PLAYFUL FEEDBACK AND THE DEVELOPING BRAIN

HEATHER C. BELL
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Department of Neuroscience
University of Lethbridge
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For the rats, without whom none of this work would have been possible.

Abstract

The prefrontal cortex (PFC) has long been thought to be the seat of social behaviours in mammals. Lesions of the orbitofrontal cortex (OFC), a subregion of the PFC, are known to cause social deficits in humans. Interestingly, social deficits are also seen in rats with OFC lesions. Rats that are deprived of peer play during development exhibit behaviour similar to OFC-ablated animals. Another subregion of the PFC, the medial prefrontal cortex (mPFC) is interconnected with the OFC. The mPFC and OFC have been shown to be reciprocally responsive to a variety of influences, in terms of dendritic morphology. It was hypothesized that social experiences are necessary for the proper development of the OFC, and that, because of the interconnectivity, the mPFC would also be sensitive to social experience. The social condition in which juvenile rats were raised was manipulated, and the OFC and mPFC were shown to be differentially responsive to specific aspects of social experience. It was already known that OFC lesions produce specific social deficits, but the contribution of the mPFC to the production of social behaviour was unknown. To investigate the contribution of the mPFC to the performance of social behaviour, animals were given mPFC lesions, and their social play behaviour was quantified. mPFC-ablated animals had altered play patterns that were distinct from those seen in the OFC-ablated animals. It was concluded that the OFC and mPFC are differentially responsive to social stimuli during development, and that the OFC and mPFC make discrete contributions to the production of social behaviour. The results were interpreted in an evolutionary context.

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Chapter 1

INTRODUCTION

Play is an almost universal behaviour of mammals, and has been noted to a lesser degree in birds, fish, reptiles, and even invertebrates. The fact that play behaviour is so ubiquitous has led to the assumption that play is evolutionarily adaptive. Just how play increases an organism's fitness, however, has not been determined. In fact, to date, more than 30 different theories about the ontogeny, function, and underlying neural circuitry of play have been suggested (Burghardt, 2005).

Historically, one of the biggest problems with developing a comprehensive theory of play is that researchers have been to be unable to agree on a definition of play. While it is intuitively obvious that a kitten chasing a ball of string is playing, it is difficult to pinpoint exactly what about this activity makes it playful. The issues surrounding the scientific study of play become more complex when the behaviour of different types of organisms is compared. For example, when comparing the rough-and-tumble play of juvenile rats with the fantasy play of human children, it is challenging to identify a common factor that underlies both activities.

The foundation of the lack of consensus among investigators of play is that what is called *play* is not just one type of behaviour, nor do the behaviours necessarily have common origins. For instance, it is likely that social play (play involving more than one organism) is etiologically different from object play (an organism and an object). In fact, there are at least three disparate types of play — social, locomotor (e.g., running, jumping) and object play (Fagen, 1981) — and some researchers have broken down the types further (e.g., Power, 2000). Additionally, different species have different play profiles — that is, one species may engage in two types of play, whereas another may use three types, and so on. Even closely-related species, such

as mice and rats, may have vastly different play repertoires (see Terranova, Laviola, de Acetis, & Alleva, 1998; Pellis & Pasztor, 1999). It is also possible to be engaged in more than one type of play at a given time. For example, two kittens vigorously chasing a ball could be engaging in locomotor play, object play, and social play.

A recent useful operational definition of play that encompasses the above types, and that has synthesized and expanded upon previous definitions, was developed by Burghardt (2005). First, the behaviour must have no immediate survival function. Second, the behaviour must be spontaneous and rewarding. Third, the behaviour can be comprised of elements that are functional in other contexts, but during *play*, these elements are incomplete, or are organized in manner that is atypical of the "serious" behaviour. Fourth, the behaviour must be performed similarly, but not in a stereotyped fashion, for at least part of the animal's development. Finally, the behaviour must occur in a *relaxed field* — that is, when the animal does not face any immediate survival threats.

Playfulness is especially prevalent among juvenile mammals. Of the various types of play found throughout the animal kingdom, the one that has been investigated the most is social play. Almost all mammals engage in some form of social play for at least a part of their lifespan (Burghardt, 2005). Many, including humans and other primates, play socially into adulthood – albeit, at a lower rate than during the juvenile phase (Pellis & Iwaniuk, 2000).

One type of social play that has been characterized more thoroughly than any other is rough-and-tumble social play, especially in the laboratory rat, *Rattus norvegicus*. In contrast to serious fighting, the target of which is biting the rump, rough-and-tumble play centres around attack and defense of the nape of the neck, which is nuzzled with the snout if contacted (Pellis & Pellis, 1987; Siviy & Panksepp, 1987; Pellis, 1988). One animal (the *attacker*) approaches the other animal (*the defender*)

and either contacts or attempts to contact the nape. At this point, the defender has a variety of defensive options. The defender may evade by pivoting away from the attacker, or the defender may pivot toward the attacker, ending up face-to-face with the attacker (a *facing defense*). The most common form of facing defense is to rotate around the long axis of the body, starting with the head and neck. In turn, this rotatory defense can involve either rotating completely, so that the rat ends by lying on its back (i.e, supine), or only partially, thus keeping one or both hind feet on the ground. From the latter position, the defender can push its hip against the attacker, or rear upright (usually ending with both animals standing on their hind legs facing one-another). Following a successful defensive maneuver, the defender may then launch a counterattack aimed at the original attacker's nape. In the event that the defender does not respond to the attacker, the attacker will nuzzle the nape for a moment and then leap away (Pellis & Pellis, 1990, 1992; Pellis, Pellis, & McKenna, 1994).

Rats engage in play throughout the lifespan, although the frequency and composition of the play changes as the animals mature. Elements of rough and tumble play in rats emerge gradually from about Postnatal Day 18 to 19, but the full complement of attack and defense tactics is present by about Postnatal Day 25 to 30 (Pellis & Pellis, 1997). The frequency of play reaches a peak during the juvenile phase – Postnatal Day 30 to 40 – and then declines after puberty (Thor & Holloway, 1984). During the juvenile phase, play bouts are reciprocal — the role of *defender* and *attacker* are adopted with approximately equal frequency among play partners. Following puberty, especially in males, play bouts become increasingly asymmetrical, and the asymmetries reflect the social status of the individuals (Pellis & Pellis, 1992). One factor contributing to reciprocity is that juvenile rats are more likely to stand on a supine partner with all four paws; however, after puberty, the hind feet are placed

on the ground when standing over a supine partner (Foroud & Pellis, 2002, 2003). Standing with four paws on the body of the defender, as opposed to two, makes the attacker less stable, and thus, more susceptible to counter-attack by the defender (Pellis, Pellis, & Foroud, 2005).

Play in rats is differentiated based on sex and status. Juvenile male rats engage in rough-and-tumble play at a higher frequency than female rats (Olioff & Stewart, 1978). Dominant male rats, at all ages, initiate fewer playful attacks than subordinate male rats, and the dominance-related difference in attack frequency becomes more extreme as the animals mature. With age, dominant males also tend to rotate to supine progressively less frequently when attacked, and to partially rotate with progressively greater frequency when attacked. In contrast, subordinate males maintain a high level of complete rotations as they mature (Pellis & Pellis, 1992). Interestingly, this shift in defensive tactics is not seen in female rats (Takahashi & Lore, 1983). Females also react to play solicitation earlier during the play bout than males. The increased distance between the attacker and the defender when the defender begins the defense allows female rats to adopt different defensive postures than males (Pellis et al., 1994).

Rats also find play highly rewarding. Access to a play partner is incentive enough for rats to learn a variety of tasks (Humphreys & Einon, 1981; Calcagnetti & Schechter, 1992; Crowder & Hutto, 1992). In fact, the most readily-observed effect of play deprivation on the behaviour of rats is the *rebound effect*. The rebound effect is characterized by an increase in the frequency of play by rats following a period of social isolation (Martin & Caro, 1985). Animals living in divided cages separated by either wire mesh or plexiglass also exhibit the rebound effect when allowed full access to their cagemates (Hole, 1991). But it appears that the rebound effect is not simply the result of a lack of exercise during isolation. When rats are allowed access to a running wheel during periods of isolation, the rebound effect remains intact (Holloway

& Suter, 2004).

Depriving rats of play altogether produces long-term behavioural deficits. Rats that are deprived of rough-and-tumble peer play as juveniles are socially incompetent as adults (Hol, van den Berg, van Ree, & Spruijt, 1999). Specifically, play-deprived rats do not express the normal identity-dependent modification of behaviour in response to a variety of social partners (Hol, Koolhaas, & Spruijt, 1994; Pellis, Field, & Whishaw, 1999). Additionally, play-deprived animals, when exposed to a novel colony, do not demonstrate normal avoidance behaviour that would reduce the risk of attack from the established residents (van den Berg et al., 1999). Isolation-raised rats are impaired at rule-learning tasks and are behaviourally more rigid than socially-reared animals (Jones, Marsden, & Robbins, 1991). Exposing socially isolated juveniles to another normal conspecific for only one hour a day, during which the animals spend much time engaged in rough and tumble play, attenuates the effects of social isolation (Einon & Portegal, 1991). Being exposed to adults or to drugged peers that do not play is not enough to compensate for the effects of isolation (Einon, Morgan, & Kibbler, 1978). The fourth and fifth weeks of life are crucial for the experience of play in rats (Einon & Morgan, 1977; Einon et al., 1978; Portegal & Einon, 1989; Hol et al., 1994). It appears that play deprivation before and after this critical period does not interfere with social behaviour. Isolation-reared animals also show deficits in the pre-pulse inhibition component of the Acoustic Startle Task (AST) (Wilkinson et al., 1994).

The lack of exposure to social peer play during development negatively affects adult behaviour. The fundamental assumption of neuroscience is that behavioural changes occur as a result of structural changes in the brain. But does play affect the structure of the brain? Byers and Walker (1995) have suggested that play developed as a method for refining the motor system during development. As evidence for this

hypothesis, they noted that several species of animals undergo cerebellar synaptic pruning and muscle fibre differentiation at approximately the same time that play peaks during the juvenile phase. Therefore, animals that engaged in play during the critical period of plasticity in the motor system were able to refine their movements according to environmental inputs. Animals that were better suited to their environments were selected for, and the propensity for animals to play during the juvenile phase was enhanced. Thus, the evolutionary benefit of play may be in its role of shaping the development of crucial brain areas, with play fighting, in particular, influencing the development of aspects of the social brain.

Play and the Brain

It has been known for some time that a wide variety of variables influence the development of the brain. Manipulating factors as diverse as diet (West & Kemper, 1976; Hamdi, Onaivi, & Prasad, 1992) tactile stimulation (Pinckney, 1976; Pauk, Kuhn, Field, & Schanberg, 1986), housing (Stern, Winokur, Eisenstein, Taylor, & Sly, 1960; Rosenzweig & Bennett, 1972; Hargreaves, 1994) and prenatal environment (Sobrian, 1977; Smith, Wills, & Naylor, 1981) has produced structural changes in the brain, as well as behavioural effects. Drugs, which produce behavioural changes, have been shown to act on the microstructure of the brain by altering neuronal morphology. For example, the administration of stimulants to rats selectively alters both spine density and dendritic length of neurons in several brain regions (Robinson & Kolb, 1999). Because excitatory synapses are located on dendritic spines (Cowan, Südhof, & Stevens, 2001), changing dendritic spine number by altering spine density or dendritic length (or both) can affect neuronal connectivity.

One pattern of neuronal plasticity that has been noted is a reciprocal relationship

between two sub-regions of the prefrontal cortex – the orbitofrontal cortex (OFC) and the medial prefrontal cortex (mPFC). The OFC and mPFC are present in the brains of many mammals, including rats and humans. The OFC is situated anteriorly, on the ventrolateral surface of the frontal lobe (Fig. 1.1). The OFC receives connections from all sensory modalities, from limbic circuitry, and weakly, from motor areas (see Kolb, 1984). The mPFC is found medially in the frontal cortex, encompassing the anterior-most portion of the anterior dorsal cingulate cortex (Fig. 1.1). The mPFC is connected primarily with motor areas, and to a lesser degree, with reward areas of the cortex (see Kolb, 1984; Heidbreder & Groenewegen, 2003). The mPFC and OFC receive afferents from the dorsomedial nucleus of the thalamus (Krettek & Price, 1977), which makes both areas part of the prefrontal cortex (Rose & Woolsey, 1948). Additionally, the OFC and mPFC are reciprocally connected with one-another (Öngür & Price, 2000; Walton, Rudebeck, Bannerman, & Rushworth, 2007).

Lesion studies suggest that the OFC and mPFC are functionally distinct. For instance, Schneider and Koch (2005a) found that rats with mPFC lesions performed better than intact animals on the AST – that is, rats with mPFC lesions were better able to inhibit their startle responses. In marmosets, it has been demonstrated that lesions of the OFC (but not mPFC) interfere with the acquisition of a new response to a conditioned reinforcer, leaving responses to primary reinforcers unchanged (Pears, Parkinson, Hopewell, Everitt, & Roberts, 2003). In rats, selective OFC lesions impair the ability to perform anticipatory reward tasks (Kesner & Gilbert, 2007). Latent inhibition is also abnormally persistent in rats with OFC lesions, but not in those with mPFC lesions, indicating that the OFC-ablated animals had difficulty forming new associative relationships (Schiller & Weiner, 2004). OFC lesions, but not mPFC lesions, also increase impulsivity in rats (Walton et al., 2007).

On the other hand, mPFC-damaged animals are impaired in movement initiation,

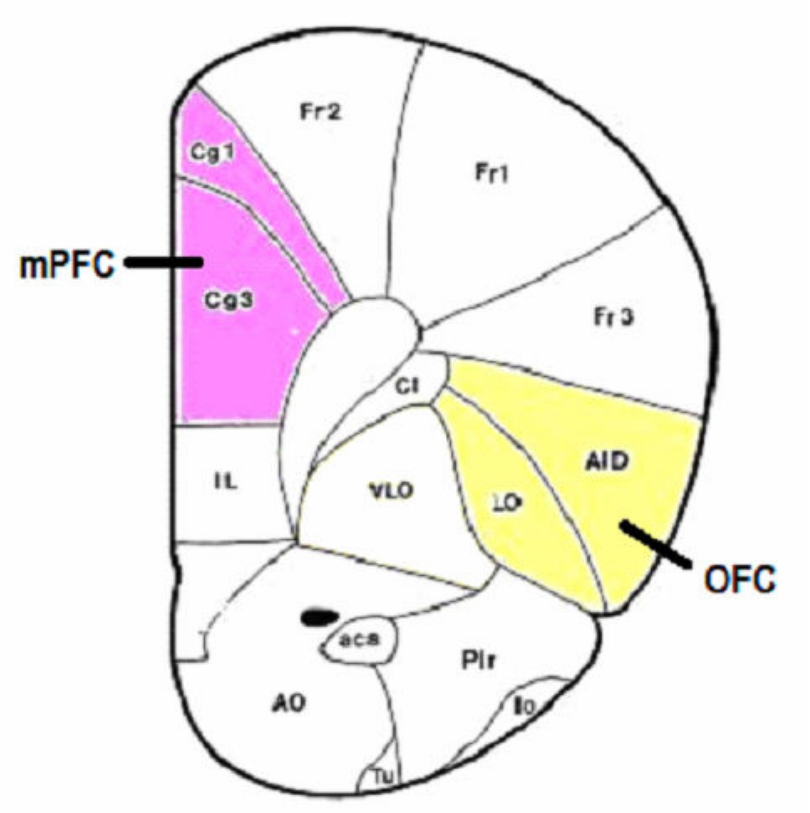

Figure 1.1: The locations of the OFC and mPFC in an anterior coronal section of the rat brain.

but not on performance of a movement once it has been initiated (Hauber, Bubser, & Schmidt, 1994). mPFC lesions, but not OFC lesions, result in rats that are less willing to expend effort in order to obtain a reward (Walton et al., 2007). Additionally, medial frontal lesions are known to disrupt both learned and "natural" sequential behaviours (Kolb & Whishaw, 1983a, 1983b). Indeed, the mPFC is thought broadly to control the temporal sequencing of movement (Heidbreder & Groenewegen, 2003). In addition, mPFC lesions disrupt working memory for egocentric responses (Ragozzino & Kesner, 2001). Generally, lesions of the mPFC have been thought to increase timidity – in contrast to lesions of the OFC, which are thought to increase aggressiveness (Kolb, 1990). However, the appearance of timidity may simply be an artifact of a general decrease in the motivation or ability for movement.

Even though the mPFC and OFC are functionally distinct, the two areas are reciprocally connected (Öngür & Price, 2000; Walton et al., 2007). There is evidence that the neuronal structure of the OFC and mPFC are altered in opposite ways by a variety of influences. Specifically, when the number of available neuronal connections increases in the mPFC in response to some factor, it decreases in the OFC, and vice versa. Substances that have produced this reciprocal plasticity include amphetamine (Crombag, Gorny, Li, Kolb, & Robinson, 2005) and morphine (Robinson, Gorny, Savage, & Kolb, 2002). Additionally, there is a baseline reciprocal sex difference between the OFC and mPFC. Female rats have more extensive dendritic arborization in the OFC than males, and less extensive dendritic arborization in the mPFC than males (Kolb & Stewart, 1991).

The reciprocity and functional distinctness between the mPFC and OFC becomes important in the context of behavioural differences. For instance, two strains of rats that have been bred for their differential responsiveness to kindling (the priming of proneness to production of seizures by electrical stimulation of the amygdala)

exhibit opposite patterns of dendritic morphology in the OFC and mPFC. FAST rats, which are susceptible to kindling, have less dendritic arborization in the OFC and more arborization in the mPFC than SLOW rats, which are resistant to kindling (C. Reinhart, unpublished data). Behaviourally, FAST rats display more juvenile-typical play behaviour throughout their lifespan, whereas SLOW rats play in a more adult-typical manner (Reinhart, Pellis, & MacIntyre, 2004).

Rats that are given amphetamine have more dendritic spines in the mPFC and fewer spines in the OFC than controls (Crombag et al., 2005). Amphetamine-treated rats solicit play less than controls, despite the fact that their overall movement increases (Thor & Holloway, 1983). At low doses, only play solicitation (attack) is reduced, but at higher doses, both attack and defense components of play fighting are diminished (Field & Pellis, 1994). Morphine, which increases spine density in the OFC and decreases spine density in the mPFC (Robinson et al., 2002), increases the frequency of all aspects of social play in rats in a dose-dependent manner (Louk, Vanderschuren, & van Ree, 1994).

It has been demonstrated that the cortex is involved in the production of play behaviour in rats (Pellis, Pellis, & Whishaw, 1992). Furthermore, specific cortical areas are involved in specific aspects of play. It has been shown that animals that are given Postnatal Day 3 orbitofrontal cortex (OFC) lesions display almost identical deficiencies in adult social competency as those seen in the play-deprived rats (Pellis et al., 2006). Pilot studies have also suggested that the neurons of the OFC become more complex in response to varied social experience (as opposed to static social experience) during development (D. Hamilton, unpublished data), and juveniles that were housed socially following weaning had more complex OFC dendritic fields than isolation-housed subjects (Bock, Murmu, Ferdman, Leshem, & Braun, 2008). The motor cortex (MC) is also important for the production of play behaviour in rats;

however, the contribution of the MC is different from that of the OFC. When animals are given Postnatal Day 10 MC lesions, they do not exhibit the age-related changes in play fighting tactics that are observed in controls (Kamitakahara, Monfils, Forgie, Kolb, & Pellis, 2007).

The Experiments

Initially, the goal of the current research was to determine how different types of juvenile social experience affect the neuronal morphology of the frontal cortex. Based on the above literature, it was hypothesized that different types of social experiences during the juvenile period — specifically, different types of play experiences — would alter the morphology of the neurons in the OFC and the mPFC. It was predicted that animals with access to peer play would have more complex OFC neurons compared to animals that did not have access to peer play, and that reciprocal alterations would be seen in the mPFC (i.e., mPFC neurons should be less complex).

The findings of the first experiment that tested the relationship between neuronal morphology and juvenile play experience resulted in a revision of the initial hypothesis. It appeared that the OFC and mPFC, although they exhibited a roughly reciprocal relationship in neuronal complexity, responded differentially to specific aspects of the social environment to which the animals were exposed. The results of the first experiment suggested that the OFC is sensitive to the number of social partners encountered during the juvenile phase, and that it becomes more complex as the number of social partners increases. In contrast, the mPFC is sensitive to the types of behaviours engaged in during the juvenile phase, and becomes more complex when peer play is not present. A second experiment, in which the number of social partners was held constant, and the presence or absence of peer play manipulated,

was designed to test the hypothesis that specific aspects of social experience during development affect OFC and mPFC differentially.

Given that the OFC is known to affect the partner-related modulation of defense tactics (Pellis et al., 2006), and given that the OFC and mPFC are affected by different aspects of social experience, a second set of experiments was conducted to assess the contribution of the mPFC (if any) to the regulation of play behaviour. The contribution of the mPFC to play was addressed by quantifying the play behaviour of animals with selective mPFC aspiration lesions both developmentally, and during adulthood. The results suggest that the OFC and mPFC make distinct contributions to the production of appropriate social behaviour.

Chapter 2

JUVENILE REARING CONDITIONS AND THE DEVELOPMENT OF THE PREFRONTAL CORTEX

Play has long been thought to offer a benefit to the animals that engage in it. However, direct evidence that playing is beneficial has been lacking. This research will assess the relationship between the experience of social play during the juvenile period of development, and the microstructure of the brain in two regions that are thought to be involved in social behaviour.

It was suspected that manipulating the social environment in which juvenile rats were raised would alter the dendritic morphology of OFC neurons. Because of the suggested link between the OFC and mPFC, corresponding morphological changes were also anticipated in the mPFC. It was anticipated that increasing the amount of peer play to which a juvenile rat was exposed during the critical period, by increasing the number of peers with which it could play, would produce an increase in the complexity of OFC neurons. The same increase in peer play was expected to have a complexity-decreasing effect in the dendritic fields of mPFC neurons. On the other hand, if juvenile rats were deprived of peer play by being raised with adults, it was expected that the OFC should be less complex when compared to those of rats raised with juveniles. Again, it was expected that the mPFC would be more complex in the absence of peer play than in the presence of peer play.

The results of the first experiment required that the initial hypothesis be refined. It also became apparent that, not only was the presence or absence of juvenile play being manipulated, but that the number of other animals to which the rat was exposed was also being altered. Therefore, in order to eliminate the confound between play and social partners, a second experiment was run in which the number of social partners

was held constant while the presence or absence of peer play was manipulated.

Method

Subjects

A total of 96 female Long-Evans hooded rats born at the University of Lethbridge, Canadian Centre for Behavioural Neuroscience, were used. All subjects were housed, beginning at approximately Postnatal Day 21, in pairs or quadrads (see below). Rats were kept in 46 cm x 25 cm x 20 cm polyethylene tubs with processed corncob as bedding, and maintained at a constant $21 - 23\,^{\circ}\mathrm{C}$ on a 12-hour light-dark cycle. Food and water were provided *ad libitum*. All animals were handled and cared for in accordance with the Canadian Council for Animal Care (CCAC) regulations of the University of Lethbridge.

Experiment 1: Brain Development and Exposure to Varying Degrees of Peer Play

To examine the neural correlates of the behavioural changes stemming from juvenile peer play experiences, 18 female subjects were placed randomly into one of three conditions at Postnatal Day 21. *No peer play* subjects were housed with one adult female (older than Postnatal Day 60), *single peer play* subjects were housed with one other juvenile female, and *multiple peer play* subjects were housed with three other juvenile females. It was expected that there would be more peer-peer play in the quadrads than in the pairs of juveniles as a result of the *contagion effect*, whereby animals are more likely to play in the presence of other animals that are playing (Hole & Einon, 1984; Pellis & McKenna, 1992). Because the period from Postnatal Day 30

to Postnatal Day 55 has been shown to be a critical period during which to experience peer play (Einon & Morgan, 1977; Einon et al., 1978; Portegal & Einon, 1989; Hol et al., 1994), subjects remained in their respective groups until Postnatal Day 60. Subjects were handled by the experimenter approximately once every three days for 10 minutes in order to familiarize the subjects with the experimenter. Due to space constraints, subjects were run in two cohorts. Three animals from each group were run in each cohort.

Histology and Anatomy

On Postnatal Day 60, subjects were deeply anesthetized using 0.6 mL of 3.4% sodium pentobarbitol, perfused with 9% percent saline and brains were harvested. All brains were prepared using the modified Golgi-Cox procedure (see Gibb & Kolb, 1998). Briefly, following extraction, brains were immersed in Golgi-Cox solution. After 14 days, brains were placed in a 30% sucrose solution for seven days. The brains were then cut into 200 μm sections using a vibrating microtome and placed on 2% gelatin-dipped glass slides. After being placed in an airtight darkened container for three days, the slides were stained, coverslipped, and left to dry for approximately 2 weeks.

Quantification of dendritic morphology was carried out by tracing Layer III pyramidal neurons (Fig. 2.1) from Zilles' (1985) AID (OFC) and CG3 (mPFC) (Fig. 1.1) onto paper using a *camera lucida*. Cells were drawn at 250x magnification. Five cells were selected from each hemisphere in each area, and the mean for each measure was taken as the data point. Each cell was drawn only if it was fully impregnated and if its processes were not overlapping the processes of other cells. The drawings were analyzed using two measures, and each analysis was applied separately to the apical and basilar dendritic fields, as it is known that the apical and basilar fields receive

information from different cortical areas (Kolb & Whishaw, 2003). Sholl analysis (Sholl, 1956) determined the total dendritic length by overlaying a transparency with concentric circles onto the drawing of the neuron and counting the number of line crosses at each circle. Branch order analysis (Coleman & Reisen, 1968), an estimate of dendritic complexity, was calculated by counting the number of bifurcations that occurred on each dendrite. Lengths of dendrites in both the apical and basilar fields were also traced at high magnification (1600x). Each section was required to be at least 20 microns (μm) long. Spine density, which is an indicator of the availability of synaptic space, was then calculated by counting the number of dendritic spines, divided by the total length of the traced dendrite in μm.

Results

Univariate Analyses

All analyses were univariate ANCOVAs, with *set* (first or second half of the experiment) as the covariate. *Group* (rearing condition) was the between-subjects factor. Because this experiment was primarily an hypothesis-generating exercise, all post-hoc analyses were unrestricted Least Significant Differences (LSDs) (Saville, 2003).

Branch Order.In the OFC, the number of basilar dendrites arising from the soma (first basilar branch order) differed significantly with respect to *group*, $F(2, 32) = 3.765, p = .034$. Post-hoc analyses showed that the *multiple peer play* group had significantly more basilar dendrites than the *no peer play* group ($p = .012$) (Fig. 2.2). The apical field did not exhibit any significant differences on any measure ($p > .05$).

In the mPFC, the apical field had a significantly different mean branch order (degree of arborization, that is, the space-filling properties of the dendritic arbor) with respect to *group*, $F(2, 32) = 5.590, p = .008$. The post-hoc analysis revealed that

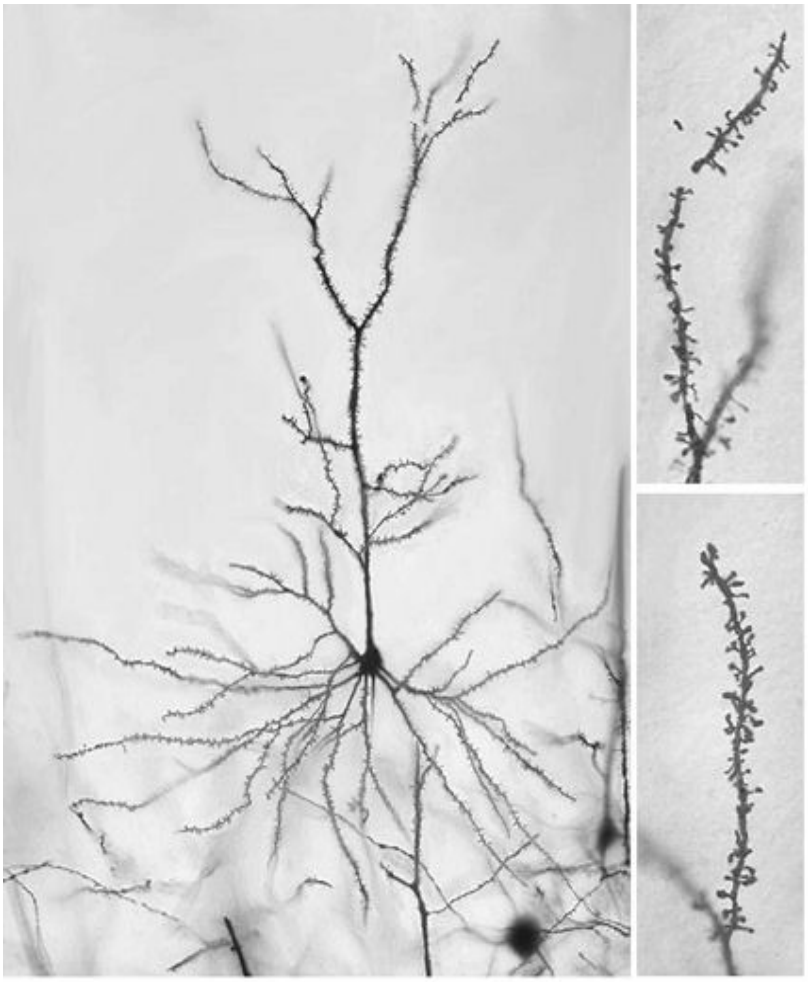

Figure 2.1: Layer II/III pyramidal neuron in the rat brain. Insets are higher-magnification sections of dendritic spines. Photo by Grazyna Gorny. Photo obtained from: `http://sitemaker.umich.edu/terryrobinson/par1_pyramidal_cell`

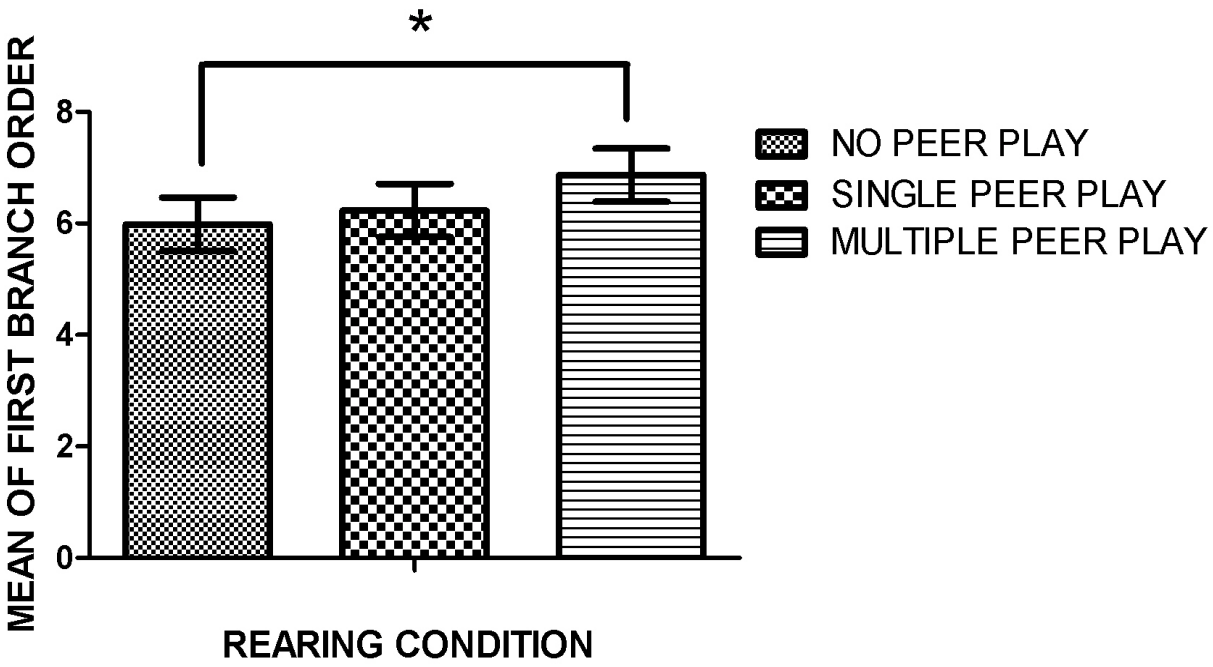

Figure 2.2: Mean number of basilar dendrites originating from the soma of OFC Layer III pyramidal neurons with respect to rearing condition. Error bars are 95% confidence intervals. * indicates difference is significant at $\alpha = .05$.

the *no peer play* group had significantly more dendritic arborization than both the *single peer play* and *multiple peer play* groups ($p = .009$ and $p = .005$, respectively), but that the latter two groups did not differ from one-another ($p > .05$) (Fig. 2.3). In the mPFC, there were no significant effects revealed in any aspect of the basilar dendritic field ($p > .05$).

Sholl Analysis.There were no significant differences with respect to Sholl analysis in any aspect of the OFC neurons ($p > .05$).

In the mPFC, the dendritic length (mean Sholl value) in the apical field differed significantly with respect to *group*, $F(2, 32) = 5.332, p = .010$. The post-hoc test showed that the *no peer play* grouped differed from the *single peer play* group ($p = .003$) (Fig. 2.4). In the mPFC, there were no significant effects revealed in any aspect of the basilar dendritic field ($p > .05$).

Spine Density.There were no significant differences in either the OFC of mPFC with respect to spine density ($p > .05$).

Repeated-Measures Analyses

Repeated-measures analyses were also undertaken. All analyses were repeated-measures ANCOVAs with *set* as the covariate. The between-subjects factor was *group*. Within-subjects factors were either *Sholl* or *branch order*. Separate analyses were carried out for *Sholl* and for *branch order*.

Branch Order.Branch order was not significant for either the OFC or mPFC ($p > .05$).

Sholl Analysis.In the OFC, there was a significant *Sholl* by *Group* interaction in the right hemisphere of the basilar field, $F(16, 112) = 9.872, p = .002$. There was also a main effect of *Sholl*, $F(8, 112) = 57.419, p < .001$ (Fig. 2.5). There was no effect of

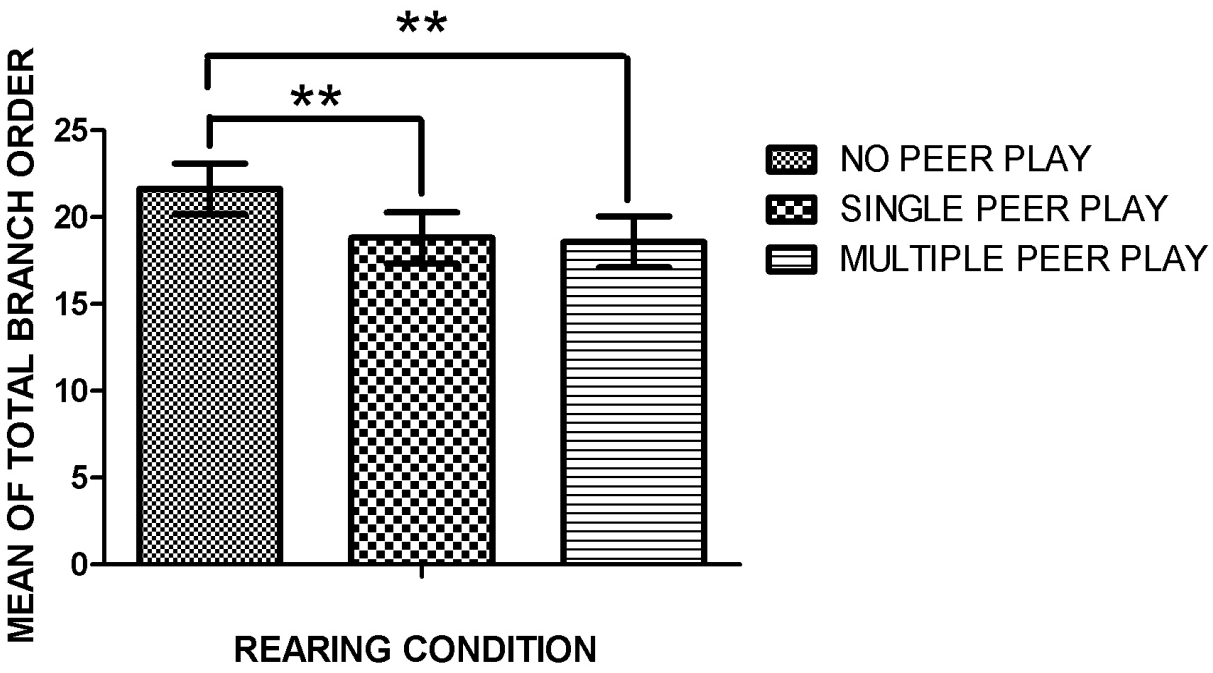

Figure 2.3: Mean degree of dendritic arborization in the apical field of mPFC Layer III pyramidal neurons with respect to rearing condition. Error bars are 95% confidence intervals. ** indicates difference is significant at $\alpha = .01$.

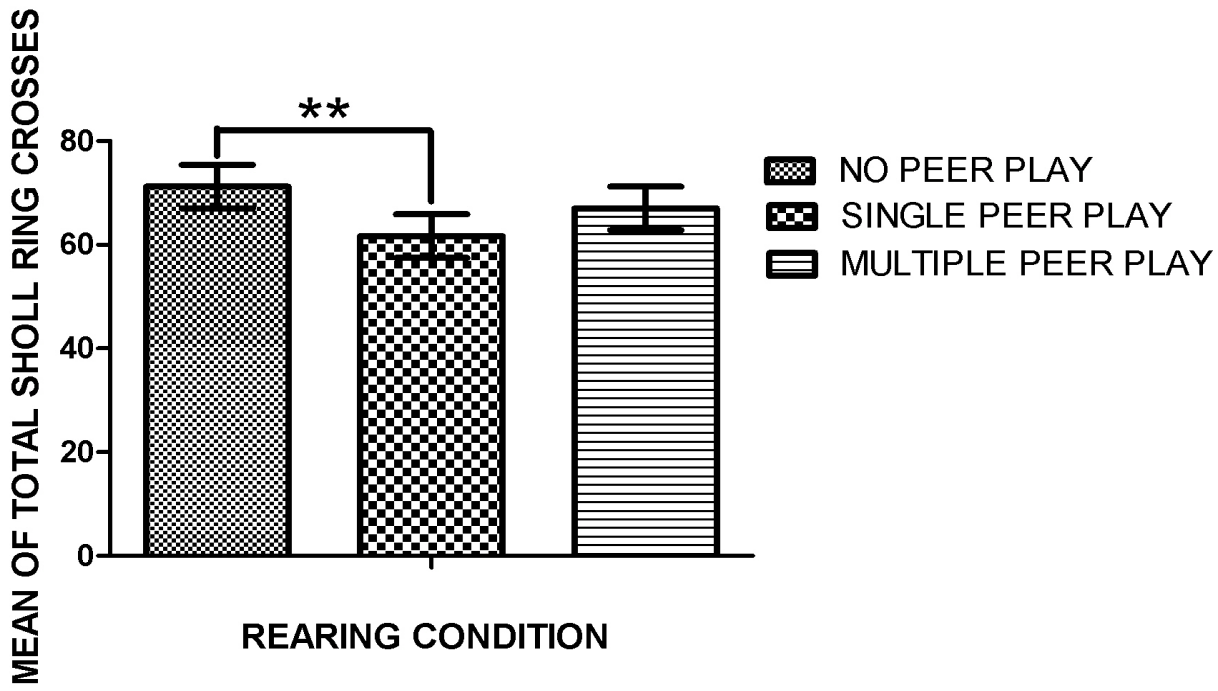

Figure 2.4: Mean dendritic length in the apical field of mPFC Layer III pyramidal neurons with respect to rearing condition. Error bars are 95% confidence intervals. ** indicates difference is significant at $\alpha = .01$.

group ($p > .05$).

Although the analysis was not significant, the pattern seen in both hemispheres of the OFC reflects that of the right hemisphere (Fig. 2.6).

There was a significant interaction of *Sholl* and *group* in the apical field of the mPFC, $F(30, 480), p < .001$. There was also a main effect of *Sholl*, $F(15, 480) = 17.577, p < .001$ (Fig. 2.7). *Group* was not significant.

Experiment 2: The Effects on Brain Development of Peer Play and Number of Social Partners

Owing to the above results, the initial hypothesis was refined to reflect a possible differential involvement of the two facets of juvenile social experience – the experience of peer play, and the number of social partners encountered in the rearing environment. The prediction arising from this hypothesis was that manipulating the presence or absence of peer play, while keeping constant the number of social partners experienced, should result in a change in mPFC but not OFC neuronal morphology. Specifically, the mPFC neurons should be less complex when peer-peer play is present than when it is absent.

Twelve female subjects were placed randomly and equally in one of two conditions at Postnatal Day 21. Both groups were comprised of quadrads of animals; however, one group, the *adult* condition contained the subject and three adult females, whereas the other group, the *juvenile* condition, contained the subject and three juvenile females. Subjects remained in their respective groups until Postnatal Day 60.

Histology and Anatomy

All procedures were identical to those in Experiment 1.

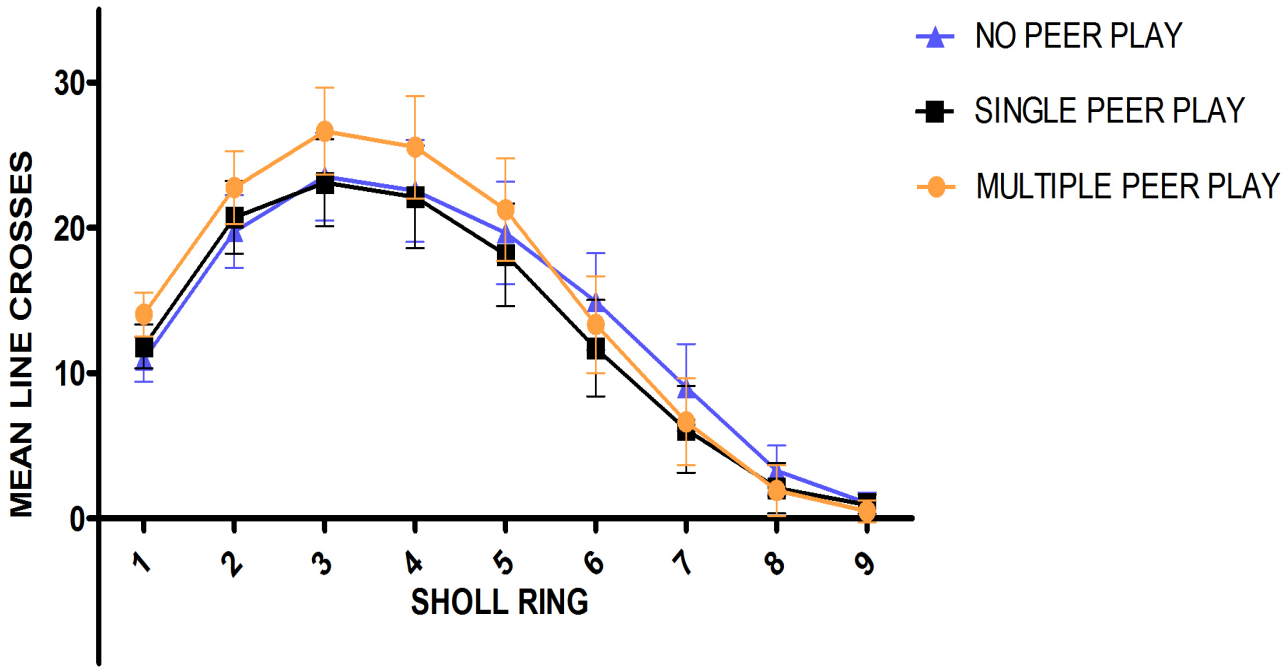

Figure 2.5: Change in mean dendritic length of the basilar field of OFC Layer III pyramidal neurons in the right hemisphere, moving distally from the soma, with respect to rearing condition. Error bars are 95% confidence intervals.

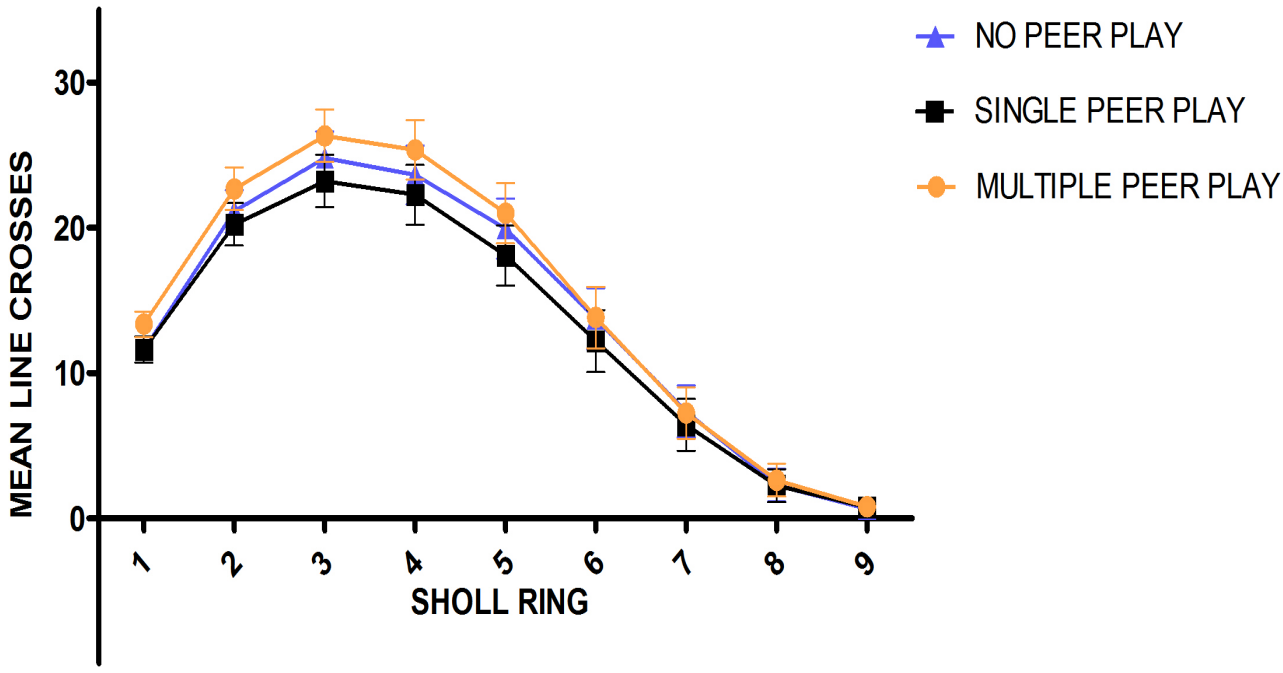

Figure 2.6: Change in mean dendritic length of the basilar field of OFC Layer III pyramidal neurons, moving distally from the soma, with respect to rearing condition. Error bars are 95% confidence intervals.

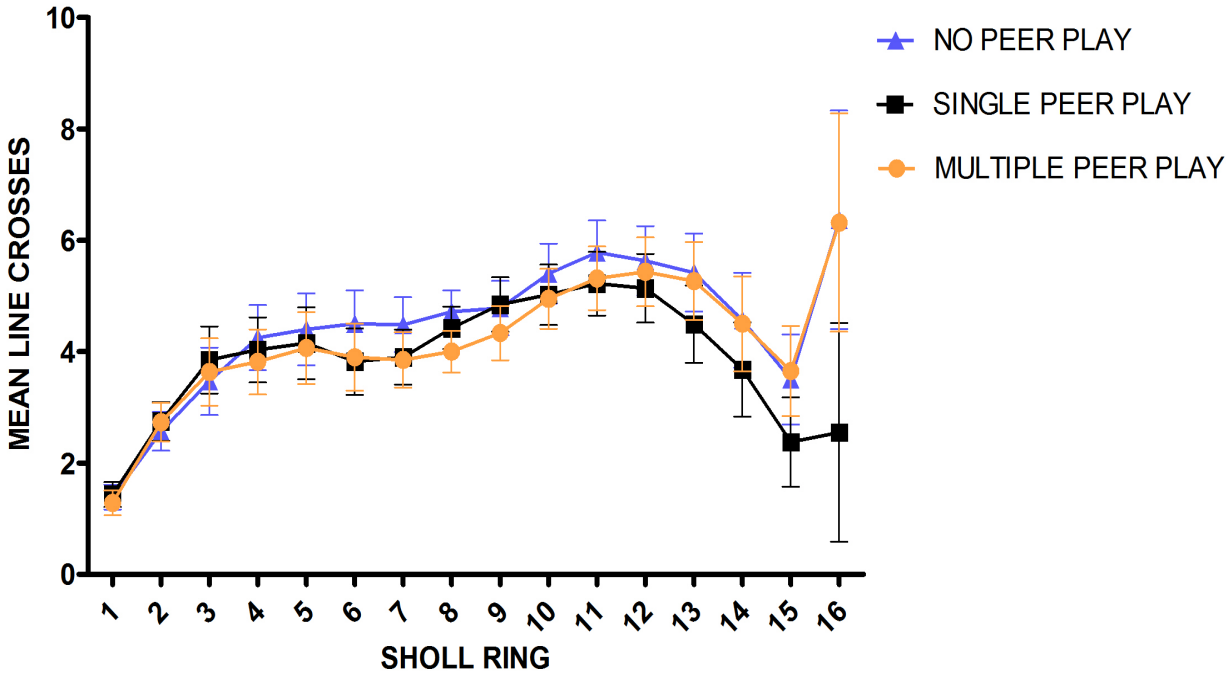

Figure 2.7: Change in mean dendritic length in the apical field of mPFC Layer III pyramidal neurons, moving distally from the soma, with respect to rearing condition. 16 represents Sholl ring 16 and beyond. Error bars are 95% confidence intervals.

Results

Independent t*-tests*

Independent-samples *t*-tests were undertaken, with *group* (rearing condition) as the between-subjects factor. Because there was an *a priori* directional hypothesis, one-tailed tests were used in the analyses of the mPFC data. Two-tailed tests were used in all other analyses.

Branch Order.With respect to the two groups, branch order was not significantly different in any aspect of the OFC ($p > .05$)(Fig. 2.8).

In mPFC, the *juvenile* condition had a greater number of apical dendrites (mean of first branch order) than the *adult* condition, $t(22) = 3.821, p < .001$ (Fig. 2.9). The *juvenile* group also had a significantly greater degree of arborization in the apical field (mean branch order) than did the *adult* group, $t(22) = 2.096, p = .024$ (Fig. 2.10). There were no significant differences in branch order analyses in the basilar field ($p > .05$).

Sholl Analysis.There were no significant differences in Sholl measures in either the OFC or the mPFC ($p > .05$).

Spine Density.In the OFC, spine density in the apical field was significantly greater in the *adult* condition when compared to that of the *juvenile* condition, $t(22) = 2.465, p = .022$ (Fig. 2.11). In addition, spine density in the basilar field was significantly greater in the *adult* condition when compared to that of the *juvenile* condition, $t(22) = 2.672, p = .014$ (Fig. 2.12). In the mPFC, the spine density of the *adult* group was significantly greater than that of the *juvenile* group in both the apical and basilar fields, $t(22) = 5.178, p < .001$ and $t(22) = 2.375, p = .027$, respectively (Fig. 2.13 and Fig. 2.14).

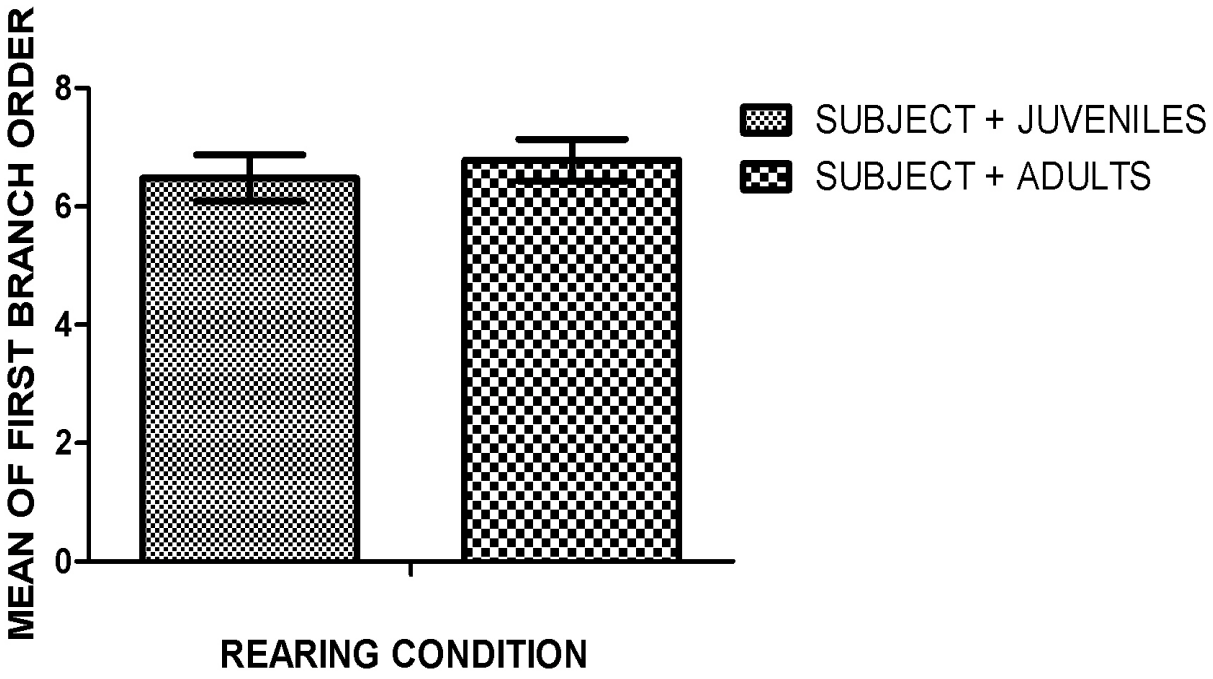

Figure 2.8: Mean number of basilar dendrites originating from the soma of OFC Layer III pyramidal neurons with respect to rearing condition. Error bars are 95% confidence intervals.

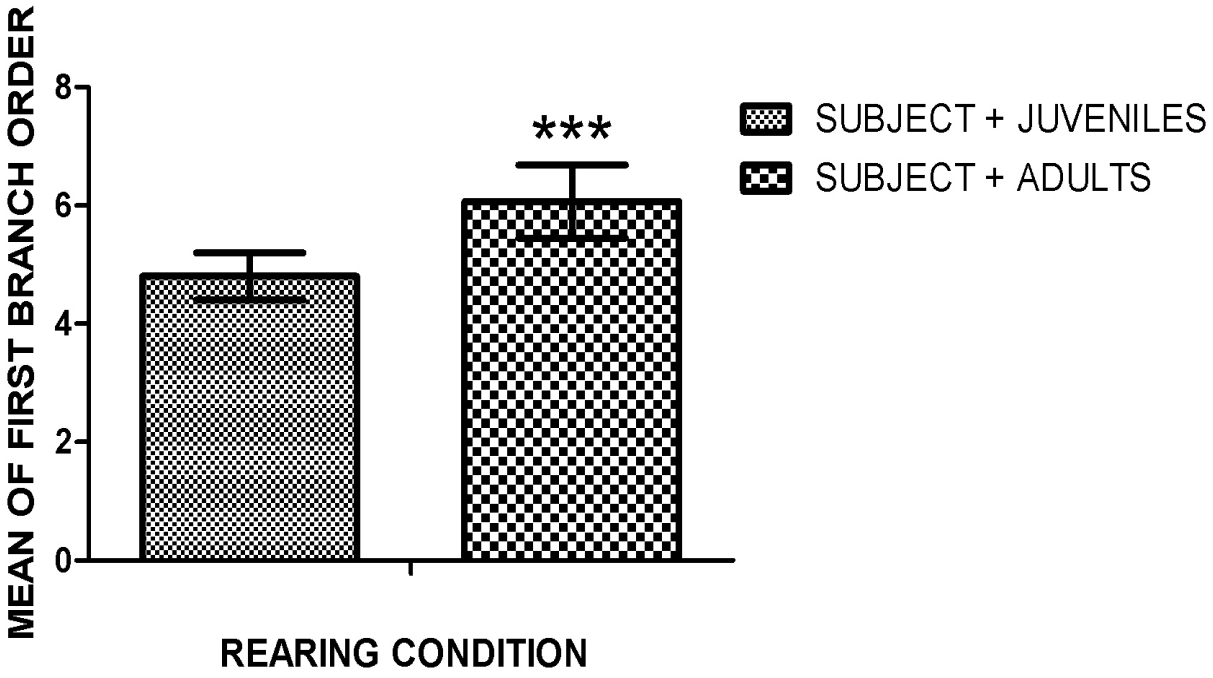

Figure 2.9: Mean number of first-order branches of dendrites in the apical field of mPFC Layer III pyramidal neurons with respect to rearing condition. Error bars are 95% confidence intervals. *** indicates difference was significant at $\alpha = .001$.

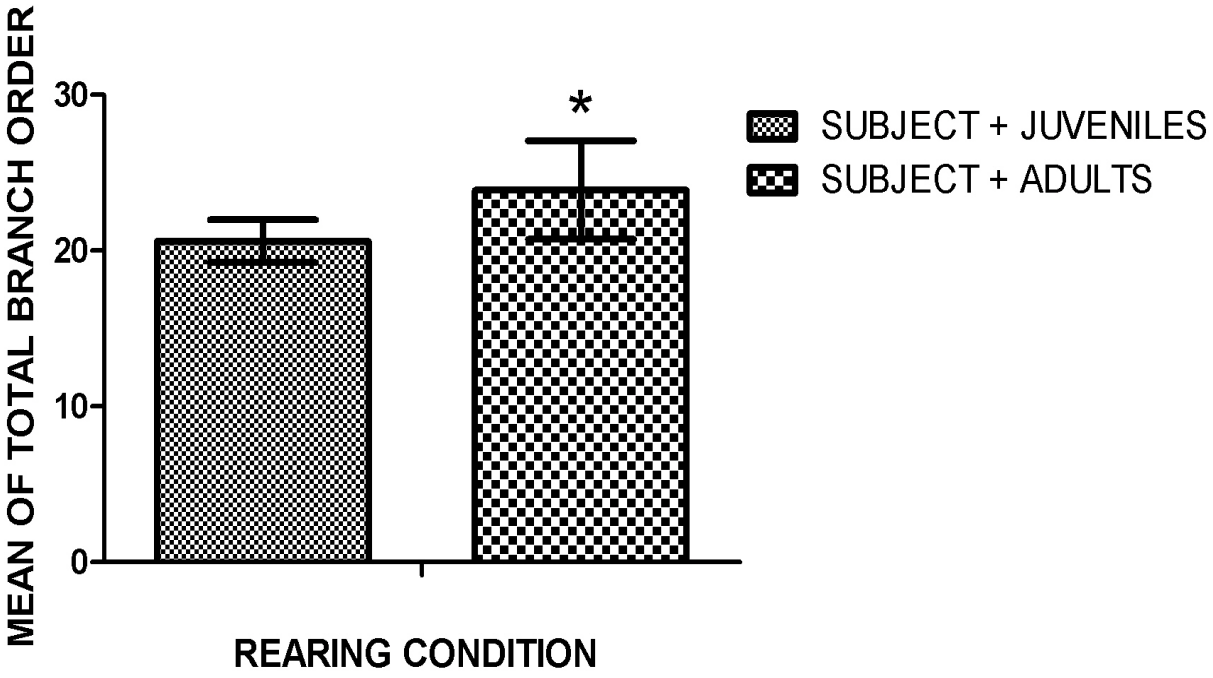

Figure 2.10: Mean total branch order in the apical field of mPFC Layer III pyramidal neurons with respect to rearing condition. Error bars are 95% confidence intervals. * indicates difference was significant at $\alpha = .05$.

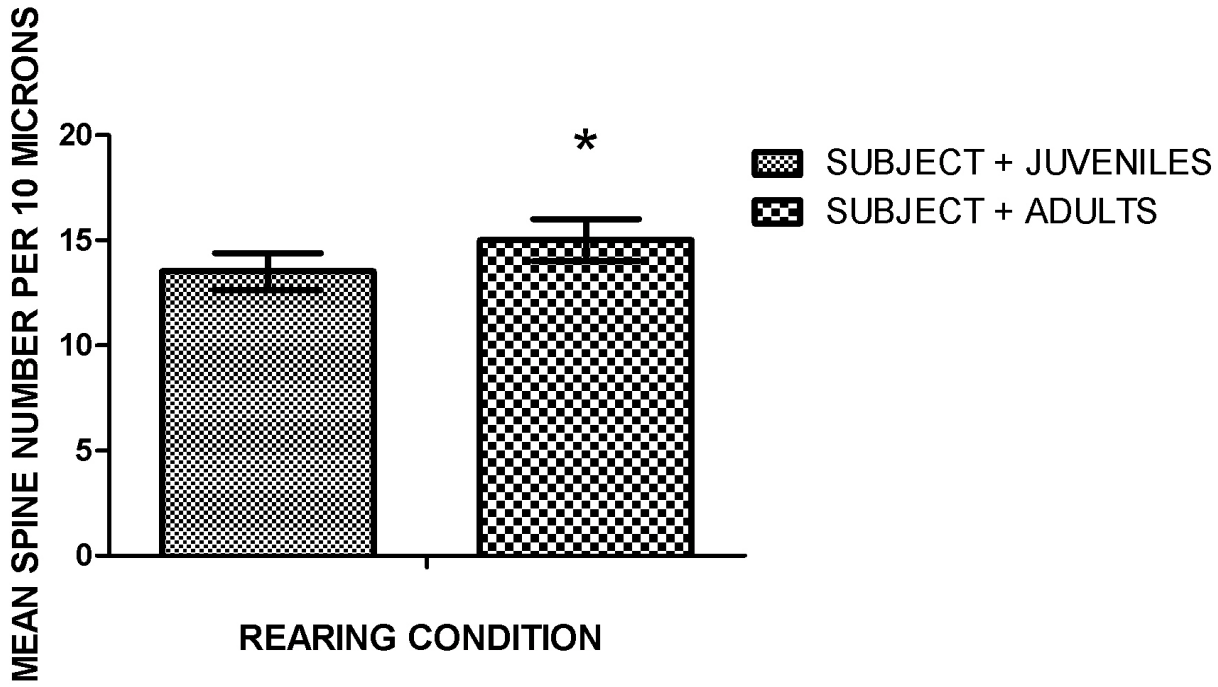

Figure 2.11: Mean dendritic spines per 10μ in the apical field of OFC Layer III pyramidal neurons with respect to rearing condition. Error bars are 95% confidence intervals. * indicates difference was significant at $\alpha = .05$.

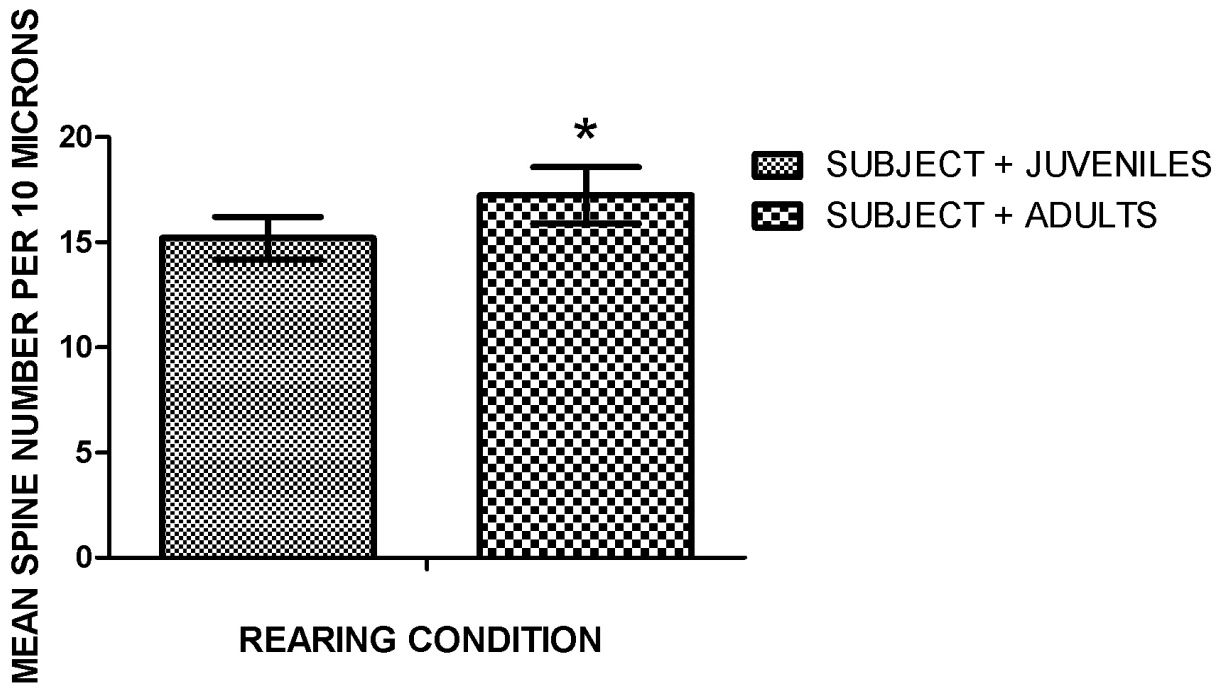

Figure 2.12: Mean dendritic spines per 10μ in the basilar field of OFC Layer III pyramidal neurons with respect to rearing condition. Error bars are 95% confidence intervals. * indicates difference was significant at $\alpha = .05$.

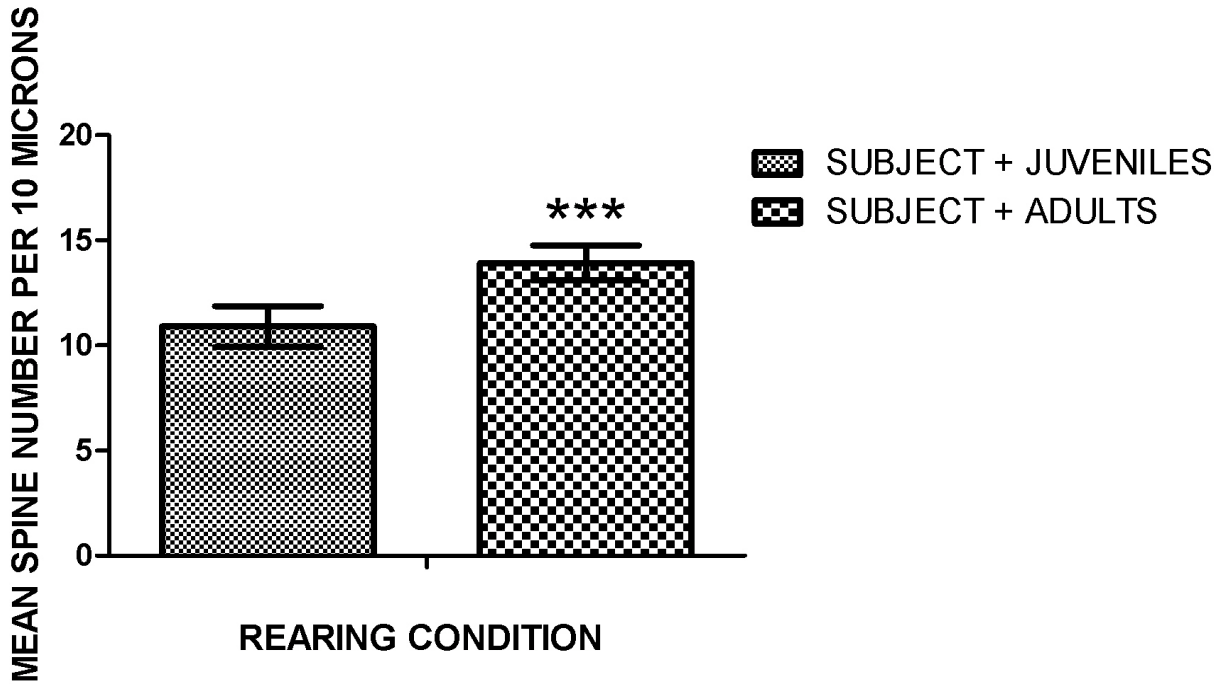

Figure 2.13: Mean dendritic spines per 10μ in the apical field of mPFC Layer III pyramidal neurons with respect to rearing condition. Error bars are 95% confidence intervals. *** indicates difference was significant at $\alpha = .001$.

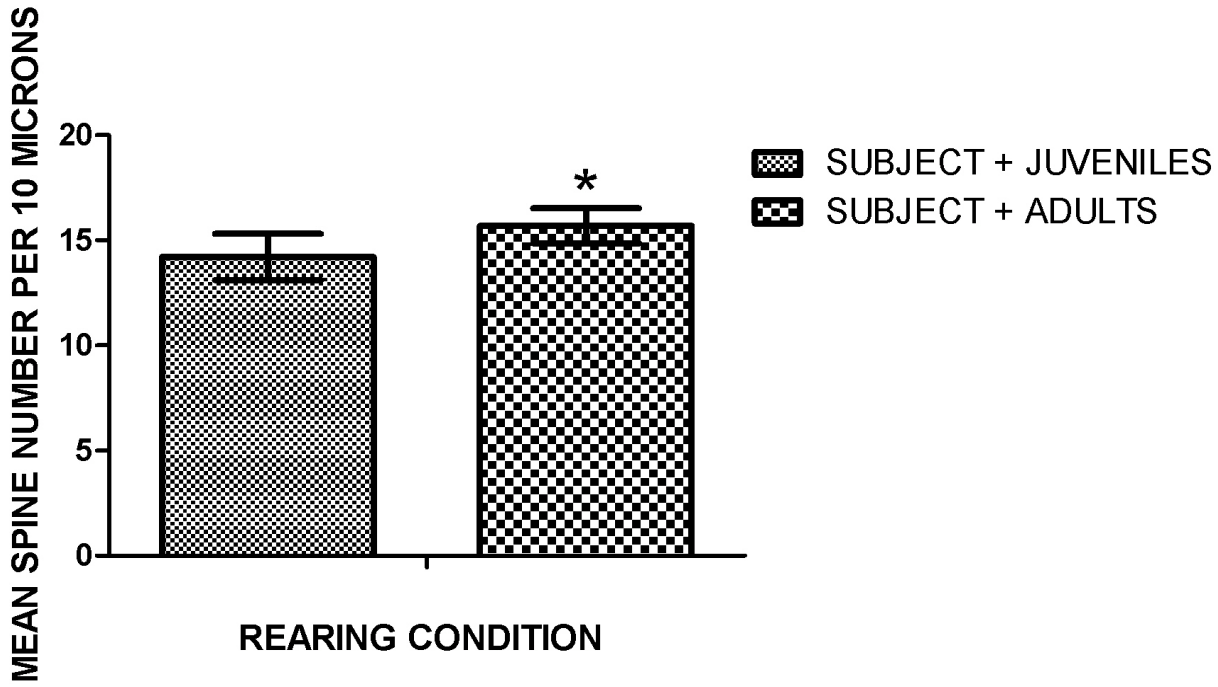

Figure 2.14: Mean dendritic spines per 10μ in the basilar field of mPFC Layer III pyramidal neurons with respect to rearing condition. Error bars are 95% confidence intervals. * indicates difference was significant at $\alpha = .05$.

Repeated-Measures Analyses

Repeated-measures ANOVAs were also used to analyze Sholl and branch order data. In these analyses, *group* was the between-subjects factor. *Sholl* or *branch order* were within-subjects factor, and separate analyses were carried out for each within-subjects factor.

Sholl Analysis. There were no significant effects with respect to *Sholl* revealed in any aspect of the OFC ($p > .05$) (Fig. 2.15).

In the mPFC, there was a significant *Sholl* by *group* interaction in the apical aspect, $F(15, 330) = 13.349, p < .001$ (Fig. 2.16). There was also a main effect of *Sholl*, $F(15, 330) = 20.252, p < .001$. There was no main effect of *group* ($p > .05$).

Branch Order. *Branch order* did not exhibit significant differences in any aspect of either the OFC or mPFC ($p > .05$).

Summary

In the first experiment, the OFC basilar dendritic field was more complex in the *multiple peer play* group than in the *single peer play* and *no peer play* groups. In contrast, the apical field of mPFC neurons was more complex in the *no peer play* group than in the *single peer play* and *enriched peer play* conditions. These results led to the refinement of the initial hypothesis to include the possible differential contributions of specific aspects of juvenile social experience to the development of the OFC and mPFC. The refined hypothesis was tested in the second experiment.

The results of the second experiment showed that there was no difference in the complexity of either dendritic field of the OFC with respect to the *adult* or *juvenile* condition. However, the spine density of both dendritic fields of the OFC was higher in the *adult* condition than it was in the *juvenile* condition. In the mPFC, spine

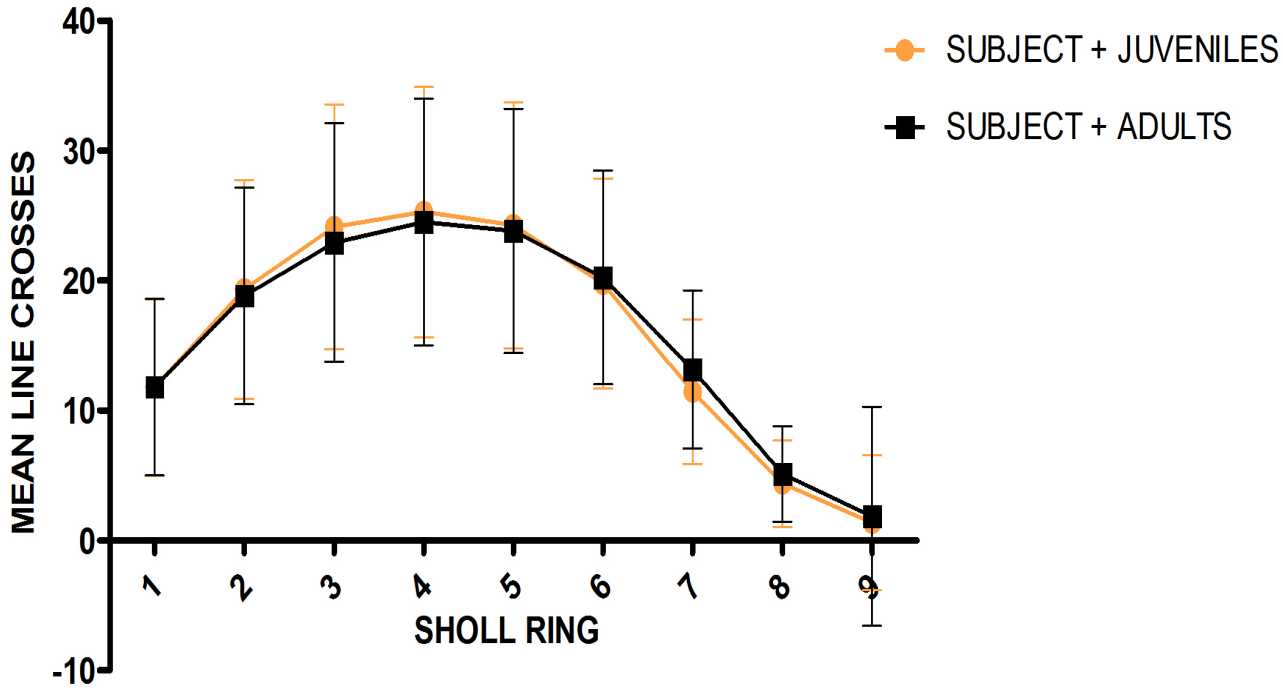

Figure 2.15: Change in mean dendritic length in the basilar field of OFC Layer III pyramidal neurons, moving distally from the soma, with respect to rearing condition. Error bars are 95% confidence intervals.

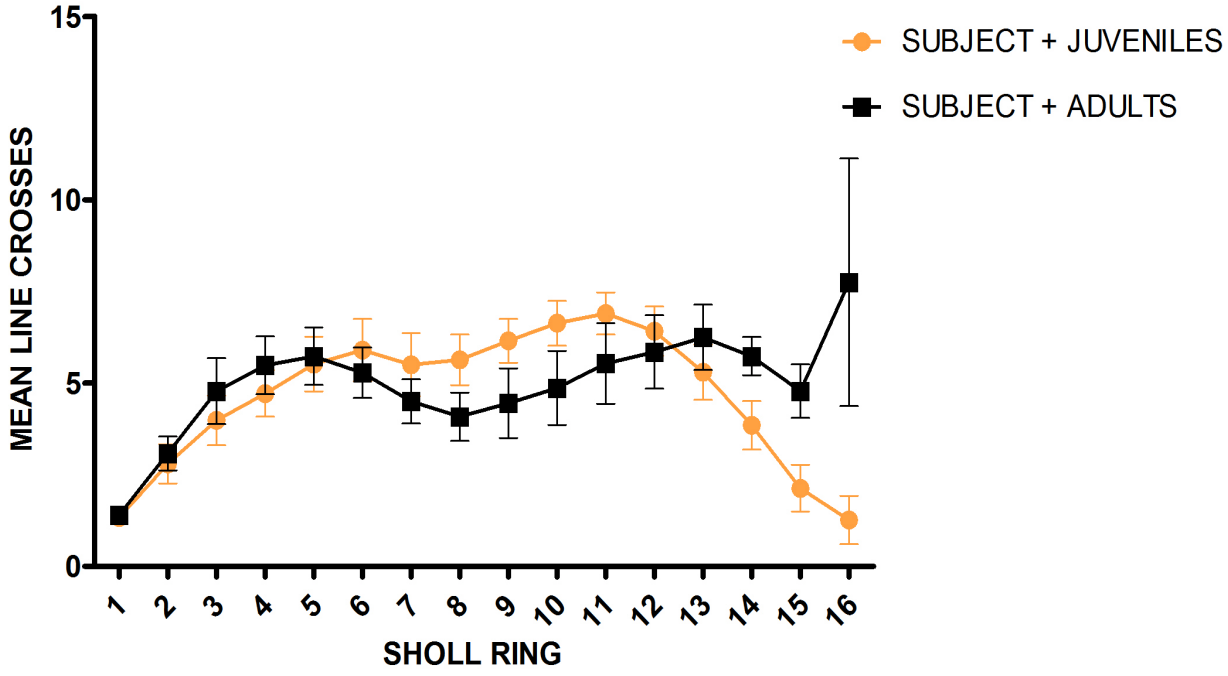

Figure 2.16: Change in mean dendritic length in the apical field of mPFC Layer III pyramidal neurons, moving distally from the soma, with respect to rearing condition. 16 represents Sholl ring 16 and beyond. Error bars are 95% confidence intervals.

density was also higher in both dendritic fields in the *adult* condition than it was in the *juvenile* condition. Dendritic complexity of the apical field in the mPFC was higher in the *adult* condition than in the *juvenile* condition. The results of the second experiment show that the OFC responds to the number of social partners encountered during the juvenile phase, and that the mPFC responds to the presence or absence of peer play during the juvenile phase. The contribution of the mPFC to playful behaviour will be explored further in the following chapter.

Chapter 3

THE ROLE OF THE mPFC IN SOCIAL BEHAVIOUR

In the previous chapter, it was shown that the medial prefrontal cortex (mPFC) of rats is responsive to the types of behaviours that are experienced in the social environment during development; however, the exact nature of the mPFC's role in the production of social behaviour is not known. This chapter will investigate the role of the mPFC in the production of social play behaviour.

It has been demonstrated that damage to specific areas of the frontal cortex interfere with discrete aspects of the production of play behaviour in rats. For example, animals with OFC lesions are unable to correctly modulate their behaviour with respect to the identity of play partners (Pellis et al., 2006). Related to this, the neurons of the OFC respond to the variety of social partners experienced during development (see Chapter 2), and rats that are deprived of social partners during development exhibit many of the same social deficits as those seen in OFC-ablated animals (van den Berg et al., 1999). In contrast, rats with motor cortex (MC) damage are able to respond to the identity of partners, but do not exhibit age-related changes in play tactics that are normally observed during development (Kamitakahara et al., 2007).

A third frontal cortex area, the medial prefrontal cortex (mPFC) is also connected to social play in rats. Specifically, animals that are not exposed to peer play during the juvenile phase have more complex neuronal morphology in the mPFC than animals that did experience peer play (see Chapter 2). The functioning of the mPFC is broadly associated with the initiation and sequencing of movement (Hauber et al., 1994; Ragozzino & Kesner, 2001; Heidbreder & Groenewegen, 2003; Walton et al., 2007). This is important because it has been suggested that play during the juvenile period prepares the motor system of animals for engagement in adult behaviours

(Brownlee, 1954). The so-called Motor Training Hypothesis asserts that animals that play during the juvenile period have motor systems that are better prepared to deal with the environmental demands faced during adulthood. Recently, support for the Motor Training Hypothesis has emerged, showing that the critical period for cerebellar pruning and motor nerve differentiation occur at roughly the same time as the peak play period during the juvenile phase in several mammalian species (Byers & Walker, 1995). Indeed, more recent comparisons of the maturation of different brain areas with different types of play in primates (Fairbanks, 2000), suggest that this may be a more general principle, rather than just related to motor function. That is, particular kinds of play may occur at particular times in development to fine-tune the maturation of a variety of brain systems. In turn, those neural changes may improve the performance of those systems, including how the animals engage in play itself.

The following experiments were done to assess the involvement of the mPFC in the performance of social play. If, in line with the Motor Training Hypothesis, the experience of peer play during the juvenile phase works to refine motor abilities, and if the mPFC controls some aspect of the movements involved in play, then one would expect that disrupting the functioning of the mPFC would disrupt the movements used in the performance of play behaviour. In order to test the prediction that the mPFC contributes to play behaviour by controlling some aspect of the movement involved in play, juvenile and adult rats were given bilateral mPFC lesions, and their social play was evaluated.

Method

Subjects

A total of 122 Long-Evans Hooded rats (96 males and 26 females) were used. Eighty-three animals were raised at the Canadian Centre for Behavioural Neuroscience at the University of Lethbridge (70 males and 13 females) and 39 animals (26 males and 13 females) were bred at Charles River Laboratories, QC. The reason for the two groups was that, owing to space constraints, the second experiment was run in two cohorts and animals were not available from the University of Lethbridge for the second cohort. Subjects were housed in pairs or triads (see below). Rats were kept in 46 cm x 25 cm x 20 cm polyethylene tubs with processed corncob as bedding, and maintained at a constant $21 - 23\,^{\circ}\mathrm{C}$ on a 12-hour light-dark cycle. Food and water were provided *ad libitum*. All animals were handled and cared for in accordance with the Canadian Council for Animal Care (CCAC) regulations at the University of Lethbridge.

Experiment 1: Developmental Effects of mPFC Lesions

Because the mPFC is connected to the MC, it is important to determine whether the behavioural alterations seen in MC-ablated animals are different from any that might be seen in mPFC-ablated animals. MC-ablated animals exhibit the full range of behavioural patterns typical of play, but they do not show age-related modulation of defensive tactics. To explore the involvement of mPFC in the development of play fighting, 20 subjects were given mPFC lesions or sham surgeries on Postnatal Day 3 (see Surgical Procedures below). Subjects were then returned to dams until weaning (Postnatal Day 21). Postnatal Day 3 was chosen to administer the lesions because

it is known that lesions made later in development (Postnatal Day 7 to 15) result in a filling in of the lesion cavity and a greater degree of functional recovery (Kolb, Petrie, & Cioe, 1996). If functional recovery were to occur, it would not allow for an accurate representation on the mPFC's contribution to play behaviour. Following weaning, each subject was housed with a normal, sex- and age-matched partner. Subjects were drawn from a total of five litters, and sham and lesion animals were distributed across litters whenever possible.

Beginning on Postnatal Day 27 - 30, subjects and their respective cagemates were habituated in pairs to a play box. The habituation sessions occurred for 1/2 hour per day for three consecutive days. The play box was a 50cm x 50cm x 50cm open-topped structure, consisting of a posterior mirrored wall, bottom and anterior plexiglass walls, and wooden side walls. Approximately 3 cm of processed corncob substrate was used during all procedures.

Following the third habituation session, subjects were isolated for 24 hours and then reunited with their cagemates in the play box. The pairs of animals were then filmed in the dark, using the infrared setting on the camera, for 10 minutes in the play box. Isolation was used because the incidence of play is known to increase following the reunion of play partners (Niesink & van Ree, 1982; Panksepp & Beatty, 1980; Pellis & Pellis, 1990). Increasing the incidence of play facilitates the collection of sufficient amounts of play data required to conduct analyses. Similarly, the pairs were filmed in the dark because overall activity, specifically social behaviour, increases in the absence of light (Vanderschuren, Niesink, & van Ree, 1997).

Experiment 2: Effects of Adult mPFC Lesions

Because OFC lesions abolish the partner-related modulation of defensive tactics in rats (Pellis et al., 2006), it was necessary to determine whether animals with mPFC lesions could distinguish among social partners. Given that the magnitude of the dominance-based partner-related modulation in playful defense can vary markedly among dyads of rats (Pellis & Pellis, 1992), within-subject designs are the most powerful methods with which to assess experimental changes in the asymmetry of response to different partners (Pellis, Castaneda, McKenna, Tran-Nguyen, & Whishaw, 1993). However, the pre-ablation baseline must first be established. Thus, in this experiment, adult rats were tested with two different partners and then were tested again following the mPFC ablation; for this reason, unlike in the first experiment, the ablations were made when the rats were adults (see Pellis et al., 2006).

Because the presence of a female (Flannelly & Lore, 1977; Martinez, Calvo-Torrent, Pico-Alfonso, & Angeles, 1998) and a large age difference among males (Berdoy, Smith, & MacDonald, 1995; Blanchard & Blanchard, 1990; Takahashi & Lore, 1983) lead to exaggerated dominance asymmetries among the males, in this experiment, colonies were formed containing two males, one 20 days younger than the other, and a female. The dominance asymmetry arising from such colonies also leads to clear asymmetries in play-fighting behavior (Pellis et al., 2006). Twenty-six colonies, each composed of a 60-day-old male, an 80-day-old male, and a 70-day-old female, were formed. Before being housed with the males, all female rats received tubal ligations (see Surgical Procedures below).

All colonies were left undisturbed for approximately 10 days following the initial housing of the animals. This was to facilitate the establishment of dominance hierarchies, and to allow the animals to become habituated to their cagemates. Following

the settling period, when the youngest male was 76 days old, animals were habituated to the play box (described above) in triads for 30 minutes per day for three days. Each triad was then separated, so that all animals were housed in isolation for 24 hours. Following isolation, the young male was videotaped for 10 minutes interacting with one of the other members of the triad. 12 of the young males were filmed with the female, and 12 were filmed with the old male. After each filming session, triads were re-established for 1 hour so that the triad member that was not in the play box would not be adversely affected by being isolated longer than the other two triad members. Triads were then isolated again for 24 hours. On the second day, the young male from each triad was filmed with the triad member that he was not filmed with on the previous day. Following the second day of filming, triads were returned to the colony room and re-united in their home cages.

The video of the play encounters was then analyzed (see Behavioural Analysis below) in order to identify the dominant and subordinate males in each triad. Because dominant males tend to weigh more, perform fewer complete rotations, and more partial rotations, as well as being playfully attacked more often than subordinate males, these measures were used to assess the status of each animal. In 25 out of 26 colonies, the younger male was subordinate. In the remaining colony, the status of the males was ambiguous (each had similar numbers of attacks, and complete and partial rotations). The young male in the ambiguous colony was assigned as subordinate and was placed in the sham condition. In several cases, there were not enough play interactions in one 10-minute session to accurately assess the dominance status of the individuals, so these individuals were isolated for 24 hours and re-filmed. The data across both 10-minute sessions were pooled to evaluate dominance status. All re-filmed animals were divided equally into sham and lesion conditions.

Following the analysis of the video, the subordinate male of each triad was given

either a mPFC lesion or a sham surgery (see Surgical Procedures below). The subjects were then allowed to recover for three days, after which the triads were again habituated to the play box and filmed as described above.

Behavioural Analysis

Subjects were identified by their coat patterns, which were both drawn and photographed by the experimenter prior to the analysis of the video data. Each 10-minute video segment was watched at full-speed, slow-motion, and frame-by-frame in order to identify the components of the play bouts. A playful attack was counted each time one of the rats put its snout near the nape of the other, or when the snout contacted the nape. Defense of a playful attack was categorized into one of four groups – *no response*, *complete rotation* (the defender rolled into a completely supine position), *partial rotation* (the defender rotated, but did not become completely supine), or *other*. The *other* category was comprised of various facing defenses that were not included in the *complete rotation* or *partial rotation* categories (e.g., rearing, boxing). Each defensive measure was analyzed as a proportion of the total number of defensive responses following playful attack. For a more thorough categorization of how play bouts were scored, see Results (below).

Surgical Procedures

Tubal Ligations in Female Rats

Prior to the establishment of the triads in Experiment 2, female rats were given tubal ligations. Females were anesthetized with isofluorane delivered through a nose cone. Each ovary was extracted from the flank, and was ligated approximately 5 mm from

the end of the oviduct. Ovaries were then returned to the abdominal cavity, and the incision was sutured with 3-0 Vicryl suture (Novartis-Ethicon, Somerville, NJ). A sub-cutaneous injection of Metacam (0.05 ml; meloxicam, Boehringer Ingelheim, St. Joseph, MO) was given to alleviate swelling and discomfort following surgery. The females were then returned to their home cages for three days prior to the establishment of the triads.

Neonatal mPFC Lesions

On Postnatal Day 3, subjects were removed from their dams and anesthetized by cooling in a Thermatron (Hauppage, NY) until their rectal temperatures were in the range of $18 - 20\,^{\circ}\mathrm{C}$. The frontal bone was cut with iris scissors and removed. The medial frontal cortical tissue was aspirated using a glass pipette. The incision was then sutured with 5-0 Vicryl. The subjects were held in the hand of the experimenter until they reverted to normal body temperature and then returned to their dams until weaning. Shams underwent the same procedure, with the exception of the removal of the frontal bone and accompanying aspiration of the cortex.

mPFC Lesions in Adults

When the youngest subject was 84 days of age, and following the analysis of the video (see Experiment 2 above), subordinate males were anesthetized with isofluorane. The anesthetic was delivered through a nose cone, and once the animals were unconscious, each was placed in a stereotaxic apparatus. The skull was drilled with a .5 mm bit in order to leave the dura intact, but to allow access with the a rongeur tip. The frontal neocortex was exposed by removing the skull with the rongeurs from the bregmoidal junction anteriorly to the frontal bone suture, and laterally about 2 mm

from the midline on each side. After the dura was retracted, the medial frontal cortex was removed via aspiration with a glass pipette, and with the aid of a surgical microscope. The incision was then sutured with 3-0 Vicryl. Subjects were given 0.25 mL intramuscular injections of buprenorphine to alleviate pain following surgery. Half of the shams were anesthetized in the same way, and were sutured with 3-0 Vicryl. The other half were left untouched.

Histology and Anatomy

At the completion of the behavioural testing, subjects with lesions were deeply anesthetized with sodium pentobarbital and perfused with 0.9% saline and then 4% formalin. The brains were harvested and placed in 4% formalin, and were then transferred to a 4% formalin in 30% sucrose solution after 24 hours. Brains were then cut on a cryostat into 40 μm coronal sections. Every fifth section was used, and sections were placed onto 1% gel, 0.2% chromium aluminium-dipped glass slides. Sections were stained with Cresyl Violet to facilitate the measurement of lesion size.

The sections were digitally photographed, and the photographs were analyzed using ImageJ (Version 1.40, National Institutes of Health, Bethesda, MD). At two planes (A = +2.20 and +1.2 mm from Bregma), lesion size and cortical thickness measurements were made. Lesion size measurements were made by recording the area, in square millimetres, of each section of the brains with and without lesions. The mean area of the brains with lesions was divided by the mean area of the brains without lesions, yielding a percentage estimate of lesion size. Cortical thickness, another indicator of lesion size, was assessed by measuring the thickness of the cortex in millimetres at medial, central and lateral points on each section. The three values were then averaged for each plane (Kolb, Cioe, & Whishaw, 2000).

Results

Anatomy

Neonatally-ablated animals had brain volumes that were 70.97%($\pm$4.56%, 95% Confidence Interval (CI)) the size of shams, and adult operates had brain volumes that were 79.04%($\pm$3.25%, 95% CI) the size of the brains of the sham animals (Fig. 3.1). With respect to cortical thickness, there was a significant effect of *condition*, $F(1, 26) = 21.799, p < .001$. There was also a significant effect of *plane*, $F(1, 26) = 10.477, p = .003$ (Fig.3.2 and Fig.3.3). There was no main effect of *age at surgery* ($p > .05$). There was no *condition* by *age at surgery* interaction; nor were there *plane* by *condition* or *plane* by *age at surgery* interactions ($p > .05$). The *plane* by *age* by *condition* interaction was also not significant.

Behaviour

Experiment 1

Lesion vs. Sham Animals.Lesion- and sham-treated animals were compared using two-way repeated-measures ANOVAs. In all analyses, *condition* (lesion- or sham- treated animal) was a between-subjects variable and *time* (Postnatal Day 30 or Postnatal Day 90) was a within-subjects variable. Because the *attack* and *defense* components of play behaviour are thought to involve separate motivational systems (Pellis & Pellis, 1991), these two features of play were analyzed separately. The measures investigated were *total attacks in a 10-minute period*, *probability of defending against a playful attack by a play partner*, *proportion of complete rotations* and *proportion of evasions*. The probability of defending against a playful attack was calculated by subtracting the number of times that the subject did not respond to

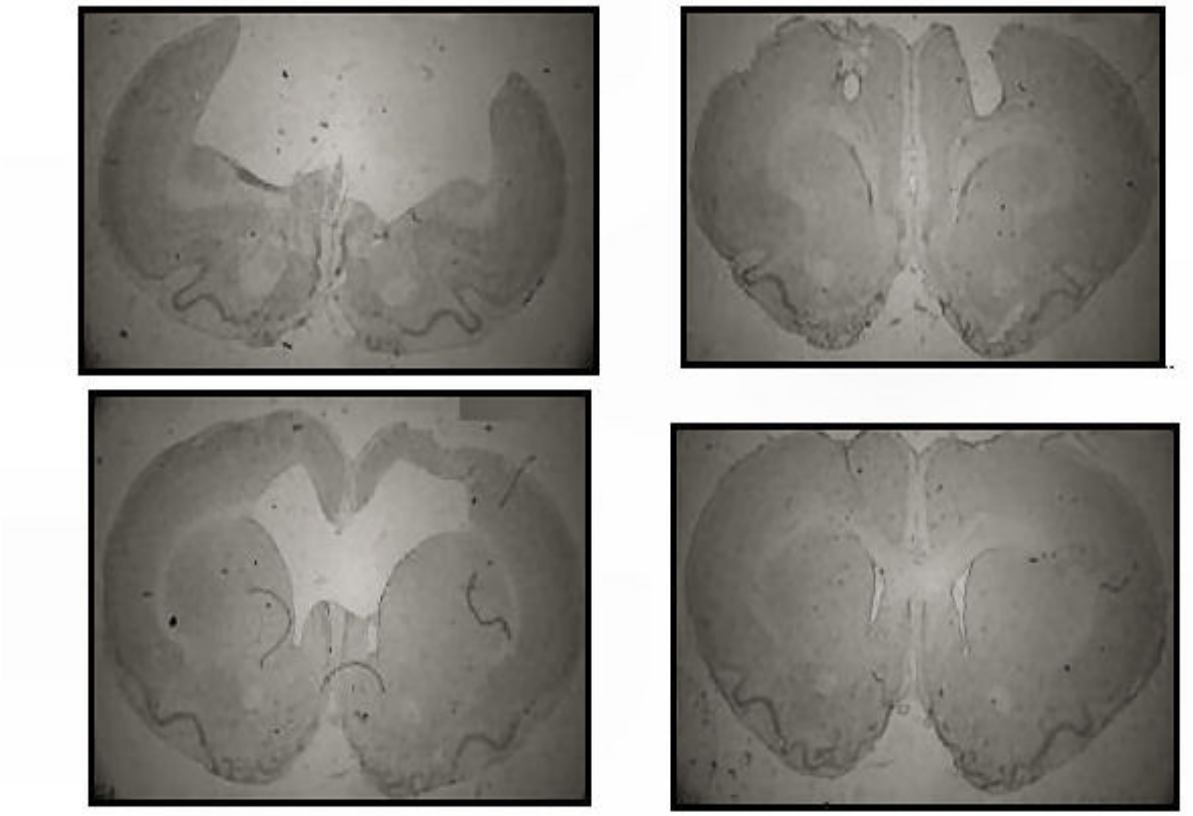

Figure 3.1: Representative sections from Postnatal Day 3 (left) and adult (right) mPFC-ablated brains.

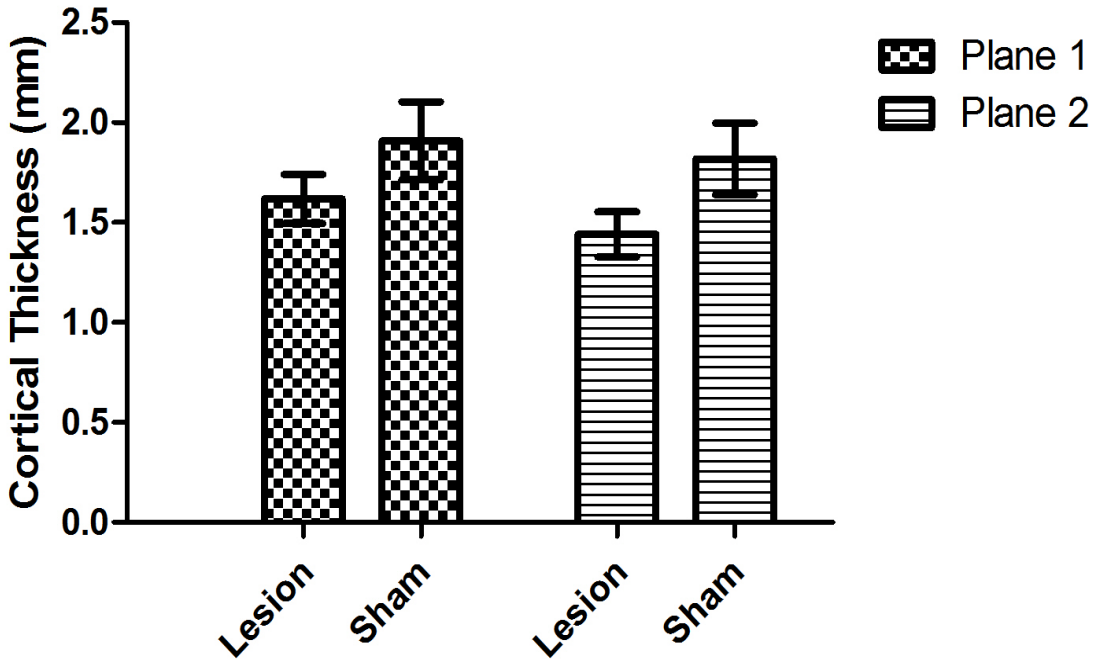

Figure 3.2: Cortical thickness in millimetres of neonatal mPFC-ablated and sham operated animals at two planes. Error bars are 95% confidence intervals.

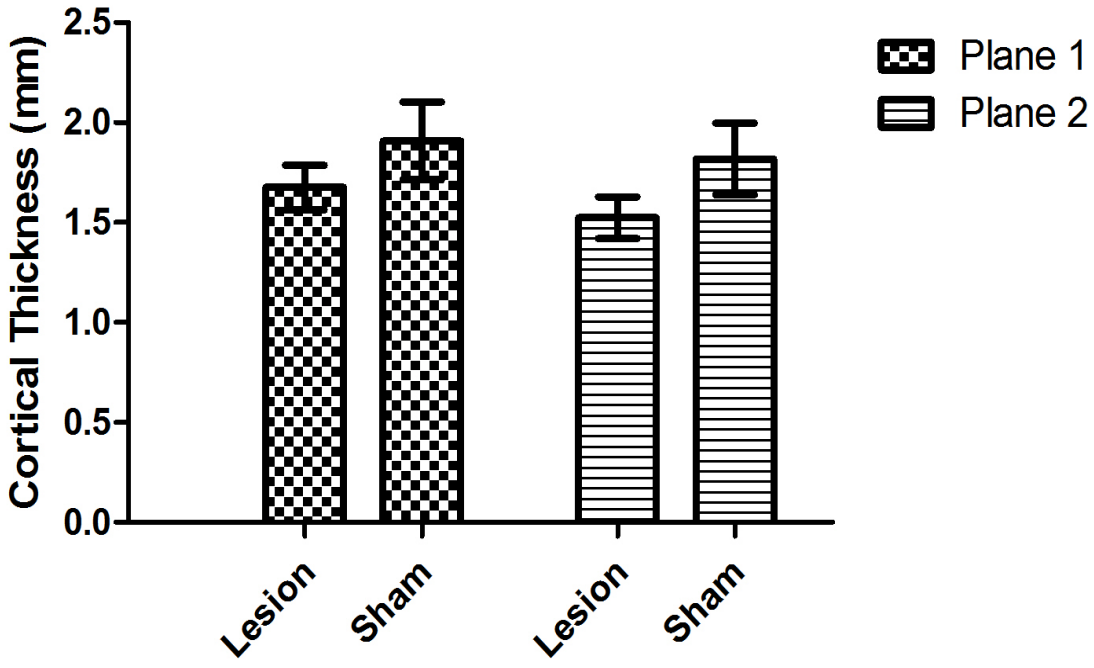

Figure 3.3: Cortical thickness in millimetres of adult mPFC and sham operated animals at two planes. Error bars are 95% confidence intervals.

playful attack from the total number of attacks launched against the subject, and dividing by the total number of attacks launched against the subject. The proportion of evasions were determined by calculating the percentage of evasion used relative to the total defenses used. The proportion of complete rotations used was determined by calculating the percentage of all non-evasive defenses that were complete rotations. The n for all groups was 11.

Briefly, mPFC-ablated animals initiated more playful attacks than shams, but had a lower response rate when attacks were perpetrated by a play partner than shams. When ablated animals did respond to a playful attack, they were less likely to use complete rotations, and more likely to evade attacks than shams.

Subjects with mPFC damage initiated playful attacks significantly more frequently than shams in a 10-minute session, $F(1, 20) = 9.90, p = .005$. There was also a significant effect of *time*, $F(1, 20) = 82.24, p < .001$. There was no interaction between *time* and *condition* ($p > .05$) (Fig. 3.4).

Subjects with mPFC lesions also exhibited a reduced likelihood of responding to a playful attack relative to shams, $F(1, 20) = 19.84, p < .001$. Probability of defense did not change relative to *time* ($p > .05$), nor was there an interaction between *condition* and *time* ($p > .05$) (Fig. 3.5).

Lesion animals adopted evasive tactics in response to playful attacks at a higher frequency than shams, $F(1, 20) = 11.716, p = .003$. The use of evasions changed with respect to *time* $F(1, 20) = 12.714, p = .002$, but there was no *time* by *condition* interaction ($p > .05$) (Fig. 3.6).

mPFC-damaged subjects were also less likely than shams to perform complete rotations in response to playful attacks, $F(1, 20) = 8.708, p = .008$. Complete rotations changed as a function of *time*, $F(1, 20) = 12.014, p = .002$, but there was no *time* by *condition* interaction ($p > .05$). Because what is of interest here is whether or not

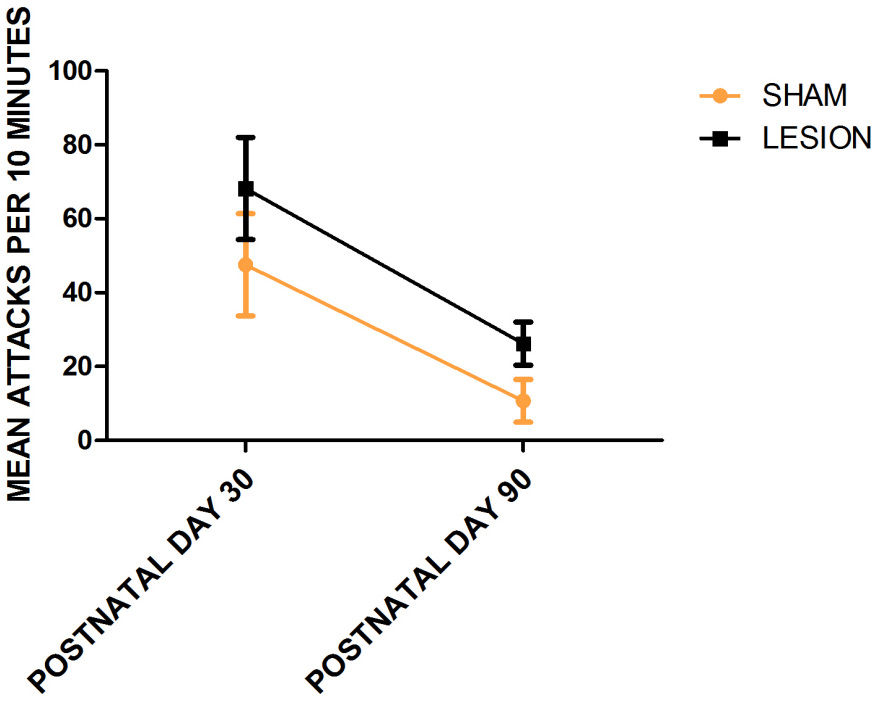

Figure 3.4: Mean number of playful attacks initiated by sham vs. lesion in a ten-minute period at two developmental timepoints. Error bars are 95% confidence intervals.

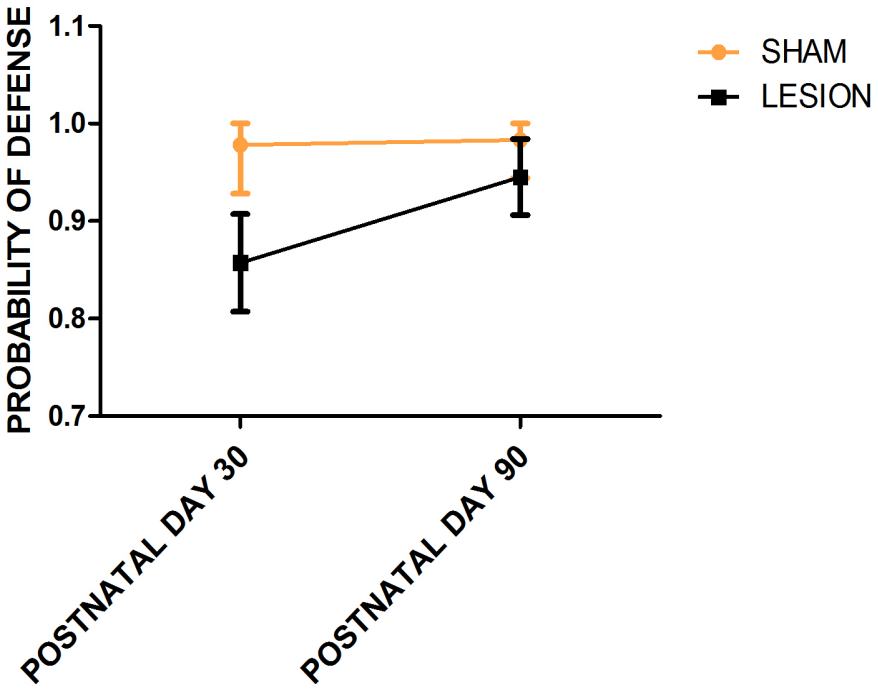

Figure 3.5: Probability of shams vs. lesions defending a playful attack at two developmental timepoints. Error bars are 95% confidence intervals.

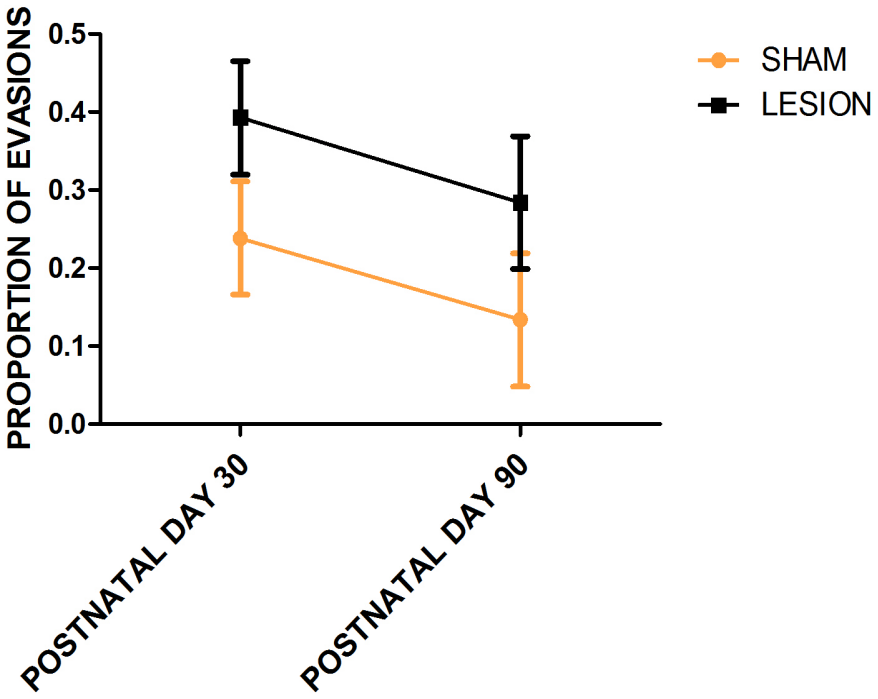

Figure 3.6: Proportion of evasions by shams vs. lesions at two developmental time-points. Error bars are 95% confidence intervals.

the lesion animals exhibited age-related changes in the use of complete rotations, the difference between the Postnatal Day 30 and Postnatal Day 90 scores was found for each animal. The difference scores were then compared using an independent-samples *t*-test. Both groups exhibited age-related changes, and there was no significant difference between the difference scores, $t(20) = 1.464, p > .05$ (Fig. 3.7).

Lesion Playmates vs. Sham Playmates. The behaviour of the play partners of the lesion- and sham-treated animals was also examined using two-way repeated-measures ANOVAs. The behaviour of play partners is important because it has previously been shown that play partners respond to abnormal subject behaviour by also behaving abnormally (Pellis et al., 2006; Kamitakahara et al., 2007). Thus, the behaviour of play partners can be used to further identify altered patterns of subject behaviour. In these analyses, *condition* was a between-subjects factor, and *time* was a within-subjects factor. The n for all groups was 11.

The playmates of lesion animals exhibited a significantly higher proportion of evasive responses to playful attacks than did the playmates of sham animals, $F(1, 20) = 10.326, p = .004$. The proportion of evasions used did not change with respect to *time*, and there was no *time* by *condition* interaction ($p > .05$) (Fig. 3.8).

Experiment 2

Lesion vs. Sham Animals. Lesion- and sham-treated animals were compared using three-way repeated-measures ANCOVAs with Bonferroni post-hoc tests. There were two within-subjects variables — *time* (pre- and post-lesion) and *sex* (sex of play partner) — and one between-subjects variable, *condition* (lesion- or sham-treated). Because the experiment was run in 2 cohorts, with the animals in each cohort originating from different sources, *cohort* was run as a covariate in all analyses.

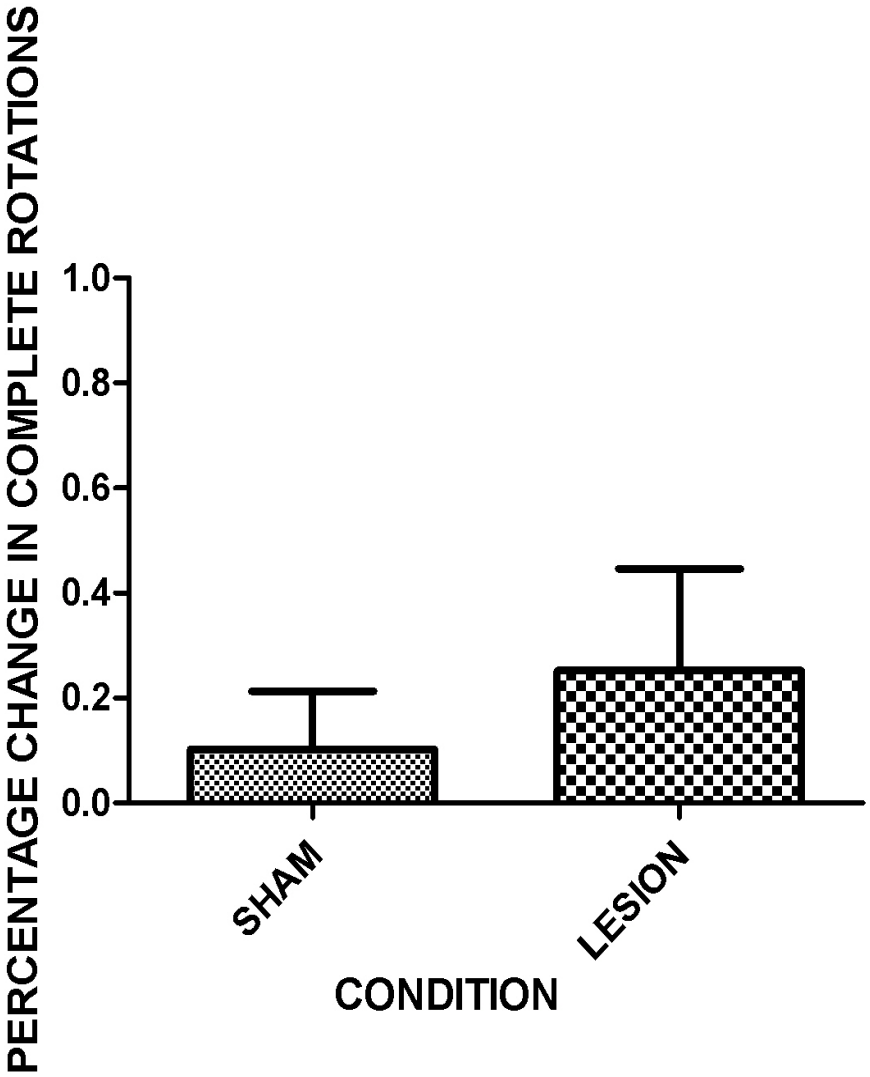

Figure 3.7: Change in the use of complete rotations between Postnatal Day 30 and Postnatal Day 90 with respect to sham and lesion animals. Error bars are 95% confidence intervals.

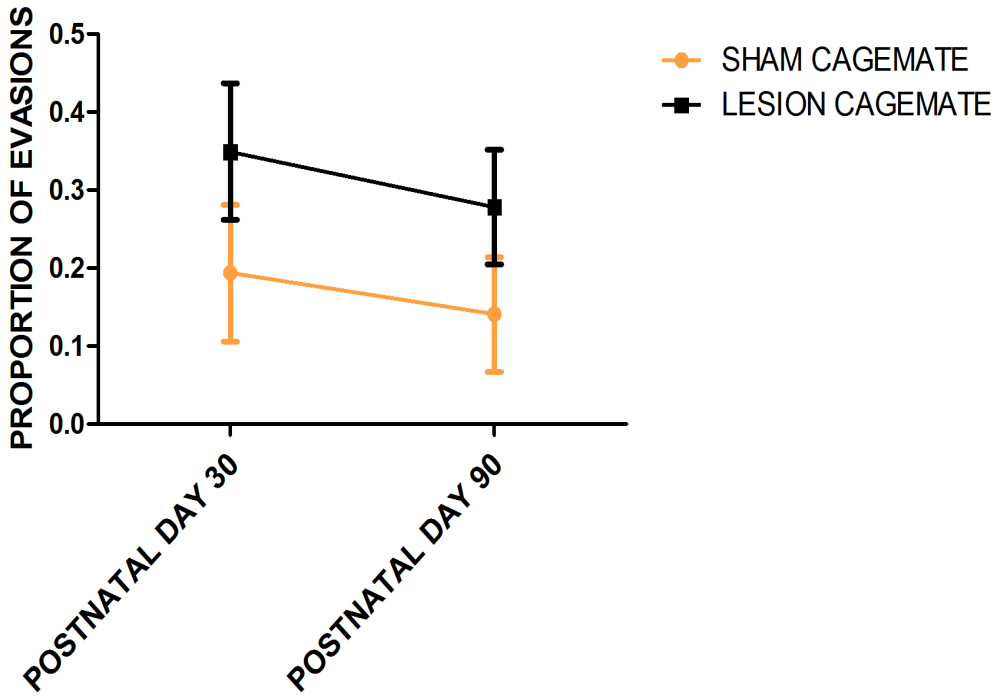

Figure 3.8: Proportion of evasions by cagemates of sham vs. cagemantes of lesion animals at two developmental timepoints. Error bars are 95% confidence intervals.

Briefly, mPFC-ablated animals were less likely to initiate playful attacks than shams. Ablated animals also evaded playful attacks more than shams, and used fewer complete rotations than shams.

There was a significant *time* by *condition* interaction in the number of playful attacks perpetrated over a 10-minute period, $F(1, 23) = 5.961, p = .026$. There was a main effect of *time*, $F(1, 23) = 4.756, p = .04$, but no main effect of *sex*, nor was there a main effect of *condition* ($p > .05$). The *time* by *sex* interaction was significant, $F(1, 20) = 15.288, p = .001$, but the *condition* by *sex* interaction was not significant ($p > .05$). The *time* by *sex* by *condition* interaction was also not significant ($p > .05$) (Fig. 3.9). Post-hoc analyses revealed that lesion animals attacked play partners less following surgery ($p = .000$). Sham animals showed no pre-post surgery changes ($p > .05$). Lesion and sham animals were not different with respect to attacks initiated on either sex prior to surgery ($p > .05$), but following surgery, lesion animals attacked both male and female partners less than shams ($p = .004$ and $p = .043$ respectively). Lesion animals attacked female and male partners at the same level prior to surgery ($p > .05$); however, following surgery, lesion animals attacked male partners significantly less often than female partners ($p = .009$).

The proportion of evasions used by lesion and sham animals also changed. There was a significant main effect of *sex*, $F(1, 23) = 6.324, p = .019$, as well as a significant *condition* by *sex* interaction, $F(1, 23) = 4.943, p = .036$. There was no main effect of *time*, nor was there a main effect of *condition* ($p < .05$). The *time* by *condition*, the *time* by *sex* and the *time* by *sex* by *condition* interactions also did not attain signficance ($p > .05$) (Fig. 3.10). Post-hoc analyses showed that lesion animals used signficantly more evasions following surgery than prior to surgery ($p = .019$). Specifically, the proportion of evasions used with female partners increased signficantly in lesion animals following surgery ($p = .017$).

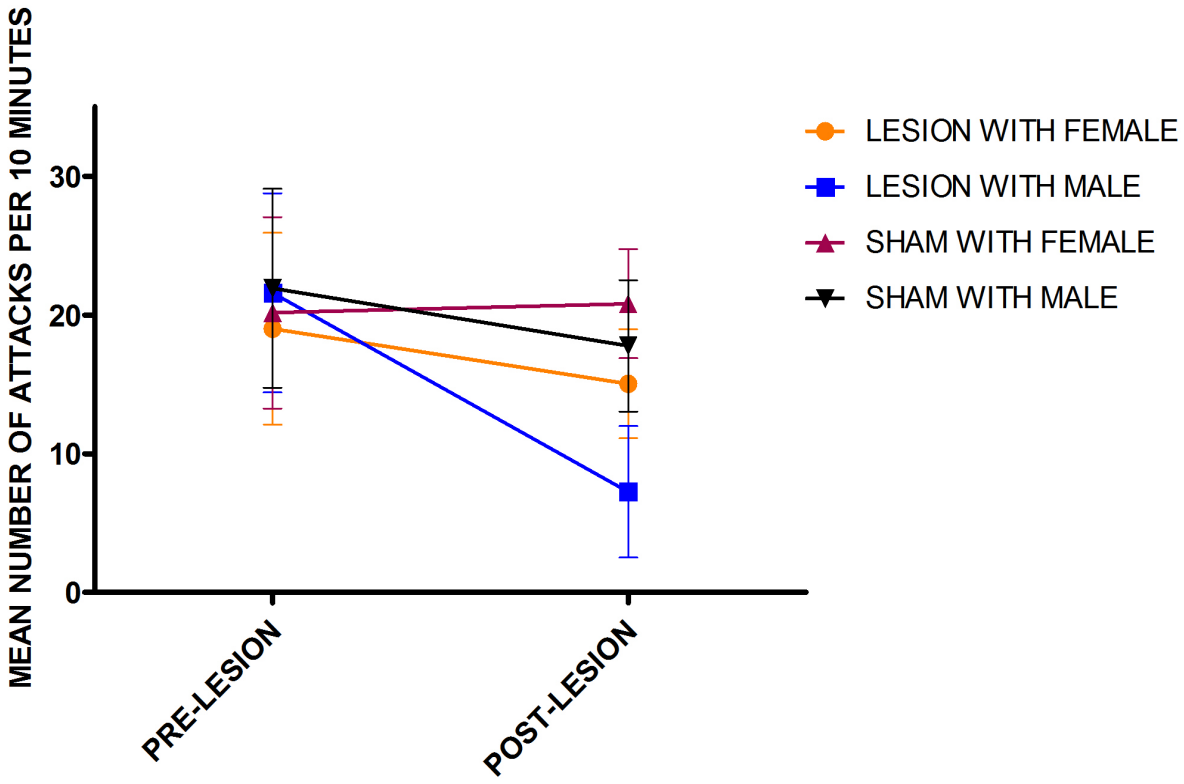

Figure 3.9: Mean number of playful attacks initiated by sham vs. lesion animals in a ten-minute period with respect to male and female play partners. Pre- and post-surgery timepoints are shown. Error bars are 95% confidence intervals.

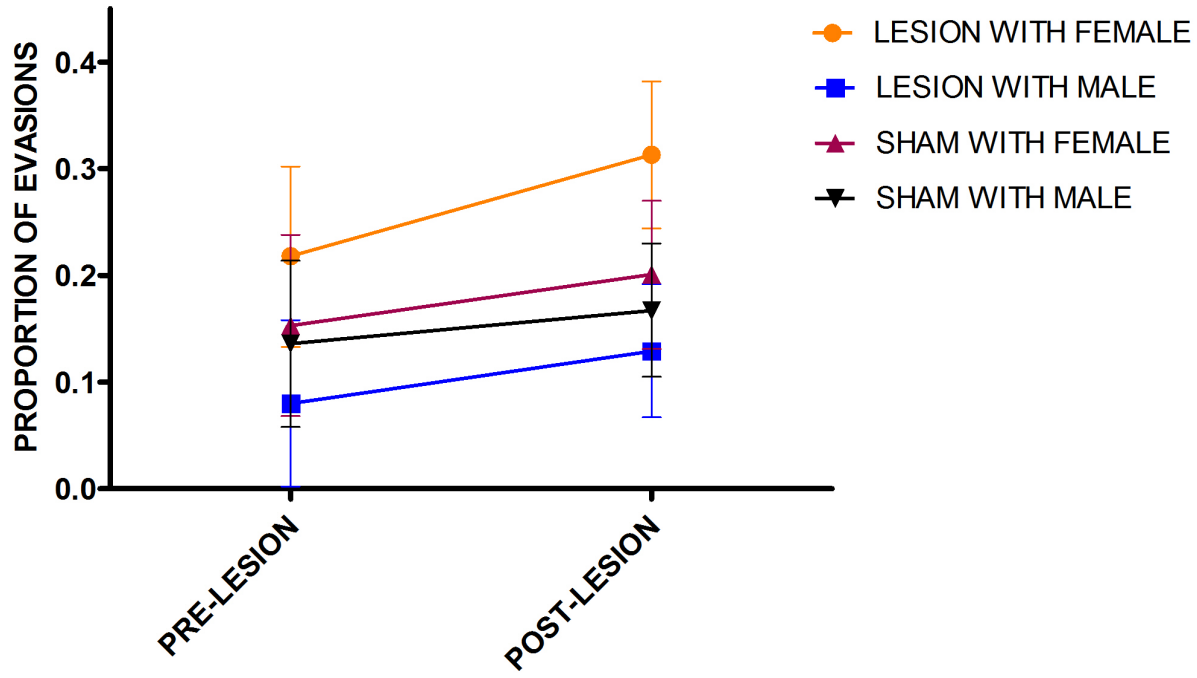

Figure 3.10: Proportion of evasions used by sham vs. lesion animals with respect to male and female play partners. Pre- and post-surgery timepoints are shown. Error bars are 95% confidence intervals.

With respect to the proportion of complete rotations used, there was a significant *time* by *condition* interaction, $F(1, 23) = 8.016, p = .009$. There was also a significant effect of *sex*, $F(1, 23) = 8.026, p = .009$. There was no main effect of *time*, nor was there a main effect of *condition* ($p > .05$). The *time* by *sex* interaction was not significant, and neither was the *condition* by *sex* interaction ($p > .05$). The *time* by *sex* by *condition* interaction also did not attain significance ($p > .05$) (Fig. 3.11). Post-hoc tests showed that lesion animals used fewer complete rotations following surgery than prior to surgery ($p = .002$), but the proportion of complete rotations used did not differ for shams across the two timepoints ($p > .05$). All animals used a higher proportion of complete rotations with male partners than with female partners ($p < .001$).

In fact, because what was of interest here was whether or not animals could still tell the difference between male and female partners, and because males use fewer complete rotations with a female versus a dominant male partner (Pellis & Pellis, 1992), the difference in complete rotations used with male and female partners prior to and following surgery was found. Two-way repeated-measures ANCOVAs were conducted with *difference score* as the dependent measure, and *time* and *condition* as independent measures. The analysis showed that the magnitude of the difference between the male and female partners prior to and following surgery did not change with respect to condition, $F(1, 23) = .306, p > .05$. Additionally, there was no main effect of time, nor a main effect of condition ($p > .05$) (Fig. 3.12).

Lesion Playmates vs. Sham Playmates. The behaviour of the play partners of lesion- and sham-treated animals was compared using three-way repeated-measures ANCOVAs with Bonferroni post-hoc tests. There were two within-subjects variables, *time* and *sex*, and one between-subjects variable, *condition*. *Cohort* was the covariate.

In the proportion of complete rotations used by play partners, there was a main

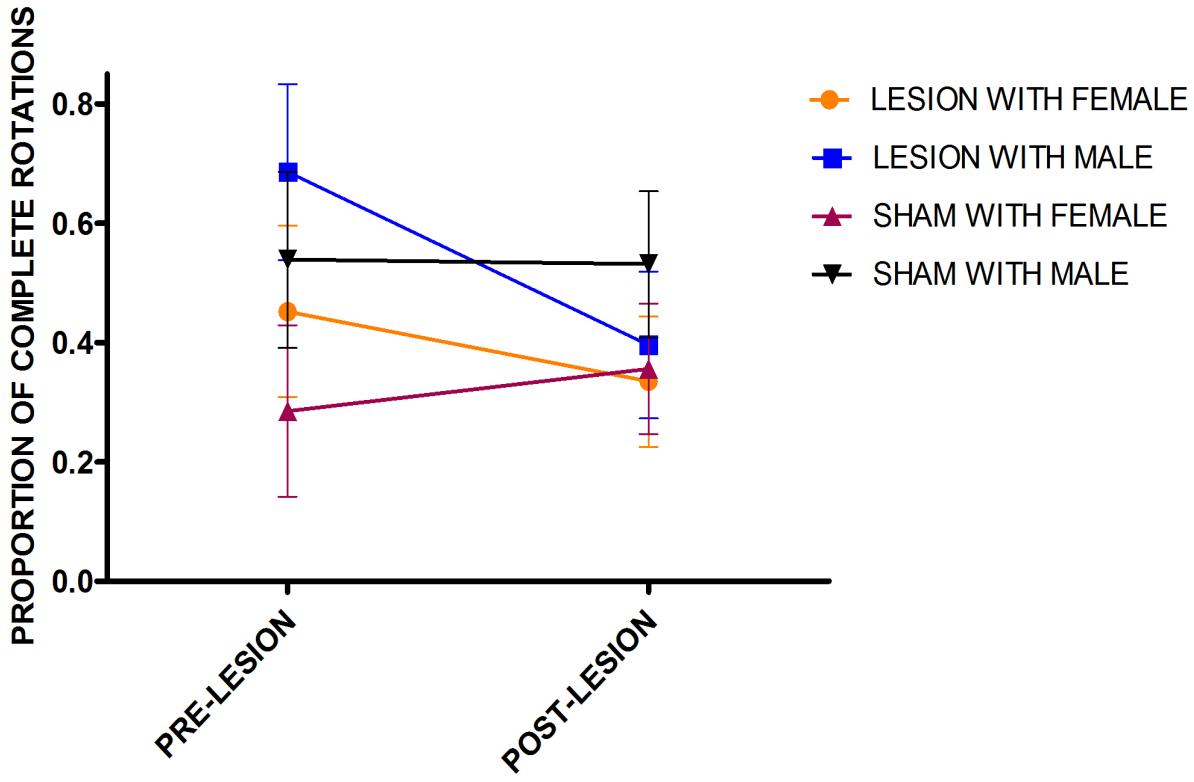

Figure 3.11: Proportion of complete rotations by sham vs. lesion animals with respect to male and female play partners. Pre- and post-surgery timepoints are shown. Error bars are 95% confidence intervals.

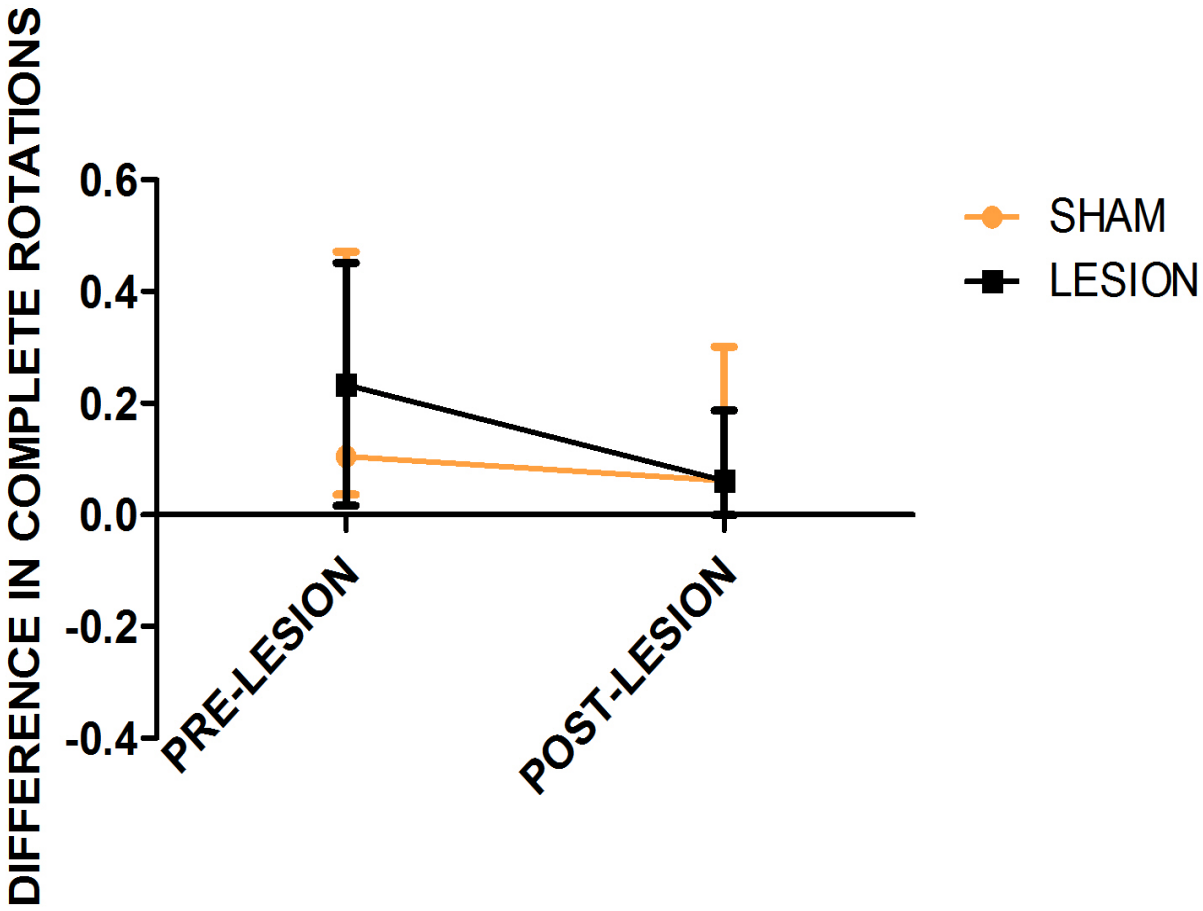

Figure 3.12: Percentage difference between complete rotations used with male vs. female partners with respect to condition. Pre- and post-surgery timepoints are shown. Error bars are 95% confidence intervals.

effect of *sex*, $F(1, 23) = 15.186, p = .001$. There were also significant *time* by *condition* and *sex* by *condition* interactions, $F(1, 23) = 24.860, p < .001$ and $F(1, 23) = 4.760, p = .04$ respectively(Fig. 3.13). Post-hoc analyses showed that female partners used more complete rotations than male partners ($p = .009$). Following surgery, the partners of lesion animals used a smaller proportion of complete rotations ($p < .001$); whereas the partners of sham animals used the same proportion of complete rotations at both timepoints ($p > .05$). Following surgery, the partners of lesion animals used a smaller proportion of complete rotations than the partners of shams ($p = .004$), but prior to surgery, both groups used the same proportion of complete rotations ($p > .05$).

Summary

When Postnatal Day 3 lesions were performed, ablated animals showed a decreased propensity to respond to playful attacks relative to sham animals. When ablated animals did respond to a playful attack, they were more likely to evade the attack, and less likely to rotate to supine than sham animals. Interestingly, mPFC-ablated animals were also more likely to initiate a playful attack than shams. Both mPFC-ablated animals and sham animals showed age-related changes in the proportion of complete rotations, and the magnitude of the changes was not significantly different across the two groups. Additionally, the play partners of neonatally-ablated animals evaded playful attacks a higher proportion of the time than the play partners of sham animals.

Adult mPFC lesion produced a slightly different pattern of behaviour than neonatal lesions. Adult mPFC-ablated animals initiated playful attacks less frequently than did shams. mPFC-ablated animals also evaded a higher proportion of playful attacks

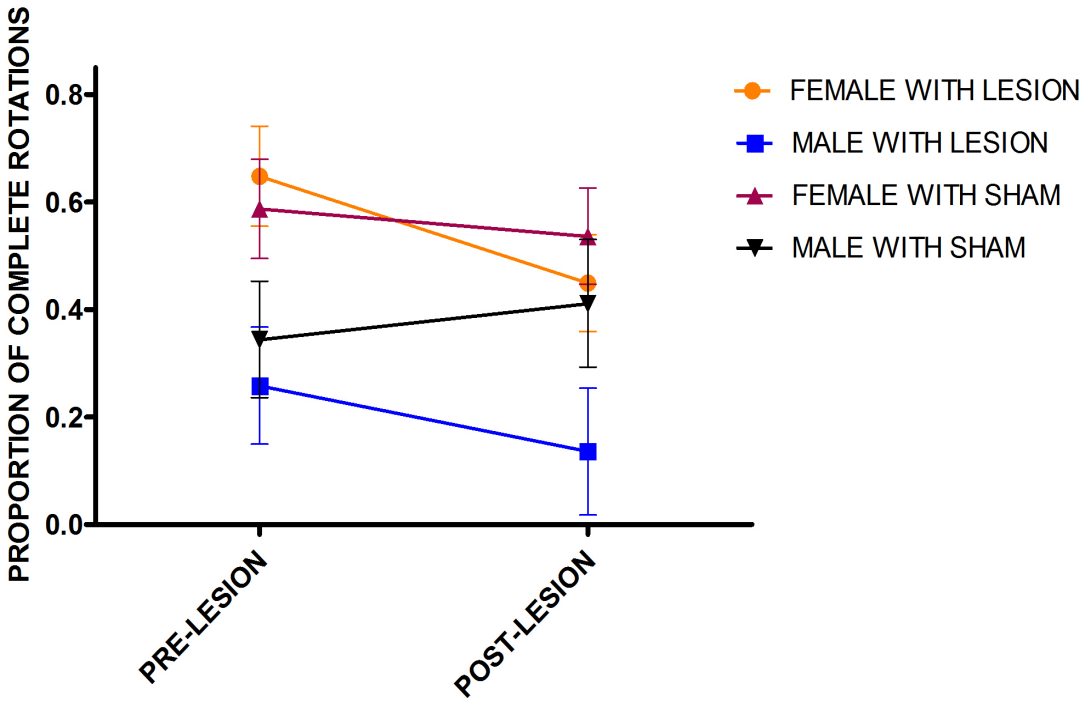

Figure 3.13: Proportion of complete rotations by male and female play partners of lesion and sham animals, pre- and post-surgery. Error bars are 95% confidence intervals.

than shams, and mPFC-ablated animals used a lower proportion of complete rotations than shams. Both mPFC-ablated and sham animals used a higher proportion of complete rotations with male partners than with female partners. Following surgery the magnitude of the response asymmetry with respect to female and male play partners did not change for either the sham or lesion animals. The play partners of ablated animals also used a smaller proportion of complete rotations than the play partners of shams.

Overall, the results suggest that the pattern of behaviour arising from mPFC ablation is different from the patterns associated with both OFC and MC ablations. The results further suggest that the proper and synergistic functioning of the OFC, MC and mPFC are required in order to produce appropriate social behaviour.

Chapter 4

DISCUSSION

The Frontal Cortex (FC) is sensitive to the social environment to which it is exposed during development. The FC is also key to the production of social behaviours; however, it is not that social behaviours are absent without the FC. Rather, the FC mediates the behavioural flexibility needed to produce behaviours in specific contexts, and to be able to make adjustments based on new information. Several subregions of the FC contribute discrete aspects of behavioural flexibility. It is not a coincidence that the FC should be sensitive to a variety of social experiences, and also that it should dictate how an animal behaves in a variety of situations. The FC, and indeed, the entire cortex, is believed to have evolved to provide such behavioural flexibility. If an animal is not exposed to a variety of experiences as a juvenile, then there is no reason to expect that there will be a variety of social experiences to be had as an adult. Therefore, the FC functions to equip the animal with the appropriate behavioural responses for its environment. Deficits associated with lesions of the FC have to do with the inability to alter behaviour based on the introduction of exogenous factors. Similarly, the various subregions of the FC must work in concert in order to produce dynamic social behaviour.

Environmental Inputs and Prefrontal Cortex Microstructure

Previous work has demonstrated that depriving juvenile rats of social play produces social incompetence in the same animals as adults (Hol et al., 1999); however, a specific mechanism by which these behavioural changes might be achieved has not been determined. Given that the functioning of the OFC has been linked to the

modulation of play-fighting tactics with respect to partner identity (Pellis et al., 2006), and given that play-deprived rats also exhibit deficits related to partner identification (Hol et al., 1999), it follows that the OFC may require the experience of behavioural variety through the performance of peer-peer play. The data presented here suggest that the OFC may not be dependent on the experience of play itself, but on another important social influence during the juvenile period, exposure to multiple partners.

In the first set of experiments, it was demonstrated that the OFC responded to the number of partners encountered during the juvenile period, irrespective of the degree of play involved with those partners. Socially-isolated animals are known to be deficient in tasks requiring discrimination, and to exhibit behavioural rigidity (Jones et al., 1991). These findings suggest that the OFC contributes to the production of appropriate social actions by pairing appropriate behavioural responses with the identity of social partners. Furthermore, the development of the ability to discriminate among different individuals is facilitated by experience with a range of different individuals. It is not the case that having experience with different animals simply allows animals to distinguish among individuals; rather, experience with a variety of individuals trains animals to behave flexibly. Through exposure to different individuals, and thus, different social situations, varying outcomes of social contact are encountered. The animal learns to alter its behaviour toward partners having specific characteristics in order to increase the probability of positive outcomes, and to reduce the probability of negative outcomes. That is, the reward-value of specific types of encounters is established.

In fact, the above interpretation is supported by work on several species. The somatic marker hypothesis (Damasio, 1994) postulates that, at least in humans, the projections that the OFC receives from the amygdala are important in order to give specific behavioural options emotional value. These emotional values facilitate appro-

priate decisions among behavioural options in specific contexts. In humans, damage to the OFC results in a variety of impairments with respect to social decisions (see Damasio, 1994; Wallis, 2007).

More generally, in marmosets, it has been demonstrated that lesions of the OFC (but not mPFC) interfere with the acquisition of a new response to a conditioned reinforcer, but responses to primary reinforcers do not change (Pears et al., 2003). In rats, OFC lesions impair the ability to perform an anticipatory reward task (Kesner & Gilbert, 2007). Latent inhibition is also abnormally persistent in rats with OFC lesions, but not those with mPFC lesions, indicating that the OFC-ablated animals have difficulty forming new associative relationships. Interestingly, the same study also showed that lesions in the basolateral amygdala affected latent inhibition in the same way as OFC ablation did, thus providing evidence that the OFC and amygdala may be working together in rats the same way that is thought to happen in humans (Schiller & Weiner, 2004). OFC lesions, but not mPFC lesions, also increase impulsivity in rats, which may be related to the diminished reward-value of behavioural options (Walton et al., 2007).

The changes that occurred in the OFC were seen only in the basilar dendritic field. Because the basilar field receives more connections from across columns and from other brain areas than the apical field, the basilar field is most likely responsible for integrating input from a variety of sources (see Stuart, Nelson, & Häusser, 1999; Douglas, Markram, & Martin, 2004). The synapses that are measured by the current measures are primarily excitatory synapses. Therefore, it is likely that, because exposure to a range of individuals requires the integration and processing of more information, one would expect to see a greater degree of complexity in the basilar field of OFC neurons in animals that were required to process a greater amount of social information.

In contrast, the mPFC responded to social housing conditions in a subtly different manner from the OFC. Instead of being sensitive to the number of individuals encountered during development, the mPFC changed with respect to the types of interactions the animal engaged in. It was seen that animals that were housed with only adults had different mPFC neuronal morphology from those that were housed with juveniles. Because juveniles that are exposed only to adults have the same social deficits as juveniles that are exposed to drugged, non-playful juveniles, and the same deficits as isolation-housed animals (Einon et al., 1978), it is likely that the difference between the juvenile-with-adult(s) condition and the juvenile-with-juvenile(s) condition is the presence or absence of peer play.

Juvenile play differs from adult play in many ways, but the most obvious is frequency. Juveniles play more than adults (Thor & Holloway, 1984). However, if it were the frequency of play that was the most important factor, then there should have been a difference between the subjects housed with one other juvenile, and those housed with three other juveniles. The frequency of play in the quadrads was higher than in the pairs because of the *contagion effect* (Hole & Einon, 1984; Pellis & McKenna, 1992). In fact, there was no difference with respect to mPFC cell morphology between the quadrad and pair conditions (see Figure 2.3). Furthermore, playing with a peer for one hour per day is sufficient to attenuate the effects of social isolation, even though there are fewer play interactions present during that hour than would occur in socially-housed animals (Einon et al., 1978).

Aside from the frequency of play, adult play differs from juvenile play in its overall structure. Whereas, juvenile play tends to be reciprocal, with partners adopting the *attacker* and *defender* roles with roughly equal frequency, rough-and-tumble play becomes asymmetrical as rats mature (Pellis & Pellis, 1992). Indeed, the organization of play in the peak juvenile period is such that reciprocation, loss of control, and the

experience of unexpected occurrences is greatly exaggerated (Foroud & Pellis, 2002, 2003; Pellis et al., 2005). That the mPFC changes with the presence or absence of peer play suggests that the specific experience of peer-peer play is different from the experience of play that occurs between juveniles and adults. It has been suggested that the reciprocal nature of juvenile play found in many mammalian species is essential training for integration into complex adult social systems (Bekoff, 2001). If correct, then it is not just experience with social partners that is required for proper adult social functioning; what the social partners *do* with one-another is also key.

The changes that were seen in the mPFC occurred only in the apical dendritic field. The apical field receives non-specific inputs, but forms more connections within columns than between columns when compared with the basilar field (see Stuart et al., 1999; Douglas et al., 2004). It is therefore more likely to be connected with other cells in the mPFC, rather than with cells outside of this area. Because the mPFC is thought to be involved with temporal sequencing of movements, it is likely that limiting the experience that an animal has with movements will result in a lesser degree of refining of those movements, and a lesser degree of refining in the connections of areas that produce the movements. Therefore, one would expect to see more complex apical fields of neurons in the mPFC of animals with less experience coordinating movements. In fact, it has been suggested that play during the juvenile period prepares the motor system of animals for engagement in adult behaviours (Brownlee, 1954). The so-called Motor Training Hypothesis asserts that animals that play during the juvenile period have motor systems that are better prepared to deal with the environmental demands faced during adulthood. Animals that are better adapted go on to produce more offspring, and thus, there is play during the juvenile period. Recently, support for the Motor Training Hypothesis has emerged, showing that the critical period for cerebellar synaptic pruning and motor nerve differentiation

occur at roughly the same time as the peak play period during the juvenile phase in several mammalian species (Byers & Walker, 1995).

One might argue that the changes seen in the OFC and mPFC neurons of juvenile rats living with adults are stress effects. In fact, there is evidence that corticosterone levels are elevated, and that stress responses are altered by circumstances of social stress during adolescence (Merrick, Secen, Helmreich, & McCormick, 2006). However, the fact that the cells of the OFC were not different, with respect to dendritic complexity, across the *adult* and *juvenile* groups in the second experiment seems to contradict the idea that stress was the primary contributor to changes (see Figure 2.8). Spine density was significantly increased in the *adult* condition when compared to the *juvenile* condition in all aspects of the OFC and mPFC that were measured (see Figure 2.11, Figure 2.12, Figure 2.13 and Figure 2.14). However, stress has been shown to reduce the spine density in the mPFC (Seib & Wellman, 2003; Radley et al., 2006; Michelsen et al., 2007) and in the OFC (Murmu et al., 2006). The results obtained in the current experiments indicate that, in direct opposition to stress effects, the OFC and mPFC microstructure remains the same in all raising conditions, except when juveniles are raised with four adults, in which case the spine density is actually increased. In fact, an increase in spine density is associated with the administration of amphetamines (Robinson & Kolb, 1997) and with the experience of complex housing environments (Kolb, Gorny, Soderpalm, & Robinson, 2003).

The OFC and mPFC are responsive, during development, to different aspects of social experience. The fact that the OFC and mPFC are interconnected leads to the speculation that the disparate types of information are shared across the two areas (see Heidbreder & Groenewegen, 2003). The OFC is altered by the number of animals to which the subject is exposed, and the mPFC is altered by the types of behaviours that are expressed. Essentially, the OFC requires exposure to a variety of animals in

order to develop the ability to distinguish among a variety of social partners. The mPFC seems to require practice with certain types of movements during development.

So, the OFC and mPFC are sensitive to specific aspects of the social environment during development, and the OFC is known to control partner-related modulations in defensive tactics used by rats. Does the mPFC also contribute to the production of social behaviour?

Contributions of the mPFC to Social Behaviour

When Postnatal Day 3 lesions were performed in the mPFC, ablated animals exhibited fewer playful responses to playful attacks than shams. For instance, if the play partner were to approach the subject in a *playful* manner (i.e., running, jumping) and then attempt to contact the subject on the nape, the ablated animal would either remain motionless, or would casually stroll away from (but not actively evade) the play partner. When the mPFC-ablated animals did respond to an attack, they were less likely to rotate completely to supine – a defensive style that facilitates the continuation of the play bout. mPFC-ablated animals also evaded playful attacks by partners a higher proportion of the time than did shams. Evasion of a playful attack serves to curtail the play bout.

It has previously been demonstrated that mPFC-damaged animals are impaired in movement initiation, but not on performance of a movement once it had been initiated (Hauber et al., 1994). Additionally, mPFC lesions produce rats that are less willing to expend effort in order to obtain a reward (Walton et al., 2007). However, aside from movement initiation and the effort involved in movements, medial frontal lesions are known to disrupt both learned and "natural" sequential behaviours (Kolb & Whishaw, 1983b, 1983a). Indeed, the mPFC is thought broadly to control temporal

sequencing (Heidbreder & Groenewegen, 2003). In addition, mPFC lesions disrupt working memory for egocentric responses (Ragozzino & Kesner, 2001). Therefore, it is possible that lesions of the mPFC disrupted the ability to appropriately package sets of movements. In turn, the inability to appropriately sequence movements might make play a less rewarding behaviour, resulting in a decreased motivation to engage in play when attacked. Normally, rats find play highly rewarding, and access to a play partner is incentive enough for rats to learn a variety of tasks (Humphreys & Einon, 1981; Calcagnetti & Schechter, 1992; Crowder & Hutto, 1992).

In contrast to the above findings, the mPFC-ablated animals exhibited an increase in the motivation to initiate play, as indicated by an increase in the number of playful attacks perpetrated. Because attack and defense components of play are thought to arise from separate motivational systems(Pellis & Pellis, 1991), an increase in attacks does not necessarily contradict the data with respect to defense. If, as suggested above, the increased number of evasions is a result of a decreased reward value for play, then it is likely that any inherent motivation to engage in play is not being satiated by the interactions experienced by the mPFC-ablated animals. Therefore, an increased propensity to initiate play may be explained by an effort to compensate for the decreased reward value associated with the altered play of mPFC-ablated animals.

If it is the case that mPFC-ablated animals are not able to properly temporally sequence responses, then, because social play is a dynamic activity requiring the behavioural response of one animal to the behavioural input from another, one would expect that the partners of mPFC-ablated animals would exhibit altered behaviour. In fact, like the mPFC-ablated animals, the partners of mPFC-ablated animals also evaded playful attacks a higher proportion of the time than did the partners of sham animals. The increase in evasions, which curtail play bouts, by the partners of mPFC-

ablated animals suggests that there is something abnormal about the play of lesion animals that makes them less attractive as play partners.

As further support for the results obtained in the current research, Schneider and Koch (2005b) recently reported that, following neonatal mPFC lesion, rats were less likely to use complete rotations during play bouts. However, Schneider and Koch (2005b) did not report a change with respect to the probability of defense, or the proportion of evasions. One difference between the current research and the Schneider and Koch (2005b) paper that could account for differing results is the age at which lesions were performed. In the current study, mPFC ablations were performed at Postnatal Day 3, whereas, Schneider and Koch (2005b) performed the surgeries at Postnatal Day 7. Animals that have mPFC lesions at Postnatal Day 3 have no filling in of the lesion cavity, and little functional recovery on tasks that are mediated by the mPFC; however, animals that are ablated at Postnatal Day 7 show a significant filling-in of the lesion cavity, which is correlated with a much greater degree of functional recovery than is seen in any other neonatally-operated age group (Kolb et al., 1996). In fact, the lesions in the Schneider and Koch (2005b) study had filled in, and were evident only in a thinning of the cortex and the presence of scar tissue. In contrast, the brains of the animals in the current research had obvious lesion cavities, and no evidence of filling-in.

Similar to neonatal mPFC lesions, adult mPFC ablations reduced the frequency of complete rotations that the operates used with their partners. It appears as though, following surgery, mPFC-ablated animals were no longer able to differentiate between male and female partners; however, the proportion of complete rotations was decreased greatly with respect to both male and female partners. Therefore, it is more likely that there is a lower limit to the proportion of complete rotations that will occur during play, and that the decrease seen in mPFC-ablated animals was so

great the the lower limit was reached for both sexes. A subsequent analysis of the proportion of complete rotations used with male versus female partners revealed that the magnitude of difference did not change for either group following surgery.

mPFC ablations performed during adulthood also increased the proportion of evasive responses used by operates. Again, the increase in evasions may be linked to a diminished reward value associated with play, or with an increase in the avoidance of effortful behaviour. Interestingly, unlike in the neonatal operates, adult mPFC lesions reduced the number of playful attacks launched by operates. It is known that the the sequelae of neonatal versus adult brain injuries are not identical (Kolb & Whishaw, 1985; Kolb, Holmes, & Whishaw, 1987), so the reduction in attacks may be indicative of a discrete deficit associated only with adult lesions of the mPFC.

The play partners of mPFC-ablated animals used a lower proportion of complete rotations following surgery relative to the partners of shams. The use of a complete rotation as a defensive tactic generally serves to lengthen the play bout, and a decrease in the use of complete rotations may reflect a diminished willingness to engage in play. Unlike the partners of mPFC-ablated animals in the developmental experiment, however, the adult partners of mPFC-ablated animals did not evade playful attacks more often than the partners of shams. There are at least two reasons why evasions may not have increased. First, the pattern of behaviour observed in the adult operates was not identical to the pattern observed in neonatal operates . Therefore, whatever the behavioural change was that resulted in an increase in evasions performed by partners of neonatally-ablated animals may not have been present in the adult-ablated animals. Second, because the surgery-to-behavioural filming interval was relatively short (about 7 days post-surgery), it is possible that the partners of the operates did not have time to develop evasive patterns of playful defense in response to the behavioural abnormalities of the operates.

The results suggest that, in line with investigations of the contributions of other areas of the cortex (Pellis et al., 1992; Kamitakahara et al., 2007; Pellis et al., 2006) animals with mPFC damage are able to perform all of the movements normally used in play fighting. However, more subtle deficits with respect to the frequency of certain behaviours are seen. Furthermore, the current research provides evidence for a triple functional dissociation with respect to the frontal cortex areas and play. Broadly, OFC lesions abolish the normal partner-related modulation of defensive tactics (Pellis et al., 2006), and MC lesions eliminate the normal age-related modulation in defensive tactics (Kamitakahara et al., 2007). In contrast, lesions of the mPFC disrupt the organization of the movements used in play. The most obvious difference between mPFC-ablated animals and shams is a global reduction in the use of complete rotations. Furthermore, de-corticated rats displayed all three of the above patterns of behaviour (Pellis et al., 1992). That the mPFC is sensitive during development to the experience of peer play, indicating that the functioning of the mPFC is refined through exposure to certain types of movements, further supports the suggestion that the mPFC regulates the movements used in play. mPFC-ablated animals behave as though social play were less rewarding, and the behaviour of the partners of mPFC-ablated animals further suggests that playing with lesion animals is not as rewarding as playing with normal animals.

Intriguingly, human children with Autism Spectrum Disorder (ASD), a condition known to be associated with mPFC abnormalities (Courchesne & Pierce, 2005), show patterns of social behaviour similar to those observed in the mPFC-ablated rats. Like the rats, children with ASD are able to perform all of the constituent movements of play; however, the children are unable to appropriately sequence play behaviours with respect to specific contexts (Boucher, 1999; Jordan, 2003). When children with ASD engage in play with partners, the improper behaviours leads the play partners to find

playing with children with ASD aversive. In response, play partners avoid playing with children with ASD. Because play partners avoid play, play also then becomes aversive to ASD children, and the children with ASD learn to avoid engaging in play if it is initiated by someone else. Interestingly, also like the rats with mPFC lesions, people with ASD still maintain the desire to engage in play (Stahmer, 1999).

The Prefrontal Cortex in a Broader Context

The functioning of the OFC and mPFC, rather than being fundamentally reciprocal, is in fact, synergistic. The OFC and mPFC respond differentially to specific types of input from the social environment during development. The specific types of input that each area responds to is related to the respective area's function in the use of social behaviours. The relationship between the requirement for specific environmental input and the production of appropriate behavioural output is evidence that experience during development is crucial for the refining of appropriate social responses. It is also evidence that the prefrontal cortex enables the behaviour of animals to be modified to suit specific environmental requirements.

Neurally, the ability to flexibly modify one's behaviour in the presence of a variety of social partners is expensive, but the expense of flexible social behaviour is compensated for by the ability to engage in co-operative behaviour, and thus, generally provides a survival advantage. If, however, a situation exists, such as a period of limited food, during which fewer other animals are encountered (especially during development), it may make adaptive sense to abolish some degree of social behavioural flexibility in favour of devoting limited neural resources to basic survival. In fact, during a period of limited resources, it may not be desirable to co-operate at all, in which case, behavioural flexibility that would allow for co-operative social behaviour

may actually be disadvantageous. Primary evidence supporting this idea is that rats that are socially isolated during development, which are socially abnormal as adults, do not differ markedly from socially-housed animals with respect to non-social tasks (Einon & Portegal, 1991; van den Berg et al., 1999); however, isolated animals do show an increase in self-directed behaviours (Hurst, Barnard, Nevison, & West, 1997). And it seems that social play is the key to developing dynamic adult social behaviour. Rats that are deprived of juvenile play are more aggressive toward conspecifics than socially-reared animals (Einon & Portegal, 1991; van den Berg et al., 1999; von Frijtag, Schot, van den Bos, & Spruijt, 2002), further suggesting that social play during development forms the foundation for co-operative adult behaviour.

Furthermore, male rats that are isolated as juveniles show aberrant sexual behaviour as adults (Gerall, Ward, & Gerall, 1966; Hole, Einon, & Plotkin, 1986). In an environment in which resources are especially limited, the reproductive fitness of males that are particularly susceptible to deprivation may be limited. One might expect that such a trait would eventually disappear from the population as animals that are more resistant to deprivation, and thus display a lesser degree of sexual incompetency in response to isolation, are more successful. However, the deficiencies seen in mating behaviour are specific to males, and thus, the trait could be passed on through the female line.

As it turns out, the *thrifty phenotype hypothesis* postulates that developmental influences permanently alter metabolic and behavioural patterns (see Wells, 2006). The rationale behind the thrifty phenotype is essentially the same as what was outlined above – animals are likely to encounter similar environments during development and during adulthood. To survive successfully in environments that are subject to occasional fluctuations, it is advantageous for organisms to be able to change aspects of their metabolic and behavioural functioning. The specific mechanisms responsi-

ble for the expression of the thrifty phenotype have been proposed to be epigenetic. A recent study in mice showed that exposure to a greater variety of peers through communal nesting during development resulted in more diverse social behaviour during adulthood; however, learning and memory were not affected (Branchi, in press). In an earlier investigation, growth factors associated with neuronal proliferation and synaptogenesis were found to be increased in the brains of adult mice that had been raised in communal nesting environments (Branchi et al., 2006). The findings of the above papers were viewed as evidence favouring the thrifty phenotype hypothesis.

The contributions of the current research to the support of the thrifty phenotype hypothesis are novel in at least 2 respects. First, evidence for the hypothesis in rats has heretofore been primarily concerned with the long-term metabolic effects of pre-natal environments (e.g., Rao, 1996; Pravenec et al., 2005). Behavioural effects resulting from perinatal exposure to social environments has not been examined in the context of the thrifty phenotype; however, the previous research showing behavioural impairments following play deprivation could now be re-interpreted in this light (as above). Second, the brain areas involved in the implementation of the thrifty phenotype have not been fully examined. Notably, except for recent un-tested speculation, the role of the neocortex has not been addressed in the development of the thrifty phenotype (Reser, 2006).

The ability of the prefrontal cortex to direct social behaviour is dependent on specific inputs during development, primarily through the experience of social play during the juvenile phase. If social play is not present in an animal's social environment during the juvenile phase, it may be reflective of a resource shortage in the environment. Alterations in adult social behaviour following play deprivation may reflect alterations in survival strategies that are necessitated during periods of limited environmental resources.

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