somewhat incompatible with the study of Northcutt and Nguyen (2014), in which Fos expression in the ventral tegmental area (VTA) increased after play behavior in female, but not male rats. This suggests that play behavior may be more rewarding for females, however, it should be noted that this increase occurred in rats that engaged in spontaneous play behavior with familiar cagemates. We (Wall et al., 2012; Grotewold et al., 2014) and others (Stack et al., 2010) have demonstrated that females engage in less social interaction than males when the stimulus rat is unfamiliar. Furthermore,

a novel social cue alone was enough to induce conditioned place preference (CPP) in males, but not females, using our procedures (Grotewold et al., 2014). However, Douglas et al. (2004) have demonstrated social CPP in both male and female adolescent rats, but social CPP was greater in males, and greatest in socially isolated males.

Our finding of increased Fos in the CEA is in contrast to those of Weatherington et al. (2012), who found no increase in Fos in the CEA in juvenile (P28) male or female rats after a single social encounter (Weatherington et al., 2012) and those of van Kerkhof et al. (2014), who found no increase in this region in juvenile males. However, and consistent with the present results, CEA Fos expression was increased in adult male rats that had experienced social defeat in another study (Martinez et al., 1998), suggesting the possibility of a developmental shift in CEA reactivity in response to social stimuli.

In the present study, there were no brain regions in which social interaction did not increase Fos expression in at least some of the groups. In contrast, in a study of male rats and using in situ hybridization for *c-fos*, Gordon et al., (2002) observed no increases in *c-fos* mRNA expression in the cingulate cortex or amygdala. Furthermore, the study of van Kerkhof et al. (2014), which also used in situ hybridization for *c-fos*, reported no activation in the CeA. These discrepancies may be due to a number of variables including difference in the expression of *c-fos* mRNA and Fos protein, differences in the ages of the subjects (early vs late adolescence), strain differences, or the different experimental protocols. In the present study, rats experienced a single 15 min test, while in the study of Gordon et al. (2002), 3 tests of 5 min each were used; one possibility is that habituation may have occurred over repeated trials. There are differences in both age (late vs early adolescence) and strain (Sprague-Dawley vs Wistar) between the present study and that of van Kerkhof et al. (2014). Null results of social interaction-induced Fos expression have also been reported in the hippocampus. No increases in Fos were observed in any hippocampal subregions in a study of male rats (von Heimendahl et al., 2012), although it is interesting to note that in that study, all rats were socially isolated for 3 weeks (beginning at P28) before experimentation, and physical contact was limited (von Heimendahl et al., 2012).

3.4. Relationships between Fos expression in the forebrain and social behavior

There were a number of negative relationships between Fos in the different brain regions assessed and social behavior. The negative correlations likely reflected the greater activation of Fos in group-reared rats, which also tended to engage in lesser amounts of the behaviors assessed. Patterns of relationships differed between males and females, and there were more significant correlations in males than in females. Chasing and aggressive grooming were more strongly related to Fos in several brain regions in males than in females, and few relationships were observed between overall social interaction than in chasing and aggressive grooming, especially in males. One possibility is that males rely on activation of these regions to inhibit aggressive behavior such as chasing and aggressive grooming. It should be noted that there is some ambiguity in assessing play versus aggressive behavior at this age in the rat, and chasing and aggressive grooming may be considered as playful, rather than aggressive. However, many of the behaviors that are manifested as play during adolescence can be interpreted as aggressive during adulthood, as social play (particularly of

the “rough and tumble” type) can be, at least in part, practice for behaviors that can be considered aggressive in adults (Palagi et al., 2015). Playful attacks decline throughout puberty (Panksepp, 1981), suggesting that PSI rats may be “stuck” in an immature stage of social development, and this may be unexpected by the stimulus rat. Pellis and Pellis (2011) have suggested that play-like social interactions that vary too far from the expected behaviors are no longer considered playful, so that conspecifics avoid interactions with the individuals engaging in these behaviors.

In the present experiment rats exposed to a social encounter were also exposed to a new, clean, standard housing cage in a different room. Thus environmental novelty alone, rather than a novel social encounter, may have contributed to the increased Fos expression observed in these brain regions. However, the primary finding of blunted activation in the forebrain of isolates is still of considerable interest, although it may generalize to responsiveness to diverse novel experiences. It is also important to note that estrous phase, which can affect forebrain function and behavior, was not assessed. However, it has been previously demonstrated that neither social interaction nor social interaction-induced *zif268* in the mPFC are dependent on stage of estrous in the rat, although both are dependent on the sex of the rat (Stack et al., 2010). In that study, both social interaction and *zif268* expression in the mPFC were greater in males than in females (Stack et al., 2010). We have previously observed sex differences in tailshock stress-induced *c-fos* mRNA, however, females in the different estrous stage groups did not differ significantly from each other (Bland et al., 2005). Figureido et al. (2002) have reported sex- and estrous-cycle dependent differences in stress-induced *c-fos* mRNA in the frontal cortex and hippocampus; females in estrus and proestrus had less *c-fos* activation after restraint stress than males and females in diestrus (Figureido et al., 2002). It remains unknown what impact, if any, ovarian hormones may have had on the present results. In accordance with McCarthy et al. (2012), here we merely sought to determine a sex difference, independent of estrous cycle, and the lack of increased variability in our females suggests that estrous phase may not have played a role in our effects.

3.5. Conclusion

A brief social encounter with a novel same-sex rat induced large increases in Fos protein expression in the forebrains of GRP rats, in some regions exclusively in males, and PSI produced marked attenuations of social encounter-induced Fos expression in several of these regions. Post-weaning social isolation is a widely used model of early-life adversity and its potential for increasing the vulnerability to psychopathology; thus, these results have implications for the important role of forebrain function in the social deficits that may be associated with psychological disorders.

4. Methods and Materials

4.1. Animals

Male (n = 32) and female (n = 32) Sprague-Dawley rats (Harlan; Indianapolis, IN) were purchased at postnatal day (P) 21 and were housed in standard Plexiglas cages either individually or in same-sex groups of 4 with food and water freely available in a 12:12

light:dark cycle. Isolated rats were exposed to the sight, sound, and smell of other rats in the colony room but were deprived of physical contact. Rats were weighed weekly but were not otherwise handled. Vaginal smears were not performed on females because regular handling during the isolation period is known to reduce the effects of isolation rearing (Rosa et al., 2005; Sfikakis et al., 1996). Experimentation took place after 4 weeks of either isolation (ISO) or group (Group) rearing, between days P49 and P52, a period corresponding to late adolescence (Saalfeld and Spear, 2015). Rats were run in squads of 16 per day, counterbalanced by rearing condition, sex, and conspecific exposure. All experiments were conducted with protocols approved by the University of Colorado Animal Care and Use Committee.

4.2. Experimental procedures

4.2.1. Social Encounter Procedure—After 4 weeks of isolation or group rearing, rats from each group (n = 8 per group) were quasi-randomly chosen for social exposure, such that cages were randomly chosen for either social exposure or no exposure and all rats within a cage were exposed individually to a novel conspecific simultaneously. This was to ensure that rats within each cage were not deprived of social exposure or unduly disturbed by having cagemates removed. These rats were placed in a cage with a novel same-sex rat (conspecific) for 15 minutes. Slightly younger (P24 to P34), naïve, group housed rats were used as stimulus rats in order to be less threatening to the experimental rats thus potentially reducing threatening and aggressive behavior (File and Seth, 2003). Exposure to novel conspecifics occurred in standard Plexiglas cages with a webcam above the cages recording each trial. Control rats were left in their home cages and all rats were sacrificed approximately 100 min later after having been returned to their home cages. This time point was chosen as an optimal time point to measure the protein product of activity-dependent genes based on the time of peak protein expression following acute stimuli for the prototypical immediate-early gene, Fos (Kovács, 2008). The social behavior (total time in social interaction, aggressive grooming, and chasing) of each experimental rat was coded from the video recordings by experimenters blind to the treatment groups and has been previously published (Wall et al., 2012). In that study, in addition to increased social interaction, we observed increases in aggressive grooming in both males and female isolates. First described by Grant and Mackintosh in 1963, aggressive grooming differs from normal social grooming by an increased use of the teeth, pulling of fur, and the vigorousness of the act. We have used the definition of Hurst et al, (1999) as “vigorous grooming by the experimental rat of the novel conspecific when it is standing, crouching, supine, or trying to escape”. Aggressive grooming has been called an agonistic behavior, though it is distinguishable from attacks (Clipperton et al., 2010), which typically include biting and lateral displays. It is important to note that we have observed very little of this behavior in group-reared rats.

4.3. Tissue harvest

Rats were deeply anesthetized with sodium pentobarbital and transcardially perfused with cold 0.9% saline followed by 4% paraformaldehyde in 0.01M PBS. Brains were postfixed for 4 hours in the same paraformaldehyde solution, cryoprotected in 30% sucrose for three days, then rapidly frozen in -30°C isopentane just prior to cryosectioning. Sections were

taken (40 μm) through the forebrain using the atlas of Paxinos and Watson (2007). Sections were stored at 4°C in cryoprotectant until immunohistochemistry was performed.

4.4. Immunohistochemistry

Immunohistochemistry was performed for Fos as previously reported (wall). Free-floating sections were first washed 3 times in 0.01 M PBS and between each subsequent step except as noted. Sections were incubated in 0.3% hydrogen peroxide, followed by 5% normal goat serum (NGS) and 0.25% Triton X in PBS. Sections were incubated overnight at RT in rabbit anti-Fos, (1:10,000, Santa Cruz Biotechnology) in PBS with 5% NGS and 0.25% Triton X. Sections were then incubated in biotinylated goat anti-rabbit secondary antibody (1:200, Jackson Labs) for 2 h, followed by incubation in avidin biotin complex (ABC kit, Vector Laboratories) for 2 h. Sections were washed 3 times in 0.1 M PB, then immunoreactivity was visualized with 3,3'-diaminobenzidine (DAB substrate kit, Vector Laboratories) and nickel ammonium sulphate as chromogens. Sections were mounted on slides using a 0.15% gelatin solution, dehydrated using a series of ethanol solutions, defatted using Histoclear (Sigma-Aldrich), and coverslipped with Permount (Sigma-Aldrich). Representative photomicrographs from each of the regions of interest are shown in Figure 5.

4.5. Cell counts

Microscopy was performed using an Olympus BX51 microscope and VisioPharm software (VisioPharm, Hørsholm, Denmark). Immunolabeled cells were counted by centering a counting frame within each subregion at 10x then shifting to a 40x objective; cell counts were performed within the 22,406 μm^2 counting frame in real-time throughout the z plane. Cell counts were performed in the posterior aspect of the cingulate cortex (CgC), somatosensory cortex (SSC), piriform cortex (PIR), agranular insular cortex (AIC), lateral septum (LS), basolateral (BLA) and central (CEA) nuclei of the amygdala, nucleus accumbens core (NAcC) and shell (NAcS), dorsolateral (DLS) and dorsomedial (DMS) striatum, lateral habenula (HAB), paraventricular nucleus of the thalamus (PVT), and hippocampal subfields CA1, CA3, and dentate gyrus (DG). Placement of the field of view for each subregion is shown in Fig 6. For each rat, 6-8 individual hemisphere (both right and left) measurements were assessed, mean values were calculated, and values were normalized as cells per mm^2 .

4.6. Statistics

Data for each subregion were analyzed separately with $2 \times 2 \times 2$ factorial ANOVAs with sex (female or male), rearing condition (GRP or ISO), and social encounter (no social or social) as between-groups variables. When significant interactions were obtained, Tukey's HSD post-hoc tests were performed to determine differences between groups. Alpha was set at .05. Bivariate Pearson correlations were performed to determine relationships between Fos immunoreactivity and behaviors, with alpha set at .05 (two-tailed). Statistical analyses were performed using SPSS (IBM SPSS Version 22, Chicago, IL).

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Highlights

1. Male and female rats were subjected to 4 weeks of social isolation after weaning.
2. Rats engaged in a single 15 min social encounter with a novel same-sex conspecific.
3. Regionally specific effects of a social encounter on Fos protein expression.
4. Housing and sex-dependent blunting of encounter-induced Fos protein in some regions.

Figure 1.

Fos expression in cortical regions in male and female rats that had experienced either Group or Isolation housing during the postweaning period, and were then exposed to a 15 min social encounter with a same-sex novel conspecific. In the PIR (A), CgC (B), and AIC (C), a social encounter produced an increase in Fos protein expression only in GRP males. In the SSC (D), a social encounter produced an increase in Fos protein expression that did not differ between groups. * $p < .05$, significantly different from male GRP home cage controls. #, main effect of Social Encounter, $p < .05$.

Figure 2.

Fos expression in limbic regions in male and female rats that had experienced either Group or Isolation housing during the postweaning period, and were then exposed to a 15 min social encounter with a same-sex novel conspecific. In the PVT (A), a social encounter produced an increase in Fos protein expression only in group housed males. In the HAB (B) and BLA (C), a social encounter produced an increase in Fos protein expression only in group housed rats. In the CEA (D), a social encounter produced an increase in Fos protein expression in all groups. * $p < .05$, significantly different from male group-housed controls. @ $p < .05$, significantly different from same-sex home cage controls. #, main effect of Social Encounter, $p < .05$.

Figure 3.

Fos expression in the hippocampus and lateral septum in male and female rats that had experienced either Group or Isolation housing during the postweaning period, and were then exposed to a 15 min social encounter with a same-sex novel conspecific. In the CA1 (A) and CA3 (B) subfields, a social encounter produced an increase in Fos protein expression in all groups, though there was a trend for a Social Encounter $\times$ Housing interaction in CA3. In the DG subfield (C) and the LS (D), a social encounter produced an increase in Fos protein expression only in group housed males and females. @ $p < .05$, significantly different from same-sex home cage controls. #, main effect of Social Encounter, $p < .05$.

Figure 4.

Fos expression in the basal ganglia of male and female rats that had experienced either Group or Isolation housing during the postweaning period, and were then exposed to a 15 min social encounter with a same-sex novel conspecific. # $p < .05$, main effect of Social Encounter.

Figure 5.

Representative photomicrographs of Fos immunoreactivity in the regions of interest. Lower case letters represent tissue from control animals, and upper case letters represent tissue from group-housed males exposed to a social encounter. (a, A): PIR; (b, B): CgC; (c, C): AIC; (d, D): SSC; (e, E): PVT; (f, F): HAB; (g, G): BLA; (h, H): CEA; (i, I): CA1; (j, J): CA3; (k, K): DG; (l, L): LS; (m, M): NAcC; (n, N): NAcS; (o, O): DLS; (p, P): DMS. The scale bar is 50 μ m.

Figure 6.

Location of the field of view (not to scale) for each of the regions of interest assessed for Fos immunoreactivity. A: cingulate cortex (CgC), B: somatosensory cortex (SSC) C: agranular insular cortex (AIC), D: piriform cortex (PIR), E: dorsomedial striatum (DMS), F: dorsolateral striatum (DLS), G: lateral septum (LS), H: nucleus accumbens shell (NAcS), I: nucleus accumbens core (NAcC), J: CA1 subfield of the hippocampus, K: CA3 subfield of the hippocampus, L: dentate gyrus (DG), M: paraventricular nucleus of the thalamus (PVT), N: lateral habenula (HAB), O: central nucleus of the amygdala (CEA), P: basolateral

nucleus of the amygdala (BLA). Plates 14 (top) and 31 (bottom) from Paxinos and Watson (2007).

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Table 1

Correlations between Fos immunoreactivity in the regions of interest with social interaction, aggressive grooming, and chasing, split by sex. The behavioral data have been reported previously (Wall et al., 2012).

Region	Social Interaction		Aggressive Grooming		Chasing	
	Female	Male	Female	Male	Female	Male
PIR	-0.389	-0.429	-0.118	-0.713**	-0.213	-0.694**
CgC	-0.214	-0.267	-0.042	-0.587*	-0.316	-0.626*
AIC	-0.200	-0.195	-0.058	-0.528*	-0.523*	-0.545*
SSC	-0.552*	-0.190	-0.464	-0.285	-0.366	-0.099
PVT	-0.496	-0.650**	-0.460	-0.779**	-0.394	-0.533*
HAB	-0.643**	-0.283	-0.441	-0.423	-0.425	-0.234
BLA	-0.290	-0.362	-0.185	-0.459	-0.565*	-0.505*
CEA	-0.498	-0.063	-0.269	-0.202	-0.560*	-0.100
CA1	-0.123	-0.111	0.066	-0.369	-0.098	-0.323
CA3	-0.355	-0.229	-0.391	-0.380	-0.331	-0.349
DG	-0.441	-0.083	-0.267	-0.306	-0.381	-0.307
LS	-0.369	-0.376	-0.129	-0.620*	-0.428	-0.662**
NACs	-0.137	0.042	0.133	-0.064	-0.136	-0.081
NACc	0.107	-0.086	0.260	-0.129	0.004	0.017
DMS	-0.114	-0.087	0.180	-0.217	-0.074	-0.203
DLS	-0.218	0.113	0.036	-0.096	-0.329	-0.121

* $P < .05$

** $P < .01$.