BEHAVIOURAL RESPONSES OF ARTIFICIALLY REARED RATS TO REWARD AND NOVELTY

by

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AUTHOR'S DECLARATION FOR ELECTRONIC SUBMISSION OF A THESIS

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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ABSTRACT

Artificial rearing of infant rats is a useful method for studying the role of early experiences in neural and behavioural development because it permits precise control over key features of the early environment without maternal influence. The present thesis examined the behavioural response of artificially reared rats towards natural and drug-mediated rewards, as well as novel environments. Male Sprague-Dawley rats were separated from their mother and litter-mates starting on post-natal day five and reared artificially (AR, n = 39), or they were reared naturally with a foster mother and litter (MR, n = 43). On post-natal day 21, half the rats from each rearing group were housed in isolation and the other half were group-housed with 3-4 rats per cage. Following three weeks in their respective housing conditions, all rats were exposed to three behavioural tests in the following order: open field, elevated plus-maze and sucrose preference. Additionally, one of the two cohorts used in adolescent behavioural testing was later tested in adulthood for conditioned place preference in response to morphine injection (intraperitoneal, 10 mg/kg). Adolescent AR rats were found to be more active in the open field and in the elevated plus-maze than MR rats. Furthermore, although there were no differences between the groups in fearfulness in the open field, in their first experience on the elevated plus-maze AR rats were more anxious than MR rats in exploring the open arms. AR rats also showed increased preference for sucrose consumption relative to chow, although their overall caloric intake during the 1h test was lower than that of MR rats. In adulthood, AR rats displayed a stronger conditioned place preference response to morphine. There were no significant effects of housing condition on any of these outcome measures. These findings support the potential of this model to contribute to the understanding of the role of early experience in the development of behavioural motivation.

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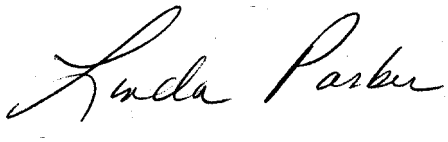
NOTES

This manuscript incorporates a measure (conditioned place preference to morphine) that is not part of the author's thesis work. This measure was performed on a cohort of rats used in the present thesis and its results contribute further information about the response to reward of artificially reared rats, thus aiding in the interpretations of the thesis findings. As indicated below, this work is included with permission from Ms. Shadna Rana and Dr. Linda Parker at Wilfrid Laurier University who collected and analyzed the data.

DECLARATION OF PERMISSION

We hereby declare that we authorize Anna Lomanowska to incorporate into her Master's Thesis the results of the conditioned place preference to morphine test conducted in our laboratory at Wilfrid Laurier University.

Shadna Rana



Dr. Linda Parker

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ABBREVIATIONS

ACTH – adrenocorticotrophic hormone

AR – artificially reared

AR-GRP – artificially reared, group housed

AR-ISO – artificially reared, isolation housed

CNS – central nervous system

CORT – corticosterone

CPP – conditioned place preference

CRF – corticotrophin releasing factor

DA – dopamine

EH – early handled

GABA – gamma-aminobutyric acid

HPA – hypothalamo-pituitary-adrenal

MR – maternally reared

MR-GRP – maternally reared, group housed

MR-ISO – maternally reared, isolation housed

MS – maternally separated

NAc – nucleus accumbens

NH – non-early handled

PND – post-natal day

PVN – paraventricular nucleus of the hypothalamus

VTA – ventral tegmental are

1 INTRODUCTION

In recent years, the formative role of early experiences in guiding brain development has become the focus of much research into the etiology of mental disorders. In particular, accumulating evidence suggests that adverse childhood experiences, such as physical and sexual abuse or neglect, are associated with increased risk of mental disorders in later life (Glaser, 2000; Heim & Nemeroff, 2001; Teicher, 2002; Brown, 2003; McEwen, 2003). Additionally, patterns of early interactions between young children and their parents or caregivers are predictive of future mental health outcomes (Bowlby, 1988; Field, 1998; Cassidy & Shaver, 1999). Studies using animal models have supported these findings, where prolonged separation from the mother and/or altered patterns of maternal care have been associated with changes in stress responsiveness and other maladaptive behavioural characteristics in the offspring (Liu et al., 1997; Plotsky & Meaney, 1993; Francis et al., 1999; Pryce & Feldon, 2003; Matthews & Robbins, 2003). These studies have also provided insight into the mechanisms by which certain aspects of the early environment, particularly maternal stimulation, affect development. Nevertheless, questions regarding the influence of other environmental factors still remain (Pryce & Feldon, 2003). Thus, the present thesis focused on an animal model that allows strict control of these early environmental factors through artificial rearing of infant rats to study the role of early experience in brain and behavioural development. The aim was to characterize the effects of artificial rearing using several measures of behavioural motivation in later life and to determine whether different post-weaning social experiences would further influence these outcomes.

The following is a review of the literature pertaining to the use of animal models in studying the role of early experience in development, including an overview of the artificial

rearing model. In addition, the neural and endocrine substrates involved in behavioural motivation as well as the experimental measures used in the present study are discussed.

2 REVIEW OF LITERATURE

2.1 Rat Models for the Study of Developmental Experiences

Several animal models, both in rodents and primates, have been developed to investigate the effects of early adverse experiences on later life functioning (Harlow et al., 1965; Seay & Harlow, 1965; Levine, 1960; Denenberg & Smith, 1963; Hall, 1975; Diaz et al., 1981). Rat models that have been commonly used include: early handling and maternal separation, adolescent isolation housing, and more recently, artificial rearing. The following sections discuss the experimental manipulations involved in these models, their consequences and hypothesized underlying mechanisms, as well as some of the complexities associated with this research. The discussion begins with an overview of the normal course of development of laboratory rats during the key stages of infancy and adolescence.

2.1.1 Characteristics of Normal Development in Laboratory Rats

Newborn rat pups are poorly developed at birth and depend solely on their mother to supply necessary nutrients in milk, to ensure thermal regulation, and to provide somatosensory stimulation essential for appropriate development (Croskerry et al., 1978; Hofer, 1994; Levine, 2001). Maternal care during the first few weeks of life involves a set of characteristic behaviours, termed nest-bouts (Leon et al., 1978; Hofer, 1983), interspersed by periods of short absence from direct contact with the litter (Stern, 1996). In the course of a nest-bout, the dam gathers the pups under her, licks and grooms them, then begins nursing. The nursing stance may take on either an active arch-back position, or a passive “blanket” position (Stern, 1996; Caldji et al., 1998). Although most rat mothers engage in these typical patterns of behaviour, natural variations in the frequency of maternal activities in laboratory rats are widely reported (Liu et al., 1997; Caldji et al., 1998). These are typically classified into two main profiles of care based on the frequency of

nest-bouts and the mother's nursing stance (see Liu et al., 1997 for review). The more active profile of care is characterized by frequent licking, grooming, and arch back nursing, whereas the more passive profile of care is characterized by significantly less licking, grooming, and arch back nursing. Importantly, variations in maternal care are associated with characteristic patterns of stress responsiveness (Francis & Meaney, 1999), as well as of maternal behaviour (Fleming et al., 2002) in the adult offspring. These observations have led to the premise that the amount of tactile stimulation received by pups regulates the development of their hypothalamo-pituitary-adrenal (HPA) axes, thereby influencing stress reactivity in adulthood. It is believed that such regulation reflects the mother's immediate environmental conditions and thus primes the offspring for the experiences that likely lie ahead (Hofer, 1983; Liu et al., 1997). For instance, fearful and anxious mothers naturally exhibit low frequencies of maternal behaviours and tend to be less maternally responsive towards their pups (Francis & Meaney, 1999; Liu et al., 1997). This prepares the pups to be more reactive to stressors, thereby increasing their chances of survival in a more demanding environment.

In addition to maternal stimulation, littermates also contribute to the early experiences of an individual rat pup (Bolles & Woods, 1964; Pryce and Feldon, 2003). During the mother's absence, pups huddle closely together in the litter to maintain their body temperature. Upon the mother's return, they compete with each other for access to milk (Bolles & Woods, 1964). In both cases, the development of an individual pup is affected by the pup's physical characteristics relative to other littermates. Litter size is also shown to affect maternal behaviour (Deviterne et al., 1990), such that mothers typically spend more time with larger litters. In fact, the induction of maternal behaviour is encouraged by the presence of many pups, where the suckling of several pups is necessary to induce an active arch-back nursing stance, to promote milk ejection, and to

enhance the quality of milk (Stern, 1996; Yagil et al., 1976). Thus, the litter plays an influential role in shaping the early development of a rat pup through thermal and tactile stimulation, and competition for resources, as well as through the manipulation of maternal behaviour.

Rats that are raised in the wild usually maintain close association with the mother for approximately 2 months after birth (Calhoun, 1962). In the laboratory setting, rat pups are typically nurtured by their mother for approximately three to four weeks after birth, at which point the young are ready to be weaned and placed into their own cages. However, if laboratory rats are permitted to remain with the mother, complete dispersion of the young does not occur before 60 days of age (Meaney & Stewart, 1981). At the time of weaning, littermates or pups of similar age are typically group-housed, which allows them to partake in repeated social interactions with their cage mates. The most frequent interaction of young rats, both male and female, during adolescence (from the time of weaning to approximately 60 days of life) involves play-fighting (Meaney & Stewart, 1981). Play-fighting, also referred to as rough-and-tumble play, is characterized by a variety of playful attack and defence activities (Pellis & McKenna, 1995). This behaviour is thought to be important in the development of adulthood dominance relationships (Pellis & Pellis, 1991; Pellis et al., 1992a). The features of play-fighting may be distinguished from aggressive encounters between adult rats; being dominated by another young rat does not result in fleeing, as it would in adult rats, but instead in the initiation of another bout of play fighting. Moreover, play fighting is marked by an absence of distress vocalizations that are often present in aggressive encounters in adult rats (Calhoun, 1962). Rough-and-tumble play between males and females is also important for the development of appropriate sexual behaviours in adulthood (Moore, 1985; Pellis et al., 1992b). This occurs in stages, starting with the onset of sexual attraction at around five weeks of life, the onset of mounting in males around

six weeks, and the progression towards adult sexual competency at approximately seven to eight weeks of life and onward (Meaney & Stewart, 1981). In sum, the social interactions of adolescent rats are influential in facilitating the development of characteristic adulthood behaviours.

2.1.2 Post-Natal Maternal Separation

The study of the long term impact of early environmental experiences in rats began approximately 50 years ago with simple manipulations of the rat post-natal environment (Levine, 1960; Levine et al., 1967; Denenberg & Smith, 1963; Denenberg et al., 1967) that simulated ethologically valid environmental circumstances (Caldji et al., 1998). In initial studies, individual rat pups were separated from their mother and litter for brief intervals of time over several days during the post-natal period. When tested in adulthood, these rats displayed a different behavioural profile compared to control rats that originated from a completely undisturbed litter (Levine et al., 1955). These findings suggested that early experiences leave a long-lasting impression on the course of behavioural development, thus providing the momentum for ongoing research in this field.

Post-Natal Maternal Separation Procedures

Since the early studies, modifications to the maternal separation procedure have involved changes to the duration, timing, and frequency of separation. Currently, the most commonly used procedures involve the daily separation of neonatal rats from the mother (and sometimes the litter as well), for either a brief separation, e.g., 15 minutes, termed early-handling (EH), or a longer period of separation, e.g., 3 hours, termed maternal separation (MS). The duration of separation during EH is shorter than the time a laboratory rat mother would normally spend away from direct contact with the litter. Conversely, separation for longer periods (more than 20 – 25

minutes) constitutes a deprivation of maternal care (see Jans & Woodside, 1990; Liu et al., 1997). The typical control groups in these separation experiments comprise rats that are not disturbed at all for the duration of the post-natal period, referred to as non-handled (NH), or rats that are only disturbed for husbandry purposes during the post-natal period, referred to as animal facility reared (AFR).

The separation procedure involved in the above manipulations consists of removing the individual pups or the entire litter from the home cage and placing them into another cage in the same or different room (placement in a different room prevents the pups and the mother from hearing each other's ultrasonic vocalizations). The new cage is kept warm by an external heat source to prevent hypothermia in the pups. During the time of separation, pups do not have access to nourishment because at this stage of development they can only obtain food from suckling the mother.

Post-Natal Maternal Separation Outcomes

Generally, EH rats exhibit different adulthood physiological and behavioural characteristics than rats that are not disturbed at all (NH) until weaning (Levine et al., 1955; Denenberg et al., 1967; Weiner et al., 1985; Meaney et al., 1989). In the open field test used to assess exploratory and affective behaviour, EH rats explore more, are more active at the start of the test, and defecate and urinate less in comparison to control NH rats, indicating reduced emotionality and fearfulness (Levine, 1960; Caldji et al., 1998, 2000a). Additionally, EH in infancy appears to have both anxiolytic and stress reducing effects in adulthood. For instance, EH rats express lower novelty induced suppression of feeding and lower acoustic startle responsiveness compared to NH rats (Caldji et al., 2000a), as well as a more rapid, less prolonged plasma corticosterone (CORT) response to a range of stressors (Levine, 1960, Meaney

et al., 1989). This pattern of stress activation is found to persist throughout the life of these rats (Meaney et al., 1988, 1991) and is generally indicative of reduced activation of the sympathetic branch of the autonomic nervous system and overall better stress regulation.

Despite the congruence of evidence regarding the differences between EH and NH rats, recent criticism addresses the appropriateness of NH rats as a control group (Lehmann & Feldon, 2000; Pryce & Feldon, 2003). It is argued that both EH and NH consist of experimental manipulations, where EH rats are handled more than typical laboratory bred rat pups, providing extra environmental stimulation, but NH rats are actually handled less than typical laboratory bred pups, resulting in environmental under-stimulation. To resolve this issue, an arguably more appropriate control group made up of AFR pups, which undergo some stimulation during regular husbandry procedures, is used in several studies (e.g. Campbell & Spear, 1999; Ladd et al., 2000; Pryce et al., 2001). When AFR rats are used as controls, the extent of the differences between the experimental (EH) and control (AFR) rats are reduced (Campbell & Spear, 1999; Ladd et al., 2000; Pryce et al., 2001), although some differences do exist (Campbell & Spear, 1999). This suggests that the favourable effects of EH on adulthood functioning are not as striking as those reported in relation to NH rats.

The long-term consequences of adverse experiences in rat pups have been further investigated using MS, the longer and theoretically more severe period of separation of pups from the mother. Animals reared in this way tend to show a behavioural and physiological profile that is similar to that of NH rats, which is thought to result from the environmental under-stimulation received by both groups (Pryce and Feldon, 2003). For instance, when adult MS rats are compared to adult NH and EH rats on the performance in the open field, EH rats are typically most active in first few minutes of the test, but habituate to the environment and show less

activity overall. On the other hand, MS and NH rats show initial fearfulness and reduced activity in this novel setting, followed by ongoing hyperactivity, which indicates their inability to habituate to the test environment (Meaney et al., 2002; Brake et al., 2004). Furthermore, in comparison to EH rats, MS rats typically exhibit increased endocrine and behavioural stress reactivity over their entire lifespan (Plotsky & Meaney, 1993; Francis & Meaney, 1999; Liu et al., 2000), including increased adrenocorticotrophic hormone (ACTH) and CORT peak and overall responses to an air puff stressor (Ladd et al., 2000), or higher levels of anxiety-like behaviour on the elevated plus-maze (Huot et al., 2001), which is similar to the endocrine and behavioural profile of NH rats.

The MS condition is also used to study the effects of early adverse experiences on the behavioural responsiveness to psychostimulants. MS rats are shown to exhibit enhanced self-administration of a high dose of cocaine and higher sensitivity to cocaine-induced locomotor activity compared to EH rats (Mathews & Robbins, 2003; Brake et al., 2004). Additionally, they show greater propensity for cross sensitization between the effects of a mild stressor and drug administration, such that repeated saline injections induce a greater sensitized response to amphetamine challenge in MS than in EH rats (Meaney, et al., 2002; Brake et al., 2004).

Although the examples presented above indicate general agreement across studies regarding the long-term behavioural consequences resulting from different early experiences, other studies report results incongruent with the pattern of outcomes presented thus far. For instance, Marmendal et al. (2004) found no differences between MS and EH rats in plasma CORT levels, exploratory behaviour, spontaneous locomotion, or voluntary ethanol intake, and Shalev & Kafkafi (2002) observed no differences between these two groups in open field exploration or in a sucrose preference test. In addition, Li et al. (2003) reported that MS rats

showed significantly less sensitization to the locomotor effects of cocaine in comparison to AFR rats, although increased sensitization would be expected in accordance with the findings of Meaney et al. (2002) and Brake et al. (2004). Lastly, in MS studies where pups are separated from both the mother and the litter, there were no significant differences found between MS rats and EH or AFR rats in CORT levels following restraint stress, in acoustic startle responsiveness, or in open field activity (Pryce & Feldon, 2003; Pryce et al., 2001). Both Marmendal et al. and Pryce and Feldon indicated that these incongruent findings may be explained partly by some methodological differences between studies, such as the duration and frequency of maternal separation, husbandry procedures across animal facilities, or the use of the light or dark period for behavioural testing. This suggests that more strict control over these factors is necessary before definite conclusions may be drawn regarding the specific long term behavioural effects of the MS condition.

Post-Natal Maternal Separation Outcomes – Mediating Mechanisms

The prominent hypothesis concerning the mediating factors behind the effects of EH and MS on developmental outcomes points towards the role of the mother in shaping the physiological development of her pups (Levine, 1975; Liu et al., 1997; Weaver et al., 2004). As previously mentioned, the interaction between the mother and her offspring are thought to regulate the development of the pups' HPA axis, where the frequency of maternal behaviour towards pups is associated with their adulthood pattern of stress responsiveness. Interestingly, the profile of maternal behaviour may actually be altered by disturbing the litter, as in the case of the handling and maternal separation studies (Liu et al., 1997). For instance, the frequency of maternal stimulation in mothers of EH pups is significantly elevated compared to mothers of NH pups, especially immediately after reunification (Liu et al., 1997). According to this hypothesis,

the altered nursing behaviour of mothers of EH pups results in better stress regulation in their offspring, which is consistent with their physiological and behavioural stress profile. In the case of prolonged MS, the lack of interaction with the mother over the course of the separation likely reduces the total amount of tactile stimulation received by the pups (de Kloet et al., 1996; Caldij et al., 2000), whereas distress experienced during the separation period may result in HPA activation. Therefore, the convergence of maternal influences and separation distress is thought to lead to the loss of homeostasis in the development of the HPA axis (Pryce & Feldon, 2003), subsequently programming the MS offspring to exhibit enhanced stress reactivity when probed in adulthood.

Recently however, other factors implicated in the separation procedure, such as the duration of separation, the disruption of feeding, or the influences of littermates, have been considered potentially influential in shaping subsequent developmental outcomes (Pryce & Feldon, 2003; Macri et al., 2004; Marmendal et al., 2004). Further research in this area is required to determine the role of different aspects of the early environment in mediating the observed developmental outcomes.

2.1.3 Post-Weaning Isolation Housing

Post-Weaning Isolation Housing Procedures

In addition to the pre-weaning experiences of rat pups, early post-weaning environmental experiences of adolescent rats are also influential in shaping their adulthood behaviour and neurochemistry (Hall, 1998). A common procedure used to manipulate the experiences of adolescent rats involves post-weaning isolation housing. In these experiments, weanling rats are typically housed individually in standard laboratory cages from where they are able to see, hear, and smell other rats, but are not able to physically interact with them (e.g. Smith et al., 1997;

Hall et al., 1997a). Control animals are housed in standard laboratory group cages, two to five rats per cage.

Post-Weaning Isolation Housing Outcomes

In tests of activity and exploration, rats housed in isolation for between two and 13 weeks after weaning tend to exhibit an overall hyperactive profile in comparison to group-housed rats. Elevated activity levels in isolation housed rats were observed during the initial period of exposure (5 – 15 minutes) to the open field situation (Del Arco et al., 2004, Molina-Hernandez et al., 2001; Schrijver et al., 2002), as well as for the total duration of the test (Del Arco et al., 2004; Domeney & Feldon, 1998; Heidbreder et al., 2000). Isolated rats were also hyperactive when tested in activity monitors (enclosed boxes smaller than the open field) for various durations of time (Bakshi & Geyer, 1999; Cilia et al., 2001; Domeney & Feldon, 1998; Hall et al., 1998a, b; Smith et al., 1997; Powell et al., 2002). However, several studies reported no differences in activity between isolated and group housed rats (Bowling & Bardo, 1994; Weiss et al., 2004; Arakawa, 2003). Others find the two groups to differ depending on the ambient light level (Hall et al., 1997b; 1998a; 2000), with greater differences becoming apparent under conditions of dim light. Furthermore, differences in activity were also noted between isolated rats of different ages (Muchimapura et al., 2003; Arakawa, 2003) or between different strains (Bakshi & Geyer, 1999; Hall et al., 1998a). Of note is the difference in the behaviour of post-weaning isolation housed rats compared to pre-weaning NH and MS rats. As mentioned earlier, MS and NH rats also display overall hyperactivity in the open field, but they typically show a fearfulness-related reduction in activity at the start of the test (Meaney et al., 2002; Brake et al., 2004). In contrast, isolation housed rats exhibit a hyperactive profile for the total duration of the

test. This difference points to potential complexities in interpreting the results of the open field test across various experimental conditions.

In terms of measures of fearfulness and anxiety, isolation housed rats appeared to be more emotional, as indicated by the lower amount of time spent in the centre of an open field in comparison to group housed rats (Arakawa, 2003; Hall et al., 1997c; 2000). In addition, isolates were found to display greater avoidance of the open arms of the elevated plus-maze (Hall et al., 1998a; Lopes Da Silva et al., 1996; Molina-Hernandez et al., 2001; Weiss et al., 2004), which is indicative of increased anxiety in this setting (Pellow et al., 1985). However, the response to this measure may be dependent on environmental conditions, such as the light level (Hall et al., 1998a), where greater differences between isolation and group housed rats became apparent under bright light conditions. Interestingly, Lodge and Lawrence (2003) reported that the neurochemical effects of anxiolytic drugs are dependent on the post-weaning rearing condition, pointing to possible neurophysiological changes associated with isolation housing. Despite the apparent behavioural differences between isolated and group housed rats, differences in endocrine stress hormones are not as clear. For instance, elevated basal and stress-induced CORT (Gambardella et al., 1994) and ACTH (Weiss et al., 2004) have been observed in isolation housed rats compared to controls. However, some studies report no differences between the two groups of rats on similar measures (Hall et al., 2000; Schrijver et al., 2002). This discrepancy may be attributed to the use of different rat strains across these studies or other methodological incongruencies, but due to the limited evidence available, this issue requires further exploration.

Several studies have also reported that isolation housing affects the responses of rats towards natural and drug-mediated rewarding stimuli. In terms of consumption of appetitive

substances, isolation housed rats have been shown to display enhanced sucrose feeding in comparison to group housed rats (Hall et al., 1997b; 1998c). Additionally, in Pavlovian conditioning experiments, isolated rats show enhanced excitatory conditioning and acquisition of conditioned inhibition (Harmer & Phillips, 1998a), as well as an enhanced acquisition of discriminative approach towards a rewarding stimulus (Harmer & Phillips, 1998b). However, when anticipatory behaviour (van den Berg et al., 1999a) or lever pressing (Harmer & Phillips, 1998b) towards a conditioned stimulus associated with sucrose was measured, the response of isolated rats was suppressed (van den Berg et al., 1999a) or the same (Harmer & Phillips, 1998b) compared to group housed rats. The behavioural response towards sucrose may be classified into two opponent processes involved in the response to reinforcing stimuli: motivational (searching, approach) and consummatory (Wilson et al., 1995; Bassareo & Di Chiara, 1997). These findings suggest that isolation housing may affect the consummatory response towards sucrose differently than the motivational response, but further studies that directly compare these two processes are required to investigate this hypothesis. In terms of sensitivity to drugs of abuse, several studies found that isolation enhanced the locomotor stimulating effects of cocaine (Phillips et al, 1994; Hall et al., 2001), amphetamine (Hall et al., 2001; Smith et al., 1997), as well as ethanol (Hall et al., 1998a). In contrast, other studies reported no such differences in locomotor excitation or sensitization in response to amphetamine (Bowling & Bardo, 1994; Weiss et al., 2001) and cocaine (Smith et al., 1997), or reduced reactions to cocaine in isolation housed rats (Boyle et al., 1991). Furthermore, isolated and group housed rats show no differences in sensitivity to place preference conditioning with amphetamine (Bowling & Bardo, 1994; Wongwitdecha & Marsden, 1995; Schenk et al., 1986), but isolated rats do show reduced sensitivity to place conditioning with cocaine (Schenk et al., 1986) or morphine (Wongwitdecha

& Marsden, 1996). Similarly, isolated rats show either lower levels of intravenous self-administration of cocaine compared to controls (Phillips et al., 1994) or the same level of intravenous self-administration of amphetamine (Schenk et al., 1988; Boyle et al., 1991), cocaine and heroine (Bozarth et al., 1989). Only in one study do isolation reared rats show greater levels of self-administration of cocaine than group housed rats (Schenk et al., 1987). The above findings indicate that the effects of drugs of abuse on isolation-housed rats may be dissociated into two levels of responding, depending on whether drug administration is direct or whether it requires the establishment of a contingency between a stimulus and drug reinforcement. Future studies are required to explore this phenomenon.

There are certain caveats that should be considered in interpreting the behavioural findings presented above, particularly with respect to the length of isolation used across the various studies. In all these reports, the start of isolation occurred directly at weaning (typically around post-natal day 21), but the length of isolation varied from approximately two weeks to 13 weeks. In most studies rats were isolated for at least six to eight weeks after weaning, but several studies used rats isolated only during the first three to four weeks after weaning, which coincides with rat adolescence (Bowling & Bardo, 1994; Lopes Da Silva et al., 1996; van den Berg et al., 1999a**;** Bakshi & Geyer, 1999; Molina-Hernandez et al., 2001; Muchimapura et al., 2003; Arakawa, 2003). This variability in the duration of isolation adds to the difficulty in interpreting the above findings; not only does it fail to account for any of the reported inconsistencies, but it also does not provide any notable clues as to an association between performance on behavioural tests and the length of isolation. Arakawa (2003) investigated the possibility of differences in activity in an open field setting in rats that were isolation housed for either two, five, or 15 weeks and found no significant differences in the pattern of performance

on the task between the three groups as compared to age-matched control groups. Nevertheless, while the duration of isolation may not significantly affect performance on different tasks, other variables that typically confound experimental results, including strain and gender, as well as the age of the rats at the time of testing, may influence these findings.

Post-Weaning Isolation Housing Outcomes – Mediating Mechanisms

As mentioned earlier, adolescent rats engage in numerous bouts of social interaction consisting largely of rough-and-tumble play, which is thought to be important for future social functioning (Pellis & Pellis, 1991; Pellis et al., 1992a; Moore, 1985; Pellis et al., 1992b). Isolation housing during this time prohibits any social contact and has been shown to alter patterns of social interactions in later life (e.g. Hol et al., 1999; van den Berg et al., 1999a; 1999b). Beyond the disruptions in learning of appropriate social responsiveness, the deficiency in contact with other rats during this early post-weaning stage may also mediate long-term physiological changes due to alterations in the functioning of the HPA axis, as well as neural changes similar to those observed following pre-weaning maternal separation. However, due to the lack of consistent evidence regarding the developmental outcomes associated with post-weaning isolation housing, hypotheses regarding the mediating mechanisms of these outcomes have been mostly speculative.

Several studies suggests that the rough-and-tumble play aspect of early social interactions may be particularly important in providing appropriate stimuli for the developing brain of adolescent rats (Pellis & McKenna, 1995; Holloway & Suter, 2004). Juveniles of most mammalian species have a play drive, which arises spontaneously and is associated with neural impulses originating from thalamic structures (Panksepp et al., 1984; Panksepp, 1998). Additionally, adolescent rats appear to find playful interactions rewarding (Pellis & McKenna,

1995; Vanderschuren et al., 1997), as demonstrated in conditioned place preference paradigms (e.g. Calcagnetti & Schechter, 1992; Douglas et al., 2004). This implies that several neurophysiological systems may be involved in mediating play behaviour and leads to the hypothesis that the functioning of these systems may be altered when the expected input is deficient, as in the case of socially isolated adolescent rats.

Furthermore, when compared to isolation housing in adulthood, early social isolation may have more wide-spread and long-lasting consequences on behaviour and neurophysiology because the brain is still undergoing developmental changes (Spear, 2000). Although isolation housing in adulthood is also associated with some changes in subsequent behavioural and physiological functioning (e.g. Arakawa, 2003; Miura et al., 2002), studies that directly compared isolation during adolescence versus isolation in adulthood reveal that early isolation housing results in more profound changes in behaviour (van den Berg et al., 1999a; Arakawa, 2003; Schenk et al., 1990). Interestingly, in a review of the characteristics of adolescent brain and behavioural manifestations, Spear (2000) indicates that adolescent brains differ from adult brains in the functional balance between mesocortical and mesolimbic brain regions, where inhibitory DA input into cortical areas (e.g. pre-frontal cortex) is at a higher level, whereas DA input into striatal regions is lower than in adult brains. The author suggests that this difference in balance may be further altered by stressors, which affect mesocortical DA projections more than mesolimbic DA systems. If isolation rearing during adolescence is viewed as a stressor, it is possible that it may lead to further alterations in these systems leading to potential long-term developmental changes.

2.1.4 Artificial Rearing

The artificial rearing technique has proven very useful in studying the effects of early dietary manipulations on the developing brain, independent of the correlated effects of diet on maternal physiology and behaviour that are inherent in other methods that involve feeding the diet to the pregnant and/or lactating dam (e.g., Smart et al., 1984; Wainwright et al., 1999). Recently, however, it has been realised that the artificial rearing procedure may also prove to be an informative model in studying the effects of adverse neonatal experiences, such as maternal deprivation, on developmental outcomes (e.g. Gonzalez et al., 2001; Lovic & Fleming, 2004).

Artificial Rearing Procedures

The artificial rearing technique was first introduced by Messer et al. (1969) and modified by Hall (1975) and Diaz et al. (1981). It was developed as a method of rearing neonatal rat pups as young as four or five post-natal days (PND) independently of their mother and littermates, and at the same time providing the basic needs of pups, including nutrition, warmth, and tactile stimulation in a strictly controlled fashion. AR rat pups are fed a rat milk substitute formula through an intragastric cannula connected to an automated infusion device (Diaz et al., 1981; Auestad et al., 1989). They are normally housed in individual plastic cups filled with bedding and floating in a water bath at 36 – 38°C to maintain steady ambient temperature. From their cups, the pups are able to smell and hear other AR rat pups, as well as their mothers if they are housed in the same room, but they do not engage in any physical interaction with other rats. Twice daily, the pups are gently stroked with a warm, moist tissue or paint brush in their anogenital region to promote urination and defecation. AR pups are typically raised in these conditions until PND 18 or 19, when they are weaned off the rat milk formula onto regular lab chow and placed in standard laboratory cages. The typical control group used in artificial rearing studies are mother reared (MR) pups. Both the AR and the MR pups are usually weighed daily

to monitor the growth trajectories of the two groups. The typical survival of AR pups for the duration of the procedure, given the initial surgery is successful, is between 75 to 100%.

Several modifications of this standard procedure are possible, including manipulating the feeding schedule, changing the ambient temperature, controlling the duration of isolation, providing extra tactile stimulation by inserting a piece of artificial fur into the housing cup or extending the duration and frequency of daily stroking, and adding other enriching stimuli to the pups environment (e.g. Smart et al., 1990; Levy et al., 2003).

Artificial Rearing Outcomes

Thus far, there have been a limited number of studies undertaken to explore the long-term effects of early maternal deprivation using artificial rearing, likely because the procedure is more demanding than the previously described methods. Out of five studies that assessed activity levels in AR rats, two studies tested the animals during the pre-weaning period (Goldenring et al., 1982; Thomas et al., 2000) and three performed tests in adulthood, between 12 and 22 weeks of age (Tonkiss et al., 1987; Kaneko et al., 1996; Gonzalez et al., 2001). During the pre-weaning period, Goldenring et al. found AR rats to be more active than MR rats in a 60 minute test of activity (apparatus not reported) on PND 12 and 15, but there were no differences on PND 19 and 25. Similarly, a more recent study by Thomas et al. found no differences in activity levels between AR and MR rats at any time during a 60 minute open-field test conducted on PND 18 and 19. Interestingly, Goldenring et al. reported that the AR rats demonstrated reduced activity at the start of the test, but remained active for the duration of the test, suggests that they may be more fearful in this test situation midway through the pre-weaning period.

Two studies of activity in adult AR rats reveal that they are more hyperactive compared to MR rats in open field tests of short duration (6 – 10 minutes) (Tonkiss et al., 1987; Gonzalez

et al., 2001). In contrast, Kaneko et al. (1996) found no differences in performance between the two groups in a five minute open field test. In terms of emotionality and fearfulness, AR rats have shown greater avoidance of the centre of the open field than control rats in one study (Kaneko et al., 1996), but reduced avoidance in another (Gonzalez et al. 2001). Thus, the inconsistencies in the above findings warrant further investigation of these effects.

In terms of stress responsiveness, only two studies provide insight into the functioning of the endocrine stress system in AR rats. Kelly et al. (1991) found no differences in stress-induced CORT levels between adult AR and MR rats of either sex, although prior exposure to ethanol during artificial rearing appeared to potentiate the CORT levels of female rats. Another study by Ward et al. (2004) reported that there were no differences between infant AR and MR rats on PND 12 in baseline or stress-induced CORT levels, leading the authors to conclude that artificial rearing does not disturb the stress hyporesponsive period (SHRP) that is typically observed in neonatal rats. Additionally, when both group were exposed to 24 h of food deprivation (maternal deprivation in the MR rats), which usually acts as a stressor during the SHRP and potentiates CORT responses to subsequent stress (Rosenfeld et al., 1991,1993; Levine et al., 1991), the baseline and injection stress-induced CORT levels were higher in MR than AR rats. The above findings suggest that some alterations in the functioning of the HPA axis of AR rats may indeed exist under certain conditions. However, due to limited evidence, further examination of the endocrine stress response profile of AR rats is required.

Despite the severe nature of social deprivation during artificial rearing, the subsequent effects of this procedure on the developmental outcomes examined thus far suggest that it does not have extremely deleterious developmental consequences. This is also supported by studies of cognitive performance and social behaviour. For instance, studies of cognitive function report

no effects of artificial rearing alone on the performance on a variety of tasks in the water maze (e.g. Girard et al., 2001; Girard & Wainwright, 2002; Levy et al., 2003). Additionally, two investigations of social communication find no differences in the acquisition of the social transfer of diet preference between adolescent AR and MR rats (Galef & Smith, 1994; Girard et al., 2003). However, some recent investigations do report differences between these two groups on the same task (Levy et al., 2003), as well as on measures of attention, including reduced pre-pulse inhibition of the startle response and poorer performance on an attentional set-shifting task (Lovic and Fleming, 2004). Interestingly, several studies have reported robust deficits in maternal behaviour of AR rats. Specifically, adult AR rats spent less time crouching over pups and displayed less pup retrieval and licking (Gonzalez et al., 2001; Gonzalez & Fleming, 2002; Lovic & Fleming, 2004). It has been shown that maternal behaviour is mediated in part by the brain's motivation circuitry (Hansen, 1994; Insel, 2003) and also that rat pups constitute a source of reinforcement to the dam (e.g., Fleming et al., 1994). These findings suggest that artificial rearing may have long-term effects on the development of the motivation system. Thus, the aim of the present thesis is to further explore this possibility by probing the responses of AR rats towards both natural and drug-mediated rewards.

2.2 Behavioural Motivation

In order to maximize the chances of survival, an animal must recognize cues in its environment that should be approached (e.g., food, a sexual partner) or avoided (e.g., a predator) and generate an appropriate behavioural response to these cues (Chambers et al., 2003). The process implicated in the assessment of cues in the external environment, as well as the internal state (e.g., hunger), and the determination of the necessary behavioural response may be referred to as behavioural motivation. The enactment of an appropriate behavioural response may be

complicated by the presence of multiple survival goals at any given time and the availability of several behavioural strategies to obtain these goals (Dorman & Gaudiano, 1998). The synthesis of information implicated in this process requires the involvement of integrated neural circuitry that assembles sensory input, selects an appropriate response, and drives the motor output. Such circuitry, encompassing cortical-striatal-thalamic-cortical pathways, has been identified in both animals and humans (Kalivas et al., 1999; Chambers et al., 2003). The following sections will outline the substrates involved in this neural motivation circuitry. Due to the potential involvement of the endocrine stress system in mediating some of the effects of early experiences on future behavioural functioning, the impact of stress activation on the motivation circuitry will also be examined.

2.2.1 Neural and Endocrine Substrates Implicated in Behavioural Motivation

Neural Substrates of Motivation

The motivation circuitry comprises ascending and descending fibres of the medial forebrain bundle, which connect to basal forebrain and midbrain structures of the central nervous system (CNS) (Nestler et al., 2001). The key brain areas implicated in this system include the nuclei of the ventral tegmental area (VTA) in the brain stem, as well as the termination site of its projections at the nucleus accumbens (NAc), located in the midbrain ventral striatum. The NAc is regarded as an integrator of inputs from limbic (basolateral amygdala and hippocampus), cortical (prefrontal cortex), and midbrain structures (mediodorsal thalamus), which are implicated in the overall function of the reward system (Kalivas & Nakamura, 1999). Another key area interconnected with this system is the ventral pallidum, which projects directly to motor nuclei and is considered as the mediator of primary behavioural output.

Several neurotransmitters function within the reward system, including glutamate, gamma-aminobutyric acid (GABA), dopamine (DA), serotonin, and opioid peptides (Nestler et al., 2001). The main substrate implicated in the reinforcement of appropriate behavioural responses to specific sensory cues is thought to be DA. Activation of DA in the mesocorticolimbic system, particularly the NAc (Piazza & LeMoal, 1996), is believed to act as a learning signal to form associations between external and internal cues and the rewarding or aversive effects of a behavioural response (Spanagel & Weiss, 1999). DA is also thought to act as an incentive signal that encourages the production of a specific behavioural response towards a rewarding stimulus once an association has been made (Wilson et al., 1995). A consummatory response related to the experience of pleasure and enjoyment associated with the presence of the reward, know as hedonic evaluation, is thought to be mediated by opioid peptide neurotransmitters (Bassareo & Di Chiara, 1997).

The Stress Response System

The stress response is primarily characterized by sympathetic nervous system activation and the successive release of hormones through the (HPA) axis (Nelson, 2000). Generally, a stress-inducing stimulus (e.g. frightful experience) is perceived by the sensory processing systems of the CNS and the nature of the stressor is assessed by various interacting brain regions, including the hippocampus, the amygdala, the prefrontal cortex, the locus ceruleus, the bed nucleus of the stria terminalis, as well as several related areas (Herman & Cullinan, 1997; LeDoux, 1994). When the stimulus is perceived as stressful, an immediate response of the sympathetic nervous system results in the secretion of epinephrine from the adrenal medulla and norepinephrine from the sympathetic nervous system (Nelson, 2000), which induce overall physiological activation and initiate the fight-or-flight response. Additionally, converging

stimulatory influence from these brain regions acts to induce the release of the corticotropin releasing factor (CRF) from the paraventricular nucleus of the hypothalamus (PVN) (Herman & Cullinan, 1997), beginning a cascade of hormone release. CRF acts on the anterior pituitary gland to induce the release of ACTH, which in turn stimulates the release of glucocorticoids from the adrenal cortex. CRF also acts back onto central targets involved in the stress response, including the amygdala and locus ceruleus, which is thought to further enhance the stress response (Lehnert, 1998; Carrasco & Van de Kar, 2003).

Adrenal glucocorticoids, cortisol in humans and CORT in animals, are the primary substrates that modulate the effects of the stress response (Piazza & Le Moal, 1997) through modification of energy metabolism and immune functioning, as well as through their action at the central nervous system. The overall effect of glucocorticoid release is thought to prevent harmful overreaction of the organism to the physiological effects of the stressor by dampening its primary response and enabling the individual to cope better (Piazza & Le Moal, 1996; 1997). Glucocorticoids also feedback onto the CNS, including the PVN and the hippocampus, in order to reduce further activation of the stress response, which eventually results in limiting the secretion of stress hormones to basal levels (Herman & Cullinan, 1997). This negative feedback system of the HPA axis is essential in preventing the potentially harmful consequences of prolonged action of elevated levels of glucocorticoids, such as immune system inhibition.

Stress Response and Reward System Interactions

Accumulating evidence suggests that certain endocrine substrates involved in the stress response may have a direct effect on the motivation system. In particular, there is strong evidence for the association between glucocorticoid levels and DA release. For instance, receptors for glucocorticoids have been found on VTA DA cell bodies (Harfstrand et al., 1986).

Additionally, the phasic increase in the secretion of glucocorticoids typically stimulates DA release, whereas adrenalectomy and the subsequent reduction in glucocorticoid levels leads to a suppression of DA release (Piazza & Le Moal, 1996). Other evidence for this association comes from studies using drugs of abuse to probe the motivation circuitry. Drugs such as cocaine, amphetamine, alcohol, nicotine or opiates chemically activate this neural system, exploiting it to produce powerful sensations of reward and pleasure (Di Chiara, 1995). Stress related potentiation of the behavioural and physiological response to these substances has been associated with glucocorticoid release (Piazza & Le Moal, 1996; 1997). For instance, endogenous CORT levels in rats are correlated with drug consumption (Piazza et al., 1989). In addition, exogenous administration of CORT enhances the reinforcing action of psychostimulants (Piazza et al., 1989), whereas adrenalectomy results in diminished response (Piazza & Le Moal, 1996). Interestingly, rats are shown to self-administer CORT alone (Deroche et al., 1993), indicating that this hormone is strongly linked to the functioning of the neural motivation circuitry. On the whole, the evidence of an interaction between the stress and the motivation systems is not surprising. Given that the induction of the stress response towards particular environmental cues alters the internal state of an animal, the interaction between these systems likely acts to mediate an appropriate behavioural response to a stressful situation.

2.2.2 Behavioural Measures of Motivation in Rats

The functioning of the motivation system may be measured using a variety of behavioural tests performed on experimental animals, where the response to both appetitive and aversive environmental cues may be probed using both natural and chemical stimulation. To assess the motivated behaviours of AR rats, four such tests were used in the present thesis. Two of the tests, sucrose preference and conditioned place preference to morphine, measured the

response to rewarding substances. The other two tests, open field and elevated plus-maze exploration, measured the response to novel environments that contained some aversive components.

Sucrose is an appetitive substance that is considered a strong natural reward to laboratory rats (Agmo et al., 1995; Delamater et al., 2000; Wyvell & Berridge, 2001). As with other natural reinforcers, its consumption and hedonic evaluation is mediated by the motivation circuitry (Levine et al., 2003). The sucrose preference test performed here was based on previous work by Sills and Vaccarino (1994), with some modifications. Over several days, rats were presented with jars filled with laboratory chow and sucrose, and were allowed to feed for an hour. On the last test day, their overall consumption of both substances and their relative preference for the more appetitive substance, sucrose, was measured. Previous work has shown that increased sucrose preference is associated with enhanced responding to DA agonist drugs, such as cocaine or amphetamine (Sills & Vaccarino, 1994; Gosnell, 2000; DeSousa et al., 2000). In this respect, the sucrose preference test may be used as an indicator of the general sensitivity of the motivation system to both natural and drug-mediated reinforcers.

Morphine is a sedative and pain-relieving drug extracted from opium with highly reinforcing and addictive properties (Nestler et al., 2001). It acts to directly stimulate opioid receptors in the brain, but it also has indirect effects on other neurotransmitter systems in the motivation circuitry, particularly DA (Nestler et al., 2001). In the present experiment, the rewarding properties of morphine were used in the conditioned place preference test to probe the motivation system with respect to the formation of associations between environmental cues and internal states (Bardo & Bevins, 2000). Morphine injections were administered in the presence of specific environmental cues over several trials. During the final probe trial, rats were exposed

to the cues associated with morphine injections as well as to the cues associated with placebo injections, and their degree of preference for the morphine-associated cues was measured. The results provide information about the strength of responsiveness of AR rats towards cues associated with a rewarding substance.

The response of AR rats to a novel environment was tested in the open field, an experimental apparatus consisting of an empty square enclosed by walls with an open top. This apparatus provides both an appetitive and an aversive environmental cues for rats (e.g., Hall et al., 1997a) and it allows for the measurement of several components of behaviour, including activity, exploration, and affective response (Roth & Katz, 1979; Seliger, 1977; Hall et al., 1997a; Gonzalez et al., 2001). While rats are typically inclined to explore the open field because of the reinforcing properties of novelty (Bardo et al., 1996; Klebaur & Bardo, 1999), the open nature of the apparatus, particularly its centre, may be fear provoking (Archer, 1973; Prut & Belzung, 2003). In this context, the frequency of entries into the centre of the apparatus is used as a measure of the propensity for exploration (Hall et al., 1997a), while the time spent in the centre is typically used to assess fearfulness and emotionality (Gonzalez et al., 2001). General activity is measured by the distance travelled in the open-field (Hall et al., 1997a).

The elevated plus-maze is another test used here to measure the response of rats to a novel environment, although this test is primarily designed to generate aversive conditions. The elevated plus-maze consists of a plus sign shaped platform that is raised above ground and has four arms that are available for exploration. Two of the arms are enclosed by walls and the other two arms are un-walled, revealing an open space surrounding the apparatus (Pellow et al., 1985). Rats tend to avoid the open arms of the maze and their degree of avoidance is considered to be a measure of the level of anxiety experienced due to the exposure to the surrounding open space

(Treit et al., 1993). The number of entries into all arms of the maze is considered a measure of behavioural activation in this context (Pellow et al., 1985). In the present study, rats were tested twice in this environment, with a 48 h interval between the first and second test. The second exposure is considered to be a measure of conditioned fear, where rats form an association between the internal state of anxiety experienced on the first trial and the open arms (e.g., File & Zangrossi, 1993; Holmes & Rodgers, 1998). Rats on the second trial tend to be more fearful, which is indicated by increased avoidance of the open arms. On the whole, in contrast to the appetitive cues provided in the sucrose preference and conditioned place preference test, the open field and the plus-maze tests are used to assess the response of AR rats to the aversive aspects of novel environments.

3 STUDY RATIONALE AND DESIGN

The reviewed literature suggests that manipulations of the early environment of infant rats alter their physiology and behaviour in adulthood. The effects of these manipulations have been typically attributed to changes in maternal behaviour towards pups, but recently, other factors of the early environment have also been considered as influential in development. By allowing the opportunity for more direct manipulation and control of these factors, the artificial rearing method provides a useful approach to this research.

Despite the seemingly severe nature of maternal deprivation associated with the artificial rearing procedure, uniform deleterious consequences have not been reported in AR rats. However, several recent investigations have found robust deficits in maternal behaviour of AR rats. As maternal behaviour is mediated in part by the brain's motivation circuitry the present thesis aimed to further probe the motivational responses of AR rats. To accomplish this, AR and control MR rats were tested for their responses to rewards and novel environments.

Figure 1 provides a graphical representation of the study design. In brief, on PND 5, two cohorts of male rats were assigned to either the AR or the MR condition, and these groups were further subdivided at weaning (PND 21) into two housing conditions, isolation or group housing. In all, four experimental groups were formed: artificially reared, isolation housed (AR-ISO), artificially reared, group housed (AR-GRP), maternally reared, isolation housed (MR-ISO), and maternally reared, group housed (MR-GRP). Following three weeks in their respective housing conditions, all rats were submitted to three consecutive behavioural tasks: the sucrose preference test, the open field test and the elevated plus-maze test. One of the two cohorts was also tested in adulthood for their conditioned place preference in response to morphine injections. This test was performed in adulthood in order to determine whether the effects of artificial rearing persist

throughout the life-span. The isolation housing procedure was used in this study to investigate whether the post-weaning social experiences of isolation or group housing would be influential in the course of behavioural development of AR rats. This procedure also served as a comparison to previously used procedures of early environmental manipulations in MR rats, thus allowing for clearer interpretation of the present findings in light of the existing literature.

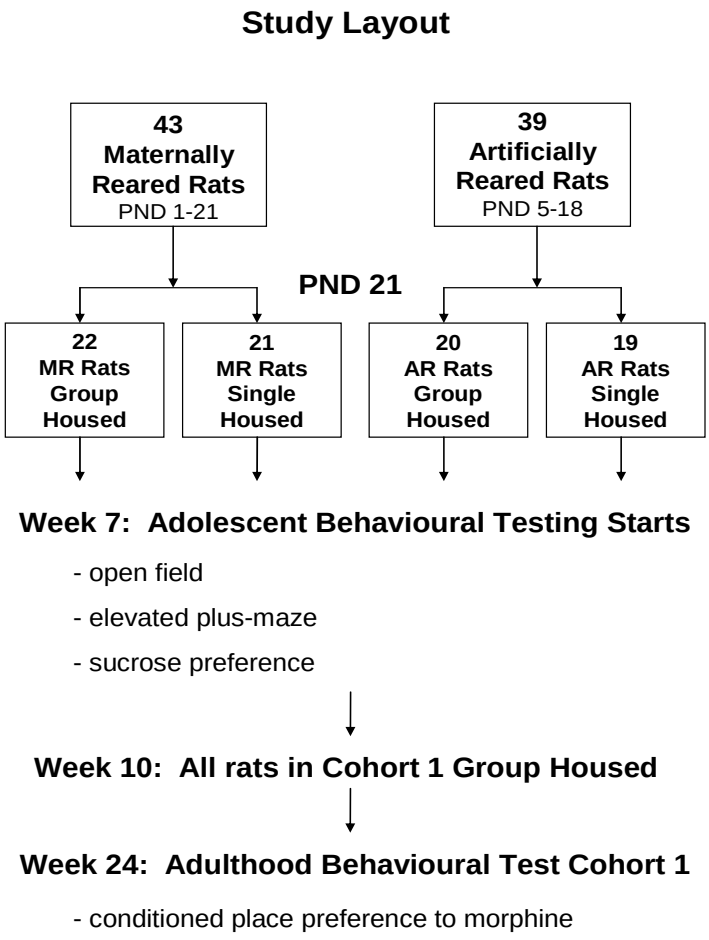


Figure 1. Study design. Rats were reared and tested in two cohorts.

The hypothesis was that AR rats would show greater sensitivity to reward than MR rats, as indicated by increased preference for sucrose and a stronger conditioned place-preference to morphine, as well as increased activity and affective responses in the novel environments of the open-field and the elevated plus-maze. It was also predicted that isolation-housed rats from both the AR and MR groups would show greater sensitivity to reward and increased activity and affective responses compared to group housed rats, but these effects would be augmented in AR rats.

4 METHODOLOGY

4.1 Animals

Ninety-one male offspring of 24 primiparous Sprague-Dawley rat dams were used in this experiment. Rats were obtained from a colony at the University of Waterloo and bred in two cohorts. Pregnant dams were housed individually in clear, Plexiglas cages (43 x 21 x 22 cm), lined with woodchip bedding ("Sani-Chips", Harlan Teklad, Madison, WI). They were maintained at 22 ± 1 °C, under a reversed 12:12hr light:dark cycle, with lights on at 1900 hr, and free access to water and lab chow (Harlan Teklad, Madison, WI). Litters were culled to ten pups within 24 hrs after birth.

Rats from both cohort 1 and 2 took part in adolescent behavioural testing, but only those from cohort 1 were tested as adults. For this latter part of the experiment, the rats from cohort 1 were transferred from the University of Waterloo to Wilfrid Laurier University. The rats continued to be maintained under the same housing conditions as described above. Animals were housed and tested in compliance with the Animals for Research Act of Ontario and the Guide for the Care and Use of Experimental Animals (Canadian Council on Animal Care, 1993).

4.2 Rearing

On post-natal day 5 (gestational day 27), each litter was removed from the mother and home cage and transferred to another cage. The male littermates were weighed and a maximum of six male pups from each litter that were closest to the litter mean weight were selected as subjects in the experiment. They were assigned randomly to one of two conditions: artificial rearing (AR) or maternal rearing (MR). Pups in the AR condition underwent a gastrostomy procedure (described below) and were reared artificially, whereas pups in the MR condition were placed with a foster dam in a litter of ten foster pups to be reared "maternally". There were 11

age-matched foster litters over the two cohorts with an average of four experimental pups per foster litter, resulting in 43 pups in the MR group. MR pups were weighed daily from PND 5 until weaning at PND 21, which involved removal of the entire litter of pups from the mother and placement in another cage for approximately 2 min, followed by individual weighing of experimental pups from that litter. For purposes of identification, the MR pups were marked initially with food coloring, which was renewed daily during weighing. On PND 10, when fur growth was significant, these pups were marked with a 1% solution of picric acid to permanently stain the fur.

4.3 Gastrostomy and Artificial Rearing

Each gastric cannula was composed of a 15 cm long piece of PE-10 intramedic tubing (Clay Adams, Parsipanny, NJ) with a small plastic flange at one end. The other end was attached to a small lead wire contained within Silastic tubing. Prior to the gastrostomy operation, pups were anaesthetized with Isoflurane (4%) via inhalation. The wire end of the cannula was lubricated with MCT oil, inserted into the mouth, guided down the esophagus and pushed out through the stomach wall. The remainder of the cannula was lubricated with MCT oil and pulled gently through the pup until the flanged end came to rest against the inside wall of the stomach. A soft plastic washer was pulled on the gastric cannula and gently pushed against the skin of the pup at the site of penetration on the exterior of the stomach wall. Neosporin antibacterial cream was applied topically at this site. The washer was held in place by a short piece of PE-50 tubing (Clay Adams, Parsipanny, NJ). Next, the wire end of the cannula was passed through a flap of skin over the shoulder and held in place by another PE-50 washer to ensure that any pulling on the cannula would create pressure on the shoulder and not on the stomach wall. All washers

were held in place with a small amount of Superglue. The operation was completed within 90 s and pups recovered from the anaesthetic within 3 – 5 min.

All gastrostomized pups were housed individually in plastic cups (11 mm diameter, 20 mm depth), lined with corn-cob bedding, floating in a water bath at 36 ± 1 °C. Their gastric cannulae were connected via PE-50 tubing to syringes filled with rat milk substitute (RMS) formula (see Ward et al., 1998). The syringes were mounted on timer-controlled infusion pumps (Model #55-4143, Harvard Apparatus, South Natick, MA), and programmed to deliver RMS formula for 20 min every 2 hr. Feeding via gastric cannulae began 1-2 hours after the gastrostomy operation. On the first day of artificial rearing, pups were fed 29% of the mean body weight of six pups per pump, with the volume increasing to 31 % of mean body weight on the next day, and up to 33% of mean body weight thereafter. Two rat pups pulled out their gastric cannulae before PND 17, thus requiring the insertion of oral cannulae. Oral cannula insertion was performed according to the same procedure as that for inserting a gastric cannula, with the exception that the site of implantation was the cheek instead of the stomach. Of the 48 pups that were assigned to the AR condition, 9 pups were euthanized in the first few days of artificial rearing due to abdominal bloating, leaving 39 pups in the AR group.

AR pups were handled twice daily. Each morning, pups were removed from the cups, weighed, and had their cannula tubing flushed with 0.1 ml of distilled water. As well, their anogenital region was stimulated for 45-60 s with a camel-hair paint brush dipped in warm water to induce urination and defecation. At this time, infusion syringes were replaced with new syringes containing fresh diet and the pumps were recalibrated according to the pups' new mean weight. In the evening, pups were once again removed from the cups, their tubing was flushed with distilled water, and their anogenital region was stimulated. This routine was followed until

the evening of PND 17 when the pups were taken off RMS formula feeding. At this time, the gastric cannulae were snipped off close to the abdomen and the pups were placed in small individual cages (20 x 10 x 10 cm) with sawdust bedding. They were supplied with a water bottle, dry food pellets, as well as mashed food consisting of ground lab chow ("Lab Diet", PMI, Richmond, IN) mixed with water. The mashed food was changed twice daily until PND 20, when only dry pellets were provided. Daily weighing of AR rats continued until PND 21.

4.4 Weaning, Housing and Testing

On PND 21, MR rats were weaned from their litters. Subsequently, rats from both rearing groups were subdivided into one of two housing conditions: isolation housing or group housing. Stratified random assignment was used to ensure that pups from each original litter were evenly represented among the groups. A total of four experimental groups were thus formed: artificially reared, isolation housed (AR-ISO, n = 19) artificially reared, group housed (AR-GRP, n = 20), maternally reared, isolation housed (MR-ISO, n = 21), and maternally reared, group housed (MR-GRP, n = 22).

Isolation-housed rats were placed individually in clear Plexiglas cages (43 x 21 x 22 cm) and group housed rats were placed into opaque Plexiglas cages (43 x 35 x 22 cm), 3-4 rats from the same rearing condition per cage. All rats were ear notched for the purpose of future identification and were provided with sawdust bedding, a black plastic tube for enrichment, and free access to water and lab chow. After PND 21, rats were handled once per week for the purpose of weighing and cage changes. Adolescent behavioural testing began following three weeks in isolation or group housing. Three tests were performed over three weeks, one test per week, in the following order: open field test, elevated plus-maze test, sucrose preference test. In the week following the sucrose preference test, isolation housed rats from Cohort 1 were group

housed, 3-4 rats per cage. At 12 weeks of age, the rats from cohort 1 were transported to the animal facility at Wilfrid Laurier University. They arrived two weeks prior to the start of the conditioned place preference testing, allowing for a week of acclimatization to the new facility, followed by a week of daily handling. All testing was done during the dark cycle when rats are normally active.

4.5 Open Field Test

The open field apparatus consisted of a wooden arena (120 x 120 x 30 cm), with a beige floor and walls and with black lines dividing the floor into 124 (10 x 10cm) squares, and an open top. The arena was illuminated by a dim red light. A video camera mounted above the apparatus was relayed to a video recorder and a computerized motion tracking system (EthoVision, Noldus Information Technology, Leesburg, VA), allowing recording and analysis of locomotor activity.

On the day of testing, each rat was brought into the experiment room from the colony room in a transport cage and allowed to acclimatize to the room for a 5 min period. Next, each rat was individually placed in the centre of the open field, facing away from the experimenter, and was allowed to explore the arena for 15 min. At the end of every test session each rat was returned to the colony room and the apparatus was cleaned with an ammonia-based cleaning solution, then sprayed with water and dried with paper towels.

Overall activity during exploration of the open field was measured based on the total distance (m) traveled in the entire arena. Fearfulness was assessed based on the time spent and the number of entries into the centre of the arena (60 x 60 cm square in centre).

4.6 Plus-Maze Test

The elevated plus-maze was a plus-shaped, black Plexiglas maze with two opposing open arms (50 x 10 cm), two enclosed arms (50 x 10 x 40 cm), and a central platform (10 x 10 cm), as

previously described by Pellow et al. (1985). The apparatus was elevated 50 cm above the floor and was illuminated by a dim red light. A video camera mounted above the apparatus was relayed to a video recorder and a computerized motion tracking system (EthoVision, Noldus Information Technology, Leesburg, VA) that allowed recording and analysis of activity in the maze.

On the day of testing, each rat was brought into the experiment room in a transport cage and allowed to acclimatize to the room for a 5 min period. Next, each rat was individually placed in the centre of the elevated plus-maze apparatus, facing towards an open arm, and was allowed to explore the maze for 5 min. At the end of every test session the rat was returned to the colony room and the apparatus was cleaned with an ammonia-based cleaning solution, then sprayed with water and dried with paper towels.

Rats were tested twice on the plus maze, with a 48 h interval between the first and second testing sessions and the testing procedure being identical on both test days. The total number of arm entries was used as the measure of overall activity in this environment. The other dependent measures were: % Open Entries = (# open arm entries/ # total entries) x 100; and % Open Time = (Time in open arms/ Time in open arms + Time in closed arms) x 100). On the first trial these are the standard measure used to assess anxiety (Pellow et al., 1985). However, it has been suggested that on the second trial they might rather be considered a measure of conditioned fear (e.g., File & Zangrossi, 1993; Holmes & Rodgers, 1998).

4.7 Sucrose Preference Test

The sucrose preference test was based on the procedure previously used by Sills and Vaccarino (1994), with some modifications. This test was conducted in the colony room to reduce the novelty of the situation, but instead of using the home cage as did Sills & Vaccarino,

each rat was placed individually into an opaque Plexiglas cage (43 x 21 x 22 cm) lined with paper towels and supplied with a water bottle. Following 5 min of acclimatization to the new cage, two identical pre-weighed jars were placed in the cage. One of the jars was filled with sucrose (table sugar, 4 kCal/g) and the other was filled with powdered chow ("Lab Diet", PMI, Richmond, IN, 4 kCal/g). To avoid spillage, the jars were fitted with metal lids with a hole in the centre and were mounted onto metal holders fastened to a black plastic tube. The position of the jars was varied across days.

Rats were allowed to feed for 1 h, following which the jars were removed and the rats returned to their home cages. Jars were subsequently weighed in order to determine the amount of sucrose and powdered chow consumed. This procedure was repeated for eight days to allow the rats to habituate to the test setting and to the taste of sucrose and powdered chow. The final indicator of sucrose preference was the consumption of sucrose on day 8 proportional to total consumption of both sucrose and chow on that day. Sills and Vaccarino included a subcutaneous saline injection as an acute stressor prior to consumption on day 8. However, as their findings revealed no substantial differences between day 7 and 8 consumption, in the present study rats were not injected on day 8. Weights were converted into kilocalories in order to account for the energy content of both substances.

4.8 Drugs

Morphine sulphate was obtained from the British Drug House (BDH; Toronto, ON, Canada) and mixed with physiological saline to produce a solution of 10 mg/ml (w/v). All injections were administered intraperitoneally at a volume of 1 ml/kg.

4.9 Place Preference Apparatus

The place preference equipment consisted of eight identical black Plexiglas rectangular boxes (60 x 25 x 25 cm) with a steel mesh top, positioned adjacent to each other. The floors of each rectangular box were removable. During conditioning trials, the entire floor of each box consisted of either small holes (1 cm in diameter arranged 1 cm apart from one another) or was lined with strips of sandpaper (2 x 25 cm) adjacent to uncovered portions of the floor producing a pattern of vertical strips of sandpaper and non-sandpapered areas. For half the rats, the hole floor served as the treatment floor and for the other half, the sandpaper (sand) floor was the treatment floor. During the testing trial, three floors were inserted into each rectangular box: one made of holes (25 x 25 cm), one made of strips of sandpaper (25 x 25 cm), and a floor made of smooth black Plexiglas (9 x 25 cm). The hole and sand floors were positioned on each side of the smooth floor and possessed equivalent tactile stimulation properties as the floors used during conditioning. A video camera mounted above the apparatus was relayed to a video recorder and a computerized motion tracking system (EthoVision, Noldus Information Technology, Leesburg, VA) that allowing recording and analysis of the movement of each rat inside each box and the duration of time spent on each of the three floors. The raw data were converted to difference scores by subtracting the time spent (min) on the morphine-paired floor from the time spent (min) on the saline-paired floor for each test.

4.10 Place Preference Procedure

4.10.1 Pre-test

All rats received a 15-min drug-free pre-test in the place preference boxes containing the three floors in order to determine baseline preference for the floors. The time spent on each of the floors was analyzed prior to the start of conditioning trials and it was determined that there

was no obvious bias among the groups for a particular floor (hole or sand). The floors of the apparatus were cleaned with soapy water and dried between rats.

4.10.2 Conditioning

There were four conditioning trial cycles, 48 h apart. During each cycle, rats were injected with morphine sulphate or saline vehicle 10 min prior to placement in the place preference box with either a hole or sand floor for a period of 30 min. Each conditioning cycle consisted of one morphine trial and one saline trial, with each trial separated by 24 h. The order of the morphine trial within a cycle and the particular floor paired with morphine were counterbalanced. After each use, the floors were removed, washed with soapy water and dried.

4.10.3 Test

Three days after the final conditioning trial, all rats were subjected to a place preference test. They were placed in the place preference box containing the three floors (hole, sand, and smooth) for 15 min, and the amount of time spent on each floor was recorded automatically. The floors were removed, washed and dried between each rat.

4.11 Data Analysis

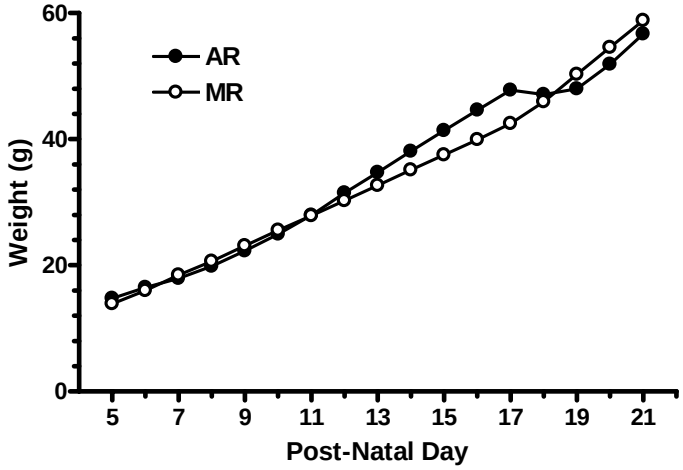
Statistical analyses were carried out using a mixed-factor analysis of variance (ANOVA) with the between-groups factor of Rearing (AR, MR) and Housing (ISO, GRP) and the within-groups factor of Trial (CPP and plus-maze) or Time (weight, open field). In the event of a significant interaction, the simple effects were further analyzed using two-way ANOVA with Rearing and Housing as the independent factors. Analyses were performed using SPSS Version 12.0 (SPSS, Inc., Chicago, IL) and the significance level for all tests was set at $p < 0.05$.

5 RESULTS

5.1 Body Weights

A repeated measures ANOVA of weight from PND 5 to 21 revealed a significant Day × Rearing interaction ($F(16, 1280) = 10032.6$, $p < 0.001$). As shown in Figure 2a, AR rats grew at a faster rate than MR rats until PND17 when AR rats were taken off RMS feeding. After PND 17, MR rats grew at a faster rate such that on PND 21, MR rats weighed more than AR rats ($F(1, 80) = 7.7$, $p < 0.01$). As shown in Figure 2b, there were no significant group differences in the subsequent rate of growth or overall weight over the three weeks before behavioural testing (Week × Rearing: $F(2, 156) = 1.7$, $p > 0.05$, Rearing main effect: $F(1, 78) = 3.2$, $p > 0.05$; Week × Housing: $F(2, 156) = 1.9$, $p > 0.05$; Housing main effect: $F(1, 78) = 1.0$, $p > 0.05$). To determine if the rate of growth was the same for the four experimental groups as they matured, cohort 2 rats were weighed after the last behavioural test (sucrose preference). A two-way ANOVA revealed that there was no effect of Rearing or Housing on body weight at this time, $F(1, 35) = 0.9$, $p > 0.05$, $F(1, 35) = 0.1$, $p > 0.05$, respectively.

a.



b.

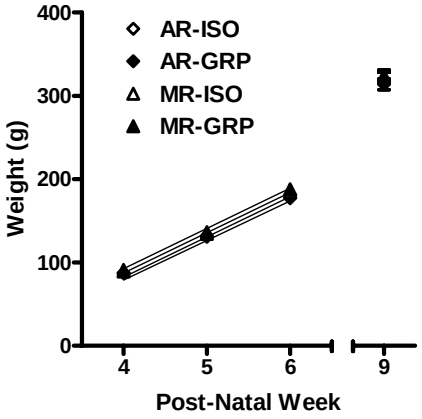


Figure 2. Weight (g, mean, ±SEM*) of AR (n = 39) and MR (n = 43) rats from post-natal day 5 to 21 (a) and during the weeks before and after behavioural testing (b). There was an overall difference in the rate of growth between the two groups, $p < 0.001$. AR rats grew at a faster rate than MR rats until day 17 when AR rats were taken off formula feeding. After day 17, MR rats grew faster. The weights of the four experimental groups equalized during the weeks before behavioural testing (week 4 - 6) and remained equal (week 9). * Error bars too small to be visible.

5.2 Open Field Test

A repeated measures ANOVA of total distance travelled revealed a significant main effect of Rearing over three 5 min intervals in the open field ($F(1, 77) = 6.1$, $p < 0.05$), but no main effect of Housing ($F(1, 77) = 0.8$, $p > 0.05$). As shown in Figure 3a, AR rats were more active in the open field test than MR rats, irrespective of Housing. There was a significant main effect of Interval ($F(2, 154) = 681.6$, $p < 0.001$), but the Interval × Rearing interaction was not significant ($F(2, 154) = 0.7$, $p > 0.5$), indicating that both AR and MR rats habituated to the novelty of the test at the same rate. There were no significant findings with respect to the percentage of time spent in the centre square of the open field or the frequency of entries into the centre square (Figure 3b and 3c). One rat from the AR-GRP group was removed from the above analyses due to technical difficulties with data collection.

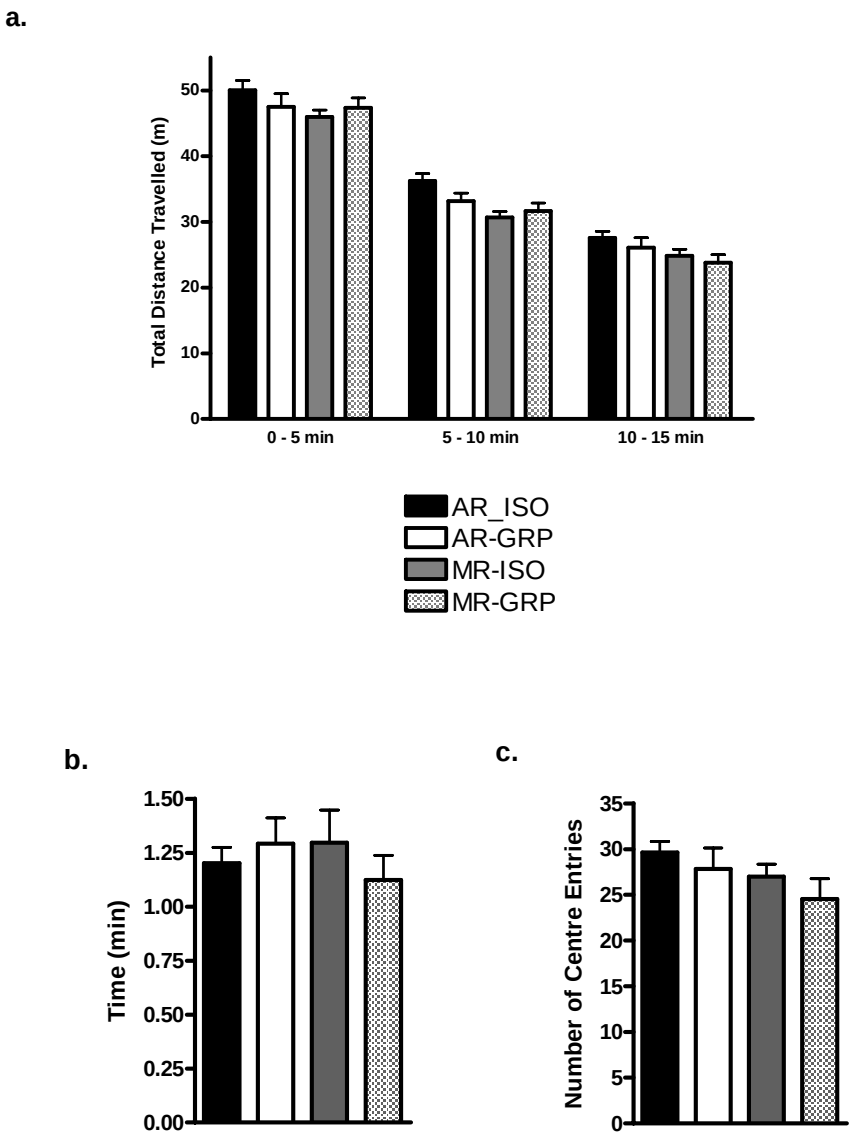


Figure 3. Distance travelled (m, mean, ±SEM) by AR-ISO (n = 19), AR-GRP (n = 19), MR-ISO (n = 21), and MR-GRP (n = 22) rats in the open field (a), time spent in the centre of the open field (b) and number of entries into the centre (c) during the 15 min test. AR rats were more active than MR as indicated by more distance travelled over the three time intervals. Both groups habituation similarly to the test situation over time and did not differ in the time spent in the centre of the open field. There were no significant effects of Housing condition.

5.3 Elevated Plus-Maze

A repeated measures ANOVA of total entries into all arms of the elevated plus-maze on both Trial 1 and 2 revealed a main effect of Rearing, $F(1, 73) = 7.6$, $p < 0.05$, but no significant Trial × Rearing interaction. As shown in Figure 4a, AR rats made more overall arm entries than did MR rats.

With respect to measures in the open arms of the plus-maze, a repeated measures ANOVA on % Open Time showed significant findings for the Trial × Rearing interaction, $F(1, 73) = 10.3$, $p < 0.01$, and the Trial × Housing interaction, $F(1, 73) = 9.9$, $p < 0.01$. A simple effects analysis on each trial revealed a significant effects of Rearing on Trial 1 for % Open Time, $F(1, 73) = 5.8$, $p < 0.05$, but not on Trial 2. As seen in Figure 4b, in Trial 1, AR rats spent less time in the open arms of the plus-maze than did MR rats, but in Trial 2, there was no difference between AR and MR rats. With respect to the Trial × Housing interaction, Figure 4b demonstrates that there were no effects of housing on the first trial, but on the second trial isolation housed rats spent more time in the open arms than did group housed rats. However, the analysis of simple effects on Trial 2 revealed that this Housing effect was not significant. In terms of % Open Entries, there was a main effect of Trial, $F(1, 73) = 5.5$, $p < 0.05$ as well as a Trial × Rearing interaction, $F(1, 73) = 4.2$, $p < 0.05$, and a Trial × Housing interaction, $F(1, 73) = 4.7$, $p < 0.05$. As with % Open Time, an analysis of simple effects on each trial showed a significant effects of Rearing on Trial 1 for % Open Entries, $F(1, 73) = 4.8$, $p < 0.05$, but not on Trial 2. As seen in Figure 4c, in Trial 1, AR rats made fewer entries into the open arms of the plus-maze than did MR rats, but in Trial 2, there was no difference between AR and MR rats. Despite the significant Housing × Trial interaction, analyses of simple effects on Trial 1 and 2 revealed no significant effects of housing on either trial. Figure 4c shows that there were no

striking differences between the housing groups over both trials, with the exception of the AR-ISO group. AR-ISO rats made fewer open arm entries than did AR-GRP rats in Trial 1 but not in Trial 2, which likely resulted in the significant Trial × Housing interaction. There were four rats excluded from the above analyses (Groups: 2 AR - ISO, 1 MR – GRP, 1 MR - ISO) because they fell off the plus-maze during Trial 1 and one rat (MR - GRP) was excluded due to lost data.

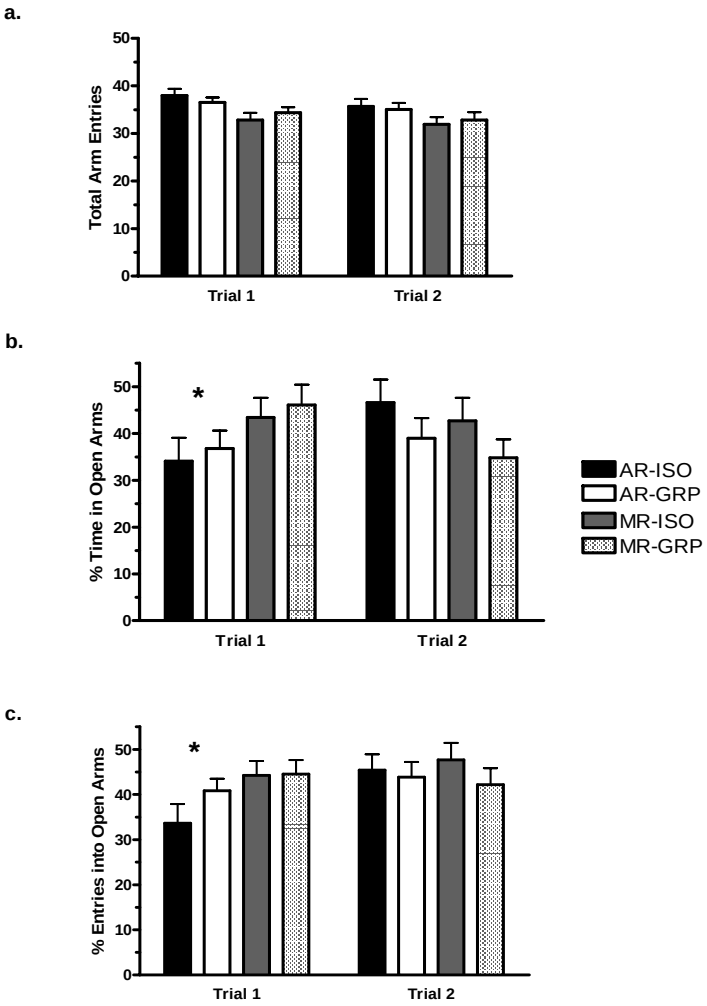


Figure 4. Behaviour of AR-ISO (n = 17), AR-GRP (n = 20), MR-ISO (n = 20), and MR-GRP (n = 20) rats in the elevated plus-maze. The graphs represent total arm entries (a), % time in open arms (b), and % open arms entries (c) over two trials 48 h apart (mean, ±SEM). AR rats made more total arm entries than did MR. In terms of % time in open arms, and % open arm entries, there was a significant interaction where AR rats spent less time in open arms and made fewer entries than did MR rats on Trial 1, but the groups did not differ on Trial 2. A significant interaction of Trial and Housing condition was found for both measures, but an independent analysis of each trial did not reveal any significant effects of Housing in either trial.

* Significant effect of Rearing on Trial 1, $p < 0.05$.

5.4 Sucrose Preference

A two-way ANOVA conducted on the probe trial on day 8 of the sucrose preference test revealed a significant main effect of Rearing for both total caloric intake, $F(1, 74) = 16.6$, $p < 0.001$, and % sucrose consumed, $F(1, 74) = 17.5$, $p < 0.001$. As is evident in Figure 5c, AR rats consumed fewer calories overall than did MR rats. Despite lower overall consumption, AR rats showed a significant preference for sucrose over chow on day 8 compared to MR rats (Figure 4d). There were no significant effects of Housing in any of the above analyses. Four rats were excluded from these analyses because they consumed less than 0.5g of either substance on each of two consecutive days during the last four days of the test (Groups: three AR-GRP, one MR-GRP).

Figures 5a and 5b show the profiles of total caloric intake and % sucrose consumed over the eight days of the sucrose preference test. These figures indicate that results reported on the probe trial were in fact evident after the first few days of exposure to the test conditions, which is supported by the statistical analyses of these data. Repeated measures ANOVAs revealed a significant Day × Rearing interaction, $F(7, 518) = 5.0$, $p < 0.001$, for total caloric intake and a significant third order interaction of Day × Rearing × Housing, $F(7, 518) = 4.8$, $p < 0.001$, for % sucrose consumed. The finding of an interaction of Housing with the other two variables likely stems from the erratic patter of % sucrose consumed over the first few days of the test. As evident in Figure 5b, over the last few days of the test these patters stabilized into the arrangement seen on the probe trial.

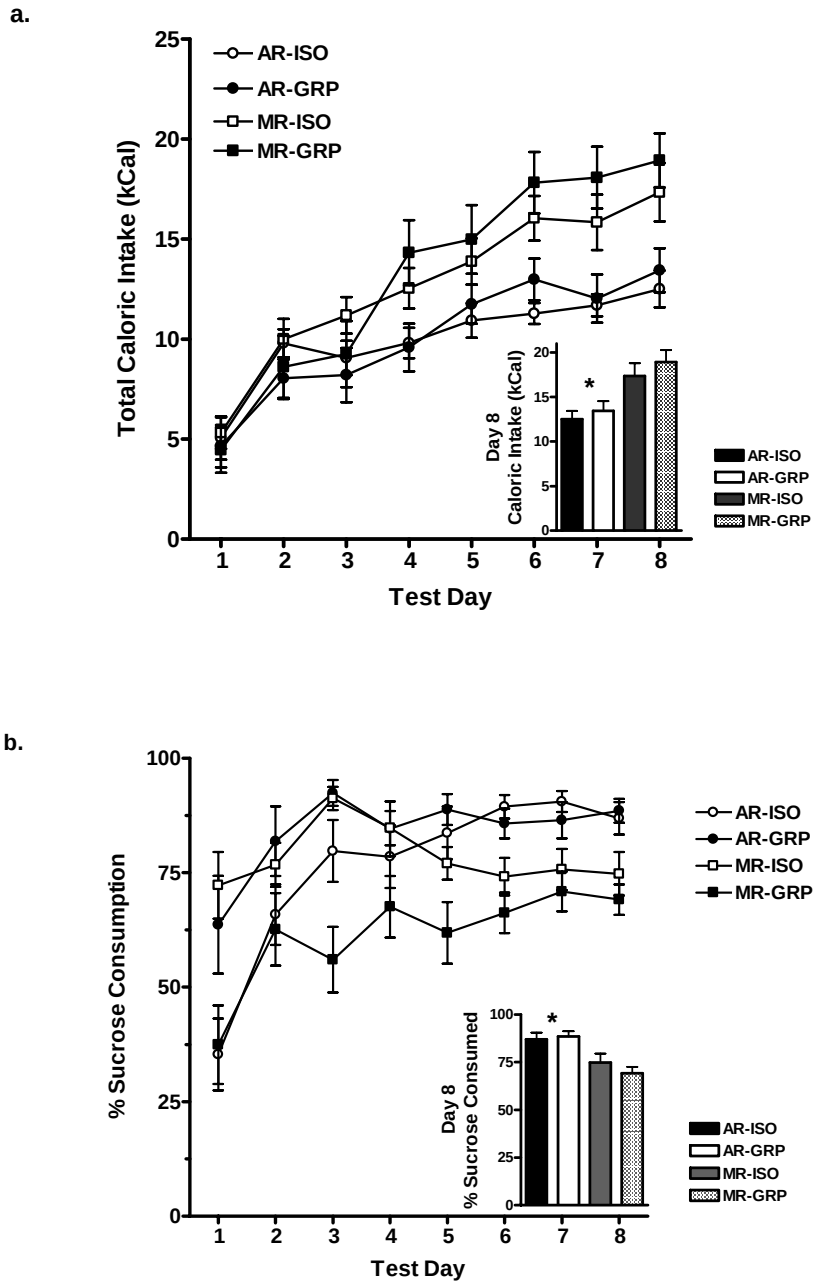


Figure 5. The profiles of overall caloric intake (a) and % sucrose consumed (b) over eight days of the sucrose preference test in AR-ISO (n = 19), AR-GRP (n = 17), MR-ISO (n = 21), and MR-GRP (n = 21) rats (mean, ±SEM). Insets show the results of the probe trial, where AR displayed a significantly greater preference for sucrose relative to chow, but showed lower overall consumption than MR rats. * Significant effect of Rearing on probe trial, $p < 0.05$.

5.5 Conditioned Place Preference to Morphine

Figure 6 represents the time spent on the morphine-paired minus the saline-paired floor during the pre-test and test. A repeated measures ANOVA revealed significant main effects of Rearing, $F(1, 41) = 4.2$, $p < .05$, and Trial, $F(1, 41) = 81.5$, $p < 0.001$, as well as a significant Rearing × Trial interaction, $F(1, 41) = 5.3$, $p < 0.05$. While AR rats did not differ significantly from MR rats on the pre-test trial, AR rats displayed a larger morphine-induced place preference on the test trial, $F(1, 41) = 8.6$, $p < 0.01$, regardless of Housing.

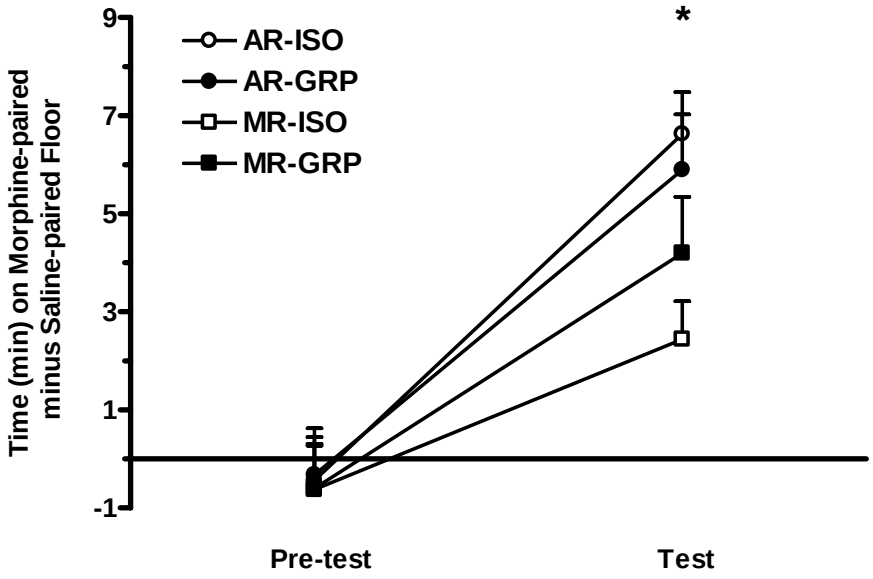


Figure 6. Time (s) spent on the morphine-paired floor minus the saline-paired floor by AR-ISO (n = 10), AR-GRP (n = 11), MR-ISO (n = 12), and MR-GRP (n = 11) rats during the pre-test and test of conditioned place preference to morphine (mean, ±SEM). AR rats did not differ significantly from MR rats on the pre-test trial, but AR rats displayed a larger morphine-induced place preference on the test trial. There were no significant effects of Housing condition.

* Significant effect of Rearing on test trial, $p < 0.05$.

6 DISCUSSION

6.1 Summary of Findings

The purpose of the present study was to investigate the responses of AR rats to reward as well as to novel environments. The results show that AR rats displayed a greater response to both natural and drug-mediated rewards than did MR rats, independently of post-weaning isolation or group housing conditions. Specifically, in the sucrose preference test, adolescent AR rats showed increased preference for sucrose consumption relative to chow. Adult AR rats also showed a greater response to a drug-mediated reward, as seen in their stronger conditioned place-preference to morphine. In addition to their response to reward, the exploratory and affective behaviours of AR rats were assessed in the novel environments of the open field and the elevated plus-maze during adolescence. As predicted, AR rats were found to be more active during a 15 minute open field exploration. However, in contrast to previous findings (Gonzalez et al., 2001), AR rats did not differ from MR rats in emotionality as measured by the time spent and the frequency of entries into the centre of the open field. There were no effects of housing on any measures recorded in the open field. In terms of the elevated plus-maze test, AR rats were found to be more active than MR rats, making more arm entries over both trials. Moreover, in their first experience on the elevated plus-maze, AR rats made fewer entries and spent less time on the open arms, indicating increased anxiety. Conversely, in their second exposure to the plus-maze 48 h later, which is thought to measure conditioned fear, they did not differ from MR rats in terms of the number of entries or the time spent in the open arms. As with the other tests, there were no significant effects of housing on behaviour in the plus-maze, with the exception of an interaction of trial with housing on both measures of open arm exploration (discussed below).

On the whole, the above findings support the premise that the motivational system of AR rats is altered by their developmental experiences.

6.2 Response to Novel Environments

With regard to exploratory and affective behaviour in AR rats, the present findings are generally in accordance with previous reports. In earlier studies (Tonkiss et al., 1987; Gonzalez et al., 2001), AR rats were found to show more exploratory activity; in this study they were also more active than MR rats in the elevated plus-maze. Although it has been previously reported that AR also displayed a reduced affective response, as indicated by enhanced exploration of the centre of the open field (Gonzalez et al., 2001), this was not shown in the present study. Nevertheless, differences between AR and MR rats were found on another measure of affective behaviour, the elevated plus-maze, but the direction of effect was opposite to that found by Gonzalez et al. in the open field. While Gonzales et al. reported less emotionality in AR rats, in the present study AR rats displayed greater avoidance of the open arms of the elevated plus-maze, which suggests greater anxiety in this situation. This discrepancy may stem from the fact that the rats tested here were adolescent males (6 weeks of age), whereas Gonzalez et al. tested adult females. Such differential effects of age (reviewed in: Spear, 2000) and sex (reviewed in: Kelly et al., 1999; Palanza, 2001) on the performance of rats in similar test situations have been commonly reported. This warrants further examination of the influence of these factors on the behavioural profile of AR rats.

With respect to the second exposure to the elevated plus-maze, neither group of rats showed the typical response of conditioned fear reported in the literature (e.g., File & Zangrossi, 1993; Holmes & Rodgers, 1998). In this situation, rats are expected to display an increased avoidance of the open arms compared to the first exposure due to the contingency formed during

the first trial between the internal state of anxiety and the open arms. However, an inspection of Figure 4b and 4c reveals that in the present study the only group that showed any increase in avoidance of the open arms on the second trial compared to the first was the MR-GRP group, whereas both AR groups showed a decrease in avoidance and the MR-ISO group showed no substantial change from one trial to the other. Interestingly, the group that displayed the expected response to this test situation is also the only group in the study that was not isolated during either the pre-weaning or the post-weaning period. These findings suggest that such isolation may interfere with the formation of the conditioned fear response in this environment and that artificial rearing and post-weaning isolation may actually have differential effects on this behaviour.

6.3 Response to Rewards

There is one concern with the findings related to sucrose preference, which is that the total caloric intake of AR rats during the 1 h test period was lower than that of MR rats. Thus, although AR rats showed a strong sucrose preference, the actual amount consumed was less than that of MR rats. The reasons for this are not clear. As the two groups did not differ in body weight at the time of testing, it is unlikely that their overall food consumption differed. One possibility is that AR rats show a larger neophobic response to a novel food (e.g. Burns et al., 1996). This is supported by the data shown in Figure 5a, where it is evident that all animals consumed fewer calories at the beginning of the eight day test period, but MR rats increased their consumption over time to a greater degree than did the AR rats. This is an issue to be explored in future studies.

It is also important to note that the present study did not explore all aspects of the motivation system in AR rats. According to Berridge and Robinson (2003), the appetitive and

consummatory response to reward involves different neural processes: motivation to obtain the reward (termed incentive salience or “wanting”) and affective response (termed hedonic impact or “liking”). With reference to the present study, the sucrose preference test and conditioned place preference test represent measures of incentive salience rather than direct measures of hedonic impact. Accordingly, the findings suggest that the incentive salience of sucrose and a morphine-paired floor are increased in AR rats, i.e., that they have an enhanced "wanting" response. However, further studies, using an established measure of liking, the taste reactivity test (Grill & Norgren, 1978) would be needed to address whether there were also associated changes in their hedonic response to sucrose. Additional experiments are also necessary to characterize the functioning of the neurotransmitter systems involved in reward processing that were not directly probed in the present study. Since morphine acts mostly on opioid receptors, the response to this drug may not reveal the full profile of behavioural responses of AR rats to drugs that act on the reward circuitry. For example, several studies have reported a positive correlation between sucrose preference and response to DA agonists, such as cocaine or amphetamine (Sills & Vaccarino, 1994; Gosnell, 2000; DeSousa et al., 2000). Drugs such as these have the potential to provide further insight into reward processing in AR rats.

6.4 Isolation Housing

As discussed earlier, isolation housing in early life has been associated with long lasting developmental consequences similar to those of MS (Hall, 1998; Weiss, et al., 2004). Therefore, the hypothesis of the present study was that isolation housing would exacerbate the effects of artificial rearing. However, the results indicate that there were no substantial effects of isolation housing evident in any of the outcome measures. This finding was surprising in the context of the existing literature, particularly with respect to the MR group, but it may be explained in

relation to the influence of handling received by these rats during daily weighing from PND 5 to 21. Previous studies have shown that some of the effects of isolation housing can be prevented by daily handling during the neonatal period (e.g., Gentsch et al., 1988; Reboucas & Schmidek, 1997), which may have also been the case in the present study. This may be confirmed in future studies by using an additional non-handled control group. With respect to the AR rats, the effects of isolation housing may have been overshadowed by the experience of artificial rearing, such that any influence of isolation housing would not have been detectable due to the ceiling effect on behavioural performance caused by artificial rearing. Alternatively, it is possible that isolation housing did not have any further influence on artificially reared rats due to their habituation to the conditions of social isolation.

6.5 Possible Developmental Mechanisms

As reviewed in the introduction, previous work has shown that rat pups which experience lower daily amounts of maternal stimulation, either naturally or through experimental manipulations such as MS, show alterations in the development of their HPA axis. This is partly evident in their enhanced glucocorticoid activation under basal conditions or in response to stressors (Francis & Meaney, 1999; Caldji et al., 2000b, Ladd et al., 2000). As glucocorticoid hormones have been implicated in the effects of stress on the motivation system (Marinelli & Piazza, 2002), one of the mechanisms by which different early experiences may influence the development of this system is through altered glucocorticoid functioning (Meaney et al., 2002; Brake et al., 2004; Hall et al., 1999). It has been reported that the adult offspring of mothers that naturally exhibit low frequency maternal stimulation towards pups display reduced glucocorticoid feedback sensitivity compared to mothers that stimulate their pups more frequently (Liu et al., 1997; Francis et al., 1999c). This is thought to result from decreased

expression of glucocorticoid receptors in these rats, which leads to less efficient binding of circulating glucocorticoids and subsequent deficits in the induction of negative feedback mechanisms that dampen the activation of the HPA axis. Recent analyses of epigenetic modification of the expression of glucocorticoid receptors in these two groups of rats support this hypothesis (Weaver et al., 2004). Similarly, MS rats have also been shown to display decreased cortical and hippocampal glucocorticoid receptor messenger RNA density when compared to EH rats (Ladd et al., 2004), which is congruent with their enhanced glucocorticoid activation (Ladd et al., 2000). These findings suggest that the degree of expression of glucocorticoid receptors in the brain may be modified by different early experiences. It has been shown that glucocorticoids alter the transmission of DA in the NAc, one of the key brain regions involved in mediating the response to reward (Barrot et al., 2000), thus changes in glucocorticoid feedback sensitivity may also impact DA functioning in this region. This possibility is supported by studies investigating the neurochemical profile of MS rats. For instance, decreased levels of DA transporter proteins, which act in the reuptake of DA molecules from the synapse following DA release, have been found in the NAc and the caudate/putamen regions of MS rats compared to EH rats (Meaney et al., 2002). Furthermore, MS rats showed enhanced release of DA in comparison to EH rats in response to both amphetamine and K+ perfusate (Hall et al., 1999), as well as a potentiated increase in NAc levels of DA following the mild stress of a tail pinch (Brake et al., 2004). Overall, the accumulating evidence points towards the role of altered HPA development in mediating the effects of different early experiences on the functioning of the motivation system.

The above findings suggest a possible mechanism for the effects of artificial rearing seen in the present study, but there is little known to date about the stress responsiveness of AR rats to allow for any specific conclusions. Unlike maternal separation, where rats are subject to less

maternal stimulation than handled rats, the artificial rearing environment, while involving complete separation from the dam, does incorporate repeated handling and stimulation by the experimenter. In this respect, the AR procedure may not lead to the same HPA axis activation seen in maternal separation. The only evidence thus far indicates that infant (Ward et al., 2004) and adult (Kelly et al., 1991) AR rats did not differ in stress-induced CORT levels from MR controls, unless they were also food-deprived for 24 h, where they showed a lower CORT response to an injection stressor compared to similarly deprived MR rats (Ward et al., 2004). There is some evidence that increasing the frequency and duration of anogenital stroking in AR rats ameliorated their hyperactivity (Gonzalez et al., 2001). This is congruent with the observation that lower frequency of maternal stimulation is associated with increased stress responsiveness in the adult offspring, whereas higher frequency of maternal behaviours is associated with decreased stress responsiveness (Francis & Meaney, 1999; Caldji et al., 2000b). However, more frequent stimulation did not reverse all of the changes seen in AR rats: although some improvements were seen in AR rats that were stimulated more frequently, their subsequent performance of maternal behaviours was not restored up to the level of control MR rats (Gonzalez et al., 2001; Levy et al., 2003). This suggests that factors other than the frequency of stimulation may also be influential in the development of motivated behaviours in AR rats. Further research in which the amount of stimulation of the AR rats is varied and in where the hormonal profile in response to a stressor is characterized over time will be needed to resolve these issues.

Another contributing factor to the altered motivational responsiveness observed in AR rats may be their impoverished or inappropriate early experience with opportunities to learn instrumental responses where the rewards are contingent on behaviour. Numerous studies

indicate that rat pups display the capacity to engage in instrumental learning involving a variety of reinforcers within a few days after birth (Johanson et al., 1984; Camp & Rudy, 1988; Flory et al., 1997). Moreover, the induction of various aspects of maternal care is encouraged by the behavioural responding of pups. For instance, the suckling of several pups is necessary to induce an active arch-back nursing stance, to promote milk ejection, and to enhance the quality of milk (Yagil et al., 1976; Stern, 1996), whereas ultrasonic vocalizations of pups prompts the dam to engage in maternal behaviours (Jans & Leon, 1983; Hashimoto et al., 2001). In this way, the actions of pups are reinforced by the rewarding properties of stimuli that are obtained from the mother, including food, tactile stimulation, or warmth, which provide incentive for instrumental learning. In contrast to rats reared with their mother, AR rats are passive in terms of their behavioural interactions with the nurturing aspects of their environment. As such, it is possible that they may form Pavlovian stimulus-reinforcer contingencies (e.g. vibration of the infusion pump predicting the delivery of food), but the conditions of artificial rearing eliminate their opportunities to learn to associate specific actions with reward. In this way, their likelihood of learning the appropriate contingencies between behaviour and reward is reduced. During this time of ongoing brain development, it is possible that such deprivation of contingent interaction with rewarding stimuli may change the course of development of the motivational system, which is then manifest in later life as altered responsiveness to reward.

6.6 Limitations

One limitation of the artificial rearing method is that it does not allow for complete maternal deprivation to occur until PND 4 or 5 (e.g., Gonzalez & Fleming, 2002; Ward et al., 2004) due to the nature of the surgery and feeding procedures. As such, it is not possible to completely control the influence of the mother and the litter during the entire neonatal period

using this method. However, the rat brain is still undergoing substantial developmental changes after PND 4 or 5, including the development of regions involved in the motivation circuitry (Clancy et al., 2001), thus the artificial rearing approach does allow for considerable influence over this process.

A further limitation of the present study design is the lack of an untreated control group to account for the effects of handling. Although the MR-GRP control group did not receive either of the two experimental treatments, rats in this group were handled daily during the pre-weaning stage from PND 5 to 21 for the purpose of weighing. As discussed earlier, such handling may have ameliorated the effects of isolation housing on the behavioural performance of MR rats, but this procedure was necessary in order to monitor the growth of the AR group by comparing it to the MR group. Although the lack of a handling control group complicated the interpretation of some of the results, it did not compromise the internal validity of the present study.

Lastly, the use of repeated testing in this study limits the generalizability of the findings. As several behavioural tests were conducted in sequence, the rats used in the present study were not naïve to testing after the completion of the open field test. However, due to the extensive resources and the time required to obtain a cohort of artificially reared rats, it would have been difficult to collect all the behavioural data on naïve rats. Additionally, although the rats received extra handling by the experimenter during repeated testing, the behavioural tests used were not extremely stressful and most likely did not have the long-lasting effects that would severely alter the performance of rats on subsequent tests.

6.7 Future Perspectives

There are numerous questions regarding the motivational responses of AR rats that require further investigation. The present results point towards several areas where future research is required to fully assess the behavioural, neurochemical, and endocrine components of these behaviours. As indicated earlier, all aspects of the motivational system in AR rats were not examined in this study because the measures used did not dissociate between the different components involved in the response to reward, including incentive salience (wanting), hedonic evaluation (liking) and the association of behaviour with reward (learning) (Berridge & Robinson, 2003). A more complete investigation would require an analysis of the AR rats' pattern of approach towards a rewarding substance, their latency to consume it, as well as their efficiency in finding it during consecutive trials. A recent study by Robinson et al., 2005 employed an interesting experimental design that allowed for simultaneous testing of these behavioural components in response to a sucrose reward in an appetitive T-maze task. Although it has been suggested that such an experiment should also employ the taste reactivity test to correctly asses liking (Berridge, 2005), a similar approach, plus the taste reactivity test, could be used in AR rats.

There is no information to date regarding the neurochemical profile of AR rats in relation to brain regions that comprise the motivation circuitry, thus analyses that measure neurotransmitter levels, their precursors and metabolites and their receptor expression in these regions would be useful in future studies. Interestingly, recent research has identified CREB (cAMP-response element binding protein) activation in the NAc as a key factor involved in place conditioning to morphine, in sucrose preference as well as in the expression of anxiety-like behaviour in the elevated plus-maze (Barrot et al., 2002; Barrot et al., 2005). This suggests that

future studies examining the motivational processes of AR rats should also include indicators of the expression of CREB in relation to both appetitive and aversive stimuli.

In order to provide more information regarding the mechanisms behind the behavioural effects of artificial rearing further studies should also focus on a detailed analysis of the endocrine profile of AR rats in relation to stress hormones. This would offer more insight into the questions of whether the observed behavioural changes are mediated by alterations in the development of the HPA axis, or whether other potential mechanisms should be considered.

7 CONCLUSION

In summary, in the present study, AR rats were found to display enhanced responsiveness to natural and drug-mediated rewards compared to MR control rats, as well as elevated activity and greater affective response during exploration of a novel environment. These findings indicate that AR rats have altered motivational responses towards both appetitive and aversive stimuli. The artificial rearing method provides a useful model for controlled investigation of the influence of various early environmental stimuli on neural and behavioural development by circumventing confounds associated with the complexities of mother-pup interactions. The present findings support the potential of this model to contribute to the understanding of the role of early experience in the development of the motivational system. Further research using artificial rearing may shed more light on the neural mechanisms underlying the relationship between early experiences and mental disorders related to behavioural motivation, including addiction, depression, and obsessive compulsive disorder.

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