The Effects of Maternal Deprivation, Through Artificial Rearing, on Impulsiveness in Rats

by

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Doctor of Philosophy, 2010
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Abstract

Mammalian brain and behaviour are plastic, particularly early in development when offspring are under the care of their mothers. Adverse early life events, such as maternal and social deprivation, have short- and long-term consequences for neurobiology and consequently for behaviour. The purpose of this thesis was to investigate the effects of maternal and social deprivation, through artificial rearing (AR), on adult impulsive behaviour.

Rats were raised in isolation from mothers and siblings (AR) or with their mothers and siblings in the nest [maternal rearing (MR)]. In addition, half of the AR rats were provided with replacement somatosensory stimulation designed to simulate mothers’ licking (see Gonzalez et al., 2001).

As adults, rats were tested on impulsive action using the differential reinforcement of low rates (DRL) operant task and locomotor activity. Both male and female AR rats were more impulsive than MR rats; they made more responses and they were less efficient at earning rewards. In addition, they displayed greater levels of locomotor activity. These effects were partially reversed by replacement somatosensory stimulation. Furthermore, impulsivity was positively correlated with locomotor activity.

Impulsive choice was then assessed using a delay discounting operant schedule. On this task, AR rats were less likely to discount the value of large-
delayed reward, suggesting that they were *better* able to tolerate delays to large reward and were *less* impulsive. However, performance on a modified version of delay discounting revealed that AR rats were less efficient at switching their responses; that is, they displayed reduced behavioural flexibility. To address this finding, impulsive *choice* was next assessed in fixed consecutive chain operant schedule of reinforcement, but there were no differences between AR and MR animals.

Finally, the relationship between impulsive action and a species-characteristic behaviour, maternal behaviour, was investigated. Consistent with the literature, AR rats were less maternal and more impulsive. Moreover, there was a negative correlation between impulsivity and maternal behaviour.

Overall, this set of studies demonstrates that maternal and social deprivation produces an increase in impulsive action without an effect on impulsive choice. Furthermore, increased action impulsiveness is a significant moderator of disrupted maternal behaviour observed in AR rats.
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<tr>
<td>5-CSRTT</td>
<td>5 choice serial reaction time task</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine – serotonin</td>
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<td>5-HTT</td>
<td>5-hydroxytryptamine transporter</td>
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<tr>
<td>5-HIAA</td>
<td>5-hydroxyindoleactic acid</td>
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<tr>
<td>AMPH</td>
<td>amphetamine</td>
</tr>
<tr>
<td>AR</td>
<td>artificial rearing or artificially reared</td>
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<tr>
<td>AR-MAX</td>
<td>artificially reared maximally stimulated</td>
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<tr>
<td>AR-MIN</td>
<td>artificially reared minimally stimulated</td>
</tr>
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<td>ASST</td>
<td>attentional set shifting task</td>
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<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
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<tr>
<td>DA</td>
<td>dopamine</td>
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<td>DD</td>
<td>delay discounting</td>
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<tr>
<td>DRL</td>
<td>differential reinforcement of low rates operant schedule</td>
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<tr>
<td>ED</td>
<td>extradimensional</td>
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<tr>
<td>FCN</td>
<td>fixed consecutive number</td>
</tr>
<tr>
<td>FSCV</td>
<td>fast scan cyclic voltammetry</td>
</tr>
<tr>
<td>HPA</td>
<td>hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>ID</td>
<td>intradimensional</td>
</tr>
<tr>
<td>mIRTs</td>
<td>mean interresponse times</td>
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<tr>
<td>NA</td>
<td>noradrenaline</td>
</tr>
<tr>
<td>MR</td>
<td>mother reared</td>
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<td>NAC</td>
<td>nucleus accumbens</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------------------------</td>
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<tr>
<td>OFC</td>
<td>orbitofrontal cortex</td>
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<tr>
<td>PKA</td>
<td>protein kinase A</td>
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<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
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<tr>
<td>PND</td>
<td>postnatal day</td>
</tr>
<tr>
<td>PPI</td>
<td>prepulse inhibition</td>
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<tr>
<td>SSRIs</td>
<td>selective serotonin reuptake inhibitors</td>
</tr>
<tr>
<td>STN</td>
<td>subthalamic nucleus</td>
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CHAPTER ONE: GENERAL INTRODUCTION

STATEMENT OF PURPOSE

Among rodents and primates alike, adverse early life experiences can have long lasting effects on adult neurobiology and behaviour. Previous research has demonstrated that maternal or social deprivation produces changes in several neurophysiological systems, including the HPA-axis, DA and 5-HT neurotransmitter systems, as well as numerous behaviours, such as social, maternal and sexual behaviour. In addition, maternal deprivation can alter cognitive abilities (e.g., attention and learning).

Maternal and social deprivation, through artificial rearing (AR)(see page 9 and Chapter Two), produces deficits in maternal and sexual behaviour (Gonzalez, Lovic, Ward, Wainwright & Fleming, 2001; E. Akbari, unpublished data). In addition, AR rats are more active and have reduced attentional capabilities (Lovic & Fleming, 2004; Lovic, Fleming & Fletcher, 2006). It has also been casually observed that artificially reared rats are more impulsive and less inhibited. They approach minor environmental stimuli more readily and often at the expense of other more relevant behaviours (e.g., being maternal). In addition, they respond faster in attentional-learning tasks, and make more errors.

Impulsive behaviour in artificially reared rats has not been investigated. In fact, virtually nothing is known about how early life factors, such as maternal and social deprivation, influence adult impulsive behaviour.

The literature defines impulsivity in at least two ways (de Wit, 2008; Evenden, 1999; Winstanley, Dalley, Theobald, & Robbins, 2004). Impulsive
action is characterized by the inability to withhold actions. Impulsive choice is characterized by poor decision making, a tendency to terminate sequenced behaviour prematurely and intolerance to delay of gratification. The primary objective of this dissertation was to investigate the differential effects of maternal deprivation, through artificial rearing, on impulsive action and impulsive choice. In addition, we were interested in assessing the relationship between impulsive behaviour and maternal behaviour in artificially and mother reared rats.

This thesis is divided into the following seven sections. Chapter One is a comprehensive review of relevant topics: maternal influences and deprivation effects on rat behavioural and neurobiological development, and on attention and impulsivity, specifically. Chapter Two is a general methods section describing the artificial rearing paradigm, which was common to all studies described in this thesis. Chapters Three through Six are the presentation of four studies designed to investigate the effects of early life maternal deprivation on impulsivity. Finally, Chapter Seven is a general discussion of the main findings of this thesis in the context of the literature, and their potential application to disorders characterized by impulsivity.
Influence of Early Experiences and Mothering on Development

It has been more than a century since Sigmund Freud started to develop his theory of personality. At the crux of it was the idea of personality dynamics and the importance of early life experiences on adult behaviour. Some fifty years later Donald Hebb (1949) also emphasized the importance of early experiences on adult behaviour, but in contrast to Freud, Hebb emphasized learning and the physiological effects of early life experiences on adult behaviours. The early life experiences of rodents and primates can have long lasting effects on neurophysiology and behaviour. These effects can be general, in the sense that they can change individuals’ responses to a wide variety of stimuli and pattern of behaviour in adulthood (Kraemer, 1997). They can be specific as well, in the sense that they can influence some biobehavioural systems while leaving others intact. Below I describe different kinds of early experience manipulations and their effects on later behaviour and neurobiology. I also describe their commonalities and points of departure, with a view to providing a rationale for our decision to explore the effects of preweaning artificial rearing on later behaviour in Sprague-Dawley rats.

Handling

In the early 1950’s animal researchers, driven by personality theories, hypothesized that early life adversity and trauma can lead to long-lasting alterations in ‘temperament’, specifically individuals’ responses to novelty, anxiety provoking situations, and stressors. Levine and colleagues (1956)
subjected rat pups to electric shock, removed them from the nest or left them with the mother. As adults, these rats were tested on their stress responses. Contrary to their predictions, pups that were shocked and those that were removed from the nest actually had ‘better’ stress responses (shorter in duration) than the individuals that were left undisturbed with the mother.

This led to several hypotheses and numerous studies exploring these effects. Initially it was suggested that these effects are driven by ‘handling’ or somatosensory input provided to the pups by the researchers (e.g., Levine & Lewis, 1959; Schaefer, Weingarten & Towne, 1962). While it was true that pups that were repeatedly and briefly removed from the litter (~15mins), and handled in the process, displayed a better stress response, this effect was conditional. If the pups were removed and placed in cooler ambient temperatures (lower than nest temperature) the effect was observed (Bovard, 1958; Schaefer et al., 1962). However, if the pups were placed in a warm environment (e.g., warm incubator) for a brief period of time, there were no beneficial effects of the handling procedure (e.g., Schaefer, 1963). This led researchers to refute the possibility that somatosensory input provided by the researchers was causing the ‘handling’ effects. Instead, they hypothesized that the effects might be driven by metabolic changes associated with a drop in pups’ body temperature experienced during brief nest absence (Borard, 1958; Schaefer et al., 1962; Schaefer, 1963).

While a drop in temperature turned out to be necessary to observe ‘handling’ effects, it was later demonstrated that the mechanisms were not metabolic in nature (see Bell, Mitshke, Gorry & Sachma, 1971; Levine 1975).
Instead, a drop in temperature was necessary for what happened to pups upon their return to the nest. Pups that are briefly separated from the mother (handled) and experience a drop in temperature, vocalize more upon their return to the nest and mother. In turn, they get licked significantly more than they otherwise would. Conversely, pups that are briefly removed from the mother, but kept in a warm environment, do not experience a drop in body temperature, and do not vocalize more upon reunion with the mother. In turn, their mother's behaviour is not different with respect to them following this separation (Bell et al., 1971; Levine 1975).

Hence, what became clear is that maternal licking, artificially manipulated through brief pup removal from the nest, can have long lasting effects on pups behaviour (e.g., responses to novelty) and physiology (e.g., stress hormones). While it became evident that maternal behaviour was the hidden regulator of handling effects (Hoffer, 1994) it was not immediately evident how altered maternal behaviour translates into altered stress responses in the offspring. Progress was made over many studies and Meaney’s group has found that handling is peripherally mediated by thyroid hormone, centrally by serotonin, and intracellularly by protein kinase A (PKA). More specifically, they have found that handling effects, which are mediated by maternal behaviour, on emotionality can be blocked by either thyroid hormone (propylthiouracil) or serotonin (ketanserin) or PKA inhibitors or blockers (Meaney, Diorio, Francis, Weaver, Yau, Chapman & Seckel, 2000). More recent evidence suggests that maternal licking is associated with the activation of genes associated with the negative feedback
system of the stress response, namely hippocampal glucocorticoid receptors (Kaffman & Meaney, 2007).

**Alternative Approaches**

**Maternal Separation and Deprivation**

In addition to handling, several other approaches have been used to study the effects of early life environment and maternal behaviour on developing offspring. Maternal separation and deprivation studies involve separation of pups from the mother and sometimes from the litter (Lehmann & Feldon, 2000; Lovic, Gonzalez & Fleming, 2002). In order to differentiate them from ‘handling’ studies, which typically involve short-term (<15 min) and repeated procedures, maternal separation and deprivation studies refer to procedures of longer periods of separation of pups from their mother (1-24 hr) acutely or repeatedly.

While maternal ‘deprivation’ and ‘separation’ terms are sometimes used interchangeably, they are both somewhat narrow in their description. While they both involve deprivation and separation from the mother, they also involve food deprivation. In addition, they may also involve a separation from the nest (i.e., siblings, nest odors, etc.). However, the term ‘separation’ is more precise as it operationally describes what is being done (i.e., pups are separated from the mother). ‘Deprivation’ is somewhat open to interpretation as it suggests that any observed effects are due to pups being deprived from the mother or maternal care. While this is a possibility, it is also possible that being separated from the mother might produce effects that are mother independent (e.g., they might be driven by changes in body temperature or they might be of purely nutritional
nature). A number of variables are relevant and are manipulated in maternal separation studies. For example, pups can be removed from the nest, singly or as a group, or the mother can be removed from the nest. Environmental temperature during separation is of particular significance. During maternal separation pups can be placed in a controlled temperature environment (usually nest like warm) or left in an ambient temperature (~21ºC). During separation pups can be placed in contact with an anaesthetized adult female or left alone. Nutrition is usually absent during periods of maternal deprivation and is of significant consequence.

Not surprisingly, findings from maternal separation studies have been inconsistent (Lehmann & Feldon, 2000). These inconsistencies can be mainly attributed to differences in procedures employed in these studies. In fact, it is becoming increasingly clear that significantly different procedures are being described as maternal separation. Overall, maternal deprivation and separation studies usually involve manipulations of more than maternal behaviour (Hoffer, 1994; Lehmann & Feldon, 2000); hence, interpretations of maternal behaviour being the driver of the observed effects are questionable. Nevertheless, these interpretations have been made (e.g., Kuhn and Schanberg, 1998).

**Natural Variations in Maternal Care**

There is considerable individual and strain variability in the intensity and quality of maternal behaviour rat mothers display (Francis, Diorio, Liu & Meaney, 1999; Moore, Wong, Daum & Leclair, 1997). Recently, Meaney's laboratory has
investigated the effects of maternal behaviour on development by assessing the effects of natural variations in maternal care on various biobehavioural systems (e.g., Cameron, Fish & Meaney, 2008; Cameron et al., 2008). In these studies, dams were observed for the amount of licking and arch-backed nursing (mother positioned above the pups with her back highly arched) that they displayed towards their pups. Observations are done over many occasions during the early postnatal period. Dams are designated as either high-licking/arched-back nursing (High LG; upper third of the population) or low-licking/arched-back nursing (Low LG; lower third of the population). Following this classification, offspring are studied during various stages of development (usually adulthood). While this approach is beneficial as it does not have confounds found in maternal deprivation/separation studies (e.g., separation from littermates, change in temperature, nutritional deprivation), the approach is correlational in nature and nothing is being manipulated per se. Furthermore, the role of genomic versus non-genomic influences on factors of interest is of relevance. That is, it is difficult to discern whether changes in maternal behaviour (e.g., licking of pups) is the sole driver of observed effects. It is possible that pups biobehavioural profiles (e.g., reduced emotionality) are a product of their genetic background rather than a product of maternal care that they receive. Hence, cross-fostering of pups (High-LG mothers rearing pups of Low-LG mothers and vice versa) is necessary. At least one study has been performed and Meaney’s group has shown, through cross-fostering, that the observed effects are non-genomic (Francis et al., 1999).
That is, maternal behaviour variation, rather than the genetic variation is the
driver of these effects (at least with respect to measures of stress and HPA axis).

However, cross-fostering is not normally done in these studies. Furthermore, even when pups are cross-fostered, somatosensory input that pups receive, through maternal licking, is not the only variable being manipulated. It is possible that High-LG mothers have a different hormonal profile of the milk that they feed the pups with (their biological pups or cross-fostered pups) and this nutritional difference might be responsible for the observed differences. It is also possible that High-LG and Low-LG mothers organize pup huddles differently. Overall, while there are many advantages to studying individual differences in maternal behaviour, the approach is non-experimental in nature, and thus does not allow for causal inferences to be made about the role of maternal behaviour on offspring development.

**Artificial Rearing**

Another approach to studying the effects of maternal behaviour and early life isolation involves rearing rat pups artificially. In this artificial rearing (AR) paradigm, pups are implanted with cheek or gastric cannula on postnatal day (PND) 2. While their siblings are reared maternally, those implanted with cannula are reared artificially in isolation. They are kept in a consistently (nest-like) warm environment and are fed regularly. Over the past decade Fleming and colleagues have used this paradigm to assess the effects of early life isolation and the role of somatosensory stimulation, used to mimic maternal
licking, on a number of biobehavioural measures (Burton, Lovic & Fleming, 2006; Fleming, Kraemer, Gonzalez, Lovic, Rees & Melo, 2002; Gonzalez et al., 2001; Lovic & Fleming 2004; Lovic, Fleming & Fletcher, 2006).

Compared to MR rats, AR rats are less maternal (Fleming et al. 2002), more active (Burton et al., 2006; Lovic et al., 2006) and show deficits in sexual behaviour (E. Akbari, unpublished data). In addition, they show reduced prepulse inhibition of the startle responses (sensorimotor gating) and deficits in the attentional set shifting task (ASST)(Lovic & Fleming, 2004). However, AR rats do not show any overt learning deficits (e.g., spatial learning and memory in the water-maze; Levy, Melo, Galef, Madden & Fleming, 2003).

While this paradigm is less natural, it allows experimenters to create stable conditions (e.g., no food deprivation, no changes in temperature) where specific factors (e.g., somatosensory stimulation) can be experimentally manipulated (Gonzalez et al., 2001). For these reasons (stable environment and experimenter induced specific manipulations) we have chosen to use the AR paradigm in the set of studies contained in this thesis document.

**Biobehavioural Systems Affected by Maternal Behaviour**

A comprehensive review of all of the biobehavioural systems affected by maternal behaviour and maternal separation is beyond the scope of this document. Hence, what follows is a brief overview of the relevant literature.

It is now well established that maternal behaviour (e.g., licking) and absence of maternal behaviour (i.e., maternal separation) both affect offspring’s
emotionality and functioning of the HPA-axis (Fish et al., 2004; Waiver, Diorio, Seckl, Szyf & Meaney, 2004). In general, pups whose mothers display less licking and arched-back nursing are more emotionally responsive and show prolonged activation of the HPA-axis following presentation of stressors. These effects are mediated by negative feedback systems of HPA-axis, namely hippocampal glucocorticoid receptors (Liu et al., 1997; Waiver et al., 2004).

Maternal behaviour also influences other behavioural systems. Some of these include cognition (Lovic & Fleming, 2004; Liu, Diorio, Day, Francis, & Meaney, 2000) sexual (Cameron et al., 2008; Cameron, Del Corpo, Diorio, McAllister, Sharma & Meaney, 2008; Cameron, Fish & Meaney, 2008), social (Melo, Lovic, Gonzalez, Madden, Sinopoli & Fleming, 2006; Parent & Meaney, 2008) and maternal behaviour (Francis, Diorio, Liu & Meaney, 1999; Fleming et al., 2002).

Numerous aspects of neurophysiology are affected, as well. Relevant to this thesis, are changes in dopamine (DA) and serotonin (5-HT) functioning associated with variations in maternal behaviour and maternal deprivation/separation. In general, in comparison to non-maternally deprived or highly licked pups, maternally deprived pups or pups of low-licking dams, grow up to become more sensitive to dopaminergic drugs such as amphetamine (Lovic et al., 2006) and show greater release of DA in response to potassium or amphetamine stimulation (Hall, Wilkinson, Humby & Robbins, 1999; Matthews, Dalley, Matthews, Tsai & Robbins, 2001). In addition, AR rats show higher baseline DA levels in the nucleus accumbens and altered DA responses to
natural stimuli such as pups (V. Afonso, unpublished data). Overall, evidence suggests that reduced maternal care is associated with increased DA tone in offspring.

Periodic maternal separation is also associated with reduced 5-HT content in the dorsal hippocampus (Lee et al., 2007; Matthews et al., 2001) reduced sensitivity of 5-HT neurons in dorsal raphe nucleus (Gartside, Johnson, Leitch, Troakes & Ingram, 2003), reduced 5-HT transporter mRNA in raphe nucleus (Lee et al., 2007), and reduced 5-HT immunoreactivity in anterior hypothalamus (Veenema, Blume, Niederle, Buwalda & Neumann, 2006). Conversely, handling procedures, which lead to increased maternal licking, are associated with increased 5-HT levels (Papaioannou, Dafni, Alikaridis, Bolaris & Stylianopoulou, 2002) and upregulation of 5-HT transporter (5-HTT) and serotonergic 1A receptor densities (Vicentic et al., 2006). Alterations in DA and 5-HT systems are of relevance as both of these neurotransmitters have been implicated in impulsivity (see below).

**Early Life Experiences**

In rodents, maternal nests are rich with various stimuli (e.g., nest odors, odor of the mother, tactile stimuli from the mother and other pups etc.). Specific experiences with the mother and within the context of the nest can have long-lasting alterations in preferences in adulthood (e.g., food preferences). For example, in rats, early life olfactory experiences with the mother can alter olfactory preferences in adulthood. Rats that experience their mother smelling of
particular scent (e.g., lemon) are maternally more responsive if pups smell of this same odor (Shah, Oxley, Lovic & Fleming, 2003). However, mere exposure to a particular odor early in life, but outside of maternal context, does not render rats more maternally responsive to pups smelling of those odors in adulthood.

Other maternal-nest experiences are relevant, as well. Recent studies suggest that the experience of being mothered has long-lasting effects on the offspring expression of maternal behaviour in adulthood. Pups that are mothered less grow up to be less maternally responsive in adulthood (Fleming et al., 2002; Lovic & Fleming, 2004; Francis et al., 1999).

Of particular relevance to this thesis, are the effects of maternal separation, through AR, on the nature of maternal behaviour deficits. In maternal as well as non-maternal settings (e.g., attentional set shifting task; ASST) AR rats tend to be less inhibited (Fleming et al., 2002; Lovic & Fleming, 2004). In the maternal context, they leave the nest often in order to investigate irrelevant environmental stimuli (e.g., another rat biting top of the cage). During the ASST, rats are presented with 2 bowls filled with a medium (e.g., sawdust bedding), and one of these bowls is baited with food. Bowls can differ along two dimensions, either texture of the bowl or odor of the digging medium. Rats are required to assess both bowls and based on trial and error learning dig only in baited bowls. Rats sometimes approach the baited bowl first, in which case they can immediately dig in that bowl. However, rats sometimes approach the unbaited bowl first, in which case they should inhibit responding (i.e., digging) and go to the other bowl. Compared to MR rats, AR rats would often approach and
respond to the closest stimulus – that is, that would not sufficiently investigate the stimulus before they made their response (Lovic & Fleming, 2004). Hence, the focus here was to investigate differences in behavioural inhibition and impulsive behaviour between AR and MR rats. A brief review of relevant impulsivity literature can be found below.

Impulsivity

**What is impulsivity?**

Impulsivity is a non-unitary, multidimensional trait that colours aspects of normal and abnormal behaviour. Hence, it is of interest to both scientists and clinicians. While it is easy to categorize particular behaviours as impulsive, it is difficult to define impulsiveness. Hence, there is a spectrum of definitions of impulsiveness. A commonality among the proposed definitions is the cognitive-behavioural profile or trait, characterized by a pattern of rapid cognitive decisions and/or behavioural responses that might be acutely rewarding but are, in general, disadvantageous in the long run (Evenden, 1999, De Wit, 2008). Most individuals have engaged in impulsive behaviours. These include quick decisions, without much forethought, to socialize with friends, make purchases, eat food, engage in sexually promiscuous activities etc. Impulsiveness is a continuum trait, as individuals can show various levels of impulsiveness, including low (self-control) or high impulsiveness (Evenden, 1999).

It should be noted that not all instances of ‘impulsive’ behaviour are disadvantageous. Indeed, some aspects of successful functioning require quick
decisions when individuals are faced with sudden opportunities in rapidly changing environments. For example, if scarce food sources suddenly appear, and for short period of time become available, it might be beneficial for animals to act quickly, without much forethought, thus gaining resources that might be transient. However, in general, persistent high impulsiveness is not adaptive in the long run. In fact, a number of psychiatric disorders are characterized by impulsiveness and have impulsiveness as one their main features/criteria (see below).

**Impulsivity and Personality**

Systematic assessment of impulsivity is rooted in personality research. Hans Eysenck’s biosocial model of personality initially consisted of two superfactors: Extraversion and Neuroticism (Eysenck, 1957). These superfactors were composed of lower level traits, which in turn were composed of habits. Within this model, impulsivity was one of the lower level traits under the umbrella of the Extraversion superfactor. Later on, Eysneck revised his model to include Psychoticism as another superfactor (Eysenck & Eysenck, 1977). Within this new model, impulsivity was placed, as a sub-trait, under the Psychoticism superfactor and not under Extraversion superfactor. Later personality theorists also struggled with the conceptualization of impulsivity as a trait, as well as how to organize it with respect to other traits. Even contemporary personality researchers cannot agree on where to place this trait within the personality models, or even how to best measure impulsivity.
**Fractioning Impulsive Behaviour**

Impulsivity, like memory and attention, is not a unitary construct and consists of several related neurochemically, neuropharmacologically and behaviourally dissociable phenomena (de Wit, 2008; Evenden, 1999; Winstanley et al., 2004). *Impulsive action* is characterized by individuals’ inability to withhold actions (sometimes referred to as motor impulsiveness or behavioural disinhibition – tantamount to saying “I acted too quickly”). Impulsiveness can also be characterized by poor decision making, tendency to terminate sequenced behaviour prematurely and intolerance to delay of gratification. This aspect of impulsivity is often termed *impulsive choice*.

**Impulsivity Tests in Rodents**

There are several behavioural tasks that measure rodent impulsive action. These include go/no-go, 5 choice serial reaction time tasks (5-CSRTT) and differential reinforcement of low rate responding (DRL). While these test procedures differ from one another, in general, successful performance relies on the ability to withhold responses that might be at later times rewarding. Specifically, in the DRL procedure, rats are rewarded for making operant responses after a certain amount of time has passed. For example, in DRL-20, rats respond on a lever and are rewarded with food. However, they have to wait at least 20 sec since their last lever response in order to be rewarded for making a response. Making premature responses resets the clock and ‘waiting time’ (Evenden, 1999; Fletcher, 1995; Monterosso & Ainslie, 1999; O’Donnell, Marek &
Seiden, 2005; Peterson, Wolf & White, 2003). Number of responses, number of reinforcers and efficiency at earning rewards is a measure of interest.

The 5-CSRTT is a rat analogue of human sustained attention tasks. It was developed to assess rats’ attentional capabilities. More recently it has also been used to assess impulsive action. In an operant box setting, rats are presented with 5 nose-poke openings and are required to respond, by nose poking the opening, once the opening is briefly illuminated (often less than 1 s). Successful responses are rewarded with food or water. However, responding in one of the boxes prior to any of them being illuminated, on a particular trial, constitutes premature responding and is viewed as impulsive action (Bari, Dalley & Robbins, 2008).

In the go/no-go task, rats are trained to respond to one stimulus (e.g., left light; go trials) and inhibit responding to a similar but different stimulus (e.g., right light; no-go trials). In a symmetrical go/no-go task rats are rewarded for correctly responding on the go trials and inhibiting responding on the no-go trials. Incorrect inhibition, (i.e., responding on the no-go trials), is an index of impulsivity (Perry & Carroll, 2008).

Impulsive decision making, or impulsive choice as it is referred to here, is most commonly measured by assessing aversion to delay of reinforcement. Specifically, in an operant setting, rats are presented with a choice of responding on two levers. Responding on one lever results in an immediate delivery of small reward (e.g., one pellet of food) while responding on the other lever results in delayed delivery of larger reward (e.g., 4 pellets). The second option is more
beneficial but the subjective value of the large reward is influenced by the time delay to receive the large reward. That is, the value of a large reward is discounted by the delay. The rate of discounting is a hyperbolic curve, where preference for large reward is based on the size of the reward and delay to that reward. The curve is described by the equation $V = A / (1 + kD)$, where $V$ is the value of reward, $A$ is size of the delayed reinforcer, $D$ is delay to the reward and $k$ is the steepness of the curve (Perry & Carroll, 2008; de Wit & Richards, 2004). Individuals differ in their tolerance of delay, and the more impulsive individual discounts the value of large delayed reward at a greater rate than the less impulsive individuals (See Figure 1).

Impulsive choice can also be measured using a fixed consecutive number (FCN) operant schedule. On this schedule, rats are presented with two levers, either intermittently or throughout the session. They have to make a number of responses (e.g., at least 8 responses on an FCN8 schedule) on one lever (chain lever) in order to get reward by responding once on the other lever (reinforcement lever). Terminating responding before a minimum of number of responses has been reached on the chain lever does not result in food delivery and it resets the number requirement (i.e., new chain needs to be started). While rats cannot count, they rapidly learn to make their chains of appropriate length. However, length of their chains varies and shorter chains are an index of impulsiveness. This tendency to switch to the other lever is considered an impulsive decision or choice (Evenden, 1998; Dellu-Hagedorn, 2006). In contrast to tests of impulsive action, acting or not acting (e.g., responding on a lever) is
not as relevant as the choice on which lever responses are made. That is, similar to DD testing, on an FCN schedule, rats have a choice between options. In the DD task rats have a choice between two options – small-immediate reward lever or large-delayed lever. On an FCN schedule, they have an option of responding on either the chain lever or the reinforcement lever.

As mentioned earlier, impulsive action and impulsive choice are behaviourally, neuroanatomically and neuropharmacologically dissociable. For example, amphetamine increases impulsive action in the DRL (Bayley, Bentley & Dawson, 1998) and 5-CSRTT (Robbins, 2002; van Gaalen, Brueggeman, Bronius, Schoffelmeer, & Vanderschuren, 2006) tasks but reduces impulsive choice in the DD (Winstanley, Dalley, Theobald & Robbins, 2003) and FCN tests (Rivalan, Gregoire & Dellu-Hagedorn, 2007).

It is important to acknowledge that both impulsive action and impulsive choice involve motor responses and inhibition, as well as decisions. In impulsive action tasks, rats have a choice of acting or not acting but they do not have a choice between different contingencies or their responses will not lead to different contingencies. In impulsive choice tasks, rats have to inhibit some responses, however, what is most relevant is where (towards which stimulus; e.g., left or right lever) they will respond. Hence, the terms impulsive action and impulsive choice represent the most salient features of what these tasks aim at assessing.
Figure 1  The figure depicts hypothetical hyperbolic curves typically observed in delay discounting experiments. The y-axis represents the preference for large reward and the x-axis represents the delay to large reward. As the delay to large reward increases, preference for large reward decreases in a hyperbolic manner.
Cognitive-Behavioural Disorders and Impulsiveness

While the Diagnostic and Statistical Manual (DSM-IV) does not classify impulsiveness as a disorder, a number of disorders are characterized by impulsiveness. These include substance abuse, paraphilias, conduct disorder, antisocial personality disorder, borderline personality disorder, attention deficit/hyperactivity disorder and mania (however, impulsivity is not explicitly stated as criteria for mania). In addition, pathological gambling, kleptomania and pyromania are classified under the heading of ‘impulse control disorders not elsewhere classified’ (APA, 1994). Therefore, increased understanding of impulsivity might provide us with greater understanding of these disorders. Furthermore, treating impulsivity, as a symptom and precursor of some of these disorders, might be beneficial in treating these disorders.

Impulsiveness has recently received considerable attention in relation to substance abuse. Loss of self-control or impulsiveness, with respect to drug use, is one of the criteria for a diagnosis of substance abuse. In fact, drug abuse and impulsivity are closely related (Jentsch & Taylor, 1999), as impulsivity is both a determinant and a consequence of drug abuse (de Wit, 2009). When given a choice between a small immediate reward and large delayed reward, heroin and cocaine abusers select the smaller immediate reward. They make these choices in response to many different types of reward (Kirby & Petry, 2004). Non-human studies have shown that impulsivity is actually predictive of future drug self-administration (Perry, Larson, German, Madden & Carroll, 2005). Highly impulsive rats will compulsively (despite negative consequences; e.g., foot
shock) self-administer drugs (Belin, Mar, Dalley, Robbins & Everitt, 2008) and they are more likely to relapse to cocaine-seeking after extinction (Economidou, Pelloux, Robbins, Dalley & Everitt, 2009). Therefore, increased impulsiveness can be viewed as a risk factor for developing substance abuse.

Attention deficit/hyperactivity disorder affects 1-5% of grade school children (Berger & Sagvolden, 1998). It is characterized by inattentiveness, overactivity and impulsiveness. Children with AD/HD are unable to refrain from responding, they show premature responding, and their responding often occurs in bursts (Berger & Sagvolden, 1998). In addition, undiagnosed children with high impulsiveness tend to have poor peer relations and difficulties with academic tasks (Snyder, Prichard, Schrepferman, Patrick & Stoolmiller, 2004). Furthermore, these children are at an increased risk for early-onset and persisting conduct problems (Moffitt, 2003).

Increased impulsiveness is also characteristic of many brain-injured individuals. The orbital prefrontal cortex (OFC) is important in self-regulation and this part of the brain is highly susceptible to traumatic brain injury. Hence, the majority of individuals with traumatic brain injury have impulse control problems (Berlin, Rolls & Kischka, 2004; Rieger & Gauggel, 2002; Fontaine, Azouvi, Remy, Bussel & Samson, 1999).

**Neuroanatomy of Impulsivity**

Impulsivity is related to several psychological concepts, such as attraction to rewards, avoidance of stressors and attention. Hence, numerous brain areas
are involved in impulsivity. The prefrontal cortex, striatum and limbic system have been implicated in impulsivity. Ever since Phineas Gage sustained damage to ventral prefrontal cortex and showed marked increase in impulsiveness, neuropsychologists have found that brain damage, particularly of the ventral prefrontal cortex, is associated with impulsiveness (Berlin, Rolls & Kischka, 2004; Damasio, Grabowski, Frank, Galaburda & Damasio, 1994).

While some non-human studies have shown that OFC lesions increase impulsive choice (Mobini et al., 2002), others have found no effect (Winstanley, Theobald, Cardinal & Robbins, 2004). In addition to the OFC lesions, nucleus accumbens core (NAC) lesions produce an increase in impulsive choice and impulsive action (Pothuizen, Jongen-Realo, Feldon & Yee, 2005; Cardinal, Pennicot, Sugathapala, Robbins & Everitt, 2001). Furthermore, lesions of the amygdala have been associated with an increase in impulsive choice (Winstanley et al., 2004) and lesions of the hippocampus have been associated with increases in both impulsive action and choice (Cho & Jeantet, 2009; Cheung & Cardinal, 2005). There is considerable overlap between the brain areas involved in impulsive action and impulsive choice; however, there are also clear dissociations. For example, lesions of the subthalamic nucleus (STN) are associated with an increase in impulsive action and decreased impulsive choice (Uslaner & Robinson, 2006). That is, STN lesions produce an increase in lever responses and decreased efficiencies on a DRL schedule. In contrast, STN lesioned rats discount the value of large-delayed rewards at slower rates compared to control rats.
Neurochemistry of Impulsiveness

In the last two decades, a number of researchers have attempted to shed light on the neurochemical correlates of self-control and impulsiveness (e.g., Winstanley et al., 2004, 2005; Evenden, 1999; Fletcher, 1995; Sourbie, 1986; see Pattij & Vanderschuren, 2008). Historically, increased disinhibition and impulsivity has been associated with decreased 5-hydroxytryptamine (5-HT, serotonin) levels (Soubrie, 1986). Dorsal and median raphe 5-HT neurotoxic lesions produce deficits in impulsive action as measured in a DRL operant schedule (Fletcher, 1995). In addition, global 5-HT depletion (>90%) produces an increase in conditioned locomotor activity (behavioural disinhibition) and impulsive action (Winstanley et al., 2004). Similarly, human volunteers whose 5-HT levels have been reduced, through tryptophan (serotonin precursor) depletion, show greater motor impulsiveness (Crean, Ricards & de Wit, 2002; LeMarquand et al., 1998). Low levels of the 5-HT metabolite, 5-hydroxyindoleactic acid (5-HIAA) in the cerebrospinal fluid (CSF), have been associated with violent suicide attempts and unprovoked aggression (Brown et al., 1982; Cremniter et al., 1999). Similarly, vervet monkeys with lower levels of 5-HIAA show greater impulsivity in the social context (aggressive and assertive interactions). Fluoxetine treatment, which increases the 5-HT tone, reverses these effects (Fairbanks, Melga, Jorgensen, Kaplan & McGuire, 2001).

However, findings that a reduction in 5-HT lead to increased impulsivity have not been universal. Depletions of 5-HT through neurotoxic lesions have no effect on impulsive choice (delay discounting) (Winstanley et al., 2004). Brown,
Fletcher and Coscina (1998) acutely depleted brain 5-HT, by giving rats amino acid loads deficient in tryptophan (5-HT precursor) and found no effects on impulsive action (DRL 20). Furthermore, tonic increases of serotonin in the prefrontal cortex actually increase impulsivity (Dalley, Theobald, Eagle, Passetti & Robbins, 2002). Altogether, these data suggest that the serotonin impulsivity story is complex. This is likely due to the widespread distribution of serotonin throughout the brain, as well as the large number of serotonin receptor types – 14 different receptor types belonging to 7 receptor families (Barnes & Sharp, 1999). For example, blockade of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors have different effects on impulsive action. While antagonism of 5-HT$_{2A}$ receptors reduces impulsive action, blockade of 5-HT$_{2C}$ receptors increase impulsivity (Fletcher, Tampakeras, Sinyard & Higgins, 2007).

In addition to serotonin, several studies have suggested that the DA system may also play a role in impulsiveness. Amphetamine (DA agonist) (Bayley et al., 1998; Evenden, 1999; Robbins, 2002) and amphetamine withdrawal (Peterson, Wolf & White, 2003) both increase impulsive action. Amphetamine potentiation of impulsive action is mediated by D2 receptors (van Gaalen et al., 2006; van Gaalen, Unger, Jongen-Relo, Schoemaker & Gross, 2009). On the other hand amphetamine reduces impulsive choice (Winstanley et al., 2003). This effect is attenuated if rats receive global 5-HT neurotoxic lesions. Hence, the ability of amphetamine to reduce impulsive choice is at least in part mediated by the 5-HT system. In fact, Winstanley, Theobald, Dalley and Robbins (2005) have elegantly shown that DA: 5-HT interactions are important in
amphetamine reduction of impulsiveness. Self-control (decreased impulsiveness) was associated with increased 5-HT activity in the medial prefrontal cortex but not in the OFC, and increased DA functioning in the orbitofrontal cortex but not in the medial prefrontal cortex.

In addition to 5-HT and DA, glutamate and noradrenaline (NA) have been implicated in impulsive behaviour. Increased NA signaling, through actions of the selective NA reuptake inhibitor atomoxetine, decreases both impulsive action and choice (Robinson et al., 2008). Antagonism of NMDA receptors increases impulsive action and choice (Higgins, Enderlin, Haman & Fletcher, 2003; Floresco, Tse & Ghods-Sharifi, 2008). Lastly, the opioid system has been implicated in impulsivity. Morphine, an opioid receptor agonist, increases impulsive action and choice (Pattij, Schetters, Janssen, Wiskerke & Schoffelmeer, 2009). Recent evidence suggests that opioid receptor subtypes might differentially influence impulsive action (Olmstead, Ouagazzal & Kieffer, 2009).

**Early Life Environment and Impulsiveness**

While research on the neuroanatomical and neurochemical correlates of impulsiveness has been prolific, especially in the last decade, virtually nothing is known about how early life environmental factors produce changes in this trait. As mentioned above, AD/HD is characterized by deficits in attention and increased impulsiveness. Although this disorder has a strong genetic component, it is becoming increasingly clear that early life psychosocial environment plays a significant role in AD/HD etiology (Taylor & Rogers, 2005).
In addition, Romanian orphans who were institutionalized early in life, show cognitive behavioural profiles that have been termed inattentive/overactive, and are characterized by behaviours that could be termed as impulsive (Kreppner, O’Connor & Rutter, 2001). For example, they might have difficulty waiting for their turn to speak or participate in games. However, it is unclear as to which specific early life factors are important and how any of these factors might be affecting impulsiveness. One of the possibilities is that early life stress, which can come in many forms including ‘deficient’ maternal care, might produce changes in the DA and 5-HT systems. As mentioned above, these two neurotransmitter systems play a prominent role in the impulsiveness trait. A number of non-human studies have shown that periodic maternal deprivation can produce changes in 5-HT (e.g., Vazquez, Eskandari, Zimmer, Levine & Lopez, 2002) and DA (e.g., Matthews, Dalley, Matthews, Tsai & Robbins, 2001). In general, reduced maternal behaviour (e.g., through periodic maternal deprivations) results in increased DA tone (e.g., Lovic et al., 2006; Matthews et al., 2001) and reduced 5-HT levels (e.g., Lee et al., 2007;Matthews et al., 2001). Therefore, one would predict that early maternal deprivation would produce changes in impulsiveness.

**Rationale and Predictions for Experiments**

There is no experimental evidence for the role of early life environmental and social factors in moderating impulsiveness. Rearing rats in isolation, through artificial rearing, is an excellent paradigm for studying the effects of several components of early life environment on later cognition and behaviour, including
impulsiveness. Alison Fleming’s research group has been successful in assessing the role of somatosensory stimulation (mimicking maternal licking) through artificial rearing, on adult maternal behaviour, attention and neurophysiological changes (e.g., Fleming et al., 2002).

Previously, it was casually observed that the motherless rearing of rats, through artificial rearing, produces increased locomotor activity and a high number of errors during the attentional set shifting task (Lovic & Fleming, 2004). A number of these responses could be interpreted to be due to impulsiveness (fast responses without significant assessment of the stimuli). This pattern of responses was less prevalent in artificially reared rats that were provided with maternal-licking like stimulation during artificial rearing. At the inception of the studies that follow, it was predicted that motherless rearing, through artificial rearing, would lead to increased impulsiveness. However, it was unclear whether this treatment should produce changes in all aspects of impulsiveness (i.e., impulsive action and impulsive choice; see above). We also predicted that maternal-licking like stimulation would reduce impulsiveness in AR rats.

To address these questions, a series of experiments was conducted. In Chapter Two, common methods across these studies, namely, artificial rearing, are described. In Chapter Three the effects of artificial rearing on impulsive action was investigated using DRL-20s operant schedule. In Chapter Four, we explored differences in impulsive choice in male and female AR and MR rats. In Chapter Five impulsive decision making in male AR and MR rats was further assessed using FCN8 schedule. Finally, in Chapter Six we assessed the
relationship between maternal behaviour and impulsive action (DRL-20s) in AR and MR rats.

The series of studies described in Chapters 3 – 6 of this thesis provides insight into the importance of early life factors in the moderation of adult impulsiveness, and the relationship between impulsiveness and maternal behaviour. In addition, artificial rearing can be viewed as a non-human model of institutional rearing, and the series of studies that follow provides additional knowledge of the potential deficits associated with institutionalization.
CHAPTER TWO: GENERAL METHODS

The methods described in the General Methods section of this thesis are common to all studies (i.e., subjects, apparatus, and artificial rearing paradigm). A description of the methods specific to each study can be found in the methods section of that chapter.

Subjects

Sprague-Dawley rats were used in these studies. All rats were born at University of Toronto Mississauga animal vivarium. The rats at this facility were originally obtained from Charles River Farms in St Constant, Quebec, Canada. At the time of weaning, rats were pair-housed in medium size Plexiglas cages (W 26 x L 38 x H 21 cm) with woodchips and ad lib access to rat Purina Chow food (unless otherwise specified) and water. The room temperature and humidity were maintained at 22°C and 40-50%, respectively. Lights were off at 2000-0800 hours.

Apparatus

Operant Conditioning Chambers

Testing was carried out in 12 identical chambers (28 x 21 x 21 cm; Med Associates Inc., St. Albans, VT). Each chamber contained 2 retractable levers (4.5 cm long), a food pellet dispenser, and a house light located on the wall opposite the dispenser. The apparatus was controlled by Med. Associates software and a Dell computer.
Artificial Rearing

Groups and treatments

Dams gave birth, and on the day of parturition (postnatal day – PND 0) their litters were culled to approximately 7-8 males and 7-8 females. On PND 2, 3 males and 3 females were removed from the nest. Two males and two females were implanted with a cheek cannula and raised artificially (AR) while the third male and female were sham-operated and returned to their mother (SHAM). One male and one female sibling were not manipulated and were designated as controls (CON). Siblings that received cheek cannulae were randomly assigned to one of two conditions: 1) artificially reared with minimal maternal-licking like stimulation (AR-MIN) and 2) artificially reared with maximal maternal-licking like stimulation (AR-MAX). Therefore, there were 4 groups of rats for both sexes: AR-MIN, AR-MAX, SHAM and CON. SHAM and CON rats are usually not statistically different from one another and are combined into 1 group – mother reared (MR) (see Statistical Analyses section below).

Cheek Cannulae Implants

All PND2 pups were weighed prior to surgery. Following local anesthesia (Lidocaine) cheek cannulae were implanted. The implantation of cheek cannulae lasted less than 1 min. SHAM rats were treated in an identical fashion to AR rats, except the cannula was not implanted. The SHAM rats were marked to distinguish them from the CON rats and returned to their nests.

Artificial Rearing of Pups
After the surgical implantation of the cannulae, pups were housed individually in plastic cups (11 cm in diameter x 15 cm deep), which fitted into second weighted cups. Both cups floated in a temperature controlled water aquarium (water maintained at 36-40°C). The housing cups contained corn-cob bedding (Bed O’ Cobs) and the tops of the cups remained open to allow cheek cannulae tubing to emerge and connect to nearby syringes containing milk formula. The infusion of milk formula (Messer diet, taken from the University of Iowa) was executed and controlled by timer-controlled infusion pumps (Harvard Apparatus Syringe, PHD 2000). The pumps were programmed to infuse the diet for 10 min every hour, 24 hr daily. The amount of milk formula the pumps delivered was based on a specific fraction of the mean pup weight. We started by giving pups formula volume equal to 33% of their body weight. This amount was increased 1% each day.

Every morning the pups were disconnected from the pumps, removed from the cups, weighed and their tubing was flushed with 0.1 cc of distilled water. New syringes containing fresh formula were set up and the pump’s infusion rate was reprogrammed according to the new pup weight per pump. AR-MIN rats were stimulated twice a day, 30 sec each (morning and night) with warm, wet, camel hair paintbrush, in order to stimulate urination and defecation. Only the pups’ anogenital region was stimulated. AR-MAX rats were stimulated 8 times a day (2 min of body stimulation) in addition to 2 regular anogenital stimulations (30 sec each). Stimulations were carried out from the day the pups were placed on the pumps (PND2) to PND 16. On PND18/19 all AR rats were taken off the
pumps, placed in mouse cages (22 cm x 15 cm x 10 cm) and provided with milk formula, regular rat chow as well as the mixture of formula and chow. They were kept in these cages until the weaning day (see below).

Weaning and Groups

On PND21 artificially reared rats were paired up with mother-reared, non-experimental, social partners with whom they remained until the adult tests. MR rats were weaned from their mother and paired together (MR-SHAM and MR-CON – from the same litter). All the rats were weighed and left undisturbed until adulthood. See Figure 2 for general experimental timeline.
Figure 2  Overview of general experimental timeline. Following birth litters were culled to 7-8 female and 7-8 male pups. If only males were used in the study, litters were culled to 5 female and 7-8 male pups. Similar ratio was applied if only female pups were used. From PND 2-21 pups were either maternally- or artificially-reared. On PND 21 all rats were pair-housed with same sex conspecific. Behavioural testing commenced after rats were at least 80 days old.
CHAPTER THREE

The effects of maternal deprivation, through artificial rearing, on impulsive action (DRL-20s)

Abstract

We have previously found that total maternal separation of dams and pups, through artificial rearing (AR), has several, long-lasting consequences for the pups. For example, compared to mother reared (MR) rats, artificially reared (AR-MIN) rats show reduced maternal licking and crouching, deficits in attention, and increased locomotor activity in response to novelty and amphetamine. These effects can be reversed or ameliorated if AR pups are provided with ‘replacement’ somatosensory stimulation (simulating maternal licking; AR-MAX). In this study we investigated impulsive action in the context of AR. Male and female rats were randomly assigned to AR and MR conditions, and half of the AR rats were provided with somatosensory stimulation, simulating maternal licking. In adulthood, rats were tested on action impulsiveness using a differential-reinforcement-of-low-rate (DRL-20s) operant schedule. In addition, rats were assessed on locomotor activity. Compared to MR rats, AR-MIN rats made more responses, earned fewer reinforcers, and had lower efficiency of responding and reduced inter-response times. Distribution of responses across time bins revealed a leftward and an upward shift in responses made by the AR rats. In addition, AR rats showed increased locomotor activity in a novel environment. Locomotor activity was not significantly related to early post-reward disinhibition.
of lever responses, but it was significantly related to responses made during later time bins.
The effects of maternal deprivation, through artificial rearing, on impulsive action (DRL-20s)

Mammalian neurophysiology and behaviour are plastic as both can be altered by experience. This plasticity is particularly apparent during the early stages of development when young mammalian offspring are usually in the care of their mothers or caregivers (Cirulli, Berry & Alleva, 2003; Hoffer, 1994; Kaffman & Menaey, 2007; Pryce & Feldon, 2003). With rats, several ‘separation’ paradigms have been developed to assess the importance of mother, litter, and nest environment on biobehavioural development, often within the domains of social behaviour, emotion, attention, and the stress systems (Hall, 1998; Pryce & Feldon, 2003). The focus of the present paper is to explore how early preweaning experiences influence adult impulsivity and response inhibition.

The effects of maternal separation vary greatly depending on several factors, including the timing, duration and frequency of separations, environmental factors, such as temperature, and social factors (e.g., presence or absence of other pups during the separation period) (Hall, 1998; Kaffman & Meaney, 2007; Pryce & Feldon, 2003).

Recently we adopted an artificial rearing (AR) paradigm as a method of studying the importance of early life factors, such as maternal licking stimulation, on a host of adult behaviours and neurophysiological outcomes. This involves complete separation of the pups from the mother and the nest, so that pups are reared with no maternal contact (Gonzalez, Lovic, Ward, Wanwright, & Fleming, 2001).
AR rats show deficits in adult maternal behaviour (Gonzalez et al., 2001) and components of the attentional set shifting task (ASST) (Lovic & Fleming, 2004). Specifically, while they were not impaired on simple discriminations, AR rats required more trials to complete reversals, intradimensional and extradimensional shifts. AR rats also displayed elevated locomotor activity (Lovic, Fleming & Fletcher, 2006; Burton, Lovic & Fleming, 2006). Providing AR pups with somatosensory stimulation that resembles maternal licking can reverse or ameliorate these effects. Findings of deprivation–induced ‘deficits’ and somatosensory stimulation being ‘beneficial’, are concordant with findings from other paradigms, such as pup-mother periodic separations and ‘handling’ studies. Maternal separation produces disruption of several neurophysiological factors, including growth hormone secretion and responses to stress. Some of these effects can be reversed or countered if pups are ‘handled’ so that their mother licks them more or if they are provided with artificial maternal licking-like stimulation (e.g., Kaffman & Meaney, 2007; Kuhn & Schanberg, 1998; Moffett et al., 2006).

During observations of maternal behaviour and testing on the ASST, we observed that AR rats, compared to mother reared (MR) rats, tend to be more active and show less inhibition. During the maternal behaviour tests, AR rats, in contrast to mother reared (MR) rats, are more likely to respond to minor environmental stimuli (often at the expense of being maternal; Gonzalez et al., 2001). For example, AR rats will leave the nest and pups to investigate (e.g., come to the front of the cage) if the door of the housing room is opened or if
another rat makes noise. MR rats are less likely to do so. In the ASST, AR rats tend to respond more rapidly and often fail to inhibit responses towards the first (incorrect) stimulus (Lovic & Fleming, 2004). In other words, they would fail to inhibit a response towards an incorrect odor or texture when this was the first exemplar they encountered.

We have also found that AR rats are more sensitive to the locomotor stimulant effect of amphetamine, suggesting an alteration in the functioning of their mesocorticolimbic dopamine (DA) systems (Lovic et al., 2006). Manipulations of the DA system have been reported to affect impulsiveness (e.g., Peterson, Wolf & White, 2003; Stoffel & Cunningham, 2008). These compounded behavioural findings of reduced inhibition and increased sensitivity to amphetamine led us to hypothesize that AR rats might be characterized by increased impulsiveness.

Impulsiveness is demonstrated by a tendency to act or terminate actions prematurely, to make decisions rapidly, and choose immediate-smaller rewards over larger-delayed rewards. Based on behavioural, neurophysiological and drug studies it appears that impulsiveness, similar to memory and attention, is not a unitary construct as there are at least two different behaviourally, neuroanatomically and pharmacologically dissociable, types of impulsiveness: impulsive action (tantamount to saying “I acted too quickly”), and impulsive choice (tantamount to saying “I didn’t think this through before I made my decision”) (de Wit, 2009; Della-Hagedom, 2006; Winstanley, Eagle, & Robbins, 2006; Evenden, 1999).
Impulsive action refers to phenomena where an organism fails to inhibit premature responses. The organism does not have to make a choice between different contingencies; rather it just has to withhold the correct response until the timing is correct as acting prematurely will not be rewarded.

Differential reinforcement of low rate responding (DRL) is one of several operant schedules of reinforcement that can be used to measure impulsive action. On a DRL schedule, rats are rewarded for making operant responses after a certain amount of time has elapsed. For example, on a DRL-20s schedule, responses are reinforced with food; however, rats have to wait at least 20 sec since their last response in order to be rewarded again. Premature responses are not followed by food reward and ‘waiting times’ are reset (Fletcher, 1995; Peterson et al., 2003). Initially, rats make many lever responses and earn few reinforcers. Thus, rats have low (efficiency) ratios between the number of reinforcers and responses. In addition, the time between their responses is initially short (mean interresponse times: mIRT). However, after several sessions, performance typically improves (i.e., response rates decrease, and efficiency ratios and mIRTs increase).

In this study, we assessed the effects of early life experience on impulsive action. Similar to our previous studies, we raised rats with mothers or without their mothers, through AR. In addition, we gave half of the AR rats maternal licking-like stimulation. As indicated above, in our previous studies this procedure has resulted in reversal or attenuation of several biobehavioural
indices associated with artificial rearing and maternal separation (Lovic, Fleming & Fletcher, 2006; Lovic & Fleming, 2004; Gonzalez et al., 2001).

In addition to assessing rats’ impulsive action on the DRL-20s operant schedule, we also assessed their locomotor activity. AR rats are more active in novel environments (Burton et al., 2006; Lovic et al. 2006). Since impulsive action in the DRL-20s operant schedule is based on the number of responses rats make, it can be hypothesized that a general increase in motor activity might influence impulsiveness scores. Thus, we examined whether rats’ general locomotor activity was related to their measures of impulsive and contributed to their performance on the DRL-20s operant schedule.

Based on previous studies, we hypothesized that AR rats would be more active and impulsive, manifested through greater number of responses, lower efficiency and mIRT scores. Furthermore, we predicted that maternal licking-like stimulation, provided to half of the AR pups, would reverse the effects associated with artificial rearing. Finally, we predicted that locomotor activity would be positively related to impulsive action in the DRL-20s operant schedule.

**Methods**

**Subjects**

Twenty-nine male (AR-MIN, n=7; AR-MAX, n=8; MR=14) and 31 female (AR-MIN, n=7; AR-MAX, n=8; MR=16) Sprague-Dawley rats were used in this study. See details in Chapter 2.
**Apparatus**

Operant Conditioning Chambers

See Chapter 2 – Methods.

Locomotor Activity Boxes

Locomotor activity was assessed in 8 transparent activity boxes (46 x 25 x 21 cm) made at Centre for Addiction and Mental Health (Toronto, Canada). A frame (2 cm above the floor), located outside the box, was equipped with 16 photocell beams (approximately 2.5 cm apart) which recorded the movement of rats. Number of photocell counts was the dependent measure.

**Procedures**

Groups and treatments

See chapter 2 – Methods.

Behavioural Procedures

DRL-20s Procedures

The procedures employed in this study were based on Fletcher (1995). Adult rats were gradually reduced to 85% of their free-feeding weights. They were trained to bar press for food (45 mg food pellets; Bio-Serv) on a continuous reinforcement (CRF) schedule during a 30 min session, on 7 consecutive days. All rats had 3 or 4 successful CRF schedule sessions (100 bar presses in 30 mins) with the final session one day prior to being switched to the DRL-20s schedule. On the DRL-20s schedule, rats were reinforced only if they responded
at least 20 s since their previous response. Responses made less than 20 s since the last response were not rewarded and the 20 s period was reset. DRL-20s testing was done over 18 days (testing was done 6 days/week). Each session began with an illumination of the house light and insertion of the left lever into the chamber. The first response was always reinforced. For each session the following measures were collected: number of responses, number of reinforcers earned, percent efficiency [(number of reinforcers earned/number of responses made) x 100], and mean interresponse time (mIRT).

**Locomotor Activity**

Following completion of the DRL-20s experiment, rats’ locomotor activity was tested over 3 consecutive days (60 min tests). Testing occurred during light hours in a dimly lit room. Boxes were cleaned with 70% alcohol in between each test. Number of photocell counts was the dependent measure.

**Data Analyses**

SHAM and CON groups were not statistically different from each other on any measures of interest. Thus, these groups were combined into one group: mother-reared (MR). The level for achieving statistical significance was set at p<0.05.

**DRL**

Data analyses for each of the 4 measures was averaged across 3 days (for a total of 6 blocks of 3 days each) and analyzed using repeated measure ANOVA (Group x Sex x Block). Dunnett post hoc tests were used in order to
assess which treatment group was different from the control group (MR). We were primarily interested in differences between the 2 AR groups against the MR group, rather than overall group differences. Therefore, we only contrasted AR-MIN with MR group and AR-MAX with MR group. Significant interactions were followed by one-way ANOVA assessing group differences for specific blocks. Since we had a priori hypotheses regarding the direction of our effects, post hoc analyses were one-tailed.

Locomotor Activity

Data was analyzed using repeated measures ANOVA (Groups x Sex x Days), followed by Dunnett post hoc tests.

Correlations

In order to examine a possible relationship between activity and impulsiveness, we conducted partial correlations, controlling for group effects, on locomotor activity measures (average of session 2 and 3) and DRL-20s measures (number of responses made, efficiency and mIRTs; average of last 2 blocks of DRL-20s testing).

Results

DRL-20s

Number of Responses

As can be seen in Figure 3, all rats showed a reduction in the number of responses over time (main effect of block; $F_{(1, 53)}= 461.77, p<0.001$). In addition,
female rats made fewer responses (main effect of sex; $F_{(1, 53)}= 4.77, p<0.05$).
Finally, there were significant group differences ($F_{(2, 53)}= 3.3, p<0.05$). Dunnett’s
post hoc analyses indicated that AR-MIN rats, but not AR-MAX rats, made more
response than the MR rats. There were no significant interactions between
variables.

**Number of Reinforcers Earned**

As can be seen in Figure 4, all rats showed an increase in the number of
reinforcers earned over blocks (main effect of block; $F_{(1, 53)}= 223.38, p<0.001$).
Female rats earned more reinforcers than male rats ($F_{(1, 53)}= 6.35, p<0.05$).
There was an overall group effect ($F_{(2, 55)}= 3.42, p<0.05$). Dunnett’s post hoc
analyses indicated that AR-MIN rats earned fewer reinforcers than MR rats
($p<0.05$). AR-MAX rats were not different from MR rats. There were no
significant interactions.
Figure 3: The figure depicts mean number of responses (+SEM) across 6 blocks of testing for male and female rats. Responses decreased across blocks ($p<0.001$). AR-MIN rats made significantly more response than MR rats ($p<0.05$). Male rats made more responses (inset; $p<0.05$). There were no significant interactions.
**Figure 4:** The figure depicts mean number of reinforcers earned (+SEM) across 6 blocks of testing for male and female rats. Rats earned more reinforcers across subsequent blocks ($p<0.001$). Female rats earned more reinforcers ($p<0.05$). Compared to MR rats, AR-MIN rats earned fewer reinforcers ($p<0.05$).
**Efficiency**

As shown in Figure 5, there was an overall increase in efficiency across the blocks ($F_{(1, 53)}= 188.22, p<0.001$). There were marginal sex differences ($F_{(1, 53)}=2.73, p=0.1$) and significant overall group differences ($F_{(2, 53)}=4.2, p<0.05$). Post-hoc analyses indicated that AR-MIN rats were significantly less efficient than MR rats ($p<0.05$). AR-MAX rats were not different from MR rats. In addition, there was a significant Block x Group interaction ($F_{(2, 53)}=3.48, p<0.05$) as groups’ efficiencies increased at different rates across blocks. Among AR-MIN rats efficiencies increased at the slower rate. This interaction was followed by one-way ANOVAs and Dunnett post-hoc tests assessing group differences during individual blocks. Compared to MR rats, AR-MIN rats were less efficient during blocks 2-6 ($p<0.05$ for all analyses).

**Mean Interresponse Times (mIRTs)**

This measure assessed the average time between responses made on the lever. As with other measures, all rats’ performances improved across sessions; that is, rats’ mIRTs increased ($F_{(1, 53)}=153.1, p<0.001$). There were no sex differences, however, there were significant group differences ($F_{(2, 53)}=3.35, p<0.05$). Post-hoc analyses indicated that AR-MIN rats had significantly shorter mIRTs than MR rats ($p<0.05$; see Figure 6). In addition, there was a marginal Group x Block interaction ($F_{(2, 53)}=3.16, p=0.051$). As with efficiency, this interaction was followed up by one-way ANOVAs investigating group differences during individual blocks and post hocs tests. Compared to MR rats, AR-MIN rats showed significantly shorter mIRTs during blocks 2-6 ($p<0.05$).
Figure 5: The figure depicts mean percent efficiency (+SEM) across 6 blocks of testing for male and female rats. Overall, efficiency improved across blocks ($p<0.001$). There were overall group differences ($p<0.05$) and post-hoc analyses indicated that AR-MIN rats had lower efficiency levels than the MR rats. In addition, there was a significant group by block interactions ($p<0.05$). *One-way ANOVAs indicated that AR-MIN rats were different from MR rats during blocks 2-6 ($p<0.05$).
The figure depicts mean inter-response times (+SEM) across 6 blocks of testing for male and female rats. mIRT significantly increased across blocks ($p<0.001$). There were overall group differences ($p<0.05$) and post-hoc analyses indicated that AR-MIN rats had shorter mIRTs than the MR rats. In addition, there was a significant group by block interactions ($p<0.05$). *One-way ANOVAs indicated that AR-MIN rats were different from MR rats during blocks 2-6 ($p<0.05$).
Distribution of Responses Across Time Bins

Distribution of lever responses across time bins was initially assessed using repeated measures ANOVAs (Group x Sex x Bins). Responses changed across bins (Bin effect; $F_{(1, 53)}=48.3$, $p<0.001$) and groups differed ($F_{(2, 53)}=3.34$, $p<0.05$). In addition, there were significant Group x Bin interactions (quadratic effects; $F_{(2, 53)}=3.3$, $p<0.05$). These significant interactions were followed up by one-way ANOVAs assessing group differences during individual bins. Analyses indicated that both AR-MIN and AR-MAX rats made more responses during the 2$^{nd}$ time bin. In addition, AR-MIN rats made more responses during 5-9$^{th}$ bin ($p$s<0.05). As depicted in Figure 7, AR-MIN rats showed both a leftward and upward shift in distribution of their responses. In other words they made more responses and these responses were made during early bins (prior to 20s time interval).

Locomotor Activity

Rats’ activity levels were measured across three 1-hour sessions. Repeated measures analyses indicated a significant decrease in activity across 3 sessions ($F_{(1, 53)}=53.13$, $p<0.05$; see Figure 8). Males were more active than females ($F_{(1, 53)}=9.18$, $p<0.01$). Finally, there were overall group differences ($F_{(2, 53)}=4.5$, $p<0.05$). Post-hoc analyses indicated that AR-MIN rats, but not AR-MAX rats, were significantly more active than MR rats ($p<0.01$). There were no significant interactions.
Distribution of Responses across Bins

Males

- AR-MIN
- AR-MAX
- MR

Females

- AR-MIN
- AR-MAX
- MR
Figure 7: Depiction of lever responses across 20 (2s) bins (averaged across last three days of testing - block 6). There was a significant Bin effect as responses changed across bins ($F_{(1, 53)}=48.3, \ p<0.001$). In addition, there was a significant Bin x Group interaction (quadratic effects; $F_{(2, 53)}=3.3, \ p<0.05$). Groups' responses across individual bins were assessed using one-way ANOVAs and followed up post-hoc tests where appropriate. * AR-MIN rats, compared to MR rats, made more response during the 2nd and 5-9th bins ($ps<0.05$). ^AR-MAX rats, compared to MR rats, made more responses during the 2nd bin ($p<0.05$).
Figure 8: The figure depicts mean number of photocell counts (+SEM) during 3 locomotor activity sessions for male and female rats. There was a significant decrease in activity across the 3 sessions ($p<0.001$). Male rats were more active than female rats ($p<0.01$). Compared to MR rats, AR-MIN rats had a significantly higher number of photocell counts across the 3 sessions ($p<0.05$).
Correlations Between Measures of Impulsivity and Locomotor Activity

Partial correlations, controlling for group effects, were conducted to assess the relationship between DRL-20s test measures (number of responses, efficiency, and mIRTs) and locomotor activity. For all of the DRL-20s test measures, data were averaged across blocks 5 and 6, when responding became stable. Two sets of locomotor activity data were used, day 1 (novelty induced locomotor activity) and averaged across sessions 2 and 3 (representing more general levels of locomotor activity). These analyses indicated that novelty induced locomotor activity was positively related to the number of lever responses ($r=0.29$, $p<0.01$). Locomotor activity was inversely related to efficiency ($r=-0.3$, $p<0.01$) and mIRTs ($r=-0.33; p<0.01$) (see Table 1 and Figure 9a-c for depiction of these relationships). However, novelty induced locomotor activity was not significantly correlated with the number of responses during either bin 2 (shortly after pellet delivery) or bin 8 (shortly before the waiting period elapsed).

Correlations were also performed between locomotor activity (session 2 and 3) and responses made immediately after reception of reward (bin 2) and responses made during bin 8 (shortly before the waiting period elapses). Activity was not significantly correlated with responses made during bin 2 ($r=0.1$, $p=0.203$). However, activity was significantly correlated with responses made during bin 8 ($r=0.3$, $p<0.5$). These relationships are depicted in Table 1.
Relationship Between Locomotor Activity and DRL-20s Measures

<table>
<thead>
<tr>
<th></th>
<th>Responses</th>
<th>Efficiencies</th>
<th>mRTs</th>
<th>Bin 2</th>
<th>Bin 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>0.29**</td>
<td>-0.3**</td>
<td>-0.33**</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Day 2-3</td>
<td>0.29*</td>
<td>-0.33**</td>
<td>-0.34**</td>
<td>ns</td>
<td>0.3*</td>
</tr>
</tbody>
</table>

Table 1  The table depicts significant correlations between locomotor activity measures (novelty induced locomotor activity Day 1; general locomotor activity: averaged Day 2 and 3 activity) and DRL-20s measures (averaged across blocks 5 and 6 when responding was more stable). *p<0.05, **p<0.01
Figure 9: Depictions of significant correlations between DRL measures (averaged data for block 5 and 6) and locomotor activity (averaged data for sessions 2 and 3). (A) Depiction of a significant relationship between number of DRL lever responses and locomotor activity ($r=0.29$, $p<0.05$). (B) Depiction of a significant relationship between DRL efficiency and locomotor activity ($r=-0.33$, $p<0.01$). (C) Depiction of a significant relationship between mIRTs and locomotor activity ($r=-0.34$; $p<0.01$).
DRL Measures with Locomotor Activity as a Covariate

We assessed group differences in the 3 DRL-20s measures, with activity levels entered into the analyses as a covariate. These analyses were conducted in order to determine whether locomotor activity was a significant factor contributing to group differences in DRL-20s performance (number of responses, efficiency, and mIRTs). Accordingly, we ran the same repeated measures ANOVA described above, this time, co-varying locomotor activity (averaged scores for sessions 2-3). The results demonstrated that groups were no longer different for the number of responses ($F(2, 53)=2.34, p=.106$), efficiency ($F(2, 53)=2.4, p=.1$) or mIRTs ($F(2, 53)=1.85, p=0.167$). We also examined group differences during bin 2 and bins 5-9 with activity as a covariate. Groups’ responses significantly differed during bin 2 ($F(2, 53)=3.3, p<.05$) but not during bins 5-9 ($F(2, 53)=2.34, p=.106$). These data indicate that general activity levels are a significant contributor to our observations of group differences in the DRL-20s operant schedule. However, locomotor activity is not a significant factor in determining group differences in groups’ lever responses shortly after the reception of reward (bin 2).

Discussion

The purpose of this experiment was to assess the effects of early life environment, specifically maternal behaviour, on adult impulsive action and locomotor activity. We found that artificial rearing produces an increase in adult
impulsive action exemplified by greater response rates, lower efficiency ratios, shorter mIRTs and greater number of responses during bin 2 and 5-9.

Analyses of responses during individual bins revealed that both AR groups made more responses shortly following the delivery of reward (2nd bin). This finding suggests that AR rats are more behaviourally disinhibited following the delivery of the reward. However, only AR-MIN rats made more responses during bins 5-9, well before 20s elapsed. The possibility that AR-MIN rats have time perception deficits is plausible – second peak responses are shifted to the left. However, based on the number of responses that they made during bins 5-9, it is likely that they are less behaviourally inhibited as well. That is, AR-MIN rats responses peak earlier and they make more responses. Not surprisingly AR-MIN rats displayed greater levels of locomotor activity outside the operant context. Importantly, when locomotor activity was covaried from analyses of responses during early post-reward period (bin 2) and later bins (bin 5-9) groups were still different for response made during bin 2 but not bins 5-9. These finding suggests that locomotor activity is a moderator of behavioural disinhibition (lever responses) but only for bins 5-9 but not a significant mediator of early post-reward disinhibition.

From these finding we can conclude that: a) AR procedures increase action impulsiveness, b) increased action impulsiveness is at least due to time perceptual deficits (leftward shift of the curve), c) increased action impulsiveness is also due in part to increased behavioural disinhibition (upward shift of the response curve – that is, AR rats make more responses), d) early post-reward
increase in behavioural disinhibition is not significantly mediated by general levels of activity, but e) later responding (bins 5-9) is mediated by general levels of activity.

Providing AR rats with maternal licking-like stimulation can significantly reduce these effects - AR-MAX rats were not significantly different from MR rats except that they made significantly more responses during the 2\textsuperscript{nd} bin. We also found that locomotor activity levels were inversely related to impulsivity measures. These data are consistent with a literature showing the amelioration of AR-induced deficits by replacement tactile stimulation (Gonzalez et al., 2001; Lovic & Fleming, 2004; Lovic et al., 2006)

Our results suggest that AR-MIN rats are more action impulsive (behaviourally disinhibited), which is consistent with our empirical observations of AR-MIN rats’ greater motor output in various other tests. Increased locomotor activity in response to novelty has been associated with elevated levels of impulsivity and drug taking (Bardo, Donohew & Harrington, 1996; Stoffel & Cunningham, 2008). Both novelty seeking and drug taking have been related to the activity of the mesolimbic DA system (Bardo et al., 1996; Dellu, Piazza, Mayo, Le Moal, & Simon, 1996; Hooks & Kalivas, 1994). We have previously found that AR-MIN rats show increased novelty and amphetamine (a DA releaser) induced locomotor activity (Burton et al., 2006; Lovic et al., 2006). In addition, preliminary \textit{in vivo} findings show that AR-MIN rats have a greater baseline release of DA in the nucleus accumbens (shell) (V. Afonso unpublished data).
Several catecholamine drugs and brain lesions produce greater impulsivity in the DRL task. In general, decreases in serotonin (5-hydroxytryptamine - 5-HT) levels and increases in DA levels are associated with DRL deficits. 5-HT agonists, such as selective serotonin reuptake inhibitors (SSRI), improve performance on the DRL task (for a review see O'Donnell, Marek & Seiden, 2003). However, SSRIs are not effective if there is a dietary depletion of the 5-HT precursor tryptophan. 5-HT depletions in the median raphe nucleus increase responding, produce a leftward shift in timing of responses and decrease efficiency in the DRL task (Fletcher, 1995). Methyphenidate, a DA reuptake inhibitor, and d-amphetamine, a DA releaser, increase the rate of responding (behavioural disinhibition) (Sabol, Richards, Layton & Seiden, 1995; Seiden, Andersen & Mikhail, 1979). While we do not have direct evidence that these two neurotransmitter systems are altered in the rats that we studied, we have indirect evidence for the ‘up-regulation’ of the DA system in AR rats. Thus, AR-MIN rats display a greater sensitivity to amphetamine (Lovic et al., 2006) and they have elevated baseline DA levels in the nucleus accumbens (Afonso & Fleming, in prep). Supporting these data several other studies have found that separations of pups from mothers, during the early preweaning periods, produce long-lasting elevated DA levels and responsiveness of DA system to potassium and amphetamine stimulation (Hall, Willkinson, Humby & Robbins, 1999; Matthews, Dalley, Matthews, Tsai & Robbins, 2001).

Although AR-MIN rats are more active and impulsive than MR rats, AR-MAX rats are not significantly different from MR rats on these same measures.
These findings suggest that maternal behaviour, specifically maternal licking, might be a significant factor influencing adult impulsiveness. Numerous studies have reported long-lasting effects of maternal separations and maternal licking on a number of neurophysiological processes (e.g., Cirulli et al., 2009; Hall, 1998). For example, rat pups that are licked more by their mothers have more ‘adaptive’ hypothalamic-pituitary-adrenal (HPA) axis stress responses (Francis & Meaney, 1999). The HPA axis influences many other systems, such as the DA system, acutely and long-term (Engele & Lehner, 1995; Tzschentke, 2001).

Maternal separations influence other systems as well. Separations of pups from the mother result in a reduction in ornithine decarboxylase (an obligatory enzyme for normal cell growth and development), DNA synthesis, abnormal neuroendocrine secretions and suppression of cell responses to trophic hormones (insulin, prolactin and growth hormone) (Kuhn & Schanberg, 1998). Consistent with the data presented in this paper, and with the literature, these effects can be reversed by providing pups with somatosensory stimulation that simulates maternal licking, during maternal separation (Kuhn & Schanberg, 1998).

Our reports of adverse early life environment leading to increased locomotor activity and impulsiveness, and ‘beneficial’ effects of somatosensory stimulation, are also relevant to and concordant with human studies. Institutionalized children, such as those in Romanian orphanages, show long term differences in cognition, behaviour and brain physiology (Eluvathingal et al., 2006; Kreppener, O’Connor & Rutter, 2001). Some of the most consistent
observations seen in institutionalized children are increased activity levels and reduced attention (Stevens et al., 2008; Tarren-Sweeney, 2008). Both of these effects are also found in AR rats. Moreover, human studies have demonstrated a relationship between response inhibition, activity levels and conduct problems in children. Similar to our finding, correlations between response inhibition and conduct problems were dependent on activity scores (Berlin & Bohlin, 2002).

While there has been much interest and research devoted to understanding neural and chemical regulation of impulsiveness, virtually nothing is known about how early life factors, such as maternal care, influence this trait. The results of our study are the first experimental findings (to our knowledge) to report increased impulsiveness as a result of early life maternal deprivation. Moreover, these effects are ameliorated by somatosensory stimulation (i.e., replacement of maternal licking). As impulsivity is not a unitary construct it is important to assess the effects of motherless rearing, through AR, on other types of impulsiveness, namely impulsive choice. These objectives were the focus of experiments presented in Chapter Four and Five.
CHAPTER FOUR

The effects of Maternal Deprivation, through Artificial Rearing, on Impulsive Choice (Delay Discounting) I

Abstract

Disruptions of mammalian mother-offspring relationships can have long-lasting effects on the offspring’s behaviour and physiology. We have found that rat pups reared without mothers, through artificial rearing (AR), have deficits in maternal behaviour, attention, and an increase in novelty-induced locomotor activity. These effects can be reversed or ameliorated if AR rats are provided with maternal licking-like stimulation (Burton et al., 2006; Lovic & Fleming, 2004; Lovic et al., 2006). AR rats also show an increase in impulsive action as measured by differential rates of low rate responding (DRL-20s) operant schedule. However, impulsivity is not a unitary construct as there are at least 2 different phenomena that this construct encompasses: impulsive action and impulsive choice. Impulsive choice can be assessed using a delay discounting (DD) operant schedule. On this operant schedule rats are presented with a choice of responding on two levers, one that is associated with a small-immediate reward and the other lever that is associated with a large-delayed reward. The purpose of this experiment was to assess the effects of AR on impulsive choice. Rats were AR and MR conditions, and half of the AR rats received maternal licking-like stimulation (AR-MAX)(see Chapter Two and Three). As adults, rats were tested on the DD operant schedule. We found that AR rats were better able to tolerate delay to larger-delayed reinforcement than MR rats. This would suggest
AR rats are less impulsive than MR rats. Alternative explanations of these effects are discussed.
The effects of maternal deprivation, through artificial rearing, on impulsive choice (delay discounting) I

Early life factors, such as maternal care, can have long lasting effects on offspring. Decreases in maternal care, usually done through maternal separation procedures, have mostly detrimental effects. For example, periodically maternally separated rats display less adaptive acute stress responses during the early postnatal period (see Levine, 2001) and in adulthood (see Kaffman & Meaney, 2007). In contrast, rats that receive a relatively greater level of maternal care (e.g., licking/tactile stimulation), through handling procedures or through natural variation in maternal care, have ‘better’ stress responses (Kaffman & Meaney, 2007), as indexed by smaller and shorter peak durations in glucocorticoid release. Furthermore, simulations of maternal licking can have beneficial effects on a number of neurophysiological factors, such as the growth hormone (Kuhn & Schanberg, 1998).

We have recently employed an AR paradigm to study the effects of maternal behaviour on offspring’s behaviour and neurophysiology (Gonzalez, Lovic, Ward, Weinwright & Fleming, 2001; Lovic & Fleming, 2004; Lovic, Fleming & Fletcher, 2006). In this paradigm rats are reared without their mothers but with other elements of their environments, such as nutrition and temperature, kept relatively constant. We found that AR rats are less maternal (Gonzalez et al., 2001), show attentional deficits (Lovic & Fleming, 2004), and display higher novelty- and amphetamine- induced locomotor activity (Lovic, Fleming &
Fletcher, 2006). These effects are reversed or ameliorated if AR rats are provided with somatosensory stimulation simulating maternal licking. These findings suggest that maternal licking is an important regulator of the motivated behaviours, such as maternal behaviour, and other general cognitive-behavioural systems, such as locomotor activity and attention.

We have also found that AR rats show behaviours that suggest that they are more impulsive than MR rats (Chapter 3). Impulsivity is not a unitary construct. Based on behavioural, neurophysiological and pharmacological manipulations, it seems that there are at least two phenomena that compose the impulsivity trait: impulsive action and impulsive choice (de Wit, 2009; Dellu-Hagedom, 2006; Winstanley, Eagle, & Robbins, 2006; Evenden, 1999).

Impulsive action refers to phenomena where an organism might be making a correct response, but their responses are inappropriately timed (tantamount to saying “I acted too quickly”). Impulsive choice refers to situations where an organism is faced with choices, each leading to distinct outcomes, and chooses the less advantageous options due to lack of foresight or ability to tolerate delays to more advantageous outcomes (tantamount to saying “I did not think this through before I made my decision”). Delay discounting can be observed in a 2 choice operant schedule of reinforcement. On this operant schedule an individual is presented with a choice between an immediate, but small reward, and a delayed, larger reward. Human and non-human subjects prefer the larger reward when the delay is equal to that of the small reward. However, as the delay for the delivery of the larger reward increases, individuals
start to show a preference for the immediate reward. For impulsive individuals, this switch in preference from the larger-delayed to small-immediate reward occurs faster. Hence, these individuals discount the value of the larger/delayed reinforcer at a faster rate than the less impulsive individuals. These discounting rates for delayed reinforcers are described by hyperbolic function curves and they are similar for pigeons, rats, and humans (Cardinal, 2002; Ho, Mobini, Chiang, Bradshaw, & Szabadi, 1999; Reynolds, de Wit, & Richards, 2002).

In line with our previous findings of AR rats showing greater impulsive action on the DRL schedule, we predicted that AR rats would show greater impulsivity on the DD schedule, as indexed by higher rates of discounting of larger delayed rewards. We also predicted that maternal licking-like stimulation provided to some of the AR rats would reverse or ameliorate these deficits. In addition to the regular delay discounting procedures, we assessed the rats’ ability to rapidly change their behaviour by switching the contingencies between the left and right lever and small and large reward. This was done after the regular delay discounting schedule procedures. The aim of this testing was to assess if rats that are less impulsive (show lower discounting rates of large delayed rewards) are sensitive to changes in reward contingencies (i.e., increasing delays to large reward).
Subjects

Twenty-six male (AR-MIN, n=7; AR-MAX, n=7; MR=12) and 28 female (AR-MIN, n=7; AR-MAX, n=8; MR=13) Sprague-Dawley rats were used. Rats were assigned to the same conditions as in Chapter Three. For procedural details see Chapter Two.

Apparatus

Operant Conditioning Chambers

See Chapter 2 – Methods.

Procedures

Groups and treatments

See Chapter 2 – Methods.

Behavioural Procedures

Delay Discounting

Operant testing was initiated when the rats were ~5 months old. Testing was carried out over 61 days and was divided into several distinct stages. Rats were tested between 0900-1800 h, 6-7 days per week.

Continuous Reinforcement Training. Adult rats were gradually reduced to 85% of their free-feeding weights and trained to press left and right (alternate days) levers for food (45 mg food pellets; Bio-Serv) on a continuous reinforcement
schedule for 7 consecutive days. Each session lasted 30 min or until rats made 100 lever presses. Rats were considered trained if they made 100 lever presses on 2 consecutive days (1 day each on left and right levers).

*Training – No Delays Across Blocks.* Testing was similar to the procedures describe above and used by others (Cardinal, Pennicott, Lakmali, Sugathapala, Robbins & Everitt, 2001). Testing occurred in blocks of 12 trials – 2 forced and 10 choice trials. Sessions started in the dark and rats had to nose-poke in the food magazine to trigger the presentation of 1 (first 2 trials within a block; see below) or both levers (10 choice trials within the block) and the illumination of the house light. When both levers were extended, rats had a choice of which lever to press. Responding on one of the levers resulted in the immediate delivery of 1 pellet, while responding on the other lever resulted in immediate delivery of 4 pellets. Responding on either one of the levers resulted in retraction of both levers and darkening of the operant chamber. Each subsequent trial was signaled by the illumination of the house light and rats had 10s to nose poke in the magazine in order to initiate the presentation of one or both levers (forced and choice trials, respectively). If the rats did not respond on one of the two levers, both levers were retracted. Each trial was 100s long and the entire session lasted 100 minutes. The purpose of this testing was to assess whether rats could discriminate between two levers (small and large reward) and whether they would have a preference for the large reward. The preference criterion was at
least 85% selection of large reward across 2 consecutive days. Rats were tested in this manner for 5 days (see results section).

*Delay Discounting – Ascending Delays Across Blocks.* Each block began with 2 forced “choice” trials – one trial for each of the two levers/reward sizes. Next came 10 choice trials. Responses on the “immediate” lever resulted in the immediate delivery of 1 pellet. Responses on the “delayed” lever resulted in the delivery of 4 pellets. The delay to 4 pellets was increased across 5 blocks from 0 to 5, 10, 20 and then 40 sec. (See figure 10). Rats were tested for 30 days in this manner and the criterion was stable performance across 3 consecutive days (i.e., no significant change in preference for large and small reward; repeated measures ANOVA).
Figure 10: Overview of delay discounting procedure. There were 5 blocks (12 trials each) of testing. The first two trials within each block were forced trials during which rats could only respond on one of the levers – one delivering one pellet immediately or 4 pellets after a variable delay (blocks 1-5, 0-40s delay respectively). The next 10 trials, within each block, were choice trials during which rats could respond on the immediate lever (1 pellet, 0s delay) or delayed lever (4 pellets, 0-40s delay). Delay to the large reward systematically increased from block 1-5.


*Delay Discounting – No Delays Across Blocks.* This testing was identical to procedures used above (Delay Discounting Training – No Delays Across Blocks). Testing lasted 8 days and the criterion was stable (at least 85% preference for large reward across 2 days) performance.

*Reversal of Small and Large Reward Levers with No Delays Across Blocks.* This testing was identical as with other sessions with no delay (initial choice procedures; see above) for large reward except that large and small contingencies were reversed between the two levers. That is, if the left lever was associated with small reward it was now associated with the large reward and vice versa. Testing lasted for 4 days.

*Data Analyses*

*Delay Discounting*

SHAM and CON groups were not statistically different from each other on any measures of interest (responding for small and large reward), therefore, they were combined into one group: mother-reared (MR). Data analyses were carried out with SPSS for Windows (version 15.0, SPSS, Chicago, IL). Percent preference for large reward was the measure of interest. Data were analyzed using repeated measure ANOVA (Group x Sex x Delay) followed by Dunnett post hoc tests in order to assess which treatment group (AR-MIN and AR-MAX) was different from the control group (MR). In cases of significant interactions between sex and other variables, data were analyzed separately for males and
females. Significant Group x Delay interactions were followed by one-way ANOVAs assessing group differences at different levels of delay for large reward. These analyses were also followed by Dunnett post-hoc tests. The level for achieving statistical significance was set at p<0.05.

**Results**

*No Delays Across Blocks*

Rats were tested for 5 days with no delays to the large reward and analysis was performed on the averaged data from the last 2 days of testing. As can be seen in Figure 11, all groups showed a high preference for the large reward. There were no significant group, sex, or block effects and no significant interactions.
Figure 11: Mean percent preference (+SEM) across 5 blocks of testing with no delays to the large reward for male and female rats (data were averaged across last two days of testing). All groups preferred (>90%) the large reward over the small reward.
Delay Discounting

Rats were tested until their responses were stable across 3 days. Stability was assessed using repeated measures ANOVA (data for individual delay intervals across 3 days). If there were no significant Day effects, we considered the rats’ performance stable. Stability was achieved by day 30 of testing. Next, data were averaged across the last 3 days of testing and analyzed using repeated measures ANOVA (Group x Sex x Delay). Initial analyses indicated significant Delay effects ($F(1, 47)=337.4$, $p<0.001$), significant Group differences ($F(2, 47)=3.95$, $p<0.05$), significant Group x Sex interaction effects ($F(2, 47)=6.2$, $p<0.05$), and a significant Group x Sex x Delay interaction ($F(2, 47)=4.1$, $p<0.05$; Figure 12).

In order to further examine group differences, without Sex effects, we ran another set of repeated measures ANOVAs (Group x Delay) separately for male and female rats. For female rats, we found significant Delay effects ($F(1, 25)=188.9$, $p<0.001$) but no significant Group differences or significant Group x Delay interactions (Figure 12).

Analyses of male rats’ data indicated significant Delay effects ($F(1, 22)=149.95$, $p<0.001$), significant Group differences ($F(2, 22)=11.78$, $p<0.001$), and a significant Group x Delay interaction ($F(2, 22)=3.5$, $p<0.05$). These overall group differences were followed by Dunnett post hoc tests comparing the 2 AR groups with the MR group. AR-MIN rats, compared to MR rats, showed a significantly higher large reward preference across the delays ($p<0.001$). AR-MAX rats were not significantly different from the MR rats. In addition, significant Group x Delay
interaction effects were followed by one-way ANOVAs assessing group differences within individual blocks. Significant group differences were found within all 5 blocks of delay (all $p$s<0.05). Dunnett post-hoc analyses indicated that AR-MAX rats were different from MR rats during the first block (0s delay; $p<0.05$). AR-MIN rats were significantly different from the MR rats across all 5 delays (all $p$s<0.05; Figure 12).
Figure 12: Mean percent preference (+SEM) for the large reward across 5 blocks (delays) of testing for male and female rats. Rats’ preference for large reward decreased across delays \((p<0.001)\). There were significant Group x Sex and Group x Delay interactions \((ps<0.05)\). Post-hoc analyses indicated that AR-MIN rats, compared MR rats, have higher preference for large reward (male rats). *One-way ANOVAS and post-hoc tests (male rats) indicated significant differences between AR-MIN and MR rats during blocks 1-5. °AR-MAX rats were different from MR rats during block 1.
No Delays Across Blocks

Following delay discounting, rats were tested again with no delays to large reward across the blocks (see above). Similar to initial testing with no delays, rats’ preferences for large reward stabilized within 7 days. All groups showed over 90% preference for large reward (data not presented).

No Delays Across Blocks – Reversal of Lever Associations with Small and Large Reward

This testing was identical to other test sessions with no delays to large reward across the blocks. However, here we reversed the association of the left and right lever with the small and large reward. For example, if the large reward was associated with the left lever now it was associated with the right lever. Rats were tested over 4 days. However, since we were interested in assessing how quickly rats can modify their responses (as they do within a single test session) we were primarily interested in rats’ initial responding (Day 1 of reversal testing) on this update in testing. Data were analyzed separately for males and females using repeated measures ANOVA (Group x Delay).

For female rats, there were significant block effects ($F(1, 25)=52.6, p<0.001$) but no group differences and no interaction effects. Analyses of male rats’ performances indicated significant block effects ($F(1, 22)=79.5, p<0.001$), significant overall group differences ($F(1, 22)=3.6, p<0.05$) and a significant Group x Block interaction ($F(2, 25)=4.6, p<0.05$). Post-hoc tests indicated that AR-MIN rats were significantly different from MR rats ($p<0.05$). Interaction effects were
followed by one-way ANOVA for individual blocks. Compared to MR rats, AR-MIN rats choice for large reward was significantly lower during last 2 blocks of the session (ps<0.05; see Figure 13). Identical analyses were done on the next reversal session. Similar results were found; AR-MIN and MR rats were different during blocks 2, 3 and 5 of the session (ps<0.05; data not depicted). By day 3 of reversal testing, there were no significant main effects or interactions.
Figure 13: Mean percent preference (+SEM) across 5 blocks (10 trials each) of testing with no delays large reward for male (A) and female (B) rats on day 1 of reversal of lever associations for large and small reward with the left and right lever. Preference for large reward increased across blocks ($p<0.0001$). There were significant group differences and significant Group x Block interactions ($ps<0.05$).

*One-way ANOVAS and post-hoc tests indicated significant differences between AR-MIN and MR rats during blocks 4 and 5.
Discussion

In the present study, we hypothesized that maternally deprived (AR-MIN) rats would display greater impulsivity by showing a greater tendency to choose small-immediate rewards. In contrast to our predictions (male) AR-MIN rats had a greater tendency to select a large reward even when it was delayed. Superficially, this would suggest that AR-MIN rats were better able to tolerate delay of large reward and are, therefore, less impulsive.

However, it is also possible that the specifics of the task did not allow for accurate assessment of tolerance to delay of reward. In our version of the delay discounting operant schedule (similar to most other published studies with delay discounting; e.g., Uslaner & Robinson, 2006; Winstanley, Theobald, Cardinal, & Robbins, 2004), delay to large reward started at 0s during the first block and increased to 40s over the subsequent blocks. All rats showed a preference for the large reward during the first block when the delay to the large reward was 0s. Over the next several blocks, MR rats discounted the value of the large reward much more rapidly then did the AR-MIN rats (AR-MAX rats were similar to MR rats). This switch in preference depends on several factors, including attention to changes in large reward properties (i.e., the delay) and ability to disengage from previously preferred responses (i.e., behavioural flexibility).

In order to test the possibility that AR-MIN rats are less able to assess changes in stimulus properties and switch their behaviour accordingly, we tested the rats with no-delays but with left and right lever associations to the large and small reward reversed. As can be seen in Figure 13, all groups showed a near
0% preference for large reward during the first block of reversal testing. This was expected as rats responded mostly on the lever that was previously associated with the large reward, but currently corresponded to the selections of the small reward. However, by the 5th block, MR rats had over 80% preference for the large reward, while the AR-MIN rats chose the large reward in less than 30% of the trials. This finding shows that MR rats were able to relatively rapidly switch their behaviour within a session. On the other hand the rate of change for AR-MIN rats was significantly slower. It took 3 reversal sessions for AR-MIN rats to show similar preference for large reward as seen with MR rats. Overall, this finding suggests that AR-MIN rats are less able to switch their responding.

While it is clear that AR-MIN rats are less able to switch their behaviour, it is not clear what psychological mechanisms are responsible for these effects. As mentioned above, these effects might be driven by impaired attention and/or perseverative tendencies (i.e., continuous selection of a previously rewarded response). We have previously reported that AR-MIN rats have deficits in prepulse inhibition of the startle response, a measure of sensorimotor gating (or early attentional filtering), and shifting of the attentional sets (Lovic & Fleming, 2004). Hence, this would suggest that AR produces deficits in attentional abilities. In addition, we have found that AR-MIN rats show reversal deficits in the attentional set-shifting task (Lovic & Fleming, 2004). However, the specifics of the task used in Lovic & Fleming (2004) did not allow for definitive conclusions about whether AR-MIN rats showed simple reversal learning deficits or perseverative responding. Those data, together with the data presented in this
paper, suggested that it is not likely that AR rats are less impulsive, but rather that they are less likely to switch their preferential responding (Figure 13).

Another factor that might have influenced increased preference for large-delayed reward in AR-MIN rats is altered perception of food reward. AR-MIN rats might have preferred a large-delayed reward if the large reward had increased motivational significance for them. Work in other labs has shown that AR rats consume more sucrose (Lomanowska, Rana, McCutcheon, Parker & Wainwright, 2006). This suggests that food might have increased incentive value for AR rats and this alteration in motivation for food might have altered their ability to tolerate delay for food. In other words, it is possible that large reward (4 pellets) had greater value to AR-MIN rats, in contrast to MR rats, and this greater value of large reward was resistant to changes in delays to large reward. While this hypothesis was not explicitly tested in the current experiment, it is unlikely that it can explain the observed effects. If AR-MIN rats were better able to tolerate delay to large reward due to their elevated motivation for food, the results observed in reversal testing (Figure 13) would not be observed. On the contrary, if the AR-MIN rats had increased motivation for the large reward, they would have switched their preference for the large reward at a faster, rather than slower, rate than MR rats.

Lastly, it is possible that our AR procedure (AR-MIN rats) speeds up subjective perception of time – in other words, time goes by faster for AR-MIN rats. If this is the case then, then it would be not be surprising that they would be better able to tolerate delays to large reward as the delay does not seem to be
that long. This hypotheses is consistent with our observation of AR-MIN rats (specifically male rats) in the DRL-20s (Lovic, Fletcher, & Fleming, in prep; Chapter 3). On DRL-20s operant schedule, 2nd peak of responding was shifted to the left in male AR-MIN rats. That is, their peak responding came several seconds before that of MR rats (and before 20s waiting period elapsed) again suggesting that their perception of when 20s has elapsed might be altered.

Our finding that AR-MIN rats showed reduced impulsive choice in the delay discounting operant schedule is in contrast to our previous finding of AR-MIN rats showing increased impulsive action on the DRL-20s operant schedule. These findings of identical treatment, in this case artificial rearing resulting in opposite effects on two tasks of impulsivity (delay discounting and DRL) are not unique. Lesions of the subthalamic nucleus and systemic amphetamine treatment produce the same effects (Uslaner & Robinson, 2006; Winstanley, Baunez, Theobald & Robbins, 2005). These amphetamine-related findings suggest that a hyperdopaminergic state can produce increased impulsive action (DRL) and reduced impulsive choice (delay discounting). We have previously found that AR rats have greater novelty- and amphetamine-induced locomotor activity (Lovic, Fleming & Fletcher, 2006). These findings are suggestive of hyperdopaminergic condition in AR rats. While we do not have direct evidence of altered DA systems in the rats used in this study, we do have preliminary data from another study showing that AR rats have elevated baseline DA output \((in \text{ \it{vivo}})\) in the nucleus accumbens (V. Afonso, unpublished data). In addition, other labs, employing other methods of mother-pup separation, have also found
elevated responsiveness of the DA system to potassium and amphetamine stimulation (Hall, Willkinson, Humby & Robbins, 1999; Matthews, Dalley, Matthews, Tsai & Robbins, 2001).

Our findings of reduced impulsive choice in maternally deprived rats are also similar to those of Hellemans, Nobrega and Olmstead (2005), who reported a decrease in impulsive choice (DD) in adult rats isolated during the post-weaning period. Hence, it seems that both, pre- and post-weaning isolation might be producing similar behavioural profiles in adulthood.

There are two other significant findings to the current study. First, AR-MAX rats were not different from the MR rats during delay discounting or reversal no-delays testing (Figure 13). This indicates that maternal licking-like stimulation has a significant effect on brain mechanisms mediating performance in the DD testing. These reversal or ameliorating effects of tactile stimulation are concordant with our previous observations of tactile stimulation reversing the effects of artificial rearing. For example, tactile stimulation reversed the effects of artificial rearing on motor impulsiveness (DRL; Lovic, Fletcher & Fleming, in prep), maternal behaviour (Gonzalez et al., 2001), attention (Lovic & Fleming, 2004) and locomotor activity (Lovic et al., 2006). These effects are also concordant with the other studies showing that maternal licking has a beneficial effect on adult stress response (see Kaffman & Meaney, 2007). Simulations of maternal licking, using a paintbrush, increase several hormones and growth factors necessary for normal development (Kuhn & Schanberg, 1998). Of significant importance for DD is the DA system. The DA releaser amphetamine
reduces impulsivity in the DD task. Several studies have shown that maternal deprivation produces enduring effects on *in vivo* DA output (Hall et al., 1999), DA profile (Matthews, Dalley, Matthews, Tsai, & Robbins, 2001), DA receptors (Brake, Zhang, Diorio, Meaney & Gratton, 2004) and DA-mediated behaviours (Brake et al., 2004). We have previously found that AR rats are more sensitive to amphetamine induced locomotor activity (Lovic et al., 2006) and DA output from nucleus accumbens (Afonso & Fleming, in prep). Importantly, providing AR-MAX pups with tactile stimulation, simulating maternal licking, reverses these effects. While these studies show that maternal deprivation and maternal licking, or simulations of maternal licking, can have an effect on the DA system, virtually nothing is known about the mechanisms of these effects.

Second, sex of the rats was a significant factor in several of our findings. Specifically, ‘reduced’ impulsiveness and slower switching of behaviour in AR-MIN group (Figure 13) was seen with male rats. However, it is important to note here that our contention is that AR-MIN rats are not less impulsive in this task (see above) but that they are less able to switch their behaviour. Hence, the fact that AR-MIN females are not more impulsive than MR rats is not surprising, although lack of group differences on the reversal task is more difficult to interpret. One possibility is that the AR procedure might be differentially impacting male and female rats with respect to brain mechanisms mediating behaviours tested in this experiment. Mother rats spend more time caring for male pups than female pups (Moore & Moralli, 1979). Hence, maternal deprivation, through artificial rearing, might have a greater impact on male pups
then on female pups. While this is a possibility, these findings of sex differences in this experiment are not consistent with our findings that both male and female AR-MIN rats are more impulsive in the DRL task.

While these findings are informative, it is not entirely clear what the nature of AR deficits is with respect to impulsive choice. Since AR rats have reversal learning deficits, delay discounting might not be an ideal operant schedule to assess the effects of AR on impulsive choice. However, there is another task, a fixed consecutive number (FCN) schedule, which can assess impulsive choice and is not potentially confounded by reversal deficits. Our next experiment (described in Chapter 5 of this thesis) assessed the effects of AR on impulsive choice using FCN.
CHAPTER FIVE
The effects of Maternal Deprivation, through Artificial Rearing, on Impulsive Choice (FCN8) II

Abstract
We have previously found that rats reared in isolation, through AR, display greater impulsive action (the differential-reinforcement-of-low-rate – DRL-20s - schedule) and reduced impulsive choice (delay discounting – DD - operant schedule). However, we have also observed, that AR rats display reduced behavioural switching that is necessary on DD operant schedule. This puts into doubt the contention of reduced decision impulsiveness in AR rats. Hence, the purpose of the current experiment was to further assess decision impulsiveness in AR and MR rats using a fixed consecutive number (FCN) schedule. Similar to the DD operant schedule, the FCN schedule assesses impulsive choice; however, in contrast to the DD operant schedule, in this operant schedule perseverative responding is unlikely to occur or influence measures of decision impulsiveness. As done previously, rats were reared with mothers or artificially, and as adults they were tested for 20 days on the FCN8 operant schedule. Consistent with our predictions, we did not find evidence of altered decision impulsiveness as a result of AR. Together with our previous findings we conclude that artificial rearing leads to increased action impulsiveness but it does not alter choice impulsiveness.
The effects of maternal deprivation, through artificial rearing, on decision impulsiveness (FCN8) II

Early life adversity, in the form of stress or social or maternal deprivation, can lead to long-lasting effects on behaviour and physiology (Kaffman & Meaney, 2007). Work in our laboratory has found that rats reared in isolation from the mother and littermates, through artificial rearing (AR), compared to mother-reared (MR) rats, display reduced maternal behaviour (Gonzalez, Lovic, Ward, Weinwright & Fleming, 2001), attentional capacities (Lovic & Fleming, 2004), and increased locomotor activity (Burton, Lovic & Fleming, 2006; Lovic, Fleming & Fletcher, 2006). These effects are reversed or ameliorated if AR rats are provided with somatosensory stimulations, simulating maternal licking (AR-MAX treatment effects). Our recent work has focused on impulsiveness in MR and AR rats. We have found that AR rats show increased responding and have lower efficiencies on the DRL-20s operant schedule, suggesting increased impulsive action (Chapter 3). In contrast, male AR rats show lower discounting rates in the DD operant schedule, suggesting greater tolerance of delayed rewards and hence reduced impulsive choice (Chapter 4). However, in the DD operant schedule we suspected that the performance of AR rats might not truly represent a reduction in impulsive choice but rather an artifact of reduced ability of AR rats to change behaviour in response to ascending changes in delays to large reward. This was indirectly tested by switching contingencies between lever associations with small and large reward. Indeed, AR rats were slower to change their behaviour. In order to further clarify the issue of the effects of AR on impulsive
choice we conducted the current experiment. The purpose of the current experiment was to further assess impulsive choice in AR and MR rats using a different test of impulsive choice.

Similar to DD, the fixed consecutive number (FCN) operant schedule assesses impulsive choice (Evenden, 1998; Mechner & Latranyi, 1963). On this operant schedule rats are presented with two levers, either intermittently, through distinct trials, or constantly throughout the session (in our case levers were present throughout the entire session). Rats are required to press one lever (referred to as the *chain lever*) at least one fixed number of times (e.g., 8 times) before their single response on the other lever (referred to as the *reinforcement lever*) results in the delivery of reward. Pressing the reinforcement lever before at least 8 responses have been made on the chain lever does not result in the delivery of reward and it resets the chain lever requirement (i.e., rats have to start a new chain). Evidence suggests that rats can not count; however, within several sessions their average chain length is close to the ideal number (e.g., 8). Rats that prematurely terminate their responses on the chain lever (e.g., < 8 lever response) before responding on the reinforcement lever are thought to be impulsive. On the contrary, greater chain lengths are thought to reflect reduced impulsiveness (Evenden, 1998; Dellu-Hagedorn, 2006).

What is shared between DD and FCN operant schedules is that rats are presented with two levers on which they can make responses. At each moment a rat can decide between these 2 options of responding. These operant schedules are in contrast to DRL-20s operant schedule where rats are presented
with only one lever (no choice between different options) but in order to get
rewarded rats have to inhibit responding and time their responses appropriately.
Responding in less than 20 s since their last response does not produce the
delivery of reward and it resets response timing. As might be suspected, this
operant schedule is greatly influenced by rats’ general levels of motor output.
That is, rats that have greater motor output are more likely to respond on the
lever more frequently and hence are more likely to respond prematurely. On the
contrary, DD and FCN are not thought to be influenced by general motor output
(except in cases of significant motor impairment that might impair responding in
general) as rats with greater motor output can express that output on either one
of the two levers and not necessarily be disadvantaged by their increased
responding.

The DRL-20s experiment revealed similar effects for male and female
rats, and the DD experiment uncovered a significant ‘reduction’ in impulsiveness
and reduced flexibility in males, only. Hence, in this experiment we decided to
explore AR effects on male rats only.

Based on our interpretations of the finding from the DD experiment, we
predicted that the AR procedure would not produce changes in impulsive choice
measured on the FCN operant schedule. Furthermore, in order to exclude the
possibility that greater motor output, typically observed in AR rats, has an effect
on performance in the FCN operant schedule, we tested the rats on general
locomotor activity, as well. General locomotor activity measures were correlated
with different aspects of FCN performance.
Methods

Subjects

Thirty-three male Sprague-Dawley (AR-MIN, n=8; AR-MAX, n=8; MR=17) rats were used in this study. Rats were assigned to the same conditions as in Chapter Three. For procedural details see Chapter Two.

Apparatus

Operant Conditioning Chambers

See Chapter 2 – Methods.

Locomotor Activity Boxes

Locomotor activity was assessed in 16 transparent activity boxes (46 x 25 x 21 cm) made at the Centre for Addiction and Mental Health (Toronto, Canada). A frame (2 cm above the floor), located outside the box, equipped with 16 photocell beams (approximately 2.5 cm apart) assessed the movement of rats. Total number of photocell counts was the dependent measure.

Procedures

Groups and treatments

See Chapter 2 – Methods.

Behavioural Procedures

Continuous Reinforcement Training

Adult rats were gradually reduced to 85% of their free-feeding weights by limiting daily food intake. They were trained to bar press for food (45 mg food
pellets; Bio-Serv) on a continuous reinforcement (CRF) schedule during a 30 min session. Rats were tested with left and right lever on alternate days. Learning criteria were at least 100 bar presses, during a 30-min session, on 2 consecutive days. All rats met this criteria within 7 days.

**FCN Training**

Next, rats were tested on FCN1 schedule (1 day) and FCN3 schedule (6 days). Each 45-min long session was initiated with the illumination of the house light and extension of both levers into the operant chamber. Rats had to make at least 1 (FCN1) or 3 (FCN3) responses on one of the levers (chain lever) and then respond once on the other (reinforcement) lever in order to get rewarded (1 pellet). Once the rats’ performance stabilized (no significant changes across days) they were switched to FCN8.

**FCN8**

FCN8 testing was identical to FCN1 and FCN3 testing except that rats had to make at least 8 responses on the chain lever before their single response on the reinforcement lever resulted in the delivery of a food pellet. Rats were tested for 20 days on the FCN8 schedule. As described below, there were several measures of interest.

**Number of Chain Lever Responses.** Total number of responses made on the chain lever during a given session.

**Number of Reinforcement Lever Responses.** Total number of responses made on the reinforcement lever during a given session.
**Number of Pellets Earned.** Total number of pellets delivered during a given session.

**Number of Chains.** Responses (at least 1) made on the chain lever followed by a response on the reinforcement lever constituted a chain. Total number of chains was the sum of all chains (of various lengths; minimum 1) during a particular session.

**Time to Complete the Chain.** This measure was based on average duration (in seconds) to complete the chain (of any length) during a particular session.

**Efficiency.** The proportion of responses made on the reinforcement lever that resulted in pellet delivery [(# of responses / # of pellets) x 100]. This measure reflected the proportion of chains resulting in reinforcement. Lower values reflect decreased efficiency.

**Average Chain Length.** The average number of responses made on the chain lever before a response was made on the reinforcement lever. Optimal responding yields an average chain length of 8 (FCN8). Shorter chain length was indicative of increased impulsivity. Conversely, longer chain length was indicative of reduced impulsivity (Evenden, 1998).

Locomotor Activity
Following FCN8 testing, rats' locomotor activity was tested over 3 consecutive days (60 min tests). Testing occurred during light hours in a dimly lit room. Boxes were cleaned with 70% alcohol in between each test. Total number of photocell counts was the dependent measure.

Data Analyses

MR-SHAM and MR-CON groups were not statistically different from each other on any measures of interest; therefore, they were combined into one group: mother-reared (MR). The level of statistical significance was set at $p < 0.05$.

FCN8. Data for each of the 6 measures (described above) was averaged across 4 days (for a total of 5 blocks) and analyzed using repeated measure ANOVA (Group x Block). Dunnett’s post hoc tests were used in order to assess which treatment group was different from the control group (MR). We were primarily interested in differences between the 2 AR groups against the MR group, rather than overall group differences. Therefore, we only contrasted AR-MIN with MR group and AR-MAX with MR group.

Locomotor Activity. Data was analyzed using repeated measures ANOVA (Groups x Days), followed by Dunnett’s post hoc tests when there was a significant effect.

Correlations. In order to examine a possible relationship between activity and impulsiveness, we conducted partial correlations, controlling for group effects, on locomotor activity and FCN measures. The level for achieving statistical significance was set at $p<0.05$. 

Results

FCN8

Number of Responses Made on the Chain Lever

All groups displayed an increase in the number of responses made on the chain lever across sessions (main effect of block; $F_{(1, 30)}=39.9, p<0.001$). In addition, there were marginal overall group differences ($F_{(2, 30)}=2.75, p=0.08$) and significant group differences between the AR-MAX and the MR rats. As can be seen in Figure 14, compared to MR rats, AR-MAX rats made more responses on the chain lever ($p<0.05$; see Figure 14).

Number of Responses Made on the Reinforcement Lever

There was no overall increase in the number of responses made on the reinforcement lever, however, there were overall group differences ($F_{(2, 30)}=6.5, p<0.01$). Dunnett’s post-hoc analyses reveled that AR-MAX group, compared to the MR group, made significantly more responses on the reinforcement lever ($p<0.01$; see Figure 15).
**Figure 14:** The figure depicts mean number of responses (+SEM) made on the chain lever across 5 blocks of testing. Responses increased across blocks ($p<0.001$). AR-MAX rats made significantly more response than MR rats ($p<0.05$).
Figure 15: The figure depicts mean number of responses (+SEM) made on the reinforcement lever across 5 blocks of testing. Responses increased across blocks ($p<0.001$). AR-MAX rats made significantly more response than MR rats ($p<0.05$).
**Number of Pellets Earned**

All groups earned more pellets over successive sessions (main effect of block; $F_{(1,30)}=76.6, p<0.001$). There were marginal overall group differences ($F_{(2,30)}=2.45, p=0.1$) and marginal differences between the AR-MAX and the MR group ($p=0.076$; see Figure 16). There were no significant interaction.

**Number of Chains**

Groups generated more chains over successive sessions (main effect of block; $F_{(1,30)}=6.9, p<0.05$) and there were overall groups differences ($F_{(2,30)}=6.0, p<0.01$). Dunnett’s post hoc analyses revealed that AR-MAX, but not AR-MIN, rats created more chains across sessions compared to the MR rats ($p<0.05$; see Figure 17).
Figure 16: The figure depicts mean number of pellets earned (+SEM) across 5 blocks of testing. There was an overall increase in the number of pellets earned across blocks ($p<0.001$). AR-MAX rats earned significantly more pellets than MR rats ($p<0.05$).
Figure 17: The figure depicts mean number of chains made (+SEM) across 5 blocks of testing. There was an overall increase in the number of chains across blocks ($p<0.001$). AR-MAX rats made significantly more chains than MR rats ($p<0.05$).
Time to Complete the Chains

Repeated measures ANOVA revealed that all groups took less time to complete their chains across sessions (main effect of block; \(F_{(1, 30)}=11.8, p<0.01\)). Groups differed significantly (\(F_{(2, 30)}=4.8, p<0.05\)). As depicted in Figure 18, it took AR-MAX rats significantly less time to complete the chains compared to the MR rats. AR-MIN rats were not significantly different from the MR rats.

Efficiency

There was a significant increase in efficiency across blocks (\(F_{(1, 30)}=82.44, p<0.0001\)) but there were no significant groups differences or interactions (see Figure 19).

Average Chain Length

Rats’ chain length increased significantly over successive blocks (\(F_{(1, 30)}=54.2, p<0.0001\)). However, as depicted in Figure 20, there were no significant group differences or interactions.

Locomotor Activity

Number of photocell counts decreased over 3 test sessions (main effect of days; \(F_{(2, 30)}=18.5, p<0.001\)). There were overall group differences (\(F_{(2, 30)}=11.2, p<0.001\)) and post-hoc analyses indicated that both AR-MAX and AR-MIN rats showed greater levels of locomotor activity compared to MR rats (see Figure 21).
Figure 18: The figure depicts mean number of seconds to complete a chain (+SEM) across 5 blocks of testing. Time to complete the chain decreased across blocks \((p<0.01)\). AR-MAX rats, compared to MR rats, took significantly less time to complete their chains \((p<0.05)\).
Figure 19: The figure depicts percent efficiency (mean + SEM) across 5 blocks of testing. There was a significant increase in efficiency ($p<0.0001$), however, there were no significant group differences or interactions.
Figure 20: The figure depicts significant increase in average chain length (mean + SEM) across 5 blocks of testing ($p<0.0001$) without any group differences.
Figure 21: The figure depicts locomotor activity (photocell counts) across 3 consecutive 1-hour sessions. There was an overall decrease in locomotor activity ($F_{(2, 30)}=18.5, p<0.0001$). In addition, both AR-MAX and AR-MIN rats made more photocell interruptions than the MR rats ($p<0.05$).
**Relationship Between Locomotor Activity and FCN Measures**

Partial correlations (controlling for group effects) between novelty induced locomotor activity (day 1 of testing) and FCN measures (averaged across blocks) were performed. No significant correlations were found. Also performed were partial correlation on measures of more general locomotor activity (averaged across day 2 and 3) and FCN measures. Again, no significant correlations were found.

**Discussion**

The current experiment was completed in the context of our 2 previous experiments (described in Chapters 3 and 4) that examined impulsive action and impulsive choice in AR and MR rats. Previously, we found that AR-MIN, but not AR-MAX rats, show reduced efficiency in the DRL-20s operant schedule (implying increased impulsive action) and lower discounting rates of large-delayed reward in the DD operant schedule (implying reduced impulsive choice). However, in the DD operant schedule we suspected that lower discounting rates of large-delayed reward, seen in AR-MIN rats, might not truly represent reduced impulsive choice but rather reduced behavioural flexibility (increased perseverative or habitual responding) propelled by more frequent responding on one lever during the begging of each session. This hypothesis was assessed within the DD operant schedule (0s delays to large reward but reversed associations between large and small reward and left and right levers), which
demonstrated that AR-MIN rats do show reduced ability to rapidly switch their behaviour. These findings were also consistent with reduced behavioural flexibility seen in AR rats in the attentional set shifting task (Lovic & Fleming, 2004). In order to further examine impulsive choice we conducted the current experiment, this time using a different operant schedule of impulsive choice. In contrast to DD operant schedule, on which rats can show perseverative responding on one of the levers and still earn some pellets, on the FCN schedule rats can not perseverate on the chain lever (or the reinforcement lever) and get rewarded.

Consistent with our predictions and conclusions drawn from the DD data in Chapter 4, in the current experiment we did not see any evidence of altered impulsive choice in AR-MIN rats. Their behaviour was in many aspects quite similar to those shown by MR rats. One question that is raised from these observations is: if the AR-MIN rats show perseverative/habitual responding, as we have suggested in the DD experiment, why are they not showing perseverative responding in the current operant schedule (FCN)? One of the key differences is that in the DD operant schedule each lever is associated with a reward (one associated with small-immediate reward and one associated with larger-delayed reward). However, all rats, including AR-MIN rats make significantly more responses early in the test session solely on the large reward lever (excluding forced trials). On the contrary, in the current experiment more responses are made on the chain lever but these responses are not directly associated with reward delivery. Hence, this operant schedule does not have
confounds inherent in the DD operant schedule, at least in the version of the DD operant schedule that we used.

However, we did find that both AR groups of rats displayed greater levels of locomotor activity and these findings are consistent with our previous studies (Burton, Lovic & Fleming, 2006; Lovic, Fleming & Fletcher, 2006). In addition, compared to MR rats, AR-MAX, but not AR-MIN rats, made more responses on both the chain and the reinforcement lever, and made more chains with less time spent making these chains. AR-MAX rats earned more pellets than MR rats. However, despite increased levels of motor activity that AR-MAX rats displayed (both on the FCN operant schedule and in locomotor activity boxes) they were not more impulsive. Their average chain length did not significantly differ from the MR rats. Hence, despite being more motorically active, AR-MAX rats did not prematurely interrupt their sequences on the chain lever.

It is not clear what psychological or neurophysiological mechanism might be responsible for the observed behaviour in the AR-MAX group. One possibility could be increased incentive value of food reward in these rats (Berridge, Robinson, & Aldridge, 2009). If AR-MAX rats ‘liked’ and ‘wanted’ food reward more so than the other rats, it would not be surprising that they were more willing to work for it with greater intensity without being impulsive. However, we do not have any evidence of altered incentive motivation for food in AR-MAX rats. Their rate of acquisition in several tasks, involving food, does not suggest changes in incentive motivation for food. Their body weights and food consumption is also not significantly different from the MR and AR-MIN rats.
While we do not have any direct evidence that AR-MAX rats have altered perception of food reward or greater ‘wanting’ of food (in food deprived state), we did find that AR-MAX made significantly more lever responses on DRL-20s operant schedule immediately following the pellet delivery (unpublished data, Chapter 3). This increased responding (greater than MR and AR-MIN rats) is suggestive of increased food reward motivation in AR-MAX rats.

Interestingly, compared to AR-MAX rats, AR-MIN rats displayed even greater levels of locomotor activity in the activity boxes, yet their motor output on the FCN operant schedule (number of responses made on either lever) did not differ from the MR rats. These findings are not surprising given that we did not find a relationship between locomotor activity levels and FCN measures. Taken together, these findings are consistent with other reports (Evenden, 1998) suggesting that FCN is not an operant schedule testing impulsive action.

Overall we have demonstrated that the AR procedure produces an increase in motor output, both within and outside the operant paradigm, however, we have found no evidence of altered impulsive choice as a result of AR treatment. In addition, these results suggest that the AR procedure, combined with replacement somatosensory stimulation (AR-MAX), might increase motivation for primary reinforcers, but without an increase in impulsive responding in an attempt to earn those reinforcers.
CHAPTER SIX

Impulsive Rats are Less Maternal

Abstract

Early life environment and maternal care can have long-lasting effects on behaviour and physiology. Previously, we found that rats reared without mothers, AR, show reduced levels of maternal behaviour. These effects can be reversed if AR pups are provided with tactile stimulation which simulates maternal licking. We also found that AR rats are more action impulsive and have reduced attentional capacities (Lovic, Fletcher & Fleming, in prep; Chapter 3; Lovic & Fleming, 2004). However, it is unknown whether increased impulsivity contributes to reduced levels of maternal behaviours. The purpose of this study was to assess the relationship between impulsivity and maternal behaviour in AR and maternally reared (MR) rats. Female rats were reared with (MR) or without mothers (AR) and half of the AR rats received additional stroking stimulation. As adults, AR and MR rats were mated and maternal behaviour towards their own pups was assessed. In addition, rats were assessed on impulsive action (differential rates of low rate responding operant schedule of reinforcement; DRL-20s). Consistent with previous findings, AR rats were both less maternal and more action impulsive than MR rats. Partial correlations revealed that impulsivity was inversely related to pup-licking—impulsive rats were less maternal.
Impulsive Rats are Less Maternal

The quality and quantity of maternal behaviour shown by mother rats have a substantial impact on the behavioural, endocrine, and neural development of their pups (see Kauffman & Meaney, 2007). Maternal deprivation and variations in maternal care produce changes in offspring’s emotional, cognitive, and social behaviour and in the development of their underlying neuroendocrine and neurotransmitter systems (Kauffman & Meaney, 2007; Hall, 1998; Hall, Wilkinson, Humby & Robbins, 1999; Matthews, Dalley, Matthews, Tsai, & Robbins, 2001; Vazquez, Lopez, Van Hoers, Watson, & Levine, 2000). In general, in comparison to non-maternally deprived or highly licked pups, maternally deprived pups or pups of low-licking dams, are more active (Brake, Zhang, Diorio, Meaney, & Gratton, 2004), more emotionally reactive (Francis, Diorio, Liu & Meaney, 1999; Francis & Meaney, 1999), exhibit altered spatial memory (Liu, Diorio, Day, Francis & Meaney, 2000), social behaviour (Parent & Meaney, 2008) and show reduced levels of both sexual (Cameron et al., 2008; Cameron, Del Corpo, Diorio, McAllister, Sharma & Meaney, 2008; Cameron, Fish & Meaney, 2008), and maternal behaviour (Francis et al., 1999). They also experience sustained elevations in their hypothalamic-pituitary-adrenal responses to stressors, through a reduction in the glucocorticoid receptor sensitivity in the hippocampus (Francis & Meaney, 1999; Liu et al., 1997) and disruptions in dopamine function (Brake et al., 2004; Hall et al., 1999; Matthews et al., 2001; Meaney, Brake & Gratton, 2002; Zhang, Chretien, Meaney & Gratton, 2005). Although maternal deprivation studies, in conjunction with
studies that compare the effects of being mothered by high and low licking mothers, strongly suggest that the predominant factor affecting the offspring is the actual amount of licking stimulation received, mothers that lick more also crouch more and may produce other differences to the entire litter situation and nest configuration that could also be affecting the individual offspring (that is there may be differences in total activity of the litter, thermal characteristics of the nest, and so on). In order to isolate the role of the licking stimulation in pup development, we adopted a different strategy to determine the effects of early preweaning experiences on pup development.

The paradigm we adopted, the pup-in-a-cup rearing regimen, involves rearing pups entirely without the mother and littermates in a controlled thermal and nutrient environment during the first three weeks of life, and providing pups with different amounts of stroking and/or social stimulation. Using this artificial-rearing or AR paradigm, we have explored many of the same aforementioned developmental outcomes in the pups, including emotional, cognitive, and social behaviours, endocrinology, and the brain function and structure. In general, we have found that many of the same deficits reported for pups of low-licking mothers are also found for pups reared without mothers altogether (Burton, Lovic & Fleming, 2006; Gonzalez, Lovic, Ward, Wainwright & Fleming, 2001; Lovic & Fleming, 2004; Lovic, Fleming & Fletcher, 2006; Melo, Lovic, Gonzalez, Madden, Sinopoli & Fleming, 2006); and for most outcomes the addition of 5-8 daily stroking episodes with a small paintbrush partially reversed the effects of isolation from mother, siblings, and nest. These findings reinforce the licking-
based interpretation of results suggested by Meaney and colleagues (Kaffman & Meaney, 2007). Other early experience factors contributing to the AR effects on behaviour and physiology continue to be investigated (Melo et al., 2006).

In our studies, we focused on the effects of AR and of somatosensory licking-like stimulation on the development of species-characteristic social behaviours, especially adult maternal behaviour. We have found, for instance, that mothers that were raised apart from their own mothers (AR) are less maternal towards their own offspring when they grow up. They show reduced levels of pup licking and time spent in lactating postures over pups (Gonzalez et al., 2001; Melo et al., 2006). Moreover, these effects can be ameliorated or reversed if during their earlier AR experience mothers were provided with somatosensory stimulation provided by periodic stroking or by cohabitation with a same aged conspecific (Gonzalez et al., 2001; Melo et al., 2006). How early AR experiences affect later maternal behaviour is not altogether clear, although a number of potential mediating mechanisms have been ruled out.

The onset of maternal behaviour at parturition is known to be under the influence of a shifting profile of gestational and parturitional hormones (Bridges, 1984). This led to the hypothesis that AR alters this endocrine profile. To assess the role of these hormones, Novakov & Fleming (2005) administered a sequence of progesterone and estrogen or control cholesterol to AR and MR adult virgin (ovariectomized) females and found, as expected, that all hormone-treated rats became rapidly maternal, showing all components of the behaviour. However, only AR rats showed a reduction in licking and crouching, similar to parturient AR
females (Novakov & Fleming, 2005). These results eliminated a role for hormonal effects of AR on mothering. A second and third hypothesis, undertaken simultaneously, was to determine whether the primary effect of AR might not be specific to the regulation of maternal ‘motivation’ directly, but may be a more general effect on other behavioural systems and physiologies that contribute to the normal execution and patterning of maternal behaviour. Hence, we explored the effect of AR on a number of the dopaminergically mediated mesolimbic and mesocortical function and systems, including those mediating attention and impulsivity (Burton et al., 2006; Lovic & Fleming, 2004; Lovic et al., 2006). The present study shows the role that general behavioural systems play in the regulation of very specific species-characteristic patterns.

During our tests of maternal behaviour we observed that although all AR mothers showed all elements of maternal behaviour and were able to rear their pups through normal weaning, they tended to be somewhat erratic and frequently disengage from pup-directed activities, attending instead to extraneous stimuli in the larger environment (e.g., door opening). From this we hypothesized that AR rats might have poor attentional and behavioural inhibition mechanisms and be more prone to distraction and that these effects of AR may account for the deficits in adult maternal behaviour. Testing these assumptions, we found that AR rats exhibit greater levels of locomotor activity, reduced attention and increased action impulsiveness (Lovic & Fleming, 2004; Lovic, Fletcher & Fleming, in prep; Chapter 3). These effects could be reversed to various degrees by providing additional licking-like stroking stimulation earlier in life during AR
(Lovic & Fleming, 2004, Lovic, Fleming & Fletcher, 2006: Lovic, Fletcher & Fleming, in prep; Chapter 3). Furthermore, the attentional performance of these rats, as measured by the attentional set shifting task and prepulse inhibition of the startle response, were positively associated with levels of pup licking by females when they gave birth (Lovic & Fleming, 2004). Mothers who were inattentive on an attention task licked their pups less (Lovic & Fleming, 2004).

The purpose of this study was to investigate the role of impulsive action, using DRL schedule, in the normal regulation of mothering behaviour in the rat and to determine whether AR deficits in mothering may also be partly attributable to the fact that these mothers have poor impulse control. We were particularly interested in assessing the effects of AR and stroking stimulation on impulsive action and maternal behaviour in the same group of rats, and the relation between these two behavioural endpoints. The question then, was to determine whether the reduced licking and crouching previously found in AR rats was due to the fact that these rats are impulsive and, hence, engage in (exploratory) behaviours that preclude attending adequately to their pups. We also explored the effect of replacement stroking or ‘licking-like stimulation’ on this relation.

**Methods**

**Subjects and Housing**

Twenty female Sprague-Dawley rats (AR-MIN, n=7; AR-MAX, n=6; MR=7) were used in this study. Rats were assigned to the same conditions as in Chapter Three. For procedural details see Chapter Two.
Apparatus

Operant Chambers

See Chapter 2 – Methods.

Procedures

Groups and treatments

See Chapter 2 – Methods.

Behavioural Procedures

Maternal Behaviour

At approximately 100 days of age, rats were mated. Twenty-one days after the introduction of males, the females were given 2 shredded paper towels as the nest building material, and placed in a room with other rats observed for their maternal behaviour. On the day of pup birth (PND0 – rats giving birth before 1700h) dam’s litters were culled to 4 males and 4 females. Maternal behaviour was assessed on PND 2, 4, 6, 8 and 10. Testing was done in the following fashion: rat cages were gently placed on the table and the pups were removed from the nest and the dam’s cage was returned to her previous position on a cage rack. The pups were weighed and 5 min later they were returned to their mother’s cage. They were placed in the diagonally opposite corner of the nest and the 10 min test was started. Observations were recorded using a computer based event recorder (NEC PC 8300). During maternal observations the following behaviours were of interest and reported
here: 1) pup anogenital licking, 2) pup body licking, 3) lactating posture – female is over pups giving them access to her ventrum, 4) time spent in nest.

**DRL-20s Procedures**

Several weeks after maternal behaviour assessment, rats were tested on the DRL-20s operant schedule. Procedures employed here were based on Fletcher (1995). Adult rats were gradually reduced to 85% of their free-feeding weights. They were trained to bar press for food (45 mg food pellets; Bio-Serv) on a continuous reinforcement (CRF) schedule during a 30 min session, on 7 consecutive days. All rats had 3 or 4 successful CRF schedule sessions (100 bar presses in 30 mins) with the final session one day prior to being switched to the DRL-20s schedule. On the DRL 20s schedule, rats were reinforced only if they responded at least 20 s since their previous response. Responses made less then 20 s since the last response were not rewarded and the 20 s period was reset. DRL-20s testing was done over 16 days (testing was done 6 days/week). Each session began with an illumination of the house light and insertion of the left lever into the chamber. The first response was always reinforced. For each session the following measures were collected: number of responses, number of reinforcers earned, and percent efficiency [(number of reinforcers earned/number of responses made) x 100].

**Data Analyses**

Maternal behaviour data was averaged across 5 days of testing (from PND 2 to 10) and analyzed using one-way analysis of variance (ANOVAs). DRL-
20s data for each of the 3 measures was averaged across 4 days (for a total of 4 blocks) and analyzed using repeated measure ANOVAs (Group x Block). Dunnett post hoc tests were used in order to assess which treatment group was different from the control group (MR). The relationships between maternal behaviour and impulsivity were assessed using partial correlations (controlling for group effects; see below).

Results

Maternal Behaviours

Pup Anogenital Licking

There were marginal overall group differences in durations of anogenital licking \( (F(2, 19)= 3.2, p=0.067) \), however, post-hoc analyses indicated that AR-MIN rats, but not AR-MAX rats, displayed significantly less anogenital licking than the MR rats \( (p<0.05) \). For all maternal behaviours see Figure 22.

Pup Body Licking

One-way ANOVAs indicated that groups displayed different durations of pup body licking \( (F(2, 19)= 4.7, p<0.05) \). Follow-up post-hoc analyses showed that AR-MIN rats differed significantly from the MR rats \( (p<0.05) \). AR-MAX rats were not different from either group.

Hovering

One-way ANOVA revealed marginal group differences in the duration of hovering over pups \( (F(2, 19)= 2.9, p=0.082) \). Post-hoc tests pointed that neither AR group was significantly different from the MR group \( (ps>0.083) \)
**Time Spent in Nest**

Overall group differences were found ($F_{(2, 19)} = 4.9, p<0.05$) and post-hoc analyses revealed that AR-MIN rats, compared to MR rats, spent significantly less time in the nest ($p<0.05$). AR-MAX rats were intermediate between AR-MIN and MR and did not differ from MR group.
**Figure 22:** The figure depicts mean time (sec) dams spent engaging in different maternal behaviours. *Significant differences between MR and AR rats (p<0.05).
**DRL-20s**

*Number of Lever Responses*

As can be seen in Figure 23, all groups showed a reduction in the number of responses across blocks (main effect of block; $F_{(1, 17)}= 40.6, p<0.0001$). Groups were significantly different ($F_{(2, 17)}= 10.7, p<0.05$) and Dunnett’s post hoc analyses indicated that both AR-MIN rats and AR-MAX rats, made more lever response than the MR rats ($p<0.05$; see Figure 23).

*Number of Reinforcers Earned*

There was a significant increase in the number of reinforcers earned across test sessions (main effect of block; $F_{(1, 17)}= 21.9, p<0.0001$). Groups were significantly different ($F_{(2, 17)}= 3.6, p<0.05$) and Dunnett’s post hoc analyses indicated that AR-MIN rats, but not AR-MAX rats, earned fewer reinforcers than MR rats ($p<0.05$; see Figure 24).

*Efficiency*

Along with number of responses, efficiency was one of the measures of impulsivity. As shown in Figure 25, there was an overall increase in efficiency across test blocks ($F_{(1, 17)}= 25.8, p<0.001$). There was also a main effect of group ($F_{(2, 17)}=9.5, p<0.05$). Post-hoc analyses indicted that AR-MIN and AR-MAX rats were significantly less efficient than MR rats ($p<0.05$). There were no significant interactions.
Figure 23: The figure depicts mean number of lever responses across four 4-day blocks of testing. There was a significant decrease in lever responding across blocks ($F_{(1, 17)} = 40.6, p < 0.001$). In addition, both AR-MAX and AR-MIN groups made significantly more responses compared to the MR rats ($p < 0.05$).
Figure 24: The figure depicts mean number of reinforcers earned across four 4-day blocks of testing. There was significant increase in the number of pellets that rats earned ($p<0.05$). In addition, compared to MR rats, AR-MIN rats earned fewer reinforcers across blocks ($p<0.05$).
Figure 25: The figure depicts mean efficiency across four 4-day blocks of testing. Efficiency increased across blocks ($F_{1,17} = 25.8, p<0.001$). MR rats were more efficient than AR-MIN and AR-MAX rats ($p<0.05$).
**Correlations Between Maternal Behaviour and Impulsivity**

In order to investigate the relationship between impulsivity (DRL-20s task) and maternal behaviours, partial correlations were performed (controlling for group effects). Efficiency scores were averaged across 4 blocks. We found a significant positive correlation between efficiency in the DRL-20s task and pup body licking \((r=0.431, p<0.05)\), hovering over pups \((r=0.6, p<0.01)\) and time spent in nest \((r=0.53, p>0.01)\). There was also a marginal correlation between efficiency and pup anogenital licking \((r=0.37, p=0.06; \text{ see Figures 26-29})\).
Figure 26: The scatter plot depicts a significant positive relationship between percent efficiency (DRL-20s) and duration of pup body licking (black regression line; $r=0.437$, $p<0.05$).
Figure 27: The scatter plot depicts a significant positive correlation between percent efficiency (DRL-20s) and the duration of hovering (black regression line; \( r=0.6, p<0.01 \)).
**Figure 28:** The scatter plot depicts a positive relationship between percent efficiency (DRL-20s) and in-nest time (black regression line; $r=0.53$, $p<0.01$).
Figure 29: The scatter plot depicts a positive relationship between percent efficiency (DRL-20s) and duration of pup anogenital licking (black regression line; r=0.37, p=0.06).
Multivariate Analyses of Maternal Behaviours with Efficiency as a Covariate

Given that there were significant group differences in time spent engaging in maternal behaviours and given that there was a significant correlation between some of the maternal behaviours and DRL-20s efficiency, we conducted multivariate analyses of maternal behaviours (separate analyses for each maternal behaviour that was different between groups) with efficiency as a covariate. Essentially, we asked a question whether group differences in maternal behaviours were driven by differences in DRL-20s efficiency. Consistent with our predictions, we found no group differences in maternal behaviours once efficiency was entered as a covariate (body licking, anogenital licking, in nest; \( ps>0.2 \)).

Discussion

The purpose of this experiment was to assess the relationship between maternal behaviour and impulsive action in MR and AR rats. Consistent with previous findings (Gonzalez et al., 2001), we found that AR rats spent significantly less time engaging in maternal behaviours than did the MR rats. Also consistent with previous findings (Chapter 3) was the observation that AR rats are more impulsive in the DRL-20s task; they made more lever responses, earned fewer reinforcers and were less efficient in earning rewards.

The data also suggest that time spent pup body licking and in lactating postures is positively correlated with efficiency in the DRL-20s task. Efficiency in the DRL-20s is an inverse measure of impulsivity. Rats that are more efficient
are thought to be more behaviourally inhibited and less impulsive. This suggests that more impulsive rats are less maternal.

We found that AR rats are less maternal than the MR rats (Gonzalez et al., 2001); however, the nature of this difference was not entirely clear. Rats can be less maternal for a variety of reasons such as reduced motivation and impaired learning. It is unlikely that AR rats are less maternal due to reduced motivation to be maternal. They readily approach pups, retrieve them and spend time with them indicating that pups are significant motivational magnets for AR rats. AR rats do not have learning impairments in either maternal or non-maternal contexts (Lovic & Fleming, 2004). While maternal behaviour in rats is initiated by parturitional hormones, maintenance of maternal behaviour is regulated through experiential-learning mechanism (Orpen & Fleming, 1987). Since AR rats continue to be maternal well after the parturitional hormones have subsided, their continued responding must be maintained by intact experiential-learning mechanisms.

However, other factors, such as reduced behavioural inhibition and attention, might impact maternal behaviour. Previously, we found a correlation between maternal behaviour and measures of attention: prepulse inhibition of the startle response (PPI) and intra-dimensional shifting (ID shift; attentional set shifting task) (Lovic & Fleming, 2004). PPI is thought to be a measure of sensorimotor gating, an ability to filter out and not respond to irrelevant environmental stimuli. Rats with lower PPI scores were less maternal as were those that took longer to make ID shifts (sustained attention); both suggesting
reduced attentional abilities are associated with reduced levels of maternal behaviours. In this study we report that reduced levels of maternal behaviour in AR rats might be driven by their impulsive action (reduced behavioural inhibition). These two findings are complementary as it is likely that reduced sensory motor gating (PPI) represents a tendency to ‘attend’ to multiple environmental stimuli and in the process the organism does not ‘focus’ on relevant environmental stimuli (e.g., pups).

While these novel findings represent a psychological mechanism of reduced maternal behaviour in AR rats, we don’t know which brain mechanisms mediate these effects. Early life adversity in the form of stress, periodic maternal deprivation, or isolation (artificial rearing) alters DA system (Brake et al., 2004; Hall et al., 1999; Lovic et al., 2006). These manipulations increase the tone of DA systems. Increased DA levels have been associated with increased impulsivity and reduced sensorimotor gating. Furthermore, given that DA systems are involved in motivation, learning and stimulus salience (Berridge, 2007), it is not surprising that AR rats’ motivation for natural rewards (food and pups) and learning are unaltered. In addition, based on their sensorimotor gating deficits and stimuli driven disinhibition, we suggest that AR rats are over-attributing salience to numerous environment stimuli and acting impulsively towards them at the expense of being maternal.

In summary, we found that impulsiveness is inversely related to the level of maternal behaviour that rat mothers will show towards their pups. Furthermore, we have found that impulsivity influences group differences in
maternal behaviours. However, since we do not have evidence of causal relationship between impulsivity and maternal behaviours, it is possible that a third variable (e.g., attention) might be influencing both dependent variables. Poor attention can lead to disengagement from focus on the pup and in turn lead to attention being diverted towards some other stimuli. This diverted attention is accentuated if rats have poor inhibitory mechanisms.
CHAPTER SEVEN

GENERAL DISCUSSION

This thesis represents a natural extension of experiments and findings previously reported in our laboratory relating to the effects of artificial rearing on attention, learning and naturally occurring behaviours (maternal and sexual behaviour) (e.g., Lovic & Fleming, 2004; Lovic, Fleming & Fletcher, 2006). Moreover, the thesis provides important and novel contributions to existing literature in that it shows that early life events, such as maternal and social deprivation, can influence adult impulsive behaviour per se. In previous work, we have shown that artificial rearing leads to a host of changes in behaviour and neurophysiology, including AR rats being less maternal, more active, more sensitive to DA agonists and having attentional deficits (Gonzalez et al., 2001; Lovic & Fleming, 2004; Lovic et al., 2006). During maternal behaviour testing, and also attentional set shifting testing, it was observed that AR rats are more active and more likely to respond, that is, less likely to inhibit responses to irrelevant or unrewarding stimuli (e.g., minor environmental noise). Hence, we hypothesized that they might be more impulsive. Impulsive behaviour was assessed using one task of impulsive action (DLR-20s) and two tasks of impulsive decision making or choice (DD and FCN8). We also assessed the relationship between impulsive action and maternal behaviour.

We found that AR-MIN, but not AR-MAX rats, make more operant responses in the DRL-20s task (Figure 3) and fewer of their responses result in rewards (lower efficiency; Figure 5). This increased impulsive behaviour can be
explained by AR-MIN rats’ increased operant responding immediately after receiving the reward (bin 2; Figure 7), as well as increased responding before the ‘wait’ period had elapsed (bin 5-9; Figure 7).

In the DD task, male AR-MIN rats preferred large delayed reward significantly more than did the MR rats. They were better able to tolerate delay of reinforcement (Figure 12). However, they were also slower to switch their behaviour when we switched the association between left and right lever and small and large reward (figure 13). Hence, they showed reduced behavioural switching, or flexibility.

In order to further explore impulsive decision making, or choice, we tested rats on the FCN8 schedule. In contrast to the DD task, behavioural flexibility is less confounding in assessing impulsive choice. AR and MR groups did not differ on measures of impulsivity – their average chain lengths were of similar values (Figure 20).

Lastly, since AR rats displayed greater impulsive action, we were interested in assessing the relationship between this measure and maternal behaviour. Female rats were tested on both maternal behaviour and impulsive action (DRL-20s) and we found a significant relationship between these variables. Rats that were more efficient (i.e., less impulsive) in the DRL-20s task were less maternal (Figures 26-29).

Overall, we can conclude that maternal and social deprivation, through artificial rearing, increased adult display of impulsive action but reduced or had
no effect on impulsive choice. In addition, impulsive action was inversely related to displays of maternal behaviour.

**Possible Mechanisms**

There are several possible mechanisms of the observed effects. The focus here will be on two critical neurotransmitters: serotonin and dopamine, which as mentioned previously, have been found to be altered by early life experiences with the mother.

**Serotonin**

The role of serotonin in DRL performance is well established. In general, the administration of selective serotonin reuptake inhibitors are associated with improvement in DRL performance. That is, increased serotonin functioning reduces operant responses and increases efficiencies in earning rewards (see O’Donnell, Marek & Seiden, 2003). Conversely, serotonin reductions decrease efficiencies at earning rewards. For example, nucleus accumbens serotonin depletions increase impulsive behaviour. Depleted rats display a leftward shift in peak responding. That is, many of their responses are made before the ‘wait’ period elapses (Fletcher, Chambers, Rizos & Chintoh, 2009). We have made similar observations with AR-MIN rats. On the other hand serotonin depletions are not associated with increased impulsive choice (Winstanley, Dalley, Theobald & Robbins, 2004).

Hence, the question is whether there is evidence that AR procedures result in a reduction of serotonin levels or functioning. While we did not measure serotonin levels in our rats, there are several studies pointing to alterations of the
serotonin system as a result of maternal separation. Specifically, maternal separation is associated with reduced serotonin content in the dorsolateral hippocampus (both sexes) and in the mPFC of male rats (Lee et al., 2007; Matthews, Dalley, Matthews, Tsai & Robbins, 2001), reduced sensitivity of 5-HT neurons in dorsal raphe nucleus (Gartside, Johnson, Leitch, Troakes & Ingram, 2003), reduced 5-HT transporter mRNA in raphe nucleus (Lee et al., 2007), and reduced 5-HT immunoreactivity in anterior hypothalamus (Veenema, Blume, Niederle, Buwalda & Neumann, 2006). Therefore, there are numerous pieces of evidence that show that maternal separation is associated with a reduction in 5-HT processes.

If the effects of maternal separation on the 5-HT system are mediated by maternal behaviour (rather than nutritional deprivation), then increased maternal behaviour should be associated with elevated serotonin functioning. While these studies cannot directly address this hypothesis, it is well demonstrated that handling, which is associated with increased maternal licking, is associated with the upregulation of 5-HTT and serotonergic 1A densities (Vicentic et al., 2006), as well as increased serotonin levels (Papaioannou, Dafni, Alikaridis, Bolaris & Stylianopoulou, 2002).

**Dopamine**

Increased dopamine functioning is associated with an increase in impulsive action (Robbins, 2002; Evenden, 1999) but a decrease in impulsive choice (Winstanley, Dalley, Theobald & Robbins, 2003). We observed similar changes in impulsive choice and action in AR-MIN rats. Hence, it is possible that
AR-MIN rats are characterized by hyperdopaminergic functioning. Supporting this notion, AR-MIN rats are more sensitive to the locomotor effects of dopamine agonist amphetamine (Lovic, Fletcher & Fleming, 2006) and also show greater basal dopamine output in the nucleus accumbens (V. Afonso, unpublished data). These findings are concordant with observations made in maternal separation experiments. Maternal separation is associated with increased dopamine levels in dorsal and ventral striatum (Matthews, Dalley, Matthews, Tsai & Robbins, 2001). Maternally separated rats show greater dopamine output in response to K+ (potassium) and amphetamine stimulation (Hall, Wilkinson, Humby & Robbins, 1999). Dopamine turnover, as measured by ratio of DA to DA byproduct DOPAC (3,4-dihydroxyphenylacetic acid), is decreased in the mPFC of maternally separated rats (Matthews et al., 2001). Together, these data suggest that the patterns of impulsive behaviour described in this thesis, are moderated/mediated by hyperdopaminergic functioning in AR-MIN rats.

Relevance

**Drug addiction**

Impulsivity has recently received much attention in the context of drug addiction research (Belin, Mar, Dalley, Robbins & Everitt, 2008; Jentch & Taylor, 1999; Perry & Carroll, 2008). Increased impulsive action is associated with compulsive drug taking in rats (Belin et al., 2008). It would be interesting and beneficial to explore drug taking in AR and MR rats. Indirect evidence suggests that early life factors are significant in determining future drug taking and
potential addiction (Meaney, Brake & Gratton, 2002). Hence, the AR model described here would be useful in delineating the role of maternal and social deprivation, as well as somatosensory stimulation on, future drug taking behaviour.

**Parallels to human studies**

The data presented in this thesis are relevant to human instances of maternal and social deprivation. They are particularly relevant to cases of institutionalized care, which is sometimes associated with extremely impoverished conditions (e.g., Romanian orphans). Many of the children described in the Romanin orphan studies have spent their early life period in institutionalized care but are often adopted into families later on. Several studies have shown that these children show persistent changes in behaviour, long after they leave the institutions. Their behaviour has been characterized by attentional deficits, hyperactivity and impulsivity (Chugani et al., 2001). However, little is know about the actual factors present or lacking in the early lives of institutionalized children, that result in lasting alterations in behaviour.

**Future Directions**

This thesis revealed important data regarding the nature of impulsivity as a result of early life maternal and social deprivation. Maternal and social isolation lead to increase in impulsive action without an increase in impulsive choice. However, the mechanism of these effects is unknown. We do not understand how maternal separation and somatosensory input, mimicking maternal licking,
alters adult expression of impulsivity. Hence, it would be important to assess the neurobiological mechanisms of these effects in adult rats. Several approaches would be beneficial here. Firstly, it would be beneficial to pharmacologically attempt to reverse increased impulsive action in AR-MIN rats. Numerous studies have shown that SSRIs can reduce responding and increase efficiency in the DRL task (O’Donnell et al., 2003). If the increased impulsive action seen in AR-MIN rats is due, at least to some degree, to reduced 5-HT levels, SSRIs might reverse these effects. Next, drugs targeting specific 5-HT receptors can be used further assess the role specific 5-HT receptors in the observed effects in AR-MIN rats.

Secondly, while we have preliminary evidence from in vivo micordialysis studies that AR-MIN rats show greater baseline DA release from the NAC, the 5-HT release profile of AR rats is unknown. Hence, it would be useful to know whether AR and MR rats’ differ in their DA and 5-HT profiles in relevant brain areas – OFC, mPFC, NAC and others.

In the opinion of the author, understanding neurotransmitter changes, namely DA and 5-HT, during assessment of impulsivity would be of greatest benefit to this body of research. In vivo microdialysis studies have been done looking at 5-HT and DA release during DD. However, in vivo microdialysis has poor temporal resolution (order of minutes) and is not necessarily useful since rats’ choices and responses occur on exponentially smaller scale (on the scale of seconds or milliseconds). Recently, fast scan cyclic voltammetry (FSCV) has been used to assess changes in DA and 5-HT release in response to brief stimuli
and in association with individual responses (e.g., single lever response) (e.g., Cheer et al., 2007; Hashemi et al., in press). FSCV has a subsecond temporal resolution. Hence, this technique would be useful in assessing dynamic DA changes (e.g., in the NAC) in association with making impulsive lever responses. One drawback of this technique is that it is limited to the assessment of neurotransmitter at the time. Furthermore, DA and NA cannot be differentiated from one another. Hence, while it is possible to make inferences about DA release from the NAC (as there is no NA in the NAC), this is not possible in the mPFC as DA and NA are colocalized there.

Previously we found that AR-MN rats show reduced attentional abilities. They show reduced PPI, an index of sensorimotor gating (early attentional filtering) and deficits in the ASST. Specifically, they required greater number of trials to criterion during ID and ED shifts. These data suggested deficits in both sustained attention and switching of attentional sets. PPI and ID shift performance was correlated with maternal behaviour. Rats with lower PPI and greater number of trials to criterion during ID shift were less maternal (Lovic & Fleming, 2004). In this thesis we found a negative correlation between impulsivity and maternal behaviour (Chapter Six). It would be beneficial to assess the relationship between attention and impulsivity in the same set of rats. 5-CSRTT would be ideal as this task allows for simultaneous assessment of attention (omissions) and impulsivity (premature responses). Rats maternal behaviour can be assessed and the relationship between all three measures (i.e., attention, impulsivity, and maternal behaviour), can be related to one another.
Impulsivity is still relatively poorly understood. For example, it is not clear how reward related cues and context modify impulsivity. It is possible that the presence of reward related cues and contexts can amplify the ‘wanting’ of rewards, which in turn can produce an increase in impulsive behaviour. Hence, it would be useful to assess how the presence of reward related cues affects impulsive behaviour in AR and MR rats. Furthermore, little is actually known about how early life factors, such as maternal deprivation, actually alters the reward system. For example, it is not clear if both aspects of reward, ‘liking’ (pleasure/hedonics) and ‘wanting’ (craving/motivation) (Berridge, Robinson & Aldridge, 2009) are affected by maternal deprivation. Alterations in one or both of these components of reward could potentially affect the expression of impulsivity. These questions could be explored by looking at progressive ratio responding, Pavlovian instrumental transfer and conditioned reinforcement.
References


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